

# SEPSIS 2010

Institut Pasteur, Paris, France

1st-3rd September, 2010

## Program & Abstract Book

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An International  
Symposium hosted by:  
The International  
Sepsis Forum



# EARLY PATIENT MANAGEMENT IN SEPSIS



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## Welcome from Konrad Reinhart

### International Sepsis Forum Chair

Welcome to Paris and to the fourth Sepsis Symposium hosted by the International Sepsis Forum (ISF).

The Symposium is designed to encourage debate and learning within this challenging subject. This years' program supports the ISF's vision and mission statements by discussing the global burden of sepsis, how and why sepsis kills, the host response, how we can help the host fight back and how we can refine the treatment of sepsis. The Symposium will also provide a review of clinical trials – past and ongoing.



Our expert panel of internationally renowned speakers are leaders from the fields of critical care, emergency medicine, infectious diseases, internal medicine and surgery. Their collective knowledge of caring for patients with severe sepsis and shock will be shared with the Symposium participants.

Sessions are plenary and roundtable, with guided poster walks throughout the Symposium. The four best abstracts will be presented on Thursday 2 September in an oral session. We hope that you enjoy the Symposium and I wish you all an informative and socially rich experience from which you will take home the information provided and bring as much as possible into your practice so that we all contribute to a better treatment of sepsis.

Konrad Reinhart  
Chair, ISF



# The International Sepsis Forum: Background



## Improving Sepsis Outcomes

The International Sepsis Forum (ISF) is a unique collaborative effort between industry and academia. It is the first initiative to focus solely on management of patients with severe sepsis. While sepsis and its sequelae are still associated with high morbidity and mortality rates, new data on patient management are emerging that may ultimately significantly improve the current situation. Such findings need to be evaluated and incorporated, when appropriate, into existing treatment protocols. Headed by a Council of international experts and opinion leaders, the ISF is focused exclusively on improving the management of sepsis and, in particular, septic shock by developing an international consensus on the latest understanding of key scientific and clinical issues, and disseminating emerging practice guidelines to researchers, intensivists, and other critical care professionals worldwide.

## Vision, Mission and Core Values

The vision of the International Sepsis Forum is to reduce the global toll of morbidity and mortality from sepsis.

The mission of the ISF is to improve the care of critically care patients with sepsis by:

- Promoting an improved understanding of the basic biology and pathology of sepsis
- Enhancing the understanding of the epidemiology of sepsis
- Improving the design and conduct of clinical research to improve the management of septic patients
- Educating health professionals in the optimal management of patients with sepsis
- Raising the profile of sepsis as a global health challenge with the public, with healthcare practitioners, with industry, and with global health agencies

## Core Values of the ISF

Integrity, responsibility and accountability to ourselves and to the patients and communities we serve.

Respectful, collegiate and transparent relationships within our group, with the scientific and clinical communities and with our academic and industry collaborators.

Courage, compassion, justice and innovative thought in combating the global challenge of severe sepsis.

## Contact details:

### Executive Director – Elaine Rinicker

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International Sepsis Forum is a Charity registered in England and Wales Number 1089944

The International Sepsis Forum Inc is currently applying for non-profit status in the USA.

## Web Site: [www.sepsisforum.org](http://www.sepsisforum.org)

[www.sepsisforum.org](http://www.sepsisforum.org) is a resource for all to use. The ISF will be adding a new feature which will include a report and web cast from Sepsis 2010.

The ISF web site also contains *Understanding Sepsis* is a booklet written for family members who are trying to understand this common and devastating disease. The booklet explains sepsis, severe sepsis and septic shock and various treatments and management techniques that may be used by physicians and other health care professionals in treating this disease.

*This version of the booklet is available in English, Spanish, Portuguese, Dutch, Italian, Japanese and French.*

## Council Members

The ISF takes its direction from a council chaired by Professor Konrad Reinhart. This prestigious group of international experts in sepsis meets regularly to identify educational needs and develop ISF activities. A group of Scientific Advisors appointed by the Council also participates in program activities, providing vital input to key clinical and research issues.

### **Konrad Reinhart      ISF Chair**

Vice Dean of Clinic for Anaesthesiology and Intensive Care, Jena, Germany

### **Edward Abraham**

Professor and Chair of the Department of Medicine, the University of Alabama, Birmingham, USA

### **Derek Angus**

University of Pittsburgh, Critical Care Medicine, Crisma Laboratory, Pittsburgh, USA

### **Gordon Bernard**

Melinda Owen Bass Professor of Medicine, and Associate Vice Chancellor for Research at Vanderbilt University School of Medicine, USA

### **Thierry Calandra**

Professor, Division of Infectious Diseases, Department of Internal Medicine Centre, Hôpitalier Universitaire Vaudois, Lausanne, Switzerland

### **R. Phillip Dellinger**

Professor of Medicine at Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, USA

### **Mitchell Fink**

Visiting Professor in the Department of Surgery at the David Geffen School of Medicine at UCLA, University of California in Los Angeles, USA

### **Simon Finfer**

Professor in the Faculty of Medicine of the University of Sydney and at The George Institute for International Health in Sydney, Australia

### **Stephen F. Lowry**

Professor and Chairman Department of Surgery at the University of Medicine and Dentistry of New Jersey - Robert Wood Johnson Medical School and Senior Associate Dean for Education New Brunswick, USA

### **John Marshall**

Professor of Surgery at St Michael's Hospital, Toronto, Canada

### **Jean-Paul Mira**

Dean of Cochin Port-Royal University and the Professor of Critical Care Medicine; Chairman of the Department of Critical Care Medicine at Cochin-St Vincent de Paul University Hospital Paris, France

### **Steven Opal      ISF Treasurer**

Professor of Medicine, Brown Medical School, Providence, Rhode Island, USA

### **Mervyn Singer**

Professor of Intensive Care Medicine at University College London, UK

### **Tom van der Poll**

Professor of Medicine at the University of Amsterdam, The Netherlands

### **Jean-Louis Vincent**

Professor of Intensive Care at the University of Brussels and Head of the Department of Intensive Care at the Erasme University Hospital in Brussels, Belgium

## Global Sepsis Alliance - GSA

The World Federation of Societies of Intensive and Critical Care Medicine (WFSICCM), the World Federation of Pediatric Intensive and Critical Care Societies (WFPICCS), the International Sepsis Forum and the Sepsis Alliance (SA), have recognized the need to elevate awareness and understanding of sepsis and have come together to form the Global Sepsis Alliance (GSA). Many leading organizations have joined the GSA and will support its efforts creating a multidisciplinary organization.

The objective of the GSA is to rally the global sepsis community in an effort to elevate public, philanthropic and governmental awareness, understanding and support of sepsis and to accelerate collaboration among researchers, clinicians, associated working groups and those dedicated to supporting them.

For more information on the GSA please visit the web site: [www.globalsepsisalliance.org](http://www.globalsepsisalliance.org)

# General Information

## Badges

Name badges will be issued to all participants on arrival at Registration. Participants are kindly requested to wear their badge at all times for security. Access to all scientific events, catering and Exhibition areas will only be possible with your own name badge. Replacement name badges may be purchased from the Registration Desk.

## Catering

Morning and Afternoon Breaks and Lunch: participants' teas and coffee after lunch will be served from the various service points within the Exhibition area. Please take time to visit the Exhibitors during the breaks. They will be pleased to see you. Participants lunch will be served in the Espace Congrès. This building is two minutes walk from the CIS building and staff will help you find your way.

## Certificates of Attendance

Each pre-registered delegate will receive a Certificate of Attendance with their delegate materials on arrival. This Conference operates on a transparency basis so that participants declare openly to their accrediting organisation how much of the Conference they attended.

## Checking out of Accommodation

Please ensure that you check-out of your accommodation by the required time (check with your hotel Reception as times vary). Please ensure that you settle any hotel room extras (telephone, meals etc) on departure.

## Cloakroom

A cloakroom is provided by the venue. This area may not be manned at all times. All bags and belongings are placed here at the owner's risk.

## Exhibition

We have two exhibits at this conference and we encourage all participants to visit the displays. For information about our Exhibitors, please see page 35.

## Feedback Forms

It is important for Organizers to have feedback to help direct future events. Please take time to fill in your Feedback Form and hand it in at the Registration Desk before departing from the Conference. Your view is valued.

## Instructions to Poster Presenters

Please note the following important details:

A listing of all Posters by title is given on pages 19–21.

Posters should be fixed to the poster boards using magnets, poster tape, or Blue Tac during the after noon of Wednesday 1 September, before the start of the opening session or during the break at 15:00 – 15:30.

Each poster has been allocated a board on which to be displayed, with a poster number. This number cannot be moved or the poster location swapped for any other. Posters have been arranged generally by theme.

All posters must be placed on the correct board no later than 15:30 hours on 1 September, be displayed for the duration of the Conference and removed at the end of the Conference.

Authors are invited to stand by their posters during the Welcome Reception and Poster Walks on Wednesday 1 September following on from the end of the program, to meet and discuss the poster with other participants. The posters will be reviewed by the Sepsis 2010 Program Committee during a series of Poster Walks.

## Please Remember To Take Your Poster Home With You!

## Liability

Neither the International Sepsis Forum, International Sepsis Forum Ltd, Index Communications Meeting Services nor the Institut Pasteur is able to take responsibility whatsoever for injury or damage to persons or property during the Conference. Please take care of yourself and your belongings.

## Messages

The telephone number for the venue is + 33 (0) 1 40 61 34 08.

It may take time for messages to reach participants, but every effort will be made.

### Mobile Phones

We appreciate that all Conference participants need to be available for calls, but the Organizers ask that all mobile phones are switched off or to mute mode in all sessions. We would like to make a polite request that any calls be made or taken in the foyer areas or outside the Conference sessions to avoid disruption to this professional meeting.

### Poster Awards

Four Posters have been selected to be Discussed Posters and this means that they also have short oral presentations within the program. Please refer to page 19 for the Conference program.

*Poster Awards are kindly supported by an educational grant from Merck & Co.*

### Publication of Abstracts

ISF has published all abstracts accepted for Sepsis 2010 in the Program Book. In addition a selection of abstracts has also been published as a supplement to the *Critical Care* journal. Abstracts published in *Critical Care* are under the editorial control of the journal and not ISF.

### Program Books

If stock allows, spare copies of the Program Book will be available to buy from the Registration Desk at the end of the Conference. If you lose your Book and need a replacement you will need to pay 30 Euros.

### Registration Desk

The Registration Desk will be situated adjacent to the Exhibition area and will be open during the following hours:

Wednesday	1 September	11:00 – 19:00 hours
Thursday	2 September	07:30 – 18:00 hours
Friday	3 September	07:30 – 14:00 hours

### Smoking

Please note that this is a No Smoking Conference.

### Social Program

#### Wednesday 1 September

#### Welcome Reception 18:00 – 19:30 hours in the Exhibition area

A Welcome Reception will be held in the Exhibition area immediately after the last session of the afternoon's program. Please ensure that you are wearing your name badge for this event. This will be an informal opportunity for delegates to meet, relax and orientate themselves ready for the next few days, as well as viewing the Poster Exhibition.

Drinks and nibbles will be served.

#### Thursday 2 September

#### Gala Dinner 19:30 hours at Bel Canto, Neuilly sur Seine

At the Bel Canto the waiters are actually students of the famous Paris Conservatoire and they burst into songs composed by geniuses like Mozart, Puccini, Verdi etc. The cuisine is typically European and the wine is good. All in all, this promises to be an atmospheric and special evening. Following a drinks reception, a 3 course dinner will be served.

**Tickets should have been prebooked.** If you have not already booked and would like to attend, please contact the Registration team for availability.

**Coach transport is provided by the Conference – please refer to your ticket for details.**

*Please ensure that you bring your ticket with you.*

Dress code: informal.

### Venue (of the Conference)

Institut Pasteur  
25 – 28 rue du Docteur Roux  
75724 Paris CEDEX 15 – France  
Tel : + 33 (0) 1 45 68 80 00  
Fax : + 33 (0) 1 43 06 98 35



# Sepsis 2010: Scientific Program

Sepsis 2010 is hosted by the International Sepsis Forum

The scientific program is endorsed by

The Australian and New Zealand Intensive Care Society (ANZICS)

The Société de Réanimation de Langue Française (SRLF)



**Program co-chairs Simon Finfer, Sydney, Australia & Jean-Paul Mira, Paris, France**

## Wednesday 1 September

## Abstract no.

13:00 – 13:15	<b>Opening Remarks</b>	
	<b>Konrad Reinhart</b> , Chair of the ISF, Jena, Germany	
13:15 – 15:00	<b>Session I The Global Burden of Sepsis</b>	
	<b>Chairs: John Marshall</b> , Toronto, Canada and <b>Konrad Reinhart</b> , Jena, Germany	
13:15 – 13:40	The burden of sepsis in the developed world <b>Derek Angus</b> , Pittsburgh, USA	S01
13:40 – 14:05	The burden of sepsis in the developing world <b>Nick White</b> , Bangkok, Thailand	S02
14:05 – 14:25	Sepsis in China: What is the difference between China and other countries <b>Du Bin</b> , Beijing, China	S03
14:25 – 14:45	Sepsis in Africa <b>Kath Maitland</b> , Kalifi, Kenya	S04
14:45 – 15:00	<i>Discussion: What can a Global Sepsis Alliance achieve?</i>	
15:00 – 15:30	<b>Break</b>	
15:30 – 16:50	<b>Session II How and Why Sepsis Kills</b>	
	<b>Chairs: Tom van der Poll</b> , Amsterdam, The Netherlands and <b>Derek Angus</b> , Pittsburgh, USA	
15:30 – 15:50	Genetic determinants in sepsis <b>Jean-Paul Mira</b> , Paris, France	S05
15:50 – 16:10	Quorum sensing and other cell signaling mechanisms between bacterial pathogen <b>Steven Opal</b> , Pawtucket, USA	S06
16:10 – 16:30	How bacteria evade innate immunity <b>Jos van Strijp</b> , Utrecht, The Netherlands	S07
16:30 – 16:50	Extracellular histones as mediators of death <b>Thierry Roger</b> , Lausanne, Switzerland	S08
16:50 – 17:50	<b>Session II, Keynote Lecture:</b> Introduced by <b>Jean-Paul Mira</b> , Paris, France Mechanisms of meningeal invasion during meningococemia <b>Xavier Nassif</b> , Paris, France	S09
18:00 – 19:30	<b>Welcome Reception and Posters</b>	



## Thursday 2 September

## Abstract no.

08:00 – 09:30	<b>Pro- or Anti-Inflammatory Treatment: Which way to go in your Patient with Severe Sepsis</b> <b>Chair: Jean-Paul Mira</b> , Paris, France <b>A bioMérieux Sponsored Session</b>	
08:00 – 08:30	Anti-inflammatory approaches to severe sepsis <b>Steven Opal</b> , Pawtucket, USA	S10
08:30 – 09:00	Immune stimulation in severe sepsis <b>Richard Hotchkiss</b> , St Louis, USA	S11
09:00 – 09:30	Individualized treatment for severe sepsis, are biomarkers ready for prime time? <b>John Marshall</b> , Toronto, Canada	S12
09:30 – 10:50	<b>Session III The Host Response</b> <b>Chairs: Jean-Paul Mira</b> , Paris, France and <b>Steven Opal</b> , Pawtucket, USA	
09:30 – 09:50	Early recognition and initial signalling <b>Tom van der Poll</b> , Amsterdam, The Netherlands	S13
09:50 – 10:00	The hormonal-bioenergetic-metabolic response <b>Mervyn Singer</b> , London, UK	S14
10:00 – 10:20	Dendritic cells <b>Jean-Daniel Chiche</b> , Paris, France	S15
10:20 – 10:40	The later phase – Immune paresis <b>Richard Hotchkiss</b> , St Louis, USA	S16
10:40 – 10:50	<i>Discussion</i>	
10:50 – 11:15	<b>Break</b>	
11:15 – 12:45	<b>Session IV How to Help the Host Fight Back</b> <b>Chairs: Simon Finfer</b> , Sydney, Australia and <b>Mervyn Singer</b> , London, UK	
11:15 – 11:35	Fluid resuscitation and cardiovascular support <b>Jean-Louis Vincent</b> , Brussels, Belgium	S17
11:35 – 11:55	Renal support and replacement in sepsis <b>Christophe Vinsonneau</b> , Paris, France	S18
11:55 – 12:15	The gut and nutrition in sepsis <b>John Marshall</b> , Toronto, Canada	S19
12:15 – 12:35	Respiratory support in ARDS and sepsis <b>Gordon Bernard</b> , Nashville, USA	S20
12:35 – 12:45	<i>Discussion: Why is the case fatality rate for severe sepsis decreasing?</i>	
12:45 – 14:15	<b>Lunch and Posters</b>	
14:15 – 15:30	<b>Session V Refining the Treatment of Sepsis</b> <b>Chairs: Jean-Louis Vincent</b> , Brussels, Belgium and <b>Gordon Bernard</b> , Nashville, USA	
14:15 – 14:35	Biomarker guided detection and treatment of sepsis <b>Konrad Reinhart</b> , Jena, Germany	S21
14:35 – 14:55	Targeting coagulation and endothelium in sepsis <b>Jean-François Dhainaut</b> , Paris, France	S22

14:55 – 15:15	Glucose control and other holistic treatments <i>Simon Finfer</i> , Sydney, Australia	S23
15:15 – 15:25	<i>Discussion:</i> Which are the most promising new treatments for sepsis?	
15:25 – 15:45	<b>Break</b>	
15:45 – 16:45	<b>Session VI      Sepsis 2010 Best Abstract Presentations</b>	
16:45 – 17:45	<b>Keynote Lecture:</b> Introduced by <i>Jean-Paul Mira</i> , Paris, France Apoptosis in sepsis- only signature of severity or therapeutic targets? <i>Richard Hotchkiss</i> , St Louis, USA	S24

**Friday 3 September**
**Abstract no.**

08:00 – 10:00	<b>Session VII      Clinical Trials in Sepsis: Lessons Learnt from the Past and Ongoing Trials</b>	
08:00 – 08:45	<b>Keynote Lecture:</b> Introduced by <i>Simon Finfer</i> , Sydney, Australia Pragmatic trials: a bold new paradigm for sepsis research <i>Stephen MacMahon</i> , Sydney, Australia	S25
08:45 – 09:15	<i>Roundtable:</i> Why do so many sepsis trials fail?	
09:15 – 10:00	<b>Session VIII      Part 1 – Update on Ongoing Clinical Trials</b> <b>Chairs:</b> <i>Mervyn Singer</i> , London, UK and <i>Derek Angus</i> , Pittsburgh, USA	
09:15 – 09:30	Use of oral lactoferrin as a preventive strategy for severe sepsis <i>Steven Opal</i> , Pawtucket, USA	S26
09:30 – 09:45	PROWESS SHOCK: Activated protein C in septic shock <i>Marco Ranieri</i> , Turin, Italy	S27
09:45 – 10:00	Activated protein C and corticosteroids <i>Djillali Annane</i> , Paris, France	S28
10:00 – 10:30	<b>Break</b>	
10:30 – 12:30	<b>Session VIII      Part 2 – Update on Ongoing Clinical Trials</b> <b>Chairs:</b> <i>Simon Finfer</i> , Sydney, Australia and <i>John Marshall</i> , Toronto, Canada	
10:30 – 10:45	ACCESS – Eritoran, a TLR4 antagonist in severe sepsis <i>Steven Opal</i> , Pawtucket, USA	S29
10:45 – 11:00	Early Goal Directed Therapy: ProCESS, ARISE and ProMISE <i>Derek Angus</i> , Pittsburgh, USA	S30
11:00 – 11:15	Fluid resuscitation in Australia and New Zealand- The CHEST trial <i>John Myburgh</i> , Sydney, Australia	S31
11:15 – 11:30	Lipopolysaccharide removal <i>John Marshall</i> , Toronto, Canada	S32
11:30 – 11:45	European revival of albumin in sepsis: EARSS and ALBIOS <i>Jean-Paul Mira</i> , Paris, France	S33
11:45 – 12:00	Fluid resuscitation in Africa <i>Kath Maitland</i> , Kalifi, Kenya	S34
12:00 – 12:15	InFACT – did we learn anything from H1N1? <i>John Marshall</i> , Toronto, Canada	S35
12:15 – 12:30	<b>Closing Remarks</b>	

# Speaker Abstracts

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## Session I: The Global Burden of Sepsis

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### S01

#### The burden of sepsis in the developed world

Derek Angus  
Pittsburgh, USA

In recent years, a number of large scale studies have estimated the incidence or prevalence of severe sepsis in many industrialized nations.

Most of the studies are focused on patients admitted to an ICU, and most report that 5-15% of ICU patients either have severe sepsis on admission or develop severe sepsis during admission. However, the number of ICU beds per population varies widely across countries, and thus the number of cases per population varies widely. Whether this wide variation represents true differences in severe sepsis or, rather, represents the fact that many countries provide care for severe sepsis at variable rates outside the ICU is unclear. Severe sepsis is defined as infection complicated by acute organ dysfunction, but many measures of acute organ dysfunction require provision of life support (e.g., acute respiratory failure is often defined as requirement for mechanical ventilation). Thus, the number of ventilators and number of staffed ICU beds will affect the number of cases that meet severe sepsis criteria. This interaction highlights both methodologic challenges when measuring the epidemiology of severe sepsis and potential public health challenges. Specifically, one wonders about the fate of patients who develop severe infections in countries with limited access to ICU beds. In my talk, I will discuss these findings and their implications. With these caveats in mind, I will also review the use of healthcare resources for severe sepsis and patient outcome.

### S02

#### The burden of sepsis in the developing world

Nick J White  
Bangkok, Thailand

Over 9.5 million people die each year because of infectious diseases – nearly all of whom live in developing countries. The majority of these preventable deaths are caused by bacterial infections, which occur in African or Asian children under 5 years of age. Accurate cause specific data are notoriously difficult to obtain, but neonatal sepsis, pneumonia and diarrhea are the main lethal diseases, and Enterobacteriaceae (neonatal sepsis), *Streptococcus pneumoniae*, *Staphylococcus aureus*, and non-typhoidal Salmonellae are the main pathogens. Pyogenic bacterial infections also contribute significantly to the mortalities attributable to malaria, HIV-AIDS, and measles. The introduction of vaccines for *Haemophilus influenzae B* and recently for *S.pneumoniae* (some serotypes) has reduced mortality from these infections, and oral rehydration solutions have provided dramatic benefit in watery diarrheas, but delays in receiving appropriate antibacterials account for the majority of preventable deaths.

Delays in recognizing the severity of illness, in referral to health facilities, and in provision of appropriate treatment all contribute to a fatal outcome. Antibiotics are freely available in developing countries, and resistance is widespread, but its contribution currently to mortality is uncertain, although it is likely to increase.

The lack of microbiology facilities in much of the tropics contributes to these many epidemiological uncertainties. Simple measures to ensure sick patients receive appropriate antibiotics as soon as possible would have a major impact on the mortality of bacterial sepsis.

### S03

#### Sepsis in China: what is the difference between China and other countries

Du Bin  
Beijing, China

Severe sepsis/septic shock remains the major cause of death among critically ill patients. The high incidence and mortality/morbidity have led to the development of the international sepsis guideline, first published in 2004 and revised in 2008. However, limited data exist with regards to the epidemiology and management of sepsis in mainland China.

The only published epidemiology of severe sepsis showed that, in 10 surgical ICUs in China, severe sepsis accounted for 8.7% (318/3665), with 28-day mortality of 44.7%. A recent observational study in 1297 patients who stayed in any of 22 ICUs in China found that, severe sepsis/septic shock was diagnosed in 484 patients (37.3%), with hospital mortality of 33.5%. Severe sepsis/septic shock was an independent predictor of mortality (OR 1.430, 95%CI 1.011 – 2.023,  $p = 0.043$ ).

Significantly regional variation in the management of severe sepsis/septic shock was reported worldwide. A survey was conducted in 2007 among 196 Chinese intensivists to describe self-reported practice in the management of early septic shock, and to compare the response with those of Canadian intensivists reported in an earlier study. Compared with Canadians, Chinese intensivists reported to use components of sepsis resuscitation bundle more frequently, including CVP (87.2% vs. 78.7%,  $p = 0.0240$ ), and both ScvO<sub>2</sub> (33.7% vs. 19.4%,  $p = 0.0012$ ) and SvO<sub>2</sub> (23.0% vs. 14.9%,  $p = 0.0374$ ). In addition, Chinese intensivists reported to use colloids more commonly than Canadian intensivists.

In a cross-sectional study in 391 ICUs across 25 countries to describe the practice and influencing factors of fluid challenge, the authors observed that, compared to Canada, odds ratios for the prescription of crystalloid, colloid, and blood products in China were 0.46 (95%CI 0.30-0.69), 1.72 (1.20-2.47) and 3.33 (2.02-5.50), respectively. A multinational descriptive study involving 1285 patients with severe sepsis/septic shock in 150 ICUs in 16 Asian countries was performed to assess the compliance to sepsis bundles and its association with hospital mortality. In the middle-income countries among which China is one of them, the compliance to sepsis bundle was only 6.9% (vs. 10.0% in high-income countries), corresponding to a significant higher mortality rate (50.6% vs. 38.7%).

All the above data showed that, severe sepsis/septic shock is very common among critically ill patients in China, with a relatively poor outcome, which might be improved in the future.

### S04

#### Sepsis in Africa

Kath Maitland  
Kalifi, Kenya

The cornerstones of the Surviving Sepsis Campaign are early identification of sepsis and prompt implementation of evidence-based treatment strategies. These present important yet obtainable goals for resource-rich countries. In malaria-endemic African in the current context of acute and emergency paediatric care they remain only a notional possibility.

Several factors including limited pre referral emergency care, low priority given to triage, poor evidence base for current guidelines and poor diagnostic facilities contribute to the current status. Even the most simple emergency interventions such as fluid resuscitation, thresholds for hypoglycaemia correction, transfusion thresholds and who and what antimicrobials to administer have not been subjected to rigorous evaluation. The term 'sepsis', which broadly implies a sepsis-like syndrome, is not even mentioned in the paediatric hospital management guidelines. Instead,

probably for historical, programmatic reasons, management treatment strategies are largely syndromic so treatment of suspected bacterial infection is covered separately in vertical and non-overlapping management guidelines. Practically, this presents challenges for bedside management as many children present with clinical features consistent with multiple syndromes.

Since between a quarter and a half of children present to hospital in their final illness the poor evidence base and limited operational use of triage and emergency care results in a high 'hidden' morbidity and mortality. This therefore contributes importantly to overall under-five mortality. If not adequately addressed, the poor standard of emergency care may be an obstacle to achievement of the Millennium Development Goal No.4 on child survival in Africa.

Whether given as single or as a complex intervention there is an urgent need for scientific evaluation and advocacy to ensure that both the evidence base is more robust for these simple interventions and that implementation research drives future practice in order to improve child survival.

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## Session II: How and Why Sepsis Kills

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### S05

#### Genetic determinants in sepsis

Jean-Paul Mira  
Paris, France

Severe sepsis continues to be a major and increasing health care burden worldwide. Despite aggressive organ support and optimal microbial therapy, sepsis remains the commonest cause of death in ICU with an overall mortality rate of 25-40%. The relative failure of conventional therapeutic strategies has stimulated a major interest in the development of new research axes, such as identification of genetic factors that influence sepsis susceptibility, therapeutic response, or outcome.

Genetically-determined differences in host immune responses against pathogens might explain why some people get sick and die when they encounter a pathogen whereas others stay perfectly healthy. Explosion of knowledge both in human genomics and in host inflammatory response explains the increasing interest in immunogenetics over the last years. Indeed, twin and adoptee studies have suggested more than 20 years ago, that host genetic factors are major determinants of susceptibility to infectious diseases in humans. Recently, candidate gene studies and human genome wide analysis have been used to identify infectious diseases susceptibility and resistance genes. Rarely, a single gene defect has been directly related to devastating consequences such as interferon-gamma receptor mutations leading to fatal infections with ubiquitous mycobacteria. For clinical practice, gene polymorphisms of specific immunological or physiological mediators appear to be of major importance. These genetic variants, which modify the regulation or function of either Pathogen Recognition Receptors or inflammatory mediators, have been associated with susceptibility and/or outcome of severe sepsis and septic shock. All steps of the host response to bacteria clearance have been shown to be potentially affected by genetic factors. However genetic studies in sepsis have produced contradictory results related in part to methodological faults. Improved adherence to published guidelines of good study design will help to ensure that genetic epidemiology contributes to a better classification of the heterogeneous septic population.

The impact of these findings on the understanding of infectious disease pathogenesis and on the design of future preventive and therapeutic strategies should also be considerable.

### S06

#### Quorum sensing and other cell signaling mechanisms between bacterial pathogens

Steven Opal  
Pawtucket, USA

Bacteria has long been known to have the capacity to sense environmental clues such as substrate availability and recognition of concentration gradients to direct motility. What has recently become apparent is the existence of extensive communication networks between bacteria of the same species and even signals between bacteria and eukaryotes,

including human tissues. These communication networks are generally referred to as quorum sensing and often function by a sudden alteration in the transcriptional programs of bacteria after a threshold of chemical mediators produced by other bacteria is reached within the micro environment of bacterial cells. There are at least four recognized signaling systems in bacterial pathogens that infect humans and numerous variations on these basic pathways are now described. An emerging field of sociomicrobiology or chemical microbial ecology has developed in an effort to understand bacterial communication. The potential exists to manipulate these pathways as a therapeutic option in invasive bacterial infections or infections of prosthetic devices. A number of quorum sensing inhibitors are in preclinical or clinical studies with the ultimate goal of using small molecule inhibitors of bacterial communication to disrupt bacterial pathogenesis. Other forms of bacterial communication probably exist including the use of electrical field currents and vibratory waves to alter behavior of bacterial populations. Whether these alternative communication systems are amenable to therapeutic manipulation remains to be demonstrated in further preclinical and clinical research.

### S07

#### How bacteria evade innate immunity

Jos van Strijp  
Utrecht, The Netherlands

### S08

#### Extracellular histones as mediators of death

Thierry Roger  
Lausanne, Switzerland

Covalent modifications of histones through acetylation of  $\epsilon$ -amino group of lysine strongly influence the structure and the function of the chromatin. Acetylation of histones loosens the chromatin structure promoting active transcription, whereas de-acetylation of histones compacts the chromatin structure preventing gene transcription. The net state of histone acetylation is regulated by the opposing actions of histone acetyltransferases and histone deacetylases (HDACs). Beside histones, non histones proteins are also modified by reversible acetylation, among which  $\alpha$ -tubulin, HSP90 and transcription regulators. An impressive number of publications arising within the last two decades have demonstrated that HDAC activity have a major impact on the development of age-associated diseases including cancer, diabetes and cardiovascular and neurodegenerative diseases. HDAC inhibitors (HDIs) were originally developed for their powerful anti-cancer activity (1, 2). Yet, recent preclinical studies suggest that HDIs possess anti-inflammatory activity (3). Based on these observations, we postulated that HDIs could impact on innate immune response to microbial infection.

Here we will discuss the results from our studies on the effect of HDIs on the innate immune system. We first performed genome-wide gene expression analyses to have a global view of the impact of HDIs on the transcriptome of resting and microbial product-stimulated primary macrophages. We then studied the influence of HDIs on key parameters (activation of the intracellular signal transduction pathways, production of cytokines and chemokines, expression of co-stimulatory molecules and chemokine receptors) of macrophages, dendritic cells and whole blood activated by a broad range of microbial products. Finally, we analyzed the impact of HDIs in preclinical models of non-severe bacterial and fungal infections, toxic shock and septic shock. Overall, these studies demonstrate that HDIs are powerful anti-inflammatory drugs that impair innate immune responses to microbial infections *in vitro* and *in vivo*. While the results suggest that HDIs may represent attractive adjunctive therapies to treat pathological situations characterized by dysregulated inflammatory responses such as severe sepsis, they also warn that HDIs may increase the risk of developing opportunistic infections and sepsis, especially in immunocompromised cancer patients.

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## Session II: How and Why Sepsis Kills

### Keynote Lecture

S09

#### Mechanisms of meningeal invasion during meningococemia

Mathieu Coureui, Xavier Nassif

Paris, France

*Neisseria meningitidis* is a commensal of the human nasopharynx that in some circumstances can invade the bloodstream, cross the blood brain barrier and invade the meninges. The bacteria enter the central nervous system following a direct interaction with the luminal side of the cerebral endothelium, which constitutes the blood–brain barrier. To breach the barriers protecting the brain, *N.meningitidis* must cross a monolayer of tight junction-expressing endothelial or epithelial cells. The limited number of pathogens capable of crossing these tight barriers and invading the meninges suggests that they display very specific attributes. For *N.meningitidis*, type IV pili have been identified as being essential for meningeal invasion by inducing the formation of microvilli like structures on the apical surface of the endothelial cells. This lecture will focus on the molecular basis of the interaction of *N.meningitidis* with human brain endothelial cells and the mechanisms by which this interaction leads to the opening of the blood brain barrier.

