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POSTER PRESENTATIONS

P1

TLR-independent activation of NK cells during systemic inflammation.

O Rasid*, J-M Cavaillon
Cytokines and Inflammation, Institut Pasteur, Paris, France

Critical Care 2014, **18(Suppl 2):**P1; doi:10.1186/cc14004

Introduction: During the course of systemic inflammation, most of the immune cell types get activated to a certain degree as part of, or contributing to, the cascade of physiopathological events. Whether for some cells, classically phagocytes of the innate immune system, it is clear that direct sensing of pathogen-associated molecular patterns leads to activation initiating systemic inflammation, the picture is not so clear for natural killer (NK) cells. While NK cells have been shown to express toll-like receptors (TLR), the role of these receptors on NKs during systemic inflammation has not been directly addressed.
Methods: To directly assess the role of TLR expression on NK cells we used an adoptive transfer model in which NKs purified from the spleens of WT, TLR4KO and TLR2/4DKO mice were transferred intravenously to $RAG2^{-/-}\gamma c^{-/-}$ (devoid of T, B and NK cells). Five days after reconstitution the mice were challenged intraperitoneally with conventional or TLR-grade lipopolysaccharide (LPS). Immune cell activation and production of IFNγ by NK cells was determined after 6 hours by FACS analysis.
Results: We observed no differences in reconstitution of the recipient mice with NK cells from different backgrounds suggesting no difference in trafficking and survival of the transferred cells. At 6 hours after LPS challenge, WT, TLR4KO or TLR2/4DKO NK cells recovered from the spleen and lungs of $RAG2^{-/-}\gamma c^{-/-}$ mice showed comparable levels of CD69 activation marker expression. Intracellular labeling for IFNγ in NK cells also revealed no significant differences.
Conclusion: Whether there is a role for direct TLR signaling on NK cells remains the objective of further investigations; however, our data show that in the course of a systemic inflammatory process, like endotoxinemia, the expression of TLR2 and TLR4 by NK cells makes no difference in terms of their activation and secretion of IFNγ

P2

Role of 6-hour, 12-hour, and 24-hour lactate clearance in mortality of severe sepsis and septic shock patients.

V Herwanto[1*], KC Lie[2], S Suwarto[2], CM Rumende[3]
[1]Department of Internal Medicine, University of Indonesia, Jakarta, Indonesia; [2]Division of Tropical Medicine and Infectious Diseases, Department of Internal Medicine, University of Indonesia, Jakarta, Indonesia; [3]Division of Respirology and Critical Care, Department of Internal Medicine, University of Indonesia, Jakarta, Indonesia

Critical Care 2014, **18(Suppl 2):**P2; doi:10.1186/cc14005

Introduction: Lactate is one of biomarkers used for risk stratification, resuscitation target, and death prediction in sepsis [1,2]. Interpretation of lactate clearance was proven more superior than single measurement to evaluate resuscitation adequacy and to determine prognosis [3,4]. This study aimed to find out whether mean differences of 6-hour, 12-hour, and 24-hour lactate clearance were observed between nonsurvivors and survivors of acute phase mortality in severe sepsis and septic shock patients.
Methods: The study design was prospective cohort. Subjects were collected by consecutive sampling from the emergency department, hospital ward, and ICU at Cipto Mangunkusumo Hospital, Jakarta. Lactate levels were measured at 6, 12, and 24 hours, and subjects were subsequently followed to evaluate 3-day mortality. To determine their association with mortality, we used mean difference analysis of those three lactate clearance periods between nonsurvivors and survivors. In addition, to determine the cutoff value, we used receiver operator curve analysis.
Results: Eighty-one subjects were included in this study. Eighty of 81 were followed until 12 hours, and 72 out of 80 were followed until 24 hours. Twenty-five subjects (31%) did not survive within 3 days of hospitalization. Only 24-hour lactate clearance had significant median difference (-17.0% in nonsurvivor vs. 15.2% in survivor group; $P = 0.034$). The best cutoff value for 24-hour lactate clearance was -6.0% (AUC 0.744, sensitivity 62.5% and specificity 87.5%, positive predictive value 58.8% and negative predictive value 89.1%, relative risk 5.39). From multivariate analysis, 24-hour lactate clearance was proven to be an independent predictor of mortality.
Conclusion: Median of 24-hour lactate clearance was significantly lower in nonsurvivors of severe sepsis and septic shock patients. Its cutoff value was -6.0%.
Acknowledgements: Gratitude to Dr Imam Subekti, head of Internal Medicine Department, Faculty of Medicine University of Indonesia, and Dr Aida Lydia, head of Internal Medicine study program, Faculty of Medicine University of Indonesia for their guidance and useful critiques of this research work. Thanks also to Dr Kuntjoro Harimurti, Dr Esthika Dewiasty, and Ms Utami for their advice and assistance in doing methodology and statistic of this study

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P3

High frequency of myeloid-derived suppressor cells in sepsis patients, with the granulocytic subtype dominating in Gram-positive cases.

H Janols[1,2*], C Bergenfelz[2], R Allaoui[2], A-M Larsson[3], L Rydén[4], S Björnsson[5], S Janciauskiene[6], M Wullt[1], A Bredberg[7], K Leandersson[2]
[1]Department of Infectious Diseases, Skane University Hospital, Lund University, Malmo, Sweden; [2]Center for Molecular Pathology, Skane University Hospital, Lund University, Malmo, Sweden; [3]Department of Laboratory Medicine, TCR, MV, Lund University, Lund, Sweden; [4]Department of Surgery, Lund University Hospital, SUS, Lund, Sweden; [5]Cytometry Laboratory and Department of Laboratory Medicine, Skane University Hospital, Lund University, Malmo, Sweden; [6]Department of Pulmonology, Hannover Medical School, Hannover, Germany; [7]Department of Medical Microbiology, Skane University Hospital, Lund University, Malmo, Sweden
Critical Care 2014, **18(Suppl 2):**P3; doi:10.1186/cc14006

Introduction: Myeloid-derived suppressor cells (MDSCs) constitute a heterogeneous population of immature myeloid cells that potently suppress immune responses. They were originally identified in cancer patients and have since been reported to occur also in chronic inflammation, autoimmunity and even bacterial infections. Human MDSCs are commonly divided into monocytic (Mo-MDSCs) and granulocytic (PMN-MDSCs) subtypes. To what extent the *bona fide* cancer MDSCs are representative of the proposed MDSCs found in other diseases is not well known. PMN-MDSCs have previously been found to be enriched among low-density granulocytes (LDGs) in density gradient centrifuged blood.
Methods: In this study we analyzed potential MDSCs in sepsis patients with different causative microorganisms, using total peripheral blood as compared to density gradient centrifuged blood.
Results: We found a high frequency of typical $CD14^{+}HLADR^{low}$ Mo-MDSCs in all sepsis patients, whereas the typical PMN-MDSCs as well as a prominent $CD14^{low}$ PMN-MDSC-like population appeared preferentially in Gram-positive cases (Figures 1 to 3). The $CD14^{low}$ PMN-MDSC variant was demonstrated to suppress T-cell proliferation *in vitro* via a ROS-dependent mechanism, to display an increased IL-10:TNFα ratio, and to present with signs of immaturity: blast morphology and low cytokine levels (Figures 4 and 5).
Conclusion: We conclude that a spectrum of cells with MDSC features are enriched in sepsis, and that microbial origin of sepsis contributes to the substantial interindividual patient variation in MDSC pattern.

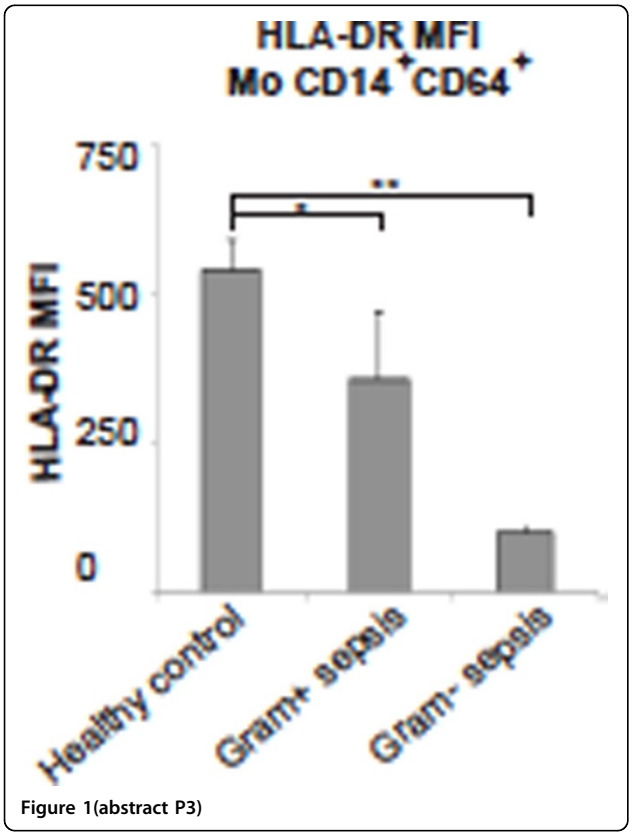

Figure 1(abstract P3)

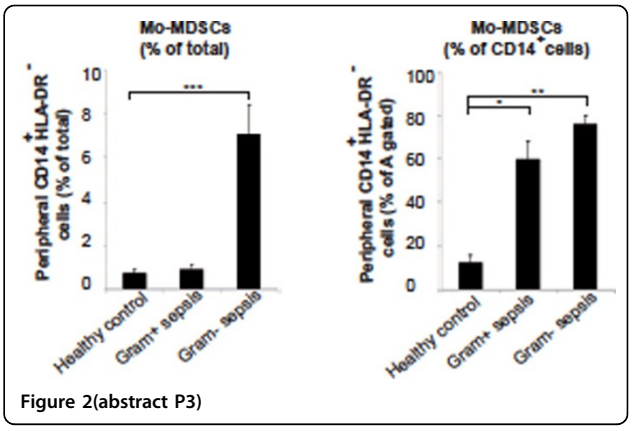

Figure 2(abstract P3)

P4

Selective decontamination using antibiotics in ICU patients: counterfactual protection versus contextual hazard toward bacteremia incidences.

JC Hurley
Melbourne Medical School, University of Melbourne and Ballarat Health Services, Ballarat, Australia
Critical Care 2014, **18(Suppl 2):**P4; doi:10.1186/cc14007

Introduction: Among methods for preventing pneumonia and possibly also bacteremia in ICU patients, selective digestive decontamination (SDD; topical with or without protocolized parenteral antibiotic) appears most effective within randomized concurrent controlled trials (RCCTs) [1]. However, whether parenteral antibiotic is required, and whether SDD actually increases pneumonia incidences in SDD RCCTs versus the broader ICU pneumonia evidence base, remain unresolved [2,3]. The purpose of this analysis is to test for counterfactual and contextual effects of the topical and parenteral SDD components on the bacteremia incidence versus the broader evidence base related to the patient group at risk of VAP.
Methods: Bacteremia incidence proportion data were extracted from component (control and intervention) groups from studies investigating antibiotic (SDD) or nonantibiotic methods of VAP prevention. Both the counterfactual and the contextual effects of SDD factorized as topical or protocolized parenteral exposures were estimated using random-effects meta-analysis of study and group level data. Studies without any prevention methods under study constituted the reference category (benchmark groups).
Results: As a counterfactual within RCCTs, SDD when given as combined topical and parenteral antibiotic appears to halve the bacteremia incidence (odds ratio (OR) 0.59; 0.48 to 0.73; n = 18 studies). As a contextual however, the mean bacteremia incidence among 27 control groups (17.1%; 13.1 to 22.1%) and 12 intervention groups receiving topical antibiotic alone (16.2%; 9.1 to 27.3%) from SDD RCCTs is double that of 36 benchmark groups (8.3; 7.0 to 10.8%) and 19 control groups from studies of nonantibiotic methods (7.7%; 5.2 to 11.1). The upward dispersion in bacteremia incidence among component groups from SDD RCCTs away from this benchmark is striking with all but two of the 27 control groups and all but two of 12 SDD intervention groups that did not receive PPAP being above this benchmark.
Conclusion: The major contextual hazard of SDD toward bacteremia among ICU patients is inapparent within individual studies. The apparent protection in SDD RCCTs is spurious as the SDD counterfactual is conflated by the strong contextual effect with partial mitigation by SDD

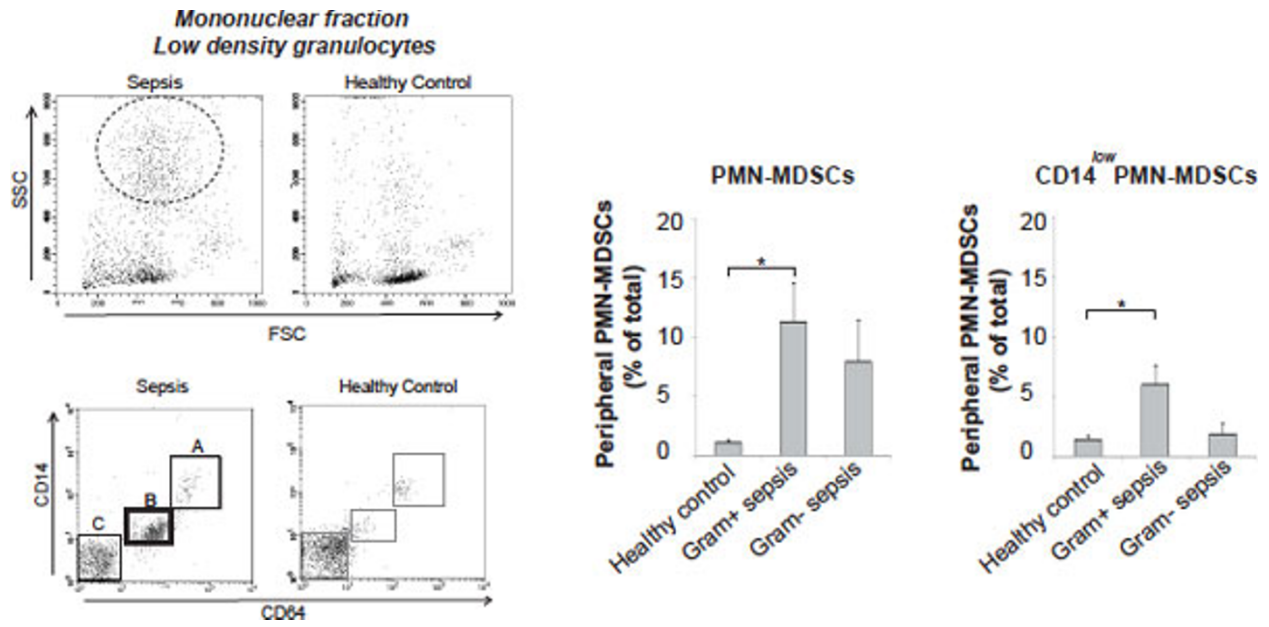

Figure 3(abstract P3)

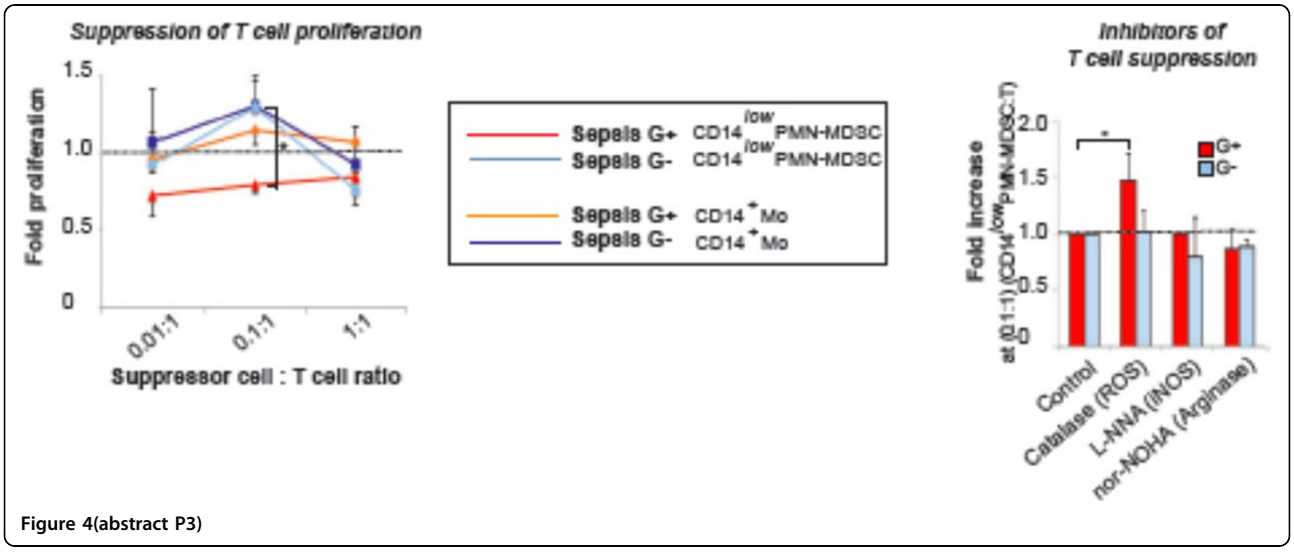

Figure 4(abstract P3)

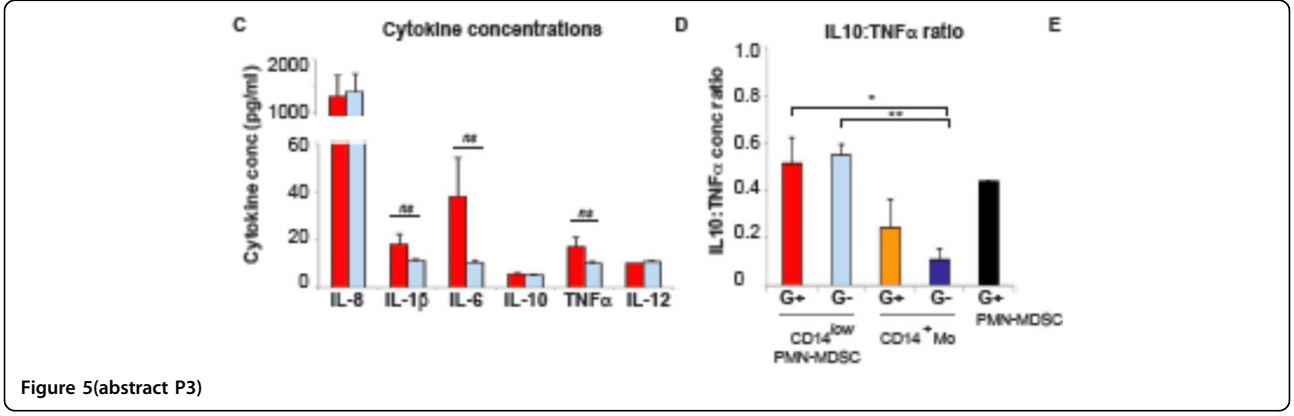

Figure 5(abstract P3)

protocolized parenteral antibiotic. Not only is the safety of SDD within the ICU environment unclear, but this SDD contextual effect may conflate the apparent SDD counterfactual effect on the incidence of bacteremia, as with VAP.

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P5

Thalidomide exerts protective immunomodulatory action during *Klebsiella pneumoniae* B5055-induced acute lung infection in BALB/c mice.

V Kumar*, S Chhibber
Department of Microbiology, Panjab University, Chandigarh, India

Critical Care 2014, **18(Suppl 2):**P5; doi:10.1186/cc14008

Introduction: Thalidomide (α-naphthylimidoglutarimide), a psychoactive drug that readily crosses the blood-brain barrier, has been shown to exhibit anti-inflammatory, anti-angiogenic, immunomodulatory properties through a mechanism that is not fully established. Keeping these properties in mind, we have tried to find out the anti-inflammatory and immunomodulatory properties of thalidomide in mouse model of acute inflammation by introducing *Klebsiella pneumoniae* B5055 in BALB/c mice via the intranasal route.
Methods: Acute lung infection (ALI) or pneumonia in BALB/c mice was induced via instillation of selected dose (10^4 CFU/ml) of bacteria (that is, *K. pneumoniae* B5055) intranasally. Mice were observed for 7 days and lungs were isolated on designated days for studying difference in bacterial load and other proinflammatory mediators using standard biochemical methods and ELISA.
Results: The intranasal instillation of bacteria in this mouse model of acute pneumonia-induced inflammation led to significant increase in neutrophil infiltration into the lungs. This was further accompanied by an increased production of proinflammatory cytokines (that is, TNFα and IL-1α) and other mediators of inflammation (that is, malondialdehyde (MDA), myeloperoxidase (MPO) and nitric oxide (NO)) in the lung tissue. The animals, which received thalidomide alone orally or in combination with augmentin, 30 minutes prior to bacterial instillation into the lungs via intranasal route, showed significant ($P \leq 0.05$) decrease in neutrophil influx into the lungs. A significant ($P \leq 0.05$) decrease in the production of proinflammatory cytokines (that is, TNFα and IL-1α) and other biochemical mediators of acute inflammation (that is, MDA, MPO, and NO) was also observed in this group. But the augmentin treatment alone did not decrease these proinflammatory mediators significantly ($P \geq 0.05$) as compared to the control group.
Conclusion: We therefore conclude that thalidomide ameliorates lung inflammation induced by *K. pneumoniae* B5055 without significantly ($P \leq 0.05$) decreasing the bacterial load in the lung tissue whereas augmentin takes care of bacterial proliferation. Hence, it can be used as an adjunct therapy along with antibiotics as an anti-inflammatory or an immunomodulatory agent in case of acute lung infection or pneumonia.

P6

Impact of purulent complications and sepsis on cardiovascular system in patients with type 2 diabetes.

E Shalaeva*, B Babadjanov, U Pulatov, N Dadabayeva, A Shalaeva
Republican Center of Purulent Surgery and Complications of Diabetes, Tashkent Medical Academy, Tashkent, Uzbekistan

Critical Care 2014, **18(Suppl 2):**P6; doi:10.1186/cc14009

Introduction: Purulent complications in patients with type 2 diabetes are usually severe, often complicated by sepsis and require emergency surgery. Noncardiac surgery is associated with a 7 to 11% complication rate and mortality of 0.8 to 1.5% [1], up to 42% are cardiac reasons [2]. After surgery, 2% of patients suffer major cardiac complications [3], and 8% show evidence of significant myocardial injury [2]. The aim of this study was to identify the impact of purulent complications and sepsis on cardiovascular system in patients with type 2 diabetes.
Methods: We analyzed 112 consecutive patients (54 men and 58 women) aged 57.2 ± 8.4 years with purulent-necrotic complications (gangrene, phlegmon, and abscess) of type 2 diabetes and sepsis in 2013. We compared laboratory and instrumental data (blood tests, ECG, echocardiography and others), which were previously obtained in the same patients receiving inpatient treatment before sepsis (2011 to 2012).
Results: Gangrene of lower extremities in 59 (52.7%) prevailed among purulent complications. After the development of sepsis we detected in all patients significantly increased heart rate, respiratory rate per minute, leukocytosis, anemia, worse glucose metabolism and renal function (Table 1). Congestive heart failure became more severe. This was confirmed by decrease of left ventricle ejection fraction (55.2 ± 5.1% before sepsis vs. 49.3 ± 4.1% after) and increase brain natriuretic peptide (291.4 ± 34.5 ng/ml vs. 395.2 ± 28.1 ng/ml, $P < 0.001$). Prior sepsis in 66 (58.9%) of patients with arterial hypertension was observed, after in 88 (78.6%). After admission to

Table 1(abstract P6) Hemodynamic parameters and blood tests in patients with purulent complications of type 2 diabetes and sepsis

Parameter	Before sepsis (n = 112)	After sepsis (n = 112)	P value
Heart rate (beats/minute)	78.4 ± 15.2	112.5 ± 18.9	< 0.001
Respiratory rate (breaths/minute)	18.0 ± 2.0	29.5 ± 5.5	< 0.001
Systolic BP (mmHg)	155.7 ± 35.4	154.2 ± 58.5	n.s
Diastolic BP (mmHg)	90.4 ± 10.3	91.9 ± 8.6	n.s
Left ventricle ejection fraction (%)	**55.2 ± 5.1**	**49.3 ± 4.1**	**0.033**
Fasting plasma glucose (mmol/l)	8.4 ± 2.5	15.4 ± 4.8	< 0.001
Two-hour plasma glucose (mmol/l)	10.2 ± 2,8	19.9 ± 3.3	< 0.001
HbA1c (%)	**8.4 ± 0.5**	**12.1 ± 0.5**	**< 0.001**
Hemoglobin (g/l)	121.5 ± 12.5	105.4 ± 11.7	0.04
White count (10^3)	6.7 ± 1.2	14.4 ± 2.1	< 0.001
Fibrinogen (mg%)	411.6 ± 103.6	715.4 ± 215.5	< 0.001
Blood urea (mmol/l)	6.1 ± 2.9	8.8 ± 2.5	0.011
Blood creatinine (mmol/l)	88.4 ± 18.5	105.6 ± 17.3	0.02
Brain natriuretic peptide (ng/ml)	291.4 ± 34.5	395.2 ± 28.1	< 0.001

BP, blood pressure; HbA1c, glycosylated hemoglobin A1c

Table 2(abstract P6) Cardiovascular comorbidity in patients with type 2 diabetes before and after purulent-necrotic complications and sepsis

Parameter	Before sepsis (*n* = 112)	After sepsis (*n* = 112)
Insulin dependence	**42 (37.5)**	**112 (100)**
Normal blood pressure (110 to 139 mmHg)	46 (41.1)	11 (9.8)
Arterial hypertension	**66 (58.9)**	**88 (78.6)**
First degree (140 to 159 mmHg)	33 (29.5)	21 (8.9)
Second degree (160 to 179 mmHg)	21 (18.6)	43 (38.4)
Third degree (>180 mmHg)	12 (10.7)	24 (21.4)
Arterial hypotension (<90 mmHg)	-	13 (11.6)
CAD, stable angina	108 (94.6)	82 (73.2)
FC I	18 (16.1)	-
FC II	29 (25.9)	18 (16.1)
FC III	52 (46.4)	45 (40.2)
FC IV	9 (8.0)	19 (17.0)
CAD, unstable angina	**4 (3.6)**	**17 (15.2)**
Acute myocardial infarction	-	**13 (11.6)**
Postinfarction cardiosclerosis	7 (6.3)	7 (6.3)
Atrial fibrillation	7 (6.3)	7 (6.3)
Supraventricular arrhythmia	3 (2.7)	12 (10.7)
Ventricular arrhythmia	14 (12.5)	36 (32.1)
Congestive heart failure	112 (100)	112 (100)
FC II (NYHA)	76 (67.8)	26 (23.2)
FC III (NYHA)	36 (32.1)	65 (58)
FC IV (NYHA)	-	21 (18.7)
Abscesses of the lower extremity	-	22 (19.6)
Phlegmon of the lower extremity	-	31 (27.7)
Gangrene of lower extremity	-	59 (52.7)

Data presented as *n* (%). CAD, coronary artery disease; FC, functional class; NYHA, New York Heart Association

the centre, patients had no signs of septic shock. In 13 (11.6%) patients, the perioperative period was complicated by acute myocardial infarction, which was accompanied by a fall in blood pressure. We detected an increase of the functional class of stable angina, congestive heart failure, 4.2 times increased incidence of unstable angina, 2.6 times ventricular and four times supraventricular extra systole after septic complications (Table 2).

Conclusion: After the development of purulent complications and sepsis in patients with type 2 diabetes, we observed increased incidence of arterial hypertension, arrhythmias, worsened severity of coronary artery disease and congestive heart failure. Perioperative risk of acute myocardial infarction amounted to 11.6%.

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P7

Severity of sepsis in patients with acute purulent destructive pulmonary disease depending on the presence of type 2 diabetes: impact on the forecast.

A Babobekov*, B Babadjanov, S Atakov, E Shalaeva
Republican Center of Purulent Surgery and Complications of Diabetes, Tashkent Medical Academy, Tashkent, Uzbekistan
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Introduction: Lung abscesses and gangrene are the most severe clinical manifestation and outcome among acute purulent destructive pulmonary disease (APDPD). Mortality ranges from 10 to 35%, and in the presence of diabetes increases up to 30 to 90% [1]. The main reason for this is the generalization of infection (sepsis), leading to the development of multiple organ failure [2,3]. The aim of this study was to identify the severity of sepsis in patients with APDPD depending on the presence of type 2 diabetes, and the impact on the forecast.

Methods: During the period 2012 to 2013, we examined 408 patients aged 48.5 ± 12.5 years (258 men/150 women) who underwent surgical treatment for APDPD. The patients were divided into two groups: 144 patients with type 2 diabetes, and controls (*n* = 246). We carried out computed tomography, ECG, echocardiography, laboratory biochemical testing, and bacteriological analysis of pathologic material and blood samples.

Results: Patients with type 2 diabetes had much more complications and cases of severe sepsis and septic shock (Table 1). Bacteriological analysis of the pathologic material showed Gram-positive bacteria in 35 to 45%, anaerobic association in 55 to 65%, pathological fungi in 50 to 60%. The patients with type 2 diabetes had much more time from the onset of the first symptoms of lung disease prior to admission (12.5 ± 3.5 vs. 7.5 ± 2.5 days, $P = 0.002$), and the duration of inpatient treatment was significantly longer (13.8 ± 5.5 vs. 7.1 ± 3.4 days, $P = 0.001$). Only 53 (36.8%) of patients with type 2 diabetes and 68 (29.5%) without it had bacteriological positive blood culture. The analysis of the distribution of pathogens in groups is presented in Figure 1. Patients with diabetes had more *Candida* spp.

Table 1(abstract P7) Clinical symptoms and severity of sepsis in patients with acute purulent destructive pulmonary disease depending on the presence of type 2 diabetes

Data	Type 2 diabetes (*n* = 144)	Control (*n* = 264)
Acute lung abscess	59 (40.9)	122 (46.2)
Necrotizing pneumonia	47 (32.6)	98 (37.1)
Lung gangrene	38 (26.4)	44 (16.7)
Empyema	88 (61.1)	81 (30.7)
Pyopneumothorax	16 (11.1)	9 (3.4)
Mediastinitis	34 (23.6)	16 (6.1)
Body temperature >38°C/<36°C	98 (68.1)/21 (14.6)	261 (98.9)/3 (1.1)
Respiratory rate >20/minute	144 (100)	264 (100)
Heart rate >90 beats/minute	138 (95.8)	242 (91.7)
$PaCO_2$ <32 mmHg	144 (100)	264 (100)
Leukocytes >12,000/<4,000 cells/mm^3	111 (77.1)/13 (9.1)	202 (76.5)/11 (4.2)
Renal failure, oliguria	42 (29.2)	34 (12.9)
Increase liver enzymes	34 (23.6)	45 (17.1)
Systolic blood pressure <90 mmHg	33 (22.9)	51 (19.3)
Sepsis	101 (70.1)	223 (84.5)
Severe sepsis	25 (17.4)	29 (11.0)
Septic shock	18 (12.5)	12 (4.5)

Data presented as *n* (%)

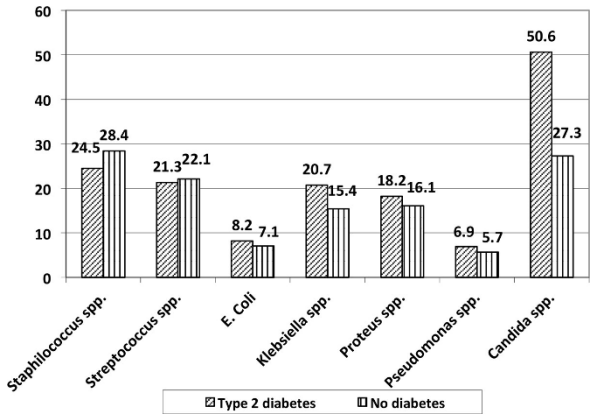

Figure 1(abstract P7)

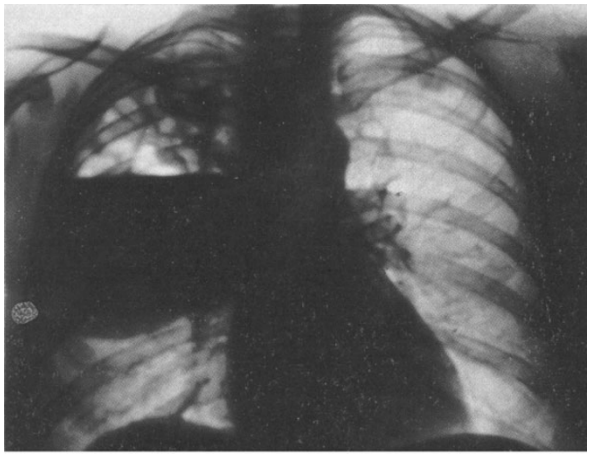

Figure 2(abstract P7) Man, 42 years old, with right lung abscess after admission to the centre

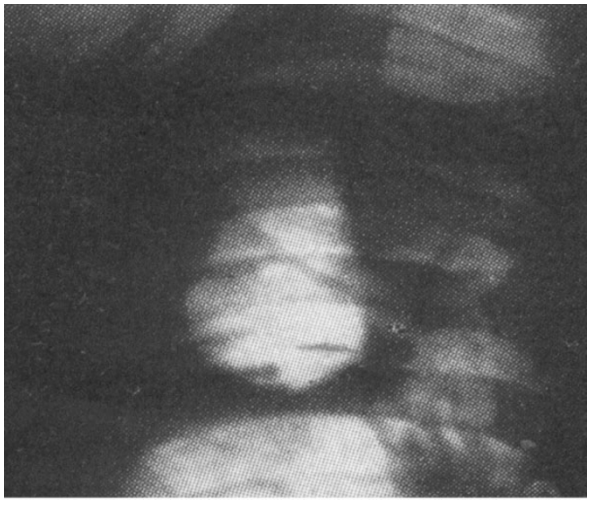

Figure 3(abstract P7) The same patient on the fourth day after drainage of the abscess

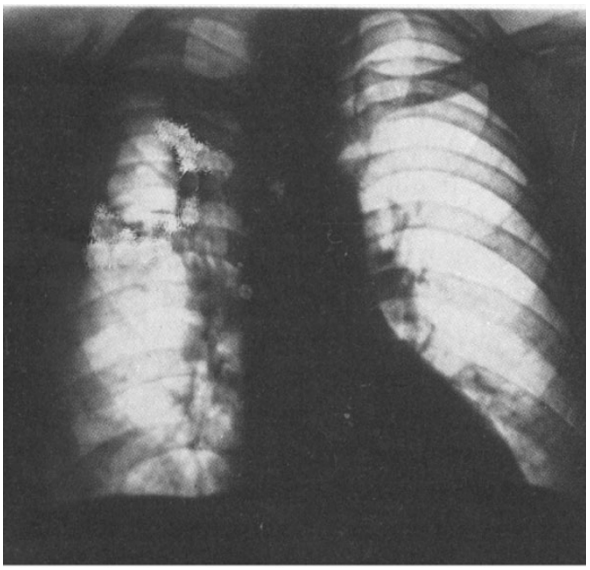

Figure 4(abstract P7) The same patient before discharge, 14 days in dynamics

(Figure 1). Figures 2, Figure 3 and Figure 4 present the X-ray dynamics of a 42-year-old man with lung abscess. Clinical recovery in patients with type 2 diabetes was significantly worthy compared to controls (45 (31.2%) vs. 153 (57.9%)), mortality rate 48 (33.3%) versus 39 (14.7%), respectively.

Conclusion: In patients with acute purulent destructive pulmonary disease and type 2 diabetes, severe sepsis and septic shock more often prevailed, inpatient mortality rate was 2.27 times higher, compared to patients with normal glucose metabolism.

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P8

Risk factors and incidence of mediastinitis in patients with lung abscess and sepsis.

E Shalaeva*, B Babadjanov, B Janabaev, A Shalaeva
Republican Center of Purulent Surgery and Complications of Diabetes, Tashkent Medical Academy, Tashkent, Uzbekistan

Critical Care 2014, **18(Suppl 2):**P8; doi:10.1186/cc14011

Introduction: Mediastinitis is a life-threatening condition, which is accompanied by high rates of mortality in cases of delayed diagnosis and inadequate treatment. The aim of the study was to identify the risk factors and incidence of mediastinitis in patients with lung abscess and sepsis.

Methods: In 2013, 218 consecutive patients (83 women and 135 men) with lung abscess and sepsis aged 45.8 ± 13.2 years were operated. They had a full range of laboratory and instrumental examinations, including echocardiography and computed tomography.

Results: Aerobic-anaerobic association in sputum was revealed in all patients with lung abscess and sepsis, *Candida* spp. in 34 (15.6%). Blood culture was positive only in 59 (27%) patients, which had not previously received antibacterial therapy (polymicrobial flora including *Staphylococcus* and *Streptococcus* specimen). Empyema was diagnosed in 123 patients (56.4%), 31 (14.2%) of them were complicated by mediastinitis. The main clinical symptoms of mediastinitis were hyperthermia (100%), dysphagia (83.8%), dyspnea (80.6%), chest pain (61.3%), orthopnea

Table 1(abstract P8) Risk factors for mediastinitis in patients with lung abscess and sepsis

	With mediastinitis (*n* = 31)	Without mediastinitis (*n* = 187)	*P* value
Gender, male/female, *n* (%)	7 (22.6)/24 (77.4)	128 (68.5)/59 (31.5)	0.001
Type 2 diabetes mellitus, *n* (%)	26 (83.9)	42 (22.5)	0.001
Body mass index	32.3 ± 5.3	27.8 ± 6.1	0.031
Hemoglobin (g/l)	87.9 ± 9.2	128.4 ± 18.4	< 0.001
Fibrinogen (mg%)	800 ± 200	533 ± 166	< 0.001
End-diastolic volume of the left ventricle (ml)	139 ± 27	108 ± 28	0.024
End-systolic volume of the left ventricle (ml)	66.1 ± 8.2	46.8 ± 10.4	< 0.001
Left ventricular ejection fraction (%)	52.4 ± 2.2	57.2 ± 3.4	0.002

(61.3%), and tachycardia (87.1%). The computer tomography revealed an increase in mediastinum size with accumulation of fluids and fluid in the pleural cavities (100%), free gas in the mediastinum (45.1%), enlarged mediastinal lymph nodes (45.1%), and fluid in the pericardium cavity (48.4%). To analyze the risk factors, we include 31 patients with lung abscess and sepsis complicated by mediastinitis in the first group, and 187 patients without mediastenitis in the second group. Groups were similar in age (46.1 ± 8.2 years vs. 45.8 ± 13.2 years). A total 77.4% of patients with mediastinitis were women who suffered from type 2 diabetes (HbA1c = 9.7 ± 1.4%), congestive heart failure and anemia. Significant differences in the groups according to the data of laboratory and instrumental studies are presented in Table 1.

Conclusion: In total, 14.2% of patients presented with lung abscess and sepsis complicated by mediastinitis, more commonly in women with diabetes mellitus, obesity, anemia and reduced ejection fraction of the left ventricle.

P9

Impact of KDO in biological activity of Re-LPS.

I Prokhorenko*, S Zubova, D Kabanov, S Grachev
Institute of Basic Biological Problems, Pushchino, Moscow Region, Russia
Critical Care 2014, **18(Suppl 2):**P9; doi:10.1186/cc14012

Introduction: The minimal biological active structure of endotoxins (lipopolysaccharides (LPS)) is Re-LPS (KDO2-lipid A), which consist of lipid A and two (or three) molecules of 3-deoxy-D-manno-2-octulosonic acid (KDO) [1,2]. Biological activity of endotoxins is defined in general by the number and distribution of acyl residues on the lipid A backbone [3]. Recently it has been reported that KDO-treated RAW 264.7 cells exhibited a gene expression pattern similar to that in LPS-treated cells. These authors revealed that free KDO participated in crosstalk between Toll-like receptors (TLR) and G protein-coupled receptors and so that regulated activators and repressors of immune signaling [4]. LPS-dependent TLR4-triggered activation of target cells leads to specific changes in the levels of surface receptors and induces synthesis of proinflammatory cytokines [5]. However, the dependence of these processes on the structural composition of LPS is not well understood. To extend our knowledge in this field, the effects of free KDO as well as KDO as covalently linked to lipid A constituent of Re-LPS on expression of TLR4, CD11b and CD14 receptors and TNFα synthesis in whole human blood have been investigated.

Methods: Human blood was incubated with Re-LPS from *Escherichia coli* JM103 or *Salmonella enterica sv Typhimurium* SL1181 (100 ng/ml) or with lipids A from *E. coli* F583 or *S. enterica* sv Minnesota R595 (80 ng/ml) or with ammonium salt of KDO (20 ng/ml) at 37°C in 5% CO_2-humidified atmosphere for 2 or 6 hours to determine receptor expression or TNFα release, respectively. Receptor expression was monitored by EPICS XL-MCL flow cytometer using Alexa Fluor 488 anti-TLR4 (HTA125), anti-CD11b (ICRF44) and anti-CD14 (HCD14) antibodies. Human TNF-α ELISA Kit II was exploited to TNFα determination.

Results: Re-LPS *E. coli* or Re-LPS *S. enterica* differentially affected receptor expression in comparison to their respective lipids A. Free KDO in the equimolar concentration as it exists in KDO2-lipid A (Re-LPS) did not influence the level of CD14 but downregulated the expression of TLR4 and CD11b (Figure 1). Tenfold increased KDO concentration did not affect further the receptor expression. The addition of KDO2 to lipid A *E. coli* - that is, applying KDO as covalently linked constituent of Re-LPS - led to upregulation of CD14 and TLR4 but downregulated CD11b expression. The expression of TLR4 was most pronounced upregulated by Re-LPS *S. enterica* but in the case of CD14 and CD11b this Re-LPS had an opposite effect in comparison to *E. coli* endotoxins (table in Figure 1). Lipid A *S. enterica* was a less potent TNFα inductor than that from *E. coli* (Figure 2). This may be explained by the differences in lipid A composition determining lipid A affinity to target receptor(s). LPS *E. coli*, as had been shown early, caused MyD88-dependent fast NF-κB degradation (rapid TNFα response) whereas LPS *S. enterica* induced MyD88-independent signaling (delayed TNFα response) [5]. In our study, free KDO did not stimulate TNFα release. KDO2 as a constituent of Re-LPS *S. enterica* increased significantly the TNFα-inducing activity of lipid A *S. enterica* but this effect was not so distinguished between Re-LPS *E. coli* and lipid A *E. coli* (Figure 2).

Conclusion: Free KDO in the used concentration was inactive in regulation of TLR4, CD11b and CD14 expression and did not induce TNFα release but its impact in biological activity was detected when KDO was applied as constituent of Re-LPS. This may be explained by the effect of KDO on the spatial conformation of Re-LPS.

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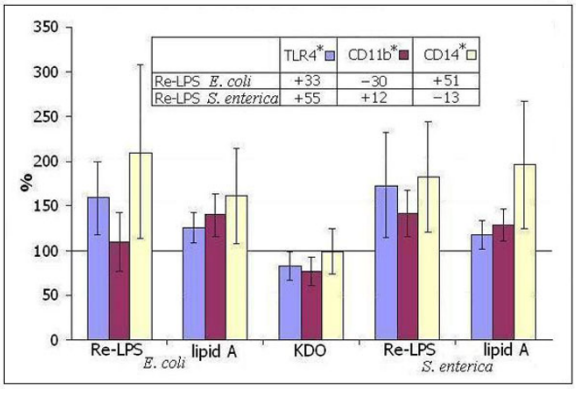

Figure 1(abstract P9) Expression of TLR4, CD11b and CD14 on monocytes after incubation of whole blood with Re-LPS, lipid A or KDO. Presented are the results of six independent experiments. Alteration in receptor expression was calculated according to the control level that had been expressed as 100%. *Changes in receptor expression were calculated as %MnIX [KDO2-lipid A] - %MnIX [lipid A]

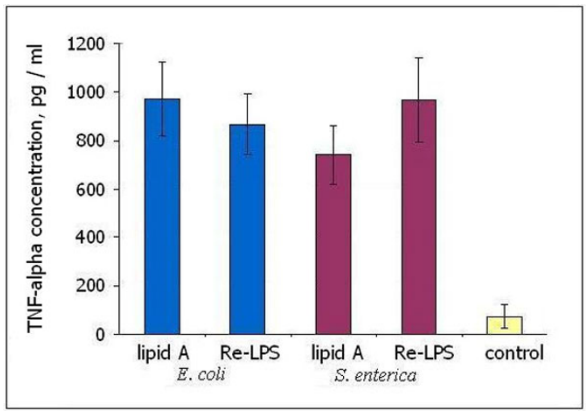

Figure 2(abstract P9) Production of TNFα after incubation of whole blood with lipid A or Re-LPS

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P10

Two novel formulae are superior to procalcitonin for prediction of sepsis in trauma patients.

H-p Liang*, H Jin, Y Xiao, Z Liu
State Key Laboratory of Trauma, Burns and Combined Injury, Research Institute of Surgery, The Third Military Medical University, Chongqing, People's Republic of China
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Introduction: The purpose of this study was to verify the predictive value of two novel formulae and compare them with that of procalcitonin (PCT) for predicting sepsis in trauma patients.
Methods: We performed a retrospective study of trauma patients treated at Daping Hospital in Chongqing, China and Affiliated Hospital of Zunyi Medical College between 2010 and 2013. Patients ≥16 years old, admitted to hospital after injury within 24 hours and with length of hospital stay ≥48 hours were included. Predictive ability of two formulae based on LD_{50} values of the Injury Severity Score (ISS) and New Injury Severity Score (NISS) were verified: ISS/LD_{50}ISS+SIRS score and NISS/LD_{50}NISS+SIRS score, and then were compared with the most common used biomarker PCT. LD_{50} values for different age groups and genders were obtained in our former study. The statistical performance of the two formulae and PCT to predict sepsis was evaluated using receiver operating characteristic (ROC) curve analysis.
Results: Two hundred and twenty-one trauma patients' data were enrolled in the study, including 44 females and 177 males. The average age of the patients was 44.77 ± 15.01 years. The performance of the ISS/LD_{50}ISS+SIRS score and the NISS/LD_{50}NISS+SIRS score was equivalent (area under the ROC curve (AUC) = 0.816 vs. 0.819, $P > 0.05$) and both performed better than PCT (AUC = 0.592, $P < 0.05$) in predicting posttraumatic sepsis. For the ISS/LD_{50}ISS+SIRS score, the cutoff value was 2.38, with a positive predictive value of 78.08%, a negative predictive value of 81.33%, a sensitivity of 89.06%, a specificity of 65.59%, a positive likelihood ratio of 2.59, a negative likelihood ratio of 0.17, a Youden index of 0.5465, an odds ratio of 15.52, and an accuracy of 79.19%. For the NISS/LD_{50}NISS+SIRS score, the cutoff value was 2.4677, with a positive predictive value of 79.70%, a negative predictive value of 75.00%, a sensitivity of 82.81%, a specificity of 70.97%, a positive likelihood ratio of 2.85, a negative likelihood ratio of 0.24, a Youden index of 0.5378, an odds ratio of 11.78, and an accuracy of 77.83%.
Conclusion: The two novel formulae ISS/LD_{50}ISS+SIRS score and NISS/LD_{50}NISS+SIRS score performed well and were both better than PCT in predicting sepsis post trauma. The value of the two formulae can be easily calculated in real time and can identify the high-risk patients susceptible to sepsis. This method may become an effective way to guide the early assessment and treatment in trauma patients.

P11

Inhibitory effects of evodiamine on zymosan-induced inflammation: inactivation of NF-κB by inhibiting IκBα phosphorylation.

X Fan*, J-y Zhu, Z Liu, J Yan, Q Ma, H-p Liang
State Key Laboratory of Trauma, Burns and Combined Injury, Research Institute of Surgery, The Third Military Medical University, Chongqing, People's Republic of China
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Introduction: Inflammation is a host defense reaction against pathogenic infection. In this process, inflammatory cytokines contribute to combat against infection, but excess cytokines will lead to tissue damage. Nonsteroidal anti-inflammatory drugs and corticosteroids are commonly used for regulating these inflammatory mediators and treatment of inflammatory disorders. But these drugs are not sufficient for clinical practice due to their adverse effects, so new anti-inflammatory drugs are still needed. Evodiamine (EVO), an important alkaloidal component extracted from the fruit of Evodiae fructus, possesses the property of analgesia, antiemesis, and vascular dilatation. Its anti-inflammatory effect and underlying mechanism were investigated using a zymosan-induced inflammatory model.
Methods: *In vitro*, RAW264.7 cells and primary peritoneal macrophages were treated with different doses of EVO (25, 50 or 100 μM) for 1 hour prior to incubation with zymosan (100 μg/ml), and the effects of EVO on protein and mRNA levels of proinflammatory cytokines were determined by ELISA and qRT-PCR, respectively. *In vivo*, peritonitis was induced in C57BL/6 mice by zymosan (500 mg/kg) injection, and the effects of EVO (10 mg/kg) on plasma cytokine levels and organ injury were evaluated. Activation of the NF-κB signal pathway was investigated by ELISA-based Trans-AM transcription factor NF-κB p65 kit, immunocytochemistry and western blotting.
Results: EVO effectively suppressed the expression of IL-1β, IL-6 as well as TNFα in both protein and mRNA level *in vitro*. It can also attenuate zymosan-induced DNA-binding activity of NF-κB, which was achieved through the inhibitory effects on the phosphorylation of inhibitory kappa B α (IκBα) and p65 nuclear translocation, but there was little association with mitogen-activated protein kinase activation. *In vivo*, treatment with EVO could ameliorate inflammatory cell infiltration and vascular ectasia induced by zymosan in both lung and intestine tissues. EVO can markedly decrease the level of TNFα and IL-6 in plasma, and effectively downregulate expression of IL-6, TNFα and myeloperoxidase in both lung and intestine. Moreover, cell apoptosis in organs was also attenuated by treatment of EVO. The underlying mechanism that a decrease in the phosphorylation of IκBα and the subsequent transcription activity of NF-κB was also confirmed.
Conclusion: Taken together, our data demonstrate that EVO displays anti-inflammatory actions *in vitro* and *in vivo* by suppressing the phosphorylation of IκBα and inactivating NF-κB, which suggests that EVO is a potential therapeutic agent against inflammatory disorder.

P12

Imaging in severe sepsis and septic shock: is early radiological identification of occult sources of infection needed?.

A Creamer*, J Keep
Emergency Department, Kings College Hospital, London, UK
Critical Care 2014, **18(Suppl 2):**P12; doi:10.1186/cc14015

Introduction: The importance of imaging in establishing the focus of infection is recognised in current guidelines for the management of severe sepsis [1], with decisions regarding timing and modality of imaging left to the physicians' clinical judgement. In the emergency department (ED), clinical assessment combined with bedside investigations of chest X-ray

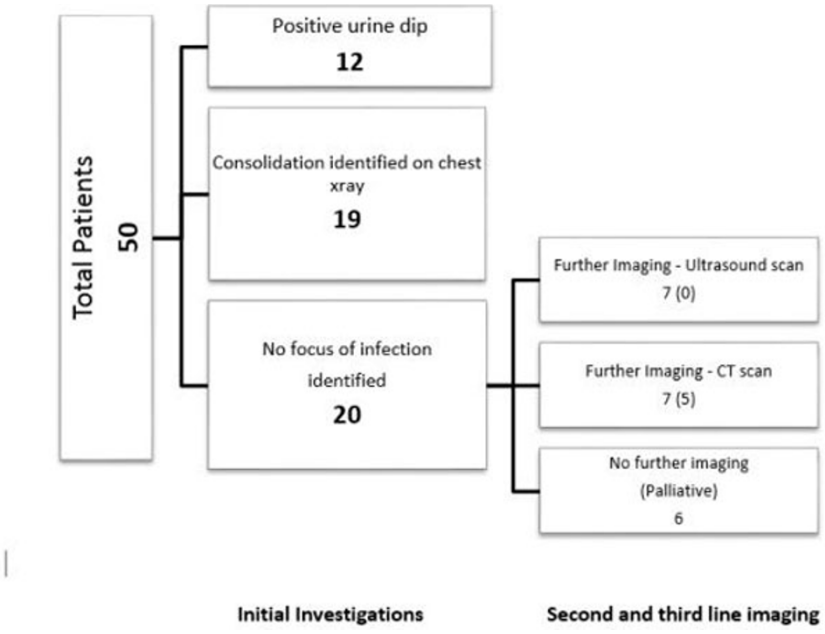

Figure 1(abstract P12) Initial and further investigations performed (number where a septic focus was identified in parentheses)

(CXR) and urine dip can be used to confirm the two most common sources [2]. However, they may fail to identify occult sources of infection, such as intraabdominal collections and abscesses, the treatment of which may require alteration of empirical treatment or be refractory to antibiotic therapy alone. Further imaging is necessary to confirm the focus so that optimal treatment can be achieved.

Methods: The study cohort was composed of 50 consecutive patients who met the criteria for severe sepsis [1] attending the ED in 2013. Electronic and paper patient records and radiology results were analysed. All radiological studies done in the first 72 hours following attendance were included in the study.

Results: CXR was performed as the initial investigation in 49 of the 50 patients (98%). The median time from arrival at the ED to initial imaging was 1 hour:00 minutes (range 0 hours:04 minutes to 4 hours:25 minutes). Initial investigations in the ED of CXR and urine dip identified a septic focus in 30 of 50 patients (60%). Fourteen of the remaining 20 went on to have one or more further imaging studies. Figure 1 outlines the second-line and third-line radiological investigations performed (number where a septic focus was identified in parentheses). The median time to first positive imaging was 0 hours: 50 minutes (range 0 hours:04 minutes to 40 hours), with the source remaining unidentified by imaging and urine testing in 15 of the 50 patients.

Conclusion: Our results indicate that simple bedside investigations are able to identify a focus of infection in 60% of patients presenting to the ED with severe sepsis. Our results support the continued use of CXR as the initial imaging modality in severe sepsis, but also demonstrate the benefit of further imaging in confirming the focus of infection and to guide definitive treatment. Instances where further imaging was delayed by several days highlight the need for guidelines detailing which investigations should be done and in what time frame.

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P13

Proinflammatory versus anti-inflammatory response in sepsis patients: looking at the cytokines.

D Anand[1*], S Ray[2], S Bhargava[1], S Das[1], A Garg[2], S Taneja[2], D Dhar[2], L Mohan Srivastava[1]
[1]Department of Biochemistry and Critical Care Medicine, Sir Ganga Ram Hospital, New Delhi, India; [2]Department of Critical Care and Emergency Medicine, Sir Ganga Ram Hospital, New Delhi, India
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Introduction: Despite improvements in supportive care, mortality rates in sepsis remain substantially high. Sepsis exhibits phases of enhanced inflammation, alternating with immune suppression with a resultant variant time point of mortality; yet no human study has correlated levels of cytokines to the timeline of mortality. Our study attempts to analyze the association of levels of proinflammatory and anti-inflammatory cytokines in sepsis with the timeline of death in terms of early (<5 days) versus late (>5 days) mortality, and day of death. We also assessed correlation of these cytokines with length of stay.

Methods: The study protocol was approved by Institutional Ethics Committee. Subjects were 74 consecutive patients with community-acquired severe sepsis/septic shock admitted to the ICU of a tertiary care superspeciality hospital. Blood samples drawn on days 1, 3 and 7 of admission were analysed for proinflammatory cytokine (TNFα) by chemiluminescent immunometric assay and anti-inflammatory cytokine (IL-10) by ELISA. Subjects were segregated on basis of: ratio of proinflammatory and anti-inflammatory mediators on day 1 of admission into patients with predominant proinflammatory or predominant anti-inflammatory response. Survival and time point of mortality into survivor, early mortality and late mortality groups. Statistical analyses were performed using SPSS version 17.

Results: There were 37 patients each in predominant proinflammatory and predominant anti-inflammatory groups. The number of deaths was 11 and 17 respectively in these groups. However, there was significantly higher early mortality in the proinflammatory group as compared to the anti-inflammatory group (7 vs. 1, $P = 0.0247$). On the contrary, late deaths were significantly higher in the anti-inflammatory group as compared to the proinflammatory (16 vs. 4 $P = 0.0017$). The ratio of proinflammatory/anti-inflammatory cytokines (TNF/IL-10) on day 1 was significantly lower in patients of late death ($n = 20$) as compared to patients of early death ($n = 8$) and survivors ($n = 46$) as shown in Table 1. Further, the median day of death

Table 1(abstract P13) TNF/IL-10 ratio in study groups at different time points

	Early death (≤ 5 days) (*n* = 8)	Late death (>5 days) (*n* = 20)	Survivors (*n* = 46)	*P* value
Day 1	1.81 (1.00 to 3.44)	0.50 (0.31 to 0.90)	1.22 (0.43 to 3.91)	0.020*
Day 3	1.12 (0.50 to 3.91)	1.01(0.20 to 2.21)	2.5 (0.90 to 3.91)	0.158
Day 7	-	1.25 (0.59 to 2.38)	1.79 (0.75 to 3.90)	0.256

Data presented as median (IQR). Kruskal-Wallis test was performed for significance between three groups. *$P < 0.05$ considered significant

was significantly delayed in patients in the anti-inflammatory as compared to the proinflammatory group (20 vs. 5, $P < 0.001$). Length of hospital stay amongst survivors was significantly longer in the anti-inflammatory as compared to the proinflammatory group (23 vs. 10 $P < 0.001$).

Conclusion: Our preliminary data suggest that in sepsis, the ratio of proinflammatory/anti-inflammatory cytokines on day 1 is significantly associated with time point of mortality; hence, this ratio can be used to particularize management. Further studies are in progress to substantiate the role of proinflammatory and anti-inflammatory cytokines in this subset of patients. Moreover, since predominant anti-inflammatory response was associated with later death, role of immunomodulators in sepsis needs to be explored.

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P14

Understanding heterogeneity in the host response to *Staphylococcus aureus* infection for prognostic biomarker discovery.

JB Dinoso[1*], J Gutierrez[2], DF Choy[1], S Kummerfeld[3], A Baruch[2], HF Chambers[4], CM Rosenberger[1]

[1]ITGR Diagnostic Discovery, Genentech Inc., South San Francisco, CA, USA; [2]Pharmacodynamic Biomarkers, Genentech Inc., South San Francisco, CA, USA; [3]Bioinformatics, Genentech Inc., South San Francisco, CA, USA; [4]Division of Infectious Disease, University of California San Francisco, San Francisco, CA, USA

Critical Care 2014, **18(Suppl 2):**P14; doi:10.1186/cc14017

Introduction: Invasive *Staphylococcus aureus* infections remain an unmet medical need with the issues of drug resistance (MRSA) and mortality. Understanding clinical trial data in the development of antibiotics to *S. aureus* is complicated by heterogeneous clinical outcomes (that is, length of hospitalization, mortality, treatment response), which makes interpretation of drug efficacy challenging. Identification of prognostic biomarkers of different biological processes that associate with clinical outcomes would aid in clinical development of novel therapies for *S. aureus* infections.

Methods: In an effort to discover biomarkers that differentiate patient populations based on clinical outcomes following *S. aureus* infection, we retrospectively analyzed published gene expression datasets of *S. aureus* infection and sepsis.

Results: We identified a leukocyte gene expression signature that positively correlated with *S. aureus* disease severity [1]. This severity signature was enriched for genes associated with neutrophils but was not solely explained by increased percentage of neutrophils. This set of genes was also associated with severity in sepsis, with higher expression in patients with septic shock compared with sepsis [2] and in nonsurvivors compared with survivors of septic shock [3]. Our *in vitro* studies revealed that the severity signature may reflect an increase in circulating immature neutrophils or band cells which has been previously reported in the context of bacterial stimuli and sepsis [4,5]. This line of evidence is consistent with a recent report by Guerin and colleagues that demonstrated that quantification of immature neutrophils by flow cytometry was prognostic for sepsis mortality [6].

Conclusion: To extend the insight gained from our retrospective analysis and *in vitro* studies, we are conducting a longitudinal, non-interventional clinical study of patients with *S. aureus* bacteremia. The goal of the study is to associate molecular metrics (gene expression, plasma protein levels, immune cell subsets) with clinical outcomes (length of hospitalization, mortality, treatment response, readmission for recalcitrant infection). Our preliminary data show an association between grade of sepsis or infection localization and increased immature neutrophils as well as monocyte subsets that can promote inflammation or immune exhaustion. Ongoing experiments are designed to understand the impact of these cellular phenotypes on disease progression and to identify robust protein or RNA biomarkers that are prognostic for clinical outcomes.

P15

miR-20a-5p mediates hypoxia-induced autophagy by targeting ATG16L1 in acute kidney injury.

I-K Wang[1,2*], C-Y Li[2,3]

[1]Graduate Institute of Clinical Medical Science, China Medical University, Taichung, Taiwan; [2]Division of Nephrology, China Medical University Hospital, Taichung, Taiwan; [3]Department of Anesthesiology, China Medical University Hospital, Taichung, Taiwan

Critical Care 2014, **18(Suppl 2):**P15; doi:10.1186/cc14018

Introduction: Autophagy could be induced under stress conditions, including starvation, infection, and hypoxia. The microRNA (miRNA) network may be critical in the regulation of autophagy. Upregulation of autophagy may be a protective response for cell survival in ischemic kidney injury. The aim of this study was to evaluate whether miRNA regulates autophagy in ischemic kidney injury and renal proximal tubular cells under hypoxic conditions.

Methods: Ischemic kidney injury was performed by clamping bilateral renal pedicles for 60 minutes in male mice. Human kidney proximal tubular (HK2) cells are incubated in a hypoxic chamber with 0.3% O_2. Bioinformatics analyses were used to select the candidate miRNA. Gain-of-function and loss-of-function methods were employed to evaluate the effects of miRNA on autophagy. Chromatin immunoprecipitation analyses and promoter luciferase reporter assays were used to evaluate the interaction of transcriptional factors with miRNA.

Results: Increase of LC3 and ATG16L1, autophagy-related proteins, and down expression of miR-20a-5p were detected in kidneys after ischemic injury and in HK2 cells under hypoxic conditions. The 3′-untranslated region luciferase reporter assays indicated that miR-20a-5p targeted ATG16L1 messenger RNA. Overexpression of miR-20a-5p reduced the expression of LC3-II and ATG16L1 in HK2 cells under hypoxic conditions, whereas antagomiR-20a reversed the inhibition. Using RNAi against hypoxia-inducible factor-1α (HIF-1α) in HK2 cells, we confirmed the inhibitory binding of HIF-1α to miR-20a-5p.

Conclusion: The signaling axis of HIF-1α, miR-20a-5p, and ATG16L1 in autophagic process might be a critical adapting mechanism for ischemic kidney injury.

P16

Evaluating the sensitivity and specificity of a severe sepsis tool utilized at a community hospital in Miami, FL.

J Hirigoyen
4 Tower Medical-Surgical Unit, Baptist Hospital of Miami, Miami, FL, USA

Critical Care 2014, **18(Suppl 2):**P16; doi:10.1186/cc14019

Introduction: Since the initial development of the Surviving Sepsis Campaign guidelines outlining the management of severe sepsis, there has been an absolute discount on the management of septic patients in medical surgical units. In efforts to improve severe sepsis, a community hospital in Miami adopted a severe sepsis screening tool (SSST) to rapidly identify severe septic patients in medical surgical units. A pilot study was conducted to evaluate the sensitivity and specificity of the SSST.
Methods: A descriptive retrospective study. There were two phases. Phase 1 evaluated the percentage of patients with sepsis criteria utilizing the SSST. Patients admitted to 4 Tower during 2013 presenting with a diagnosis of sepsis syndrome and admitted to 4 Tower presenting without sepsis syndrome were reviewed. Phase 2 evaluated the sensitivity and specificity of SSST from August 2013 to January 2014. Total number of patients admitted to 4 Tower: of those patients, total number with discharge diagnosis of sepsis, total number who screened positive >1 time during hospital stay, and total number who screened negative during hospital stay; there were five missing cases. The receiver operating curve (Figure 1) and the respective area under the curve were calculated. Utilizing a 2 × 2 design, the sensitivity and specificity of the tool was calculated.
Results: Phase 1: a total of 220 patients records were reviewed, a frequency distribution was utilized (Table 1), demonstrating that the SSST identified those patients with sepsis criteria 76 % (n = 167) of the time. Phase 2: a total of 1,555 patients were included during phase 2. A 2 × 2 design (Table 2) was utilized: 78 patients were identified as true positive and 1,233 patients were identified as true negative. The study yielded a sensitivity of 41.49% and a specificity of 90.53%. The positive predictive value of the tool was estimated at 37.68%, negative predictive value was estimated at 91.81% and disease prevalence was 12.13%. Area under the receiver operating curve (Table 3) was 0.66.
Conclusion: A two-phase retrospective chart review study demonstrated that the SSST utilized at a community hospital in Miami had a sensitivity value of 41.49% and a specificity value of 90.53% when evaluating medical surgical patients. These results indicate the tool is accurate in detecting patients that are not septic; however, it is not reliable in identifying patients who are truly septic. Further studies need to be conducted to validate the sensitivity and specificity of the SSST; changes will be recommended in an effort to improve sensitivity.
Acknowledgements: Thanks to Eve Butler and Andrea Prentiss from Baptist Research Department, 4 Tower team. Special thanks to Melanie Santos, Luz Lorenzo, Disney Granado, Katiuska Diaz, Sandra Benitez, Magdely Perez, Viviana Castillo and Ofelia Cabrera.

P17

Surviving Sepsis Campaign 2012 3-hour bundle in the emergency department: compliance and impact of pathway of care before and after implementation.

J Masse[1*], A Filali[2], O Nigeon[1], N Van Grunderbeeck[2], P Gosselin[3], L Tronchon[2], J Mallat[2], D Thevenin[2]
[1]Emergency Department, Hospital Center of Lens, France; [2]Intensive Care Unit, Hospital Center of Lens, France; [3]Emergency Department, Lille University Hospital, Lille, France

Critical Care 2014, **18(Suppl 2):**P17; doi:10.1186/cc14020

Introduction: Compliance with the Surviving Sepsis Campaign 2012 (SSC) bundle in the emergency department (ED) is a key point to improve outcome of severe sepsis and septic shock [1,2]. Before and after education of ED staff, we registered compliance and timing of lactate dosing, blood culture sampling, empiric antibiotic therapy (ATB) and fluid resuscitation, the 3-hour (H3) bundle. Survival and compliance according to the initial pathway of care were also studied.
Methods: A monocentric study before and after education of ED staff about SSC bundles (courses, posters, pocket guides). We looked at compliance of the H3 bundle items in a retrospective and a prospective cohort, timing of realisation, day 28 survival, overall severity (SAPS2, SOFA and RISCC scores), impact of prehospital medical management, and initial pathway of care. Statistical analysis was performed with Fisher exact test and Mann-Whitney test. Multivariate analysis of factors associated with survival was made through logistic regression.
Results: Eighty-nine patients were included in the prospective cohort, 65 in the retrospective cohort. Patterns of patients in the retrospective and prospective cohort were respectively: sex ratio M/F 29/39 and 39/47 (NS);

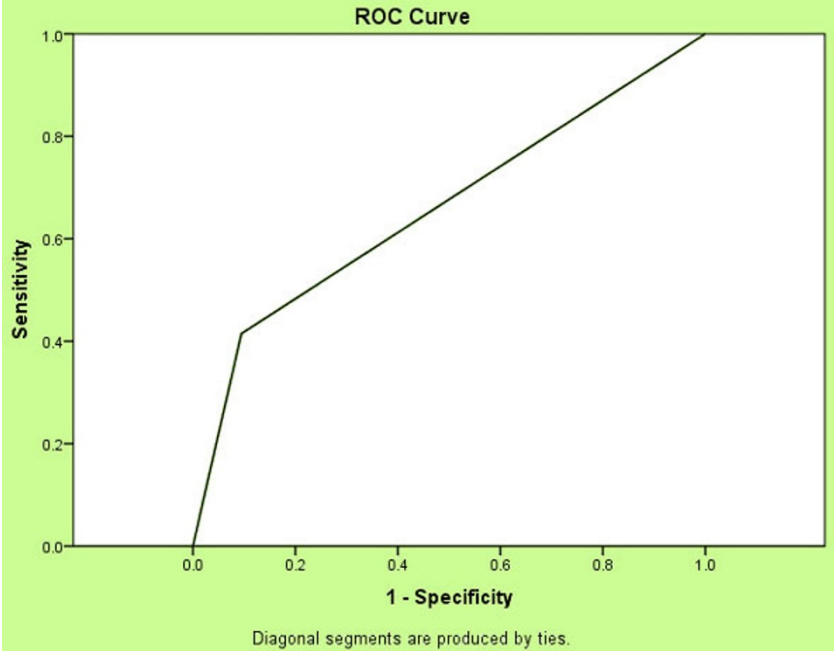

Figure 1(abstract P16) ROC curve

Table 1(abstract P16) Frequency distribution

Statistics	Diagnosis on admission	Sepsis tool identifies sepsis		
Valid	220	220		
Missing	0	0		
Frequency table	**Frequency**	**Percent**	**Valid percentage**	**Cumulative percentage**
Diagnosis on admission				
Valid	220	100.0	100.0	100.0
Sepsis tool identifies sepsis				
Not valid	53	24.1	24.1	24.1
Valid	167	75.9	75.9	100.0
Total	220	100.0	100.0	

Table 2(abstract P16) The 2 × 2 design

	Sepsis present	Sepsis absent
Positive	78 true positive	129 false positive
Negative	110 false negative	1,233 true negative

Table 3(abstract P16) Area under the curve

Test result variable(s):screen
Area
0.660

median age 63.29/61.38 (NS); SAPS2 44/40 (P = 0.019); SOFA 4/3 (P = 0.005); RISCC 9/12.5 (P = 0.002). Compliance with the H3 bundle items before and after intervention was: lactate 72.1% vs. 81.4% (NS); blood cultures 61.8% vs. 67.4% (NS); ATB 29.3% vs. 52.3% (P = 0.005); fluids 52.9% vs. 59.3% (NS). Median delays before and after implementation were (in minutes): lactate 56 vs. 40 (P = 0.024); blood cultures 68 vs. 75 (NS); ATB 229 vs. 160 (NS); fluids 100 vs. 74 (NS). Survival was superior after intervention 67.6 vs. 81.4% (P = 0.049), and associated with a low SAPS2 score in multivariate analysis. Admission through a prehospital medical team was associated with a stronger H3 ATB compliance before intervention (P = 0.032). Within the ED, initial orientation to the acute care unit was associated with a better H3 ATB compliance compared to standard care before and after staff education (P = 0.001; P = 0.003), and with better overall compliance (P = 0.004; P = 0.026).

Conclusion: Compliance with the SSC H3 bundle was increased but still needs to be improved. There is an impact of the initial pathway of care on compliance of the bundle, and on timing of ATB injection. Differences in healthworker/patient ratio in the units of care could explain these disparities [3]. Improvement could be obtained through optimizing early screening, correct initial guidance, or with dedicated teams.

Acknowledgements: The authors would like to thank the members of the Unit of Clinical Research of the Hospital Center of Lens for their great help in data recovery

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P18

Benefit of achieving lactate clearance versus central venous oxygen saturation target as microcirculation end point resuscitation in severe sepsis and septic shock.

R Sinto*, S Suwarto, KC Lie, D Widodo, HT Pohan
Division of Tropical and Infectious Disease, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia
Critical Care 2014, **18(Suppl 2):**P18; doi:10.1186/cc14021

Introduction: In severe sepsis and septic shock patients, lactate clearance >10% and central venous oxygen saturation ($ScvO_2$) >70% are accepted parameters of tissue oxygenation adequacy. There is controversy of which parameters better associate with early mortality, and thus should be implemented as the microcirculation end point resuscitation [1-3]. This study was aimed to address the association of achieving either one or two targets of microcirculatory end point resuscitation and early mortality in severe sepsis and septic shock patients.

Methods: A retrospective cohort study was conducted in severe sepsis and septic shock patients (aged 18 years and older) hospitalized in the ICU, Cipto Mangunkusumo Hospital, Indonesia. Patients' early outcomes were observed during first 120 hours of hospitalization. Cox's regression analysis was used to analyse risk of early mortality in subject groups achieving lactate clearance target only, $ScvO_2$ target only, both targets, and not achieving any target in 6 hours after onset of resuscitation.

Results: Subjects consisted of 268 patients. Early mortality developed in 70 subjects. Fifty-four subjects achieved lactate clearance target only, 16 achieved $ScvO_2$ target only, 138 achieved both targets, 60 did not achieve any target. Subjects who achieved both targets had a significant lowest early mortality risk (P = 0.104 compared with subjects achieved lactate clearance target only and P = 0.000 compared with remaining subject groups) (Figure 1). In subgroup analysis of subjects who achieved lactate clearance or $ScvO_2$ target only, failure to achieve lactate clearance target associated with higher early mortality risk (hazard ratio 5.92; 95% CI 2.18 to 16.01).

Conclusion: Achieving both lactate clearance and $ScvO_2$ targets in 6 hours after onset of resuscitation associates with lowest early mortality risk in severe sepsis and septic shock patients. Patients who achieve lactate clearance target only have a significant lower early mortality risk compared with those who achieve $ScvO_2$ target only.

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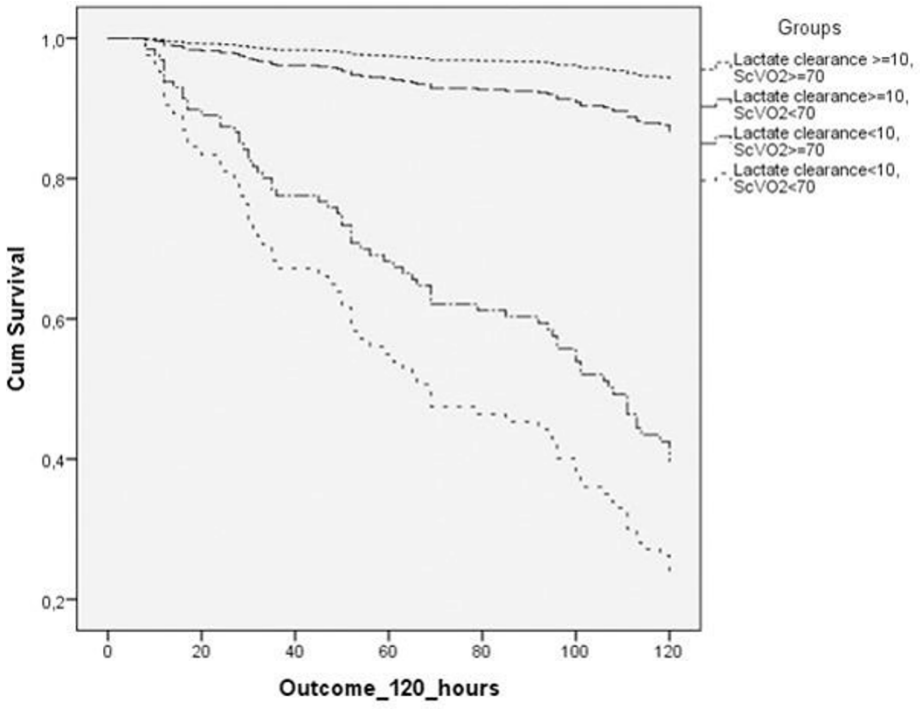

Figure 1(abstract P18) Survival analysis of groups based on target achievement

P19

The PRESEP score: an early warning scoring system to identify septic patients in the emergency care setting.

O Bayer[1*], CS Hartog[1,2], D Schwarzkopf[2], C Stumme[1], A Stacke[1], F Bloos[1,2], C Hohenstein[3], B Kabisch[1], C Weinmann[1], J Winning[1], Y Sakr[1], JK Reinhart[1,2]
[1]Department of Anaesthesiology and Intensive Care Medicine, Jena University Hospital, Jena, Germany; [2]Center for Sepsis Control & Care, Jena University Hospital, Jena, Germany; [3]Emergency Department, Jena University Hospital, Jena, Germany

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Introduction: Many patients present with sepsis through emergency services [1]. Their outcome could be improved if sepsis could be detected already in the prehospital setting. This study aims to develop and evaluate a score to detect prehospital early sepsis.

Methods: A retrospective study of 375 patients admitted to Jena University Hospital emergency department (ED) through emergency medical services (EMS). Sepsis was present in the ED in 93 (24.8%) patients, of which 60 (16.0%) had severe sepsis and 12 (3.2%) had septic shock. The following predictors for sepsis based on consensus criteria were extracted from the EMS protocol: body temperature (T), heart rate (HR), respiratory rate (RR), oxygen saturation (SaO_2), Glasgow Coma Scale, blood glucose and systolic blood pressure (BP). Sepsis predictors were determined based on inspection of loess graphs. Backward model selection was performed to select risk factors for the final model. The PRESEP score was calculated as the sum of simplified regression weights. Its predictive validity was compared to the modified Early Warning Score (MEWS) [2], the Robson screening tool [3] and the BAS 90-30-90 [4].

Results: Backward model selection identified T, HR, RR, SaO_2 and BP for inclusion in the PRESEP score. Its AUC was 0.93 (CI 0.89 to 0.96). The cutoff based on maximum Youden's Index was ≥4 (sensitivity 0.85, specificity 0.86, PPV 0.66, NPV 0.95). The PRESEP score had a larger AUC than the MEWS (0.93 vs. 0.77, $P < 0.001$) and surpassed MEWS and BAS 90-60-90 concerning sensitivity (0.74, 0.62), specificity (0.75, 0.83), PPV (0.45, 0.51) and NPV (0.91, 0.89). The Robson screening tool had a higher sensitivity and NPV (0.95, 0.97) was better, but its specificity and PPV lower (0.43, 0.43).

Conclusion: The PRESEP score can be easily applied in the emergency setting and could be a valuable tool to identify septic patients in the case of suspected infection.

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P20

Simultaneous targeting of interleukin-1 and interleukin-18 is required for protection against inflammatory and septic shock.