### bioMérieux Sponsored Session

#### Pro- or Anti-inflammatory Treatment - Which way to go in your Patients with Severe Sepsis

S10

#### Anti-inflammatory approaches to the treatment of sepsis

Steven Opal

Pawtucket, USA

The early events that follow the invasion of the patient by microbial pathogens are characterized by localized activation of the innate immune response. If these local defenses are breached, generalized activation of the innate and adaptive immune response follows. This initial host response is often accompanied by classic signs of inflammation followed by hemodynamic sequelae, activation of the coagulation system, tissue hypoperfusion, multi-organ failure and death. Interventions designed to stop this process in the early phases of excessive inflammation that has formed the basis of numerous immunomodulatory strategies tested over the last three decades.

The therapeutic benefits of anti-inflammatory agents have been extensively documented in animal models of sepsis but have largely failed to show efficacy in clinical trials of human sepsis. While there is a number of potential explanations for the failure of translational research to bring these agents into clinical practice, one of the fundamental issues remain the difficulty in verifying the inflammation hypothesis in a large number of patients enrolled in sepsis trials. It has become increasingly clear that many patients have only transient hyper-inflammation followed by prolonged periods of relative immune refractoriness. In some patients there may be no excessive inflammatory state at all and the response to systemic infection is predominantly immunosuppression.

If anti-inflammatory strategies are to be successful, identification of patients with various stages of immunosuppression and avoid the use of anti-inflammatory agents in such patients. The state of immune activation or suppression cannot be distinguished by SIRS criteria alone. A reliable set of immunologic biomarkers need to be identified and utilized to inform the clinical investigator of the state of the patient's immune response before intervening with experimental anti-inflammatory agents. The failure to develop such assays will impede further progress in this field of sepsis research.

S11

#### Immune stimulation in severe sepsis

Richard Hotchkiss

St Louis, USA

Sepsis is a highly lethal disorder that is responsible for over 210,000 deaths annually in the U.S alone. Developing new therapies for sepsis has been particularly frustrating and over thirty trials of new agents have failed. This failure has been due in part to a lack of understanding of the critical pathogenic mechanisms driving sepsis. Sepsis initiates a complex immunologic response that varies over time. Although recent studies show that both a hyper-inflammatory and an anti-inflammatory response are triggered nearly simultaneously in sepsis, the early net result is characterized by a hyper-inflammatory response of varying magnitude due to many factors including the number and virulence of the pathogens and host co-morbidities. The majority of patients survive the hyper-inflammatory phase and enter a stage of protracted immunosuppression which has been termed "immunoparalysis". This immunosuppression in sepsis is manifest by loss of delayed type hypersensitivity response to positive control antigens, failure to clear the primary infection, and development of new secondary infections. The clinical relevance of this immunosuppressed state is evidenced by the frequent occurrence of infection by relatively avirulent and often multidrug-resistant bacterial, viral, and fungal pathogens such as *Stenotrophomonas*, *Acinetobacter*, *Candida* and *Pseudomonas* species, enterococci, and cytomegalovirus.

Sepsis can be considered as a race to the death between the pathogens and the host immune response. Pathogens seek an advantage by incapacitating various aspects of host defenses. For example, they induce the apoptotic depletion of immune effector cells, suppress the expression of MHC Class-2 molecules, increase expression of negative co-stimulatory molecules, increase anti-inflammatory cytokines, and augment levels of T-regulatory and myeloid-derived suppressor cells. Sepsis-induced apoptosis is particularly noteworthy because there is profound depletion of cells of both the innate and adaptive immune system.

Furthermore, uptake of apoptotic cells further impairs host immunity by inducing an anti-inflammatory phenotype in phagocytic cells which consume the cellular corpses. Prevention of this sepsis-induced apoptosis apparently attenuates the immunosuppressive cascade and leads to sustained immunity.

Perhaps the most important implication of this evolving concept of immunosuppression as a major pathogenic mechanism in sepsis relates to potential new therapies. The fundamental problem in these patients is loss of immune competence; eradicating a particular class of pathogens will likely result in super-infection with other microorganisms. In addition to developing clinical practices to avoid infections, attention should be directed at methods to enhance or restore immune function in critically ill patients. Although there is risk of exacerbating the early hyper-inflammatory phase of sepsis, methods to quantify the immune competence of each patient and appropriately time immunotherapy should minimize this danger. A number of potential new immunotherapies will be discussed.

S12

#### Individualized treatment for severe sepsis, are biomarkers ready for prime time

John Marshall

Toronto, USA

A biomarker is an objective biological parameter that facilitates patient stratification on the basis of diagnosis or prognosis, and permits an evaluation of response to therapy. Although the word carries a connotation of the arcane, exotic, or exceedingly costly, the reality is that the practice of intensive care in general, and the management of patients with sepsis in particular, is highly dependent on the use of biomarkers.

Early resuscitation of the septic patient, for example, is guided by both physiologic parameters and biomarkers suggestive of tissue ischemia – the ScVO<sub>2</sub> and serum lactate levels. Both serve not only to diagnose otherwise occult tissue hypoxia, but to enable the clinician to titrate therapy by monitoring the patient's response to intervention. A decision to transfuse is informed by the hemoglobin level, and the selection of appropriate antimicrobial agents is guided by the results of culture and sensitivity.

The unmet need for, and evolving science of biomarkers for sepsis lies in three broad domains. The first of these is the development of tools that

can establish a diagnosis more rapidly than is possibly using contemporary technology. Since effective early antibiotic therapy is an important determinant of outcome in sepsis, tools such as PCR-based assays for microbial DNA may permit earlier identification and management of the specific infection responsible for sepsis in the individual patient. A second need is for tools demonstrating greater sensitivity to an abnormal state – identifying regional tissue hypoxia, for example, before it can be detected on the basis of increased lactate release.

The most compelling need, however, is for biomarkers that can identify patients who are likely to experience differential responses to treatment. For example, treatments targeting endotoxin are most likely to be efficacious in patients who are endotoxemic, while the replacement of activated protein C is most likely to be of most use in those patients who are protein C deficient, or who lack the capacity to convert protein C to its activated form. It is implausible that these patient populations are identical, nor that they will be readily identified on the basis of physiologic criteria of sepsis syndrome or SIRS alone. Moreover just as insulin therapy is titrated to the blood glucose level, so both the dose and duration of mediator-directed therapy will be optimized by titration to levels of valid biomarkers.

Identifying and validating biomarkers that can inform therapeutic decisions is difficult, for it is predicated on the availability of an effective treatment. An evolving body of data indicate that procalcitonin can be used to guide the duration of antibiotic therapy. Studies on the use of protein C or endotoxin levels to guide therapies targeting protein C or endotoxin are in progress. Rapid assay of genetic polymorphisms may enable identification of patients most likely to benefit from treatment with activated protein C. Yet while the need is great and the promise compelling, the reality of 2010 is that biomarkers have yet to have a proven role in the adjuvant therapy of sepsis.

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### **Session III: The Host Response**

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#### **S13**

##### **Early recognition and initial signaling**

Tom van der Poll

*Amsterdam, The Netherlands*

The innate immune system is able to sense the presence of potential pathogens by the expression of a limited number of pattern-recognition receptors (PRRs). PRRs recognize highly conserved, soluble motifs derived from essential structures expressed by pathogens but absent in higher eukaryotes. These microbial elements are collectively referred to as pathogen-associated molecular patterns (PAMPs). Recent evidence suggests that PRRs also serve as early warning systems or danger signals as they have the capacity to recognize endogenous mediators released during tissue damage, independent of actual invasive infection. These endogenous danger signals are released in burns, trauma and tissue necrosis and have been termed “alarmins” or DAMPs (danger-associated molecular patterns).

Toll like receptors (TLRs) are critically important PRRs in the initiation of the innate immune response to bacteria. During an evolving infection distinct TLRs recognize different PAMPs expressed by pathogens, whereby the relative contribution of different TLRs at least in part depends on the bacterial burden. In addition, the recognition of bacteria by TLRs may be hampered by thick capsules, which may shield TLR ligands expressed by pathogens. Moreover, during progressed sepsis TLRs may amplify inflammatory responses by the interaction with DAMPs released after tissue injury. As such, the role of TLRs in sepsis is complex, contributing to responses that can be either beneficial or detrimental to the host.

This lecture will present recent experimental data on the function and cooperation of distinct TLRs during severe bacterial infection *in vivo*. The lecture will focus on clinically relevant pathogens (*Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Escherichia coli*) and the two most common infections causing sepsis (pneumonia and peritonitis).

#### **S14**

##### **The hormonal-bioenergetic-metabolic response**

Mervyn Singer

*London, UK*

The inflammatory septic insult triggers a phasic response in downstream pathways. The initial acute phase response requires the organism to ‘fight the foe’. It does so through neural, hormonal and cytokine signalling that (i) mobilises substrate and increases energy production to cope with the increase in metabolic demands (including a thermal response), (ii) diverts resources away from organs less vital for self-defence, and (iii) facilitates entry of immune cells to the infected area. In many patients, recovery quickly ensues and normal activity recommences. However, a proportion of patients fail to quench the inflammatory fires and progress to suffer organ dysfunction and failure. This occurs as a consequence of impaired tissue perfusion, a failure to generate sufficient energy (bioenergetic failure) and, possibly, a programed metabolic shutdown analogous to hibernation/estivation. Multi-organ failure thus appears to be driven by a combination of direct mitochondrial inhibition/damage/oxygen lack, by alterations in hormonal profile that depress mitochondrial and metabolic activity, and by a decrease in expression of genes encoding for mitochondrial proteins. Recovery from organ failure is thus dependent on a reversal of these processes. To what extent we can therapeutically modulate these pathways represents one of the major challenges of the next 10-20 years.

#### **S15**

##### **Dendritic cells**

Jean-Daniel Chiche

*Paris, France*

#### **S16**

##### **The later phase-immune paresis**

Richard Hotchkiss

*St Louis, USA*

Sepsis is a highly lethal disorder that is responsible for over 210,000 deaths annually in the U.S alone. Developing new therapies for sepsis has been particularly frustrating and over thirty trials of new agents have failed. This failure has been due in part to a lack of understanding of the critical pathogenic mechanisms driving sepsis. Sepsis initiates a complex immunologic response that varies over time. Although recent studies show that both a hyper-inflammatory and an anti-inflammatory response are triggered nearly simultaneously in sepsis, the early net result is characterized by a hyper-inflammatory response of varying magnitude due to many factors including the number and virulence of the pathogens and host co-morbidities. The majority of patients survive the hyper-inflammatory phase and enter a stage of protracted immunosuppression which has been termed “immunoparalysis”. This immunosuppression in sepsis is manifest by loss of delayed type hypersensitivity response to positive control antigens, failure to clear the primary infection, and development of new secondary infections. The clinical relevance of this immunosuppressed state is evidenced by the frequent occurrence of infection by relatively avirulent and often multidrug-resistant bacterial, viral, and fungal pathogens such as *Stenotrophomonas*, *Acinetobacter*, *Candida* and *Pseudomonas* species, enterococci, and cytomegalovirus.

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## Session IV: How to Help the Host Fight Back

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### S17

#### Fluid resuscitation and cardiovascular support

Jean-Louis Vincent  
Brussels, Belgium

### S18

#### Renal support and replacement in sepsis

Christophe Vinsonneau  
Paris, France

### S19

#### The gut and nutrition in sepsis

John Marshall  
Toronto, Canada

Despite its relatively simple anatomic architecture and its humble role in the basic physiologic functions of eating and elimination, the gastrointestinal tract is a highly complex organ that exerts a fundamentally important role in metabolic and immunologic homeostasis. Derangements in gut function are common in critical illness, and play an important, though largely occult role in the characteristic pathologic derangements of sepsis.

The normal human GI tract houses an extraordinarily diverse microflora, comprising 1000 or more distinct microbial species, and outnumbering the cells of its human host by a factor of 10 to 1. This flora plays a key role in the development of the gut microvascular architecture, in the expression of key digestive enzymes, and in the development and maturation of the systemic immune response. The complexity of this role is underlined by the fact that interactions between the endogenous gut flora and the host enable the host to mount a rapid and effective response to invasive microorganisms, while maintaining a state of mutual tolerance at the microbial-mucosal interface in the gut wall. Cell wall constituents of gut luminal bacteria, including LPS and peptidoglycan, can be absorbed through the gut wall, and modulate systemic immunity. It has recently been shown, for example, that normal neutrophil killing activity requires induction of Nod1 by peptidoglycan from bacteria in the gut lumen.

Critical illness is associated with a reduction in the diversity of the gut microbiota (particularly the anaerobic flora), and overgrowth of the upper gut by opportunistic species such as *S. epidermidis*, the *Enterococcus*, *Pseudomonas*, and *Candida* – the same species that predominate in ICU-acquired infections. Moreover stress in the host induces enhanced virulence of organisms such as *Pseudomonas*, potentially transforming this bacterium from a saprophyte into a pathogen. Critical illness is also associated with increased rates of translocation of viable organisms and bacterial cell wall products across the gut wall. Multiple factors contribute to gut dyshomeostasis in the critically ill including ileus resulting from bed rest

and narcotics, reduced splanchnic blood flow, ablation of gastric acidity, the disruptive effects of systemic antibiotics, and a lack of enteral feeding.

Luminal nutrition serves both local and systemic roles. Locally enteral feeding stimulates gut peristalsis and provides nutrients to maintain the integrity of the gut wall. On a systemic level, and in part because of its local effects on the gut, enteral nutrition reduces infectious risk and attenuates inflammatory responses. Systematic reviews indicate the superiority of the enteral route of feeding, and suggest additional benefit for the use of feeding formulations enriched in antioxidants. This latter hypothesis is the focus of ongoing large clinical trials.

### S20

#### Respiratory support in ARDS and sepsis

Gordon Bernard  
Nashville, USA

The use of positive end-expiratory pressure (PEEP) in the treatment of the acute respiratory distress syndrome (ARDS) was introduced by Ashbaugh and Petty more than 40 years ago. Since then a massive literature has accumulated addressing various methods of PEEP titration, along with other methods of modulation of airway pressure during mechanical ventilation, yet substantial controversy remains. Few practitioners question the effect PEEP has on oxygenation and oxygen delivery in most ARDS patients, but how much to use and the optimal means of titration are far from established.

The size of the compulsory tidal volume provided to critically ill patients requiring mechanical ventilation was recommended to be in the range of 10-15 ml/kg of body weight in the early days of the development of intensive care units and substantial evidence to reduce it as a routine practice was not available until 30 years later.

Various modes of ventilatory support and weaning from mechanical ventilation was approached through a number of disparate methods and some components of current techniques remain controversial and under further study. Both due to these improvements and in spite of the lack of evidenced-based improvements in some areas, survival from sepsis induced ALI has improved substantially over the past quarter century. Evidence to support the most common approaches to mechanical ventilation in sepsis-induced ALI will be presented along with some data-supported ancillary techniques thought to be important in the outcome of this population.

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## Session V: Refining the Treatment of Sepsis

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### S21

#### Biomarker guided detection and treatment of sepsis

Konrad Reinhart  
Jena, Germany

Sepsis is difficult to diagnose and often treated too late which is related to an increase in mortality rate. On the other hand, time of anti-microbial treatment is often too long which may result in an increase of adverse effects and contribute to antimicrobial resistance. At the bedside, a sepsis marker is only useful if it adds value to the physician's clinical judgment. Ideally, an infection/sepsis marker should meet the following demands: to shorten the time to and improve the diagnosis, to facilitate the differentiation between infectious and non-infectious causes of inflammation and its sequelae organ dysfunction or shock, to allow the differentiation between viral and bacterial infections, to represent the effectiveness of antimicrobial treatment and other measures of source control more accurately than conventional clinical and laboratory signs.

In the meanwhile it could be demonstrated in a number of well-designed RCTS that the use of PCT in anti-biotic stewardship results in a reduction of antibiotic use by a range of 45 – 50% without interfering with patient outcome. A proof of concept study demonstrated in severe sepsis patients that this might also be possible for these patients. A large RCT with 1,000 patients by the SepNet clinical trials group is ongoing in Germany to test this hypothesis. However, PCT has some limitations as infection and sepsis marker. It may increase also for other reasons such as trauma, burns, major surgery, birth stress; some forms of cancer and by

treatment of patients with some immunosuppressive drugs such as OKT3 and antilymphocyte sera.

Pangenomic as well as targeted microarrays allow monitoring effects of pathogen associated molecular patterns on the host-cell transcriptome. Thus, transcriptomic signatures potentially can differentiate infectious from non-infectious host response thereby differentiating SIRS from sepsis. There is first data that suggest the RNA derived molecular fingerprints may overcome some of the limitations of PCT as a sepsis marker. However, this needs to be tested in further clinical trials.

## S22

### Targeting coagulation and endothelium in sepsis

Jean-François Dhainaut

Paris, France

## S23

### Glucose control and other holistic treatments

Simon Finfer

Sydney, Australia

In acutely ill patients, including those treated in intensive care units, the occurrence and degree of hyperglycaemia are associated with increased morbidity and mortality in a variety of patient groups. As a result, Van den Berghe and colleagues studied the impact of intensive insulin therapy designed to maintain the normoglycaemia, on the outcome of critically ill patients. In their first study published in 2001, patients in a surgical intensive care unit treated with intensive insulin therapy had a reduced mortality rate and the authors stated that the greatest reduction mortality "involved deaths due to multiple-organ failure with a septic focus".[1] These data had great face validity as patients with diabetes mellitus are known to be at increased risk of infection and commonly in use to treatments such as catecholamine infusions and corticosteroids may further impair glucose control in patients with severe sepsis. Subsequent to the landmark study in 2001, Van den Berghe and colleagues and other investigators have reported additional trials studying the impact of intensive insulin therapy in a number of populations of critically ill patients. In the VISEP study, Brunkhorst and colleagues investigated the impact of intensive insulin therapy in patients with severe sepsis admitted to 18 intensive care units in Germany.[2] they reported no improvement in outcome in patients treated with intensive insulin therapy. In 2006, Van den Berghe and colleagues reported the combined results of their trials in patients treated in the surgical and medical ICUs in Leuven,[3] in that paper they reported a post-hoc analysis analysing the impact of intensive insulin therapy on the outcome of 950 patients classified in as having severe sepsis, 172 of 471 (33.4%) patients treated with conventional insulin therapy died in hospital compared with 160 of 479 (33.4%) patients treated with intensive insulin therapy (odds ratio 0.87; 95% CI 0.67 – 1.14). In the NICE-SUGAR Trial which randomly assigned 6104 patients treated in ICUs in Australia, New Zealand, Canada or the USA to an intensive blood glucose control target of 4.5 – 6.0 mmol/L or a conventional target of less than 10.0 mmol/L,[4] 1299 patients were classified as having a severe sepsis at baseline. In these patients the effect of intensive glucose control was almost exactly the same as in patients who did not have severe sepsis, odds ratio for death 1.13 (0.89 - 1.44) compared to 1.15 (1.01 - 1.31), P value for heterogeneity 0.93. Most recently Annane and colleagues reported that intensive insulin therapy did not improve the outcome of patients with severe sepsis being treated with corticosteroids.[5]

Most commentators and organisations such as the American Diabetes Association do not now recommend intensive glucose control for adult patients treated in intensive care units.[6] Despite the appealing theoretic rationale, there are no robust data to suggest that patients with severe sepsis respond differently to this treatment than do other critically ill patients. In patients with sepsis, as in critically ill patients in general, the conflict between the results of the studies conducted in Leuven and elsewhere leaves many questions to be answered.

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## Session VI

### Keynote Lecture

## S24

### Apoptosis in sepsis- only signature of severity or therapeutic targets?

Richard Hotchkiss

St Louis, USA

Sepsis is a highly lethal disorder that is responsible for over 210,000 deaths annually in the US alone. Developing new therapies for sepsis has been particularly frustrating and over thirty trials of new agents have failed. This failure has been due in part to a lack of understanding of the critical pathogenic mechanisms driving sepsis. Sepsis initiates a complex immunologic response that varies over time. Although recent studies show that both a hyper-inflammatory and an anti-inflammatory response are triggered nearly simultaneously in sepsis, the early net result is characterized by a hyper-inflammatory response of varying magnitude due to many factors including the number and virulence of the pathogens and host co-morbidities. The majority of patients survive the hyper-inflammatory phase and enter a stage of protracted immunosuppression which has been termed "immunoparalysis". This immunosuppression in sepsis is manifest by loss of delayed type hypersensitivity response to positive control antigens, failure to clear the primary infection, and development of new secondary infections. The clinical relevance of this immunosuppressed state is evidenced by the frequent occurrence of infection by relatively avirulent and often multidrug-resistant bacterial, viral, and fungal pathogens such as *Stenotrophomonas*, *Acinetobacter*, *Candida* and *Pseudomonas* species, enterococci, and cytomegalovirus.

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ill patients. Although there is risk of exacerbating the early hyper-inflammatory phase of sepsis, methods to quantify the immune competence of each patient and appropriately time immunotherapy should minimize this danger. A number of potential new immunotherapies will be discussed.

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## Session VII: Clinical Trials in Sepsis- Lessons from the Past and Ongoing Trials

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### Keynote Lecture

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#### S25

##### **Pragmatic trials: a bold new paradigm for sepsis research**

Stephen MacMahon  
Sydney, Australia

Over the past few decades, the treatment of several acute vascular conditions including myocardial infarction and ischaemic stroke has been revolutionised and case-fatality greatly reduced, primarily as a consequence of findings from "mega-trials". For example, it was mega-trials (and their meta-analyses) that proved the value of thrombolysis for both acute myocardial infarction and ischaemic stroke. It was also mega-trials that proved the value of aspirin and clopidogrel in acute coronary syndromes. Typically, these trials included tens of thousands of patients among whom a thousand or more primary outcome events were observed. Such trials were able to detect improvements in mortality as small as 10-15% with a single treatment, and on that basis packages of care have been developed that have collectively more than halved case-fatality rates.

The practical success of such mega-trials is importantly dependent upon simplicity. To recruit such large numbers and ensure full follow-up with realistic financial resources requires broad entry criteria, minimal data collection and straightforward outcomes (such as total mortality). However, this pragmatic approach is often at odds with prevailing clinical views that favour seeking much larger – though probably implausible – treatment benefits in trials with much smaller numbers of patients. In numerous areas of medicine, this has resulted in treatments, now known to be essential, going unrecognised for much longer than was necessary, with many lives lost that would otherwise have been saved. Indeed, it is inevitable that many treatments believed today to be ineffective are actually worthwhile but have been studied in trials that were too small to detect moderate but worthwhile benefits.

Sepsis is an enormous global health problem for which effective treatments are desperately needed. The lack of proven therapies may represent unique challenges posed by the condition. However, it may also represent inadequate evaluation of promising treatments in trials that were not large enough to reliably detect the real benefits. It is therefore a priority for the sepsis research community to build a global network in which mega-trials of promising approaches to sepsis care can be tested robustly. While the effort and resource required is substantial, the need is far greater.

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## Session VIII: Update on Ongoing Clinical Trials

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#### S26

##### **Use of oral lactoferrin as a preventive and therapeutic strategy for severe sepsis**

Steven Opal  
Pawtucket, USA

Lactoferrin is an antimicrobial protein that is found in high concentrations within the specific granules of neutrophils and in mammalian breast milk. Lactoferrin has numerous salutary effects in controlling bacterial populations including: potent LPS neutralizing ability through its specific LPS binding domain, iron sequestration, direct antibacterial effects by permeabilizing cell membranes, promoting bactericidal activity of phagocytic cells, neutrophils in the clearance of both gram-negative and gram-positive bacteria, activation of dendritic cells and antigen presenting cells, and

promotion of the activity of gut-associated lymphatic tissue. It has long been considered a potential preventive treatment against invasive bacterial pathogens. Recent clinical studies now support the potential therapeutic utility of lactoferrin as a preventative and strategy in therapeutic human sepsis. A multicenter clinical trial with bovine-derived lactoferrin has been tested against placebo in a large multicenter trial for the prevention of neonatal sepsis (n=472). Oral administration of lactoferrin in high risk neonates statistically significantly reduced the incidence of late onset sepsis, reduced mortality, prevented necrotizing enterocolitis and was particularly useful in the smallest premature babies weighing less than 750 grams. A recombinant human form of lactoferrin (talactoferrin) has also been tested in a multicenter phase II trial in adult sepsis. The oral administration of talactoferrin in this study was shown to statistically significantly reduce the overall 28-day mortality rate in high risk patients in this 190 patient clinical trial from 26% (placebo) to 14.6% (treatment). Phase III testing of this, non-absorbed, human anti-bacterial protein is planned in the near future.

#### S27

##### **Prowess shock: activated protein C in septic shock**

Marco Ranieri  
Turin, Italy

#### S28

##### **Activated protein C and corticosteroids**

Djillali Annane  
Paris, France

#### S29

##### **ACCESS- Eritoran, a TLR4 antagonist in severe sepsis**

Steven Opal  
Pawtucket, USA

Eritoran (E5564) is a synthetic analogue of the lipid A form of LPS from a non-toxic gram-negative bacterium known as *Rhodobacter sphaeroides*. Eritoran is a potent inhibitor of MD2-TLR4 signaling and blocks LPS mediated pathologic events in a variety of preclinical models of endotoxin-induced shock. Eritoran is a competitive inhibitor for lipid A binding within the hydrophobic pocket of MD2. If Eritoran occupies this MD2 pocket, lipid A from pathogens cannot bind to the MD2-TLR4 signaling complex and this effectively terminates LPS signaling in the host tissues. Eritoran itself lacks LPS agonistic activity as the molecule is tetra-acylated rather than hexa-acylated and lacks a critical myristic acid at the R2 position necessary to activate the ecto-domain of TLR4. Eritoran has been extensively studied in phase I and phase II clinical testing and is the first toll-like receptor inhibitor that has advanced to phase III clinical testing in a multinational sepsis trial. This 2000-patient trial should be completed in September of 2010 and results of the study should be available within the first quarter of 2011.

#### S30

##### **Early goal directed therapy: ProCESS, ARISE and ProMISE**

Derek Angus  
Pittsburgh, USA

Ten years ago, Rivers et al published a highly interesting single center study suggesting that an algorithm to protocolize resuscitation, with decisions guided by central venous pressure and oxygen saturation, could significantly improve mortality from septic shock. The algorithm, dubbed 'Early Goal Directed Therapy' (EGDT) drove decisions for the first six hours of care, initiated in the Emergency Department. EGDT included a number of traditional steps relating to use of fluids and vasopressors. In addition, it also involved use of a proprietary central venous catheter which allowed continuous measurement of central venous oxygen saturation, and the oxygenation parameters drove instructions for blood transfusion and use of inotropes, steps that are a little less traditional for septic shock. In the wake of the trial, many centers around the world have attempted to adopt EGDT, often reporting success in before-and-after study designs. However,

there were no confirmatory randomized trials. In response, three large government-funded randomized trials have been initiated. In Australia and New Zealand, EGDT is being tested against usual care for septic shock in 1300 patients (650 per arm) in a trial known as ARISE. In the US, EGDT is being tested in a three-arm trial (ProCESS) against usual care and a protocolized usual care arm that does not involve use of the central venous catheter and does not include blood transfusion or inotrope instructions.

This trial will also study 650 patients per arm. Finally, in the UK, a trial entitled ProMiSe, EGDT is being tested against British usual care in a 2-arm trial similar in size to ARISE. All 3 trials will administer EGDT via a standardized trained team, akin to trauma teams. All 3 trial investigator groups have collaborated to ensure similar data collection and delivery of EGDT, thus facilitating a post-trial individual patient meta-analysis. In this talk, I will review the design and status of these trials.

### S31

#### **Fluid resuscitation in Australia and New Zealand: the CHEST study**

John Myburgh  
Sydney, Australia

Fluid resuscitation is a fundamental component of critical care medicine and one of the most common interventions administered to critically ill patients. The selection of resuscitation fluids by clinicians is largely determined by personal and institutional preferences based on purported physiological benefits, availability, cost and the influence of marketing. Crystalloids and colloids are used equivalently on a global basis although there is marked regional variation in their use, particularly with colloids.

The largest randomised-controlled trial of fluid resuscitation (n=6997) was conducted in Australia and New Zealand where albumin is widely used. The Saline vs Albumin Fluid Evaluation (SAFE Study) demonstrated no difference in 28-day mortality in patients resuscitated with albumin or saline in the Intensive Care Unit.<sup>1</sup> Differences mortality rates were observed in pre-defined subgroups, specifically in patients with traumatic brain injury where albumin was associated with a significant increase in relative risk of death at 2 years (41.8% vs 22.2%; RR 1.88, 95%CI 1.31-2.70, p<0.001)<sup>2</sup> and a trend to improved survival in patients with severe sepsis (30.7% vs 35.3%, RR 0.87, 95%CI 0.74 to 1.02, p=0.09). Whilst interpretation of subgroup analyses requires caution, these data suggest that the selection of fluid for resuscitation may have a direct impact on patient-centred outcomes in select populations.

Globally, synthetic colloids, specifically hydroxyethyl starches (HES) are the most commonly prescribed resuscitation fluids. The safety of HES has been questioned following a factorial randomised-controlled trial that compared fluid resuscitation with a high molecular weight, highly substituted HES (10% pentastarch 200/0.5) to Ringers lactate in patients with severe sepsis.<sup>3</sup> This study was stopped early and whilst demonstrating no difference in 28 day mortality, the use of HES was associated with a significant increase in the incidence of acute renal failure and the requirement for renal replacement therapy. Newer preparations of HES (6% HES 130/0.4) may be associated with a reduced risk of acute renal injury, although this has not been demonstrated in a high-quality randomised-controlled trial.

The crystalloid vs hydroxyethyl starch trial (CHEST) commenced recruitment in Australia and New Zealand in 2009 (Clinicaltrials.gov record GI-CCT24378). This study is modelled directly on the SAFE study and will compare resuscitation of patients with 6% HES (130/0.4) to saline on 90 day mortality (n=7000). Pre-defined subgroups include patients with acute renal injury/failure, severe sepsis, trauma and non-haemorrhagic traumatic brain injury. Completion of this trial is expected by the end of 2012.

A subsequent individual patient meta-analysis of the SAFE and CHEST databases is planned to provide a definitive comparison between the two colloids to saline, potentially providing a definitive answer to the overall safety and efficacy of these solution and specific subgroups, particularly patients with severe sepsis.

1. SAFE Study Investigators. A comparison of albumin and saline for fluid resuscitation in the intensive care unit. *N Engl J Med* 2004;350(22):2247-2256.
2. SAFE Study Investigators. Saline or albumin for fluid resuscitation in patients with traumatic brain injury. *N Engl J Med* 2007;357(9):874-884.
3. Brunkhorst FM, Engel C, Bloos F et al. Intensive insulin therapy and pentastarch resuscitation in severe sepsis. *N Engl J Med* 2008;358(2):125-139.

### S32

#### **Lipopolysaccharide removal**

John Marshall  
Toronto, Canada

Lipopolysaccharide (LPS) or endotoxin – an integral component of the cell wall of Gram-negative bacteria – is a potent trigger of an innate immune response. Extensive investigation in both animal models and human volunteers has shown that LPS alone can evoke the characteristic features of sepsis including, with higher doses, organ dysfunction and death. Endotoxemia – the presence of elevated LPS levels in the blood – is common during Gram-negative infection. But endotoxemia can occur in association with Gram-positive infection, as well as in a variety of non-infectious inflammatory states such as ischemia reperfusion injury, shock, multiple trauma, pancreatitis, burns, and splanchnic ischemia. Indeed a majority of patients admitted to an ICU show increased endotoxin levels on the day of ICU admission, suggesting that gut or environmental reservoirs may be numerically more important than invasive infection in the etiology of endotoxemia.

Multiple strategies for LPS removal or detoxification have been studied; the results to date have been muted. Endotoxin can be neutralized using a monoclonal antibody, or through the administration of endogenous neutralizing proteins such as bactericidal-permeability increasing protein or HDL. It can be detoxified through the activity of the enzyme alkaline phosphatase. Alternatively, its interaction with cell surface receptors can be blocked through the use of antibodies directed at these, or through the use of a non-toxic synthetic competitive antagonist, eritoran. Finally, the antibiotic polymyxin B binds endotoxin with high affinity, and extracorporeal removal of LPS using hemoperfusion against a polymyxin B column has gained popularity – particularly in Japan where the technique was developed and popularized.

Pooled data from multiple small studies of variable methodologic quality have shown that polymyxin B hemoperfusion can improve survival for patients with severe sepsis. Cruz and colleagues in a small multicenter Italian study of patients with severe sepsis secondary to peritonitis also reported that polymyxin B hemoperfusion resulted in a survival benefit.

A more definitive test of the efficacy of polymyxin B hemoperfusion is currently in progress in a North American study that will recruit 300 patients. Entry into the EUPHRATES trial requires both clinical evidence of distributive shock and an elevated endotoxin level of greater than 0.6 endotoxin activity units as measured using the chemiluminescent Endotoxin Activity Assay®. Efficacy will be measured as survival over 28 and 90 days.

LPS is an attractive target in sepsis, both because of its well-established role in the pathogenesis of the clinical syndrome, and because of its prevalence in a population of septic patients. However the assumption that endotoxemia per se is necessarily detrimental is likely to be overly simplistic, and the probability of success for future work will be increased by targeting patients with documented endotoxemia and titrating therapy to as yet unidentified optimal levels.

### S33

#### **European revival of albumin in sepsis: EARSS and ALBIOS**

Jean-Paul Mira  
Paris, France

The efficacy of albumin administration on survival in the critical care setting remains controversial. Hence, several meta-analyses have reported negative, neutral, or beneficial effects of albumin administration. To clarify this controversy, the Saline versus Albumin Fluid Evaluation [SAFE] study has been performed, comparing the effects of 4% albumin vs. saline for volume replacement in critically ill patients. The results demonstrated no differences in 28-day mortality between the two randomized groups, but confirmed the safety of albumin administration in acutely ill patients. Furthermore, a predefined subgroup analysis has shown a trend of longer survival in albumin-treated septic patients treated. Moreover, hypoalbuminemia is common in critically ill patients such as septic patients and is associated with worse outcomes. However, it is not known if hypoalbuminemia is just an excellent biomarker or if correcting hypoalbuminemia has beneficial effects on survival of septic patients, because albumin has several important physiological effects with potentially beneficial effects

in critical illness. Thus, further studies are needed to clarify what precise role albumin has in today's ICU.

Two current large multicenter European clinical trials try to understand the place of 20% albumin in severe sepsis treatment. These studies have similar end-points but different methodologies. Their main common objective is to analyze if 20% albumin administration in the early course of severe sepsis/septic shock reduces the 28th day mortality with a further control at 90th day. The secondary objectives are to verify the differences in organ dysfunctions (as assessed by the Sequential Organ Failure Assessment score), hospital and intensive care unit (ICU) length of stay between the treated and control group. The French study (EARSS) will include 800 patients and compare administration of 300 mL 20% albumin per day from D1 to D3 of septic shock to the same volume of saline. Albumin administration will not be allowed in the control group during the study. The Italian study (ALBIO) aims to verify whether volume replacement with 20% albumin and its maintenance within plasmatic physiologic range (equal or above 30 g/l) improves survival of patients with severe sepsis of septic shock, as compared to crystalloids. ALBIO will include about 1350 patients with severe sepsis or septic shock, which will be randomized to receive either albumin or crystalloids as fluid therapy. Volume replacement will be performed for both groups according to the early-goal directed therapy. Treated group will receive 60 gr albumin infusion after randomization, and 40-60 gr albumin daily infusion to maintain serum album level equal or above 30 g/l. Control group will receive crystalloids for the entire study; albumin administration will be allowed only when daily serum albumin level will be lower than 15 g/l. Patients will be treated until the 28th day after randomization or until ICU discharge.

The results of these two European studies will be available in 2010/2011 and will clarify the effects of 20% albumin in the settings of severe sepsis/septic shock.

### S34

#### Fluid resuscitation in Africa

Kath Maitland  
Kalifi, Kenya

Child survival programs have largely ignored the role of triage and emergency care in reducing child mortality, despite the fact that these interventions may be highly cost-effective. Over 50% of childhood deaths in African hospitals occur within 24 hours of admission, and shock complicates many of these cases. Adoption of simple treatment algorithms for the treatment of hypovolaemia and prevention of shock (irrespective of aetiology) could be of great benefit, but their effectiveness and safety have not been rigorously evaluated. Moreover, definitive evidence based guidelines for paediatric fluid resuscitation are hampered by the lack of adequate trials to influence choice of fluid. The prevailing controversy over crystalloid versus colloids solutions for treating paediatric septic shock also requires evaluation.

The FEAST (Fluid Expansion as a Supportive Therapy) trial is a multi-country pragmatic randomised controlled trial based at 6 hospitals in East Africa comparing the effect on mortality of volume expansion using 0.9% saline or human albumin solution (4%) with current practice (no bolus) in children less than 12 years of age. Interventions are administered at hospital admission, following rapid clinical assessment and randomisation. The trial includes 2,880 eligible children, it commenced in January 2009, and by July 2010 2200 children had been recruited. Children are assessed over 48 hours, and further fluid boluses given in strict accordance with the protocol.

The primary endpoint is in-hospital mortality at 48 hours post randomisation. Secondary endpoints include mortality at 4 weeks after

randomisation; neurological sequelae at 4 weeks after randomisation; persistent neurological sequelae at 6 months; development of hypotensive shock within 48 hours of randomisation; adverse events within 48 hours of randomisation (pulmonary oedema; intracranial hypertension; severe allergic reaction in those receiving albumin).

### S35

#### InFACT- did we learn anything from H1N1?

John Marshall  
Toronto, Canada

The index human case of influenza caused by a novel H1N1 swine origin strain was identified in rural Mexico in March 2009; by June of the same year, cases had been documented in more than 180 countries worldwide, and the WHO declared the first level 6 pandemic of the 21<sup>st</sup> century. On the basis of the rapidity of spread of the virus, and the severity of illness for some patients, the WHO predicted in the summer of 2009 that upwards of 2 billion people might ultimately be infected, and that between 10 and 100 million of these would require ICU care. This number vastly exceeded available resources, and confronted the discipline of intensive care with an unprecedented challenge.

Working independently in the early stages of the pandemic, investigator-led research groups in Canada, Australia and New Zealand, Europe, England, and the United States launched observational cohort studies to characterize the epidemiology of severe H1N1 infection. In July of 2009, representatives from these various groups began to explore the possibility of global collaboration under the auspices of the International Forum for Acute Care Trialists (InFACT) – a fledgling network of investigator led critical care research consortia.

The InFACT collaboration evolved rapidly, and with an unprecedented spirit of collegiality and collaboration. It involved 21 investigator-led groups, and received endorsement from 22 separate professional societies. As outlined in a commentary in the *Lancet*, the initiative encompassed five specific programs. Investigators from each of the national or regional registries agreed to work together to harmonize data sets and pool results to enhance the power of these observational studies. A clinical trials working group collaborated on the launch of 3 trials of statins or steroids for severe H1N1 infection, focusing their efforts on inexpensive interventions that would also benefit infected patients in the developing world. An ethics working group prepared and published a white paper on the ethical dimensions of pandemic research. A working group on care in the developing world developed a program to catalog global capacity for care and research during a pandemic. And a biomarkers working group focused on developing studies to better characterize the biology of severe H1N1 infection.

The second wave of H1N1 infection in the fall of 2009 proved much briefer, less severe, and less widespread than originally predicted, with the result that InFACT H1N1 research initiatives were stalled for lack of patients.

Nonetheless the legacy of H1N1 and the critical care professional response promises to be substantial. The clinical patient database now includes some 5000 patients with severe H1N1 infection, and secondary analyses are ongoing. The focus of clinical trials has shifted to a better understanding of the barriers to the rapid implementation of research during a pandemic, and the effort to catalog global research capacity is continuing to gain momentum. Perhaps most importantly, the experience revealed the common needs of clinical researchers around the world, and galvanized a commitment to greater international commitment to address these needs. InFACT was informally inaugurated earlier this year with a working steering committee and an inaugural membership of 15 groups; its formal launch is planned for later this year or early 2011.

# Sepsis 2010 Best Abstract Awards

Poster awards are kindly supported by an educational grant from Merck & Co.

Authors of four best abstract awards will present their work as oral presentations on Thursday 2 September at 15:45 – 16:45.

## P9

### A novel molecular biomarker diagnostic for the early detection of sepsis

Deon Venter<sup>1</sup>, Mervyn Thomas<sup>2</sup>, Jeffrey Lipman<sup>4</sup>, Benjamin Tang<sup>5</sup>, Anthony McLean<sup>5</sup>, Randal Pascoe<sup>3</sup>, Gareth Price<sup>1</sup>, Thu Nguyen<sup>1</sup>, Richard Brandon<sup>2</sup>, Allison Sutherland<sup>2</sup>

<sup>1</sup>Mater Adult Hospital, Pathology, Brisbane, Australia; <sup>2</sup>Athlomics Pty Ltd, Immunobiology and Bioinformatics, Brisbane, Australia; <sup>3</sup>Wesley Hospital, Intensive Care Medicine, Brisbane, Australia; <sup>4</sup>Royal Brisbane & Women's Hospital, Intensive Care Medicine, Brisbane, Australia; <sup>5</sup>Nepean Hospital, Intensive Care Medicine, Sydney, Australia

## P32

### Two chromogranin A-derived peptides, chromofungin and catestatin induce neutrophil activation via a store-operated channel-dependent mechanism

Marie Hélène Metz-Boutigue<sup>1,2</sup>, Dan Zhang<sup>1</sup>, Thomas Lavaux<sup>2,3</sup>, Francis Schneider<sup>3</sup>, Dominique Aunis<sup>1</sup>

<sup>1</sup>University of Strasbourg, Inserm Z575, Strasbourg, France; <sup>2</sup>University of Strasbourg, University Hospital, Strasbourg, France; <sup>3</sup>University of Strasbourg, Inserm U977, Strasbourg, France

## P34

### Cardiovascular hyporesponsiveness in sepsis is associated with G-protein receptor kinase expression via a nitric oxide-dependent mechanism

Daniela Dal Secco<sup>1</sup>, Vania Olivon<sup>2</sup>, Thiago Corrêa<sup>1</sup>, Mara Rubia Celes<sup>3</sup>, Monica Abreu<sup>3</sup>, Marcos Rossi<sup>3</sup>, Ana Maria Oliveira<sup>2</sup>, Fernando Cunha<sup>2</sup>, Jamil Assreuy<sup>1</sup>

<sup>1</sup>Federal University of Santa Catarina, Pharmacology, Florianopolis, SC, Brazil; <sup>2</sup>University of Sao Paulo, Pharmacology, Ribeirao Preto, SP, Brazil; <sup>3</sup>University of Sao Paulo, Pathology, Ribeirao Preto, SP, Brazil

## P43

### Soluble TLT-1 is a naturally occurring TREM-1 inhibitor and protects mice from hyperresponsiveness and death during sepsis

Marc Derive<sup>1</sup>, Youcef Bouazza<sup>1</sup>, Frédéric Massin<sup>2</sup>, Corentine Alauzet<sup>3</sup>, Bruno Levy<sup>1,4</sup>, Pierre-Edouard Bollaerts<sup>5</sup>, Sébastien Gibot<sup>1,5</sup>

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# Sepsis 2010: Poster Listing

- P1 Epidemiology of sepsis in pediatrics: first Colombian multicenter pilot survey**  
J Camilo Jaramillo-Bustamante, A Marín-Agudelo, M Fernández-Laverde, J Bareño-Silva
- P2 Randomized controlled trials are not designed to prove the safety of third-generation hydroxyethyl starch for resuscitation: results from a systematic review**  
CS Hartog, M Kohl, K Reinhart
- P3 Effect of canine hyperimmune plasma on TNF $\alpha$  and inflammatory cell levels in a lipopolysaccharide-mediated rat air pouch model of inflammation**  
BE Essien, M Kotiw, H Butler, D Strunin
- P4 Development of Klebsiella pneumoniae B5055-induced mouse model of sepsis-associated brain inflammation in BALB/c mice**  
V Kumar, A Sharma
- P5 Withdrawn**
- P6 Lipopolysaccharide is required for leukocyte adhesion to Toraymyxin® filters used in the treatment of sepsis**  
EL Martin, B Assenzio, VM Ranieri
- P7 Risk of neonatal septicemia associated with neonatal–maternal–bacterial determinants**  
M Douraghi, MN Rostami, H Goudarzi, M-M Soltandallal, M Radfar, H Zeraati
- P8 Age-associated changes in the inflammatory response to Gram-positive challenge of the lung**  
HM Linge, K Ochani, K Lin, EJ Miller
- P9 A novel molecular biomarker diagnostic for the early detection of sepsis**  
D Venter, M Thomas, J Lipman, B Tang, A McLean, R Pascoe, G Price, T Nguyen, R Brandon, A Sutherland
- P10 In vivo and in vitro role of cholecystokinin in nitric oxide**  
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- P11 Sepsis induces platelet mitochondrial uncoupling and a gradual increase in respiratory capacity that is negatively associated with clinical outcome**  
F Sjövall, S Morota, MJ Hansson, H Friberg, E Gnaiger, E Elmér
- P12 New sepsis related marker: endotoxin activity assay**  
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- P13 A fast and accurate diagnostic test for severe sepsis using model-based insulin sensitivity and clinical data**  
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- P14 Serum procalcitonin as a diagnostic tool of bacteremia**  
C Gartzonika, E Priavali, N Zotos, A Kallinteri, I Katsoula, H Sakkas, E Papapetrou, E Kapsali, G Vrioni, A Mavridis, G Nakos, S Levidiotou
- P15 Neonatal immune challenge impairs endotoxemic shock-induced hypotension: potential role for vasopressin**  
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- P16 Neonatal LPS exposure reduces stress fever in adult rats: modulation by glucocorticoids and PGE2**  
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- P17 Innate immunity and inflammation in sepsis: mechanisms by which acute ethanol exposure alters the course of sepsis and the effect to TLR4 signaling**  
B Jan, W Tan, X Deng, M Gadson, S Pruett
- P18 Lipopolysaccharide alters expression of incretin receptors in monocytic and hepatocytic cell lines**  
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- P19 The new sepsis marker, sCD14-ST, induction mechanism in the rabbit sepsis models**  
K Shirakawa, K Naitou, J Hirose, M Nakamura, T Takeuchi, Y Hosaka, S Furusako
- P20 Impact of delayed antimicrobial therapy in septic ITU patients**  
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- P21 Estimating coagulopathy in an ovine acute lung injury model of sepsis using a disease progression model**  
BW Footer, S Rehberg, P Enkhbaatar, DM Parish, HM Linge, LD Traber, EJ Miller, DL Traber, JJ Schentag
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- P23 CCR2 drives neutrophil infiltration and elicits tissue damage in remote organs during sepsis**  
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- P24 Prospective multicenter study of the effect of early fluid resuscitation on trends in IL-6 and TNF $\alpha$  levels in severe sepsis**  
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- P25 Severity of illness scoring systems in community-acquired Legionella pneumonia**  
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- P26 Increased survival after a cecal ligation and puncture-induced sepsis in mice consuming oleic acid**  
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- P27 Anti-inflammatory effect of procalcitonin on in vitro LPS-stimulated human PBMC**  
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- P28 Clinical effects of adsorption of lipopolysaccharide in the treatment of Gram-negative severe sepsis**  
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- P29 Withdrawn**
- P30 Clinical impact of a multiplex real-time PCR assay (SeptiFast®) for the rapid detection of pathogens in patients with end-stage heart failure bridged to heart transplantation with ventricular assist devices**  
A Chaidaroglou, E Manoli, A Gouziouta, P Gourzi, M Pantou, G Saroglou, P Alivizatos, P Sfyakis, D Degiannis
- P31 Extracellular metabolic alterations in critically ill septic patients studied by adipose tissue microdialysis**  
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- P32 Two chromogranin A-derived peptides, chromofungin and catestatin, induce neutrophil activation via a store-operated channel-dependent mechanism**  
MH Metz-Boutigue, D Zhang, T Lavaux, F Schneider, D Aunis
- P33 Withdrawn**
- P34 Cardiovascular hyporesponsiveness in sepsis is associated with G-protein receptor kinase expression via a nitric oxide-dependent mechanism**  
D Dal Secco, V Olivon, T Corrêa, MR Celes, M Abreu, M Rossi, AM Oliveira, F Cunha, J Assreuy
- P35 Kinetics of TREM-1 expression on canine neutrophils after in vitro and in vivo stimulation with microbial products**  
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- P36 Severe sepsis and its impact on outcome in old and very old patients admitted to the intensive care unit**  
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- P37 Chromogranin A expression in plasma of critically ill patients**  
T Lavaux, F Schneider, C Bach, J-E Herbrecht, D Aunis, M-H Metz-Boutigue
- P38 Chlamydomonas pneumoniae infection in macrophages and in lung epithelial cells: IL-10 and the innate immunity response**  
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- P39 Influence of hydroxyethyl starch and gelatin versus crystalloids on renal function, fluid balance, and ICU length of stay in patients with severe sepsis**  
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- P40 Study of the Helicobacter pylori infection in Iranian patients with multiple sclerosis**  
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- P41 Incidence of bacteremia at the time of ICU admission and its impact on outcome**  
D Juneja, O Singh, G Singh, P Bajaj
- P42 Differential kinetics of endothelial cell activation biomarkers E-selectin and endocan during nonlethal endotoxemia in 129Sv mice: a role for PMN-derived serine proteases in the transient decrease of circulating endocan levels**  
J Pastré, N De Freitas Caires, M Delehedde, A Scherpereel, E Parmentier, D Mathieu, P Lassalle
- P43 Soluble TLT-1 is a naturally occurring TREM-1 inhibitor and protects mice from hyperresponsiveness and death during sepsis**  
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- P44 Monoamine-oxidase-A function and potential benefit of its inhibition in sepsis**  
GP Otto, M Sossdorf, J Lemm, S Scholz, RA Claus, M Bauer
- P45 Transcripts coding the VWF cleaving protease are decreased under proinflammatory conditions, which is reversed by co-incubation with activated protein C and selenate**  
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- P49 Modeling sepsis induced by methicillin-resistant Staphylococcus aureus infection: a human/ovine approach**  
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- P51 Building up an infection control strategy based on the e-health concept**  
C Palos, A Pedroso, J Mapril, C Alves
- P52 Elimination of cytokine and soluble cytokine receptors by carbon sorbents from blood**  
N Anisimova, M Kiselevsky, E Gromova, L Kuznetzova
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## MEETING ABSTRACTS

# Sepsis 2010

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### P1

#### **Epidemiology of sepsis in pediatrics: first Colombian multicenter pilot survey**

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**Introduction** In 2002, the Surviving Sepsis Campaign defined a strategy that aimed to reduce the high mortality due to sepsis. One point of this strategy was a recommendation to recognize that sepsis is a frequent cause of death and high economic costs in the pediatric intensive care unit. Knowledge of the disease is the first step to impact it. There are few studies on pediatric sepsis epidemiology in the world and none in Colombia.

**Hypothesis** The epidemiological features of Colombian children are different from other countries.

**Methods** We constructed a website where 14 intensive care units across the country reported in a prospective way the epidemiological features of children with sepsis using an electronic process [1]. We asked for sociodemographics, microbiological data, sepsis classification, complications, and outcome.

**Results** We collected 253 patients from March to May 2009. Fifty-five percent of the cases were male and 45% were female; 53% were less than 1 year old. A total of 67.2% came from urban areas and 33% came from rural villages. Eighty-five percent were very poor (score 1 and 2 over 6 used in Colombia as socioeconomic classification). Forty-five percent have government-supported insurance. In total, 23.72% of the population presented with sepsis; 30.04% with severe sepsis; and 46.5% with septic shock. The infection origin was respiratory in 54.55%, followed by abdominal in 17.39%. In 50.2% no cause was identified. A total of 75.1% required mechanical ventilation. The mortality rate was 20.4%.

**Conclusions** Sepsis, severe sepsis, or septic shock is a common diagnosis in Colombian intensive care units. The majority of pediatric patients are 2 years or younger and from the poorest communities. It affected males more. In the majority, the process starts in the respiratory system. We had difficulty identifying the cause. The disease causes high mortality and cost for a developing society. We need a complete survey to find a correct approach to the problem.

#### **Reference**

1. Sepsis en Columbia [www.sepsisencolombia.com]

### P2

#### **Randomized controlled trials are not designed to prove the safety of third-generation hydroxyethyl starch for resuscitation: results from a systematic review**

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*Critical Care* 2010, **14**(Suppl 2):P2 (doi: 10.1186/cc9105)

**Introduction** Hydroxyethyl starch (HES) is widely used for volume therapy in intensive care. In critically ill and sepsis patients, HES use was

dose-dependently associated with increased renal failure, tissue storage with organ failure, and increased long-term mortality. There are other safety concerns with regard to coagulopathy, pruritus, and mortality. However, third-generation HES 130/0.4 is considered to have an improved risk profile. Therefore, we wanted to assess whether published studies on HES 130/0.4 resuscitation are sufficiently well designed to draw conclusions about the safety of this compound.

**Methods** We derived clinically relevant outcome parameters to analyze safety outcomes from the literature and provided exemplary power calculations. Randomized controlled trials (RCT) on fluid resuscitation with HES 130/0.4 were systematically searched and analyzed for clinical condition, sample size, study duration, cumulative dose, control fluids, endpoints, and colloid-crystalloid volume ratios in studies with a goal-directed fluid regimen. Due to the heterogeneity of included studies, all analyses were descriptive (SPSS 17.0).

**Results** A total of 56 RCTs were included. Only two studies included severe sepsis patients, 80% were from the elective surgical setting and one study from the emergency surgical setting. In general, studies were underpowered (median sample size 25 patients in HES 130/0.4 groups, range 10 to 90 patients); of short duration (median study period 12 hours, range 0.5 to 144 hours) and with low cumulative HES doses (median 2,465 ml, range 328 to 6,229 ml). Sepsis studies ( $n = 2$ ) included 18 patients (median, range 10 to 26 patients), study period was 96 hours (median, range 72 to 120 hours) and total fluid volumes was 3,000 ml in one study. Sixty percent of control fluids were synthetic colloids (other starches, gelatins, or dextran) that carry a similar risk profile. Primary endpoints with power calculation (in 87% of studies) were mostly unspecific or clinically irrelevant. Only one sepsis study provided a primary endpoint, which was extravascular lung water. This did not differ in comparison with the albumin 20% control group.

**Conclusions** There is no reliable evidence from published clinical data that third-generation HES 130/0.4 is safe in septic patients or in the emergency or elective surgical setting.

### P3

#### **Effect of canine hyperimmune plasma on TNF $\alpha$ and inflammatory cell levels in a lipopolysaccharide-mediated rat air pouch model of inflammation**

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*Critical Care* 2010, **14**(Suppl 2):P3 (doi: 10.1186/cc9106)

**Introduction** Unregulated elevated levels of serum TNF $\alpha$  have been associated with proinflammatory cytokine cascades, which are characteristic in diseases such as septic shock. Endotoxic shock, which has a poorer prognosis than found with other forms of septic shock, is mediated by lipopolysaccharide (LPS), a molecule that is released from the outer membrane of Gram-negative bacteria. LPS is a potent stimulator of TNF $\alpha$  secretion by serum monocytes and tissue macrophages. While the use of monotherapeutic TNF $\alpha$  antagonists has been trailed, none has been registered for use in patients with sepsis.

**Objective** The purpose of this study was to test the effect of canine hyperimmune frozen plasma (HFP), which is known to contain elevated levels of soluble TNF $\alpha$  receptor 1 (sTNFR1), on TNF $\alpha$  and inflammatory cell levels in a LPS-mediated rat air pouch model of inflammation.

**Methods** A dorsal air pouch in 175 to 200 g Sprague–Dawley rats was formed by 20 ml subcutaneous infusions of sterile air. Prophylactic subcutaneous injections of canine HFP, canine fresh-frozen plasma (FFP), or carprofen

were administered daily for 3 days into the lateral flank of the right foreleg at doses recommended by the manufacturers ( $n = 10$  for each treatment group). Pouch fluid was harvested by syringe at 1, 6, 12, 24, and 48 hours post LPS administration and subjected to histological and cytokine/cytokine receptor analysis. TNF $\alpha$  and sTNFR1 levels were determined by ELISA and an immunofluorescent dot blot assay.

**Results** Pouch fluid analysis: maximal effects were detected at 6 hours post LPS administration. TNF $\alpha$  levels were significantly depressed in animals dosed with HFP, but not in animals treated with FFP or carprofen ( $P < 0.05$ ). sTNFR1 levels were significantly elevated in HFP, but not in FFP or carprofen-dosed animals ( $P < 0.05$ ). Neutrophil numbers were significantly depressed in HFP-dosed, but not in FFP-treated or carprofen-treated animals ( $P < 0.05$ ).

**Conclusions** There appears to be a correlation between elevated levels of sTNFR1 and depression of TNF $\alpha$  and neutrophil levels in the pouch fluid of HFP-dosed rats ( $r = -0.73$ ,  $P < 0.0001$ ). The data suggest that canine HFP, which has been demonstrated to contain elevated levels of sTNFR1 compared with FFP, has a direct effect on depressing TNF $\alpha$  levels and neutrophil sequestration in the rat air pouch model of inflammation. These data suggest that HFP may be worthy of further investigation to determine whether such preparations have a therapeutic potential for treatment of acute inflammatory diseases in which TNF $\alpha$  is implicated.

#### P4

##### Development of *Klebsiella pneumoniae* B5055-induced mouse model of sepsis-associated brain inflammation in BALB/c mice

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**Introduction** Incidence of sepsis is continuously increasing in the developing as well as the developed world. Severe sepsis is associated with the development of multiorgan dysfunction syndrome. In addition to other vital organs (that is, lungs, kidneys, heart, or liver), the brain is one of the severely affected organs in sepsis. Autopsy studies from septic patients reveal various cerebral lesions including ischemia, hemorrhage, microthrombi, microabscesses, multifocal necrotizing leukoencephalopathy, and bacterial invasion of the nervous system. Until now no animal model of sepsis has been developed that in a true sense represents the brain inflammation associated with evolving sepsis originating from Gram-negative bacteria. This study comprises development of a mouse model of sepsis-induced brain inflammation.

**Methods** A mouse model of sepsis-associated brain inflammation was developed by directly placing a selected dose ( $10^2$  cfu) of *Klebsiella pneumoniae* B5055 entrapped in a fibrin-thrombin clot into the peritoneal cavity of mice, while in control group animals only a sterile fibrin clot was kept. Various inflammatory cytokines (that is, IL-1 $\alpha$  and TNF $\alpha$ ) and other inflammatory markers (that is, malondialdehyde, myeloperoxidase and nitric oxide) in serum and brain were estimated by ELISA, biochemical methods, and histopathology in both the experimental groups.

**Results** Bacterial colonies were found to be established in brain on the very first day of sepsis induction in experimental group but no bacteria were observed in sham-operated animals. Along with this, a significant ( $P < 0.05$ ) increase in neutrophil infiltration into the brain along with significantly ( $P < 0.05$ ) increased levels of proinflammatory cytokines (TNF $\alpha$  and IL-1 $\alpha$ ) and other inflammatory mediators like nitric oxide, malondialdehyde, and myeloperoxidase were observed in animals with sepsis. Also the septic animals survived until the 5th day of post-sepsis development, while 100% survival was observed in the sham-operated group on all experimental days without any inflammatory change in the brain as observed by histopathologic examination and estimating the above-mentioned inflammatory parameters.

**Conclusions** This mouse model of sepsis-induced brain inflammation may prove helpful to study immunopathogenesis of brain inflammation observed during Gram-negative bacterial sepsis and may also prove helpful to study neuroimmunology of sepsis along with behavioral changes associated with sepsis.

#### P5

##### Abstract withdrawn

#### P6

##### Lipopolysaccharide is required for leukocyte adhesion to Toraymyxin® filters used in the treatment of sepsis

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Critical Care 2010, **14**(Suppl 2):P6 (doi: 10.1186/cc9109)

**Introduction** Extracorporeal hemoperfusion with polymyxin B is a novel septic treatment, shown to improve hemodynamics, organ dysfunction, and mortality through the removal of circulating lipopolysaccharide (LPS). This therapy can also remove activated leukocytes, which likely contributes to reduced inflammation and improved patient outcome; however, the mechanistic role of LPS in the removal of leukocytes remains unclear.

**Objective** To determine whether the presence of LPS and/or activation of leukocytes by LPS alters their ability to bind to polymyxin-bound filters used for extracorporeal hemoperfusion of septic patients.

**Methods** Toraymyxin® filters were opened under sterile conditions and 2 cm<sup>2</sup> sections were incubated for 2 hours under various conditions. Experiment 1: filters were exposed to (1) whole blood collected from a health volunteer, (2) blood with 700 ng/ml LPS (*Escherichia coli* 0127:B8), or (3) blood pre-incubated for 2 hours in 700 ng/ml LPS. Experiment 2: filters were pre-exposed to LPS then incubated with blood alone or blood with LPS. Experiment 3: filters were exposed to blood containing increasing LPS concentrations (1 pg/ml to 500 ng/ml) or TNF $\alpha$  (15 pg/ml to 10 ng/ml). In all experiments, following incubation, filters were washed, stained (methylene blue + eosin) and the number of adhered leukocytes were counted by light microscopy. Endotoxin activity of the collected whole blood in both the absence and presence of LPS was determined by an endotoxin activity assay (EAA™).

**Results** The presence of LPS significantly increased ( $3.77 \pm 0.54$ -fold,  $P = 0.005$ ) the number of adhered leukocytes to Toraymyxin® filters. Moreover, pre-incubation of the blood with LPS, to activate inflammatory cells, further increased leukocyte adhesion ( $7.59 \pm 1.08$ -fold increase vs. control,  $P = 0.002$ ,



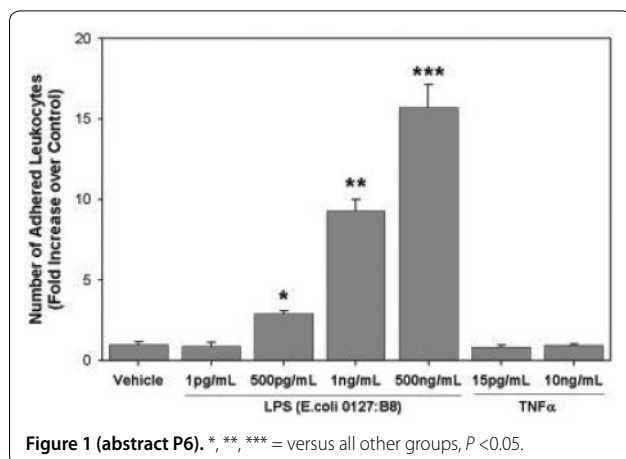


Figure 1 (abstract P6). \*, \*\*, \*\*\* = versus all other groups,  $P < 0.05$ .

or vs. non-incubated LPS,  $P = 0.03$ ). Pre-exposure of Toraymyxin® filters to LPS versus vehicle control increased leukocyte adhesion, both for blood alone ( $7.60 \pm 1.51$ -fold increase,  $P = 0.004$ ) or blood incubated with LPS ( $24.43 \pm 5.32$ -folds vs.  $7.59 \pm 1.08$ -fold increase,  $P = 0.019$ ). Moreover, while the presence of TNF $\alpha$  or low levels of LPS did not induce leukocyte binding to Toraymyxin® filters, increasing LPS concentrations induced a dose-dependent increase in adhesion (Figure 1). Sterile blood was confirmed by EAA to have low endotoxin activity (EAA™ <0.3), while blood containing 700 ng/ml LPS had high endotoxin activity (EAA™ = 0.8).

**Conclusions** While leukocyte activation by LPS increases their adhesion to Toraymyxin® filters, the activation of leukocytes by TNF did not alter binding, indicating the essential need for the presence of LPS possibly as a bridging molecule in the mechanism responsible for the removal of leukocytes during extracorporeal hemoperfusion with Toraymyxin® filters.

## P7

### Risk of neonatal septicemia associated with neonatal-maternal-bacterial determinants

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**Introduction** The triad interaction of neonatal-maternal-bacterial determinants plays a crucial role in the increased incidence of bacterial sepsis during the neonatal period. This study was undertaken to determine whether neonatal-maternal predisposing factors and bacterial pathogens affect the risk of early or late onset sepsis.

**Methods** Three hundred neonates in the NICU of two hospitals in Tehran were studied. Blood cultures from neonates with suspected sepsis were performed on BHI broth followed by identification of isolates and testing for their susceptibility to antimicrobial agents. Collectively, neonatal and maternal risk factors such as birth weight, gestation age, PROM, Apgar score, and others were studied in the cultures of proven cases of neonatal sepsis. In univariate binary logistic regression models, the impact of neonatal and maternal factors on sepsis risk was estimated in terms of odds ratio (OR) with 95% confidence interval (CI).