T Vanden Berghe[1,2*], D Demon[1,2,3], P Bogaert[1,2], B Vandendriessche[1,2], A Goethals[1,2], B Depuydt[1,2], M Vuylsteke[4], R Roelandt[1,2], E Van Wonterghem[1,2], J Vandenbroecke[1,2], SM Choi[1,2], E Meyer[3], S Krautwald[5], W Declercq[1,2], N Takahashi[1,2], A Cauwels[1,2], P Vandenabeele[1,2]
[1]Inflammation Research Center, VIB, Ghent, Belgium; [2]Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium; [3]Department of Plant Systems Biology, VIB, Ghent, Belgium; [4]Lab

Aquaculture & Artemia Reference Center, Faculty Bioscience Engineering, Ghent University, Belgium; [5]Department of Nephrology and Hypertension, University Medical Center Schleswig-Holstein UKSH, Kiel, Germany
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Introduction: Sepsis is one of the leading causes of death around the world. The failure of clinical trials to treat sepsis demonstrates that the molecular mechanisms are multiple and still insufficiently understood. The objective is to clarify the long disputed hierarchical contribution of several central inflammatory mediators, namely IL-1β, IL-18, CASP7, CASP1 and CASP11, in septic shock, and to explore their therapeutic potential.
Methods: LPS-induced and TNF-induced lethal shock, as well as cecal ligation and puncture (CLP), were performed in genetically or pharmacologically targeted mice. Body temperature and survival were monitored closely, and plasma was analyzed for several markers of cellular disintegration and inflammation.
Results: Interestingly, deficiency of both IL-1β and IL-18 additively prevented LPS-induced mortality. The detrimental role of IL-1β and IL-18 was confirmed in mice subjected to a lethal dose of TNF, or to a lethal CLP procedure. Although their upstream activator, CASP1, and its amplifier, CASP11, are considered potential therapeutic targets because of their crucial involvement in endotoxin-induced toxicity, CASP11 or CASP1/11 deficient mice were not, or hardly, protected against a lethal TNF or CLP challenge. In line with our results obtained in genetically deficient mice, only the combined neutralization of IL-1 and IL-18, using the IL-1 receptor antagonist Anakinra and anti-IL-18 antibodies, conferred complete protection against endotoxin-induced lethality.
Conclusion: Our data point towards the therapeutic potential of neutralizing IL-1 and IL-18 simultaneously in sepsis, rather than inhibiting the upstream inflammatory caspases.
Acknowledgements: DD and PB contributed equally to this work. AC and PV share senior authorship. The authors are grateful to R Flavell (Howard Hughes Medical Institute, Chevy Chase, USA) and A Zychlinsky (Max Planck Institute, Berlin, Germany) for respectively the CASP1 knockout mice and the IL-1β/IL-18 double knockout mice. They thank B Lambrecht for fruitful discussions. TVB holds a postdoctoral fellowship from the FWO, PB is paid by VIB. Research in the Vandenabeele group is funded by European grants (FP6 ApopTrain, MRTN-CT-035624; FP7 EC RTD Integrated Project, Apo-Sys, FP7-200767; Euregional PACT II), Belgian grants (Interuniversity Attraction Poles, IAP 7/32), Flemish grants (Research Foundation Flanders, FWO G.0875.11, FWO G.0973.11 and FWO G.0A45.12N, FWO G.0787.13N, Methusalem), Ghent University grants (MRP, GROUP-ID consortium), a grant from the Foundation against Cancer, F94 and grants from Flanders Institute for Biotechnology (VIB). PV holds a Methusalem grant (BOF09/01M00709) from the Flemish Government. The authors declare no conflict of interest.

P21

Sepsis electronic surveillance and clinical outcomes: impact over mortality of a sepsis early detection electronic rule implemented in the emergency department.

P Martin-Rico[1*], A Valdivia-Perez[2], MD Marco-Lattur[1], J Chorda-Ribelles[1], N Lozano-Cortell[1], P Olcina-Lloret[1], R Andres-Navarro[1], E Mateo-Sanchis[1], M Jordan-Lluch[1], O Esparcia-Rodriguez[3], J Magraner-Egea[3], J Lacalle-Martinez[4], A Barcelo-Lopez[5]
[1]Internal Medicine (Infectious D. Unit), Hospital de Denia, Denia Alicante, Spain; [2]Preventive Medicine, Hospital de Denia, Denia Alicante, Spain; [3]Microbiology Department, Hospital de Denia, Denia Alicante, Spain; [4]IT Department, Hospital de Denia, Denia Alicante, Spain; [5]Emergency Department, Hospital de Denia, Denia Alicante, Spain
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Introduction: Severe sepsis and septic shock (SS/SS) have a high mortality. Therapeutic guidelines can improve mortality, but early recognition and timely implementation of these requires a proactive attitude that can be electronically supported.
Methods: From May 2013 our hospital implemented a Sepsis Code (SC) based on an early detection electronic rule developed by our multiprofessional sepsis team: clinicians and IT engineers (EMR Cerner Millennium platform) and a standardized order set plus systematic follow-up by our sepsis team. We performed a before-after study to assess the impact over mortality of this strategy. Time-series analysis of sepsis admissions and mortality from January 2011 to December 2013, before and after SC implementation. (Analysis by STATA.) All urgent admissions recorded in the minimum basic data set in patients over 14 years from 1 January 2011 to 31 December 2013 were included. Inclusion criteria: patients with ICD-9 sepsis-associated codes in the principal diagnosis or patients with infection-associated codes in the principal diagnosis together with sepsis-associated codes in secondary diagnosis. Medical records were manually reviewed by clinicians to confirm SS/SS diagnosis. Temporal series analysis (Poisson regression). First analysis: sepsis admissions in relation to total urgent admissions. Second analysis: deaths due to SS/SS related to admissions in this group. In both cases we compared results before SC activation (28 months) and after that (first 2 transitional months and 6 consolidated months). The multivariate adjustment in both analyses included year, month of the year, and months with activated rule. Graphic analysis estimated predictions for the last 8 months based on the previous 28 months, comparing both observed and predicted sepsis and deaths.
Results: A total of 24,118 urgent admissions were included, 5,657 in the postalert period. Mean monthly admissions: 652 (SD 47) (570 to 740). In total, 408 and 178 SS/SS were identified in the prealert and postalert period, respectively. After SC implementation we observed no significant changes in sepsis admission risk but a clear downward trend in sepsis mortality: in the first 2 transitional months we did not observe major changes, while in the last 6 months the risk of death does fall 36% reaching statistical significance (IRR 0.64 (95% CI 0.43 to 0.97, $P = 0.036$)) (Table 1 Figures 1 and Figure 2). Both antibiotic door-to-needle time and adequacy significantly improved in sepsis cases where the alert was triggered.
Conclusion: Implementation of a SC triggered by an electronic detection alert, compared to the prealert period, decreased mortality risk by 36% (IRR 0.64 (95% CI 0.43 to 0.97, $P = 0.036$)) with the rule fully implemented.

P22

Assessing the value of a real-time electronic screening algorithm for early detection of severe sepsis in the emergency department.

P Martin-Rico[1*], A Valdivia-Perez[2], JM Lacalle-Martinez[3], J Chorda-Ribelles[1], MD Marco-Lattur[1], N Lozano-Cortell[1], M Jordan-Lluch[1], E Mateo-Sanchis[1], R Andres-Navarro[1], P Olcina-Lloret[1], JD Garcia-Pedro[1], O Esparcias-Rodriguez[4], J Magraner-Egea[4], A Barcelo-Lopez[5]
[1]Internal Medicine (Infectious D. Unit), Hospital de Denia, Denia Alicante, Spain; [2]Preventive Medicine, Hospital de Denia, Denia Alicante, Spain; [3]IT Department, Hospital de Denia, Denia Alicante, Spain; [4]Microbiology Department, Hospital de Denia, Denia Alicante, Spain; [5]Emergency Department, Hospital de Denia, Denia Alicante, Spain
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Introduction: In severe sepsis/septic shock (SS/SS), early recognition and timely implementation of treatment is critical for survival, and this could be electronically supported. We assess the value of an electronic automatic algorithm based on EMR data as a screening tool for early detection of sepsis.
Methods: Our multiprofessional sepsis team (clinicians and IT engineers) developed an electronic algorithm using data from our EMR (Cerner Millennium platform) aimed at the automatic, real-time recognition of two or more systemic inflammatory response syndrome components + one or more organ failure parameter (according to sepsis definition) in every patient attended in the ER. The firing of this sepsis rule issues an alert to the responsible clinician to confirm an infectious etiology and opens an electronic standardized order set according to sepsis bundles. The alert database (from its start in May 2013 to December 2013) was cross-matched with the minimum basic data set for urgent admissions (>14 years) during this same period. We selected, based on an *ad hoc* syntaxes, those admissions due to sepsis. Medical records were manually reviewed for confirmation of SS/SS. We assessed sensibility, specificity, negative and positive predictive value of the electronic rule, considering the confirmed clinical diagnosis at discharge as the gold standard.
Results: In total, 37,323 patients were seen in the ER, 5,657 emergency admissions took place and 178 were due to SS/SS. Alert fired in 1,190 (3.2%) total emergencies and in 754 emergency admissions (13.3%). Data analysis after alert implementation identifies a global sensitivity of 80%, which improved after the first 2 months of transition. In the last 6 months (consolidated period) it was between 85 and 90%. Global specificity 89%, NPV of 99% and PPV of 19% for a global prevalence at admission of 3.2 cases/100 (Table 1). The mean door-to-alert time was 167 minutes (SD 193).

Table 1(abstract P21) Risk of death in sepsis admissions

Variable	Category	IRR	95% CI	*P* value
Month	January	2.11	0.80 to 5.55	0.130
	February	2.55	1.04 to 6.24	0.040
	March	2.45	1.00 to 6.01	0.051
	April	1.0 (reference)		
	May	2.24	0.86 to 5.88	0.100
	June	1.45	0.53 to 4.01	0.470
	July	2.15	0.82 to 5.66	0.121
	August	2.62	1.00 to 6.85	0.050
	September	3.74	1.47 to 9.53	0.006
	October	2.31	0.89 to 6.02	0.085
	November	2.69	1.09 to 6.65	0.032
	December	1.89	0.71 to 5.01	0.203
Alert	Previous	1.0 (reference)		
	Transition	1.26	0.64 to 2.47	0.506
	Implemented	0.64	0.43 to 0.97	0.036

Median door to needle (antibiotic) time in the alert-sepsis group was 259 minutes vs. 417 minutes in the group where alert did not fire. In the alert + order set group, antibiotic treatment was adequate in 70% vs. 35% in the nonalert sepsis group (Fisher's exact = 0.001). Overall mortality among alert-fired admissions not due to sepsis was 15% (RR for death during admission: 5.33, 95% CI: 3.73 to 7.59; $P < 0.0001$).

Conclusion: This pioneering sepsis algorithm identified 80% of SS/SS. The alert prompted adequate antibiotic treatment and shortened door to needle time from 7 to 4 hours. Furthermore, in patients without sepsis the algorithm identifies a poor-prognosis subset (RR for death 4.75 (95% CI: 3.72 to 6.07).

P23

Histidine-rich glycoprotein prevents septic lethality through neutrophil regulation.

M Nishibori[1*], H Wake[1], S Mori[2], K Liu[1], Y Morioka[1], K Teshigawara[1], M Sakaguchi[3], K Kuroda[4], H Takahashi[5], A Ohtsuka[6], T Yoshino[7], H Morimatsu[4]
[1]Department of Pharmacology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan; [2]Department of Pharmacology, Faculty of Pharmaceutical Science, Shujitsu University, Okayama, Japan; [3]Department of Molecular Biology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan; [4]Department of Anesthesiology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan; [5]Department of Pharmacology, Faculty of Medicine, Kinki University, Osakasayama, Japan; [6]Department of Human Morphology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan; [7]Department of Pathology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan
Critical Care 2014, **18(Suppl 2):**P23; doi:10.1186/cc14026

Introduction: Although there have been many clinical trials for treatment of sepsis, no drugs are available at present. Sepsis is thought to be complex disorders; activation/lesion of vascular endothelial cells, accelerated coagulation and platelet aggregation, leukocyte activation or paralysis, and hypercytokinemia. To treat the pathological status of sepsis, it must be necessary to control these plural disorders simultaneously or sequentially. In the present study, we identified and characterized a plasma protein histidine-rich glycoprotein (HRG) as a factor that decreases dramatically in septic condition and maintains neutrophil's shape and functional quiescence. We clarified the involvement of HRG in septic pathogenesis and propose a novel therapy for sepsis based on that.

Methods: Sepsis was induced in mice by cecal ligation and puncture. The mice were treated with HRG purified from human plasma after confirming the marked decrease in plasma HRG. We evaluated the beneficial effects of HRG administration on survival rate, lung inflammation, and the state of circulating neutrophils using *in vivo* imaging. Purified neutrophils from human blood were treated with HRG and analyzed with respect to neutrophil shape, adhesiveness to vascular endothelial cells, passage through microcapillaries, production of reactive oxygen species, and cytoskeleton rearrangement with relevant signal transduction.

Results: Supplementary treatment of septic mice with exogenous HRG for the decrease in plasma HRG improved the survival of mice remarkably. HRG treatment reduced the number of neutrophils in the lung, on which platelet aggregation and fibrin deposit were observed. HRG also inhibited the expression of mRNAs of IL-6, TNFα, iNOS, and PAI-1 in the lung. In contrast, knockdown of HRG by siRNA exacerbated lethality. Purified human HRG reversibly induced morphological changes in human neutrophils *in vitro*; induction of spherical shape with reduced microvilli and adhesiveness to vascular endothelial cells. HRG maintained the passage of neutrophils through microcapillaries and abolished the production of reactive oxygen species whereas HRG had no effect on the expression of adhesion molecules including CD11b and CD62L.

Conclusion: We show that plasma protein HRG is a crucial factor that keeps the circulating neutrophils quiescent and prevents unnecessary activation in bloodstream using a cecal ligation and puncture model in

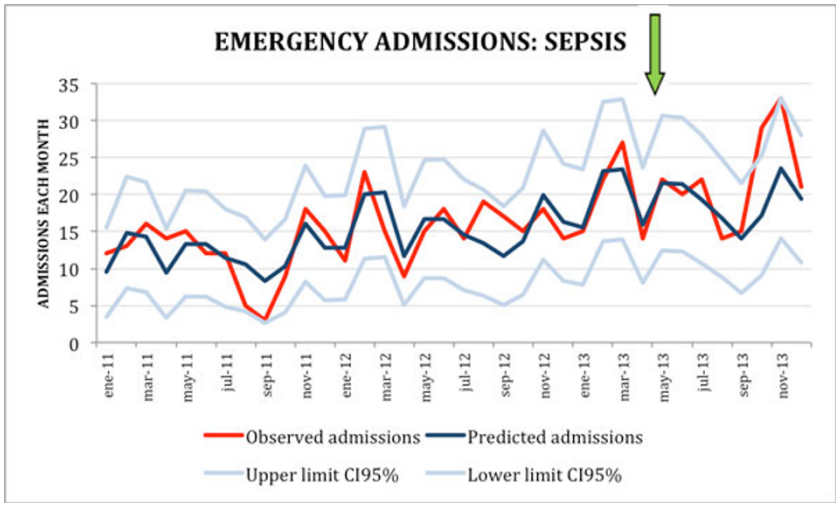

Figure 1(abstract P21) Emergency admissions for sepsis

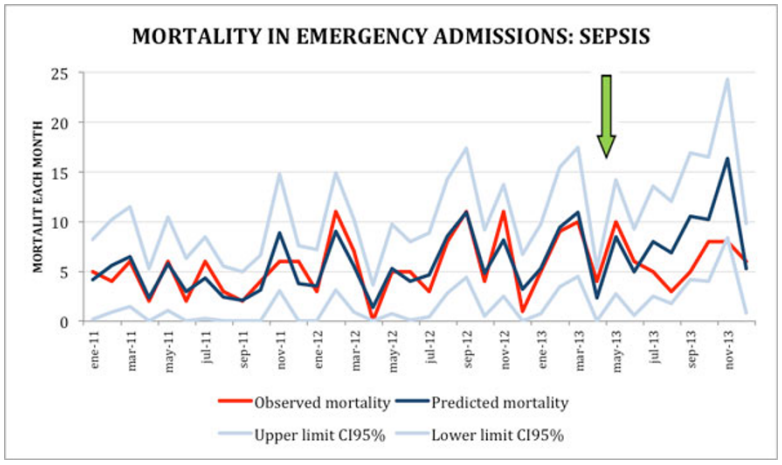

Figure 2(abstract P21) Mortality in emergency admissions for sepsis

Table 1(abstract P22) Prevalence of SS/SS upon admission and sensitivity, specificity, and predictive values of the sepsis rule

Month	Prevalence	Sensitivity	Specificity	PPV	NPV
May	3.1 (1.8 to 4.5)	72.7 (51.8 to 93.6)	88.3 (85.8 to 90.7)	16.7 (8.7 to 24.6)	99.0 (98.1 to 99.9)
June	3.0 (1.6 to 4.3)	55.0 (30.7 to 79.3)	89.2 (86.7 to 91.6)	13.4 (5.4 to 21.4)	98.5 (97.4 to 99.6)
July	3.0 (1.7 to 4.3)	86.4 (69.8 to 100)	89.8 (87.5 to 92.1)	20.7 (11.8 to 29.5)	99.5 (89.9 to 100)
August	1.8 (0.8 to 2.8)	78.6 (53.5 to 100)	87.8 (85.4 to 90.2)	10.7 (4.2 to 17.1)	99.6 (99.0 to 100)
September	2.2 (1.0 to 3.4)	86.7 (66.1 to 100)	88.0 (85.4 to 90.5)	14.0 (6.4 to 21.6)	99.7 (99.1 to 100)
October	4.3 (2.7 to 5.9)	82.8 (67.3 to 98.2)	90.6 (88.3 to 92.9)	28.2 (18.1 to 38.4)	99.2 (98.3 to 100)
November	5.4 (3.5 to 7.2)	84.9 (71.1 to 98.6)	88.9 (86.2 to 91.5)	30.1 (20.3 to 40.0)	99.1 (98.1 to 100)
December	2.9 (1.6 to 4.2)	90.5 (75.5 to 100)	88.4 (85.9 to 90.8)	19.0 (10.8 to 27.2)	99.7 (99.1 to 100)
Total	3.2 (2.7 to 3.6)	80.1 (73.9 to 86.3)	88.8 (88.0 to 89.7)	19.0 (16.1 to 21.8)	99.3 (99.0 to 99.5)

Results of each period are indicated in percentages with confidence intervals at 95%

mice and human neutrophils. The attachment of neutrophils on the vascular wall under reduced concentration of HRG was accompanied by microthrombus formation in the lung, leading to ARDS. Supplementary therapy with HRG may thus provide a novel strategy for the treatment of septic patients through neutrophil regulation.

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P24

Usefulness of intravenous immunoglobulin administration to sepsis-induced coagulopathy in ICU patients.

N Matsumoto*, H Ishikura, Y Nakamura, J Tanaka, M Mizunuma, Y Kawano, S Morimoto, H Kaneyama, A Murai
Department of Emergency and Critical Care Medicine, Faculty of Medicine, Fukuoka University, Fukuoka, Japan
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Introduction: Intravenous immunoglobulin (IVIG) has been used as adjuvant therapy for severe sepsis patients expecting the anti-inflammatory effect. However, the effects of IVIG have been controversial. The majority of ill patients with SIRS had coagulation abnormalities. In addition, inflammation and coagulation play pivotal roles in the pathogenesis of sepsis. Moreover, the evidence of extensive cross-talk between these two systems has been increasing. The aim of this study is to investigate the effects of IVIG treatment for inflammation and hemostatic abnormality in sepsis patients.

Methods: This prospective single-center observational study was conducted in our ICU between January and July 2013. We enrolled 41 patients (≥18 years, admitted to the ICU diagnosed for sepsis, and more than 7 days of ICU stay) and divided them into two groups: IVIG-treated group (IVIG group) and non-IVIG-treated group (non-IVIG group). After that, we compared inflammatory molecule markers (WBC, CRP, procalcitonin (PCT), and interleukin-6 (IL-6)), coagulation/fibrinolysis markers (platelet counts, PT-INR, APTT, D-dimer, TAT, PIC, soluble fibrin (SF), and plasminogen activator inhibitor-1 (PAI-1)), and Japanese Association for Acute Medicine Disseminated Intravascular Coagulation (JAAM DIC) score and positive rate at the admission period (day 1) and days 4 to 7 between two groups. Moreover, the 28-day mortality rate was investigated.

Results: Nineteen patients were treated with IVIG (IVIG group) and 22 patients were without (non-IVIG group). In the IVIG group, PCT and IL-6 were significantly higher than that in the non-IVIG group at day 1 ($P < 0.01$, and $P < 0.01$). In this group, CRP, PCT, and IL-6 were significantly decreased at days 4 to 7 rather than that at day 1 ($P < 0.01$, $P < 0.01$, $P < 0.01$, respectively). Moreover, the JAAM DIC score was decreased at days 4 to 7 than that at day 1 significantly ($P < 0.05$) in this group. We also confirmed that PT-INR, APTT, TAT, SF, and PAI-1 were significantly improved between day 1 and days 4 to 7. On the other hand, in the non-IVIG group there was significantly decreased IL-6 and TAT only, but not CRP, PCT, PT-INR, APTT, SF, PAI-1 and JAAM DIC score between day 1 and days 4 to 7. For the 28-day mortality rate, the IVIG group was lower than that of the non-IVIG group (IVIG group, 5.3%; non-IVIG group, 18.2%).

Conclusion: Our study demonstrated that IVIG treatment significantly improved the inflammatory and hemostatic abnormalities in sepsis patients.

P25

Impact of fluid management during the three ICU days after admission in patients with ARDS.

A Murai*, H Ishikura, N Matsumoto, Y Nakamura, D Ohta, K Muranishi, Y Izutani, T Nishida
Department of Emergency and Critical Care Medicine, Faculty of Medicine, Fukuoka University, Fukuoka, Japan
Critical Care 2014, **18(Suppl 2):**P25; doi:10.1186/cc14028

Introduction: The optimal evaluation of fluid resuscitation in patients with acute respiratory distress syndrome (ARDS) remains to be clearly elucidated. The purpose of this study was to clarify the influence of fluid overload in patients with ARDS.
Methods: Two hundred and seven ARDS patients admitted to the ICUs of 23 tertiary referral hospitals in Japan were enrolled in this study. These patients were divided into survivor (survival group, n = 137) or nonsurvivor (nonsurvival group, n = 70) groups according to the 28-day mortality. All patients received mechanical ventilation and also underwent transpulmonary thermodilution monitoring. The data for analysis were collected for three consecutive days from time of admission.
Results: On the second hospital day, the extravascular lung water index of the nonsurvival group was significantly higher than that of the survival group (18.6 ± 9.4 ml/kg vs. 15.4 ± 6.3 ml/kg, P = 0.03). Moreover, regarding the first 3-day cumulative fluid balance, the nonsurvival group was significantly higher than survival group (5.1 ± 4.3 l vs. 3.5 ± 0.4 l, P = 0.015). We suspected that these results might be related to the cardiovascular and/or renal function. We therefore excluded any patients with a score of three or more regarding the cardiovascular and/or renal criteria about the Sequential Organ Failure Assessment score. Thereafter, we confirmed the results to be similar for the first 3-day cumulative fluid balance between the two groups (3.8 ± 1.6 l vs. 2.2 ± 4.0 l, P = 0.0339). A stepwise logistic regression analysis identified the 3-day cumulative fluid balance to be an independent risk factor for the 28-day mortality (adjusted odds ratio: 1.0001, 95% CI: 1.000017 to 1.000217, P = 0.0252).
Conclusion: Our findings suggest that excessive fluid resuscitation may therefore have a negative impact on the 28-day mortality for patients with ARDS.
Acknowledgements: In this study, we retrospectively analyzed the database of a multicenter observational study (The PiCCO Pulmonary Edema Study)

P26

Candida albicans versus *Mycobacterium tuberculosis*: infection-specific human immune responses.

JPLF Saraiva[1,2*], R König[1,2]
[1]Network Modelling, Hans-Knöll-Institut, Jena, Germany; [2]Centre for Sepsis Control and Care, Jena University Hospital, Jena, Germany
Critical Care 2014, **18(Suppl 2):**P26; doi:10.1186/cc14029

Introduction: Systemic infection of the human host can arise from several pathogenic bacteria as well as from fungal species, and it is of high clinical relevance to trace back the nature of infection from the host response. Amongst these systemic infections are sepsis inducers *Candida albicans* and *Mycobacterium tuberculosis*, responsible for a great amount of worldwide deaths [1]. Sepsis, however, does not necessarily originate from infection. Traumas and non-infection-based injuries can also trigger an unbound inflammatory response from the human host most commonly known as systemic inflammatory response syndrome. Both cases, infection and non-infection, nevertheless display similar clinical symptoms with significantly better recovery times of patients included in the latter. Despite the clinical similarities, studies have suggested that distinct infection-induced host responses from different pathogens occur, namely at the signalling pathway level [2].
Methods: Studies have been performed focused on common gene expression profiles between several pathogens [3]. However, understanding the difference in immune responses between these two pathogens, but not exclusively, might lead to better diagnostic tools and treatment decision-making. Relying on systems biology concepts and bioinformatics tools, we use gene expression data to distinguish *C. albicans* and *M. tuberculosis* infections in the human host; for example, cellular regulation and communication between host immune cells and pathogens [4,5].
Results: We have, in a first analysis, focused on the cytokine-cytokine signalling pathway due to its role in inflammation response towards infections. Genes belonging to the IL-2 cytokine family are only expressed when facing infection by *C. albicans* in comparison to *M. tuberculosis* suggesting a tendency towards B-cell proliferation and production of antibodies. However, during infection caused by *M. tuberculosis*, no significant changes in gene expression occur in this pathway that indicates a specific immune response for this pathogen. Further analysis of additional signalling pathways might highlight other human infection-specific immune responses in regards to the pathogens considered.
Conclusion: Next we will focus on developing a mathematical model capable of simulating such immune responses and possibly identifying genes and pathways which might clarify how these inflammation responses can be targeted, countered and moderated [6].
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P27

Vasoactive intestinal peptide inhibits the production of Salmonella-induced inflammatory cytokines by human monocytes.

B Askar*, P Barrow, N Foster
Animal Infection and Immunity, School of Veterinary Medicine and Science, University of Nottingham, UK
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Introduction: Systemic salmonella infection is a frequent cause of Gram-negative sepsis. Bacterial lipopolysaccharide in blood triggers immune response by monocytes, which results in overwhelming production of proinflammatory cytokines and pathology in peripheral organs such as the liver, heart and lungs. The mortality rate due to sepsis remains high even after chemotherapeutic clearance of pathogens, due to sustained production of inflammatory mediators. Therefore, anti-inflammatory therapy as an adjunct to antibiotics could reduce the mortality from sepsis.
Methods: To date, several studies have evaluated the role of vasoactive intestinal peptide (VIP) as a therapeutic agent in sepsis both *in vivo* and *in vitro* since it possesses several desirable biological properties. The peptide, acting via VIP cell receptors, mediates effects by altering production and secretion of inflammatory mediators such as cytokines. VIP has been shown to decrease production of proinflammatory cytokines, ameliorate histopathological changes and inhibit mortality in mice rendered septic by LPS administration. Nothing is known about the effect of VIP on production of proinflammatory cytokines in human monocytes infected by virulent Salmonella.
The aim of the current study, therefore, was to investigate the effect of vasoactive intestinal peptide on the production of inflammatory cytokines in human monocytes exposed to *S. Typhimurium* 4/74.
Results: Our finding demonstrates that freshly isolated human monocytes produce proinflammatory cytokines such as TNFα, IL-6, IL-1β and also anti-inflammatory cytokines such as IL-4 and IL-10 following bacterial challenge.
Conclusion: However, co-culture of infected monocytes with VIP (10-7 M) significantly reduced (P < 0.05) production of TNFα, IL-6, IL-1β but significantly increased (P < 0.05) concomitant production of anti-inflammatory IL-10.

P28

Mortality reduction in patients with severe sepsis and septic shock through a comprehensive sepsis initiative.

K Nguyen[1*], L Cook[2], EP Greenlee[3]
[1]Performance Improvement Department, El Camino Hospital, Mountain View, CA, USA; [2]Emergency Department, El Camino Hospital, Mountain View, CA, USA; [3]Clinical Effectiveness, El Camino Hospital, Mountain View, CA, USA
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Introduction: The Integrated Nurse Leadership Program (INLP), a regional quality improvement initiative to reduce deaths from sepsis, began in 2008. Nine participating hospitals were provided networking opportunities and training on: care of the septic patient, program and leadership development, and data collection and analysis. El Camino Hospital (ECH) applied these concepts during implementation of the early goal-directed therapy bundle as recommended by the Surviving Sepsis Campaign and the Institute for Healthcare Improvement. This paper describes the implementation process and presents data analysis from the 22-month project, focusing on severe sepsis and septic shock mortality.

Methods: A multidisciplinary team approach guided the sepsis initiative. Lean methodologies such as: root cause analysis, process mapping, data collection and direct observation were applied. Frequent meetings with executive personnel, frontline staff and physician leaders occurred to evaluate current practices. Policies and procedures were created, including standardized screening tools and order sets for early identification and management. Training materials (modules, lectures, handouts and simulations) were developed. Extensive training occurred at all levels, with experts presenting to large groups of clinicians. Ongoing data analysis included screening tool and bundle element compliance as well as ICD-9-based mortality among patients with severe sepsis and septic shock (excluding patients <18 years old, pregnant, or designated 'Do Not Resuscitate' within 24 hours of presentation). Length of stay (LOS) data were compared with data from a large healthcare collaborate quality alliance (Premier) using US Medicare Severity Diagnosis-Related Group sepsis codes (MSDRG 870, 871, 872).

Results: Prior to implementation of the program (April 2009) through April 2014, ECH achieved a relative reduction of 68% in the mortality rate among those with severe sepsis and septic shock ($P = 0.03$). This equates to 1,456 lives saved (Figure 1) [1-3]. During the initiative ECH maintained a lower than expected average LOS compared to other hospitals within the Premier database (Figure 2).

Conclusion: The significant and sustained decrease in mortality among those with severe sepsis and septic shock was achieved through the structure and support of multisite collaboration with the INLP and robust internal operations [1,3]. The focus is now on sustainability; including key elements pertaining to accountability, affordability, compassionate care and systematic excellence [4].

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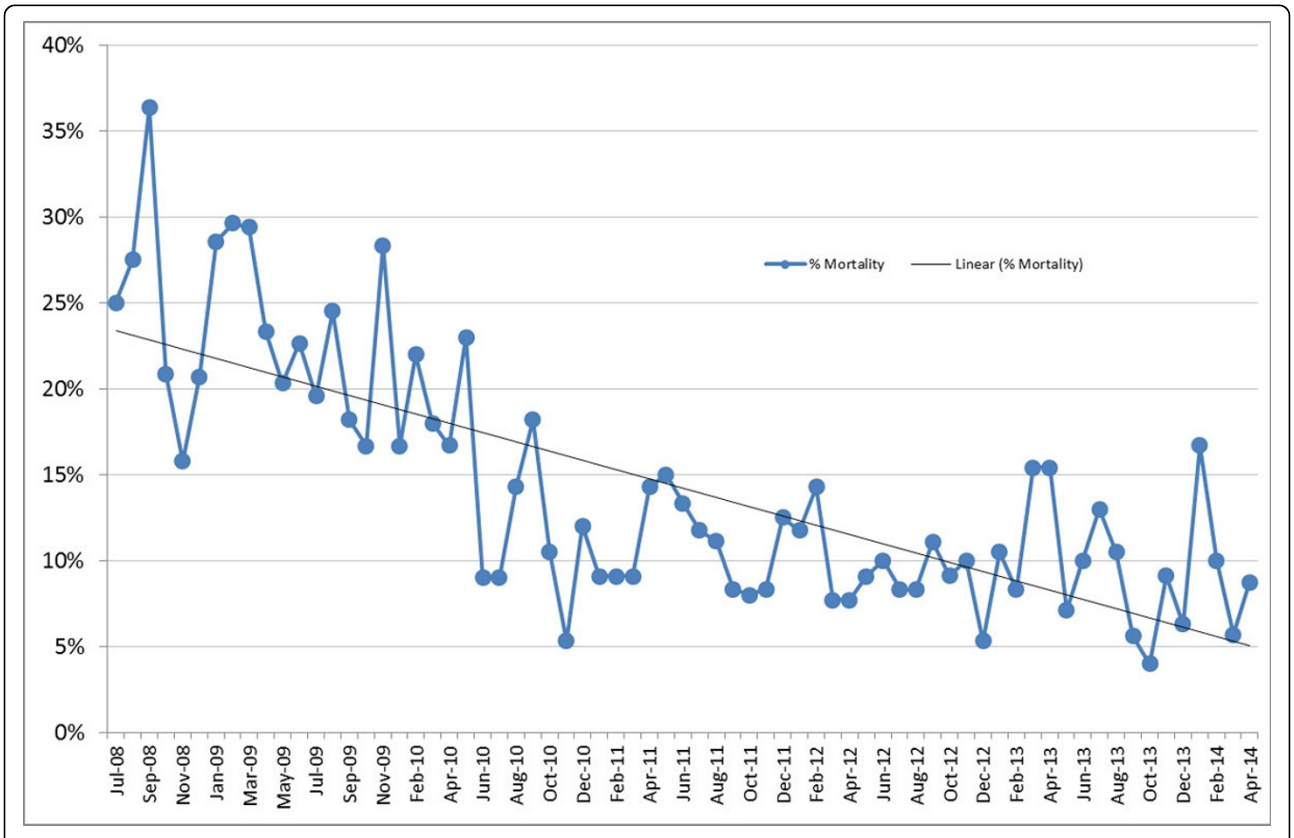

Figure 1(abstract P28) Mortality among patients with severe sepsis and septic shock; excluding patients <18 years of age, pregnant females and patients designated 'Do Not Resuscitate' within 24 hours of presentation

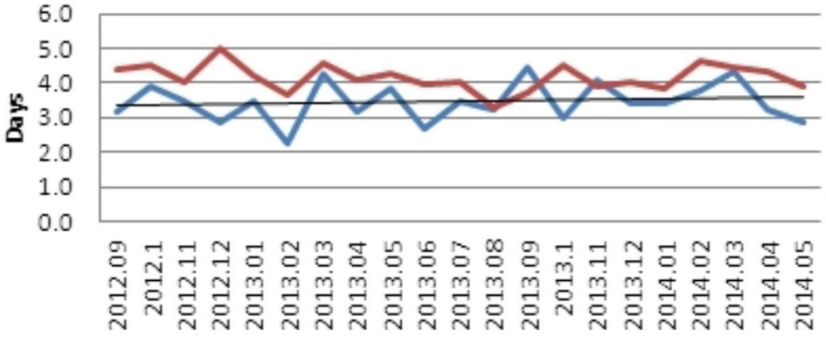

Figure 2(abstract P28) Average length of stay for patients with sepsis codes (MSDRG 870, 871, 872), comparing El Camino Hospital to expected LOS from the Premier database. Red line, Premier database expected LOS for sepsis patients; blue line, El Camino Hospital LOS for sepsis patients.

P29

Presence of bacterial infection and duration of antibiotic therapy in patients with standardized sepsis detection in the emergency department.

TC Minderhoud[1*], C Spruyt[1], M Lomax[1], S Huisman[1], SCE Schuit[2], M-D Levin[1]
[1]Department of Internal Medicine, Albert Schweitzer Ziekenhuis, Dordrecht, the Netherlands; [2]Department of Internal Medicine and Emergency Medicine, Erasmus University Medical Center, Rotterdam, the Netherlands
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Introduction: In 2002 the international Surviving Sepsis Campaign was initiated. Following this, Dutch authorities introduced a nationwide safety campaign, encouraging screening for sepsis and advocating early treatment. In 2010, the Albert Schweitzer hospital, a large community hospital, introduced a screening program for sepsis in the emergency department (ED) [1]. The goal of this study was to evaluate the bacterial outcome in patients targeted with this campaign.

Methods: All patients 18 years and older visiting the ED were screened using the criteria of systemic inflammatory response syndrome (SIRS). Patients with more than two SIRS criteria and a clinical suspicion for infection were eligible for prompt antibiotic administration, after a short assessment by an ED physician. Patient data were collected prospectively, but a retrospective analysis was conducted using a cohort of patients presenting in the ED in the first 6 months of 2011. The definitions for sepsis severity were derived from the guidelines of the Surviving Sepsis Campaign in 2008 and criteria to define bacterial infection were derived from an article by Limper and colleagues [2].

Results: A total of 269 patients were included in the study. Review of infectious outcomes showed no evidence of bacterial disease in 79 (29.4%) patients. Of these patients, 70.9% were in the lowest category of sepsis (SIRS and clinical suspicion of infectious disease). Patients in the lowest category were less likely to suffer from bacterial infection than patients ($P = 0.046$, see Table 1). In the patients without objective bacterial infection, the median duration of antibiotic treatment was 7 days (IQR 4 to 10). Overall mortality was 7.8 %, which is low compared to current literature regarding (severe) sepsis [3,4], but comparable to literature addressing SIRS and fever in the ED settings [5-8].

Conclusion: Much effort has been put into promoting early antibiotic treatment for (bacterial) sepsis. However, overtreatment has hardly been addressed and no optimal screening strategy has been identified. Evaluation of our screening protocol using SIRS criteria showed that almost 30% of patients did not suffer from bacterial infection but did receive antibiotic treatment for a median duration of 7 days. Future investigations should address the possible negative effects of overtreatment.

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Table 1(abstract P29)

	No bacterial infection ($n = 79$)	Bacterial infection proven/probable ($n = 190$)	*P* value
Culture-proven infection	-	97 (51.1%)	-
Positive blood cultures	-	51 (26.8%)	-
Probable bacterial infection	-	93 (48.9 %)	-
Sepsis category			
Sepsis	56 (70,9%)	110 (57.9%)	0.046[a,b]
Severe sepsis	20 (26,3%)	57 (30.0%)	0.549[a]
Sepsis-induced hypotension	2 (2,6%)	18 (9.5%)	0.053[a,b]
Septic shock	1 (1,3%)	5 (2.6%)	0.506[a]
Antibiotic treatment duration (days)[c]	7 (4 to 10)	10 (7 to 14)	0.000[b,d]

[a]Chi-square test. [b]Test reached statistical significance. [c]Data provided as median (IQR). [d]Mann-Whitney *U* test

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P30

Clinical audit system in implementing Surviving Sepsis Campaign guidelines in patients with peritonitis.

RC Valiveru[1*], NK Maroju[1], K Srinivasan[1], A Cherian[2]
[1]Department of Surgery, Jawaharlal Institute of Postgraduate Medical Education and Research, Dhanvantari Nagar, Pondicherry, India; [2]Department of Anesthesiology and Critical Care, Jawaharlal Institute of Postgraduate Medical Education and Research, Dhanvantari Nagar, Pondicherry, India
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Introduction: Sepsis is the predominant cause of morbidity and mortality in patients with peritonitis [1-6]. The Surviving Sepsis Campaign (SSC) is an international effort in reducing mortality based on evidence-based guidelines [7-13]. This study aims to assess the impact of audit-based feedback in a Plan-Do-Study-Act (PDSA) format on improving implementation of the SSC guidelines in patients with generalised peritonitis at our centre.
Methods: This prospective observational study was conducted in four audit cycles in PDSA format. Multidepartmental inputs were taken to suggest modifications in practice. A questionnaire-based analysis of reasons for noncompliance was done to find out the opinions and reasons for noncompliance. Morbidity, mortality, the ICU and hospital stay among these patients were also analysed.
Results: The baseline compliance with i.v. bolus administration, CVP-guided fluids and inotrope supports when indicated were 100%. Over the course of the three audit cycles, statistically significant improvement in compliance was noted for antibiotic administration within 3 hours of presentation (46% to 90%) (Table 1 Figure 1), obtaining blood cultures before antibiotics (13.8% to 72.5%) (Table 1 Figure 2) and serum lactate measurement (0% to 78.2%) (Figure 3). Overall bundle compliance improved from 9.2% to 64.7% (Table 2 Figure 4) by the end of Audit III. The mortality rate decreased from 32.3% to 20% (Table 2 Figure 5).
Conclusion: This study demonstrates that audit-based feedback is a dependable means of improving compliance with SSC guidelines. It brings about improvement by educating users, by modifying their behaviour through feedback and also enhances process improvement by identifying and correcting systemic deficiencies in the organisation.