**Results** The present study revealed the impact of bacterial pathogens and neonatal and maternal predisposing factors on sepsis as follows. **Bacterial pathogens:** 14/300 (4.7%) of neonates developed septicemia. Among infected neonates, 64.3% and 35.7% were considered with early-onset and late-onset sepsis, respectively. The most isolated Gram-negative organism was *Stenotrophomonas maltophilia* (42.8%) followed by *Klebsiella pneumoniae* (28.6%), *Escherichia coli* (21.4%) and *Serratia liquefaciens* (7.2%). **Neonatal factors:** the mean age of neonates  $\pm$  SD with early-onset sepsis ( $1.56 \pm 0.88$ ) was lower than that of those with late-onset sepsis ( $10.40 \pm 5.50$ ) and

this difference was statistically significant ( $P < 0.05$ ). Low birth weight (LBW) <2,500 g increased the risk of sepsis to more than twofold (OR = 2.9, 95% CI = 1.17 to 9.86;  $P < 0.01$ ). Gestation age (GA) <29 weeks was significantly associated with sepsis ( $P < 0.01$ ). The septicemia, in turn, increased the risk of death up to more than fivefold (OR = 5.5; 95% CI = 1.98 to 15.3;  $P < 0.01$ ). More than one-half of septic neonates had positive result for CRP whereas only 1.9% of neonates with sepsis were CRP-negative, and this difference was statistically significant ( $P < 0.001$ ). **Maternal factors:** PROM affected the sepsis risk to more than threefold (OR = 3.8; 95% CI = 1.37 to 10.56;  $P < 0.05$ ).

**Conclusions** The present study reveals that specific neonatal and maternal factors are associated with increased risk of sepsis. Among the studied factors, prematurity of neonates explained as GA and LBW are the most important contributors to morbidity in neonate who suffered from sepsis. Furthermore, PROM as a maternal risk factor predisposes a child to neonatal sepsis.

## P8

### Age-associated changes in the inflammatory response to Gram-positive challenge of the lung

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Critical Care 2010, **14**(Suppl 2):P8 (doi: 10.1186/cc9111)

**Introduction** Mortality from sepsis is greater in the elderly than in the young although incidence only increases slightly. Pulmonary infections caused by *Staphylococcus aureus* that progress into sepsis are a major cause of death in elderly patients. Bacterial pneumonia is a common precipitating cause of sepsis. Gram-positive bacteria are increasing as causative agents of pneumonia in the elderly.

**Objective** To investigate age-dependent changes in the intrapulmonary response to staphylococcal challenge.

**Methods** Cell wall components lipoteichoic acid (LTA, 200 ng) and peptidoglycan (PGN, 660 ng) from *S. aureus* were instilled intratracheally to young (3 to 4 months) and old (>18 months) C57Bl6 mice ( $n > 5$ /group). Controls received saline alone. After 6 hours, mice were euthanized by exsanguination, and blood saved for analysis. One-sided lavage was performed, the nonlabeled lung tissue collected and total RNA isolated.

**Results** Using this relatively mild challenge of LTA and PGN, we observed significant and age-dependent differences in the inflammatory response. Macrophage migration inhibitory factor (MIF) protein was significantly and age-dependently increased in BAL and plasma. A trend toward lower levels of total cells and neutrophils in the lung was noted in the old following stimulation, although the variation of the response was large. The dynamics of MIF at a transcriptional level in the lung was age-dependently altered, with a marked downregulation in the young mice after stimulation, whereas levels of MIF mRNA remained unchanged in the old mice. Transcriptional changes were also noted for the anti-inflammatory cytokine IL-10 and other mediators involved in lymphocyte, macrophage and neutrophil recruitment. Interestingly, explanted lung cells from young and old mice showed a similar expressional pattern, with atypical expression levels in cells originating from old mice lungs.

**Conclusions** The findings support a hyperinflammatory response in the older individual at the measured time point. Interestingly, the differences were sustained *in vitro* in cells explanted from young and old mice. Together the data suggest an altered inflammatory response to infectious challenge of the aged lung. Explanted cells from old animals may be a valuable tool in determining age-dependent differences in inflammatory response and identifying novel targets for intervention.

## P9

### A novel molecular biomarker diagnostic for the early detection of sepsis

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**Introduction** Sepsis is a complex immunological response to infection characterized by a sinusoidal pattern that represents early hyperinflammatory

signals [1] followed by severe and protracted immunosuppression, suggesting that a multimarker approach has the greatest clinical utility in early detection within a clinical environment focused on SIRS differentiation. Preclinical research using an equine endotoxemia model identified a panel of gene expression biomarkers that define the aberrant immune activity during early sepsis. Thus, the primary objective was to apply these gene expression biomarkers to distinguish patients with sepsis from those who had undergone major open surgery and had clinical outcomes consistent with systemic inflammation due to physical trauma and wound healing.

**Methods** This was a multicenter, prospective clinical trial conducted across four tertiary critical care settings in Australia. Sepsis patients were recruited if they met the 1992 Consensus Statement [2] and had clinical evidence of systemic infection based on microbiology diagnoses ( $n = 27$ ). Participants in the post-surgical (PS) group were recruited preoperatively and blood samples collected within 24 hours following surgery ( $n = 36$ ). Healthy controls (HC) included hospital staff with no known concurrent illnesses ( $n = 19$ ). Each participant had minimally 5 ml PAXgene blood collected for RNA isolation and gene expression analyses. Affymetrix Exon array and multiplex tandem (MT)-PCR studies were conducted to evaluate gene expression using a set of molecular markers that had been identified *a priori*. A LogitBoost algorithm was used to create a machine-learning diagnostic rule in which to predict sepsis outcomes.

**Results** Based on preliminary exon array analyses comparing HC and sepsis groups, a panel of 42 gene expression markers was identified that linked to key innate immunity, cell cycle, endothelial, coagulation, and apoptotic pathways. When sepsis and PS groups were combined, the test had an ROC area >95%. Using subsets of these biomarkers in the MT-PCR assay, the ROC AUC for sepsis prediction was between 85 and 90%.

**Conclusions** This novel molecular biomarker test has a clinically relevant sensitivity and specificity profile, and has the capacity for early detection of sepsis via the monitoring of critical care patients.

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## P10

### *In vivo* and *in vitro* role of cholecystokinin in nitric oxide

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*Critical Care* 2010, **14**(Suppl 2):P10 (doi: 10.1186/cc9113)

**Introduction** Nitric oxide (NO) plays a key role in innate immune system controlling microbial infection; however, during septic shock its exacerbate formation is associated with several deleterious complications. Cholecystokinin (CCK) was first described as a gastrointestinal hormone, but immune cells express their receptor, suggesting a possible involvement of this hormone in modulation of inflammatory response. Our aim was to evaluate the role of CCK on NO production during endotoxemia in rats as well as lipopolysaccharide (LPS)-stimulated macrophages.

**Methods** Male Wistar rats received an intravenous injection of CCK (0.4 and 40 µg/kg) 10 minutes before LPS (1.5 mg/kg) administration. The mean arterial pressure was monitored during 6 hours after endotoxin injection. Blood was collected for plasma nitrate level and vasopressin measurement at 2, 4 and 6 hours after LPS. Thioglycollate-elicited macrophages were obtained by peritoneal lavage and cultured in RPMI 1640 medium, supplemented with 10% fetal bovine serum and antibiotics. Macrophage culture was treated with CCK ( $10^{-14}$ ,  $10^{-12}$ ,  $10^{-10}$ ,  $10^{-8}$ ,  $10^{-6}$  M) 30 minutes before LPS stimulation (1 µg/ml) and supernatant nitrite concentration was determined at 6, 24 and 48 hours. The iNOS expression was evaluated by quantitative real-time PCR and the amount of gene transcription was measured using the delta-delta method. The presence of iNOS was analyzed by indirect immunofluorescence at 12 and 24 hours after LPS incubation.

**Results** The LPS-induced hypotension was reverted by the pretreatment with CCK only at the lower dose. Moreover, CCK increased vasopressin levels at 2 and 4 hours after LPS administration and reduced nitrate levels during 2 and 6 hours. LPS-stimulated macrophages increased rapidly nitrite levels in supernatant and also iNOS expression. The pretreatment with CCK at all tested concentrations significantly reduced nitrite levels at 6, 24 and 48 hours after LPS stimulation when compared with the LPS group ( $P < 0.05$ ). The iNOS/GAPDH expression ratio were also lower in CCK-treated cells at 6 and 24 hours ( $P < 0.001$ ). The qualitative analysis of iNOS protein was assessed at 12 and 24 hours after LPS stimulus by immunocytochemistry. In CCK-treated macrophages, a reduction of fluorescence emission in comparison with the LPS group was observed. In control groups (without LPS), fluorescence was not observed, suggesting the absence of iNOS protein in non-inflammatory conditions.

**Conclusions** These data suggest that CCK restores hypotension and reduces NO formation during endotoxemia in rats. Furthermore, CCK regulates negatively iNOS expression and also NO synthesis in LPS-activated peritoneal macrophage culture.

## P11

### Sepsis induces platelet mitochondrial uncoupling and a gradual increase in respiratory capacity that is negatively associated with clinical outcome

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**Introduction** Mitochondrial dysfunction has been suggested as a contributing factor in the pathogenesis of multiple organ dysfunction syndrome (MODS) and sepsis is the leading cause of MODS. Also, restoration of mitochondrial function, known as mitochondrial biogenesis, has been implicated as a key factor for the recovery of organ function in patients with sepsis. Here we investigated platelet mitochondrial respiratory function in patients with sepsis during the first week after disease onset.

**Methods** Platelets were isolated from blood samples taken from 18 patients with severe sepsis or septic shock within 48 hours of their admission to the intensive care unit. Subsequent samples were taken on days 3 to 4 and days 6 to 7. Eighteen healthy blood donors served as controls. Platelet mitochondrial function was determined by high-resolution respirometry. Endogenous respiration of intact platelets suspended in their own plasma or PBS glucose was determined and, in order to investigate the activity of individual complexes of the respiratory system, platelets were permeabilized with digitonin and stimulated with complex-specific substrates and inhibitors.

**Results** There was a significant increase in maximal respiratory capacity of platelets from days 1 to 2 to days 6 to 7 as well as compared with controls in both intact platelets and permeabilized platelets oxidizing complex I and/or II linked substrates. Platelets suspended in their own septic plasma exhibited increased leak respiration compared with platelets suspended in PBS glucose and to controls. No inhibition of respiration was detected in septic patients compared with controls. Mortality at 90 days was 33% (6/18). Nonsurvivors had a significantly more elevated respiratory capacity at days 6 to 7 as compared with survivors. No correlation between respiratory capacity and severity of disease as measured by APACHE II, SAPS II, SOFA or noradrenaline dose were found. Platelet content of mitochondria-specific cytochrome *c* increased significantly, but no change in mitochondrial DNA was detected over the time interval studied.

**Conclusions** The results indicate the presence of a soluble plasma factor in the initial stage of sepsis inducing uncoupling of platelet mitochondria but not inhibition of oxidative phosphorylation. Further, the mitochondrial uncoupling was paralleled by a gradual and substantial increase in respiratory capacity that may reflect mitochondrial biogenesis as a response to severe sepsis or septic shock. The enhanced respiratory capacity developing over the first week seems to reflect the severity of the condition and may be used as a prognostic marker of mortality.

**Acknowledgements** EG is the founder of Oroboros instruments, Austria and has developed the oxygraph used in the present study.

## P12

### New sepsis-related marker: endotoxin activity assay

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**Introduction** The endotoxin activity assay (EAA) is a rapid whole-blood chemiluminescent test for endotoxin that has proven clinical utility in the detection and risk stratification of clinically ill patients with suspicion of sepsis.

**Methods** The EAA was studied in a cohort of 153 septic patients admitted to the ICU. At the same time, IL-6 (chemiluminescent enzyme immunoassay), C-reactive protein (CRP), procalcitonin (PCT, chemiluminescent enzyme immunoassay) and plasminogen activator inhibitor-1 (PAI-1, latex photometric immunoassay) were measured within 24 hours after ICU admission. The patients were divided into the following three groups: L group:  $EAA < 0.4$ , M group:  $0.4 \leq EAA < 0.6$ , H group:  $0.6 \leq EAA$ . Nonrepeated-measures ANOVA was used to compare over three groups or conditions. Statistical significance was assumed for values of  $P < 0.05$ . Normally distributed data are presented as mean  $\pm$  SD, and abnormally distributed data are presented as median values.

**Results** Of the 153 patients, the L group contained 61 patients, M group 41 patients, and H group 51 patients, respectively. On the day of ICU admission, the rate of  $EAA \geq 0.4$  was 60.1% (MEDIC study: 57.2%). APACHE score in the L group was  $21.0 \pm 7.9$ , M group  $24.8 \pm 8.4$ , H group  $26.4 \pm 8.9$ , and SOFA score in the L group was  $8.2 \pm 4.3$ , M group  $8.9 \pm 4.1$ , H group  $9.5 \pm 4.3$ , respectively. There was no statistically significant difference among the groups. The median value of PCT in the L group was 1.1 ng/ml, M group 5.9 ng/ml, H group 8.5 ng/ml, respectively. PCT values of the M and H groups were significantly higher than those of the L group. Median IL-6 level of the H group was significantly higher than that of the L group (H group: 2,635 pg/ml, L group: 177 pg/ml).

**Conclusions** EAA has no significant correlation with other sepsis-related markers, but may be associated with body insults (inflammation or infection).

## P13

### A fast and accurate diagnostic test for severe sepsis using model-based insulin sensitivity and clinical data

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**Introduction** Severe sepsis occurs frequently in the ICU and is a leading cause of admission, mortality, and cost. Management guidelines define treatment objectives within the first 6 hours of clinical syndrome presentation. However, blood culture test confirmation may return in up to 48 hours, with only 30 to 50% of presentations having positive blood cultures. Early treatment compliance has demonstrated a decrease in sepsis mortality. Thus, there remains a serious need for an early and accurate diagnostic test for severe sepsis. Insulin sensitivity (SI) is known to decrease with worsening condition and inflammatory response, and could thus be used to aid clinical treatment decisions. Some glucose control protocols are able to accurately identify SI in real time, without high rates of hypoglycemia [1]. This research explores the diagnostic test properties of a real-time test for severe sepsis.

**Methods** A diagnostic biomarker for severe sepsis was developed from retrospective SI and concurrent temperature, heart rate, respiratory rate, blood pressure, and SIRS score from 36 adult patients with sepsis. Patients were identified as having severe sepsis based on a clinically validated sepsis score (ss). Kernel density estimates were used for the development of joint probability density profiles for  $ss \geq 2$  and  $ss < 2$  data hours (213 and 5,858, respectively, of 6,071 total hours) and for classification. From the receiver operator characteristic (ROC) curve, the optimal probability cutoff values for classification were determined, as well as AUC, positive and negative likelihood ratios (LHR), predictive values, and diagnostic odds ratios (DOR) for in-sample and out-of-sample estimates, respectively.

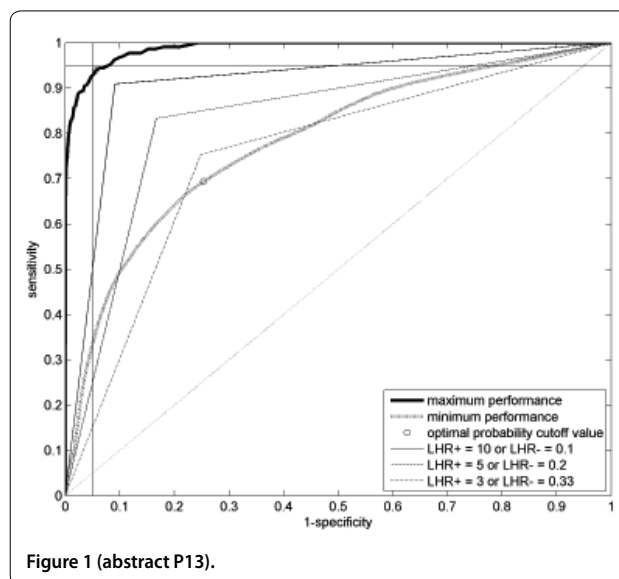


Figure 1 (abstract P13).

**Results** A biomarker including concurrent insulin sensitivity and clinical data for real-time diagnosis of severe sepsis ( $ss \geq 2$ ) achieves 69 to 94% sensitivity, 75 to 94% specificity, 0.78 to 0.99 AUC, 3 to 17 LHR+, 0.06 to 0.4 LHR-, 9 to 38% PPV, 99 to 100% NPV, and 7 to 260 DOR for optimal probability cutoff values of 0.32 and 0.27 for in-sample and out-of-sample data, respectively. The overall result lies between these minimum and maximum error bounds. See Figure 1.

**Conclusions** The clinical biomarker shows good to high accuracy and may provide useful information as an early real-time diagnostic test for severe sepsis.

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## P14

### Serum procalcitonin as a diagnostic tool of bacteremia

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Critical Care 2010, **14**(Suppl 2):P14 (doi: 10.1186/cc9117)

**Introduction** Procalcitonin (PCT) is a highly specific marker of severe bacterial infections and organ failure due to sepsis. The aim of the present study was to determine the diagnostic value of serum PCT in ICU patients with bacteremia caused by either Gram-negative or Gram-positive bacteria.

**Methods** During this prospective study, PCT levels were measured in 163 adult patients with proven systemic monobacterial infections. Bacteremia was defined as the recovery of any bacterial species and for coagulase-negative Staphylococci (CNS) the species that were included were those harboring the same antibiotic pattern grown from at least two consecutive samples. Blood for PCT levels and culture was drawn simultaneously at the onset of bacteremia. Eighty-eight episodes of bacteremia were caused by Gram-positive bacteria: *Staphylococcus aureus* 12, CNS 56, *Enterococcus* spp. 13, *Streptococcus pneumoniae* 3, *Clostridium perfringens* 1 and *Corynebacterium acnes* 3. The remaining 75 episodes of bacteremia were caused by Gram-negative bacteria: *Escherichia coli* 16, *Klebsiella pneumoniae* 19, *Pseudomonas aeruginosa* 15, *Acinetobacter baumannii* 24, and *Serratia marcescens* 1. Serum PCT was estimated with an assay based on immunochemiluminescence (BRAHMS Diagnostica, Berlin, Germany).

**Results** According to our results, PCT levels in all patients with bacteremia caused by Gram-negative bacteria (75/75) were >2 ng/ml. In more details in 41 patients with Gram-negative bacteremia (54.7%) the PCT levels were 2 to 10 ng/ml and in 34 patients (45.3%) were >10 ng/ml while in patients with CNS bacteremia the PCT levels were >2 ng/ml only in 14% (6/56). In addition, in all patients with bacteremia caused by *S. aureus* the PCT levels were >2 ng/ml and by *Streptococcus* spp., *C. perfringens*, and *C. acnes* the PCT levels were 2 to 10 ng/ml.

**Conclusions** PCT levels were markedly higher in patients with bacteremia associated with Gram-negative bacteria than in those with Gram-positive bacteremia, especially caused by CNS. Future research is needed to confirm our results.

#### P15

##### Neonatal immune challenge impairs endotoxemic shock-induced hypotension: potential role for vasopressin

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Administration of bacterial cell wall component lipopolysaccharide (LPS) stimulates the immune and endocrine systems, inducing acute phase of sickness and stress responses. Neonatal LPS exposure has been shown to alter many aspects of adult physiology, including neuroendocrine, neurochemical, and febrile responses. The aim of this study was to evaluate the effects of neonatal immune challenge on adults during septic shock-like condition assessing mean arterial pressure and heart rate, plasma vasopressin (AVP) concentration, body temperature (Tb), and macrophage nitric oxide (NO) synthesis. Male Wistar rats were exposed to LPS (100 µg/kg i.p.; nLPS) or saline administration (nSal) 14 days after birth (P14). On day 50 after birth, endotoxemic shock was induced by intraperitoneal injection of 10 mg/kg LPS, on rats previously implanted with polyethylene catheters in the femoral artery and loggers for Tb measurements. A different set of animals was used to assess the effect of neonatal LPS exposure on NO synthesis by peritoneal macrophage *in vitro*, with (1 µg/ml) or without LPS, added to the culture. In nSal rats, LPS injection induced a transitory increase in AVP plasma concentration, a decrease in mean arterial pressure with a concomitant increase in heart rate, which were statistically significant from 1 hour ( $P < 0.01$ ) up to 6 hours ( $P < 0.001$ ) after treatment. LPS-induced hypothermia ( $P < 0.05$ ) was observed for 2 hours after LPS administration, and was followed by an increased Tb ( $P < 0.01$ ). We also observed a significant increase in nitrate plasma concentration as well as in macrophage culture medium after LPS stimulation. In nLPS rats we observed an attenuation to the development of hypotension, no significant change in heart rate ( $P < 0.05$ ), an increased hypothermia, and a decreased febrile response, and further increased ( $P < 0.01$ ) AVP plasma levels were observed, in response to LPS administration. Interestingly, nitrite released in the culture medium was attenuated in nLPS animals. Neonatal exposure to LPS induces attenuation in hypotension during septic shock-like conditions and this response may involve an increased AVP release.

#### P16

##### Neonatal LPS exposure reduces stress fever in adult rats: modulation by glucocorticoids and PGE<sub>2</sub>

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Critical Care 2010, **14**(Suppl 2):P16 (doi: 10.1186/cc9119)

Immune challenges during the neonatal period may permanently program immune responses later in life, including endotoxin fever. We tested the hypothesis that neonatal endotoxin exposure affects stress fever in adult rats. In control rats (treated with saline as neonates; nSal) body temperature peaked ~1.5°C during open-field stress, whereas in rats exposed to endotoxin (lipopolysaccharide, LPS) as neonates (nLPS) stress fever was significantly attenuated. Following stress, plasma corticosterone levels significantly increased from  $74.29 \pm 7.05$  ng/ml to  $226.29 \pm 9.87$  ng/ml in nSal rats, and from  $83.43 \pm 10.31$  ng/ml to  $324.7 \pm 36.87$  ng/ml in nLPS rats. Animals treated with LPS as neonates and adrenalectomized 1 week before experimentation no longer displayed the attenuated febrile response to stress. This attenuated stress fever caused by an increased corticosterone secretion is likely to be

linked to an inhibitory effect of glucocorticoids on cyclooxygenase activity/PGE<sub>2</sub> production in the preoptic/anteroventral third ventricular region (AV3V) since stress failed to cause a significant increase in PGE<sub>2</sub> in nLPS rats, and this effect was reverted by adrenalectomy. Altogether, the present results indicate that endogenous glucocorticoids are key modulators of the attenuated stress fever in adult rats treated with LPS as neonates, and they act downregulating PGE<sub>2</sub> production. Moreover, our findings also support the notion that neonatal immune stimulus affects programming of stress responses during adulthood, despite the fact that inflammation and stress are two distinct processes mediated largely by different neurobiological mechanisms.

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#### P17

##### Innate immunity and inflammation in sepsis: mechanisms by which acute ethanol exposure alters the course of sepsis and the effect to TLR4 signaling

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**Introduction** Alcohol consumption is a significant risk factor for mortality in patients with sepsis. Alcohol is the most widely abused substance worldwide and numerous studies have revealed that it has widespread effects on the immune system and leaves abusers at increased risk of a variety of infections. An increased predisposition to infection among patients with alcohol use problems may also mediate an association with sepsis.

**Objective** The present study was carried out to investigate the mechanisms by which acute ethanol exposure alters the course of sepsis and the effect of TLR4 signaling.

**Methods** Two different strains of mice, C3H/HeJ (TLR4-mutants) and C3H/HeOuJ (wildtype), were treated with a dosage of 6 g/kg ethanol, which yields a blood-ethanol concentration of ~0.4%, similar to the blood-ethanol levels that occur in ethanol-dependent humans. Viable, indigenous *Escherichia coli*, log-phase, grown in LB broth was administered intraperitoneally. The dosage of *E. coli* was  $2 \times 10^8$  per mouse, which serves as a model for loss of intestinal integrity and release of bacteria in large numbers. Blood samples were obtained retro-orbitally while the animal was under halothane anesthesia. After euthanasia, peritoneal lavage was performed and samples of this fluid were used to quantify bacteria by making serial dilutions in LB agar, and for cell-counting, for cytospin and cytokine and chemokine study. Spleen was also harvested from all the mice for carrying out bacterial quantification, RNA analysis, and flow-cytometry analysis.

**Results** Ethanol administration decreases resistance to *E. coli* and causes a decrease in the ability to clear bacteria both from the peritoneal cavity as well as the spleen. At early time points, ethanol also suppresses the production of proinflammatory cytokines (for example, IL-1, IL-17, IFN $\gamma$ , TNF $\alpha$ , and so forth) and chemokines (for example, Eotaxin, RANTES, MIP-1, MIG, LIX, and so forth). Most (80 to 90%) of the cells in the peritoneal cavity were found to be macrophages (full of bacteria) and hardly any neutrophils could be found. See Figure 1.

**Conclusions** Ethanol decreases clearance of bacteria in the peritoneal cavity and increases mortality. Ethanol also decreases production of most proinflammatory cytokines and chemokines. A large number of macrophages in the peritoneal fluid indicates decreased attraction of neutrophils to the

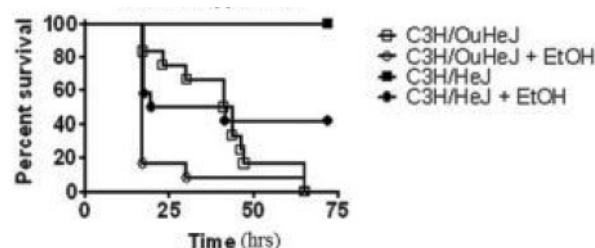


Figure 1 (abstract P17). Survival of TLR4 mutant (HeJ) versus wildtype mice.

peritoneal cavity, decreased clearance of bacteria by macrophages and neutrophils in the peritoneal cavity, and, hence, increased mortality. TLR4 is dispensable for survival in *E. coli* sepsis but it also contributes to lethality in wildtype mice. Although TLRs have been implicated as an important element of host defense against infections, evidence indicates that these receptors may also play a crucial role in the pathophysiology of sepsis.

**P18**

**Lipopolysaccharide alters expression of incretin receptors in monocytic and hepatocytic cell lines**

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*Critical Care* 2010, **14**(Suppl 2):P18 (doi: 10.1186/cc9121)

**Introduction** Sepsis hyperglycemia is poorly understood. It is not known whether there is a role in sepsis hyperglycemia for glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP), crucial for normal glucose metabolism. We developed an *in vitro* model of sepsis employing monocytes (crucial cells in mediating sepsis) and hepatocytes (crucial cells in carbohydrate homeostasis) to clarify the role of the incretin system in sepsis.

**Objective** To establish an *in vitro* model of sepsis employing monocytic (U937) and hepatocytic (HUH7) cell lines by co-incubation with lipopolysaccharide (LPS) and to determine whether receptor expression for GIP, GLP-1, and insulin (INS) was altered.

**Methods** U937 (monocyte cell line) and HUH7 (hepatocyte cell line) cells were cultured with different concentrations of LPS for 24 hours. Real-time RT-PCR quantitation of gene expression was used to compare the rates for relative expression.

**Results** U937 and HUH7 cells expressed mRNA GIPR (including GIPR protein expression in HUH7 cells), and INSR, but only HUH7 expressed GLP-1R. There was an inverse relationship between the LPS dose and mRNA expression for GIPR ( $P < 0.05$ ). For example at 5 µg/ml LPS, the expression of GIPR was reduced to 86% and INSR 72% of control in U937; while in HUH7 cells at 1 µg/ml LPS, the GIPR expression was decreased to 63%, GLP-1R 95% and INSR 89% compared with control ( $P < 0.001$ ). A direct significant relationship between LPS and inflammatory cytokines IL-1 ( $P < 0.05$ ) and IL-6 ( $P < 0.05$ ) in both cell lines validated our model.

**Conclusions** We not only show for the first time GIPR mRNA expression on U937 cells and expression of GIPR and GLP-1R on hepatocyte cell line, but also their downregulation with LPS. The LPS-mediated alteration in incretin receptor expression on these cell lines may be relevant to changes in cytokine secretion and carbohydrate metabolism in sepsis.

**P19**

**The new sepsis marker, sCD14-ST, induction mechanism in the rabbit sepsis models**

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*Critical Care* 2010, **14**(Suppl 2):P19 (doi: 10.1186/cc9122)

**Introduction** Soluble CD14 subtype (sCD14-ST) is a fragment of CD14 and is markedly increased in sepsis patients. We developed a new immunoassay to detect sCD14-ST and evaluated the efficacy of this marker for diagnosis of sepsis. For developing the strategies of sCD14-ST as a sepsis diagnostic marker, the induction mechanism must be known.

**Methods** To determine the kinetics of sCD14-ST in the rabbit endotoxin shock model and the cecal ligation and puncture (CLP) model, we prepared the rabbit sCD14-ST immunoassay. Induction by inflammatory inducers and inhibition of sCD14-ST production were assessed using rabbit abdominal cavity granulocytes. Fragmentation of CD14 by *N*-aspartic protease was analyzed by western blot analysis and immunoassay.

**Results** sCD14-ST was induced in the CLP model. However, sCD14-ST was not induced in the endotoxin shock model. These results suggested that sCD14-ST was not induced after stimulation by physiologic activating agent but induced by bacterial infection. sCD14-ST was not induced after stimulation of rabbit granulocytes by LPS, IFN $\gamma$ , FMLP, and PMA. In contrast, it was induced by adding *Escherichia coli*, indicating that sCD14-ST is produced

by phagocytosis rather than inflammation. The phagocytosis inhibitors cytochalasin D and wortmanin inhibited the production of sCD14-ST *in vitro*. Additionally, *N*-asparagin protease inhibitor inhibited the production of sCD14-ST from granulocytes. Additionally sCD14-ST was detected from recombinant CD14 digested supernatant by cathepsin D enzyme.

**Conclusions** These data suggested that induction mechanism of sCD14-ST is dependent on the phagocytosis and cathepsin D is one of the enzymes for fragmentation of CD14. This mechanism is strong evidence for explanation of the production of sCD14-ST in sepsis patients.

**P20**

**Impact of delayed antimicrobial therapy in septic ITU patients**

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*Critical Care* 2010, **14**(Suppl 2):P20 (doi: 10.1186/cc9123)

**Introduction** There is evidence that early delivery of antibiotics following the recognition of severe sepsis leads to decreased morbidity and indeed mortality. It is estimated that 36,800 people die annually in the UK as a result of severe sepsis, claiming more lives than bowel and breast cancer combined [1]. Patients admitted to ICUs with severe sepsis have a 39.8% risk of death [2], and for each hour delay in antibiotic administration, a 7.6% increase in mortality [3]. The Surviving Sepsis Campaign 2008 recommends that appropriate antimicrobial therapy be administered within 1 hour following recognition of severe sepsis [4].

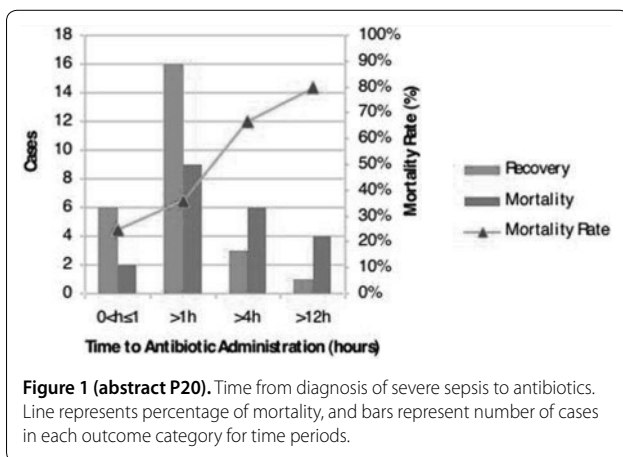
**Methods** We conducted a prospective audit of consecutive patients with severe sepsis admitted to an ITU between February and June 2010. The patients were identified as those who fulfilled two or more components of the systemic inflammatory response syndrome (SIRS) criteria, and had evidence of organ dysfunction requiring critical care. Compliance to the Surviving Sepsis Campaign's antibiotic care bundle was audited. The relationship between time of antibiotic administration and mortality was also determined.

**Results** During the study period, 33 patients out of 187 admissions met the inclusion criteria. The population demographics are illustrated in Table 1. The mean time from fulfilling SIRS criteria to delivery of antibiotics was 4.32 hours. Only eight (25%) of the patients received antibiotics within the

**Table 1 (abstract P20). Demographic characteristics of 33 patients with septic shock treated in an ICU**

Variable	Number (%)
Mean age (years)	62.1
Male gender	20 (65)
Deaths	11 (33)
Source of sepsis	
Chest	21 (75)
Urinary tract	5 (15)
Intraabdominal	3 (9)
Soft tissue	2 (6)
Other	2 (6)
SIRS criteria	
Temperature >38 or <36°C	16 (49)
HR >90 bpm	33 (100)
RR >20/min or PCO <sub>2</sub> <4.3 kPa	26 (78)
WBC >12 or <4 c/mm <sup>3</sup>	24 (72)
>2 SIRS criteria	33 (100)
Systolic BM <90 mmHg	17 (51)
Lactate >4 mol/l	5 (15) (unrecorded 36%)
Organ dysfunction	28 (64)





hour, with the mortality rate for this group being 25%. Those patients who received antibiotics after 4 hours had a lower mortality rate than the group that received antibiotics after 12 hours (67% vs. 80%). See Figure 1.

**Conclusions** Our results support published evidence that a delay in antibiotic delivery greater than 1 hour is associated with increased mortality in patients treated in the ITU. As a result of this study we have developed a standardized sepsis protocol to integrate into the AE triage *pro forma*, as well as a pathway to help instigate treatment earlier to those patients identified as septic on the wards. Recruitment period has not concluded. More data analysis will be presented later.

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#### P21

##### Estimating coagulopathy in an ovine acute lung injury model of sepsis using a disease progression model

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*Critical Care* 2010, **14**(Suppl 2):P21 (doi: 10.1186/cc9124)

**Introduction** Acute lung injury (ALI) caused by smoke inhalation with or without bacterial pneumonia remains a significant cause of morbidity and mortality among burn patients. Bacterial pneumonia in an ALI patient is particularly worrisome because it often leads to sepsis. Although much of the literature surrounding ALI and pneumonia-induced sepsis has rightfully focused on pulmonary and endothelial changes, a major consequence of ALI and an area of continued research and drug development is coagulopathy. The objective, therefore, of our study was to determine whether coagulopathy differs between types of ALI and whether the dysregulation can be estimated using a disease progression model.

**Methods** Nineteen sheep with acute lung injury were incorporated into this pneumonia-sepsis model. Pneumonia was induced by inoculating the airway with  $\sim 2.5 \times 10^{11}$  colony-forming units (CFUs) methicillin-resistant *Staphylococcus aureus* (MRSA), while smoke injury was created through inhalation of cotton smoke. The injury groups studied were as follows; MRSA and smoke inhalation (M+S), MRSA untreated (M), MRSA treated (M+T), and smoke inhalation only (S). Data were modeled over 24 hours. First, all

the sheep were modeled together to determine a rank-order of the injury groups. After rank-ordering the groups, the groups became model inputs and in conjunction with other clinical and laboratory variables were used to estimate the output parameter, prothrombin time (PT). In order to minimize overparameterization of a small patient population, the model was allowed to estimate PT using only two parameters.

**Results** The number of sheep in each group was as follows; seven M+S, three M, three M+T, and six S. The rank-order of injury from least to greatest severity was M+T, S, M, M+S. The two highest-ranking parameters in estimating PT were calcium and injury. When using calcium and injury alone, the model estimate agreement with measured PT was  $r^2 = 0.70$  and  $r^2 = 0.36$ , respectively. Allowing the model to combine the inputs did not improve the model estimate ( $r^2 = 0.70$ ) compared with when calcium was used alone.

**Conclusions** The progression model allowed all individual sheep to be characterized as to the severity of resulting coagulopathy and identified some important co-factors. Acute lung injury can lead to systemic coagulopathy even without MRSA infection, but the extent and severity is greater with infection.

#### P22

##### Overexpression of PD-1-related molecules is associated with lymphocyte anergy, mortality, and development of nosocomial infections in septic shock patients

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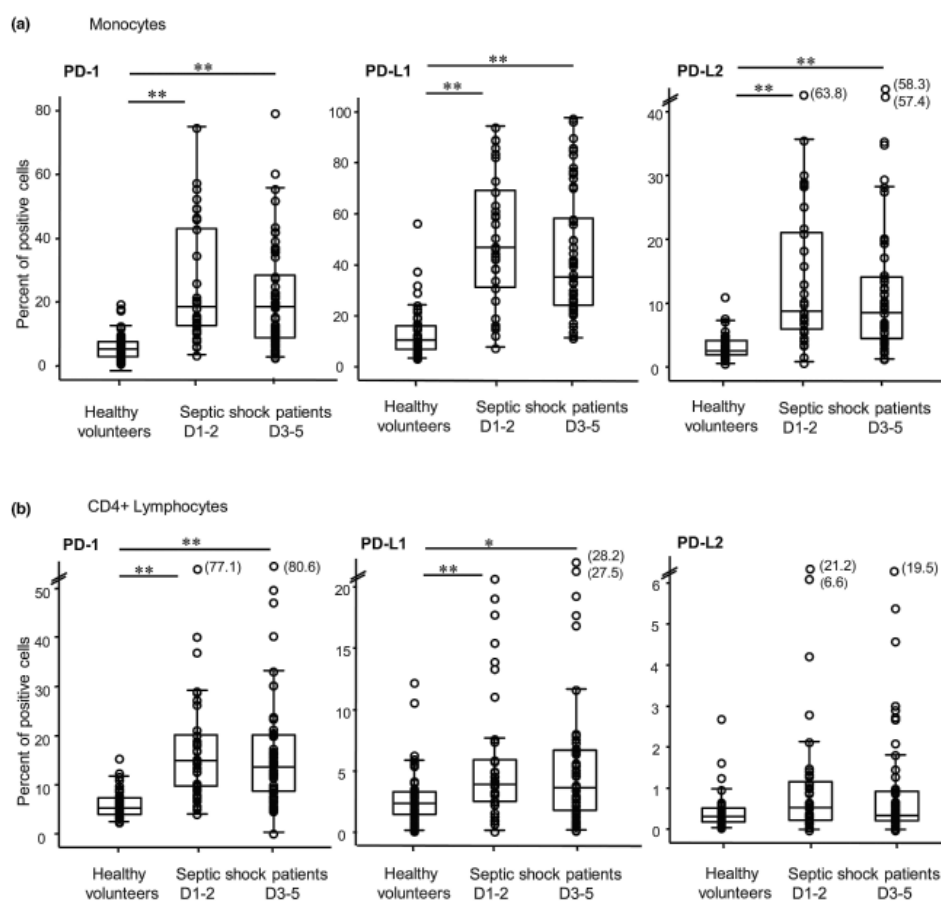
*Critical Care* 2010, **14**(Suppl 2):P22 (doi: 10.1186/cc9125)

**Introduction** Septic syndromes are a culmination of multiple partially understood dynamic processes. However, it is now established that, after a transient exacerbated proinflammatory response, a counter-regulatory phase develops, rapidly inducing immune alterations that are thought to play a major role in patients' mortality and susceptibility to nosocomial infections. Programmed Death-1 (PD-1) receptor and its ligands PD-L1 and PD-L2 constitute a newly described pathway that negatively controls immune responses. Recently, improved bacterial clearance and decreased mortality were observed in PD-1 knockout mice [1]. The objective of the present study was to investigate PD-1-related molecule expressions in septic shock patients.

**Methods** PD-1-related molecule expressions were measured by flow cytometry on circulating leukocytes from 64 septic shock patients and 49 healthy individuals. Severity scores (SAPS II, SOFA), clinical events (28-day mortality, occurrence of nosocomial infections) and the usual biomarkers of sepsis-induced immunosuppression (monocyte HLA-DR expression, lymphocyte phenotyping including Treg, plasmatic IL-10 concentration) were assessed. *Ex vivo* functional assays such as lymphocyte proliferation ( $^3\text{H}$  thymidine incorporation) in response to phytohemagglutinin, and cytokine release (TNF $\alpha$  and IL-10 assessed by Bio-Plex technique) after overnight LPS incubation, were performed in the presence of blocking antibodies against PD-1-related molecules.

**Results** Patients presented with typical features of sepsis-induced immunosuppression (decreased mHLA-DR expression, increased Treg percentage, decreased LPS-induced TNF $\alpha$  release). At days 1 to 2 and days 3 to 5 after the onset of shock, patients displayed increased PD-1 and PD-L1 expressions on CD4<sup>+</sup> T lymphocytes and enhanced PD-1, PD-L1 and PD-L2 expressions on monocytes. See Figure 1 overleaf. Non-survivors presented with increased monocyte PD-L1 expression while enhanced monocyte PD-1 or PD-L2 expressions were associated with the occurrence of secondary nosocomial infections. In addition, decreased mitogen-induced lymphocyte proliferation was negatively correlated with increased lymphocyte PD-1 and PD-L1 expressions whereas monocyte PD-1-related molecule expressions were highly correlated with increased circulating IL-10 concentration. No beneficial effects of anti-PD-1-related molecule antibodies were observed.

**Conclusions** We describe here for the first time the overexpression of PD-1-related molecules on circulating leukocytes in septic shock patients. Importantly, these increased expressions were significantly associated with the occurrence of immune dysfunctions, secondary nosocomial infection, and death after septic shock. Taken together, our results suggest that PD-1-related molecules may constitute an additional regulatory system involved



**Figure 1 (abstract P22).** PD-1, PD-L1 and PD-L2 measurements on circulating monocytes and CD4<sup>+</sup> lymphocytes in septic shock patients and healthy volunteers. PD-1-related molecule expressions were measured on (a) circulating monocytes and (b) CD4<sup>+</sup> lymphocytes in whole blood from healthy volunteers ( $n=40$ ) and septic shock patients at D1 to D2 ( $n=37$ ) and at D3 to D5 ( $n=56$ ) after the onset of shock. Results presented as percentages of positive cells among total population of monocytes or CD4<sup>+</sup> lymphocytes and as boxplots and individual values. \* $P<0.020$ , \*\* $P\leq 0.002$  (Mann-Whitney test).  $P<0.025$  was considered statistically significant (with correction by the number of tests).

in sepsis-induced immune alterations. This may offer innovative therapeutic perspectives for the treatment of this hitherto deadly disease.

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#### P23

##### CCR2 drives neutrophil infiltration and elicits tissue damage in remote organs during sepsis

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**Introduction** The severe form of sepsis is associated with multiple organ dysfunction syndrome (MODS), but the precise mechanisms by which MODS develops remain unclear. Neutrophils are essential cellular components of the innate immune system that have conserved roles in bacterial containment. Paradoxically, however, neutrophils also mediate tissue injury in varied human diseases, including sepsis.

**Objective** In the present study, we investigated the role of chemokine receptor CCR2 in driving neutrophil infiltration and eliciting tissue damage in remote organs during sepsis.

**Methods and results** We demonstrated that neutrophils, which are normally unresponsive to CCR2 chemokines, acquired substantial chemotaxis to CCL2 and CCL7 when exposed to LTA (4.33-fold and 3.02-fold increase, respectively) or LPS (4.67-fold and 3.29-fold increase, respectively). Moreover, consistent with the functional response, we found that TLR2 and TLR4 signaling through the MyD88/NF- $\kappa$ B pathway mediates the upregulation of CCR2 and chemotactic responsiveness to CCR2 ligands on neutrophils. *In vivo*, intravenous injection of TLR ligands or induction of cecal ligation and puncture (CLP)-induced sepsis triggered chemotaxis of circulating neutrophils to CCR2 chemokines, which was completely abolished in MyD88-deficient mice. Notably, CCR2-deficient (CCR2<sup>-/-</sup>) or WT mice treated with CCR2 antagonist (RS504393, 2 mg/kg) showed a significant increased survival rate after CLP when compared with WT mice. Deficiency or pharmacology blockade of CCR2 attenuated neutrophil infiltration (by myeloperoxidase activity) into the lungs, heart, and kidneys, which was associated with reduction of serum biochemical markers of organ injury/dysfunction. Importantly, neutrophils from septic patients ( $n=19$ , prospectively survivors (S) and nonsurvivors (NS)) showed an increase of median of fluorescence intensity (MFI) of CCR2 by flow cytometry (S =  $5.76 \pm 2.30$  vs. NS =  $9.12 \pm 1.72$ , MFI), which was related to the chemotactic response to CCL2 (S =  $6.35 \pm 0.68$  vs. NS =  $10.56 \pm 2.38$ , neutrophils/field). Furthermore, there was a positive correlation between SOFA scores with the neutrophil response to CCL2 ( $r^2 = 0.62$ ,  $P < 0.01$ ).

**Conclusions** Collectively, our study identified CCR2 as an important receptor that drives the inappropriate infiltration of neutrophils into remote organs during sepsis. Therefore, CCR2 blockade could be an adjuvant therapeutic strategy for treatment of sepsis-induced MODS.

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P24

**Prospective multicenter study of the effect of early fluid resuscitation on trends in IL-6 and TNF $\alpha$  levels in severe sepsis**

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*Critical Care* 2010, **14**(Suppl 2):P24 (doi: 10.1186/cc9127)

**Introduction** The prognostic capability of TNF $\alpha$  and IL-6 is limited in septic shock. Previous studies were performed prior to publication of current therapeutic guidelines recommending aggressive early resuscitation. The objective of the present study was to evaluate the impact of early fluid resuscitation on serial TNF $\alpha$  and IL-6 levels and its association with mortality in severe sepsis.

**Methods** This is a substudy of a previously completed prospective, observational multicenter investigation of patients with severe sepsis. Inclusion criteria were age >17, infection with  $\geq 2$  SIRS, hypotension despite fluid challenge, treatment with a standardized quantitative resuscitation protocol, and identification within 3 hours of treatment initiation. Blood samples were obtained at enrollment, 6 hours, and 24 hours. Therapeutic amounts of intravenous crystalloid fluid was defined by  $\geq 5$  l and <5 l over 24 hours (initial 2 l fluid challenge over 4 hours followed by 150 ml/hour for 20 hours). Data analysis compared absolute levels of TNF $\alpha$  and IL-6 at each time point between survivors and nonsurvivors. The magnitude and direction of serial cytokine levels was quantified by the percentage difference of each marker for each patient between 0 and 6 hours and 0 and 24 hours. Statistical

analysis was performed using the Wilcoxon-rank-sum test or the Student *t* test.

**Results** Forty patients were enrolled; 11 died. Vasopressors were required in 60% of all patients. Absolute values of IL-6 (pg/ml) were higher in nonsurvivors than survivors at enrollment (5,479 vs. 710); 6 hours (4,180 vs. 405), and 24 hours (5,710 vs. 377) ( $P < 0.05$ ). There was no difference in TNF $\alpha$  values between the two groups ( $P = NS$  at 0, 6, 24 hours). Nonsurvivors had a larger percentage (difference) in both TNF $\alpha$  and IL-6 than survivors at 24 hours. See Figure 1. Treatment with  $\geq 5$  l intravenous fluid over 24 hours was associated with a 32% decline in IL-6 compared with a 64% increase in IL-6 with <5 l fluid therapy. See Figure 2.

**Conclusions** In the context of a quantitative protocol for the treatment of severe sepsis, high-volume fluid resuscitation is associated with a decline in the percentage difference of IL-6. Trends in the percentage difference of both TNF $\alpha$  and IL-6 differentiate survivors from nonsurvivors. Further investigation is needed into the impact fluid resuscitation has on decreasing the inflammatory insult and the use of serial cytokine measurements as a measure of therapeutic effectiveness.

**Acknowledgements** Conducted within the Emergency Medicine Shock Research Network (EMShockNet). RA has no financial disclosures relevant to this study but has received research funding from Hutchinson Technologies. The present study was supported in part by a grant from the Shock Society/Novo Nordisk research grant for Hemorrhagic Shock and Hemostasis to ST. AJ's effort is supported by a grant from the National Institutes of Health/National Institutes of General Medical Sciences K23GM076652. NS is supported in part by grants from the National Institutes of Health L091757 and GM076659.

P25

**Severity of illness scoring systems in community-acquired *Legionella* pneumonia**

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**Introduction** Prognostic and severity-of-illness scoring systems are valuable tools for predicting mortality and choosing the site of care for patients with community-acquired pneumonia (CAP) [1]. Legionnaires' disease (LD) is a pneumonia caused by *Legionella* spp. and carries a higher mortality rate (5 to 30%) than CAP of most other etiologies. The aim of our study was to evaluate five scoring systems commonly used in CAP for predicting mortality in patients with *Legionella pneumophila* serogroup 1 infection admitted during a large LD outbreak [2,3].

**Methods** Patients with microbiologically verified LD ( $n = 103$ ) and CAP patients with epidemiological association to the outbreak with no other bacteriological etiology identified ( $n = 32$ ) were included. A clinical protocol was initiated during an early phase of the outbreak, and clinical and biochemical data were collected from patients on admission to the regional hospital. The five evaluated scoring systems were: pneumonia severity index (PSI), CURB-65 (confusion, uremia, respiratory rate  $\geq 30$ , low blood pressure, age  $\geq 65$ ) and CRB-65 score, the modified American Thoracic Society (ATS) score, and the IDSA/ATS guidelines. The endpoint was defined as 28-day mortality.

**Table 1 (abstract P25). Sensitivity, specificity and predictive values for mortality prediction of five severity-of-illness scoring systems in 132 outbreak patients with confirmed and presumptive Legionnaires' disease**

	Sensitivity ( $n = 16$ ) (%)	Specificity ( $n = 119$ ) (%)	PPV (%)	NPV (%)
PSI class IV and V	94	44	19	98
CURB-65 score $\geq 2$	88	47	19	97
CRB-65 score $\geq 2$	81	58	21	96
Modified ATS	31	91	31	91
IDSA/ATS	63	77	26	94

PPV, positive predictive value; NPV, negative predictive value.

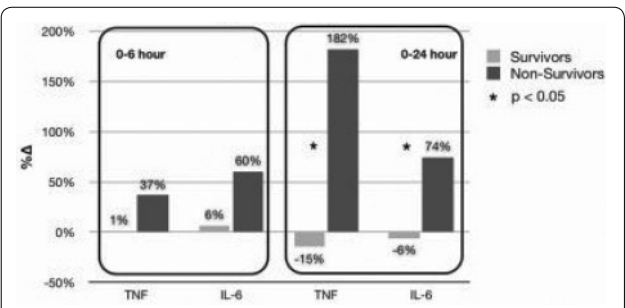


Figure 1 (abstract P24). %Δ IL-6 and TNF.

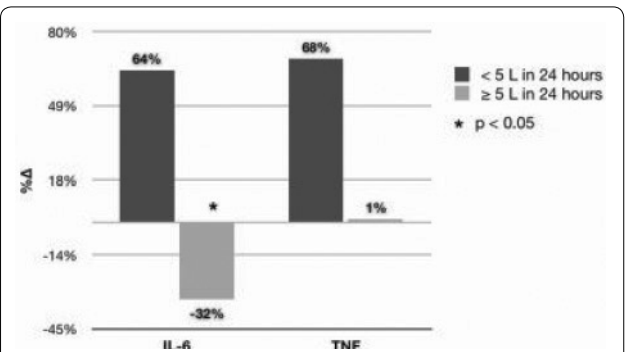


Figure 2 (abstract P24). %Δ at 24 hours.

**Results** The overall mortality rate was 12% (16/135), and 19% (25/135) were admitted to the ICU. The discriminatory power was highest for PSI, CURB-65 and CRB-65 with area under the receiver operator characteristic curve (AUC) of 0.79, 0.78, and 0.75, respectively. The AUC of the modified ATS score and IDSA/ATS guidelines were 0.61 and 0.69, respectively. Table 1 shows that a PSI class IV or V, and a CURB-65 and CRB-65 score  $\geq 2$  yielded the highest sensitivity for prediction of mortality, but the specificity and positive predictive value was low.

**Conclusions** The PSI, the CURB-65 and CRB-65 scores proved sensitive in predicting mortality in patients with *Legionella* pneumonia admitted during an LD outbreak, but the low specificities and positive predictive values necessitate thorough clinical judgment in patients with a high severity score. The modified ATS score and IDSA/ATS guidelines, which are decision recommendations for ICU admission, were not sensitive in predicting mortality from LD in this study.

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#### P26

##### Increased survival after a cecal ligation and puncture-induced sepsis in mice consuming oleic acid

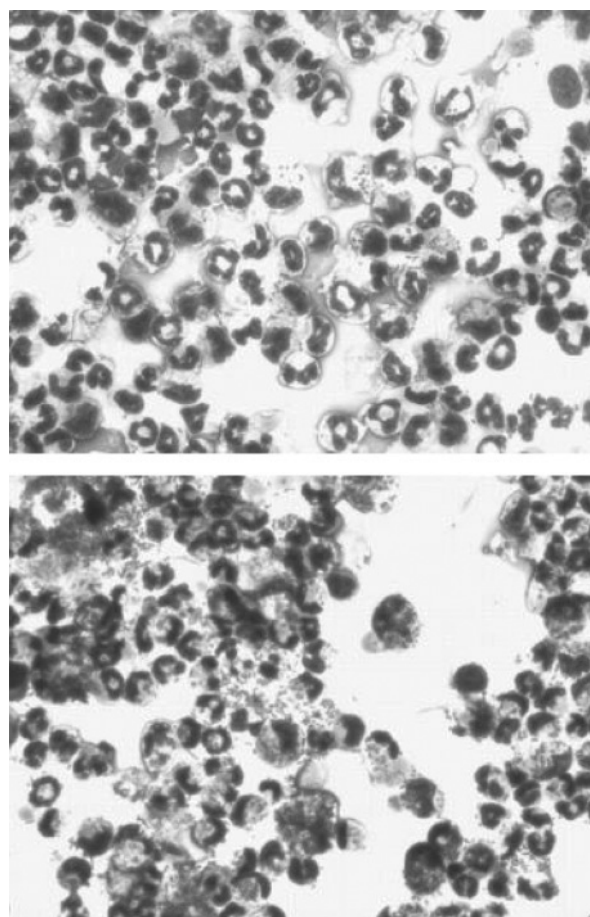
IM de Moraes, F Magno, C Campbell, P Estevam, C Araújo, P Bozza, C Gonçalves-de-Albuquerque, A Silva, HCF Neto  
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**Introduction** Sepsis accounts for a huge number of deaths in ICUs worldwide. Sepsis describes a complex clinical syndrome that results from an infection, setting off a cascade of systemic inflammatory responses that can lead to multiple organ failure and death. Leite and colleagues have shown that mice fed for 6 weeks with an olive oil diet were resistant to endotoxic shock, with 60% survival at 168 hours [1]. Olive oil is composed of different polyunsaturated fatty acids such as omega 3 and 6, but the monounsaturated fatty acid omega 9, also known as oleic acid (OA), that is the main component of olive oil, is highly consumed in the Mediterranean diet.

**Objective** We aim to investigate the role of OA in an experimental model of sepsis.

**Methods** Swiss mice were given daily doses (orally) of OA, at 282  $\mu\text{g}/\text{animal}$ , for 15 days. Control animals received saline. On the 16th day, polymicrobial sepsis was induced by cecal ligation and puncture (CLP). Immediately after the procedure, all mice received volemic reposition and after 6 hours animals were given imipenem. Twenty-four hours after surgery, mice were euthanized and the peritoneal cavity was rinsed with sterile saline. Total leukocyte counts were performed in a Neubauer chamber and differential leukocyte were stained with May-Grünwald Giemsa. The supernatant and plasma were collected for cytokine quantification. In another set of experiments, the survival rate was determined daily for 7 days in separate groups of 10 animals for each condition.

**Results** Mice fed with OA were resistant to sepsis, with a 64% survival rate at 168 hours compared with saline-treated mice (33%). OA supplementation in CLP-subject animals led to a significant decrease in the total leukocyte counts ( $10.69 \times 10^6 \pm 1.71$ ), mainly neutrophils, compared with mice that received saline ( $20.30 \times 10^6 \pm 2.69$ ). However, in mice that consumed OA the levels of TNF $\alpha$ , IL-10 and IL-6 were not significantly different from mice fed with saline submitted to CLP. Interestingly, preliminary data showed that mice fed with OA had a lower level of bacteria in the peritoneal lavage leukocyte compared with mice submitted to CLP. See Figure 1.



**Figure 1 (abstract P26).** Bacterial count in the peritoneal lavage leukocyte is lower in oleic acid-treated mice submitted to CLP.

**Conclusions** Our data suggest that treatment with OA reduces mortality in an experimental model of sepsis and attenuates inflammation. One mechanism involved may be due to an increased bacterial clearance in mice fed with OA. More data are required to clarify this mechanism of increased survival.

**Acknowledgements** This presentation was made possible by partial support from CNPq, FIOCRUZ and FAPERJ.

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#### P27

##### Anti-inflammatory effect of procalcitonin on *in vitro* LPS-stimulated human PBMC

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**Introduction** Sepsis is a leading cause of death in critically ill patients and is characterized by a marked increase of the host proinflammatory cytokine release that is precipitated by infectious agents. The sepsis response to therapy has not appreciably improved. The procalcitonin (PCT) concentration is increased in the serum samples of septic patients and correlates with severity of the illness. LPS is a pivotal bacterial product involved in pathogenesis of sepsis and septic shock. Preventing the beginning of inflammatory systemic cascade by means of LPS modulating agents might have a valuable effect in the control of such deadly illness. The aim of the present study was to

evaluate the potential effect of procalcitonin on *in vitro* LPS-induced release of cytokines from human PBMC.

**Methods** *S. typhimurium* LPS was preincubated with human PCT and then was added to freshly isolated human PBMC cultures in RPMI 1640. In such cultures the final concentrations of LPS and PCT were 10 ng/ml and either 5,000 or 500 ng/ml respectively. A panel of cytokines was evaluated on culture supernatants by Biochip microarray (Randox) and PCT was tested by ELFA. Data analysis was carried out by a nonparametric method (Mann-Whitney U test, Graph Pad Prism version 4.03) to establish statistical differences between groups.

**Results** Both of the PCT concentrations used significantly ( $P < 0.05$  vs. LPS-stimulated PCT-free controls) reduced TNF $\alpha$  (after 4-hour incubation with LPS) and MCP-1 (24 hours following LPS challenge). The lower concentration of PCT was also able to significantly ( $P < 0.05$  vs. LPS-stimulated PCT-free controls) decrease the TNF $\alpha$  and IL-2 levels in the 24-hour samples.

**Conclusions** PCT, besides the well-known role as marker of sepsis, could be a potentially useful molecule to control systemic inflammatory mediators in both sepsis and septic shock.

## P28

### Clinical effects of adsorption of lipopolysaccharide in the treatment of Gram-negative severe sepsis

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Critical Care 2010, **14**(Suppl 2):P28 (doi: 10.1186/cc9131)

**Introduction** Sepsis is a major cause of morbidity and mortality in intensive care clinics and the incidence is continuously increasing. Estimated mortality rates are over 30% for patients with severe sepsis and over 50% for patients with septic shock. Endotoxins, or lipopolysaccharides (LPSs), are a major component of the cell membrane of Gram-negative bacteria. LPS is well known to induce a strong response from the immune system leading to release of inflammatory mediators and occasionally sepsis, or even septic shock. The aim of our study is to investigate the clinical effects of adding treatment with the Alteco® LPS Adsorber (Alteco Medical AB, Lund, Sweden) to standard therapy for patients with septic shock.

**Methods** Our study included 12 patients with septic shock and endotoxemia randomized 1:1 to standard therapy plus LPS adsorption (adsorber group (AdG),  $n = 6$ ) or standard therapy alone (reference group (RefG),  $n = 6$ ). Randomization of patients will be performed using sealed envelopes.

This included five women and seven men; mean age  $47.3 \pm 24.8$  years. All patients needed inotropic support and mechanical lung ventilation. The mean APACHE II score at start of treatment was  $26.6 \pm 2.3$ . Both groups in the study received standard therapy (Surviving Sepsis Campaign International Guidelines 2008) for patients in septic shock. For patients in the study group, treatment with the Alteco® LPS Adsorber was added to standard therapy. The adsorber treatment was initiated immediately after inclusion in the study; that is, as soon as possible after onset of septic shock. The duration of Alteco® LPS Adsorber treatment was 120 minutes repeated twice within 24 hours. Hemofiltration/hemoperfusion in the study group was not carried out during the perfusion. Samples for endotoxin and PCT analysis shall be taken from the arterial line. Hemodynamic parameters are registered according to routines at the clinic (PICCO technology).

**Results** Hemodynamic data, laboratory results, and blood gas analysis are summarized in Table 1. LPS was significantly lower in all patients in the study group after treatment. No complications related to the use of the Alteco® LPS Adsorber were seen and all patients in this group were discharged from the ICU after 45 to 68 days, respectively.

**Conclusions** We have received a statistically significant improvement in hemodynamics, oxygenation, and reduced markers of endotoxemia in group therapy with Alteco® LPS Adsorber compared with traditional therapy. These effects were attributed with the removal of endotoxin from the systemic circulation. Only in one case using hemofiltration for acute renal failure in the study group (in the reference group in all patients), 28-day mortality was 16.7% and 66.7% respectively. Negative effects were negligible.

## P29

### Abstract withdrawn

Table 1 (abstract P28). Results from analyses of samples

Parameter	Stage							
	I		II		III		IV	
	RefG	AdG	RefG	AdG	RefG	AdG	RefG	AdG
LPS (EU/ml)	1.4 $\pm$ 0.0	1.4 $\pm$ 0.0	1.4 $\pm$ 0.2	0.8 $\pm$ 0.2	1.4 $\pm$ 0.6	0.4 $\pm$ 0.3	1.4 $\pm$ 0.2	0.2 $\pm$ 0.0
PCT (ng/ml)	15.6 $\pm$ 2.8	16.0 $\pm$ 2.5	16.4 $\pm$ 2.2	11.6 $\pm$ 3.7	14.3 $\pm$ 2.8	4.2 $\pm$ 2.2	13.6 $\pm$ 3.8	1.4 $\pm$ 0.9
CI (l/min/m <sup>2</sup> )	2.7 $\pm$ 0.8	2.9 $\pm$ 0.7	2.9 $\pm$ 0.8	3.9 $\pm$ 0.9	3.1 $\pm$ 0.6	3.0 $\pm$ 0.7	2.9 $\pm$ 0.5	4.7 $\pm$ 1.9
MAP (mmHg)	60.6 $\pm$ 4.3	59.1 $\pm$ 5.8	66.9 $\pm$ 5.5	79.7 $\pm$ 4.6	69.4 $\pm$ 1.4	81.1 $\pm$ 2.9	69.1 $\pm$ 3.2	83.8 $\pm$ 5.7
Pa <sub>2</sub> (mmHg)	88.2 $\pm$ 2.1	84.5 $\pm$ 12.1	89.9 $\pm$ 14.4	108.7 $\pm$ 16.1	88.3 $\pm$ 9.4	129.4 $\pm$ 12.2	91.7 $\pm$ 7.8	130.6 $\pm$ 9.2
FI <sub>O<sub>2</sub></sub> (%)	60.0 $\pm$ 0.0	50.0 $\pm$ 4.1	50.0 $\pm$ 0.0	40.1 $\pm$ 4.3	50.0 $\pm$ 0.0	37.4 $\pm$ 3.5	50.0 $\pm$ 0.0	35.0 $\pm$ 0.0
ELWI (ml/kg)	12.9 $\pm$ 3.3	13.2 $\pm$ 1.2	13.1 $\pm$ 2.2	9.2 $\pm$ 3.1	11.0 $\pm$ 2.7	7.1 $\pm$ 1.1	9.7 $\pm$ 2.1	4.3 $\pm$ 2.1
PAO <sub>2</sub> /FI <sub>O<sub>2</sub></sub>	146.4 $\pm$ 12.1	168.4 $\pm$ 16.5	179.2 $\pm$ 10.1	270.1 $\pm$ 24.3	176.3 $\pm$ 9.5	345.0 $\pm$ 21.1	183.2 $\pm$ 12.1	373.5 $\pm$ 14.3
Dopamin (μg/kg/min)	12.0 $\pm$ 1.7	13.3 $\pm$ 3.9	14.2 $\pm$ 2.2	8.1 $\pm$ 2.1	13.5 $\pm$ 3.1	3.3 $\pm$ 1.4	11.7 $\pm$ 1.9	3.1 $\pm$ 0.3
SaO <sub>2</sub> (%)	87.3 $\pm$ 2.2	86.7 $\pm$ 3.1	86.9 $\pm$ 1.9	94.9 $\pm$ 2.8	89.5 $\pm$ 3.5	98.2 $\pm$ 0.9	93.6 $\pm$ 5.4	98.5 $\pm$ 0.4
SVO <sub>2</sub> venous (%)	79.8 $\pm$ 2.5	80.0 $\pm$ 1.5	82.2 $\pm$ 2.3	76.0 $\pm$ 3.4	85.3 $\pm$ 3.1	71.3 $\pm$ 2.4	79.8 $\pm$ 2.9	72.8 $\pm$ 3.7
Lactate (mol/l)	4.9 $\pm$ 2.1	4.8 $\pm$ 1.7	5.5 $\pm$ 1.3	3.2 $\pm$ 1.1	5.3 $\pm$ 1.3	2.4 $\pm$ 0.9	5.4 $\pm$ 0.9	1.7 $\pm$ 0.6
APTT (sec)	41.5 $\pm$ 5.9	42.1 $\pm$ 4.6	46.6 $\pm$ 7.7	58.4 $\pm$ 5.7	48.8 $\pm$ 12.1	66.3 $\pm$ 8.5	50.2 $\pm$ 11.8	62.3 $\pm$ 3.4

I, baseline, prior to first adsorber treatment; II, prior to second adsorber treatment/day after first treatment; III, 2 days after second adsorber treatment/3 days after first adsorber treatment.