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P31

Introduction of bundle of care and effect on surgical site infections in patients taken for elective surgical procedures.

A Waheed[1*], S Bhat[2], M Parvez[3]
[1]Department of Anesthesia, District Hospital Doda (Deemed Medical College), Jammu and Kashmir, India; [2]Department of Gynecology, District Hospital Doda (Deemed Medical College), Jammu and Kashmir, India; [3]Department of Surgery, District Hospital Doda (Deemed Medical College), Jammu and Kashmir, India
Critical Care 2014, **18(Suppl 2):**P31; doi:10.1186/cc14034

Introduction: Surgical site infection (SSI) previously termed postoperative wound infection is defined as that infection presenting up to 30 days after a surgical procedure if no prosthetic is placed and up to 1 year if a prosthetic is implanted in the patient. Several recommendations have been published (skin preparation, surgical antibiotic prophylaxis, control of the OR environment and improvements in the surgical technique) to improve the patient safety and quality of care in the OR. Use of the bundle of care to improve patient outcomes is becoming more widespread; however, their use is more common internationally than in India.
Methods: A surveillance study for SSI after routine surgical procedures was conducted from September 2012 until August 2014. A bundle of care consisting of five elements covering the surgical process was introduced in September 2013. The elements of the bundle were perioperative antibiotic prophylaxis, hair removal before surgery, perioperative normothermia, perioperative euglycemia and operating room discipline. Normothermia was defined as a temperature between 36.0 and 38.0°C. Euglycemia was defined as blood glucose <180 mg/dl. Antibiotic prophylaxis was given 15 minutes before the incision. Hair removal whenever needed was done with clippers. Theatre discipline was counted for following points: permanent wearing of scrub suits, surgical cap and mask covering the nose and mouth by all persons in the OR during the surgical procedure. The incidence of SSI was studied as a primary outcome. Morbidity/mortality for next 3 months was studied as the secondary outcome.
Results: Implementing the bundle of care led to a decline in infection rate from 15% before the intervention to 11.4% after the introduction of bundle of care, a fall of 27%, which is significant ($P < 0.001$). No significant difference in 3-month mortality was found. The compliance to this bundle of care also steadily increased from 10% in September 2013 to 55% in August

Table 1(abstract P30) Compliance with obtaining blood cultures before antibiotics and antibiotic administration within 3 hours

Number of patients	Pre audit (n = 65)	Audit I (n = 55)	Audit II (n = 50)	Audit III (n = 51)
Blood cultures obtained before antibiotics	9 (13.6%)	18 (32.7%)[a]	30 (60%)[b]	37 (72.54%)[b]
Antibiotics given within 3 hours	30 (46.1%)	30 (67.2%)[c]	40 (80%)[d]	46 (90.1%)[b]

[a]$P = 0.016$. [b]$P < 0.0001$. [c]$P = 0.463$. [d]$P = 0.0002$

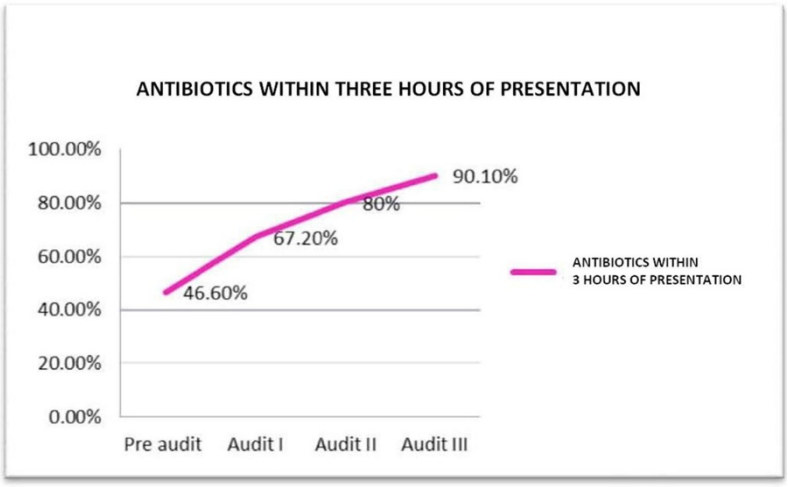

Figure 1(abstract P30) Compliance with antibiotic administration within 3 hours of presentation

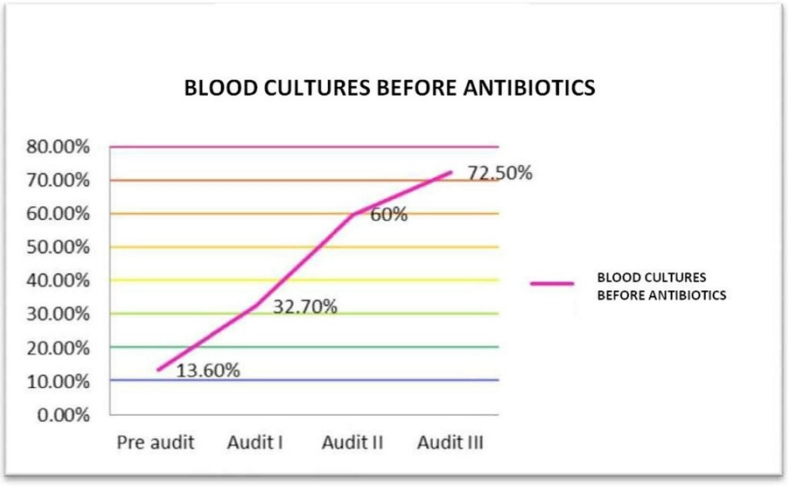

Figure 2(abstract P30) Compliance with obtaining blood cultures before antibiotic administration

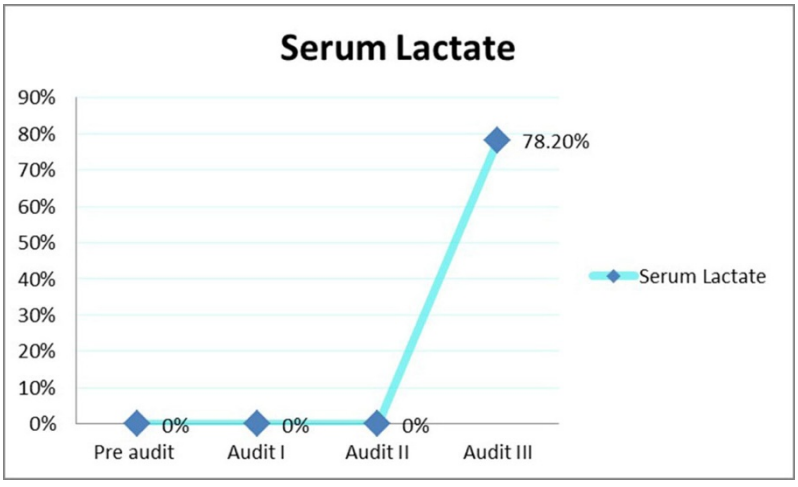

Figure 3(abstract P30) Compliance with measurement of serum lactate

Table 2(abstract P30) Total bundle compliance

Total number of bundle components performed	Pre audit (*n* = 65)	Audit I (*n* = 55)	Audit II (*n* = 50)	Audit III (*n* = 51)
6	0	0	0	33[a] (64.7)
5	6 (9.2%)	13 (23.6%)	27 (54%)	3 (5.8%)
4	26 (40%)	29 (52.7%)	16 (32%)	11 (21.5%)
3	33 (50.7%)	13 (23.6%)	7 (14%)	4 (7.8%)
2	0	0	0	0
1	0	0	0	0

[a]Serum lactate was available in the hospital only during audit cycle III

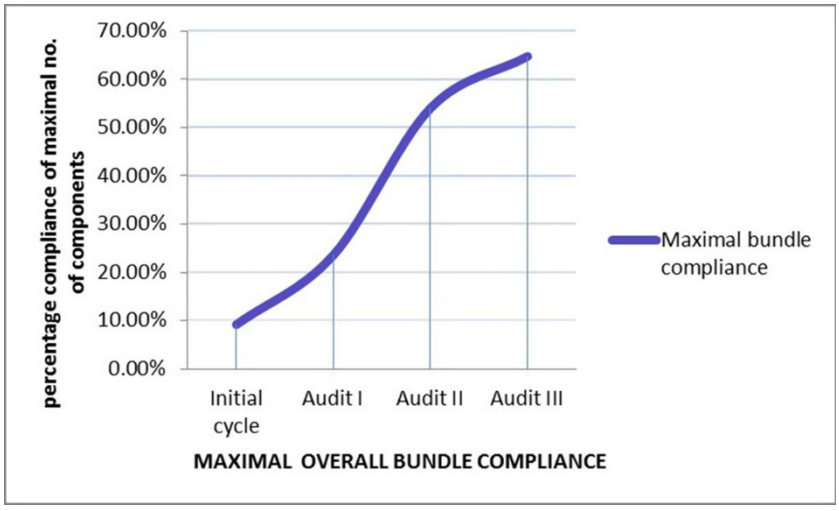

Figure 4(abstract P30) Overall bundle compliance

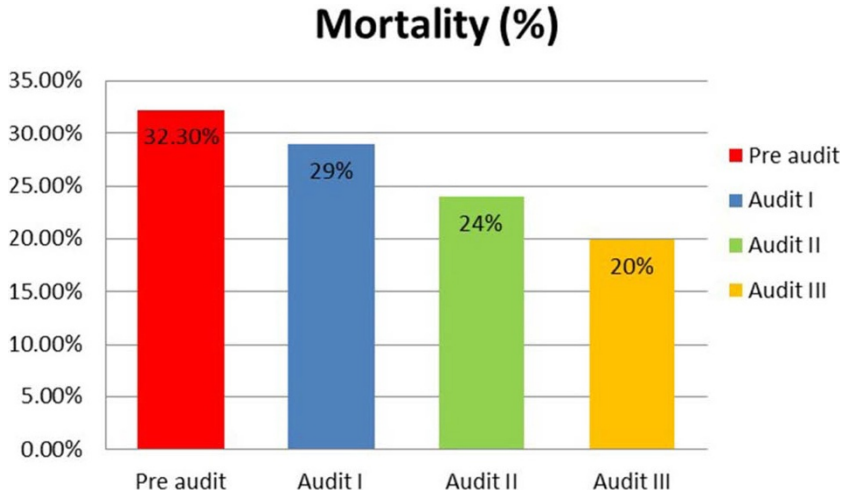

Figure 5(abstract P30)

Table 3(abstract P30) Overall mortality

	Pre audit (*n* = 65)	Audit I (*n* = 55)	Audit II (*n* = 50)	Audit III (*n* = 51)
Overall mortality in percentage	21 (32.3%)	16 (29%)	12 (24%)	11 (20%)
P value (compared with the initial cycle)		0.843	0.407	0.143

2014. For 542 surgical procedures during the study period, 62 SSI (11.4%) occurred as compared to 102 cases for 680 in the control period (15%). The adjusted odds ratio of the SSI rate was 0.7319 and was found to be 27% lower post intervention.
Conclusion: The implementation of the bundle was associated with improved compliance over time and a significant reduction of the SSI rate. This makes the bundle an important tool to improve patient safety.

P32

More effective use of polymyxin-B hemoperfusion for nonoperation cases.

M Yahata*, Y Sakamoto, S Inoue, T Iwamura, R Fujita, H Koami, T Miike, H Imahase, S Narumi, A Goto, M Ohta
Emergency and Critical Care Medicine, Saga University, Saga City, Japan
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Introduction: Direct hemoperfusion with polymyxin-B has been reported to improve hemodynamics in postsurgical patients. In 2012, the Japanese Guidelines for the Management of Sepsis were published and mention the efficacy of polymyxin-B direct hemoperfusion. But how to use and the target patients are varied by facilities. We investigated the effective use of polymyxin-B direct hemoperfusion in nonsurgical patients.
Methods: We analyzed retrospectively all septic shock patients who were treated with polymyxin-B hemoperfusion between January 2008 and December 2012.We checked their mean arterial pressure (MAP), and vasopressor requirement every 30 minutes until stopping treatment.
Results: There were 32 patients under treatment and 11 patients did not need surgical treatment. Even in the nonsurgical group, hemodynamic states and vasopressor requirement was improved after polymyxin-B hemoperfusion started. And the effects were continued over 120 minutes. A second plolymyxin-B hemoperfusion treatment underwent in nine patients. In second treatment, MAP increased in the nonsurgical group greater than in the postsurgical group.
Conclusion: Polymyxyn-B direct hemoperfusion improves hemodynamic status even in nonsurgical patients. A second polymyxin-B direct hemoperfusion is effective especially in nonsurgical septic shock patients. And if its hemodynamic effect was significantly, long-time treatment should be considered.

P33

Sepsis modulates the human hematopoietic stem cell compartment in peripheral blood and bone marrow.

T Skirecki[1,2*], U Zielińska-Borkowska[2], J Kawiak[1], G Hoser[1]
[1]Laboratory of Flow Cytometry, The Center of Postgraduate Medical Education, Warsaw, Poland; [2]Department of Anesthesiology and Intensive Care, The Center of Postgraduate Medical Education, Warsaw, Poland
Critical Care 2014, **18(Suppl 2):**P33; doi:10.1186/cc14036

Introduction: Efficient fight with infection requires robust production of immunocompetent cells. This response is called emergency hematopoiesis and depends on the proliferation of progenitor cells and awakening dormant hematopoietic stem cells (HSCs) into cycling. As during sepsis an altered immune system is often observed, it seems important to reveal the impact of this syndrome on HSCs. Recent discoveries have shown that HSCs circulate in the peripheral blood and may boost local immune response via paracrine mechanisms and differentiation into myeloid cells. Altogether, these rationales led us to investigate the circulating HSCs in septic patients and in the bone marrow (BM) of septic 'humanized mice' transplanted earlier with human HSCs.
Methods: Samples of peripheral blood were collected from 23 patients with sepsis (on days 1 and 3) and 20 healthy volunteers. The following antigens were analyzed by flow cytometry: CD34, CD38, Ki-67, CD133, Lin and CD45. In order to investigate HSCs in their microenvironment, a model of cecum ligation and puncture (CLP) was performed on the NOD.Cg-Prkdc/scidIL2rγ mice that were transplanted with human cord blood $CD34^+$ cells 8 weeks earlier. BM cells were analyzed 24 hours after CLP by colony-forming unit assay with medium supporting growth of human cells.
Results: Septic patients had a significantly increased (threefold, $P < 0.01$) number of $CD34^+CD38^-$ HSCs on the third day of the disease. Also, the $CD133^+$ HSC number was increased in septic patients, while $CD34^+CD45^+Lin^-$ progenitors were detected at much lower level than in controls. Interestingly, $Ki\text{-}67^+CD34^+Lin^-$ cells were fourfold higher in septic patients. Patients with higher number of $CD133^+$ HSCs had significantly lower likelihood of 60-day survival ($P < 0.05$). Analysis of human HSCs from BM of septic mice revealed significantly compromised hematopoietic colonies output (248 vs. 125 in sham animals). CLP caused also expansion of $CD34^+CD38^-$ HSCs in BM and absolute increase of $Ki\text{-}67^+CD34^+Lin^-$ cells (1.5-fold).
Conclusion: In this work we have observed significant changes in circulating HSCs during sepsis. During the disease, dormant HSCs enter the cell cycle (measured by Ki-67 expression) and are mobilized to the peripheral blood. However, the progenitor cells disappear from circulation. Novel use of humanized mice confirmed expansion of early human HSCs in BM during the sepsis model. Despite expansion of the HSC pool, the amount of functional progenitors in BM is decreased in this model. We suggest that HSCs play a significant role in the course of sepsis and may become a new prognostic and therapeutic target.
Acknowledgements: Supported by 'Innovative Methods of Stem Cell Applications in Medicine', EU Innovative Economy Operational Program, POIG 01.02-00-109/09.

P34

Physiological changes after fluid bolus therapy in sepsis: a systematic review of the contemporary literature.

NJ Glassford[1,2*], GM Eastwood[1,3], R Bellomo[1,2]
[1]Department of Intensive Care, Austin Hospital, Heidelberg, VIC, Australia; [2]ANZICS Research Centre, School of Public Health and Preventative Medicine, Monash University, Melbourne, VIC, Australia; [3]School of Nursing and Midwifery, Faculty of Health, Deakin University, Burwood, VIC, Australia
Critical Care 2014, **18(Suppl 2):**P34; doi:10.1186/cc14037

Introduction: Fluid bolus therapy (FBT) is a ubiquitous intervention in intensive care. However, the physiological effects in the critically ill are poorly understood. Therefore, we systematically reviewed the contemporary literature to determine the current practice and effect of FBT in the management of severe sepsis and septic shock.
Methods: We interrogated the MEDLINE, CENTRAL and EMBASE electronic reference databases using a combination of terms to define a set of records of studies of fluid administration in patients with severe sepsis or septic shock. To achieve contemporary relevance, results were limited to English-language studies in adults between 2010 and 2013.
Results: We identified 22 prospective observational studies, four retrospective observational studies, two quasi-experimental studies, and five randomised controlled trials (RCTs), 41 boluses in total. No RCT compared FBT with alternative interventions. The median fluid bolus was 500 ml (range: 100 to 1,000 ml) administered over 30 minutes (range: 10 to 60 minutes) and 0.9% sodium chloride solution was the most commonly administered. Although 17 studies describe the temporal course of physiological changes after FBT in 31 patient groups, only three studies describe the physiological changes at 60 minutes, and only one study beyond this point (Figure 1). No studies related the physiological changes after FBT with clinically relevant outcomes.
Conclusion: There is a need for obtaining randomised controlled evidence for the physiological effects of FBT in patients with severe sepsis and septic shock beyond the period immediately following its administration.

P35

Forty percent of hospitalizations after severe sepsis are potentially preventable.

HC Prescott[1*], KM Langa[1,2,3], TJ Iwashyna[1,2,3]
[1]University of Michigan, Department of Medicine and Institute of Healthcare Policy and Innovation, Ann Arbor, MI, USA; [2]VA Center for Clinical

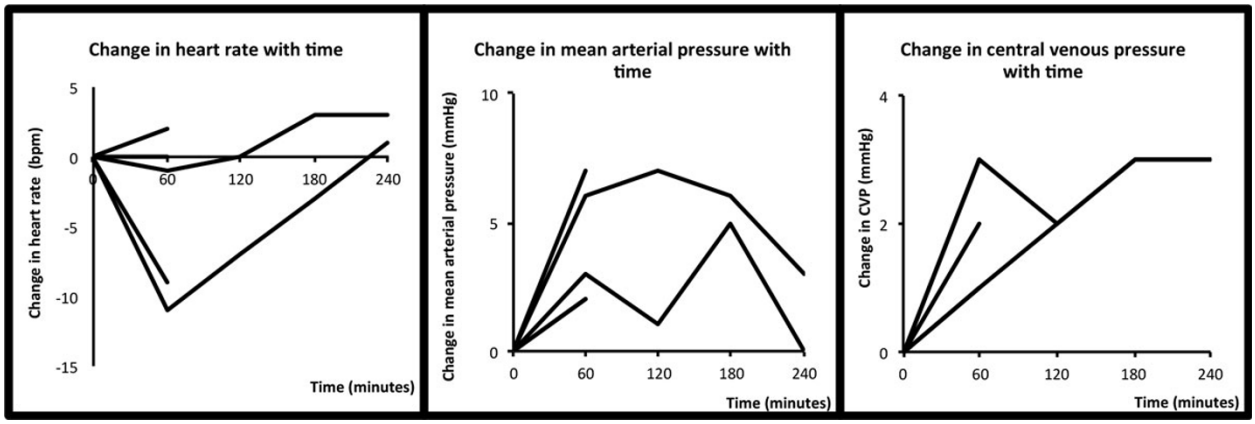

Figure 1(abstract P34) Haemodynamic changes following FBT at 1, 2, 3 and 4 hours

Management and Research, Ann Arbor, MI, USA; [3]Institute of Social Research, Ann Arbor, MI, USA
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Introduction: Patients are frequently rehospitalized in the 90 days after severe sepsis. The rate of readmission exceeds patients' baseline rate of hospitalization, and also exceeds the rate after matched nonsepsis hospitalizations [1]. We sought to determine the most common readmission diagnoses after severe sepsis, the extent to which readmissions may be preventable, and whether the pattern of readmission diagnoses differs from that of nonsepsis hospitalizations.

Methods: We studied participants in the US Health and Retirement Study with linked Medicare claims (1998 to 2010) [2]. Using validated methods [3,4], we identified severe sepsis and nonsepsis hospitalizations, then measured 90-day readmissions in each cohort. Using Healthcare Cost & Utilization Project's Clinical Classification Software [5], we determined the 10 most common readmission diagnoses after severe sepsis. We measured rates of 'potentially preventable' readmissions using published definitions [6]. We compared rates of all-cause, potentially preventable, and cause-specific hospitalizations between survivors of severe sepsis and nonsepsis hospitalizations using chi-squared tests.

Results: We identified 3,703 severe sepsis and 44,840 nonsepsis hospitalizations, of which 3,036 (82.0%) and 43,539 (93.1%) survived to discharge, respectively. In the next 90 days, 43.6% of severe sepsis survivors were rehospitalized, compared to 34.8% of nonsepsis survivors, $P < 0.001$. The top readmission diagnoses following severe sepsis (Table 1) included several recognized potentially preventable diagnoses: heart failure, pneumonia, exacerbation of chronic obstructive pulmonary disease (COPD), and urinary infection. Also common were readmissions for sepsis, acute renal failure, and aspiration pneumonitis, diagnoses that could plausibly be prevented or treated early to prevent hospitalization. Patterns of readmission differed in severe sepsis survivors; rates of readmission for sepsis, renal failure, respiratory failure, device complication, and aspiration pneumonitis were higher and accounted for a greater proportion of the total readmissions. Potentially preventable hospitalizations - infection (sepsis, pneumonia, urinary tract, and skin or soft tissue), heart failure, COPD exacerbation, acute renal failure, and aspiration pneumonitis - accounted for 40.5% of all readmissions after severe sepsis (compared to 25.8% following nonsepsis admission, $P < 0.001$), and 19.6% of severe sepsis survivors experienced a readmission for one of these diagnoses (compared to 9.5% following a nonsepsis admission, $P < 0.001$).

Conclusion: Forty percent of hospitalizations after severe sepsis occur for diagnoses that may be preventable. A few disease categories account for a relatively large proportion of the hospitalizations after severe sepsis, suggesting the feasibility of tailoring postdischarge interventions to patient's personalized risk for these common postsepsis diagnoses.

Acknowledgements: The authors declare financial support from the US National Institutes of Health and the US Department of Veteran's Affairs. There are no potential conflicts of interest. The views expressed in this article are those of the authors and do not necessarily reflect the position or policy of the Department of Veterans Affairs or the US government

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Table 1(abstract P35) Top ten hospitalization diagnoses in the 90 days following severe sepsis

Rank	Diagnosis category	Proportion of all 90-day admissions (%)	Survivors with 90-day admission (%)
1	Congestive heart failure, nonhypertensive	10.4	5.7*
2	Septicemia	9.5*	6.5*
3	Pneumonia	5.4	3.5*
4	Rehabilitation care	5.1	3.2
5	Acute and unspecified renal failure	4.6*	3.2*
6	Respiratory failure	4.1*	2.5*
7	Complication of device, implant, or graft	3.5*	2.3*
8	COPD and bronchiectasis	3.1	1.8
9	Urinary tract infection	3.1	1.8*
10	Aspiration pneumonitis	2.8*	1.8*

*Value greater than that of nonsepsis survivors, $P \leq 0.001$ for each comparison

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P36

Aryl hydrocarbon receptor activation increases survival in polymicrobial sepsis.

A de Freitas*, P Barbim Donate, F Vargas e Silva Castanheira, V Borges, D Carvalho Nascimento, J Talbot, JC Alves-Filho, F de Queiróz Cunha
Department of Pharmacology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil
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Introduction: Sepsis is a systemic inflammatory response resulting from the inability of the host to restrict the infection locally. Studies realized in our laboratory demonstrated that the high mortality observed in severe sepsis (S-CLP) correlates with the failure of the neutrophil migration to infectious focus and infection dissemination. However, animals subjected to a model of local infection (NS-CLP) present an efficient neutrophil recruitment that constrains the spreading of infection. In this context, we demonstrated a direct action of IL-17 mediating the neutrophil recruitment [1]. Recently, it was demonstrated that aryl hydrocarbon receptor (AhR) activation has a role in immune response. The AhR is expressed by Th17 cells and also by innate immune system cells and is important for their effectors functions, including IL-17 and IL-22 production [2]. However, the function of the AhR in sepsis remains uncertain. Herein, we investigate the role of AhR in polymicrobial sepsis induced by cecal ligation and puncture (CLP).
Methods: C57BL/6 mice were subjected to NS-CLP or S-CLP sepsis. The protein expressions of AhR, CYP1A1 (indicator of AhR activation) and AhRR (AhR repressor) were determined in the spleen, liver and lung by western blot, 18 hours after sepsis. AhR and CYP1A1 were also evaluated, 6 hours after surgery, by RT-PCR in peripheral blood mononuclear cells (PBMC). Mice were pretreated subcutaneously with 30 μg/kg FICZ, high-affinity AhR agonist, 12 hours and 1 hour before the induction of moderate sepsis (M-CLP). Intraperitoneal neutrophil migration, bacteremia, kidney function and AhR activation were determined 6 hours after surgery. The survival rate was assessed every 12 hours up to 120 hours.
Results: We observed reduced expression of AhR, CYP1A1 and increased AhRR expression in all analyzed organs of the S-CLP group compared with the NS-CLP group. Moreover, AhR and CYP1A1 were not detected in PBMC after S-CLP. Our results also demonstrated that activation of Ahr, through FICZ pretreatment, increased the neutrophil recruitment to the peritoneal cavity of mice subjected to M-CLP. Consequently, these animals presented reduced bacteremia and kidney injury, resulting in increased survival rate.
Conclusion: These data suggest that during severe infection the AhR expression and activity is reduced and can contribute to the mortality observed in this process.
Acknowledgements: Financial support by #2012/04076-9 and 2013/08216-2 from FAPESP
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P37

Epidemiology, management and clinical outcomes of ICU-acquired enterococcal bacteraemias.

DSY Ong[1,2,3*], K Safdari[2], PMC Klein Klouwenberg[1,2,3], C Spitoni[1,4], JF Frencken[2,3], E Witteveen[5], J Horn[5], MJM Bonten[1,3], OL Cremer[2]
[1]Department of Medical Microbiology, University Medical Center Utrecht, the Netherlands; [2]Department of Intensive Care Medicine, University Medical Center Utrecht, the Netherlands; [3]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, the Netherlands; [4]Department of Mathematics, Utrecht University, the Netherlands; [5]Department of Intensive Care, Academic Medical Center, University of Amsterdam, the Netherlands
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Introduction: Enterococcal bacteraemias are associated with high mortality rates, ranging from 23 to 48% in critically ill patients. However, it remains uncertain whether these events are causes or merely markers of disease severity and mortality. Our aim was to describe the epidemiology, management and clinical outcomes of ICU-acquired enterococcal bacteraemia in comparison to other frequently isolated pathogens. Furthermore, we aimed to estimate the population attributable fraction of ICU mortality caused by enterococcal bacteraemia.
Methods: Between January 2011 and March 2013 we included consecutive patients with an ICU length of stay of at least 3 days in two tertiary care centres in the Netherlands. ICU-acquired bacteraemia was defined as a first positive blood culture occurring at least 3 days after ICU admission. Enterococcal bacteraemias were compared to other frequently isolated pathogens with respect to patient characteristics, their management and outcomes. We used competing risk survival regression, a multistate model and cumulative incidence functions to estimate the population attributable fraction of ICU mortality due to enterococcal bacteraemia.
Results: Out of a total of 3,108 patients, 222 (7.1%) patients were responsible for 272 events of ICU-acquired bacteraemia, of which 76 were due to enterococci, 124 to coagulase-negative staphylococci and 40 to Gram-negative bacteria. Patients with enterococcal bacteraemia were more severely ill compared to patients with bacteraemia caused by other pathogens. In comparison to patients with coagulase-negative staphylococci, those with enterococci were more frequently managed with renal replacement therapy and also had more intravascular or orthopaedic hardware. Although crude ICU mortality was higher in patients with bacteraemias due to enterococci compared to coagulase-negative staphylococci, this association disappeared after adjustment for confounders (subdistribution hazard ratio 1.04, 95% confidence interval 0.65 to 1.68). The population attributable fraction of ICU mortality due to enterococcal bacteraemia was 4.7%.
Conclusion: Bacteraemias with enterococci occur in more severely ill patients, but their virulence seems comparable to that of coagulase-negative staphylococci. Furthermore, the population attributable fraction of ICU mortality due to enterococcal bacteraemia is low. Therefore, enterococcal bacteraemias are more likely to be markers than causes of increased disease severity and mortality.
Acknowledgements: This research was performed within the framework of CTMM, the Center for Translational Molecular Medicine (http://www.ctmm.nl), project MARS (grant 04I-201).

P38

Systemic inflammatory response in the pediatric emergency department: a common phenomenon that does not predict severe illness.

CPZ Foo[1*], G Sangha[1,2], J Seabrook[2,3], J Foster[1,2]
[1]Department of Paediatrics, Western University, London, Canada; [2]Children's Health Research Institute, London Health Sciences Centre, London, Canada; [3]Division of Food and Nutritional Sciences, Brescia University College, London, Canada
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Introduction: Systemic inflammatory response syndrome (SIRS) was defined in pediatrics primarily to aid in research subject identification. There is a paucity of data on the clinical utility of SIRS in the pediatric population. We aimed to determine the incidence of SIRS in children presenting to the pediatric emergency department (PED) of a mid-sized Canadian tertiary care

center and, secondarily, to examine the sensitivity and specificity of SIRS for predicting infection, the range of clinical entities presenting with SIRS, and outcomes of children with SIRS.
Methods: We conducted a prospective cohort study of all children from birth to 18 years presenting to the PED on 16 days distributed evenly over 1 year. Charts of all patients presenting to the PED on study days were reviewed to determine the presence of SIRS and sepsis. Three pediatricians adjudicated all cases of presumed infection. All patients were followed for 1 week to determine outcomes.
Results: The incidence of SIRS was 14.3% (n = 202 of 1,416). The rate of documented or presumed infection in the entire population was 37.1% (n = 525), and in SIRS patients was 81.2% (n = 164). Therefore, 11.6% of the population (n = 164) met criteria for sepsis, one patient had severe sepsis, and there were no cases of septic shock. Sensitivity and specificity of SIRS for predicting infection was 31.2% (95% CI: 27.3 to 35.4%) and 95.7% (95% CI: 94.2 to 97.0%) respectively. No difference in sensitivity or specificity was seen when cases were separated by age. Patients with SIRS had a higher risk of requiring hospital admission with a relative risk of 2.8 (95% CI: 2.0 to 4.1, P < 0.0001). Although children with SIRS stayed in hospital statistically longer (P < 0.001), the median length of stay for both groups was ≤1 day. However, 28.7% (n = 47) of patients meeting definition of sepsis had nonsevere infection types (for example, acute otitis media, viral upper respiratory tract infection); 72.3% of patients with SIRS and 75% of patients meeting sepsis criteria were discharged home from the PED without return visits.
Conclusion: While SIRS has a high specificity for infection, its poor sensitivity suggests a risk of missing infection if used as a screening tool. Although patients with SIRS have an increased risk of admission, the current definition of sepsis may result in over-inclusion of patients with nonsevere disease.
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P39

Early bacterial spreading and inflammatory profile in a pneumosepsis model.

M de Carvalho Gonçalves*, V Horewicz, J Assreuy
Department of Pharmacology, Universidade Federal de Santa Catarina, Florianopolis, Brazil
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Introduction: Pneumonia is the major cause of sepsis, responsible for almost one-half of all sources of infection [1]. Sepsis and septic shock lead to organ failure and death. Spreading of microorganisms and their toxins through the blood could contribute to the organ dysfunction. The heart, liver and kidney are examples of organs damaged during the systemic infection and organ failure predicts poor prognosis in patients with sepsis [2]. However the correlation, if any, between bacterial spreading and organ injury is unclear. Thus, the aim of this study was to study the bacterial systemic spreading from a localized infection along with time and the target organ inflammatory profile using proinflammatory cytokines as surrogate markers.
Methods: Pneumosepsis was induced by *Klebsiella pneumoniae* as described in [3]. The number of viable bacteria inoculated was 10^9 colony-forming units to achieve a mortality rate of ~50% by 48 hours. Blood, lung, heart, liver, spleen, kidney and brain were aseptically harvested, homogenized and plated on Müeller-Hinton agar dishes, for 18 hours at 37°C. Plasma and the tissues homogenates were assayed for interleukin-6 (IL-6) and interleukin-1β (IL-1β) using commercially available enzyme-linked immunosorbent assay kits according to the manufacturer's recommendations (PeproTech Inc., Rocky Hills, NJ, USA).
Results: Lung inoculation with *K. pneumoniae* evolved to systemic spreading of bacteria to all organs, mostly the liver and kidney. Surprisingly, bacteria were found as early as 30 minutes in vital organs such as the brain and heart. The infection in the organs rose steadily up to 24 to 48 hours. Significant increases in IL-6 and IL-1β were found in the plasma, 24 hours after infection. However, cytokine levels in the organs were as high as fivefold the plasma levels.
Conclusion: Our data show that the early bacterial dissemination may be important for the onset of organ inflammation. Assuming that the higher organ cytokine level is a marker of an ongoing inflammation, this may explain organ dysfunction during sepsis. Thus, our study suggests that systemic (meaning blood) parameters may not reflect the severity of inflammation/dysfunction in target organs, and that might be the determinant to sepsis outcome.
Acknowledgements: Brazilian financial support from CNPq, CAPES, FAPESC and FINEP

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P40

Effects of splenectomy and GTS-21, a selective α7 nicotinic acetylcholine receptor agonist, on the development of septic ileus in mice.

S Nullens[1*], JG De Man[1], PA Pelckmans[1,2], BY De Winter[1]
[1]Laboratory for Experimental Medicine and Pediatrics, Division of Gastroenterology and Hepatology, University of Antwerp, Belgium; [2]Department of Gastroenterology and Hepatology, Antwerp University Hospital, Antwerp, Belgium
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Introduction: Sepsis remains a leading cause of mortality in our ICUs. Ileus, defined as the inhibition of the propulsive motility in the gastrointestinal (GI) tract, together with mucosal barrier dysfunction will maintain sepsis by the translocation of intestinal bacteria. Preliminary data in our group showed that the administration of GTS-21, a selective alpha7 nicotinic acetylcholine receptor (α7nAChR) agonist, ameliorates inflammation and GI motility disturbances during sepsis [1]. Activation of the α7nAChR is the final step in the vagal anti-inflammatory pathway, a spleen-dependent and macrophage-dependent pathway that dampens inflammation. We aimed to study the effects of splenectomy (SPLX) combined with GTS-21 on GI motility and on local colonic and systemic inflammation, as the role of the spleen in the vagal anti-inflammatory pathway is currently under debate.
Methods: Septic ileus was induced in Swiss-OF 1 mice by cecal ligation and puncture (CLP). One week prior to CLP, mice underwent SPLX or sham surgical incision (sham). GTS-21 (8 mg/kg) or vehicle was administered intraperitoneally 1 hour before CLP, and once every 24 hours after the procedure. Mice were sacrificed 48 hours following CLP. Motility was assessed by measurement of GI transit of beads, with calculation of percentage of gastric emptying (%GE) and geometric center (GC). Serum samples were analyzed with cytometric bead array (CBA) for the proinflammatory cytokines IL-6 and TNFα. Supernatants of centrifuged homogenized colonic samples were assessed with CBA for IL-6 and TNFα
Results: Administration of GTS-21 resulted in a significant improvement in GI motility in septic mice, as was demonstrated by an increase in the %GE and GC (Table 1). GTS-21 significantly decreased the serum concentration of IL-6, but not TNFα, in septic mice treated with GTS-21 compared to vehicle-treated mice. Prior SPLX protected mice from developing ileus following CLP. SPLX + vehicle mice demonstrated a significantly lower serum concentration of IL-6 and TNFα compared to sham + vehicle, and colonic TNFα levels declined significantly following SPLX. Administering GTS-21 in SPLX mice did not result in an additional benefit on GI motility, nor on systemic or local colonic inflammation (Table 1).
Conclusion: Splenectomy protects mice from developing ileus following CLP, an animal model of polymicrobial sepsis, as does the preventive administration of the selective α7nAChR agonist GTS-21. Splenectomy resulted in a reduction of systemic TNFα and IL-6 levels, indicating that the spleen is a major source of proinflammatory cytokines. GTS-21 ameliorates CLP-induced septic ileus, but did not lead to a complimentary benefit in addition to splenectomy.

Table 1(abstract P40) %GE, GC, serum cytokine levels (pg/ml) and colonic cytokine levels (pg/g colon) in mice that underwent splenectomy or sham surgery, followed by the CLP procedure and administration of vehicle or 8 mg/kg GTS-21

GI transit study (n = 11)	%GE	GC
Nonseptic animals	*88.96 ± 8.13*	*4.86 ± 0.45*
Sham + vehicle + CLP	53.27 ± 10.32	2.53 ± 0.42
Sham + GTS-21 8 mg/kg + CLP	83.62 ± 5.91*	4.26 ± 0.35*
SPLX + vehicle + CLP	79.49 ± 8.98*	3.86 ± 0.44*
SPLX + GTS-21 8 mg/kg + CLP	77.64 ± 11.81*	3.55 ± 0.52
Cytokine level in serum (n = 8 to 11)	**IL-6**	**TNFα**
Nonseptic animals	*2.57 ± 0.48*	*4.95 ± 0.29*
Sham + vehicle + CLP	243.56 ± 54.89	63.72 ± 21.25
Sham + GTS-21 8 mg/kg + CLP	90.26 ± 31.31*	54.23 ± 25.14
SPLX + vehicle + CLP	74.31 ± 17.45*	11.52 ± 1.99$^{\#}$
SPLX + GTS-21 8 mg/kg + CLP	64.98 ± 7.15*	12.81 ± 3.91$^{\#}$
Cytokine levels in colonic tissue (n = 5 to 11)	**IL-6**	**TNFα**
Nonseptic animals	*20.43 ± 4.87*	*41.30 ± 4.47*
Sham + vehicle + CLP	1281.85 ± 932.10	137.15 ± 61.36
Sham + GTS-21 8 mg/kg + CLP	134.88 ± 36.15	46.46 ± 5.03*
SPLX + vehicle + CLP	214.22 ± 75.88	29.97 ± 8.60*
SPLX + GTS-21 8 mg/kg + CLP	487.49 ± 343.87	46.03 ± 17.22*

Data presented as mean ± SEM. Two-way ANOVA or one-way ANOVA as appropriate with SNK *post hoc* testing; *$P \leq 0.05$ versus sham + vehicle, $^{\#}P \leq 0.05$ significant effect of splenectomy (SPLX)

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P41

Biobanking in the emergency department: implementation of the Mayo Clinic Emergency Department Sepsis Biorepository.