### P30

#### Clinical impact of a multiplex real-time PCR assay (SeptiFast®) for the rapid detection of pathogens in patients with end-stage heart failure bridged to heart transplantation with ventricular assist devices

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*Critical Care* 2010, **14**(Suppl 2):P30 (doi: 10.1186/cc9133)

**Introduction** Implantable ventricular assist devices (VADs) are widely used in patients with end-stage heart failure as a bridge to heart transplantation (HTx) or as destination therapy (DT); however, their use is associated with increased postoperative infection-related morbidity and mortality. Rapid identification of responsible organisms is imperative for the initiation of appropriate treatment and for lowering mortality due to sepsis. Direct detection of pathogens in blood samples by nucleic acid amplification is a sensitive and fast alternative to blood cultures. The multiplex real-time PCR system SeptiFast® (Roche Diagnostics) allows for rapid detection and identification of the 25 most common pathogens (Gram-positive and Gram-negative bacteria and fungi) in blood, in less than 6 hours. The aim of this study was to evaluate the usefulness of SeptiFast® in patients with implanted VADs.

**Methods** The study included 103 blood samples from 38 VAD patients analyzed over a period of 24 months (January 2008 to December 2009), using SeptiFast® in parallel with blood cultures. Blood samples were obtained only from patients suspected of harboring an infection, and in case of positive results follow-up samples were obtained on a weekly basis. PCR was performed according to manufacturers' description (SeptiFast®) using MagnaLyser for extraction of DNA from 1.5 ml peripheral blood and LightCycler 2.0 (Roche Diagnostics) for amplification and detection.

**Results** SeptiFast® and blood cultures yielded concurrent negative results in 76% of the samples and positive results in 7.3% of them. There was a 75% concordance in species identification. Diverging results were obtained in 10.3% of the samples where SeptiFast® only was positive and in 6.4% of the samples where blood cultures only were positive. In cases with concurrent positivity, acceptance of the SeptiFast® results could have led to an earlier targeted treatment.

**Conclusions** The PCR-based SeptiFast® test in combination with traditional microbiological methods may facilitate fast and specific antibiotic treatment and may contribute to reduction of sepsis progressing infections in VAD patients.

### P31

#### Extracellular metabolic alterations in critically ill septic patients studied by adipose tissue microdialysis

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*Critical Care* 2010, **14**(Suppl 2):P31 (doi: 10.1186/cc9134)

**Introduction** Tissue metabolic alterations during critical sepsis have not been well characterized.

**Objective** To investigate the tissue metabolic alterations during sepsis.

**Methods** A microdialysis (MD) catheter was inserted into the subcutaneous adipose tissue of the upper thigh in 65 (39 men) septic critically ill patients upon sepsis onset. Dialysate samples were analyzed for glucose, lactate, pyruvate, and glycerol. The lactate/pyruvate (L/P) ratio was calculated. Sampling was performed six times per day for a maximum of 6 days. The daily mean values of MD measurements were calculated for each patient. Eleven (five men) critically ill nonseptic patients served as controls.

**Results** Septic patients were older ( $66 \pm 17$  vs.  $45 \pm 20$  years,  $P < 0.001$ ), and had a higher APACHE II score ( $21 \pm 5$  vs.  $14 \pm 6$ ,  $P < 0.001$ ) along with a higher

SOFA score ( $8 \pm 3$  vs.  $3 \pm 3$ ,  $P < 0.001$ ) compared with nonseptic patients. Septic patients had a high tissue glucose ( $>4.6$  mmol/l), lactate ( $>2$  mmol/l), pyruvate ( $>120$  μmol/l), L/P ratio ( $>25$ ), and glycerol ( $>200$  μmol/l) during almost the entire observation period. Septic patients had higher tissue glucose ( $P = 0.02$ ) and glycerol ( $P = 0.04$ ) levels than nonseptic patients during the whole study period. They also tended to have higher lactate ( $P = 0.14$ ) concentrations. In contrast, the two groups had similar tissue pyruvate ( $P = 0.35$ ) and L/P ratios ( $P = 0.80$ ).

**Conclusions** Critical sepsis is characterized by an excessive release of extracellular glucose, lactate, and glycerol, with the latter reflecting probably increased lipolysis. These mirror the well-known sepsis-related blood metabolic alterations. Thus, chemical monitoring with subcutaneous MD is accurate in severely ill septic patients.

### P32

#### Two chromogranin A-derived peptides, chromofungin and catestatin, induce neutrophil activation via a store-operated channel-dependent mechanism

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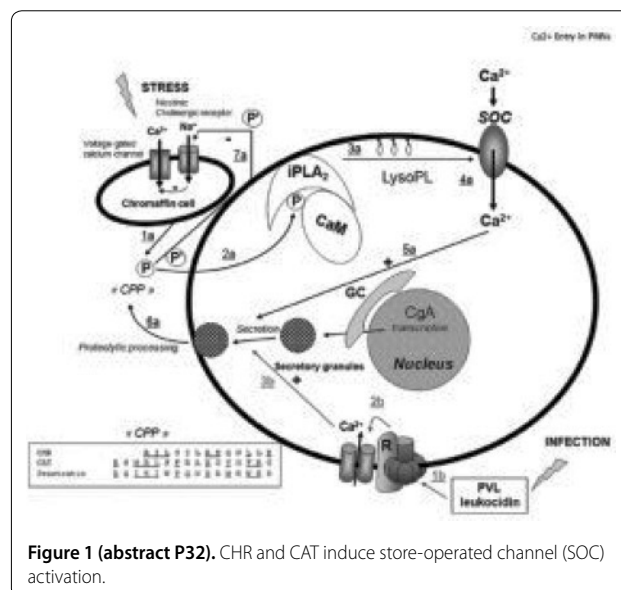
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**Introduction** New endogenous antimicrobial peptides derived from the natural processing of chromogranin A (CgA) are co-secreted with catecholamines upon stimulation of chromaffin cells. Since PMNs play a central role in innate immunity, we examine responses by PMNs following stimulation by two antimicrobial CgA-derived peptides.

**Methods** PMNs were treated with different concentrations of CgA-derived peptides in the presence of several drugs. Calcium mobilization was observed using flow cytometry and calcium imaging experiments. Immunocytochemistry and confocal microscopy were performed to analyze the intracellular localization of the peptides. The calmodulin-binding and iPLA2-activating properties of the peptides were shown by surface plasmon resonance and iPLA2 activity assays. Finally, a proteomic analysis of the material released after PMN treatment with CgA-derived peptides was performed using HPLC and nano-LC MS-MS.

**Results** Using flow cytometry we first observed that after 15 seconds, in the presence of extracellular calcium, chromofungin (CHR) or catestatin (CAT) induce a concentration-dependent transient increase of intracellular calcium. In contrast, in the absence of extracellular calcium the peptides are unable to induce calcium depletion from the stores after 10 minutes of exposure. Treatment with 2-aminoethoxydiphenyl borate, a store-operated channel blocker, inhibits completely the calcium entry, as shown by calcium imaging. We also showed that they activate iPLA2 as the two CaM-binding



**Figure 1 (abstract P32).** CHR and CAT induce store-operated channel (SOC) activation.

factors (W7 and cmZ) and that the two sequences can be aligned with the two CaM-binding domains reported for iPLA2. We finally analyzed by HPLC and nano-LC MS-MS the material released by PMNs following stimulation by CHR and CAT. We characterized several factors important for inflammation and innate immunity. See Figure 1.

**Conclusions** For the first time, we demonstrate that CHR and CAT penetrate into PMNs, inducing extracellular calcium entry by a CaM-regulated iPLA2 pathway [1]. Furthermore, new experiments show that CAT penetrates quickly into immune cells such as dendritic cells and macrophages. To conclude, this study highlights the role of two CgA-derived peptides in the active communication between neuroendocrine and immune systems.

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#### P33

**Abstract withdrawn**

#### P34

##### Cardiovascular hyporesponsiveness in sepsis is associated with G-protein receptor kinase expression via a nitric oxide-dependent mechanism

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**Introduction** Septic shock is characterized by cardiac collapse and decreased peripheral resistance due to systemic resistance vessel dilatation, generally induced by large nitric oxide (NO). G-protein-coupled receptor (GPCR) kinases (GRKs), specific kinases interacting with GPCR proteins, induce receptor phosphorylation and thereby signal desensitization in the continuing agonist presence. Then, an increased expression of GRKs could augment adrenergic receptor desensitization and in turn reduce cardiovascular responses. Thus, we hypothesized that the hyporesponsiveness observed in sepsis could result from signal adrenergic receptor desensitization mediated by GRK2 via a NO-dependent mechanism.

**Methods** C57Bl/6 mice were submitted to cecal ligation and puncture (CLP) surgery and sham-operated animals as controls. The cardiovascular responsiveness activity was evaluated in aorta rings or in cardiac ventricles. Aorta rings were contracted with phenylephrine (Phe; 1 µM), whereas ventricles were contracted with isoproterenol (Iso; 1 µM). The tissues responsiveness was evaluated 6, 12, and 24 hours after CLP surgery in the presence or absence of NO synthesis inhibitor (1400W; 100 µM; 30 min). GRK2 expression was analyzed on heart and aorta 6, 12, and 24 hours after CLP from sham, septic, and 1400W (1 mg/kg)-treated septic mice by immunofluorescence analysis. The procedures have been approved by the Animal Use Ethics Committees of UFSC (PP003).

**Results** The vascular responsiveness to vasoconstrictor Phe was significantly reduced in aorta rings from septic mice evaluated 6 hours (55%), 12 hours (57%), and 24 hours (78%) after CLP. However, the 1400W incubation prevented this vascular hyporesponsiveness 6 and 12 hours after CLP. The cardiac responsiveness to Iso was significantly reduced in ventricles from septic mice evaluated 12 hours (73%) and 24 hours (88%) after CLP. Conversely, the 1400W incubation prevented this cardiac hyporesponsiveness 12 hours after CLP. Moreover, high expression of GRK was detected in aorta 6 hours (65%), 12 hours (70%), and 24 hours (88%), and heart of septic mice 12 hours (52%) and 24 hours (63%) after CLP. The 1400W treatment reduced the GRK high expression on the aorta (75%) and heart (79%) of septic mice.

**Conclusions** Our findings identify that NO seems to activate GRK, which may induce adrenergic receptors' desensitization to agonists, contributing to severe cardiovascular hyporesponsiveness observed during septic shock. Therefore, the results suggest that GRK could be a new potential target to sepsis pharmacotherapy.

**Acknowledgements** The present study was supported by CNPq, CAPES, FAPESP, and FAPESC.

#### P35

##### Kinetics of TREM-1 expression on canine neutrophils after *in vitro* and *in vivo* stimulation with microbial products

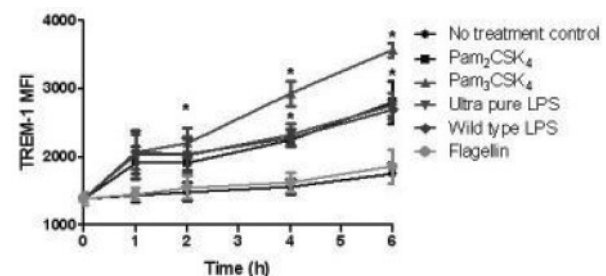
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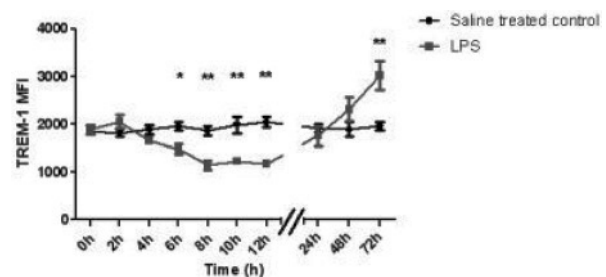
*Critical Care* 2010, **14**(Suppl 2):P35 (doi: 10.1186/cc9138)

The triggering receptor expressed on myeloid cells-1 (TREM-1) is a recently discovered cell surface molecule expressed on neutrophils, mature monocytes, and macrophages [1]. Activation of TREM-1 synergistically enhances proinflammatory cytokine production induced by toll-like receptor (TLR) stimulation [2]. A soluble form of TREM-1 has shown promise as a sensitive and specific biomarker for sepsis in humans [3-5]. Expression and function of TREM-1 in the dog has yet to be characterized. We hypothesize that the expression of canine TREM-1 will be upregulated after stimulation with TLR agonists. We assessed TREM-1 expression on canine neutrophils after exposure to TLR agonists *in vitro* and *in vivo* after i.v. LPS administration. *In vitro*, expression of TREM-1 on neutrophils is significantly upregulated by stimulation with microbe-derived agonists against TLR2/6 (Pam2CSK4), TLR1/2 (Pam3CSK4), and TLR4/MD2 (ultrapure LPS and wildtype LPS) (paired *t* test, *P* < 0.05). The TLR5 agonist flagellin did not significantly upregulate TREM-1 expression at any time point. See Figure 1. In contrast, i.v. administration of LPS to dogs resulted in a significant decrease in both TREM-1 expression and the percentage of TREM-1-positive neutrophils from 6 hours through 12 hours post LPS administration. See Figure 2. The disparity between *in vitro* and *in vivo* effects of LPS suggest other factors, such as systemic and local cytokine production and neutrophil turnover, may influence expression and shedding of TREM-1 on canine neutrophils. We suggest that naturally occurring sepsis in the dog represents the ideal model for defining diagnostic biomarkers and discovering efficacious therapeutics for use in human sepsis.

**Acknowledgements** This presentation was supported by Morris Animal Foundation and ICARE.



**Figure 1 (abstract P35).** Kinetics of TREM-1 expression after TLR agonist stimulation *in vitro*.



**Figure 2 (abstract P35).** Kinetics of TREM-1 expression post LPS administration *in vivo*.

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## P36

### Severe sepsis and its impact on outcome in old and very old patients admitted to the intensive care unit

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**Introduction** Older patients comprise an increasing proportion of ICU admissions. Advanced age and multiple co-morbidities compromise their immunity and hence they may be more prone to succumbing to severe infection and have poorer outcome.

**Objective** To assess the impact of severe sepsis on mortality in the old and very old subgroups of patients admitted to a medical ICU.

**Methods** All patients admitted to a medical ICU of a tertiary care institute with severe sepsis or septic shock were prospectively included. Patients were divided into young (age below 60 years), old (age between 60 and 80 years), and very old (age above 80 years) groups. Data regarding baseline patient characteristics, admission APACHE II score, and ICU course including need for organ support and ICU length of stay were noted. Qualitative data were analyzed using the chi-squared test or Fisher exact test as appropriate and quantitative data were analyzed using Student's *t* test. Inter-group and intra-group comparison for quantitative data was done by one-way ANOVA. The primary outcome measure was ICU mortality.

**Results** Of 387 patients who were admitted with signs of SIRS or sepsis during the study period of 20 months, 132 patients who fulfilled the criteria for severe sepsis/septic shock were included in the analysis. The most common suspected site of infection was the lungs (60 patients, 45.5%), followed by the urinary tract (28 patients, 21.2%) and the abdomen (22 patients, 16.7%). ICU mortality in younger patients was 45.6% as compared with 60.7% in old patients and 78.9% in very old patients ( $P = 0.035$ ). The odds ratio (OR) and relative risk (RR) for dying in the old age group was 1.32 (95% CI = 0.655 to 2.659) and 1.125, respectively, and OR and RR for dying was 3.313 (95% CI = 1.035 to 10.6) and 1.487 in the very old age group. There was an increased need for organ support in the old and very old population as compared with the younger population. See Table 1.

**Table 1 (abstract P36)**

	Young (<60 years)	Old (60 to 80 years)	Very old (>80 years)
Number of patients ( <i>n</i> = 132)	57 (43.2%)	56 (42.4%)	19 (14.4%)
Sex, males (%)	36 (63.2%)	38 (67.9%)	10 (52.6%)
Mean age, years ( $\pm$ SD)	44.4 $\pm$ 9.8	68 $\pm$ 5.4	86.7 $\pm$ 5
Mean APACHE II score ( $\pm$ SD)	10.6 $\pm$ 6.4	10.5 $\pm$ 7.3	13.7 $\pm$ 10.1
Inotropic support	51 (89.5%)	45 (80.5%)	16 (84.2%)
Renal support	18 (31.6%)	23 (41.1%)	5 (26.3%)
Mechanical ventilation	33 (57.9%)	41 (73.2%)	16 (84.2%)
Length of ICU stay, days ( $\pm$ SD)	4.56 $\pm$ 5.8	4.11 $\pm$ 5.4	3.47 $\pm$ 2.5
ICU mortality	26 (45.6%)	34 (60.7%)	15 (78.9%)

**Conclusions** The risk of dying from severe sepsis is considerably higher in the old and very old subgroup of patients. Hence, early aggressive care to recognize and manage severe sepsis is required to improve outcome.

## P37

### Chromogranin A expression in plasma of critically ill patients

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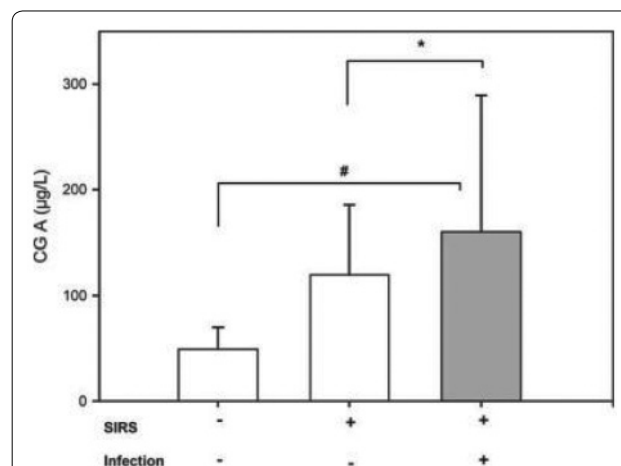
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**Introduction** Risk assessments of patients should be based on objective variables, such as biological markers that can be measured routinely. Such a prediction remains a difficult challenge. The acute response to stress causes the release of catecholamines from the chromaffin cells of the adrenal medulla accompanied by numerous proteins, peptides such as chromogranin A (CGA) and its natural fragments. To date, no study has evaluated the prognostic value of CGA in critically ill ICU patients.

**Methods** We conducted a prospective study of ICU patients, admitted for a life-threatening condition with at least two organ failures, by measuring plasma procalcitonin (PCT), C-reactive protein (CRP), the Simplified Acute Physiological Score II (SAPS II), and CGA on the 24th hour after admission. Continuous data are reported as the median (interquartile range), and group differences were evaluated with the Mann-Whitney U test or the Kruskal-Wallis test. A Cox proportional hazards regression model was used to evaluate the effect of the logarithmically transformed CGA concentration on the endpoint and to calculate hazard ratios (HRs) with 95% CIs. All statistical analyses were performed with the SPSS statistical package (SPSS for Windows version 11.5).

**Results** In 120 consecutive patients, we found positive correlations between CGA and the following: CRP ( $r^2 = 0.216$ ;  $P = 0.02$ ), PCT ( $r^2 = 0.396$ ;  $P < 0.001$ ), SAPS II ( $r^2 = 0.438$ ;  $P < 0.001$ ). Nonsurvivors had significantly higher CGA concentrations than survivors (median (interquartile range): 293  $\mu$ g/l (163 to 699  $\mu$ g/l) vs. 86  $\mu$ g/l (54 to 175  $\mu$ g/l), respectively;  $P < 0.001$ ). Serum CGA concentrations were significantly increased in SIRS patients with a median value of 115 mg/l (68 to 202), when compared with healthy controls ( $P < 0.001$ ). In cases where infection was associated with SIRS, patients had the highest increase in CGA with a median value of 138 mg/l (65 to 222;  $P < 0.001$ ). See Figure 1. In a multivariable linear regression analysis, creatinine ( $P < 0.001$ ), age ( $P < 0.001$ ), and SAPS II ( $P = 0.002$ ) were the only significant independent variables predicting CGA concentration ( $r^2 = 0.352$ ). A multivariate Cox regression analysis identified three independent factors predicting death: log-normalized CGA concentration (hazard ratio (HR), 7.25;



**Figure 1 (abstract P37).**

95% confidence interval (CI), 3.00 to 17.50), SAPS II (HR, 1.05; 95% CI, 1.03 to 1.07), and cardiogenic shock (HR, 3.92; 95% CI, 1.73 to 8.88).

**Conclusions** The admission plasma CGA concentration is increased in the most severe critically ill patients; it correlates with the SAPS II measured after 24 hours and with inflammatory/infectious markers (PCT and CRP). It may be useful in establishing an early stratification for severity in nonselected critically ill patients with organ failures.

### P38

#### **Chlamydomphila pneumoniae infection in macrophages and in lung epithelial cells: IL-10 and the innate immunity response**

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Critical Care 2010, **14**(Suppl 2):P38 (doi: 10.1186/cc9141)

**Introduction** The genus of Chlamydiae comprises obligate intracellular pathogens that occasionally can disseminate and even cause septic infections. *Chlamydomphila pneumoniae* causes acute and chronic respiratory tract infections from sinusitis to severe pneumonia, and phagocytes can transmit the bacteria from the lungs to the vasculature. We have previously shown in IL-10 knockout mice that IL-10 limits the severity of inflammation but prolongs the clearance of the *C. pneumoniae* pneumonia [1].

**Objective** Although IL-10 could contribute to the resolution of *C. pneumonia* infection by regulating the T-helper cell balance (Th1/Th2), we were interested in the direct effects of IL-10 and the IL-10-regulated genes in modulating *C. pneumoniae* growth in macrophages and in respiratory epithelial cells.

**Methods** We investigated the effect of IL-10 and the expression of an IL-10-responsive anti-inflammatory factor on mRNA and protein level in *C. pneumoniae* infected human monocyte/macrophage (MonoMac6 and Thp-1) and in lung epithelial adenocarcinoma (A549) and in HL (human lung) cell lines. We also applied a luciferase promoter assay to study the regulation of the anti-inflammatory gene expression during the *C. pneumoniae* infection.

**Results** In agreement with the previous studies, *C. pneumoniae* proliferated in epithelial cells, while in monocyte/macrophages the infection was often nonproductive and aberrant forms of bacteria were observed. The IL-10 responsive anti-inflammatory factor was differentially regulated at transcriptional level in A549 and MonoMac6 cells in response to *C. pneumoniae* infection, which could potentially affect the outcome of infection. The luciferase promoter assay showed that the transcription was mediated via the E-box regulatory element of the gene.

**Conclusions** Our results imply that the anti-inflammatory response to intracellular *C. pneumoniae* infection varies in different cell types and ongoing studies are needed to clarify the role of IL-10 response in limiting *Chlamydia* growth in these cells.

**Acknowledgements** JTK was financially supported by Drug Discovery Graduate School, Finland.

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### P39

#### **Influence of hydroxyethyl starch and gelatin versus crystalloids on renal function, fluid balance, and ICU length of stay in patients with severe sepsis**

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**Introduction** In a smaller sample of patients with severe sepsis, resuscitation with the synthetic colloids hydroxyethyl starch (HES) as well as gelatin (GEL)

increased the occurrence of acute kidney injury (AKI) in comparison with crystalloids (CRY). We now performed the analysis in a large-scale sample with over 1,100 septic patients on our ICU.

**Methods** A prospective controlled before-and-after study in patients with severe sepsis on a mixed ICU. AKI was defined by RIFLE criteria [1]. Statistical analysis was performed using SPSS 18.0.

**Results** A total of 1,165 patients with severe sepsis were included. At baseline, the three groups had similar age, SAPS2 and SOFA scores and serum creatinine levels. Between January 2004 and January 2006, patients received fluid resuscitation with HES (median cumulative dose 81 ml/kg (IQR 38 to 157),  $n = 391$ ), mainly as 6% HES 130/0.4 (in 75% of patients) or 10% HES 200/0.5. Between February 2006 and March 2008 patients received 4% GEL (40 ml/kg (IQR 18 to 71),  $n = 396$ ), and between April 2008 through April 2010 patients received only CRY ( $n = 387$ ). AKI by any criteria (risk, injury or failure) was 34% after CRY, 55% after HES, and 47% after GEL ( $P < 0.001$  for HES or GEL vs. CRY). Renal replacement therapy (RRT) was 28% after CRY compared with 34% after HES ( $P = 0.04$ ) or 39% after GEL ( $P = 0.002$ ). Median cumulative fluid input during ICU stay was 659 ml/kg (IQR 269 to 1,250) after HES, 526 ml/kg (IQR 174 to 817) after GEL and 360 ml/kg (IQR 174 to 817) after CRY ( $P < 0.001$  HES vs. CRY,  $P = 0.003$  GEL vs. CRY). Patients receiving synthetic colloids had a significantly longer median length of stay in the ICU (HES: 17 (IQR 8 to 29) days; GEL: 13 (IQR 6 to 24) days vs. CRY: 11 (IQR 5 to 20) days (HES vs. CRY  $P < 0.001$ , GEL vs. CRY  $P = 0.001$ )). ICU mortality was 35% (HES), 32% (GEL), and 30% (CRY,  $P =$  not significant).

**Conclusions** Patients with severe sepsis have a higher risk to develop AKI if they receive fluid resuscitation with synthetic colloids (HES or gelatin). Interestingly, the need for RRT under fluid therapy with mainly 6% HES 130/0.4 was higher than in the VISEP study under therapy with 10% HES 200/0.5 (RRT: 31.1%,  $n = 261$ ) [2].

**Acknowledgements** KR has in the past received honoraria from B Braun (Melsungen, Germany).

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### P40

#### **Study of the Helicobacter pylori infection in Iranian patients with multiple sclerosis**

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Critical Care 2010, **14**(Suppl 2):P40 (doi: 10.1186/cc9143)

**Introduction** Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS), the etiology of which is believed to have both genetic and environmental components. During recent years, attention was paid to the role of bacterial infections in the process of MS development. *Helicobacter pylori* infections have been linked to peripheral neuropathies as they may trigger cellular and humoral immunity due to the sharing of similar epitopes present in the nervous tissue. We have investigated one of the candidate bacteria for the environmental component of MS, *H. pylori*.

**Objective** To evaluate the roles of *H. pylori* in tendency toward MS in Iranian patients.

**Methods** In a prospective case-control study, we studied 78 patients with MS and 123 healthy controls for viral DNA detection and antibody assay. DNA extracted from serum and real-time PCR was employed to detection of *H. pylori* genome. The levels of anti-*H. pylori* IgG were measured in samples by ELISA in Dr Ahmadi's medical laboratory.

**Results** We found *H. pylori* DNA in 84% and 71% of patients and healthy controls, respectively. Furthermore, higher levels of anti-*H. pylori* IgG were detected in patients in contrast with healthy controls. Moreover, the genome copy number of *H. pylori* was significantly increased in patients. Results are expressed as the mean  $\pm$  standard deviation and analyzed using Student's *t* test for comparison between two groups. Results were considered significant when  $P < 0.05$ .

**Conclusions** We did not observe significant correlation between prevalence of *H. pylori* DNA and development of MS in selected patients, but active *H. pylori* infection was found in patients more than in controls. These results support the hypothesis that *H. pylori* may contribute to the MS disease thought to establish active infection process and induce immune response. The role of *H. pylori* in the modulation of MS requires further study.

# P41

## Incidence of bacteremia at the time of ICU admission and its impact on outcome

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**Introduction** Blood culture is routinely taken at the time of admission to the ICU for all patients suspected to have any infection, even though it may be positive only in a few patients. Moreover, the impact of a positive blood culture in such a patient population is not clear.

**Objective** To find the incidence of bacteremia at the time of ICU admission, to assess its impact on the outcome, and to analyze which factors are related to poorer outcomes in these bacteremic patients.

**Methods** A retrospective cohort study over a 2-year period. Data from all the admissions to a medical ICU, in a tertiary care hospital, with suspected infection in whom blood cultures were sent at the time of admission were analyzed. Data regarding patient demographics, probable source of infection, previous antibiotic use, and ICU course were recorded. Severity of illness on admission was assessed by APACHE II score. Qualitative data were analyzed using the chi-square test or Fisher exact test and quantitative data were analyzed using Student's *t* test. Primary outcome measure was ICU mortality.

**Results** A total of 567 patients were included in the analysis. A significant proportion of these patients, 238/567 (42%), were already on antibiotics. Three hundred and sixty-three (64%) patients were direct ICU admissions from casualty, 61 (10.76%) were shifted from hospital wards, 35 (6.17%) from other ICUs in the hospital, and 108 (19.05%) were transfers from other hospitals. Blood cultures were positive in only 60/567 patients (10.6%). Mortality was significantly higher in patients with positive blood cultures (27/60, 45% vs. 69/507, 13.6%; *P* = 0.000). Univariate analysis for assessing the risk factors for ICU mortality among bacteremic patients was done in which age (*P* = 0.061), sex (*P* = 0.253), type of admission (*P* = 0.203), type of organism, severity of illness (*P* = 0.234), and site of infection (*P* = 0.250) were analyzed, but only previous antibiotic use was statistically associated with higher mortality (*P* = 0.011). Bacteremic patients who were already on antibiotics had a significantly higher mortality (54.2% vs. 8.3%) (OR 12.9, 95% CI: 1.6 to 100). Mortality was higher in patients with *Pseudomonas bacteremia* (72.7%) although it was not statistically significant (*P* = 0.08). See Tables 1 and 2.

**Conclusions** Blood cultures may be positive in only a minority of patients with suspected infection admitted to the ICU as most of these patients may already be taking antibiotics. Nevertheless, the prognosis of those patients

with positive blood culture is worse, especially if culture is positive in spite of the patient being on antibiotics.

# P42

## Differential kinetics of endothelial cell activation biomarkers E-selectin and endocan during nonlethal endotoxemia in 129Sv mice: a role for PMN-derived serine proteases in the transient decrease of circulating endocan levels

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**Introduction** Severe septic syndrome remains one of the most frequent causes of death in ICUs. One of the main players in this pathology is the endothelium integrity. Our laboratory has demonstrated in preliminary clinical studies among the various biomarkers of endothelial dysfunction that blood levels of endocan (ESM-1), a pulmonary vascular endothelial cell-specific molecule participating in the control of endothelial-leukocyte interactions, are associated with the severity and evolution of septic states. On the other hand, we showed *in vivo* that antiprotease therapy is associated with a decrease of the leukocyte rolling and firm leukocyte adhesion to endothelium in sepsis. This decrease in the leukocyte-endothelial cell contacts was associated with an increase of blood endocan levels, suggesting a linkage between leukocyte proteases, endocan, and inflammation during sepsis.

**Methods** In order to characterize this linkage, we set up a mouse model of nonlethal shock induced by endotoxin (LPS), in mice genetically deficient in cathepsin G (CG<sup>-/-</sup>) or double deficient in cathepsin G and elastase (CGEL<sup>-/-</sup>). The neutrophil and endothelial activation biomarkers included myeloperoxidase, sE-selectin, and endocan ELISAs in mouse serum. *In vitro* tests of endocan proteolysis were also performed.

**Results** During the nonlethal endotoxemia, clinical scores as well as E-selectin levels were maximal at 24 hours and progressively returned to the baseline. Circulating myeloperoxidase also increased early at 24 hours but remained elevated until 72 hours. By contrast, circulating endocan decreased early at day 1, remained undetectable at days 2 and 3, and then normalized at day 5. A strong inverse correlation was observed between endocan and myeloperoxidase levels. Similar findings were observed CG<sup>-/-</sup>. However,

Table 1 (abstract P41)

	Overall (n = 567)	Blood culture positive (n = 60)	Blood culture negative (n = 507)	P value
Sex, males (%)	332 (58.6%)	41 (68.3%)	291 (57.4%)	0.137
Mean age, years (± SD)	59.2 ± 8.5	59.6 ± 19.4	59.2 ± 18.4	0.859
Mean APACHE II score (± SD)	16.6 ± 8.5	18 ± 9.4	16.5 ± 18.4	0.184
Previous antibiotics	238 (46.9%)	48 (80%)	190 (37.5%)	0.000
Inotropic support	158 (27.9%)	35 (58.3%)	123 (24.3%)	0.000
Renal support	86 (15.2%)	19 (31.7%)	67 (13.2%)	0.000
Mechanical ventilation	164 (28.9%)	34 (56.7%)	130 (25.6%)	0.000
ICU length of stay, days (± SD)	5.4 ± 5.6	5.18 ± 7.4	5.4 ± 5.4	0.782
ICU mortality	96 (16.9%)	27 (45%)	68 (13.4%)	0.000

Table 2 (abstract P41)

Organism	Number of patients (n = 60)	Need for inotropes	Need for renal support	Need for mechanical ventilation	ICU mortality
<i>E. coli</i>	27 (45%)	15 (55.6%)	9 (33.3%)	13 (48.1%)	13 (48.1%)
<i>P. aeruginosa</i>	11 (18.3%)	9 (81.8%)	4 (36.4%)	9 (81.8%)	8 (72.7%)
<i>K. pneumoniae</i>	7 (11.7%)	2 (28.6%)	2 (28.6%)	4 (57.1%)	1 (14.3%)
<i>S. aureus</i>	5 (8.3%)	3 (60%)	2 (40%)	2 (40%)	2 (40%)
Others	8 (13.3%)	6 (75%)	2 (25%)	6 (75%)	3 (37.5%)



CGEL<sup>-/-</sup> gained 1 day in health recovery, and showed less important reduction in endocan levels. Incubation of mouse endocan with PMN supernatants from WT, CG<sup>-/-</sup>, or CGEL<sup>-/-</sup> generated a major proteolytic fragment of 14 kDa. The proteolytic activity was inhibited by  $\alpha_1$ -antichymotrypsin.