CM Clements*, JR Anderson, J Uhl, MI Rudis, FR Cockerill
Department of Emergency Medicine, Mayo Clinic, Rochester, MN, USA
Critical Care 2014, **18(Suppl 2):**P41; doi:10.1186/cc14044

Introduction: Biomarker discovery research has not focused on the emergency department (ED) due to perceived lack of access to ED patients for study, difficult patient identification, and preemption by time-critical clinical needs. The aim of this study was to use an automated process to accrue, within the ED, a biobank of patients presenting at risk for severe sepsis.
Methods: Our group has previously derived an algorithm for identification of at-risk patients of which about 45% will be severely septic [1]. Here, we utilize that algorithm to identify a prospective cohort to be included in our ED sepsis biobank. Patients are excluded if they are pregnant, <18 years old, a vulnerable adult, or imprisoned. An electronic notification system identifies patients and automatically pages phlebotomy and laboratory processing personnel directly. Blood draws occur immediately after patient identification and at 6 and 24 hours into hospitalization. We use an IRB-approved delayed consent model that minimizes the time between identification and blood draw. Plasma extracts and cellular isolates are processed and stored immediately. Clinical data are extracted for relevant patient outcomes, severe sepsis, and indicators of critical illness.
Results: In 1 year of accrual, the alert algorithm identified 1,773 eligible patients for screening. Blood was drawn from 873 patients based on phlebotomy availability and laboratory processing staffing. A total of 642 patients completed blood draws and consented for inclusion, with the balance of patients having been discharged, transferred, or deceased with no identifiable legally authorized representative for consent. The median time from alert to first blood draw was 25 minutes (range 5 to 60 minutes). In an observation period, during which 227 consecutive patients had blood drawn, only 12 patients or families (5.2%) declined consent after the initial blood draw.
Conclusion: We have successfully developed and implemented a biobank in the ED that functions within practice limitations and has high patient accrual. Automation is key for efficient implementation in the ED. Using automated design improvements, we obtain blood samples from sepsis patients presenting to the ED faster than previous studies. Prompt biobank collection within the ED allows research into mechanisms of sepsis earlier in a patient's hospital course than has heretofore been possible and enables biomarker discovery for testing development directly relevant to emergency providers.

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P42

Defining fever: likelihood of infection diagnosis as a function of body temperature in the emergency department.

E Small*, CM Clements
Department of Emergency Medicine, Mayo Clinic, Rochester, MN, USA
Critical Care 2014, **18(Suppl 2):**P42; doi:10.1186/cc14045

Introduction: Fever predicts an infection cause of SIRS/sepsis more specifically than other commonly used hemodynamic criteria. Traditionally, a body temperature of 38.0°C has been used to define fever, but this cutoff is based on limited research. Furthermore, it has been proposed that fever responses are blunted in older adults, limiting the utility of fever in infection. This study determines the likelihood that a body temperature will predict diagnosis of infection in the emergency department (ED).
Methods: This was a retrospective cohort analysis of adult patients (>18 years old) presenting to a large academic emergency department from September 2010 to December 2012. Patient age, emergency physician diagnosis, final disposition, and initial body temperature were examined for each patient. Likelihood of a diagnosis of infection was calculated for all temperature ranges. Sensitivity and specificity of fever thresholds for a

diagnosis of infection were calculated and receiver operating characteristic (ROC) curves were generated. Confidence intervals were determined using Newcombe-Wilson hybrid scoring with continuity correction.

Results: We identified and analyzed records from 121,587 patients, including 37,933 persons >65 years old. Overall, 15.9% of patients received a diagnosis of infection in the ED with those >65 years old having a higher rate of infection at 18.3%. Likelihood of infection varied by temperature in a nonlinear relationship (see Figure 1). In adults of all ages, temperature >38.0°C had a specificity >99% for a diagnosis of infection in the ED although sensitivity was relatively poor. Specificity decreases only slightly with lower fever cutoffs (37.2 to 37.9°C) while sensitivity is increased to a greater degree (see Table 1).

Conclusion: The likelihood of a diagnosis of infection increases at the extremes of body temperature and is higher overall among older adults (≥65 years old). In our population, elevated temperature was at least as sensitive for a diagnosis of infection in older people as in younger adults. The use of body temperature for predicting diagnosis of infection has an overall low sensitivity but high specificity. Changing the current practical definition of fever from ≥38.0°C to ≥37.5°C significantly increases sensitivity of predicting infection without greatly impacting the specificity. Only 2.3% of all patients had a temperature between 37.5 and 37.9°C, and these patients were four times more likely to have a diagnosis of infection than those with temperature of 36.0 to 36.9°C.

P43

Ghrelin: an anti-inflammatory theurapeutic agent in septic rats.

GA Uluçay[1*], E Özkök[2], H Yorulmaz[3], İ Aydın[4], Ş Tamer[1]
[1]Department of Physiology, Faculty of Medicine, University of Istanbul, Turkey; [2]Institute of Experimental Medicine Research, Department of Neuroscience, University of Istanbul, Turkey; [3]Faculty of Health Science, University of Halic, Turkey; [4]Department of Histology, Faculty of Medicine, University of Istanbul, Istanbul, Turkey
Critical Care 2014, **18(Suppl 2):**P43; doi:10.1186/cc14046

Introduction: Sepsis is a life-threatening systemic inflammatory syndrome (SIRS), which affects many organ systems, that leads to hemodynamic changes in the presence of suspected or proven infection, advancing to organ dysfunction and failure [1-3]. In recent studies, 377 out of 100,000 cases of sepsis were observed while $14,600,000,000 has been determined as the average annual hospital costs. Although there are many studies, the molecular mechanism is not yet clearly elucidated [2,3]. Lipopolysaccharide (LPS) is the lipid molecule which the outer membrane of Gram-negative bacteria used in designing the experimental sepsis model [4]. Ghrelin was discovered in 1999 as a specific ligand for growth hormone secretagogue receptor and then pleiotropic effects such as anti-inflammatory, antioxidant, and so forth were found. Ghrelin was released in many tissues and organs in which there also was its receptor. The liver is an organ which has ghrelin receptors and was affected by sepsis primary [5,6].

Methods: In our study, male Wistar albino rats of average body mass 200 to 250 g were separated into four groups including: Control (n = 10), LPS (*E. coli* 055:B5, 5 mg/kg, n = 10), ghrelin (10 nmol/kg i.v., n = 10), LPS + ghrelin LPS

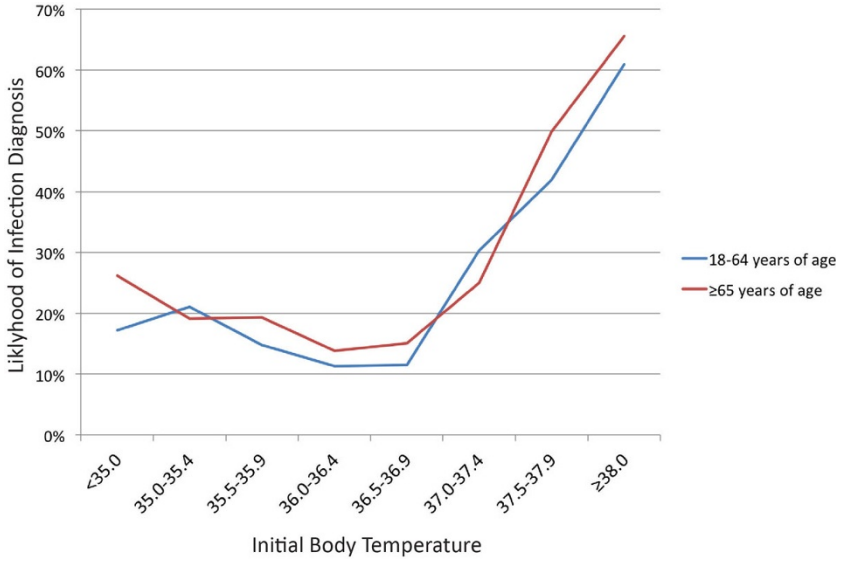


Figure 1(abstract P42) Likelihood of infection diagnosis as a function of initial body temperature

Table 1(abstract P42) Sensitivity and specificity of body temperature for a diagnosis of infection

	18 to 64 years of age		≥65 years of age	
	Sensitivity (95% CI)	**Specificity (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
≥37.0	33.5% (32.6 to 34.3)	81.9% (81.6 to 82.2)	33.3% (32.2 to 34.4)	85.7% (85.3 to 86.1)
≥37.1	25.8% (25.0 to 26.6)	89.4% (89.1 to 89.6)	27.9% (26.8 to 29.0)	91.2% (90.8 to 91.5)
≥37.2	20.4% (19.7 to 21.2)	93.4% (93.2 to 93.5)	23.4% (22.4 to 24.4)	94.0% (93.7 to 94.3)
≥37.3	17.1% (16.4 to 17.8)	95.6% (95.5 to 95.8)	20.3% (19.3 to 21.2)	95.7% (95.5 to 95.9)
≥37.4	14.6% (14.0 to 15.3)	96.9% (96.8 to 97.0)	17.8% (16.9 to 18.7)	96.6% (96.4 to 96.8)
≥37.5	12.6% (12.0 to 13.2)	97.8% (97.7 to 97.9)	15.9% (15.0 to 16.7)	97.4% (97.2 to 97.6)
≥38.0	6.48% (6.06 to 6.93)	99.3% (99.2 to 99.3)	8.77% (8.12 to 9.47)	99.0% (98.9 to 99.1)

(5 mg/kg, ghrelin 10 nmol/kg i.v., n = 10). Rats were decapitated 24 hours after first injection. We aimed in this study to investigate effects of ghrelin in sepsis which is created by LPS with sepsis descriptive parameters such as body temperature and leukocyte count with proinflammatory cytokine TNFα and anti-inflammatory cytokines IL-10 by ELISA, and hematoxylin and eosin stain for observed morphological changes.

Results: We detected the increase of leukocyte number, hypothermia, proinflammatory and anti-inflammatory cytokines such as TNFα and IL-10 as developing inflammatory reactions, resulting in hemodynamic and metabolic changes in rats treated with LPS. Also in groups with ghrelin treatment, ghrelin affects leukocyte numbers, body temperature, proinflammatory and anti-inflammatory cytokine levels and histological changes in controls and the LPS group (Figures 1 to 4). As a result of histologic examination, the curative effect of ghrelin partially on liver tissue damage is observed (Figure 5).

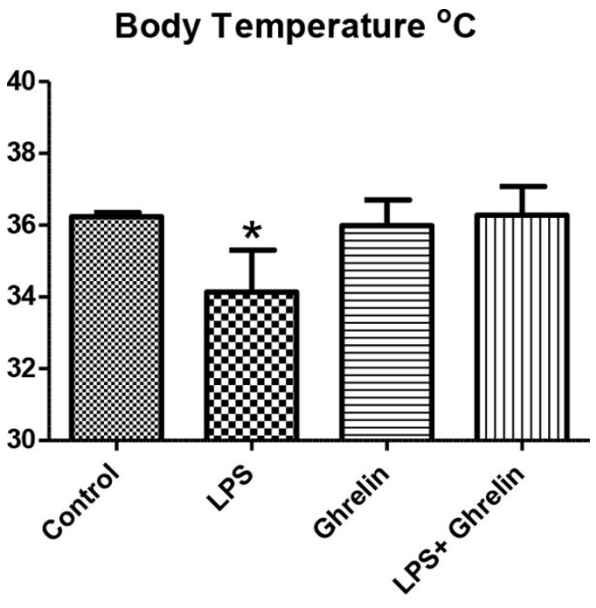
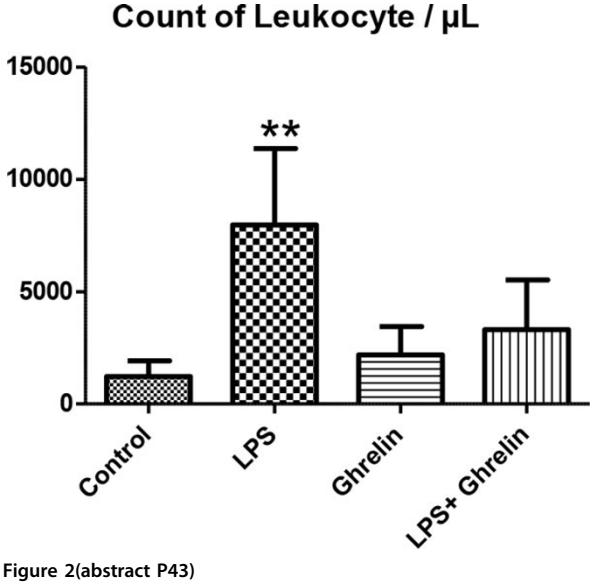


Figure 1(abstract P43)



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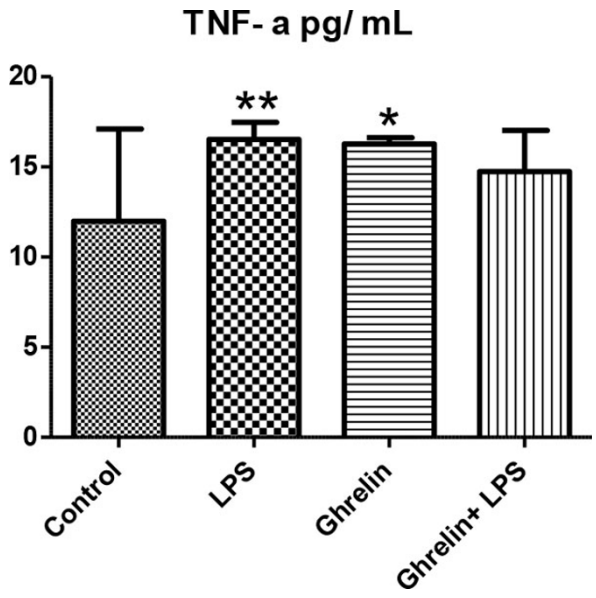

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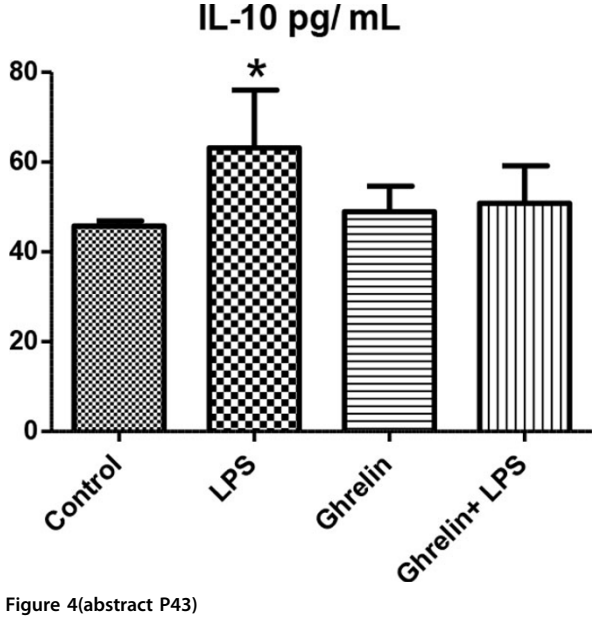

Figure 4(abstract P43)

Conclusion: We think the results of ghrelin may be affected depending on the dose and duration. Also partial healing effects of ghrelin in our results on this topic at the molecular level will contribute to other studies.

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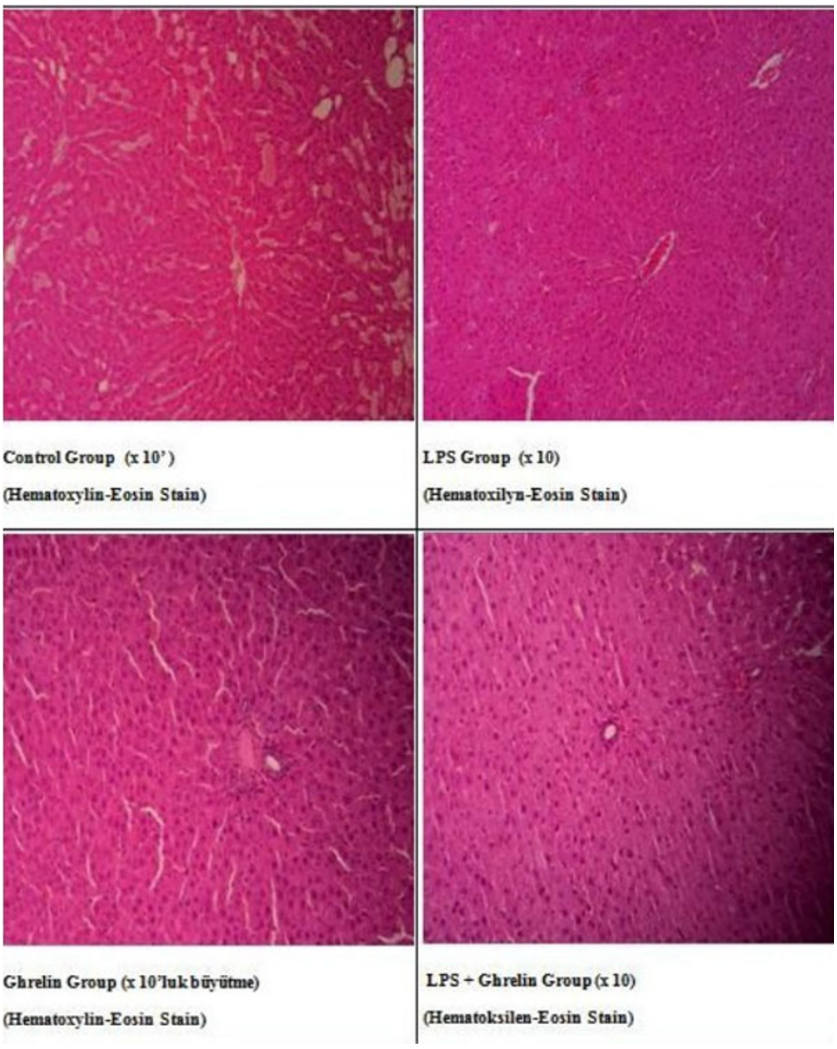

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P44

Restrictive parenteral fluid therapy in infants and children presenting with acute severe viral pneumonia in the PICU: a single-center experience.

S Kandasamy*, N Vijayakumar, T Sankaralingam, V Varadarajan, N Krishnamoorthy
Division of Pediatric Intensive Care, Department of Pediatrics, Mehta Children's Hospital Pvt Ltd, Chennai, India
Critical Care 2014, **18(Suppl 2):**P44; doi:10.1186/cc14047

Introduction: Acute viral (RSV and non-RSV) pneumonia is one of the most common causes of lower respiratory tract infection in infants and children. Although only 2 to 3% of infants with bronchiolitis require hospitalized management based on published literature, in recent years we see more children requiring hospitalization in high-dependency units. Most of these children require intravenous fluid therapy and some form of oxygen supplementation. As there is no standardized evidence-based parenteral fluid therapy protocol specific for acute severe viral pneumonia available, we decided to retrospectively analyze the result of our restrictive fluid therapy protocol.

Methods: All children less than 5 years age admitted to our PICU from June 2013 to December 2013 with etiological evaluation confirmed acute severe viral (RSV and non-RSV) pneumonia who received a minimum 48 hours of parenteral fluid therapy were enrolled and the data retrieved from the case sheet. The data analyzed were duration of PICU stay, hospital stay, oxygen therapy requirement, duration of oxygen therapy and their relationship to fluid balance on restrictive fluid regimen.

Results: A total of 32 children met the criteria for inclusion in the study period, 23 boys (71.9%) and nine girls (28.1%). The median age of children was 9.5 months (IQR 3.3 to 27.3). Of 32 children, 4/32 (12.5%) required invasive mechanical ventilation; 5/32 (15.6%) required NP-CPAP; 12/32 (37.5%) were on HHFNC; and nasal prong oxygen supplementation in 26 (81.3%) children. The median duration of ICU stay was 68.9 hours (IQR 39.2 to 80.2), that of hospital stay was 116.5 (IQR 92 to 179.2). Fluid balance at the end of 72 hours of ICU stay did not significantly differ the need for ventilation ($P = 0.45$) or the duration of ventilation ($P = 0.60$). Fluid balance in the first 72 hours correlated positively with change in serum sodium

levels indicating a fall in sodium levels with more positive fluid balance (Spearman coefficient of 0.452 (P = 0.16)). There was no significant correlation between fluid balance and duration of PICU/hospital stay (P = 0.58/0.75). eGFR calculated using modified Schwartz formula did not correlate with treatment parameters like duration of ICU/hospital stay, mechanical ventilation, and CPAP.

Conclusion: Positive fluid balance may not influence the duration of PICU stay, hospital stay, or oxygen therapy in children with severe viral pneumonia when initiated on restrictive (70% of Holliday Segar calculation) parenteral fluid therapy. eGFR at admission did not influence the fluid balance when they were uniformly initiated on restrictive parenteral fluids. Further studies with calculated sample size may help to confirm the observations.

P45

Blood sugar variation during the first 48 hours of hospitalization for patients with sepsis was associated with in-hospital mortality.

K-F Chen*, C-C Wu
Chang Gung University, Taoyuan, Taiwan and Chang Gung Memorial Hospital at Keelung and Linkou, Taiwan
Critical Care 2014, **18(Suppl 2):**P45; doi:10.1186/cc14048

Introduction: Blood sugar control for patients with sepsis remains controversial. We aimed to test the hypothesis that the variation of blood sugar level is associated with patient outcome in this study.

Methods: A retrospective cohort study on nontraumatic adult patients who visited the ED of a tertiary hospital in 2010 and had a clinical diagnosis of severe sepsis was conducted. Patients with two sets of blood culture ordered by emergency physicians and at least two blood sugar tests results available during the first 48 hours of hospitalization were included. The coefficients of variation (CoV, the ratio of the standard deviation to the mean) of the blood sugar level were analyzed with multivariate logistic regression models to test the association between in-hospital mortality.

Results: Of the 1,537 patients included, most were older than 70 years of age (median; 71, IQR: 59 to 80), male (54%), without a diagnosis of severe sepsis (63%) and had a previous diagnosis of diabetes (84%). The initial blood sugar levels of patients with and without previously diagnosed diabetes were 259 ± 9.9 and 154 ± 5.7, respectively (mean ± SEM). The CoV of the consecutively monitored blood sugar level during the first 48 hours of admission for patients with and without previously diagnosed diabetes were 29.4 ± 0.5% and 21.0 ± 0.5%, respectively. Patients with CoV lower than 10% and higher than 30% tended to have higher mortality rate, compared to patients with 10 to 30% CoV level (11% vs. 12% and 7%, respectively, Figure 1). In the multivariate logistic regression model adjusting for age, initial blood sugar level, severity of sepsis, previous diagnosis of diabetes and diagnosis of severe sepsis, the higher CoV level (>30%) was found to be associated with 60% increased odds of in-hospital mortality (aOR: 1.61 ± 0.34); while the previous diagnosis of diabetes was found to be associated with 45% lower odds of in-hospital mortality (aOR: 0.55 ± 0.13, Table 1).

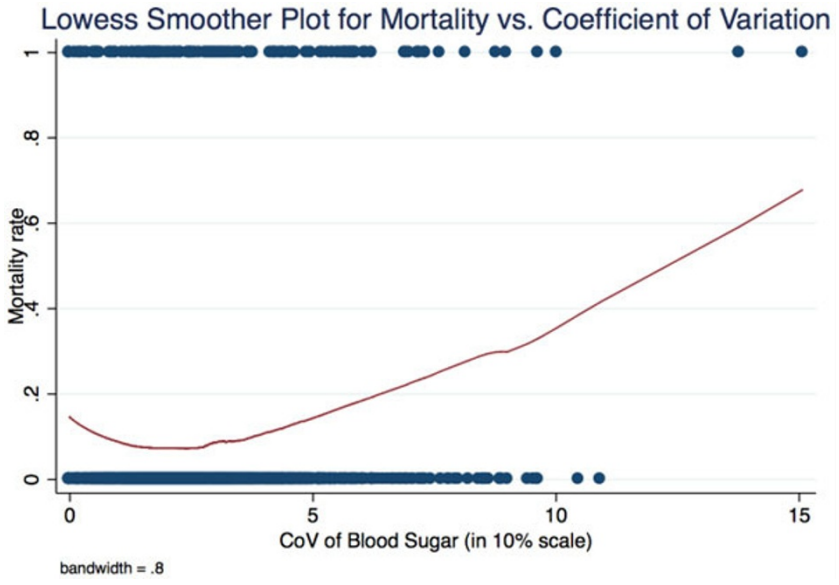

Figure 1(abstract P45) Lowess smoother plot for mortality versus coefficient of variation

Table 1(abstract P45)

	Odds ratio	Standard error	*P* value	95% CI	
Sugar CoV <10%	1.51	0.44	0.15	0.86	2.67
Sugar CoV >30%	1.61	0.34	0.02	1.06	2.43
Initial sugar level <100	0.98	0.01	0.00	0.97	0.99
Initial sugar level ≥ 100 and <500	1.00	0.00	0.67	1.00	1.00
Initial sugar level higher ≥ 500	1.00	0.00	0.54	1.00	1.00
Severe sepsis	2.43	0.48	0.00	1.65	3.56
Previous diagnosis of diabetes	0.55	0.13	0.01	0.34	0.88
Severity of sepsis	1.14	0.04	0.00	1.07	1.21
Age	1.01	0.01	0.49	0.99	1.02

Conclusion: In this retrospectively cohort study, we found that increased blood sugar variation was associated with worse patient outcome. However, further study is merited to test the possible causal relationship between variation of blood sugar level and patient outcome.

P46

Association of initial intracellular signalling pathway and cytokine level with early mortality in severe sepsis patients.

KC Lie[1*], D Widodo[1], S Lardo[2], Eppy[3], R Sinto[1]
[1]Division of Tropical and Infectious Diseases, Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia; [2]Department of Internal Medicine, Gatot Subroto Indonesia Central Army Hospital, Jakarta, Indonesia; [3]Department of Internal Medicine, Persahabatan Hospital, Jakarta, Indonesia
Critical Care 2014, **18(Suppl 2):**P46; doi:10.1186/cc14049

Introduction: Sepsis occurs as a result of systemic inflammatory process. The release of bacterial components to the systemic circulation leads to activation of inhibitor kappa B kinase (IKK)-β and nuclear factor kappa B (NF-κB) through phosphorylation and ubiquitination. Excessive inflammatory process with maladaptive host's immune response leads to organ dysfunctions and death [1,2]. This study was designed to investigate association of the initial level of intracellular signalling pathway and cytokine involved in sepsis pathophysiology (that is, IKK-β, NF-κB, tumour necrosis factor (TNF)α) and 72-hour (early) mortality in severe sepsis patients.
Methods: A prospective cohort study was conducted in severe sepsis patients (aged 18 years and older) admitted to the Emergency Unit of Cipto Mangunkusumo Hospital, Persahabatan Hospital, and Gatot Subroto Indonesia Central Army Hospital, Jakarta, Indonesia. All blood samples for intracellular signalling pathway (that is, IKK-β, total NF-κB, phospho NF-κB) and cytokine (that is, TNFα) were collected and measured using the ELISA method during first 24 hours of inclusion. Patients' outcome was observed during first 72 hours after inclusion. Mann-Whitney test was used to analyse the median difference of IKK-β, total NF-κB, phospho NF-κB level in two groups based on 72-hour survival. The *t* test was used to analyse the mean difference of TNFα levels in both groups.
Results: Subjects consisted of 90 patients. Early mortality developed in 27 subjects. Baseline characteristics of survival and death subjects are shown in Table 1. The initial intracellular signalling pathway and cytokine parameters level are shown in Table 2. There was a significant higher median first 24 hours IKK-β and total NF-κB level in survival subjects compared with death subjects ($P = 0.025$ for median IKK-β and $P = 0.036$ for median total NF-κB). There was no significant difference of initial phospho NF-κB and TNFα level in survival and death subjects.
Conclusion: There is a significant lower initial IKK-β and total NF-κB level in severe sepsis patients surviving on 72-hour observation. There is a tendency of lower initial phospho NF-κB and TNFα level in severe sepsis patients surviving on 72-hour observation.

Table 1(abstract P46) Baseline characteristics of the subjects

Characteristic	Survival subjects (*n* = 63)	Death subjects (*n* = 27)
Mean age (years)	52.98 ± 15.38	50.19 ± 13.46
Sex		
Male	23 (36.5)	12 (44.4)
Female	40 (63.5)	15 (55.6)
Sepsis severity		
Severe sepsis	42 (66.7)	13 (48.1)
Septic shock	21 (33.3)	14 (51.9)
Mean APACHE II score	13.03 ± 5.27	14.37 ± 6.55
Comorbidity		
Without comorbidity	4 (6.3)	2 (7.4)
With comorbidity(s)	59 (93.7)	25 (92.6)
Source of infection		
Respiratory tract	45 (71.4)	24 (88.9)
Intraabdominal	4 (6.3)	1 (3.7)
Skin and soft tissue	5 (7.9)	1 (3.7)
Urinary tract	2 (3.1)	1 (3.7)
≥ 2 sources	7 (11.3)	0
Mean procalcitonin (pg/ml)	21.47 ± 44.30	30.26 ± 17.29
Mean baseline lactate (mmol/l)	3.10 ± 1.77	4.89 ± 2.57

Data presented as *n* (%) or mean ± standard deviation. APACHE, Acute physiology and chronic health evaluation

Table 2(abstract P46) Initial intracellular signalling pathway and cytokine level based on 72-hour survival

Parameter	Survived subjects (*n* = 63)	Death subjects (*n* = 27)	*P* value
IKK-β[a]	0.10 (0.03)	0.12 (0,03)	0.025
Total NF-κB[a]	0.08 (0.08)	0.12 (0.08)	0.036
Phospho NF-κB[a]	0.10 (0.38)	0.33 (0.38)	0.579
TNFα[b]	9.10 (7.80)	10.91 (11.74)	0.393

[a]Median (interquartile range) in optical density, Mann-Whitney test. [b]Mean (standard deviation) in pg/ml, *t* test

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P47

Sepsis and neutropenia in hematological malignancies.

Y Labiad[1*], L Farnault[2], B Loriod[1], R Costello[1,2], C Nguyen[1]
[1]Technological Advances for Genomics and Clinics U1090, INSERM, Marseille, France; [2]Service d'hématologie de La Conception, Assistance publique - Hôpitaux de Marseille, France
Critical Care 2014, **18(Suppl 2):**P47; doi:10.1186/cc14050

Introduction: The pre-existing inflammatory state influences the behavior of infection, a first peak of inflammation may be the risk factor during infection and predispose to a severe form of the disease [1,2]. This hypothesis is frequently accepted but has never been tested. Patients with hematological malignancies treated by chemotherapy in case of autologous stem cell transplantation (ASCT) are exposed to a neutropenic state: an abnormal low number of neutrophils. In the neutropenic patient, sepsis is more frequent than in the non-neutropenic patient. The severity of infection depends on many factors, particularly the pre-existing inflammatory state, the genetic factors, and the duration and the depth of neutropenia. In the literature, the factors involved in inflammation and infection are often confused. On the other hand, no predictive transcriptomic signature of sepsis was found. Our objective is to identify predictive genes that influence the outcomes of the infection in neutropenic patients.
Methods: To test our hypothesis, high-throughput transcriptomic analysis and bioinformatics will be combined, laying on the modulation of gene expression of the peripheral blood mononuclear cells of patients with hematological malignancies treated by chemotherapy in case of ASCT.
Results: A preliminary study on a reduced number of patients has allowed us to assess the feasibility of the project. After a statistical analysis, 615 genes are differently expressed between patients who developed sepsis and those who have not developed with a false discovery rate of 5%. These 615 genes are potentially predictive of sepsis 2 days before the development of the infection. Furthermore, 28 genes are differentially expressed between patients who developed sepsis and those who have

not developed with a false discovery rate of 5%, and these 28 genes are potentially predictive of sepsis 7 days before the development of the infection.

Conclusion: The ongoing transcriptomic study with a larger effect will allow us to test our hypothesis and confirm the results of our preliminary study. The microarray results should be strengthened with quantitative PCR assays, than validated with protein assays. Finally, we will transfer the experimental data to clinical practices.

P48

Histopathological changes in septic acute kidney injury in critically ill children: an observational analytical study of postmortem renal biopsies.

R Rameshkumar[1*], S Mahadevan[1], RN Ganesh[2], P Narayanan[1], V Bhat[1]
[1]Department of Pediatrics, Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry, India; [2]Department of Pathology, Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry, India
Critical Care 2014, **18(Suppl 2):**P48; doi:10.1186/cc14051

Introduction: Critically ill children often manifest acute kidney injury (AKI) as part of their disease process with incidence up to 82% and it is independently associated with poor outcome [1]. Sepsis is the most common contributing factor and little is known about the pathogenesis of septic AKI. There are no consistent histopathological changes in human or experimental septic AKI [2]. Hence, understanding of the structural changes associated with its occurrence is therefore important in the management of AKI in critically ill children.

Methods: Children aged less than 12 years who died with septic AKI were screened for percutaneous USG-guided kidney biopsy after obtaining written informed consent from July 2012 to June 2014. Three cores of kidney tissue were taken for histopathological evaluation by light, immunofluorescence and electron microscopic examination. Sepsis and AKI was defined using international pediatric sepsis consensus conference and pRIFLE criteria respectively. Events related to death, laboratory parameters and microbiological details 24 hours preceding death were recorded.

Results: A total of 40 kidney biopsies were done during the study period. Median (IQR) age was 12 (2 to 36) months and PRISM-III was 14 (12 to 18). The most common change was normal histology in 37.5% (n = 15) followed by tubular change alone in 22% (n = 10), glomerular change alone in 5% (n = 2) and blood vessel change in 2.5% (n = 1) of specimens. Twelve specimens were showing a combination of changes (tubular + glomerular + interstitium = 5; tubular + glomerular + blood vessels = 3; tubular + glomerular = 2; tubular + interstitium = 1; tubular + glomerular + interstitium + blood vessels = 1). All tubular changes were consistent with acute tubular necrosis (ATN) and changes involved from 5 to 15% of cortex. Thrombotic microangiopathy was diagnosed in 12.5% (n = 5) of specimens. There were no significant difference between ATN versus non-ATN groups with respect to baseline characteristics and events related to mortality.

Conclusion: The most common change in septic AKI in critically ill children is normal histology followed by ATN. This is consistent with published literature [2].

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P49

Impact before and after introduction of a multifaceted quality improvement intervention on device-related infections in a pediatric ICU in India: a single-centre experience.

R Rameshkumar[1*], A Jagadeesh[1], M Kedarnath[1], S Mahadevan[1], P Narayanan[1], KN Harikrishnan[1], S Sistla[2]
[1]Department of Pediatrics, Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry, India; [2]Department of Microbiology, Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry, India
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Introduction: Healthcare-associated infections (HAI) are a significant problem in the pediatric intensive care unit (PICU). Apart from contributing to mortality, they also increase the coast of PICU care [1]. Surveillance and prevention of HAI by multifaceted quality improvement intervention among critically ill children remain part of the standard of care [2].

Methods: The study was conducted in the 19-bed PICU of a tertiary care referral academic institute. Data regarding ventilator-associated pneumonia (VAP) and central line associated bloodstream infection (CLABSI) using the CDC definition were collected prospectively from July 2013 to June 2014. Multifaceted quality improvement intervention consisting of infection control nurse and physician and hand hygiene education module and wearing a gown and mask during the care of critically ill children was introduced from January 2014. Incidence of VAP and CLABSI was compared before (July to December 2013) and after (January to June 2014) introduction of the intervention.

Results: Before the intervention period, the incidence of VAP was 28.5 per 1,000 ventilation-days and CLABSI was 13.7 per 1,000 catheter-days. After the intervention period the incidence of VAP was 13.3 per 1,000 ventilation-days and CLABSI was 8.3 per 1,000 catheter-days. The proportion of patients ventilated for more than 48 hours who had VAP was significantly less after intervention as compared to before the intervention period (14.2%, n = 25/176 vs. 25.2%, n = 29/155; P = 0.012, odds ratio (OR), 95% CI 0.49, 0.28 to 0.86). Both groups were similar with respect to age, sex ratio, severity (PRISM-III), device utilization rate and grade of infection. No significant difference occurred in overall PICU mortality before and after intervention (28.2% vs. 28.9%).

Conclusion: Multifaceted quality improvement intervention results in significant reduction of the healthcare-associated infection rate although it was higher than reported from developed countries [1-3].

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P50

Diagnostic and prognostic evaluation of soluble CD14 subtype for sepsis in critically ill patients: a preliminary study.

A Foca[*], C Peronace, V Marano, GS Barreca, AG Lamberti, N Marascio, A Giancotti, R Puccio, MC Liberto
Department of Health Sciences, Institute of Microbiology, University 'Magna Graecia' of Catanzaro, Italy
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Introduction: Sepsis, a leading cause of death in critical care patients, is the result of complex interactions between the infecting microorganisms and the host responses that influence clinical outcomes [1]. Reliable biochemical markers that enable early diagnosis are still needed. A new marker, presepsin (soluble CD14 subtype, sCD14-ST) is a circulating fragment of 13 kDa of soluble CD14 that originates from the cleavage of the CD14 expressed on the surface membranes of monocytes/macrophages. sCD14-ST has been shown to increase significantly in patients with sepsis [2]. In our study we compare diagnostic performance of presepsin, C-reactive protein (CRP) and procalcitonin (PCT).

Methods: Critical patients with suspected sepsis admitted to the Unit of Intensive Care of the University Hospital of Catanzaro (Italy) were recruited into this study; healthy volunteers were also included as controls. Plasma samples in EDTA from each patient were collected at multiple time points; samples were tested for CRP, PCT and presepsin.

Blood cultures were also evaluated and processed by a BacT/Alert 3D system (bioMérieux, Italy); CRP was measured by immunonephelometry (Siemens Healthcare Diagnostic, Italy) and PCT was assayed by an enzyme-linked fluorescent assay (VIDAS BRAHMS PCT, bioMérieux, Italy); presepsin levels were measured by rapid automated PATHFAST immunoanalyzer (kindly provided by GEPA SRL, Italy), based on chemiluminescent enzyme immunoassay. A statistical analysis was carried out by Mann-Whitney test.

Results: Presepsin and PCT levels were significantly higher in culture-positive subjects versus negative controls; such difference was found even at the admission time. The presepsin values in worsening/dead patients exhibited a significantly higher level at admission time. On the contrary, in the same group of patients, PCT exhibited a decrease of its level. In poor prognosis patients CRP showed a quite irregular kinetic, although in such a group the admission value was higher than the same marker in live subjects.

Conclusion: In this preliminary study, presepsin and PCT levels exhibited substantial higher values in culture-positive patients. The kinetic curves of presepsin, obtained from both survival and worsening/dead subjects, revealed the optimal performance of this biomarker, particularly in severely ill patients, as also shown in other studies. During sepsis, increase of presepsin levels may be a more reliable marker, indicating an unfavorable outcome [3,4]. Furthermore, high presepsin levels could alert clinicians not to suspend antibiotic treatments even after clinical symptoms have improved and PCT levels have returned to normal.

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P51

Sphingosine-1 phosphate promotes thymic atrophy during sepsis progression.

L Kuchler[1*], LK Sha[1], AK Giegerich[1], T Knape[2], A Weigert[1], B Brüne[1], A von Knethen[1]

[1]Faculty of Medicine, Institute of Biochemistry I - Pathobiochemistry, Goethe-University Frankfurt, Germany; [2]Project Group Translational Medicine & Pharmacology TMP, Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Frankfurt, Germany

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Introduction: T-cell depletion is a marker of the hypo-inflammatory phase of sepsis [1,2]. Systemic T-cell loss could be caused i.a. by thymus involution during sepsis, thus contributing to immune paralysis [3]. Usually, thymic T-cell egress is mediated by a sphingosine-1-phosphate (S1P) gradient. That is, T cells leave the thymus towards an increased S1P-level in the periphery (blood/lymph) [4]. A disruption of this S1P gradient blocks T-cell emigration. S1P is produced by sphingosine kinases 1 and 2 (SPHK1 and SPHK2), which are ubiquitously expressed [5]. Apoptotic cells are known to release S1P [6]. We suggest that apoptotic events in the thymus during sepsis increase S1P levels, thereby disrupting the gradient and preventing egress of T cells from the thymus.

Methods: We used a murine polymicrobial sepsis model to analyze thymus involution. Sepsis is induced in wildtype mice, SPHK1$^{-/-}$, or SPHK2$^{-/-}$ mice by cecal ligation and puncture (CLP). Thymus involution was determined by analyzing the T-cell amount 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, and wildtype mice treated with a SPHK1 inhibitor were used to characterize whether T cells in these mice have a higher rate of emigration compared to control mice. Thymic and serum S1P levels were quantified by LC-MS/MS. Apoptosis was determined by annexin V FACS staining. SPHK mRNA levels were determined by qPCR.

Results: 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, CLP causes apoptosis of thymocytes and increases SPHK1 mRNA expression. Concomitantly S1P levels are increased in the thymus and consequently decreased in serum following CLP. The knockout of the S1P producing sphingosine-kinases SPHK1 or SPHK2 and the pharmacological inhibition of SPHK1 restores T-cell egress as indicated by an increase of double-positive immature T cells compared to wildtype mice.

Conclusion: Our data suggest that inhibition of SPHK1-mediated S1P generation during sepsis restores thymic T-cell egress, which might improve septic outcome. Therefore, understanding mechanisms of thymus involution during sepsis may help to reconstitute thymic function, finally improving immune reactions in the hypoinflammatory phase of sepsis.

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P52

Activation of the peroxisome proliferator activated receptor γ counteracts sepsis-induced T-cell cytotoxicity towards alloantigenic target cells.

A von Knethen[1*], LK Sha[1], T Knape[2], L Kuchler[1], AK Giegerich[1], M Schulz[1], IA Hauser[3], B Brüne[1]

[1]Institute of Biochemistry I - Pathobiochemistry, Faculty of Medicine, Goethe-University Frankfurt, Germany; [2]Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Project Group Translational Medicine & Pharmacology TMP, Frankfurt am Main, Germany; [3]Department of Nephrology, Medical Clinic III, Frankfurt am Main, Germany

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Introduction: Sepsis originates from an uncontrolled inflammatory response. Despite intensive research, sepsis remains a major cause of death in ICUs. Therefore, new therapeutic approaches are mandatory. Taking into account that during sepsis progression cytotoxic T cells (CTL) are activated in an autoimmune fashion contributing to multiorgan damage, it remains unclear whether CTL are activated towards alloantigenic cells as well. This is especially important for patients receiving an immune suppressive therapy to permit organ transplantation and thus known to be at high risk for developing sepsis. Therefore, we analyzed whether sepsis activates CTL towards alloantigenic target cells and whether this can be inhibited by PPARγ activation, known to block T-helper cell responses.

Methods: To characterize whether sepsis activates CTL and whether this can be inhibited by PPARγ activation, we used an *ex vivo* cytotoxicity assay to analyze CD8^{+} T-cell-dependent cytotoxicity. Responder CD8^{+} T cells were isolated from C57Bl/6N PPARγ wildtype (PPARγfl/fl) and T-cell specific knockout (Tc-PPARγ$^{-/-}$) mice (haplotype H2Kb) following cecal ligation and

puncture (CLP) versus sham treatment. P815 mastocytoma cells, a cell line originally derived from DBA/2 mice (haplotype H2Kd), were used as alloantigenic target cells. Pharmacological inhibition and/or activation of PPARγ *in vivo* and *ex vivo* was performed to clarify the impact of PPARγ in blocking CTL-dependent cytotoxicity. *In vivo*, PPARγ activity in wildtype mice was pharmacologically inhibited by the irreversible antagonist GW9662 or induced by the thiazolidinedione rosiglitazone. Systemic application of both compounds was performed intraperitoneally. A classic splenocyte-driven stimulation protocol to activate CTL was carried out as control.

Results: CTL isolated from septic mice showed enhanced cytotoxicity towards alloantigenic P815 target cells. Enhanced cytotoxicity was effectively reduced by both PPARγ activation *in vivo* and *ex vivo*. In line, in CTL isolated from T-cell-specific PPARγ knockout (Tc-PPARγ$^{-/-}$) mice PPARγ activation was ineffective, strengthening a PPARγ-dependent mechanism. At the molecular level *in vivo* and *ex vivo* activation of PPARγ reduced Fas and granzyme B expression in activated CTL, which might explain reduced cytotoxicity.

Conclusion: Our study therefore suggests PPARγ activation *in vivo* to attenuate CTL-dependent alloantigenic cytotoxicity to possibly inhibit acute organ rejection.

P53

Using process mapping to identify barriers to effective management of sepsis in a cancer hospital: lessons for successful implementation of a whole hospital pathway.

K Thursky[1*], G Haeusler[1], B Teh[1], D Comodo[2], N Dean[2], C Brown[2], J Willis[2], K Morris[2], S Lingaratnam[3], M Slavin[1], L Worth[1]
[1]Department of Infectious Diseases, Peter MacCallum Cancer Centre, Melbourne, Australia; [2]Nursing, Peter MacCallum Cancer Centre, Melbourne, Australia; [3]Pharmacy Department, Peter MacCallum Cancer Centre, Melbourne, Australia
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Introduction: Infection and sepsis are common problems in cancer management affecting up to 45% of patients. However, international guidelines focus on the management of neutropenic fever, and fail to address the recognition and resuscitation of patients who meet sepsis criteria. Peter MacCallum Hospital is a 100 inpatient-bed tertiary cancer hospital with hematology, medical oncology, cancer surgery and radiation oncology, as well as a medical and chemotherapy day unit, apheresis, and large ambulatory service but no emergency department. Records showed that up to 25% of all in-hospital Medical Emergency Team (MET) calls were attributable to sepsis with in-hospital mortality rates of up to 25%. We aimed to identify barriers to effective management of inpatient sepsis at Peter MacCallum Cancer Centre and to implement a hospital-wide sepsis pathway.

Methods: A sepsis working party was formed with the antimicrobial stewardship team, clinicians, and senior ambulatory and inpatient nurses. Each member undertook direct observation and focus group interviews in an allocated clinical area. Three key areas were examined: issues relating the identification of sepsis, issues relating to clinical review of the patient, and issues relating to timely administration of first dose of antibiotic. Inpatient and outpatient issues were graphically represented in a process map (Figure 1).

Results: Process mapping revealed significant gaps in knowledge in medical and nursing staff and structural barriers to rapid resuscitation of patients. There were significant knowledge gaps in the awareness of sepsis diagnostic criteria, the role of lactate, effective fluid resuscitation, and the need for early clinical review and referral to the ICU. Examples of structural barriers to effective resuscitation included lack of availability of nurse cannulators, availability of antibiotics, rapid intravenous fluid infusers and sufficient after-hours medical and ICU liaison support. Knowledge and structural barriers were systematically addressed during the implementation of the clinical sepsis pathway. The sepsis pathway was designed as a medical record form to be used across all clinical areas, and supports nurse initiation. Following pathway implementation in March 2013 there have been substantial improvements in the number of patients who have had a lactate taken, received appropriate fluid resuscitation and time to first dose of antibiotics. Administrative data shown in Figure 2 demonstrate increased ascertainment of cases, and a fall in all-cause sepsis mortality after the pathway was commenced.

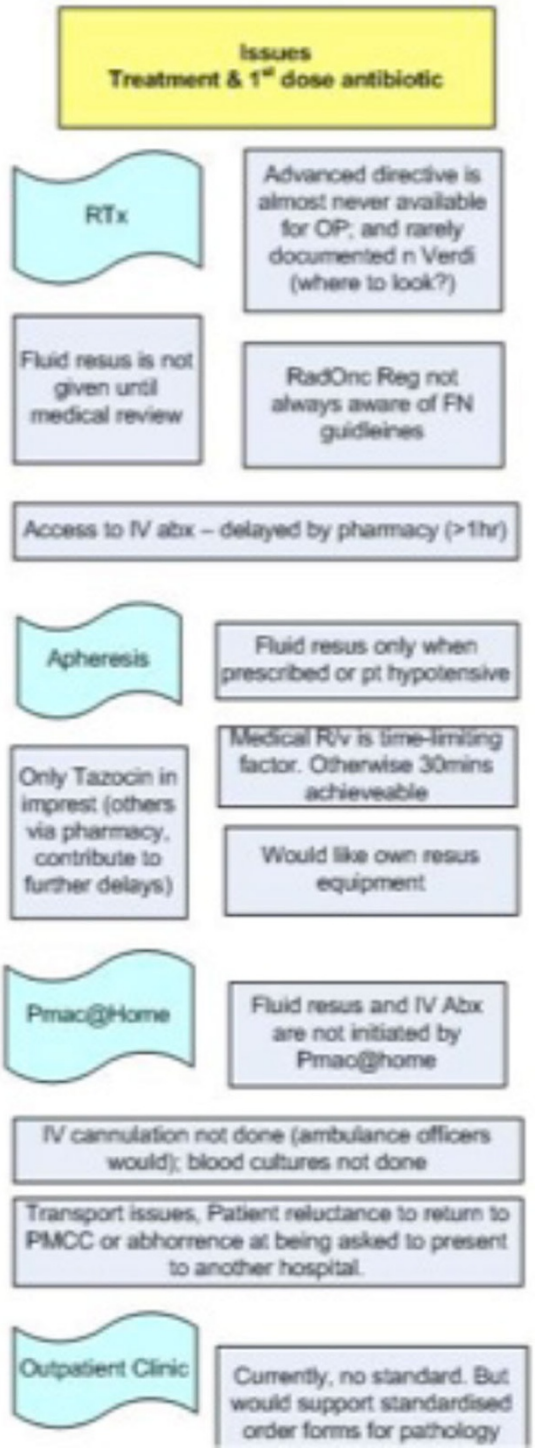

Figure 1(abstract P53) Portion of process map identifying barriers to timely administration of antibiotics in the ambulatory setting. RTx, radiotherapy department.

Conclusion: Identifying knowledge gaps and structural barriers using process mapping led to the successful design and implementation of a sepsis program. The figures show that despite increased rates of coded sepsis cases, mortality and ICU admission rates have not increased. Mortality

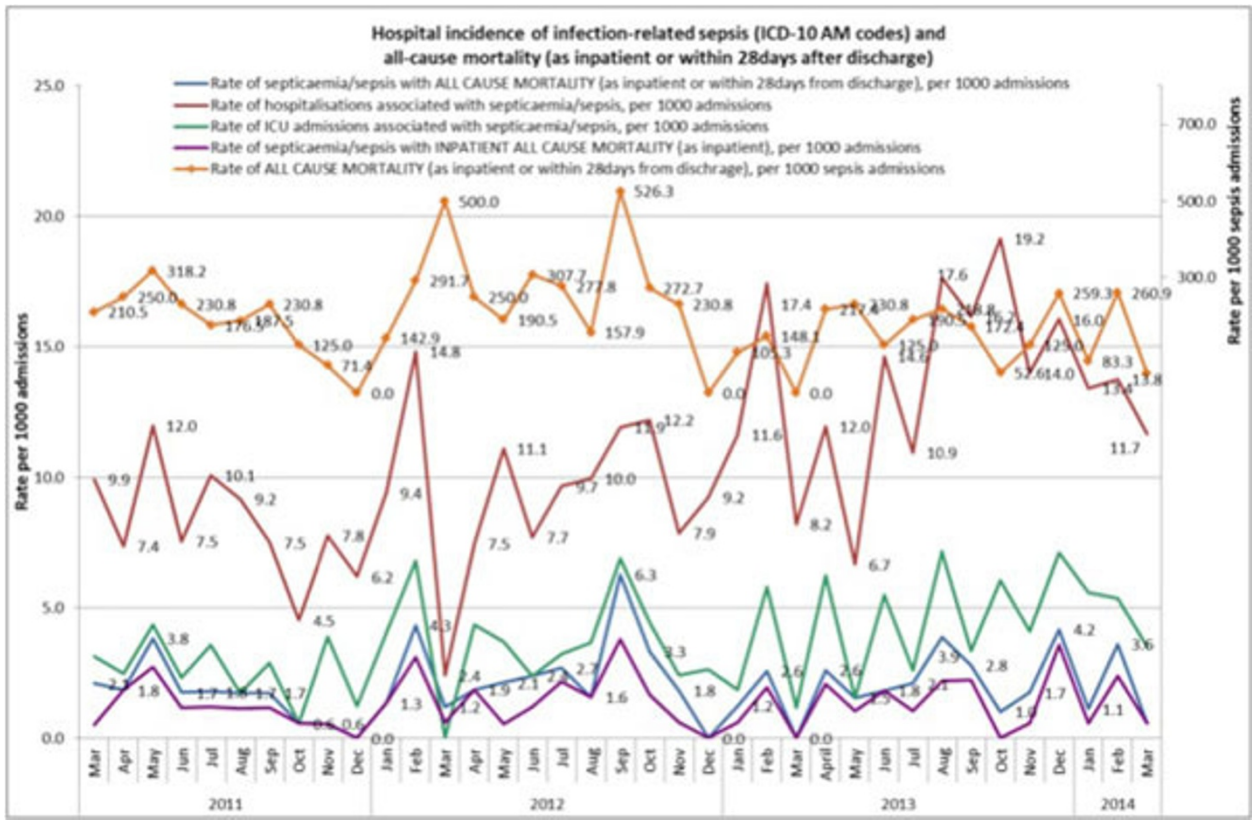

Figure 2(abstract P53) Increased rate of sepsis at Peter MacCallum Cancer Centre from March 2013, coinciding with improved identification of patients with sepsis.

of patients coded for sepsis March to October 2012 (13/56 (23.2%)) compared with those for March to October 2013 (10/121 (8.3%)).

P54

Dopamine mediates vagal modulation of the immune system by electroacupuncture.

R Torres Rosas[1,2*], P Morcillo[2], L Ulloa[2,3]
[1]Medical Research Unit on Immunochemistry, National Medical Center Siglo XXI, Mexico City, Mexico; [2]Laboratory of Anti-inflammatory Signaling, Department of Surgery, Rutgers University New Jersey Medical School, Newark, NJ, USA; [3]Center of Immunology and Inflammation, Rutgers University New Jersey Medical School, Newark, NJ, USA
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Introduction: Previous anti-inflammatory strategies against sepsis, a leading cause of death in hospitals, had limited efficacy in clinical trials, in part because they targeted single cytokines and the experimental models failed to mimic clinical settings [1-3]. Neuronal networks represent physiological mechanisms, selected by evolution to control inflammation, that can be exploited for the treatment of inflammatory and infectious disorders [3].
Methods: Animal procedures were approved by the Institutional Animal Care & Use Committee of the New Jersey Medical School of Rutgers University. All animal experiments were performed in 6-week-old to 8-week-old (~25 ± 5 g) male mice without any exclusion criteria. Experimental sepsis: endotoxemia and CLP were performed as we previously described. LPS was dissolved in sterile pyrogen-free PBS and sonicated for 30 minutes immediately before use. Mice received a LD_{50} dose of LPS (6 mg/kg body weight i.p.). LPS was added to the whole blood to a final concentration of 250 ng/ml for the *in vitro* procedures. Selective neurectomies and electrical stimulations: all selective neurectomies and electrical stimulations were performed in mice anesthetized with ketamine and xylazine. The electrical stimulation in electroacupuncture and direct nerve stimulation (sciatic and vagus nerves) was performed with a continuous-mode stimulation for 15 minutes with a electrical potential difference of 4 V, an electric current of 40 mA, a pulse width of 50 μs and a frequency of 10 Hz using an electrostimulator.
Results: Here, we report that sciatic nerve activation with electroacupuncture controls systemic inflammation and rescues mice from polymicrobial peritonitis. Electroacupuncture at the sciatic nerve controls systemic inflammation by inducing vagal activation of aromatic L-amino acid decarboxylase, leading to the production of dopamine in the adrenal medulla. Experimental models with adrenolectomized mice mimic clinical adrenal insufficiency [4], increase the susceptibility to sepsis and prevent the anti-inflammatory effects of electroacupuncture. Dopamine inhibits cytokine production via dopamine type 1 (D1) receptors. D1 receptor agonists suppress systemic inflammation and rescue mice with adrenal insufficiency from polymicrobial peritonitis. Our results suggest a new anti-inflammatory mechanism mediated by the sciatic and vagus nerves that modulates the production of catecholamines in the adrenal glands.
Conclusion: From a pharmacological perspective, the effects of selective dopamine agonists mimic the anti-inflammatory effects of electroacupuncture and can provide therapeutic advantages to control inflammation in infectious and inflammatory disorders. Preliminary results in human clinical trials indicate that electroacupuncture attenuates the postsurgical inflammatory response decreasing the serum levels of inflammatory cytokines.

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P55

Immunomodulation and infection: identification of small molecule TLR3 blockers to combat deleterious inflammation in pneumonia.

F Sônego[1*], B Pouzet[1], F Pecceu[2], L Leotard[1], B Martin[1], P-E Rouby[1], L Goullieux[1], C Ravinet-Trillou[1], J-M Bras[3], C Serradeil-Le Gal[1]
[1]Sanofi TSU ID, Toulouse, France; [2]SCP LGCR, Toulouse, France; [3]Biologics, Toulouse, France

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Introduction: Pneumonia is a common cause of death worldwide and the leading cause of sepsis and shock in the ICU. Controlling excessive immune stimulation with deleterious consequences for the host organs is a major strategy in severe infections. In addition to viral dsRNA, the immune Toll-like receptor 3 (TLR3) senses RNA released from injured tissues and necrotic cells promoting excessive inflammatory cytokine release in pulmonary epithelial, endothelial and immune cells. Thus, as a potent regulator of the lung immune response, TLR3 represents a specific target to control damaging inflammation in severe lung infections in combination with antimicrobials. Here, we aimed to characterize potent small molecules displaying *in vitro* and *in vivo* TLR3 blocker activities and develop several preclinical models to investigate the role of TLR3 in pneumonia.

Results: We identified compounds with anti-TLR3 activity (IC_{50} = 50 nM) through random screening and structure-activity relationship analysis by testing small molecule libraries on recombinant hTLR3-HEK293 cells. Selected compounds interacted with mouse and human TLR3, were devoid of effect on TNFα-induced activation at 1 μM on HEK293 cells and exhibited good early-ADME properties. Hit molecules also counteracted PolyAU (PAU)/Poly(I:C) (PIC)-induced cytokine release (IL6, IL8, IP-10) on human bronchial epithelial cells (BEAS-2B) expressing native TLR3 receptors. Interestingly, sustained stimulation of BEAS-2B by PAU/PIC (0.1 to 10 μM) to mimic viral activation induced overexpression of TLR3 mRNA, which was dose-dependently inhibited by these compounds, as evidenced by RT-qPCR. *In vivo*, we confirmed the anti-TLR3 activity (3 to 30 mg/kg i.p.) against PAU/PIC (100 μg/mouse i.v.) but not flagellin-induced plasma cytokine release in CD1 mice. To study these TLR3 inhibitors under pathological situations, we developed models of *Streptoccocus pneumoniae* (ATCC6303) or *Pseudomonas aeruginosa* (PAO-1)-induced pneumonia post influenza A virus (IAV PR/8/34 H1N1) infection. Mice previously challenged with intranasal IAV showed enhanced susceptibility to lung bacterial infections with a dramatic mortality rate, whereas separately these pathogens are not lethal. In addition, IAV/*S. pneumoniae* co-infected mice showed increased lung and blood bacterial loads with severe inflammation signs evidenced by elevated systemic IL-6 and KC levels and important lung tissue damages. Currently, TLR3 blockers are ongoing evaluation in these models.

Conclusion: Immunomodulation and personalized treatments are becoming relevant approaches to manage severe infections. Here, we discovered the first small molecule probes targeting mouse and human TLR3. These compounds efficiently control TLR3-triggered proinflammatory response *in vitro* and *in vivo* and shape TLR3 overexpression in human lung epithelial cells. They represent valuable pharmacological tools to study the contribution of TLR3 in pneumonia and to propose new adjuvant immunotherapies.

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P56

Similarity of inflammatory response in epileptic seizures and sepsis: does the sensitivity to sepsis in epileptic patients increase?.

G Üzüm[1*], K Akgün-Dar[2], A Kandil[2], N Bahçekapılı[1], I Albeniz[2]
[1]Department of Physiology, Medical Faculty of Istanbul, Istanbul University, Istanbul, Turkey; [2]Department of Biology, Faculty of Science, Istanbul University, Istanbul, Turkey

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Introduction: It is known that the systemic response during sepsis is caused by proinflammatory mediators such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα). The inflammatory response in sepsis causes disorders in the brain in addition to multiorgan dysfunctions including the kidneys and liver [1]. Inducible nitric oxide synthase (iNOS) is induced in hepatocytes by sepsis and mediates hepatic injury [2]. Matrix metalloproteinases (MMPs) play an important role in the formation of sepsis and mediate inflammatory response and tissue damage [3]. On the other hand, there are some cases in which systemic inflammatory response occurs without the presence of infection such as epilepsy. Cytokines are well-known inflammatory mediators in the brain, and they increase following seizures. We previously demonstrated that pentyleneterazol (PTZ)-induced generalized epileptic seizures significantly increased inflammatory markers (TNFα, IL-1β, IL-6) in the brain and S100B in serum [4]. In this preliminary study, we aimed to investigate the MMP2, MMP9, NOS, and myeloperoxidase activity in the liver and kidney and levels of serum proinflammatory cytokines following PTZ-induced generalized clonic-tonic seizures.

Methods: Adult Sprague-Dawley rats were divided into two groups as Control and PTZ groups. The Control group was given saline and the PTZ group was given 80 mg/kg PTZ i.p. Two hours after seizures, the rats were decapitated and a cardiac blood sample was drawn, and liver and kidneys were removed. Proinflammatory markers (IL-1βN, TNF-α, IL-6) were investigated in serum by ELISA. eNOS, iNOS, MMP2, and MMP9 levels were analyzed immunohistochemically in the liver and kidney.

Results: Proinflammatory markers significantly increased in the serum of rats after PTZ-induced seizures (Table 1). iNOS reaction was markedly increased while eNOS reactions were decreased (Figure 1) in the liver of rats after PTZ-induced seizures. MMP2 in the central vein of the liver and connective tissue areas of liver and kidney tissues in the PTZ group were markedly increased (Figure 2). MMP9 immune reaction in the PTZ group slightly increased in the kidney and liver (Figure 3). MPO reactions, which are an indicator of inflammatory activity, were markedly increased in both tissues (Figure 4).

Conclusion: The first findings show that long-term generalized clonic-tonic seizures markedly increase markers that mediate inflammation (iNOS, especially MMP2, MMP9, MPO) in the liver and kidney such as sepsis. In addition, proinflammatory markers (TNFα, IL-1β, IL-6) were found significantly high in serum. Thus, it is concluded that it will be worthwhile to determine whether epileptic seizures cause sensitivity to sepsis.

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P57

Early phases of sepsis: effects of simvastatin on mitochondrial enzyme activities in kidney tissue in rats.

E Özkök[1*], H Yorulmaz[2], G Ateş[3], İ Aydın[4], AŞ Tamer[3]
[1]Department of Neuroscience, The Institute for Experimental Medicine, Istanbul University, Istanbul, Turkey; [2]School of Health of Science, Halic University, Istanbul, Turkey; [3]Department of Physiology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey; [4]Department of Histology and Embryology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey

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Introduction: Acute kidney injury (AKI) is a frequent and serious complication of sepsis. Moreover, there is strong evidence that AKI in patients with severe sepsis is associated with a higher mortality rate. The

Table 1(abstract P56) Proinflammatory cytokines in control versus experimental groups

pg/ml	Control	PTZ
IL-1β	0.117 ± 0.042	0.775 ± 0.064
TNFα	0.062 ± 0.010	0.783 ± 0.044
IL-6	0.160 ± 0.012	0.0654 ± 0.026

*P < 0.001, compared to control one-way ANOVA and Benferroni for *post hoc* were used. n = 7

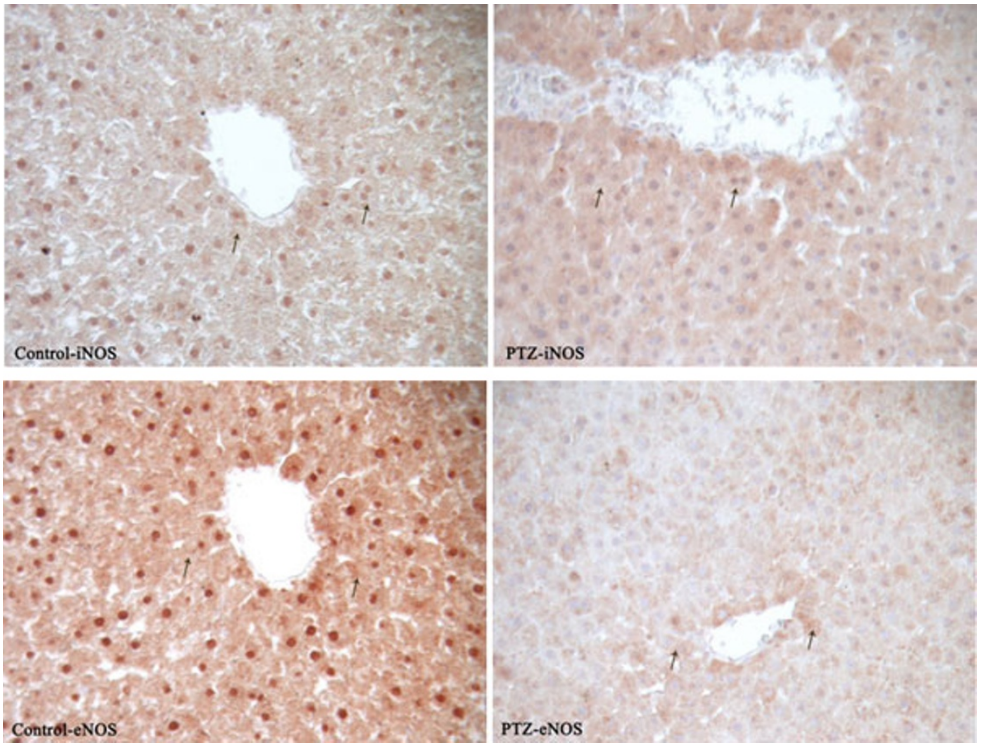

Figure 1(abstract P56) Immunohistochemical detection of iNOS and eNOS staining (arrows) in liver sections in control and experimental groups (bar: 50 μm)

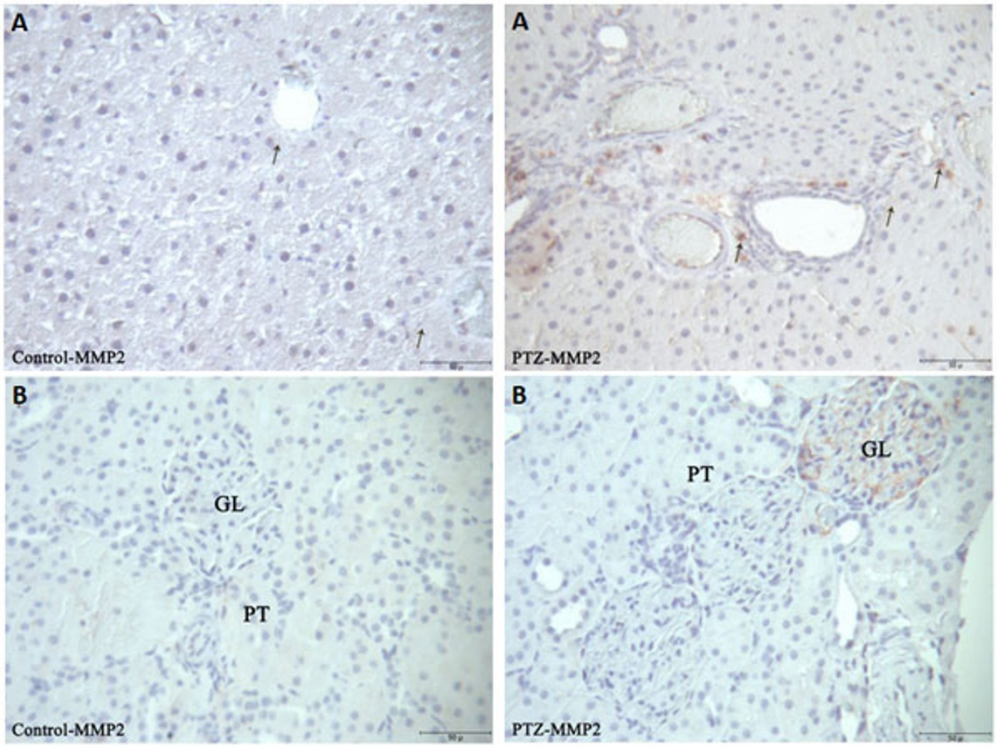

Figure 2(abstract P56) Immunohistochemical detection of MMP2 staining (arrows) in liver (A) and kidney (B) sections in control and experimental groups. PT, proximal tubule; GL, glomerulus (bar: 50 μm)

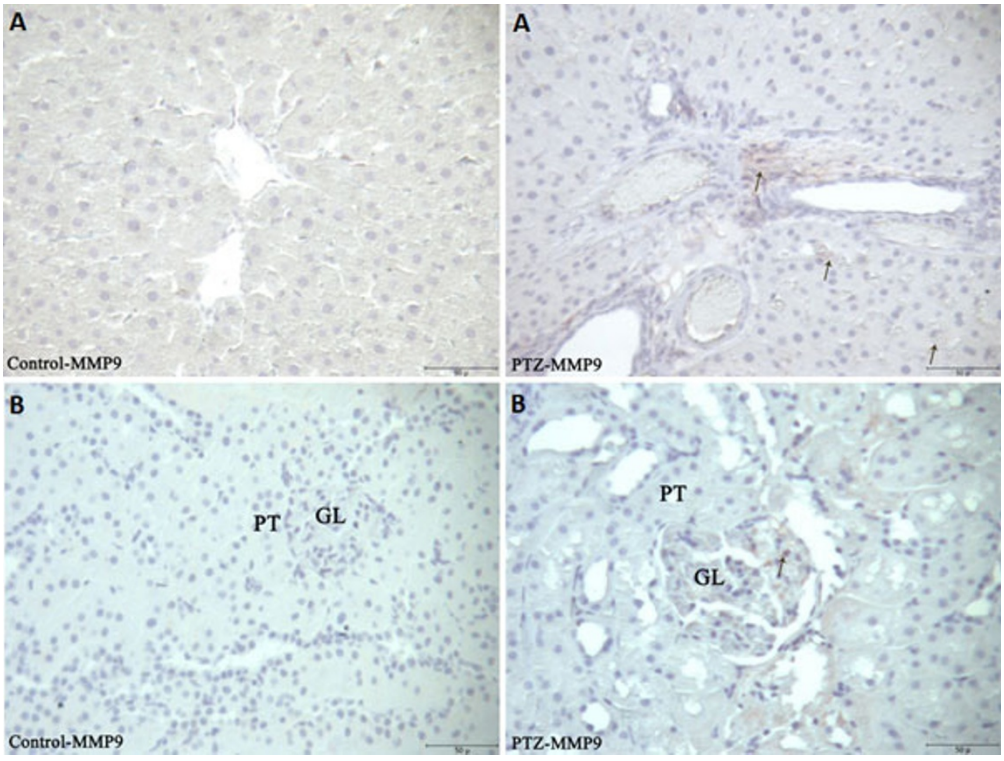

Figure 3(abstract P56) Immunohistochemical detection of MMP9 staining (arrows) in liver (A) and kidney (B) sections in control and experimental groups. PT, proximal tubule; GL, glomerulus (bar: 50 μm).

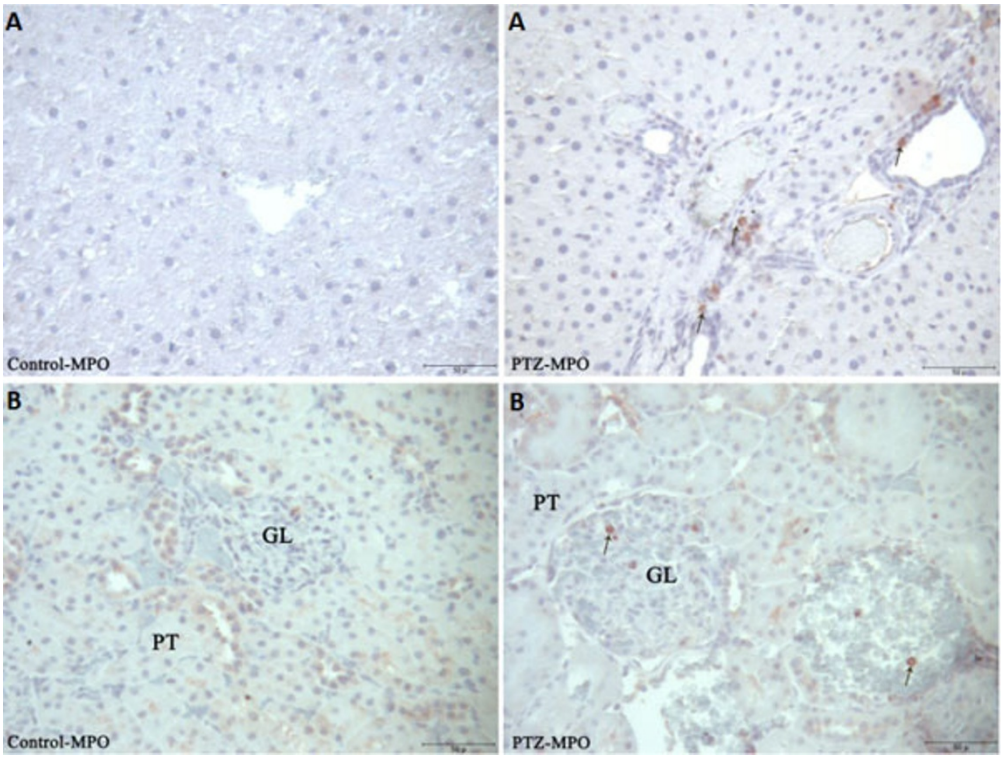

Figure 4(abstract P56) Immunohistochemical detection of MPO staining (arrows) in liver (A) and kidney (B) sections in control and experimental groups. PT, proximal tubule; GL, glomerulus (bar: 50 μm)

devastating effects of Gram-negative sepsis are largely based on the effects of lipopolysaccharide (LPS), also known as endotoxin. Mitochondrial dysfunction has been suggested to contribute to the development of organ dysfunction and failure in sepsis. The mitochondrial electron transport chain consists of four complexes (CI to CIV) and its function can be assessed with different approaches. Statins, such as simvastatin and atorvastatin, are hypocholesterolemic drugs that possess pleiotropic effects, including antioxidant and anti-inflammatory properties, that are either dependent on or independent of 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) inhibition. In addition, *in vitro* and *in vivo* studies have demonstrated that simvastatin has an anti-inflammatory effect in patients with predialytic chronic kidney disease, and may play an important role in counteracting the mechanisms involved in pathogenesis of inflammation. We aimed to investigate the effects of prior simvastatin on mitochondrial enzyme activities in kidney tissue of the early phase of sepsis.

Methods: We used male adult Wistar albino rats weighing 200 to 250 g in the experiments. The rats were divided into four groups, each composed of eight rats: control group, LPS group, Simvastatin group, Simvastatin + LPS group. Lipopolysaccharide (LPS) from *Escherichia coli* O127:B8 (Sigma, St. Louis, MO, USA) was injected intraperitoneally at a daily dose of 20 mg/kg, Simvastatin (20 mg/kg) was given p.o. via oral gavage for 5 days. In the Simvastatin + LPS-treated group, LPS was given 1.5 hours after the fifth dose of simvastatin. Mitochondrial electron transport chain enzymes citrate synthase, NADH-cytochrome c reductase (complex I + III), and NADH dehydrogenase (complex I) were measured kinetically in spectrophotometer from kidney tissue homogenate. The kidney tissue samples were fixed in 10% buffered formalin and embedded in paraffin for hematoxylin and eosin (H&E) staining. Data were expressed as mean ± standard deviation (SD) and analyzed during analysis of variance.

Conclusion: There were no changes in activities of citrate synthase, complex I, complex I + III in tissue homogenate ($P > 0.05$) (Figures 1 to Figure 3). In the LPS + Simvastatin group, we found decreased activity of complex I compared with those of the LPS and Simvastatin groups ($P = 0.05$, $P = 0.07$, respectively). As a result of the light microscopic examination with H&E stained sections, we observed tubule lumens widened and partially damaged in the epithelium. In the Simvastatin group was seen partially widened tubular structure and even in some areas tubular structure was found the same as control sections. Moreover, the damage in tubular width and proximal epithelium was observed to continue (Figure 4).