**Conclusions** In nonlethal endotoxemia, both endothelial cells and PMN are activated. The kinetics of PMN activation matched with the decrease of circulating endocan. *In vitro*, the PMN-derived serine proteases induce endocan cleavage, which may relate to the decrease of circulating level of endocan. Our results detail for the first time the kinetics of endothelial cell and PMN activation markers in a mouse model of sepsis and revealed that a PMN-derived serine protease is involved in the degradation of endocan that differs from CG or EL.

#### P43

##### Soluble TLT-1 is a naturally occurring TREM-1 inhibitor and protects mice from hyperresponsiveness and death during sepsis

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**Introduction** Triggering receptor expressed on myeloid cells-1 (TREM-1) and TREM-like transcript 1 (TLT-1) belong to the TREM family. TREM-1 is expressed on neutrophils and monocytes/macrophages, and plays a crucial role during the onset of sepsis by cooperating with pattern recognition receptors in a synergistic way, thus amplifying the host immune response. TLT-1 is selectively expressed on activated platelets and is known to facilitate platelet aggregation through binding to fibrinogen. Interestingly, TLT-1 null mice displayed higher plasma cytokines concentrations and death rates than WT mice during experimental sepsis. We identified a 17 amino acid peptide derived from the extracellular part of TLT-1, named LR17, which is responsible for TLT-1 anti-inflammatory properties.

**Methods** To quantify cellular activation, human neutrophils were isolated from whole blood by density gradient. After stimulation with LPS,  $\alpha$ TREM-1

(a TREM-1 agonist) and/or LR17, p38-MAPK and ERK1/2 phosphorylation was quantified by western blot; NF- $\kappa$ B activity and cytokine release by ELISA; mRNA levels of various gene of interest by quantitative RT-PCR; and ROS production by flow cytometry. The effect of siRNA-induced Trem-1 silencing was studied on purified human monocytes. *In vivo* studies were performed on a CLP mouse model of sepsis.

**Results**  $\alpha$ TREM-1-induced or LPS-induced cytokine/chemokine production by human neutrophils or monocytes was dose-dependently reduced in the presence of recombinant TLT-1 or LR17, both at the gene (mRNA) and protein levels (ELISA). This decrease involves a broad set of cytokines and chemokines: TNF $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, IL-16, GRO- $\alpha$ , MCP-1, MIP-1 $\beta$ , and RANTES. Exploration of intracellular signalling showed that LR17 also reduced  $\alpha$ TREM-1-induced or LPS-induced p38-MAPK and ERK1/2 phosphorylation, NF- $\kappa$ B activation and then ROS production in neutrophils (Figure 1a to e). On Trem-1-silenced monocytes, TREM-1 agonist did not induce cytokine production and LR17 did not show any effect (Figure 1f). As a result of this activity, both early and late LR17 administration to septic mice modulated the proinflammatory cascade triggered by infection with a decrease of plasma, bronchoalveolar lavage and peritoneal fluid cytokine concentration, as well as of cytokine mRNA levels in the lung and liver. TLT-1 also prevented organ damage and coagulation abnormalities and finally improved survival by more than 60% versus controls (Figure 2 overleaf).

**Conclusions** TLT-1 plays a pivotal role during sepsis, linking haemostasis and inflammation.

#### P44

##### Monoamino-oxidase-A function and potential benefit of its inhibition in sepsis

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**Introduction** The use of hydrocortisone (HC) for treatment of septic shock is controversially discussed. Microarray data from the CORTICUS trial showed the transcript encoding monoamino-oxidase-A (MAOA) as one of the

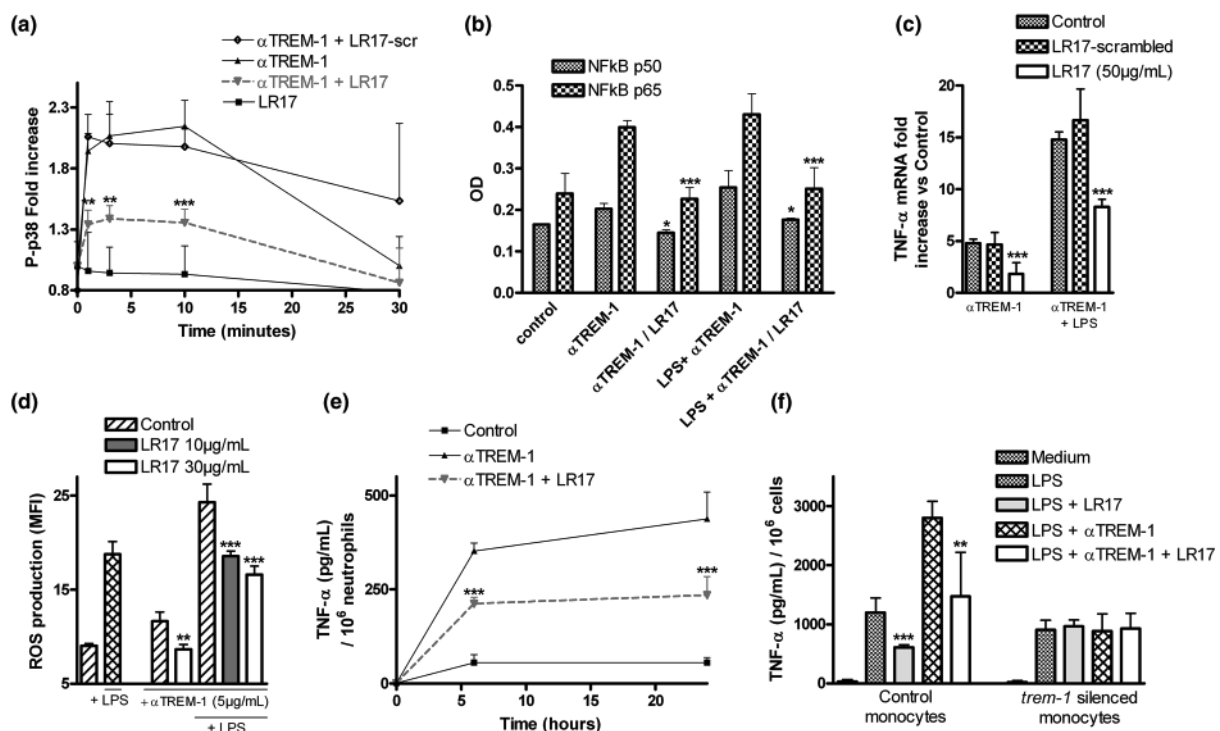
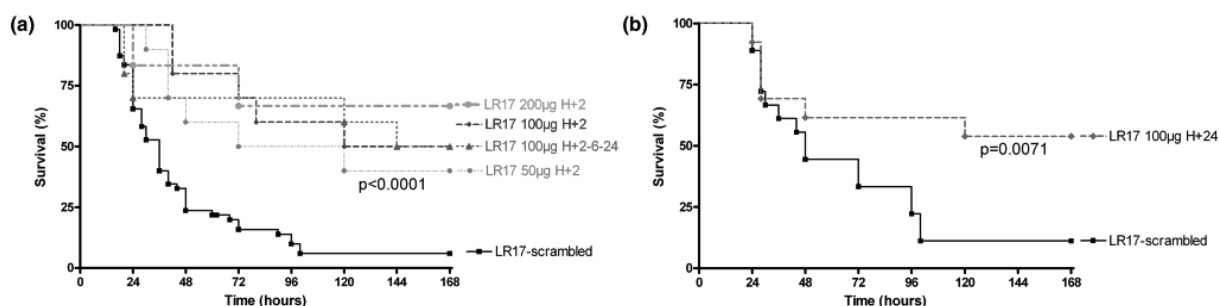


Figure 1 (abstract P43). LR17 decreases LPS-associated and  $\alpha$ TREM-1-associated human neutrophil activation.



**Figure 2 (abstract P43).** LR17 protects mice from caecal ligation and puncture (CLP)-induced mortality. **(a)** Dose–response curves. **(b)** Delayed LR17 administration.

strongest upregulated genes. Due to its involvement in generating reactive oxygen species (ROS) and apoptosis, MAOA might play an important role in infection and development of organ dysfunction in these patients. Blocking MAOA with the specific inhibitor clorgylin (CL) may provide a new therapeutic intervention for sepsis. The present study investigates the function of MAOA in sepsis and the potential benefits of its inhibition.

**Methods** A total of 15 patients with severe sepsis or septic shock were enrolled and compared with 10 healthy controls. As a polymicrobial sepsis model, 16-week-old C57BL6 mice were injected intraperitoneally with 5 µg/g BW characterized human feces. Animals were treated once a day with 35 ml/kg BW saline and 4 mg/kg BW HC + 0.25 mg/kg BW CL. MAOA mRNA was quantified by quantitative PCR, and protein levels were measured by flow cytometry. Phagotests determining phagocytotic activity were performed following the manufacturer's instructions. For *ex vivo* stimulation, whole blood of healthy individuals was incubated with HC (50 µg/ml) and LPS (100 ng/ml). For laboratory and microbiological analyses, routine laboratory procedures were used. ROS were measured by difluorodihydrofluorescein diacetate and flow cytometry.

**Results** Quantitative PCR showed a significant increase of MAOA mRNA expression in patients with sepsis versus healthy controls (eightfold,  $P < 0.05$ ). The same is true for protein levels of MAOA (1.5-fold,  $P < 0.05$ ). Blocking of MAOA by CL enhanced phagocytosis *ex vivo* (140%,  $P < 0.05$ ). In the animal model after MAOA inhibition, the survival rate was significantly higher (risk reduction 40%,  $P < 0.05$ ) and less bacterial burden was found in the blood, lung, and liver (1 log,  $P < 0.05$ ). Furthermore, less organ damage shown by LDH, ASAT and ALAT was observed ( $P < 0.05$ ). These results were associated with less ROS production in granulocytes ( $P < 0.05$ ).

**Conclusions** MAOA is strongly upregulated during severe sepsis on RNA as well as on protein level. In septic mice, higher survival rates were observed by blocking MAOA. Inhibition of MAOA might have potential in sepsis and so provide a novel method for therapeutic intervention.

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#### P45

##### **Transcripts coding the VWF cleaving protease are decreased under proinflammatory conditions, which is reversed by co-incubation with activated protein C and selenate**

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**Introduction** In sepsis, the severity-dependent decrease of the VWF-cleaving protease ADAMTS13 is a common phenomenon, which may contribute to aggregation of platelets/platelet consumption and the development of sepsis-associated thrombotic microangiopathy (TMA) and organ failure. Up to now, hepatic stellate cells (HSC) are considered to function as the primary source of ADAMTS13 protein. The underlying mechanisms of the decrease in sepsis remain unclear.

**Methods** We present data obtained in *in vitro* experiments using cultured human HSC (LX2-line) and microvascular endothelial cells (HMEC) stimulated under proinflammatory conditions. Monolayers were exposed to cytokines

known to be plasma abundant/relevant during systemic inflammation (TNF, IL1β, IFNγ), to bacterial endotoxin (100 ng/ml), to a mixture of cytokines/endotoxin, or to freshly prepared serum obtained from patients ( $n = 12$ ) with severe sepsis/septic shock.

**Results** Both cell lines expressed ADAMTS13 mRNA as quantitated using quantitative PCR normalized to a set of unvaried genes. Overall, incubation with cytokines resulted in a decrease of ADAMTS13 mRNA to different extents ranging between 40 and 80% of the basal transcription rate in between 24 hours. Furthermore, in endotoxin-treated cells, ADAMTS13 declined to 60% (HSC) or 65% of basal levels. This effect was more pronounced by the mixture of cytokines/endotoxin to levels of 55% (HSC) or 40% (HMEC). In monolayers treated with serum from patients with sepsis, only 10% (HSC) or 49% (HMEC) of the basal level was determined. Both the trace element selenium and activated protein C, which are used in the supportive therapy of patients with sepsis, ameliorates the decrease in serum-treated HSC and increased the level of ADAMTS13 transcript in endothelial cells. Continuous infusion adapted to body weight also abolished the decrease of ADAMTS13 expression in hepatic tissues during the course of polymicrobial sepsis in mice.

**Conclusions** We found that mRNA coding ADAMTS13 protein is also present in endothelial cells. Also we observed a marked decrease in both cell lines undergoing proinflammatory stimulation. This mechanism may contribute to the decline of proteolytic activity of ADAMTS13 in patients with sepsis and sepsis-associated TMA. Furthermore, the amelioration of this effect by selenate and APC may function as mechanisms resulting in the more favorable outcome observed in a number of clinical studies.

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#### P46

##### **Gastrin-releasing peptide receptor antagonist induces a protection from lethal sepsis: involvement of toll-like receptor 4 signaling**

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**Introduction** In lethal polymicrobial sepsis, toll-like receptor 4 (TLR-4) mediates a critical role in impeding the migration of neutrophils to infectious

foci, thereby favoring increased bacteremia and ultimately leading to mortality. We have previously shown that the selective gastrin-releasing peptide receptor antagonist RC-3095 can reduce organ dysfunction in experimental sepsis. Thus the aim of the present study is to report a novel link between GRPR and TLR-4 signaling and its relationship with inflammatory parameters in *in vitro* and *in vivo* experimental models as well as in sepsis patients.

**Methods** For the *in vitro* experiment, RAW 264.7 macrophages were stimulated with LPS and treated with RC-3095 for RT-PCR analyses of TLR-4 mRNA, immunoblotting of pERK1/2, pJNK, pAkt, and EMSA of NF- $\kappa$ B and activator protein 1 (AP-1). In the *in vivo* studies, male Wistar rats were divided and submitted, into sham surgery, cecal ligation and puncture (CLP) surgery, and CLP plus RC-3095. Six hours after, all rats were anesthetized and sacrificed by cardiac puncture. Blood was collected for bacterial count and cytokine analyses; bronchoalveolar lavage fluid for cell count, levels of TLR-4 and cytokines; peritoneal lavages for bacterial count; and lung tissue for levels of TLR-4 and RT-PCR analyses of TLR-4 mRNA. In a human study 12 patients, admitted to an adult medical ICU with a clinical diagnosis of septic shock, received a continuous infusion with RC-3095 over a period of 12 hours and concentrations of IL-6 and IL-10 in plasma were determined. Results are expressed as means  $\pm$  SD. Differences between groups were determined by ANOVA, followed by Tukey's *post hoc* test. Differences between two groups were determined by *t* test.

**Results** RC-3095 inhibited expression of TLR-4 and reduced phosphorylation of extracellular signal-regulated kinase (ERK-1/2), c-Jun NH2-terminal kinase (JNK), and Akt, leading to decreased activation of NF- $\kappa$ B and AP-1 in macrophages. In a rat model of sepsis, RC-3095 treatment decreased lung TLR-4 content, reduced the migration of inflammatory cells to the lung, reduced systemic cytokine levels, and attenuated bacterial dissemination. Continuous infusion with RC-3095 for 12 hours decreased IL-6 plasma levels in septic patients, but did not significantly affect IL-10 plasma levels.

**Conclusions** These findings demonstrate the beneficial action of GRPR antagonists in controlling the inflammatory response in sepsis through a mechanism involving inhibition of TLR-4 signaling.

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#### P47

##### System biology of multiple organ dysfunction: formation of a ceramide-enriched macro-domain in SIRS/sepsis

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Generation of bioactive lipids such as ceramide (Cer) and the formation of Cer-enriched macrodomains are regarded as mediators of SIRS and the development of multiple organ failure. Therefore, we addressed the question of whether there is a difference in the plasma activity of the secreted isoform of the Cer-forming enzyme sphingomyelinase (SMPD1) in patients with various degrees of SIRS/sepsis of different origin as well as in a murine loss of function model. We found plasma activity in critically ill patients (median 262.3 pmol/ml\*hour) was significantly higher than age-matched controls (123.6). In patients with fatal outcome, activity increased (+77.4) in comparison with survivors (-252.1). A severity-dependent increase was also analyzed in patients with multiple organ dysfunction syndrome (MODS) following elective cardiac surgery. Beyond immunological detection of increased pSMPD1 in septic patients, we found an increase in Cer-enriched macrodomains in endothelial cells after stimulation with patients' plasma, endotoxin, or TNF.

We also found formation of Cer-enriched macrodomains by immunostaining using specific antibodies directed against Cer, CD14, and Fas. In a loss of function model, we identified 315 transcripts differentially regulated in circulating white blood cells, liver, and lung by use of microarray technology as well as in the cytokine pattern/organ function parameters following polymicrobial cavity infection. Furthermore, host responses in knockout mice were more pronounced with respect to bacterial load in lung, liver,

and blood, plasma cytokine levels, thrombocytopenia as well as delayed migration of neutrophils into hepatic tissue.

In conclusion, the results provide demonstration of a biofunctional relevant activity of SMPD1 resulting in altered signal transduction in SIRS, which may contribute to the development of MODS.

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#### P48

##### Predicting organ failure at 24 hours from early clinical data in an ovine pneumonia-sepsis model

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**Introduction** Having the ability to predict organ failure could impact treatment decisions and potentially lessen the consequences of acute lung injury (ALI) and sepsis. While Acute Physiology and Chronic Health Evaluation (APACHE II) and the Pneumonia Severity Index are useful in predicting the risk of morbidity and mortality, they do not predict the risk of developing organ failure. Currently no accepted practice allows for the prediction of the development of organ failure. The objective, therefore, of our study was to predict the development of organ failure at 24 hours using only the data available from the first 4 hours post inoculation.

**Methods** This pneumonia-sepsis model included 19 sheep with ALI. Inoculation of  $\sim 2.5 \times 10^{11}$  colony-forming units methicillin-resistant *Staphylococcus aureus* (MRSA) induced pneumonia, while smoke injury was created through inhalation of cotton smoke. Four different groups were studied and are as follows: MRSA and smoke inhalation (M+S,  $n = 7$ ), MRSA untreated (M,  $n = 3$ ), MRSA treated (M+T,  $n = 3$ ), and smoke inhalation only (S,  $n = 6$ ). In order to use the injury group as a model input, all the sheep were modeled independent of group and a rank order of severity was determined. Additional inputs included a number of clinical and laboratory parameters. Only the first 4 hours of data were allowed to be used as an input. The model outputs were prothrombin time (PT) and mean arterial pressure (MAP) over the entire 24-hour time frame. To minimize overparameterization, only two inputs per output were used for prediction.

**Results** The rank order of injury group from least to greatest severity was M+T, S, M, M+S. PT was best predicted by calcium and injury. The agreement between predicted and measured PT using only calcium as the input was  $r^2 = 0.24$ . Adding the second input, in this case injury group, improved the model's predictive ability ( $r^2 = 0.48$ ). MAP was best predicted by lactate with an agreement between predicted and measured of  $r^2 = 0.64$ . Unlike PT, the model was not able to better predict MAP by adding a second input ( $r^2 = 0.64$ ).

**Conclusions** Our model was able to provide an accurate prediction of MAP using only the first 4 hours of data, while PT was less accurately predicted. However, this early study suggests that continued refinement of the progression model could provide a viable tool to predict organ failure in sepsis.

#### P49

##### Modeling sepsis induced by methicillin-resistant *Staphylococcus aureus* infection: a human/ovine approach

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**Introduction** Methicillin-resistant *Staphylococcus aureus* (MRSA) is an invasive pathogen in critically ill patients, and is commonly the cause of nosocomial pneumonia. Infection with MRSA can lead to bacteremia, septic shock, and multisystem dysfunction. Previous work has demonstrated that a

MRSA pneumonia-sepsis model in sheep mimics the vascular state in human sepsis. In the present study, we sought to combine sheep and clinical data from humans to determine whether common parameters existed across sepsis with regard to coagulopathy. Secondly, we wanted to model and provide estimates of MRSA bacterial load in both species.

**Methods** Nineteen sheep with acute lung injury and 14 human patients were incorporated into this sepsis model. In sheep, pneumonia was induced by inoculating the airway with  $\sim 2.5 \times 10^{11}$  colony-forming units (CFU) MRSA. Thirteen of the sheep had smoke injury induced through inhalation of cotton smoke. All human patients were retrospectively studied and were bacteremic with MRSA from varying primary infection sites. Initial bacterial load in humans was modeled using clinical and microbiologic data available at the start of sepsis, while the initial load in sheep was the inoculating amount of bacteria. Load continues throughout the study period and is modified by vital signs and antibiotic coverage. The bacterial load as well as clinical and laboratory parameters are inputs, with the output parameter being prothrombin time (PT). In order to minimize overparameterization of the population, the model was allowed to estimate PT using only three parameters. Data were modeled for 24 to 48 hours.

**Results** Bacterial load was estimated to range from between  $10^8$  and  $10^{11}$  CFU, with the high end of the range being similar to the inoculum used to induce pneumonia in the sheep. The highest-ranking parameters in estimating PT were calcium, potassium, and bacterial load. When using calcium alone, the model estimate agreement with measured PT was  $r^2 = 0.25$ . Combining calcium and potassium improved agreement ( $r^2 = 0.34$ ), while using all three parameters further improved the estimate ( $r^2 = 0.37$ ).

**Conclusions** Through progression modeling we were able to provide prediction of coagulopathy and bacterial load across two different species of animals infected with the same organism.

## P50

### Identification of immune modulators using a phage library displaying *Staphylococcus aureus* secreted proteins

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Understanding the mechanisms of bacterial immune modulation will teach us more about pathogenesis of infection and could lead to new strategies in anti-inflammatory therapy during sepsis. Various approaches are already used to identify bacterial immune modulating proteins. However, these are inefficient and time consuming. Immune modulating proteins need to be secreted in order to act on their targets outside the bacterial cell. In the present study, phage display technology was used to specifically identify secreted immune modulating proteins with high efficacy.

Phage display technology is a technique to express a protein fused to a coat protein of a filamentous phage. The most widely used coat protein is pIII encoded in the phage genome by gIII. This gene contains a signal sequence that is essential for production of stable phage particles. When a bacterial genome is randomly fragmented and these fragments are inserted into a phage vector containing gIII lacking the signal sequence, intact phage particles are formed only when the inserted bacterial genomic fragment contains a signal sequence. This allows for selective expression of a bacterial secretome since secreted proteins also contain a signal sequence. The resulting secretome phage library can be used to select displayed proteins that specifically bind to various components of the immune system. This powerful technique has several advantages: it can be used for different Gram-positive and Gram-negative bacterial genomes. There is no need for extensive culturing of bacteria so it can be used for difficult or slow-growing bacteria. Expressed proteins are not hampered by solubility problems.

There is a direct relation between expressed protein and coding gene that allows for rapid identification of selected proteins. As a proof of principle, a *Staphylococcus aureus* library was constructed. The goal is to evaluate the proportion of previously described secreted immune modulators that can be recovered and to identify new immune modulators. In order to express most of the 300 secreted proteins encoded by this microorganism, the library reached a diversity of 108 clones. The secretome phage library was screened for interaction with leukocytes and for modulation of the coagulation and complement pathways, which are highly activated in sepsis process.

## P51

### Building up an infection control strategy based on the e-health concept

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**Introduction** Healthcare-associated infection (HAI) control is one of the most important challenges in quality and safety. Defined as the intensive use of the information and communication technologies, e-health can be a major part of this matter. Aiming for implementation of an infection control strategy based on the e-health concept in a 280-bed, paper-free, general hospital, the Infection Control Committee (ICC), along with the Quality and Antibiotics Committees and the IT team, has been working on the implementation of new tools for widespread use.

**Methods** The authors used the electronic medical record, a special program for infection surveillance and data-mining, and some additional resources, such as messages and alerts sent by email or by SMS, as a global approach for improving infection control. On the electronic medical record (Soarian®; Siemens Medical Solutions), interventions are made at different levels. On patient admission, through the fulfillment by the physician of a questionnaire on detection of increased risk for infection/colonization, several protocols regarding isolation and screening testing are automatically activated and the ICC is informed via email, thus minimizing the spread of epidemiologically important microorganisms. During hospitalization, new templates for prescribing microbiological tests are in progress to improve the availability of clinical information to the laboratory and to the Vigiguard® (Biomérieux) program. New context-sensitive templates for antimicrobial prescription are being implemented to improve the quality of such therapeutics. Alerts to the pharmacy are sent when there is some inaccuracy in terms of the chosen antimicrobial or the duration of therapy, thus reducing the emergence of new drug resistances and minimizing costs. New fields were created to individualize infection control issues in the patient's history, and to generate an automatic note on epidemiologically important issues at the transfer or discharge of the patient, thus complying with the recommendations for information transfer. Finally, surveillance of several infections/colonizations will be obtained in real time from Vigiguard®, a tool with a data-mining engine.

**Conclusions** The authors hope that the application of the e-health concept to the infection control policy in a paper-free hospital will improve quality of and reduce the risk of HAI.

## P52

### Elimination of cytokine and soluble cytokine receptors by carbon sorbents from blood

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**Introduction** As has been seen in several studies, many authors describe details of different cytokines' elimination from blood serum by carbon sorbents. Some data have been published about carbon immobilization of cytokines bounded and nonbounded in complexes with specific cytokine receptors. The aim of the present study was to research cytokine and soluble cytokine receptor elimination by carbon sorbents from blood.

**Methods** The blood samples from 28 cancer patients with sepsis before and after extracorporeal detoxification by Adsorba 300C (Gambro, Sweden) were analyzed. The sorbent washouts were also tested. We evaluated cytokine levels (IL-1β, IL-6, TNFβ) and their soluble receptors (sIL-1RII, sIL-6R, sTNFRI) in the samples.

**Results** Our experiments showed that, after hemoperfusion, the cytokine levels in blood decreased or did not change compared with the initial cytokine level. At the same time the soluble cytokine receptor level increased considerably after the procedure (from 1.7 to 2.6 times). The cytokine level in the sorbent washouts was also very high. Therefore, the soluble receptor level was lower in the washouts than in the serum.

**Conclusions** These results can partially be explained by the ability of carbon sorbent to eliminate cytokine molecules more actively than cytokine receptors. Therefore, it is tempting to suppose that carbon hemosorption leads to a considerable reduction of serum cytokines, bounded and soluble, but preserves the soluble receptors. These peptide molecules play an important role in the formation of adequate anti-inflammatory response.

# P53

## Regulation of neutrophil chemotaxis by toll-like receptor 9 is important for sepsis survival

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**Introduction** Successful clearance of bacterial infection depends on efficient neutrophil migration to infected tissues [1]. Chemotaxis is a crucial event for neutrophil migration to local infection and is controlled mainly through activation of G-protein-coupled receptors. Furthermore, the functionality of these receptors is regulated by G-protein-coupled receptor kinases (GRKs) [1]. Impaired chemotactic responses in sepsis was correlated with dysregulated neutrophil toll-like receptor (TLR) signaling, TLR2 and TLR4, while TLR9 inhibition in dendritic cells was associated with reduction of mortality in polymicrobial sepsis [2]. Despite the TLR9 expression, the role of this receptor in neutrophil chemotaxis has not been studied. Thus, the aim of the present study was to verify the importance of TLR9 activation on neutrophil migration during sepsis.

**Methods** C57BL/6 wildtype (WT) and TLR9<sup>-/-</sup> mice were submitted to the cecal ligation and puncture (CLP) sepsis model and the survival rate was evaluated over 7 days. Also, neutrophil migration to the peritoneal cavity was measured 6 hours after CLP. Chemotaxis of blood neutrophils to CXCL2 in the Boyden camera, CXCR2 expression, and GRK2 induction on blood neutrophils, measured by flow cytometry and immunofluorescence, respectively, were performed 2 hours after CLP. All experiments were developed in accordance with the ethical guidelines of the School of Medicine of Ribeirão Preto, University of São Paulo (protocol number 150/2009).

**Results** TLR9<sup>-/-</sup> mice submitted to CLP had an enhanced survival rate when compared with WT mice ( $P = 0.096$ ), and these knockout mice had increased the neutrophil migration to infectious focus ( $P = 0.0445$ ). Investigating the mechanism by which the deficiency of TLR9 could recover neutrophil migration, it was observed that neutrophil derived from TLR9<sup>-/-</sup> CLP-treated mice had restored the ability to migrate *in vitro* (chemotact assay) toward MIP-2, CXCR2 ligand ( $P = 0.0133$ ). Moreover, the recovery in neutrophil chemotaxis was associated with an enhancement in CXCR2 expression on the neutrophil surface and a reduction in GRK2 induction.

**Conclusions** In sepsis, TLR9 activation, similar to that previously observed with TLR2 and TLR4, can also be harmful to control bacterial growth, because it impairs neutrophils from reaching the infection focus.

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# P54

## Disruption of sarcolemmal dystrophin and $\beta$ -dystroglycan may be a potential mechanism for myocardial dysfunction in severe sepsis

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**Introduction** Evidence from our laboratory has shown alterations in myocardial structure in severe sepsis/septic shock. The morphological alterations are heralded by sarcolemmal damage, characterized by increased plasma membrane permeability caused by oxidative damage to lipids and proteins. The critical importance of the dystrophin-glycoprotein complex

(DGC) in maintaining sarcolemmal stability led us to hypothesize that loss of dystrophin and associated glycoproteins could be involved in early increased sarcolemmal permeability in experimentally induced septic cardiomyopathy. **Methods and results** Male C57BL/6 mice were subjected to sham operation and moderate (MSI) or severe (SSI) septic injury induced by cecal ligation and puncture (CLP). Using western blot and immunofluorescence, a downregulation of dystrophin and  $\beta$ -dystroglycan expression in both severe and moderate injury could be observed in septic hearts. The immunofluorescent and protein amount expressions of laminin- $\alpha_2$  were similar in SSI and sham-operated hearts. Consonantly, the evaluation of plasma membrane permeability by intracellular albumin staining provided evidence of severe injury of the sarcolemma in SSI hearts, whereas antioxidant treatment significantly attenuated the loss of sarcolemmal dystrophin expression and the increased membrane permeability.

**Conclusions** The present study offers novel and mechanistic data to clarify subcellular events in the pathogenesis of cardiac dysfunction in severe sepsis. The main finding was that severe sepsis leads to a marked reduction in membrane localization of dystrophin and  $\beta$ -dystroglycan in septic cardiomyocytes, a process that may constitute a structural basis of sepsis-induced cardiac depression. In addition, increased sarcolemmal permeability suggests functional impairment of the DGC complex in cardiac myofibers. *In vivo* observation that antioxidant treatment significantly abrogated the loss of dystrophin expression and plasma membrane increased permeability supports the hypothesis that oxidative damage may mediate the loss of dystrophin and  $\beta$ -dystroglycan in septic mice. These abnormal parameters emerge as therapeutic targets, and their modulation may provide beneficial effects on future cardiovascular outcomes and mortality in sepsis.

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# P55

## Endocan (endothelial cell-specific molecule-1) as a pertinent biomarker of endothelial dysfunction in sepsis

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**Introduction** One of the main players in the severity of sepsis is the endothelium integrity. Endocan, also called endothelial cell-specific molecule-1 (ESM-1), was shown to be preferentially expressed in lung vasculature. Structurally, endocan/ESM-1 is a 50 kDa proteoglycan that can interact with ICAM-1 and LFA-1 integrins and consequently prevents inflammatory events. In an experimental rat endotoxemic shock model, we previously showed that a decrease in the leukocyte-endothelial cell contacts (induced by drugs) is clearly linked to an increase of blood endocan levels. Blood levels of endocan/ESM-1 were also shown to be associated with the severity and evolution of septic states in preliminary studies.

**Methods** We have designed a prospective observational larger clinical study with 125 septic patients recruited to assess endocan/ESM-1 blood levels concomitantly with a comparison with survival at D10, severity score (SAPS II) at D2 and D7, and other biomarkers such as procalcitonin, CRP, and interleukins. ICU patients were followed over a 28-day period. Time course kinetics of serum endocan/ESM-1 at D0, D2, and D7 were performed using an ELISA assay (EndoMark H1; Lunginnov).

**Results** Our preliminary results for 39 patients showed that endocan/ESM-1 blood levels were increased at ICU admission in patients with poor prognosis (severe sepsis and septic shock). The monitoring of plasma endocan/ESM-1 at D2 and D7 revealed sustained elevated endocan levels in patients deceased within D10 ( $n = 12$ ). By contrast, the endocan levels fall down as early as D2 in patients who survived at D10 ( $n = 27$ ). Among the other molecules evaluated in this study, only anti-inflammatory IL-10 presented similar variations to endocan/ESM-1. These results suggest that both endocan/ESM-1 and IL-10 may have potent predictive values for patient follow up.

**Conclusions** We have demonstrated that a simple, accurate, and blood-based biomarker such as endocan/ESM-1 (EndoMark H1; Lunginnov) could assess the initial severity and closely follow the inflammatory events and endothelial dysfunction in patients, and therefore would be hugely helpful for clinicians to predict outcome and to select more appropriate therapeutic strategies.