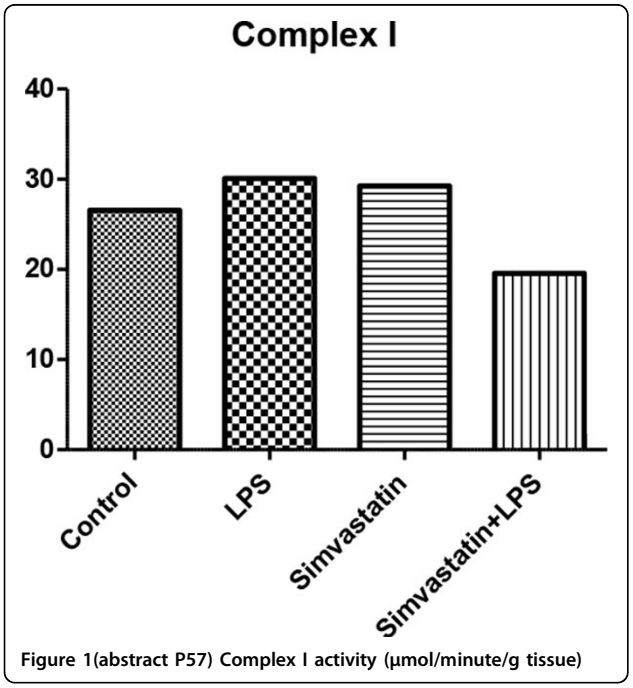

Figure 1(abstract P57) Complex I activity (µmol/minute/g tissue)

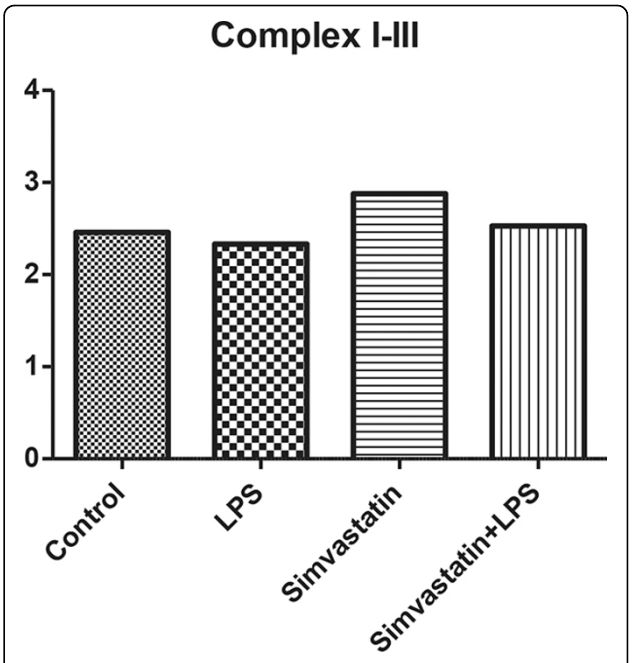

Figure 2(abstract P57) Complex I + III activity (µmol/minute/g tissue)

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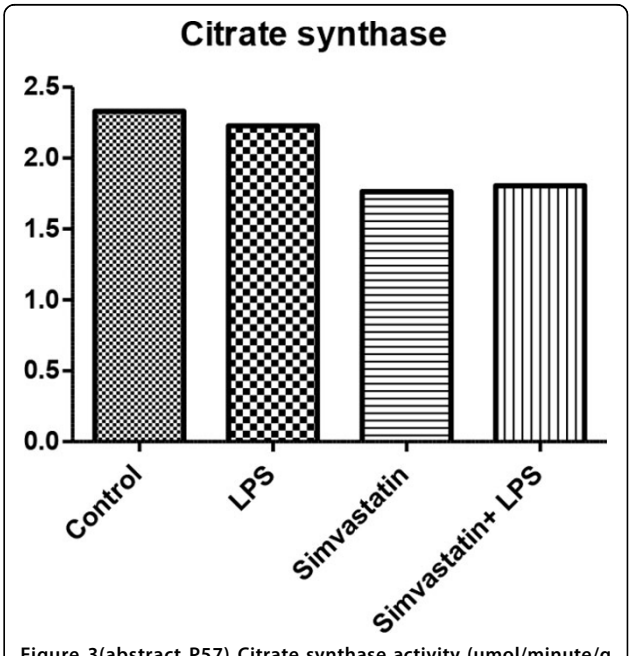

Figure 3(abstract P57) Citrate synthase activity (µmol/minute/g tissue)

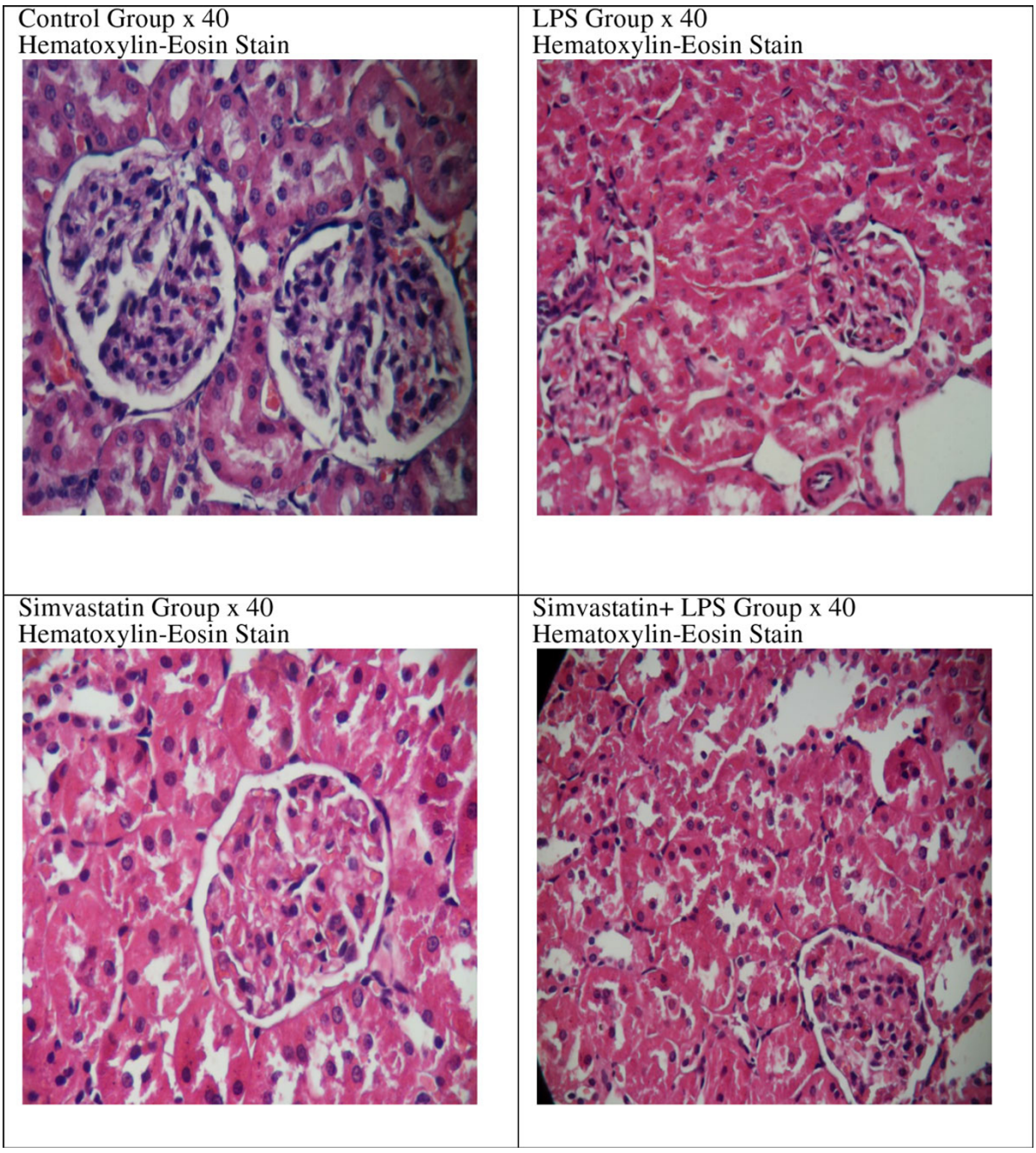

Figure 4(abstract P57) Hematoxylin and eosin staining in all experimental groups

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P58

Characterization of a murine model of septic cachexia.

D Restagno[1*], J-M Bonnet[1], K Mouchain[2], P Brune[2], C Paquet[1], T Delale[3], V Louzier[1]

[1]VetAgro sup, campus vétérinaire, Marcy L'Etoile, France; [2]Bertin Pharma, CEA Saclay, Gig sur Yvette, France; [3]Alizé Pharma, Espace Européen, Ecully, France

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Introduction: Muscular loss is a characteristic phenomenon induced by a massive inflammation as occurring in sepsis. Indeed, the early phase of sepsis is responsible for the expression of proinflammatory cytokines, namely TNFα and IL-6, known to be effectors of cachexia. Although cachexia is a morbidity factor in human, there is to date no animal model of septic cachexia. The goal of this study is to create and characterize a murine model of septic cachexia and evaluate endogenous ghrelin variations. Among the factors involved in both sepsis and muscular protection, current research highlights a potential role for ghrelin in muscle protection but there are conflicting data regarding its variations

during experimental sepsis. In this study we evaluate its two circulating forms (AG, acylated ghrelin and UAG, unacylated ghrelin).
Methods: Sixty male C57Bl6 mice (20 to 25 g, 6 to 8 weeks) were used for these experiments. Sepsis is induced by a mild CLP (survival ≃ 80%). After anesthesia, cecum is isolated, ligated at 1/3, and punctured twice (21G needle). A laparotomy is performed without ligation or puncture. Mice weight is followed every day, blood collections and hind limb muscles dissection are realized at 2 hours, 6 hours, 1, 2, 3, 5 and 13 days after CLP. Plasmatic cytokines (TNFα, IL-6) and ghrelin levels are evaluated via the Luminex technique and ELISA assays respectively and muscles are weighed.
Results: After surgery, weight loss in CLP mice significantly decreases from D1 to D11 with a peak at D3 (20% vs. 10% for the Sham). On D12, septic mice reach their original weight, which becomes identical to the Sham on D13. Despite a similar weight, CLP mice muscles are lighter. Circulating TNFα and IL-6 levels increase 2 hours after CLP and remain higher than for the Sham operated mice. Ghrelin concentrations, regardless of its form, increase after the surgery: AG rises on D1 and D2 while UAG increases earlier, from 2 to 24 hours.
Conclusion: We finalize a model of muscular and weight loss due to septic conditions. Wasting syndrome and Inflammation are two of the three conditions for cachexia. The next step is to determine proteolysis in our model to confirm that we performed a murine model of septic cachexia. The role of increased circulating levels of ghrelin in this model remains to be elucidated.

P59

Mice survival in a two-hit model of sepsis depends on intratracheal *P. aeruginosa* bacterial load.

D Restagno[1*], J-M Bonnet[1], A Kodjo[1], F Venet[2], C Paquet[1], L Freyburger[1], V Louzier[1]
[1]VetAgro sup, campus vétérinaire, Marcy l'Etoile, France; [2]Groupement Hospitalier Edouard Heriot, Laboratoire d'immunologie cellulaire, Lyon, France
Critical Care 2014, **18(Suppl 2):**P59; doi:10.1186/cc14062

Introduction: Sepsis is a systemic reaction in the presence of an infection characterized by an early systemic inflammatory response syndrome followed by a compensatory anti-inflammatory response syndrome. The first side of this typical immune response is responsible for widespread damage organ whereas the second part leads to an immunoparalysis increasing patients' susceptibility to secondary infections. The goal of this study is to finalize a murine model of sepsis to understand the physiopathology of sepsis and eventually test new therapeutic approaches.
Methods: Male C57Bl6 mice (20 to 25 g, 6 to 8 weeks) were used. Sepsis is induced by a mild CLP (survival ≃ 80%). Cecum is isolated, ligated (1/3), and punctured twice (21G needle). A laparotomy is performed without ligation or puncture. Blood is collected 2 hours, 6 hours, 1, 2, 3, 5 and 13 days after the CLP. Plasmatic cytokines (TNFα, IL-6, IL-10) are evaluated via the Luminex technique. Five days after CLP, splenocytes are collected and used for immunological assays. Mice are intratracheally instilled with *P. aeruginosa* 5 days after CLP (5×10^6, 2×10^7 and 10^8 CFU) to evaluate survival.
Results: Circulating TNFα, IL-6 and IL-10 levels increase 2 hours after CLP and remain high until the end of the experiment, as compared to Sham operated mice. Five days after CLP, total T cells (T-CD3$^+$) population and proliferation significantly decrease as compared to the nonseptic condition whereas the T-reg cell (T-CD4$^+$/CD25$^+$) population significantly increases whereas it remains low in the Sham operated animals. TNFα induction by LPS in cultured splenocytes increases both for CLP and Sham operated mice but remains lower in septic mice. Survival following a secondary infection decreases while the quantity of instilled *P. aeruginosa* increases. No mortality is observed for 5×10^6 CFU, 50% of CLP die with 2×10^7 CFU and 100% of mortality is observed for both CLP and Sham with 10^8 CFU. Two days after instillation, *P. aeruginosa* is detected in the lungs of CLP and Sham but in the spleen and liver only for CLP mice.
Conclusion: This study demonstrates that immunosuppression following CLP increases mice susceptibility to secondary *P. aeruginosa* intratracheal infection. However, this susceptibility depends on the bacterial load.

P60

Tyrosine metabolism disorder and the potential capability of anaerobic microbiota to decrease the value of aromatic metabolites in critically ill patients.

N Beloborodova[*], V Moroz, A Osipov, A Bedova, Y Sarshor, A Vlasenko, A Olenin
Negovsky Research Institute of General Reanimatology, Russian Academy of Medical Science, Moscow, Russia
Critical Care 2014, **18(Suppl 2):**P60; doi:10.1186/cc14063

Introduction: Metabolism of tyrosine can be switched in conditions of hemodynamic instability and tissue hypoperfusion. The violation of the oxygen-dependent metabolism of tyrosine must be accompanied by the activation of alternative pathways (Figure 1). Previously, we found a high level of content in the blood of aromatic intermediates p-hydroxyphenyllactic acid (p-HPLA) and p-hydroxyphenylacetic acid (p-HPAA) in patients with sepsis [1,2]. We assume that the anaerobic microbiota can take an important part in biodegradation of excess alternative metabolites of aromatic amino acids [3,4].
Methods: Serum samples were collected from critically ill patients (n = 65) with surgical diseases (n = 32), brain injury (n = 22), and lung diseases (n = 11). Patients were included in the study on the day of admission to the ICU. The median of age was 62 (IR 42 to 77) years, the APACHE II score was 17 (IR 11 to 29). The level of p-HPLA and p-HPAA were measured in serum using GC-FID. Anaerobic bacteria (Figure 2) were cultured in Shedler media, and the level of aromatic metabolites were measured before and after 48 hours of incubation using GC-MS. Data were compared by Mann-Whitney U test, $P < 0.05$ considered significant (IBM SPSS Statistics 22).
Results: In surviving patients (n = 24) the total level of p-HPLA and p-HPAA (4.47; 3.24 to 8.35 μM) was less ($P < 0,001$) than in patients who died (n = 41) (13.67; 5.78 to 52.26 μM). The severity of organ dysfunction on a SOFA scale correlates ($r_s = 0.7$, $P < 0.001$) with the total level of the p-HPLA and p-HPAA. Also the total level of aromatic compounds correlates with lactate ($r_s = 0.6$; $P < 0.001$), BE ($r_s = -0.5$, $P < 0.001$) and perfusion blood pressure ($r_s = -0.5$, $P < 0.001$). ROC analysis revealed that p-HPLA has the largest area under the curve (0.78; CI 0.67 to 0.90, $P < 0.001$). In experimental studies, anaerobic bacteria significantly reduced the level of p-HPAA and p-HPLA (Figure 2).
Conclusion: High level of p-HPLA and p-HPAA correlate with severity and mortality of patients. Hypoxia can be one of the leading mechanisms of tyrosine metabolism disorders in critically ill patients. *Bacteroides* spp. are able to consume p-HPLA and p-HPAA and consequently may be involved in the elimination of these intermediates from the human body mutually with endogenous mechanisms of detoxification.
Acknowledgements: This work was supported by the Russian Foundation for Basic Research (project number 1304-01758/13)
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P61

RADICAL study: rapid diagnosis of suspected bloodstream infections from direct blood testing using PCR/ESI-MS.

D Brealey[1*], N Libert[2], J Pugin[3], M O'Dwyer[4], K Zacharowski[5], M Mikaszewska-Sokolewicz[6], MP Maureau[7], DJ Ecker[7], R Sampath[7], M Singer[1], J-L Vincent[8]
[1]University College Hospital, London, UK; [2]Military Hospital du Val-de-Grâce, Paris, France; [3]Hôpitaux Universitaires de Genève, Geneva, Switzerland; [4]The Royal London Hospital Barts, London, UK; [5]Universitätsklinikum Frankfurt, Germany; [6]Child of Christ Hospital, Warsaw, Poland; [7]Ibis Biosciences, Abbott,

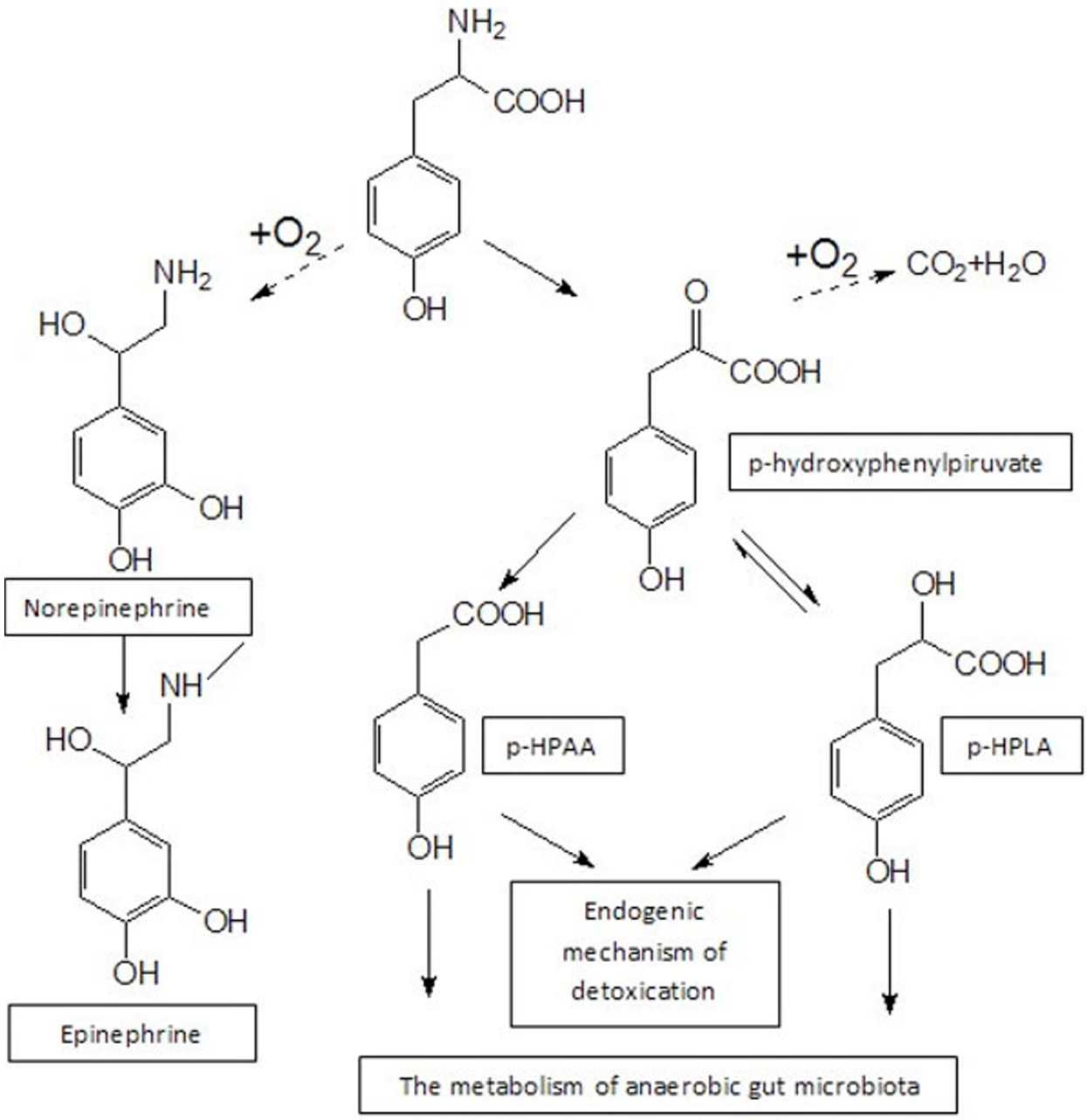

Figure 1(abstract P60)

Carlsbad, CA, USA; [8]Erasme University Hospital, Université Libre de Bruxelles, Belgium, Belgium
Critical Care 2014, **18(Suppl 2):**P61; doi:10.1186/cc14064

Introduction: Survival from sepsis and bloodstream infections (BSI) often depends upon rapid identification of the infecting pathogen and expeditious antimicrobial therapy. We report findings from the final analysis of RADICAL, a multicenter observational study that compared results from direct blood specimen testing using PCR/ESI-MS to standard microbiology in critically ill patients.

Methods: Eight ICUs in six European countries participated. Patients with suspected infection plus ≥2 new onset SIRS criteria were enrolled and had an extra blood specimen taken for PCR/ESI-MS direct analysis. Results were compared to standard of care microbiology. All patients were followed up to 28 days or until inpatient death or discharge. A panel of three independent physicians reviewed a summary of each case to determine the potential impact upon patient management if results had been available for decision-making.

Results: A total of 609 direct blood specimens from 543 patients meeting inclusion criteria were tested. Patient demographics and organ failure criteria observed were consistent with previously published sepsis studies.

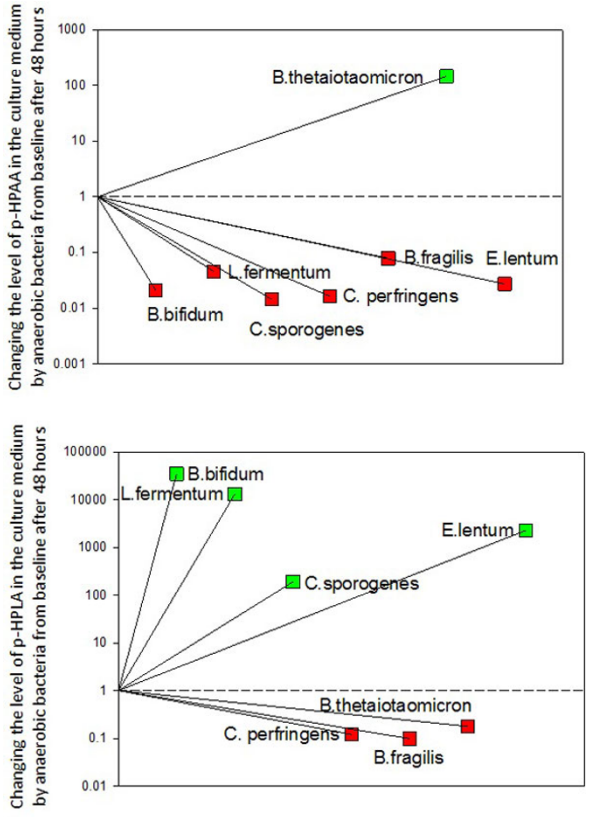

Figure 2(abstract P60)

Culture/PCR comparisons were as follows: +/+ 54; +/- 13; -/ + 169; and -/- 393, respectively, for a sensitivity of 81%, specificity of 69%, PPV of 24% and NPV of 97%. The distribution of the organisms in the culture-/PCR+ group was similar to the culture+/PCR+ group and was reproducible in replicate testing with an independent blood draw. Analysis of additional sample types (lower respiratory tract or sterile fluids and tissues) corroborated direct blood findings in 58% of cases where multiple specimens from the same patients were analyzed. The independent expert panel would have considered a different course of care in 57% of the cases when PCR/ESI-MS was positive, 41% of which would have resulted in altered, instituted, or ceased antibiotic therapy, earlier.

Conclusion: These results suggest that PCR/ESI-MS accurately detects the infecting pathogen in critically ill patients with BSI, and that its use might often lead to a different treatment. The test's ability to rule in or rule out infection approximately within 8 hours of the blood draw has added potential clinical and economic benefit, as it might minimize unnecessary use of antibiotics.

Acknowledgements: RADICAL Study Team

P62

Subgroup analysis of the lipid infusion and patient outcomes in sepsis trial (LIPOS) reveals benefit in a subgroup not treated with stress replacement doses of corticosteroids.

TS Parker[1,2*], DM Levine[1,2], BR Gordon[1,3], SD Saal[1,3]
[1]The Rogosin Institute, New York, NY, USA; [2]Department of Biochemistry, New York Presbyterian Hospital and Weill Cornell Medical College, New York, NY, USA; [3]Department of Medicine, New York Presbyterian Hospital and Weill Cornell Medical College, New York, NY, USA
Critical Care 2014, **18(Suppl 2):**P62; doi:10.1186/cc14065

Introduction: Lipidose (formerly GR270773) is a protein-free phospholipid emulsion intended for the treatment of hospitalized patients with suspected or confirmed Gram-negative severe sepsis. Lipidose contains phosphatidylcholine, triglyceride and sodium cholate formulated to optimize delivery of the phospholipid component to the surface of high-density lipoprotein (HDL) and other lipoproteins, thereby enhancing the capacity of the patient's circulating lipoprotein pool to bind and neutralize microbial toxins. When Lipidose is infused into blood, the cholic acid is adsorbed onto serum albumin and the phospholipid selectively associates with lipoproteins. Bound and neutralized toxins are removed from the circulation by the liver and excreted along with the cholic acid into the bile. The LIPOS trial enrolled 1,400+ patients at 235 study centers in 31 countries to access Lipidose treatment at two dose levels. The LIPOS headline data presented only a small mortality benefit for the lower dose and no benefit from the higher dose [1]. A subgroup analysis was carried out to test the hypothesis of benefit in the subgroup with adequate liver function, using serum albumin levels as a measure of liver function, and adequate pre-existing HDL or total lipoprotein to accept phospholipid as predicted by the mechanism of action.

Methods: Albumin, cholesterol and HDL were measured in stored serum samples. The response to treatment and interactions with baseline covariates specified in LIPOS were tested after exclusion of subjects in the lowest biomarker quartiles (AlbTC25 and AlbHDL25).

Results: Subjects above the lowest quartile of albumin cleared Lipidose significantly faster than those in the lowest quartile ($P < 0.003$). Interactions between treatment and planned use of intravenous stress replacement doses of corticosteroids (IVCST) were found in the AlbTC25 and AlbHDL25 subgroups ($P < 0.05$). Exclusion of these subjects revealed strong relationships between treatment benefit and cholesterol or HDL that were used to select optimal biomarker thresholds. Requiring albumin ≥1.5 g/dl and either cholesterol ≥1 mM or HDL ≥0.5 mM selected 59% and 36%, respectively, of the LIPOS population. Treatment with Lipidose reduced mortality in these subgroups by 6.6% ($P < 0.025$) or 10.8% ($P < 0.005$) respectively. The treatment benefits persisted for at least 1 year.

Conclusion: A strong negative interaction with IVCST may have masked a significant treatment benefit in LIPOS. This interaction may be related to the ability of bile acids to slow clearance and raise concentrations of corticosteroids [2,3]. Biomarkers can be used to select subjects with early severe septic shock responsive to treatment with Lipidose.

Acknowledgements: TSP, DML, BRG and SDS are listed as inventors on patents filed by and/or assigned to Sepsicure, L.L.C

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P63

Presence of infection in patients with presumed sepsis at the time of ICU admission.

PMC Klein Klouwenberg[1,2,3*], OL Cremer[1], LA van Vught[4], DSY Ong[1,2,3], JF Frencken[1,3], MJ Schultz[5], MJ Bonten[2,3], T van der Poll[4]
[1]Department of Intensive Care Medicine, University Medical Center Utrecht, the Netherlands; [2]Department of Medical Microbiology, University Medical Center Utrecht, the Netherlands; [3]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, the Netherlands; [4]Center for Experimental and Molecular Medicine and Division of Infectious Diseases, Academic Medical

Center, University of Amsterdam, the Netherlands; [5]Department of Intensive Care Medicine, Academic Medical Center, University of Amsterdam, the Netherlands
Critical Care 2014, **18(Suppl 2):**P63; doi:10.1186/cc14066

Introduction: A clinical suspicion of infection is mandatory for diagnosing sepsis in patients with a systemic inflammatory response syndrome. Yet the accuracy of categorizing critically ill patients presenting to the ICU as being infected or not is unknown. We therefore assessed the likelihood of infection in patients who were treated for sepsis upon admission to the ICU, and quantified the association between plausibility of infection and mortality.
Methods: We studied a cohort of critically ill patients admitted with clinically suspected sepsis to two tertiary ICUs in the Netherlands between January 2011 and December 2013. The likelihood of infection was categorized as none, possible, probable or definite by *post hoc* assessment. We used multivariable competing risks survival analyses to determine the association of the plausibility of infection with mortality.
Results: Among 2,579 patients treated for sepsis, 13% had a *post hoc* infection likelihood of 'none', and an additional 30% of only 'possible'. These percentages were largely similar for different primary suspected sites of infection. In crude analyses, the likelihood of infection had no impact on ICU mortality, but was associated with increased length of stay and complications. In multivariable analysis, however, patients with an unlikely infection had a higher mortality rate compared to patients with a definite infection (subdistribution hazard ratio 1.23; 95% confidence interval 1.03 to 1.49).
Conclusion: This study is the first prospective analysis to show that the clinical diagnosis of sepsis upon ICU admission corresponds poorly with the presence of infection on *post hoc* assessment. A higher likelihood of infection does not adversely influence outcome in this population.
Acknowledgements: This research was performed within the framework of CTMM, the Center for Translational Molecular Medicine (http://www.ctmm.nl), project MARS (grant 04I-201).

P64

Aetiology of community-acquired pneumonia in the ICU setting and its effect on mortality, length of mechanical ventilation and length of ICU stay: a 1-year retrospective review.

D Ryan*, R Connolly, J Fennell, G Fitzpatrick
The Adelaide and Meath Hospital, incorporating the National Children's Hospital (AMNCH), Tallaght, Dublin, Ireland
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Introduction: Community-acquired pneumonia (CAP) is the most common infectious reason for admission to ICUs and has mortality of up to 37% [1]. Highest mortality rates are in Gram-negative infections with lower rates in *Streptococcus pneumoniae* and viral infections [2]. Microbiology is difficult to establish with most prospective studies identifying agents in only 50% of cases [3]. We analysed microbial aetiology of CAP in ICU over 1 year and assessed its effects on inpatient mortality, length of mechanical ventilation and length of ICU stay.
Methods: We retrospectively reviewed admissions to AMNCH ICU between February 2013 and February 2014 catalogued as having pneumonia from chest radiograph and clinical findings on the internal audit system ($n = 91$). After chart review, 28 were excluded as hospital-acquired pneumonias, 12 due to insufficient information and 21 due to primary diagnosis other than pneumonia. Thirty patients were selected on the basis that CAP was the likely reason for ICU admission.
Results: Pathogens were detected in 73% of patients by culture, antigen detection or molecular methods: *S. pneumoniae* ($n = 6$; 20%), influenza ($n = 4$; 13%), viral with superimposed bacterial infection ($n = 4$; 13%), viruses other than influenza ($n = 3$; 10%), Gram-negative bacilli ($n = 2$; 7%), *Legionella* spp. ($n = 2$; 7%) and *Haemophilus* spp. ($n = 1$; 3%). No organism was identified in 27%. Gram-negative infection had highest mortality (100%), average length of mechanical ventilation (57 days) and average ICU stay (64 days). The mean age of affected patients was 68.2. Mortality was lower in influenza/other viral infections (75/50%), patients spent less time mechanically ventilated (mean 6.6/13.3 days) and less time in ICU (mean 4/16.3 days), reflecting the younger mean age of patients (53.2/51.4). Of patients infected with *S. pneumoniae*, *Legionella* spp. or *Haemophilus* spp. alone, all survived to discharge. However, of note, when superimposed on viral infection, these pathogens carried a higher mortality (50%). Infection with pneumococcus alone occurred in younger patients (mean age 50.6) and was associated with a shorter ventilation period (mean 2.66 days) and ICU stay (mean 6 days).
Conclusion: Microbial aetiology was identified in a high proportion of patients (72%) admitted to ICU with CAP, reflecting timely collection of appropriate specimens. Infection with Gram-negative organisms had the highest mortality, length of mechanical ventilation and length of ICU stay, while the pathogens usually seen in CAP were associated with more favourable outcomes.

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P65

In vivo study of endothelial barrier-related GTPase expression in the kidney and liver during the acute phase of nonlethal sepsis.

BC Zychar[1*], MA Cenezede[2], AMA Liberatore[1], NOS Câmara[2], IH Koh[1]
[1]Department of Surgery, UNIFESP/EPM, São Paulo, Brazil; [2]Laboratory of Cellular Immunology Experimental, UNIFESP/EPM, São Paulo, Brazil
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Introduction: Maintenance of the integrity of the endothelial barrier is crucial in pathological inflammatory/infectious conditions [1]. The endothelial barrier dysfunction leading to increased vascular permeability and leukocyte transmigration in the systemic inflammatory state has been intrinsically related to the multiple organ dysfunction syndrome. The Rho GTPases family regulates the organization of the actin cytoskeleton, and plays a fundamental role in maintaining homeostasis and function of the endothelial barrier [2]. The study of the mechanisms of intracellular signaling related to the integrity of the endothelial barrier may help in understanding the hemodynamic changes during systemic infection. Thus, this study aimed to correlate the pattern of genic expression of GTPases during the initial periods of sepsis in order to understand the kinetic of these molecular mechanisms in organs often affected in sepsis.
Methods: Wistar rats weighting 200 to 250 g were submitted to: nonlethal sepsis (2 ml *Escherichia coli* 10^7 UFC/ml i.v. inoculation [3,4], $n = 5$, S7 group); minor trauma (cervical incision with catheter implantation in jugular, injection of 2 ml saline, $n = 5$, SHAM group); without any procedure ($n = 5$, NAIVE group). After 2 and 6 hours, the liver and kidney were collected to determine RhoA, Rac1 and Cdc42 gene expression by quantitative real-time PCR.
Results: Low expression of RhoA was observed in both organs (Figures 1A and Figure 2A). Rac1 and/or Cdc42 showed higher expression in both organs in animals submitted to systemic infection (S7 group) compared to the SHAM and NAÏVE groups in both periods (Figures 1B, C and Figure 2B, C). Although both organs showed a similar pattern, the kidney showed a statistically significant increase of Cdc42 and Rac1 as compared to the liver, showing that GTPase expression might differ, possibly due to endothelial heterogeneity of each organ to perform its specific function. These results suggest that the infectious process leads to a higher gene expression of Rac1 and/or Cdc42 in relation to a minor surgical trauma inflammation. The RhoA and Rac1 gene expression were inversely correlated, as reported in the literature [5].
Conclusion: Rac1 and Cdc42 possibly act in favor of preserving the integrity of the endothelial barrier by their attributed stabilization and protection of microvessel endothelial cells, by gathering the cell junctions during the infectious state. Studies are being conducted to better understand the mechanisms of intracellular signaling of the endothelial barrier in different organs of animals submitted to varying degrees of systemic infections.
Acknowledgements: FAPESP.

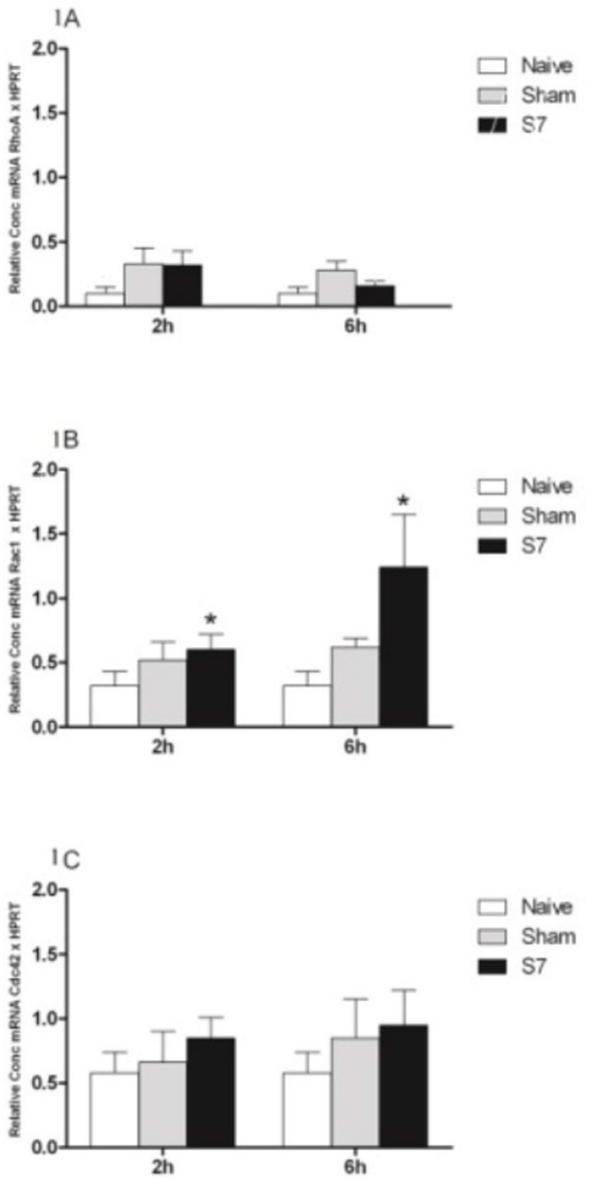

Figure 1(abstract P65) Gene expression of GTPases RhoA (A), Rac1 (B) and Cdc42 (C) in the liver of rats. Animals were subjected to nonlethal sepsis 2 ml *Escherichia coli* 10^7 UFC/ml i.v. inoculation (S7 group), minor trauma 2 ml saline i.v. inoculation (SHAM group), or animals without inoculation were used as control group (NAÏVE group). After 2 or 6 hours the kidney was isolated. Total RNA was extracted and transcribed to cDNA. Gene expression was quantified by real-time PCR. Graph bars show the relative concentration of each cDNA compared with the NAIVE group and the housekeeping gene HPRT. Results are mean ± SEM (n = 5 animals/group) and analyzed in triplicate. *P < 0.05 compared to NAÏVE group

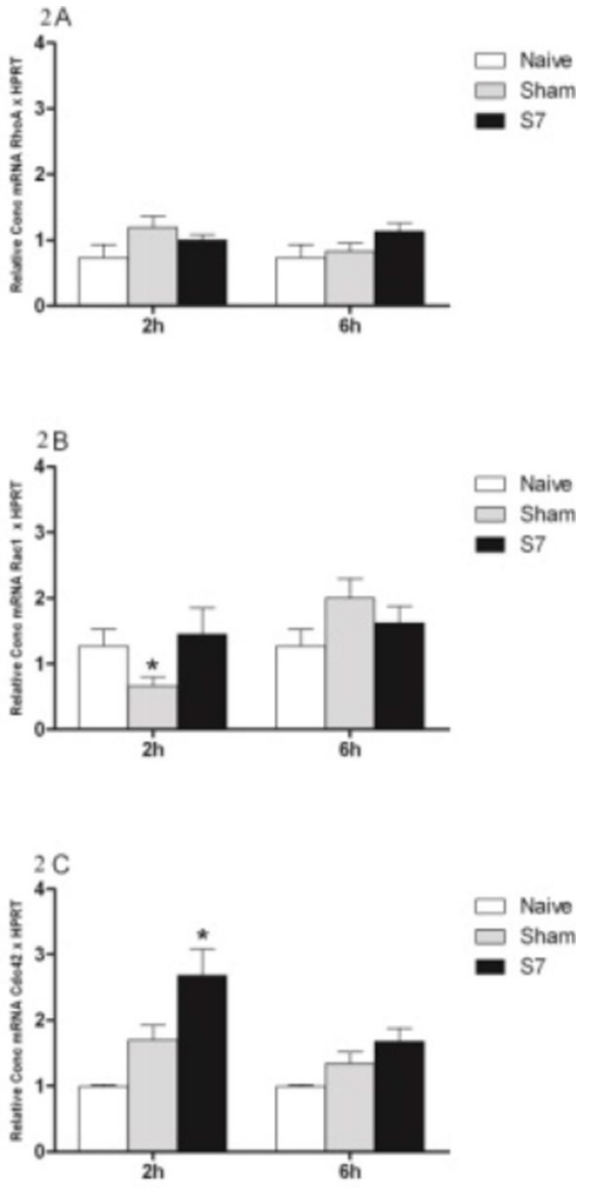

Figure 2(abstract P65) Gene expression of GTPases RhoA (A), Rac1 (B) and Cdc42 (C) in the kidney of rats. Animals were subjected to nonlethal sepsis 2 ml *Escherichia coli* 10^7 UFC/ml i.v. inoculation (S7 group), minor trauma 2 ml saline i.v. inoculation (SHAM group), or animals without inoculation were used as control group (NAÏVE group). After 2 or 6 hours the kidney was isolated. Total RNA was extracted and transcribed to cDNA. Gene expression was quantified by real-time PCR. Graph bars show the relative concentration of each cDNA compared with the NAIVE group and the housekeeping gene HPRT. Results are mean ± SEM (n = 5 animals/group) and analyzed in triplicate. *P < 0.05 compared to NAÏVE group

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P66

Direct bacterial identification from blood culture by matrix-assisted laser desorption-ionization time of flight mass spectrometer using a simplified protocol.

A García-Tapia*, I Guerrero, T Trujillo, F Galán, P Marín, C Fernández, M Rodriguez-Iglesias
Clinical Microbiology Laboratory, Puerta del Mar University Hospital, Cadiz, Spain
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Introduction: Throughout the world, the number of patients at risk for bloodstream infections (BSIs) continues to rise. BSIs are associated with high rates of morbidity and mortality, and they markedly increase the costs of hospital care. Prompt identification of the causative agent(s) and rapid initiation of appropriate antimicrobial therapy are critical for reducing mortality, especially in patients with septic shock. Matrix-assisted laser desorption-ionization time of flight (MALDI-TOF) equipment is increasingly used in the microbiological laboratory. The goal of the present investigation was to apply mass spectrometry directly from positive blood cultures in order to detect and identify bacterial strains using a simplified homemade protocol.
Methods: One-hundred and sixty-six positive blood cultures (Bactec FX; Becton Dickinson) were analyzed. Gram and conventional plates were used and the identification was done by Wider System (Soria Melguizo). In the MALDI-TOF protocol, positive blood cultures were processed as follow: 5 ml sample of positive broth was extracted and centrifuged at 1,000 rpm for 10 minutes in order to remove blood cells. The supernatant was collected and centrifuged at 4,000 rpm for 15 minutes, then the supernatant was removed and the pellet was placed directly onto a MALDI-TOF target plate and dried at room temperature, subsequently adding 1 µl HCCAMatrix solution (10 mg/ml cyano-4-hydroxycinnamic acid) and drying. Mass spectra were acquired with a Microflex LT mass spectrometer using FlexControl 3.3 software (Bruker Daltonics GmbH).
Results: One-hundred and forty-nine (89.7%) blood cultures were correctly detected. Thirty-three bacterial species were identified as being *Escherichia coli* (47), the most frequent, followed by *Pseudomonas aeruginosa* (14), *Enterobacter* spp. (11), *Klebsiella* spp. (9), *Staphylococcus aureus* (9), coagulase-negative staphylococci (9), *Enterococcus* spp. (8), *Bacteroides* spp. (7) and streptococci (6). Bacteria fastidious as *Burkholderia, Raoultella, Delftia, Listeria, Acinetobacter* or *Stenotrophomonas* were correctly identified. Only one strain of *Streptococcus pyogenes* and 14 strains of coagulase-negative staphylococci cannot be identified. In hemolyzed samples, it was not possible to identify two strains of *S. aureus*.
Conclusion: Using MALDI-TOF MS for bacteria identification directly from blood cultures represents an advance in the treatment of septic patients. Compared with conventional culture-based methods, this approach can improve species-level identification of bloodstream isolates in terms of time, accuracy, and costs. We conclude that this technique is simple, fast and reliable for direct bacterial detection from positive blood cultures.

P67

Approaches combining mice and *Drosophila melanogaster* models to decipher human sepsis.

R Ramy*, S Garnier, L Pradel, L Röder, B Loriod, A Defaye, L Perrin, P Rihet, C Nguyen
Aix-Marseille Université, Inserm, UMRS 1090, TAGC, Parc scientifique de Luminy, Marseille, France
Critical Care 2014, **18(Suppl 2):**P67; doi:10.1186/cc14070

Introduction: Sepsis is a complex and heterogeneous syndrome in which inflammatory and infection mechanisms are implicated. Thus, it is difficult to differentiate those mechanisms in mammals where inflammation is a highly complex biological process. In our laboratory, besides studying sepsis in human blood biological samples, we choose to investigate a murine model in ordered to describe a global transcriptome overview of critical events occurring in the blood, brain and lung during an induced non-infection inflammation. We decided to complete our view with another animal model where the inflammatory process is less complex and mainly achieved through innate immunity *Drosophila melanogaster*.
Methods: Female C57BL6/J mice received an intravenous oleic acid (OA) injection to induce a controlled inflammation. Lung, brain and peripheral blood mononuclear cell (PBMC) expression patterns were analyzed using an Agilent 60K cDNA mouse microarray. The enrichment of canonical pathways revealed marked changes in pathways involving the immune and inflammatory responses. The inflammatory process in *D. melanogaster* shares common mechanisms in innate immunity with mammals (Toll pathway/TLR). We decided to set up a model, called the double-hit model, where drosophila females aged from 7 to 10 days have been injured with a needle (inflammatory hit) before being infected with a needle that has been dipped in a bacterial solution of *Pseudomonas entomophila* (infectious hit). The next step will be to investigate the transcriptome in order to highlight the differences and have a global view of biological mechanisms involved; and also look for genetic factors involved in this experiment. The DGRP (Drosophila Genomic Reference Panel) project is a panel of 200 inbred fly lines that have been fully sequenced.
Results: Strikingly, in mice models, all significant pathways identified in the brain were also significant in the lung. The inflammatory responses oscillated between proinflammatory and anti-inflammatory response in both the lung and brain, the time course, however, being different in the two organs. In PBMC, we observed a significant response delay after OA injection and the pathways identified differed from those identified in the lung and brain. Our second objective will be to use this model of the expression to set up a tool to analyze precisely the effect of an infection in a second hit during the sterile inflammation activation/repression time courses. In *D. melanogaster*, we recorded their survival in comparison with flies that only received the infectious hit and observed a difference of ~40%. Flies that have received a non-infectious double-hit showed a 10% decrease in survival rate. We will perform the double-hit experiment with all the lines of the DGRP and then through genome-wide association study. We expected to identify single nucleotide polymorphisms that are associated with resistance or susceptibility to the double-hit.
Conclusion: In mice, we assessed gene expression profiles in mouse lung, brain and PBMC associated with a lung inflammation during a 24-hour time course. Overall, our microarray analysis provides a global and detailed overview of critical transcriptional events occurring in the blood, brain and lung. The analysis of gene functional annotation revealed several major features. First, many genes were upregulated or downregulated over the time in the lung, brain, and blood. Second, the analysis revealed pathways clearly related to inflammation mainly in the lung and brain. The strong inflammation observed in the brain, and a limited inflammation in the blood, indicates that blood gene expression profiles poorly reflect those observed in the lung and brain. Third, all the pathways identified in the brain were also identified in the lung, although a few common genes were characterized; it should also be stressed that there was a delay response in the brain.

P68

Role of nonpneumoniae mycoplasma in the pathogenesis of ventilator-associated pneumonia: an *in vitro* assessment.

TJ Nolan*, AC Morris, A Rossi, T Walsh
Centre for Inflammation Research, Queens Medical Research Centre, Little France, Edinburgh, UK
Critical Care 2014, **18(Suppl 2):**P68; doi:10.1186/cc14071

Introduction: Mycoplasma organisms are the smallest bacteria capable of self-replication [1] and include species capable of causing disease (for example, *Mycoplasma pneumoniae, Mycoplasma genitalium*) as well as those that are generally thought to exist synergistically with their human host (for example, *Mycoplasma salivarium*). The Edinburgh critical care group (Prof TW/ACM) has recently identified a high prevalence of *M. salivarium* in the bronchoalveolar lavage washings from patients with confirmed and suspected ventilator-associated pneumonia (VAP) (Figure 1) [2]. The aim of this study was to examine the effect of *M. salivarium* on human immune cells *in vitro*. Specifically, we measured cytokine production and phagocytosis activity in response to *M. salivarium* exposure.
Methods: Whole human blood was obtained from healthy donor volunteers and cell types were isolated using diffusion gradients and magnetic labeling as appropriate. Monocytes and macrophages were incubated with *M. salivarium* for 24 hours before a subsequent LPS stimulus.

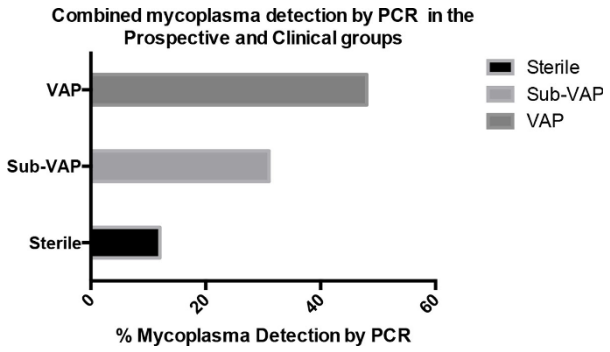

Figure 1(abstract P68) Combined mycoplasma detection by PCR in the prospective and clinical groups

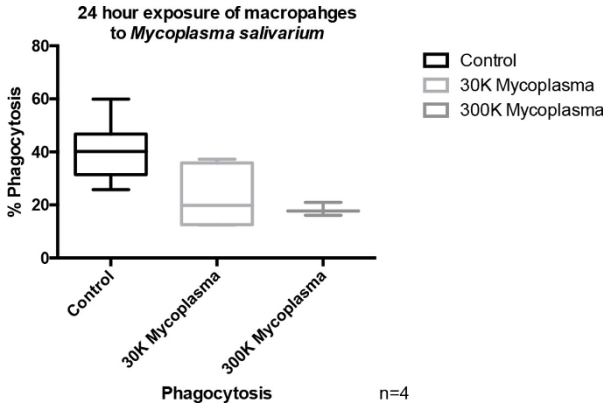

Figure 2(abstract P68) Twenty-four-hour exposure of macrophages to *Mycoplasma salivarium*.

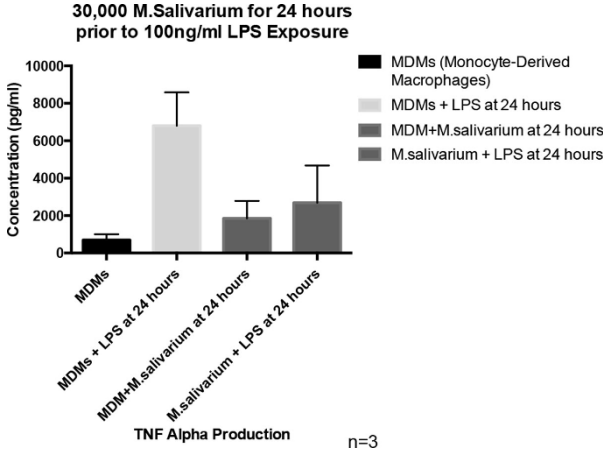

Figure 3(abstract P68) *Mycoplasma salivarium* (30,000) for 24 hours prior to 100 ng/ml LPS exposure

Macrophage phagocytosis assays were conducted after exposure times of 60 minutes and 24 hours to *M. salivarium*. Cytokines were measured using ELISA and human cytokine bead array kits.

Results: There was a statistically significant decrease in phagocytosis between control cells and the macrophages exposed to both a low titer of *M. salivarium* (*P* value 0.018) and a medium titer of *M. salivarium* (*P* value 0.011) after 24 hours of exposure (Figure 2). There was a statistically significant decrease in phagocytosis activity between the macrophages exposed to the medium titer of *M. salivarium* for 24 hours versus 60 minutes (*P* value 0.013). Exposure of macrophages to mycoplasma resulted in decreased release of TNFα after a subsequent LPS stimulus (Figure 3). To our knowledge, this is the first time extracellular traps have been induced in macrophages in response to *M. salivarium* (Figure 4).

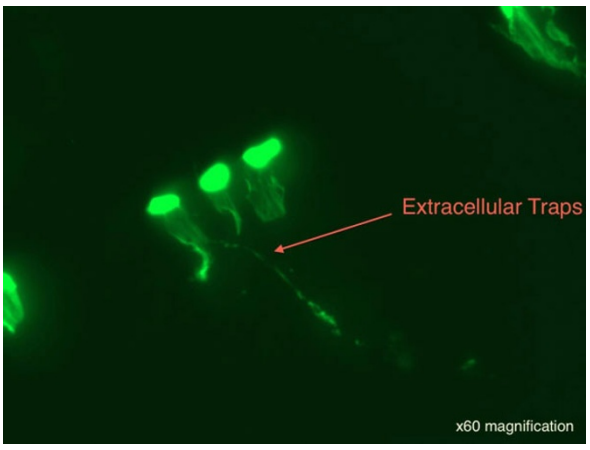

Figure 4(abstract P68)

Conclusion: Although further research is needed, it is interesting that the presence of *M. salivarium* caused an anti-inflammatory effect as well as impaired antigen presentation secondary to impaired phagocytosis. This could be consistent with the better outcome in mechanically ventilated patients that did not have *M. salivarium* bacteria detected in their bronchoalveolar lavage washings. Extracellular traps contribute to microbial containment by forming a physical barrier composed of chromatin and cytoplasmic proteins to enhance antimicrobial synergy while minimizing damage to host tissues [3]. It is interesting that *M. salivarium* induced extracellular traps.

Acknowledgements: Thanks to supervisors ACM and Prof AR for their support and expertise. Thanks also to all the staff in the Centre for Inflammation Research for their help throughout the year and for being so willing to help when help was needed

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P69

Probiotic pretreatment improves survival and prevents gut mucosal barrier dysfunction in sepsis.

KL Calisto*, ACAP Camacho, FC Mittestainer, MCS Mendes, AC Santos, MJA Saad
Department of Internal Medicine, Faculty of Medical Sciences, UNICAMP Campinas, Brazil

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Introduction: The gut is the largest immune organ and plays a central role in the promotion of systemic inflammatory responses [1]. Perturbations of intestinal epithelial homeostasis during sepsis include increased proinflammatory cytokine production, increased intestinal permeability and apoptosis [2-6]. Healthy gut is essential to promote host health and prevent organ dysfunction in sepsis. Probiotics seem to keep gut homeostasis through different pathways, such as the modulation of microbial activity, energy regulation, anti-inflammatory cytokine production, gene expression

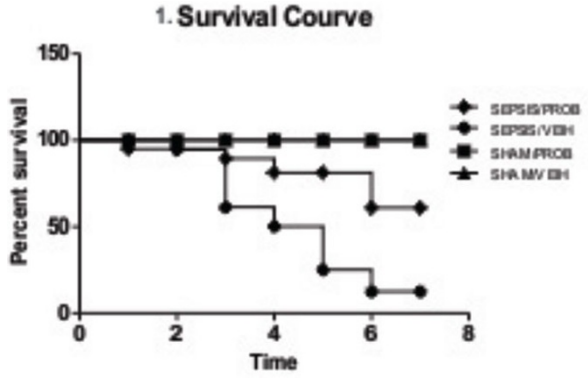

Figure 1(abstract P69)

and cell differentiation [7]. Probiotics have been shown an effective treatment in various clinical conditions, although the potential benefits of probiotic treatment in sepsis remain largely undefined. The aim of the present study was to investigate the effect of probiotic treatment on gut dysfunction and inflammatory signaling in septic rats.

Methods: Sepsis was induced by cecal ligation and puncture (CLP) in Wistar male rats (8 weeks old). They were pretreated with probiotics or vehicle once a day during 7 days before CLP. The chosen probiotic mixture contained 10×10^7 CFU *Bifidobacterium longum*, 10×10^6 CFU *Lactobacillus bulgaricus*, 10×10^6 CFU *Lactobacillus acidophilus*. Colonic tissue and serum samples were collected 24 hours after CLP for ELISA and protein expression analysis by western blotting.

Results: Our data demonstrate that probiotic pretreatment improved survival of septic rats (Figure 1) and this effect is accompanied by a marked decrease of IL-1β and TNFα (Figure 2A,B). Sepsis leads to severe intestinal epithelial damage with a decrease in claudin 2 and occludin protein expression (Figure 3A,B); probiotic pretreatment reversed these alterations in parallel with an increase in Hsp72 and Hsp25 activation (Figure 4A,B). In intestinal epithelial cells, the inducible Hsp have been shown to preserve tight junction and barrier function. The maintenance of epithelial barrier integrity induced by probiotic pretreatment, in parallel with an activation of cytoprotective pathway, may culminate in the restoration of the intestinal epithelial function.

Conclusion: Our results show that probiotics pretreatment fulfills a dual function at the intestinal mucosa: in addition to preventing intestinal permeability disruption, it also attenuates proinflammatory cytokine release, diminishing the exacerbate host's reaction to infection and offering a novel prophylactic strategy to sepsis.

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P70

Positive fluid balance and prognostic factors of ICU mortality in patients admitted with septic shock.

W Koonrangsesomboon[1*], B Khwannimit[2]
[1]Department of Internal Medicine, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand; [2]Division of Critical Care Medicine, Department of Internal Medicine, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand

Critical Care 2014, **18(Suppl 2):**P70; doi:10.1186/cc14073

Introduction: The amount of fluid during resuscitation of septic shock is important. Too little fluid may result in tissue hypoperfusion; however, too much fluid may result in volume accumulation. Several recent studies have demonstrated that a positive fluid balance in critical illness is associated with deteriorating outcomes. However, some studies have shown opposite results. The objective of this study was to determine whether initial fluid balance in septic shock patients is correlated with ICU mortality.

Methods: This is a retrospective study of septic shock patients admitted to a mixed medical-coronary care unit of Songklanagarind hospital from 2005 to 2011. Multivariate logistic regression analysis was used to identify predictors of mortality.

Results: A total of 1,048 patients admitted to ICU for septic shock was divided into two groups: in-ICU survivors (n = 555 (53%)) and nonsurvivors (n = 493 (47%)). Median survival time was 10 days (95% CI: 8 to 12 days). The respiratory tract was the most common site of infection (47.6%). Community-acquired infections accounted for 59.6%. Survivors were older than nonsurvivors (62 vs. 56 years, P = 0.016). Nonsurvivors were more severely ill and had shorter ICU stays (2 vs. 5 days, P < 0.001). Nonsurvivors received albumin and steroid more than survivors. Median cumulative fluid at 24, 48 and 72 hours of septic shock onset were 4.2, 7.7 and 10.5 l respectively. Nonsurvivors had significantly larger median cumulative fluid intake at 24 hours (4.6 vs. 3.9 l, P < 0.001), at 48 hours (8.2 vs. 7.1 l, P < 0.001) and at 72 hours (11.4 vs. 9.9 l, P < 0.001). Nonsurvivors also had

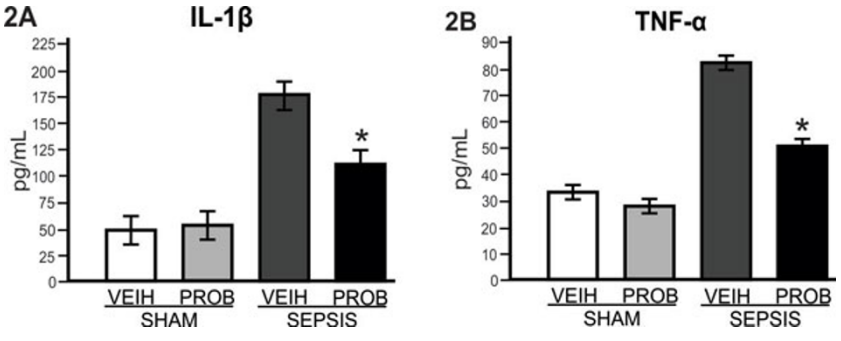

Figure 2(abstract P69)

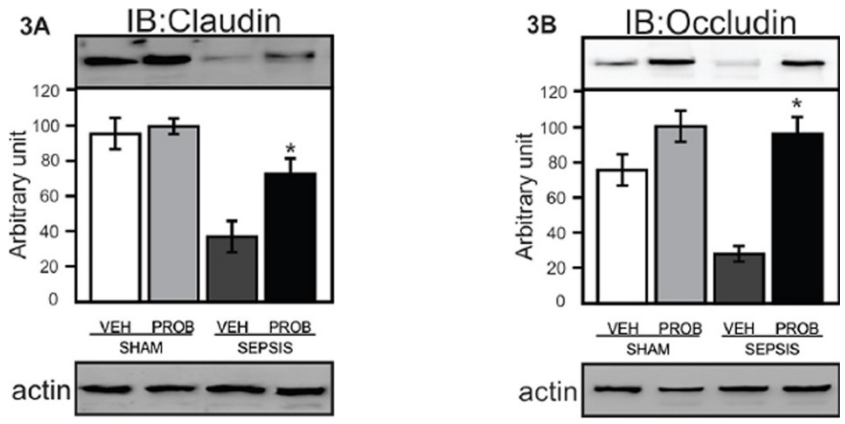

Figure 3(abstract P69)

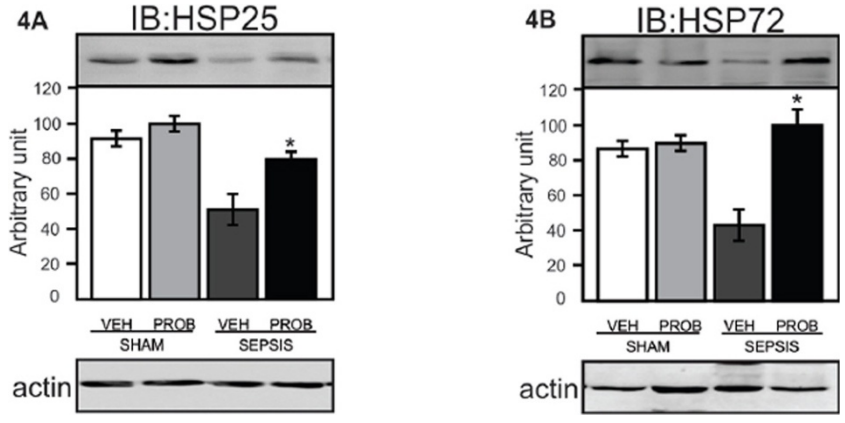

Figure 4(abstract P69)

significantly larger fluid balance (5.4 vs. 4.4 l, $P < 0.001$) and mean fluid balance (2.8 vs. 1.6 l, $P < 0.001$) within 72 hours. In multivariate logistic regression analysis, factors significantly associated with ICU mortality were mean fluid balance, APACHE II score, SOFA score, length of ICU stay, ARDS, steroid use, parenteral nutrition use and source of infection.

Conclusion: A more positive cumulatively fluid balance over 3 days is associated with ICU mortality in septic shock. Multivariate analysis found not only nonmodifiable factors such as severity score, source of infection, length of ICU stay and ARDS, but also modifiable factors such as parenteral nutrition use, steroid use and mean fluid balance were significantly associated with mortality.

P71

Alpha lipoic acid attenuates oxidative stress-induced damage macromolecules in the brain of rats with sepsis-associated encephalopathy.

LG Danielski*, M Michels, D Florentino, A Viera, A Lauriano, Fabrícia Petronilho
Laboratório de Fisiopatologia Clínica e Experimental (LAFICEXP), Programa de Pós, Graduação em Ciências da Saúde, Universidade do Sul de Santa Catarina (UNISUL), Tubarão, SC, Brazil
Critical Care 2014, **18(Suppl 2):**P71; doi:10.1186/cc14074

Introduction: Pathophysiological mechanisms of sepsis-associated encephalopathy involve oxidative stress. This imbalance between the pro-oxidant and antioxidant causes damage to macromolecules such as lipids and proteins, thus the employment of antioxidants becomes an attractive proposition. Alpha lipoic acid (LA), a potent antioxidant, is able to cross the blood-brain barrier, and is an important cofactor in enzymatic and cellular energy metabolism. We aimed to determine the use of AL in oxidative damage and neutrophil infiltration in rat brain 12 and 24 hours after induction of sepsis model by cecal ligation and puncture (CLP).

Methods: Male Wistar rats (250 to 350 g) were subjected to CLP model, with sham control. Groups were divided into sham + saline, sham + AL, CLP + saline and CLP + AL (200 mg/kg orally with single administration after CLP), $n = 10$. At 12 and 24 hours, rats were euthanized, the hippocampus, striatum, cerebellum, cortex and prefrontal cortex removed, lipid peroxidation assessed by TBARS, damage to proteins by protein carbonylation, myeloperoxidase activity (MPO) and the formation of nitrite and nitrate. Data were analyzed by ANOVA with *post hoc* Tukey test and log-rank test with $P < 0.05$.

Results: In 12 hours compared with the CLP group, the CLP + AL group showed a reduction in lipid peroxidation in the striatum, in the protein carbonylation in the cortex and hippocampus, in the MPO activity in the striatum and hippocampus, and decreased formation of nitrite and nitrate in the hippocampus and cortex.

Conclusion: While differences were not observed in 24 hours, TBARS found protein carbonylation in a reduction of damage to the CLP + AL group over the cerebellum, MPO in the striatum, hippocampus and prefrontal, and hippocampus, cerebellum, and prefrontal to nitrite/nitrate. AL may be an important therapeutic target in reducing neurologic complications in animal models of sepsis.

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P72

Effects of mesenchymal stromal cells on human umbilical vein endothelial cells in *in vitro* sepsis models.

K Lund[1*], J Peltzer[1], F Montespan[1], N Oru[2], E Vicaut[2], J Duranteau[3], J-J Lataillade[1]
[1]Unité de Thérapie Cellulaire et Réparation Tissulaire, Institut de Recherche Biomédicale des Armées/Centre de Transfusion Sanguine des Armées, Clamart, France; [2]Laboratoire d'étude de la microcirculation, Université Paris - Diderot, Paris, France; [3]Service d'Anesthésie-Réanimation Chirurgicale, Centre Hospitalier Universitaire de Bicêtre, Le Kremlin-Bicêtre, France
Critical Care 2014, **18(Suppl 2):**P72; doi:10.1186/cc14075

Introduction: Septic shock is a medical emergency that, despite the medical advances that have been made, still remains a major cause of hospital deaths. Cell therapy is an innovative field of research that could provide a therapy for sepsis. Mesenchymal stromal cells (MSC) are promising in cell therapy and more importantly for sepsis because of their immunosuppressive capabilities [1]. MSC have been shown by several groups to have a positive effect against sepsis *in vivo* [2-4]. It has been predicted that the MSC interact with macrophage to release IL-10 that in turns reduces inflammation [4]. Other groups have focused on the use of stimulated MSC to ameliorate their immunosuppressive capabilities [5]. The main stimulation of MSC has been the use of inflammatory stimulants like IFNγ. Our work focuses on the identification of effective MSC donors, whether primed with IFNγ or naïve, and the development of *in vitro* models that will predict how an MSC donor will act *in vivo*. We also want to eliminate the use of cells completely and use their secreted microvesicles as a therapy. The hypothesis is that the *in vitro* models will eliminate a noneffective MSC donor and allow us to identify the MSC donor that will have the greatest effect.
Methods: We developed two *in vitro* models that are similar to what happens *in vivo* with WBC as they circulate in a septic patient. The first test is the adherence of WBC to a layer of HUVECs in the presence of MSC or microvesicles. The second is a permeability test to determine MSC ability to block the permeability of a HUVEC layer.
Results: Our preliminary results have shown that we are able to identify, using our two *in vitro* models, which MSC donor would be an effective MSC for cell therapy.
Conclusion: MSC and their paracrine factors have to the potential to be an effective therapy for sepsis, but one needs to identify an effective donor before use in cell therapy.

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P73

Endotoxin Activity Assay levels correlate with the microbiological results of Gram-negative organisms in septic patients.

M Tsunoda*, M Kang, M Saito, N Saito, M Namiki, T Harada, M Takeda, A Yaguchi
Department of Critical Care and Emergency Medicine, Tokyo Women's Medical University, Tokyo, Japan
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Introduction: The Endotoxin Activity Assay (EAA™; Spectral Diagnostics Inc., Toronto, Canada) is a useful diagnostic test for sepsis due to Gram-negative infection and is based on the reaction of neutrophils to endotoxin complexed with an anti-endotoxin antibody. However, the relations between values of EAA and microbiological data have not been elucidated. Our hypothesis is that EAA values correlate to the results of microbiological cultures and also severity.
Methods: From July 2008 to July 2013, all adult patients with suspected sepsis admitted to our medico-surgical ICU in whom EAA was measured were included in this study. Data collected included age, gender, ICU mortality, white blood cell (WBC) count, C-reactive protein (CRP), procalcitonin (PCT), EAA levels, SOFA score and results of microbiological culture. Patients with no microbiological data were excluded. Data were analyzed by Kruskal-Wallis test, Mann-Whitney *U* test and multivariate logistic regression. $P < 0.05$ was considered significant.
Results: Of 569 patients (353 men and 216 women; mean age 66.0 ± 17.4 years), 283 patients had Gram-negative infection and 286 patients had no Gram-negative infection. Of 283 patients with Gram-negative infection, 65 patients had Gram-negative organisms in blood. EAA levels were significantly different between patients with Gram-negative blood, in other infectious sites and no Gram-negative infection (0.45 ± 0.21 vs. 0.39 ± 0.17 vs. 0.36 ± 0.15, $P = 0.03$). The odds ratio (95% confidence interval (CI)) of EAA levels for Gram-negative infection and Gram-negative bacteremia were 3.89 (1.44 to 10.4) ($P = 0.007$) and 3.36 (2.16 to 40.6) ($P = 0.003$), respectively. The odds ratio and CI of age and SOFA score for ICU mortality were 1.03 (1.01 to 1.04) ($P = 0.0003$) and 1.33 (1.26 to 1.41) ($P < 0.0001$), respectively, while gender, WBC, CRP, PCT and EAA levels had no relations with ICU mortality. SOFA score was significantly higher in patients with Gram-negative infection than in patients with no Gram-negative infection (8.0 ± 4.6 vs. 6.7 ± 4.2, $P = 0.0003$).
Conclusion: EAA levels related to the detections of Gram-negative organisms in cultures. Thus, a high EAA level may show the existence of Gram-negative organisms in patients' sites. EAA levels had relations with SOFA score but no relations with ICU mortality.

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Early diagnosis of sepsis due to Gram-negative infection with the Endotoxin Activity Assay.

M Kang*, M Tsunoda, M Saito, N Saito, M Namiki, M Takeda, T Harada, A Yaguchi
Department of Critical Care and Emergency Medicine, Tokyo Women's Medical University, Tokyo, Japan
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Introduction: The Endotoxin Activity Assay (EAA™; Spectral Diagnostics Inc., Toronto, Canada) is a rapid *in vitro* diagnostic test of the neutrophil reaction to endotoxin and reflects the endotoxemia. A higher value of EAA (>0.60) has been shown to correlate with developing severe sepsis and a high mortality in other previous studies. We hypothesize that a value of EAA more than 0.55, not >0.60, may be useful to earlier diagnose sepsis due to Gram-negative organisms and to assess the severity.
Methods: The present study is a single-center retrospective observational analysis of adult septic patients in whom EAA was performed from July 2008 to July 2013. Patients were divided into two groups: (1) EAA >0.55 and (2) EAA < 0.54. Age, sex, days of ICU stay, ICU mortality, body temperature, WBC, CRP, procalcitonin (PCT), SOFA score, and microbiological data were compared between two groups. Values are expressed as mean ± SD. Data were analyzed by chi-square test and Mann-Whitney U test. $P < 0.05$ was considered significant. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and odds ratio were also evaluated.
Results: Five hundred and ninety-four patients (377 men and 217 women; mean age 65.0 ± 17.5 years) were studied. There were (1) 104 patients with EAA >0.55 and (2) 490 patients with EAA < 0.54. ICU mortality (39.4 vs. 26.6 %, $P = 0.01$), PCT (17.2 ± 36.0 vs. 11.8 ± vs. 30.6 ng/ml, $P = 0.04$), SOFA score (8.1 ± 4.9 vs. 6.2 ± 4.6, $P = 0.04$) and positive Gram-negative organisms in cultures (57.7 vs. 45.5 %, $P = 0.02$) were significantly higher in group (1) than group (2). Age, sex, body temperature, WBC and CRP were not significantly different between two groups. Using detections of Gram-negative organisms in cultures, the sensitivity, the specificity, the PPV and the NPV were 22.1, 85.2, 57.7 and 54.6%, respectively. The odds ratio was 1.64.
Conclusion: ICU mortality and severity were higher in patients with EAA >0.55 than in patients with EAA < 0.54. There is a possibility that an EAA

value more than 0.55 is a meaningful value for early diagnosis of sepsis due to Gram-negative infection.

P75

Effect of estimated glomerular filtration rate and fluid balance on clinical course and outcomes of children admitted with severe dengue.

N Vijayakumar[1,2*], S Kandasamy[2], T Sangaralingam[1], VV Varadarajan[2], N Krishnamoorthi[1,3]
[1]Department of Pediatrics, Mehta Children's Hospital, Chennai, India; [2]Department of Pediatric Intensive Care, Mehta Children's Hospital, Chennai, India; [3]Department of Academics and Research, Mehta Children's Hospital, Chennai, India
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Introduction: Dengue fever is one of the most important seasonal epidemics in Asia Case fatality rates vary from 1 to 5% [1]. Mortality from severe dengue may range from 26% in DHF to as high as 47% in DSS [2,3]. The pathogenesis of shock in dengue fever (DF) is centered on increased capillary permeability in the critical phase leading to hypovolemia and shock in severe dengue. There have been multiple studies that compare fluid regimens in the management of dengue [4,5]. These studies do not assess the child's renal function and ability to handle the fluid loads. GFR <60 ml/minute indicates a significant decrease in the renal functioning and there are no pediatric studies that examine their association with in-hospital stay and outcomes in children with severe dengue. With this introduction we formulated this study protocol to examine that association. The objectives were to measure the estimated glomerular filtration rate (eGFR) at admission and fluid balance in the first 36 hours of ICU stay and assess their effect on disease course and outcomes in severe dengue.
Methods: This was designed as a retrospective descriptive study in a tertiary-level pediatric ICU in South India. Case records of all children fulfilling the WHO case definition of severe dengue were included, those who received intravenous fluid for less than 12 hours were excluded. Primary parameters measured included fluid balance in the first 36 hours measured every 12 hours, durations of oxygen requirement, mechanical ventilation, ICU stay and total hospital stay. Outcomes measured were death and survival.
Results: Twenty-six children were enrolled, 14 boys and 12 girls. The median duration of ICU stay was 60 hours, and that of hospital stay 109 hours. eGFR was less than 60 ml/minute in six patients (83.3% expired and 16.7% survived). eGFR, measured by modified Schwartz's formula, at the time of admission correlated inversely with requirement of oxygen therapy and mechanical ventilation ($P < 0.05$) and fluid balance in the first 36 hours. Positive fluid balance (FO > 15%) in the first 36 hours was significantly higher in children who expired ($P = 0.011$). eGFR <90 ml/minute at admission had 100% sensitivity and 79% specificity to predict the possible occurrence of fluid overload >15% (area under curve = 0.882).
Conclusion: Fluid balance in the first 36 hours had a significant positive correlation with mortality and negative correlation with eGFR. Children with admission eGFR <90 ml/minute may require restrictive fluid therapy to improve survival.

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P76

Audit of antibiotic prophylaxis at a district general hospital.

K McGrath*, J King-Robson, A Hayes, F Dashti, S Patel
Department of Microbiology, Kingston Hospital NHS Trust, Kingston-upon-Thames, UK
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Introduction: This audit evaluates the adherence to the prophylactic surgical antibiotic policy at Kingston Hospital. With a greater understanding of the current use of prophylactic surgical antibiotics comes the ability to improve patient care while minimising the development of antibiotic resistance. Surgical site infections are a major source of hospital-acquired infections, causing significant morbidity and mortality. In appropriate cases, surgical antibiotic prophylaxis is essential in preventing such infections; however, this comes with increased risks of antibiotic resistance and antibiotic-associated diarrhoea. Consequently, this institution has extensive guidelines as to the cases in which antibiotic prophylaxis is required and what antibiotics should be administered. This audit examines the adherence to these guidelines. We audited against our local antibiotic prescribing policy, named the Blue Book guidelines.
Methods: Notes were audited retrospectively for 80 patients undergoing surgery between 19 August and 18 December 2013. These were audited against local antibiotic guidelines (Blue Book guidelines) and analysed using descriptive statistics.
Results: Only 57% of operations were compliant with Blue Book antibiotic prophylaxis guidelines. For operations where administration of antibiotics was appropriate, 24% of patients received the incorrect choice, 19% were given the incorrect dose, 28% were given antibiotics for an inappropriate time relative to the procedure, and 38% received antibiotics for the incorrect duration. For operations not requiring prophylaxis, 48% of patients incorrectly received antibiotics.
Conclusion: Compliance to local guidelines for prophylactic antibiotics is extremely poor. This not only risks increased morbidity and mortality from surgical site infections but also risks the development of antibiotic resistance and *Clostridium difficile* colitis.
We suggest enhancement of the antibiotic guidelines, and education of those using them. This will improve the provision of antibiotic prophylaxis in the Trust, and ultimately improve patient care.

P77

Presepsis biomarker: high-density lipoprotein.

S Al-Zaidawi
Surgery Department, Burn Unit, Al-Sadr Teaching Hospital, Maysan, Iraq
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Introduction: Delay in diagnosis and initiation of antibiotic treatment has been shown to increase mortality. Biomarkers can play an important role in diagnosis and prognosis of sepsis. We aimed to evaluate the correlation between septicemia and high-density lipoprotein (HDL) level in burned patients.
Methods: A prospective study conducted at Al-Sadr teaching hospital, Maysan, Iraq, during the period from April to September 2013. Blood samples were collected from patients every other day to measure the level of HDL and triglycerides. Other blood samples were collected in blood culture tubes for culturing to verify septicemia depending on the clinical evidence.

Table 1(abstract P77) Characteristics of patients

Patient characteristic	Average	Range
Age (years)	17	1 to 85
TBSA%	33.5%	15 to 95%
Sex of patients	Female 61%	Male 39%
Burn type	Scalds 48%	Flame 52%

Most patients were female (61%) with average age 17 years and a wide range of burned surface area (15 to 95%)

Table 2(abstract P77) Lipid profile for all 75 patients at the onset of thermal injury during the first day of admission: all patients were with a normal range of HDL, triglycerides and cholesterol

Lipid profile	Range (mg/dl)	Average (mg/dl)	Mode (mg/dl)	Normal range (mg/dl)
HDL	30 to 56	39	38	39 to 59
Triglycerides	37 to 148	70	58	0 to 149
Cholesterol	46 to 155	78	86	0 to 199

Table 3(abstract P77) Lipid profile at the onset of sepsis showed that HDL level dropped to less than 15 mg/dl with range (4 to 13 mg/dl): elevation in triglyceride level out of normal range with no significant change in cholesterol level

Lipid profile	Range (mg/dl)	Average (mg/dl)	Mode (mg/dl)	Normal range (mg/dl)
HDL	4 to 13	7.6	4	39 to 59
Triglycerides	133 to 435	214.5	180	0 to 149
Cholesterol	56 to 139	82.8	76	0 to 199

Table 4(abstract P77) Levels of urea, creatinine, albumin, WBC, platelets during onset of sepsis: most patients developed hypoalbuminemia and thrombocytopenia

	% TBSA	WBC (×1,000/μl)	Platelet count (×1,000/μl)	Blood urea (mg/dl)	Serum creatinine	Serum albumin (g/dl)	Time to get sepsis (days)
Average	55	11.86	154.9	14	0.51	2.0	7
Mode	45	9.04	121	12	0.37	1.8	10
Minimum	27	2.27	32	9	0.32	1.4	2
Maximum	95	15.03	535	21	0.73	3.1	20

Table 5(abstract P77) Comparison between level of lipid profile before and after sepsis showed the significant dropping in HDL level during onset of sepsis

	HDL	Triglycerides	Cholesterol	*P* value
Burn onset	39	70	78	< 0.01
Sepsis onset	7.6	214.5	76	< 0.01

Results: Seventy-five patients (Table 1) were admitted consecutively into the burn unit, 35 of them (46%) developed septicemia and 11 of the 35 patients died. All dead patients had HDL value <5 mg/dl 1 or 2 days before dying since our blood samples were collected every 2 days (Tables 2 and 3). Other laboratory tests such as WBCs, platelet account, albumin level, and so forth were made to confirm sepsis (Table 4). A comparison between the level of lipid profile before and after sepsis showed a significant drop in HDL level during the onset of sepsis (Table 5). We also found that patients with HDL value <15 mg/dl were at high risk of developing sepsis.

Conclusion: There was a strong correlation between HDL level and septicemia in burn patients. The HDL value is a good biomarker for sepsis; it decreases below normal level and continues to diminish and reach an immeasurable level at the advanced stage of septicemia.

Cite abstracts in this supplement using the relevant abstract number, e.g.: Al-Zaidawi: Presepsis biomarker: high-density lipoprotein. *Critical Care* 2014, **18(Suppl 2)**:P77