**P56**

**Epidemiological situation of Crimean-Congo hemorrhagic fever**

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**Introduction** Crimean-Congo hemorrhagic fever (CCHF) is a viral zoonotic disease with up to 50% mortality rate in humans and belongs to the *Nairovirus* genus and *Bunyaviridae* family. CCHF manifests with four distinct phases including incubation, prehemorrhagic, hemorrhagic, and convalescence. The virus is transmitted to humans by infected tick bite, handling of infected blood or tissues, or nosocomially. In the present study, serological and molecular epidemiology of CCHF infection was surveyed among the Iranian population during the past decade.

**Methods** From 2000 to 2010 (30 May), probable sera of the human population throughout the country were collected. Then, the sera were analyzed through serological (IgM and IgG specific ELISA) and molecular (gel-based and real-time RT-PCR) testing.

**Results** As the results show, among 1,377 human probable sera collected from different parts of the country, 544 human cases were confirmed for CCHF and 79 CCHF death cases were reported to date. Sistan and Baluchistan (383 confirmed cases), Isfahan (44), Fars (26), Tehran (17), and Khorasan (12)

were the most infected provinces, respectively. Slaughterers, butchers, and farmers, with 21.6%, 17.64%, and 17.46%, ranked the highest among professions, respectively. Also, 52.2% of confirmed cases were in an age range of 21 to 40 years and, interestingly, CCHF infection was shown in males (77.5%) more than females (22.5%).

**Conclusions** Although CCHF has been confirmed in 23 out of 30 provinces of Iran, the disease has occurred with the highest grade in Sistan and Baluchistan during the past decade, certainly because of its proximity to Pakistan and Afghanistan, two countries with endemic CCHF. In the present study, it was demonstrated that CCHF was seen much more in the active age range and is more common in high-risk professions related to livestock such as butchers, slaughterers, and farmers. Therefore, it seems, informing the groups of high-risk professions has been efficient. Fortunately, with precise surveillance and laboratory detection, the mortality rate has been remarkably decreased recently.

**Cite abstracts in this supplement using the relevant abstract number, e.g.:** Chinikar S, Ghiasi SM, Moradi M: Epidemiological situation of Crimean-Congo hemorrhagic fever. *Critical Care* 2010, **14**(Suppl 2):P56.



## P57

**The effect of gender on cytokine syntheses and outcome of patients with severe sepsis**

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**Objectives** Men are at greater risk for sepsis than are women. Numerous experimental data suggest enhanced immune response and better outcomes in females with severe sepsis compared to males. Nevertheless, clinical data in humans are conflicting. The aim of the present study was to evaluate the gender differences in patients with severe sepsis in terms of survival and pro- as well as anti-inflammatory cytokines.

**Patients and methods** The study included 56 patients with severe sepsis. The patients were divided into 2 groups according to gender: females (group F, n = 27) and males (group M, n = 29). Severe sepsis was defined as the presence of confirmed infection and >2 of the following criteria: (a) a temperature >38 °C or <36 °C, (b) heart rate >90 beats/min, (c) respiratory rate of >20 breaths/min (d) WBC >12,000 or <4,000 cells/mm<sup>3</sup> plus at least 1 organ dysfunction indicated by the following: (a) hypotension, (b) PaO<sub>2</sub> <75 mmHg without evidence of primary respiratory tract disease, (c) pH <7.3 or a base deficit of >5 meq/liter, (d) urine output, <30 ml/h, (e) liver dysfunction, (f) acute alteration of mental status, or (g) DIC. The severity of sepsis was classified by the sepsis-related organ failure assessment score (SOFA). Levels of the proinflammatory cytokines TNF- $\alpha$  and IL-6 and anti-inflammatory cytokine IL-10, as well as TGF- $\beta$  1 were measured within 24 hours after admission (mean  $\pm$  SEM, pg/ml).

**Results** Group F had similar age (70.8  $\pm$  2.3 vs 70.5  $\pm$  3.5 y) and SOFA score (3.3  $\pm$  1.0 vs 3.4  $\pm$  0.5) with group M. All women were at menopause. Patients of both groups had similar levels of IL-6 (101.3  $\pm$  27.2 vs 120.2  $\pm$  18.6, p = 0.4) and similar levels of IL-10 (31.9  $\pm$  13.1 vs 26.5  $\pm$  14.8, p = 0.7). There was no difference in TNF- $\alpha$  levels (32.8  $\pm$  7.0 vs 39.5  $\pm$  5.7, p = 0.4) and TGF- $\beta$ a (32.8  $\pm$  7.7 vs 19.7  $\pm$  2.6, p = 0.2) between the two groups. Seven out of 27 females (5.5%) and 5 out of 28 males (7.2%) patients died (difference not significant).

**Table**

	Group F n = 27	Group M n = 28	P
IL-6 (pg/ml)	101.3 $\pm$ 27.2	120.2 $\pm$ 18.6	0.4
IL-10 (pg/ml)	31.9 $\pm$ 13.1	26.5 $\pm$ 14.8	0.7
TNF- $\alpha$ (pg/ml)	32.8 $\pm$ 7.7	19.7 $\pm$ 2.6	0.4
TGF-b (pg/ml)	32.9 $\pm$ 7.7	19.7 $\pm$ 2.6	0.2
mortality (%)	7/27 (5.5%)	5/28 (7.2%)	

**Conclusion** Male patients with severe sepsis do not seem to have a different immune profile and prognosis from females. Further work is needed to determine the influence of gender on outcome of severe sepsis. Studies must pay careful attention to matching the males and females for the many potential confounding variables and evaluating the role of sex hormones in sepsis.

## P58

**Withdrawn**

## P59

**Success stories about pneumonia caused by Pantan-Valentine *Staphylococcus aureus***

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**Background** Pantan-Valentine leukocidin (PVL) is a pore-forming toxin secreted by some *Staphylococcus aureus* strains and associated with

necrotizing pneumonia in healthy adults. We herein describe 3 cases of life-threatening pneumonia in immunocompetent adults caused by MSSA strain in the first and MRSA strain in the other 2 cases. In all cases, strains were producing PVL. **Case 1.** A 17-year-old male was admitted to the ICU with severe community-acquired pneumonia. He presented acute respiratory distress, severe hypoxia, and hemodynamic instability. *Staphylococcus aureus* methicillin sensitive was isolated from his tracheal secretions. He received linezolid and clindamycin. After a temporary amelioration, he presented a ventilator-associated pneumonia due to MDR *Klebsiella pneumoniae* strain, which was cured with colistin. New sepsis episodes due to pleuritic fluid collection were cured with the evacuation of the pleuritic fluid. He was discharged from the ICU after 25 days. **Case 2.** A 33-year-old woman was admitted to the ICU due to a necrotizing pneumonia caused by a strain of a PVL methicillin - resistant strain of *Staphylococcus aureus*. She received linezolid and clindamycin and became afebrile after 15 days. A tracheostomy was needed because the weaning from mechanical ventilation was difficult. She was discharged from the ICU to the step-down unit after 34 days. **Case 3.** A 32-year-old male was admitted to the ICU due to a necrotizing pneumonia with empyema and bacteremia due to a MRSA strain of *Staphylococcus aureus* producing PVL. The patient developed ARDS and septic shock. He was treated with linezolid, daptomycin, and a thoracic decortication. The patient was discharged a month later from the ICU.

**Results** Necrotizing pneumonia due to PVL often affects healthy young adults causing life threatening situations. PVL has been well described in CA-MRSA while the PVL gene is found much less frequently in MSSA.

**Conclusions** Most cases of severe necrotizing pneumonia are caused by community - acquired MRSA or MSSA. Empirical therapy for these conditions should cover this organism. Early treatment is necessary for a good outcome.

## P60

**Clinical characteristics and coagulative state in patients with sepsis complicated with cavernous sinus thrombosis**

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**Introduction** Cavernous sinus thrombosis is usually a rare condition, but is an important consideration because of potential morbidity. It is typically septic in origin and facial infections, most notably nasal furuncles, sphenoidal and ethmoidal sinusitis, dental infections, etc. are the source of pathogen.

**Objectives** To study clinical cause and blood coagulation state of patients with sepsis who developed cavernous sinus thrombosis and identify possible complications.

**Methods** Retrospective data analysis of patients who were admitted to Antisepsis Center of Georgia with cavernous sinus thrombosis as a complication of sepsis over the last 20 years. All the patients included in the research had no preceding disease before developing infection and their age ranged from 15 to 35 years.

**Results** 12 patients were admitted to Antisepsis Center with sepsis and cavernous sinus thrombosis. In 83% of cases mechanical irritation of furuncles in nasal and perioral area served as a place of bacterial invasion in the bloodstream; 17% were maxillary sinusitis. Disease had a rapid onset and all patients soon developed septic fever, chills, exophthalmos, and pneumonia. Additional complications were also reported: meningoencephalitis in 75% of cases, pneumothorax 16%, retrobulbar abscess 8%. Hemoculture and serological tests confirmed *Staphylococcus aureus* as a pathogen. Blood coagulation tests reflected disseminated intravascular coagulation (DIC) with rapidly declining platelet count, prolonged PT and PTT, fibrin degradation products in plasma, and low levels of antithrombin III. Overall mortality rate was 25%, actual cause of fatal outcome being septic shock.

**Conclusions** Early recognition and immediate initiation of proper treatment was associated with positive outcome. According to our results,

cavernous sinus thrombosis should be suspected in every case when sepsis is a complication of facial infection and when soon after the onset of disease the patient develops palsy of one or more cranial nerves. Proper treatment should be initiated after confirmation of diagnosis by neuroimaging and blood tests. All cases of fatal outcome were the result of late diagnosis and delayed start of treatment, which caused severe complications: multiple microthrombi in different organs secondary to DIC, meningoencephalitis, and septic shock.

## P61

### Withdrawn

## P62

### The expediency of different biomarkers' use for diagnostics and monitoring of perinatal sepsis treatment of newborns with surgical pathology

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**Background** The main role in perinatal sepsis appearance is played by intranatal and contamination infections, both bacterial and fungal, and immature immune biologic activity of an organism. The redundant secretion of cytokines (interleukins) happens under the influence of bacterial, viral, and fungal toxins; also the humoral and cell immunity is disordered due to discharge of bacterial antigens and of complement system and coagulatory hemostasis disorder.

**Materials** Treatment of newborns with postoperative and bone and joint sepsis showed that during 3 days of the disease critical values of coagulatory tests such as APTT - > 50 sec, TT - clot absence, PDF >8., and Fn < 1 g/l occurred. Also the correlation of these tests and level of procalcitonin (PCT) was observed.

**Results** The level of PCT > 10 µg/l was observed during intestinal perforation, peritonitis, aspiration pneumonia; it exceeded 2 µg/l during pyelonephritis development against the background of different kidney malformations or tumors. The high level of C-reactive protein (CRP > 24 mm/l) was observed in early stages of the disease in cases of severe sepsis and only in 2 weeks after the beginning of the disease in case of SIRS. The undertaken monitoring shows that normalization of coagulatory tests is connected with effective surgical treatment, adequate antibacterial therapy, introduction of specific immunoglobulin, and, to a lesser extent, with using protease inhibitors. The main point in diagnostics and treatment of perinatal sepsis is the determination of the etiologic factor of bacterial, viral, or fungal origin by serologic and PCR methods. The determination was made during the treatment of all patients and informed adequate therapy. This investigation showed that bacterial infections caused sepsis in 90% cases and viral in 10%. The 30% of clinical observations showed the presence of fungal infections as well.

**Conclusions** The coagulatory tests, the evaluation of humoral immunity by the presence of specific antibodies, and the continuous evaluation of PCT level are the most effective for early diagnosis of perinatal sepsis in newborns with surgical pathology.

## P63

### The experience of treatment of postoperative Gram-negative sepsis using specific immunoglobulin

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**Background** Fifty to 60% of newborns operated on for congenital malformation of gastrointestinal tract organ development have postoperative sepsis. Despite use of modern antibiotics and antifungal medications, the mortality of this group of patients is about 60 to 70%.

**Materials and Methods** When observing 60 infants with postoperative sepsis after surgery for esophageal atresia, necrotic enterocolitis, peritonitis, bowel obstruction, and making bacteriological and serological investigations, we have discovered that the etiology was Gram-negative infection with *Pseudomonas.aeruginosa*, *Escherichia coli*, or *Enterobacter aerogenes*, which, in 63% of cases, was combined with MRSA. The level of sepsis severity was determined by hemocoagulation tests and procalcitonin level. The prolongation of prothrombin time, increase of products of fibrin and fibrinogen degradation, and abrupt reduction of platelet level <50 000 were typical for severe sepsis. At the same time, the level of procalcitonin increased rapidly, especially in newborns with peritonitis and hollow organ perforation (> 10 µg/ml). Initial antibioticogram showed high resistance of the infection. We used specific immunoglobulin-containing antibodies against *Ps.aeruginosa*, *E.coli*, and MRSA for above-mentioned newborns and infants. The medicine was introduced in dose 0.2 ml/kg 1 time in 2-3 days, on average 3-4 doses.

**Results** Increase of complementary activity of blood, recovery of bacterial flora sensitivity to standard antibiotics was observed in all infants. The use of immunoglobulin specific to Gram-negative microflora promoted patients' recovery.

**Conclusions** Accurate determination of the etiologic factor requires use of serological methods. The most accurate determination of sepsis severity level is made by changes of hemocoagulation system and level of procalcitonin. Using specific immunoglobulin in treatment of postoperative sepsis in infants allows improvement of the treatment results.

## P64

### Bacteremia caused by *Enterococcus faecium* and modifications in the antimicrobial resistance profile during the past 5 years

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**Introduction** Enterococci are becoming an important cause of nosocomial infections, including bacteremia, endocarditis, and surgical wound infections. Optimal antimicrobial therapy requires the use of synergistic combinations of a cell wall-active agent such as penicillin or a glycopeptides and an aminoglycoside. Enterococci with ampicillin resistance and high level aminoglycoside resistance (HLAR) are the cause of considerable therapeutic problems, aggravated by acquired vancomycin resistance. The aim of the present study was to investigate how prevalent ampicillin resistance is in bacteremia caused by *Enterococcus faecium* and the modification of antimicrobial resistance profile.

**Methods** From January 2005 through April 2010, 52 strains of *E. faecium* were isolated from blood cultures that were consecutively received in the Puerta del Mar University Hosp. (Cádiz, Spain) from hospitalized patients corresponding to medical area (34.6%), surgery (30.7%), ICU (19.2%), and pediatric area (15.3%). In this period Enterococci bacteremia in our hospital was 8% corresponding to *E. faecium* in 20.8% of the isolates. The blood cultures were done in Versatrek System and the strains were identified by automatized method (Wider, Soria Melguizo) following manufacturer's indications.

**Results** Fifty-two strains of *E. faecium* were isolated from blood cultures during the study period. The ampicillin resistance was high (84.6%) but we did not find glycopeptide resistance (vancomycin and teicoplanin). HLAR was 75.0% for streptomycin (HLAR) and 13.4 % for gentamycin (HLGR). Concomitant resistance to both aminoglycosides was rare (11.5%). Only 3 and 1 strains were resistant to quinupristin/dalfopristin and linezolid, respectively. The resistance to levofloxacin was high (80.7%). We have detected a predominant resistance profile that includes non-susceptibility to ampicillin, quinolones, and HLAR, with susceptibility to gentamycin and glycopeptides. This profile is increasing its frequency from 50% in 2005 to 85.7% in the first third of 2010.

**Conclusions** Treatment with fluoroquinolones, cephalosporins, or carbapenems has been described as a risk factor for the development of an *E. faecium* infection. An increasing consumption of these antimicrobials agents

has also been observed in our hospital during the past 5 years. In conclusion, bacteremia caused by ampicillin-resistant *E. faecium* is a problem in our area. This may necessitate a change in the treatment of Enterococcal infections from ampicillin to vancomycin, which in turn increase the risk of spread of vancomycin resistance now not present in our hospital.

## P65

### Sepsis and acute lung injury

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**Background** The most frequent respiratory complication associated with sepsis is ALI (Acute Lung Injury). At the same time ALI total cases account about 40% of severe infections. The crucial factors for development of ALI are: the type of infectious agent, genetic background, condition of immune system of the patient, coexisting disorders, systematic inflammatory reactions, and multi-organic insufficiency.

**Materials and Methods** We present a retrospective study containing the analysis of histories of patients with sepsis and other severe bloodstream infections who were hospitalized and were being treated in Akad.V.Bochorishvili named Antisepsis Center of Georgia over 4 years. We studied 264 and 191 cases of patients from adult and gynecological departments, respectively. The major cause of hospitalization of all patients was bloodstream infection. The patients were divided into 2 groups according to their health condition before hospitalization: healthy individuals and individuals suffering from chronic/acute respiratory disorders.

**Results** 168 of 264 patients (41%) from the adult department were reported to have pulmonary/pleural complications; more precisely, 44% of affected patients were admitted with pneumonia, 36% - bronchopneumonia, 23%-infiltration pneumonia, 16%-destructive pneumonia, 31%-pulmonary stroke, 31%-lung infarction/infarct-pneumonia, 5%-solitary/multiple pulmonary abscess. 69 of 191 patients (36%) from the department of gynecology have had a history of pleuro-pulmonary complications: 35% of them developed pneumonia, 21%-bronchopneumonia, 17%-destructive pneumonia, 14%-pyothorax, 7%-pyogenic pleuritis, 6%-pneumothorax.

**Conclusion** Respiratory complications are the most common complications of sepsis (38%). This category of patients has demonstrated acute pulmonary disorders. The most frequent clinical manifestation in the patients from the second group was lobal pneumonia. On the other hand, the bronchopneumonia was the disorder most frequently reported in the patients from the first group. 8% of patients from both groups developed pleuritis, which was associated with pneumonia. The cases of primary pleuritis were not reported. The infarct-pneumonia developed during septic endocarditis ( $P < 0.005$ ). ALI resolution depended on the clinical evolution of disease as well as on the involvement of other organs in the pathologic process.

## P66

### *Mycobacterium fortuitum* endocarditis in 3 children after ventricular septal defect closure with bovine pericardial patch

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**Introduction** *Mycobacterium fortuitum*, a rapidly growing mycobacteria, is a well-known causative agent of cutaneous infections, post-surgical wound infections, and other health care-associated infections. Endocarditis due to this bacterium is rare and only sporadic cases have been reported so far.

**Materials and Methods** Herein we describe the cardiac surgery-related outbreak of endocarditis caused by *M. fortuitum* involving 3 pediatric patients. Over a 2-week period, 8 consecutive pediatric patients

underwent surgery for correction of ventricular septal defect (VSD). Patch closure of the VSD was performed by using a commercial bovine pericardial patch in all patients. The postoperative hospital stay was uncomplicated for 5 patients. However, 2 months after the surgery diagnosis of infective endocarditis was established in 3 patients. Transthoracic echocardiography revealed vegetations on the tricuspid valve in all 3 patients. Two sets of cultures of blood were taken from each of patient and they yielded acid-fast organisms on incubation Day 5. Reoperation was required in only 1 patient and bovine grafts, as well as vegetations, were submitted for bacteriologic analyses. These samples also yielded acid-fast organisms. All isolates were preliminarily identified as rapid growing mycobacteria and empiric therapy with vancomycin and ceftriaxone was switched to amikacin, ciprofloxacin, and imipenem. The conservative treatment as well as reoperation in 1 patient had favorable outcome and all 3 children are currently doing well.

**Results** It should be noted that cultural characteristics and susceptibility patterns of all isolates of acid-fast bacteria obtained were indistinguishable. The isolates were finally identified as *M. fortuitum* by 16S rRNA sequencing. In order to explore possible clonal relatedness of the strains, the isolates were genotyped. ERIC PCR was used since it had been proven as a sufficiently discriminative method for investigation of outbreaks caused by *M. fortuitum*. Identical patterns were obtained for all isolates, confirming that they are clonally related. Several cardiac surgery-related outbreaks caused by NTM, including *M. fortuitum*, have been reported. Most of these outbreaks were surgical wound outbreaks.

**Conclusion** To our knowledge, the 3 related cases we describe present the first cardiac surgery-related outbreak of endocarditis caused by *M. fortuitum*. Cases of nosocomially acquired *M. fortuitum* endocarditis were previously reported, but only sporadic cases in adult patients and they were usually fatal. In contrast, we describe the 3 obviously related cases of *M. fortuitum* endocarditis in pediatric patients with favorable outcome.

## P67

### Impact of severity of sepsis at time of ICU admission on ICU mortality

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**Background** Mortality of patients admitted to the ICU depends on various factors with severity of infection being one of them. But there is a dearth of data regarding ICU mortality based on severity grading of sepsis.

**Aims** To assess the impact of severity of sepsis at the time of admission on ICU mortality.

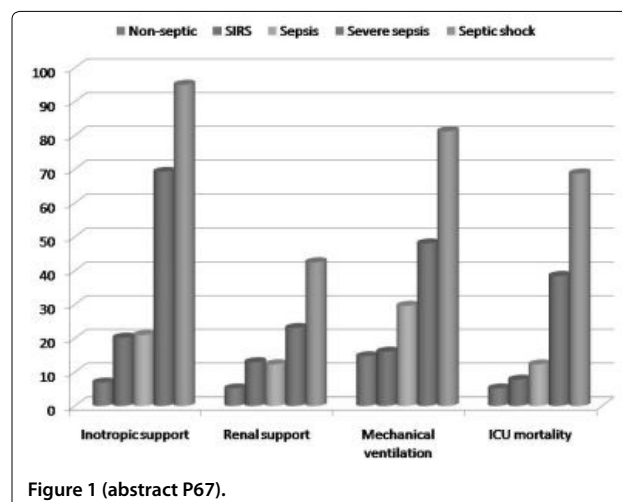


Figure 1 (abstract P67).

**Table 1 (abstract P67). Sensitivity, specificity and predictive values for mortality prediction of five severity-of-illness scoring systems in 132 outbreak patients with confirmed and presumptive Legionnaires' disease.**

Severity of sepsis	Mean age $\pm$ SD	Sex, males (%)	Mean ICU stay, days $\pm$ SD	Vasopressor support	Renal support	Mechanical ventilation	ICU mortality
Overall (n = 718)	58.4 $\pm$ 18.3	415 (57.8%)	4.95 $\pm$ 5.3	201 (28%)	103 (14.35%)	203 (28.27%)	122 (17%)
No sepsis (n = 231)	54.3 $\pm$ 19.6	122 (52.8%)	6 $\pm$ 6.3	16 (6.93%)	12 (5.19%)	34 (14.71%)	12 (5.2%)
SIRS (n = 193)	55.5 $\pm$ 16.6	110 (57%)	5.2 $\pm$ 5.5	39 (20.2%)	25 (12.95%)	3 (16.06%)	15 (7.8%)
Sepsis (n = 162)	65.9 $\pm$ 17	99 (61.1%)	4.2 $\pm$ 5.1	34 (21%)	20 (12.34%)	48 (29.63%)	20 (12.3%)
Severe sepsis (n = 52)	60.88 $\pm$ 17.2	36 (69.2%)	4.1 $\pm$ 3.1	36 (69.3%)	12 (23.1%)	20 (38.5%)	20 (38.5%)
Septic shock (n = 80)	60.2 $\pm$ 17.2	48 (60%)	3.4 $\pm$ 2.4	76 (95%)	34 (42.5%)	55 (68.75%)	55 (68.75%)

**Methods** Prospective cohort study in an 8-bed medical ICU of a tertiary care hospital over a period of 20 months. Data regarding patient demographics and ICU course including need for organ support and length of stay were recorded. Severity of sepsis was graded according to international classification. Qualitative data were analyzed using chi-square or Fisher Exact test as appropriate and quantitative data were analyzed using student's t-test. Inter- and intragroup comparison for quantitative data was done by one-way ANOVA. Primary outcome measure was the ICU mortality, which was compared in patients with different grades of sepsis.

**Results** Data from 718 patients were analyzed. There were 231 (32.2%) nonseptic patients, and 193 (26.9%), 162 (22.56%), 52 (7.24%), and 80 (11.1%) patients with SIRS, sepsis, severe sepsis, and septic shock, respectively (table). The most common suspected site of infection was lungs (232/487 patients, 47.6%), followed by urinary tract (102/487 patients, 20.9%) and abdomen (95/487 patients, 19.5%). The ICU mortality according to the severity of sepsis was nonseptic 5.2%, SIRS 7.8%, sepsis 12.3%, severe sepsis 38.5%, and septic shock 68.8%.

**Conclusions** Sepsis is common at the time of ICU admission and its severity can have a major impact on ICU outcome. Early grading of sepsis can help in identifying patients who may require organ support and those who are at higher risk of dying during their ICU stay.

## P68

### Obstetric gynecological sepsis in Georgia

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**Background** Sepsis remains a significant problem of modern health care. Despite the outstanding achievements of antimicrobial therapy, incidence of severe sepsis and septic shock is rising.

**Materials and Methods** The study was retrospective. We have studied the records of patients that were hospitalized with a diagnosis of sepsis during the past 6 years in our OB/GYN department.

**Results** A total of 310 patients were hospitalized with a diagnosis of puerperal and postabortion sepsis in OB/GYN department. The age of patients varied from 16 to 40 years. In 40% of patients, sepsis developed after spontaneous delivery, in 36% after C-section, and in 28% after abortion. A statistically significant relationship between patients' age and development of complications was not observed. Disease was observed without complications in 44% of patients. In the other 56% one or more organ failures developed. Correlation between incidence of organ failure and mortality rate was found. The most common complication of OB/GYN sepsis was pneumopleural (36%), represented by lobar pneumonia, bronchopneumonia, destructive pneumonia, etc. Second by frequency was renal insufficiency (17%). In 20% of patients with renal failure severe sepsis developed, in 63%, septic shock. Seventy percent of patients with septic shock died.

**Conclusion** In most cases OB/GYN sepsis developed with one or more organ failures. Pneumopleural complications were most prevalent.

## P69

### Mental disorders in patients with sepsis

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**Background** Patients with sepsis are at risk of developing altered mental status. In the present research possible triggers of psychiatric manifestations were studied on the example of patients with no preceding mental disorders who developed febrile schizophrenia secondary to sepsis and whose treatment was directed against generalized infection but did not receive psychotropic drugs.

**Objective** The aim of our research was to determine the possible cause of febrile schizophrenia and to identify the effective strategy of treatment.

**Materials and Methods** Retrospective data analysis of 50 patients (70% women and 30% men) who were admitted to the Antisepsis Center of Georgia over the last 10 years with the diagnosis of febrile schizophrenia. Patients included in the study had no preceding psychiatric disorder and their age ranged from 17 to 43 years. Eighty percent of female patients developed symptoms postpartum and all cases of male patients were cryptogenic. Sepsis diagnosis was confirmed by clinical and laboratory tests with isolation of *Staphylococcus epidermidis* and *aureus* from the blood culture and positive anatoxin skin test.

**Results** All patients had intermitted episodes of depression and excitation during the prodromal phase. Psychoneurological irregularities were manifested as disorientation to place, time and environment; symptoms of encephalitis; delirium and schizophrenic symptoms. In 40% of cases mental symptoms preceded fever, 35% of cases were characterized by delayed onset of psychiatric symptoms, and in 25% of cases fever and manifestation of mental disorders had simultaneous onset. The treatment of the patients aimed for eradication of infection and correction of homeostatic disturbances and no psychoactive drugs were used. Psychiatric manifestations disappeared with the cure of sepsis. Data of our patients suggest higher correlation of psychiatric symptoms with the generalization of infection than developing high fever. It could be suggested that psychiatric syndromes, including febrile schizophrenia, could be triggered by infectious diseases, particularly sepsis. Of course a combination of diseases (schizophrenia and sepsis) cannot be ruled out, but in our practice combination of diseases was not confirmed.

**Conclusion** Staphylococcal sepsis can cause mental disorders including schizophrenic syndromes manifested as amiental-catatonic or excited-hallucinatory state. In management of any patient with preceding normal psyche diagnosed with febrile schizophrenia it is important to determine cause of the fever. On the basis of our data if the underlying cause of febrile schizophrenia is staphylococcal sepsis, treatment directed against generalized infection is effective against its mental symptoms too and all the psychiatric symptoms will resolve with the cure of sepsis without any use of psychoactive drugs.

## P70

**Recombinant human soluble thrombomodulin (rhs-TM) may have an efficacy for sepsis not only for DIC**

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**Background** In Japan, rhs-TM (Recomodulin<sup>®</sup>, Asahi Kasei Pharma Co., Tokyo) administration has been approved to treat disseminated intravascular coagulation (DIC) in adults since May 2008<sup>1</sup>. Although thrombomodulin is one of components of the protein C anticoagulant pathway, it also has known anti-inflammatory activities to inhibit activated kinases and NFκB responses in endothelium<sup>2</sup>. In the previous study, we reported rhs-TM might have a role of anti-inflammation in severe sepsis. The purpose of the present study is to demonstrate how rhs-TM affects in sepsis with DIC.

**Materials and Methods** Five severe septic patients (3 women, 2 men; age range 45-82 years) with rhs-TM therapy for DIC in our ICU were investigated. In effect, rhs-TM therapy consists of 380 U/kg intravenously every 24 hours for 6 days. Serum TM, protein C (PC), protein S (PS), endothelial protein C receptor (EPCR), antithrombin III (ATIII) activity, plasminogen activator inhibitor-1 (PAI-1), WBC, CRP, IL-6, C3 (Complement 3) were measured daily from the start of rhs-TM therapy until they finished the therapy. SOFA score and DIC score were used to assess for the severity. TM, EPCR, IL-6, and PS were measured by enzyme-linked immunosorbent assay. PAI-1 and PC was measured by latex photometric immunoassay. C3 was by turbidimetric immunoassay. Data were analyzed by Kruskal-Wallis test and Mann-Whitney U test. Pearson's correlation coefficient and Spearman's correlation coefficient by rank test were used for correlations. A  $P < 0.05$  was considered as statistically significant.

**Results** There were 3 survivors and 2 nonsurvivors. No side effects of rhs-TM occurred. TM values were statistically significantly increased ( $3.7$  vs  $19.4$  ng/mL,  $P = 0.01$ ) and CRP values were significantly decreased ( $20.8$  vs  $7.4$  mg/dL,  $P = 0.005$ ) after rhs-TM therapy compared with before that therapy. There were no significant differences in other values between before and after rhs-TM therapy. TM values correlated with ATIII activity ( $r = 0.3$ ), CRP ( $r = 0.48$ ), PAI-1 ( $r = 0.46$ ), and PS ( $r = 0.34$ ). There were no statistically relationships between TM and EPCR, PC, IL-6, WBC, C3. SOFA score and DIC score were improved but did not reach to statistical significances.

**Conclusions** This study showed a close association could exist between TM and CRP, PAI-1. There is a possibility rhs-TM may also have an efficacy for anti-inflammation not only for DIC in sepsis.

**References**

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2. Conway EM et al. *J Exp Med.* 2002;196:565-577.

## P71

**Septic complications in hematological ICU: Epidemiology and antimicrobial resistance**

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**Background** Sepsis is among the main causes of death in oncohematological patients, receiving chemo- and immunosuppressive therapy and hematopoietic stem cell transplantation (HSCT). There are some epidemiological, clinical, and laboratory features that complicate its diagnosis in this group of patients. Unfortunately, this problem is still not well explored. The aim of our study was to characterize the epidemiology of sepsis and antimicrobial resistance in patients after intensive chemotherapy and HSCT.

**Materials and Methods** Microbiological cultures were sampled from 52 patients admitted to the specialized ICU clinic for hematology and transplantology with confirmed diagnosis of sepsis (17.3%), severe sepsis (38.5%), and septic shock (44.2%). Both children and adults were included

(range 0.5-76 years, median age 14 years). In 27 (52%) cases patients received conventional chemotherapy; in 10 (19%) cases HSCT; and in 13 (25%) cases immunosuppressive treatment of "graft-vs-host" disease after HSCT. Two (4%) patients did not receive any specific therapy (1 with severe combined immune deficiency and 1 with myelodysplastic syndrome). Microbiological analysis antibiotic resistance detection were provided using BacT/Alert<sup>®</sup> microbial detection system and Vitek<sup>®</sup> 2 automated identification susceptibility system.

**Results** Microbiological confirmation of sepsis was received in 39 (75%) patients. The most common organisms which cause septic complications were Gram-negative bacteria - 60.86% (*K. pneumoniae* - 30.43%, *P. aeruginosa* - 13.04%, *E. coli* - 6.52%, other - 10.87%). Gram-positive cocci were identified in 26.09% (*S. epidermidis* - 8.7%, *Enterococcus spp.* - 6.52%, other - 10.87%). In 13.05% fungal sepsis was diagnosed (*Candida spp.* - 8.7%, *Aspergillus spp.* - 4.35%). Antibiotic resistance analysis shows high efficacy of carbapenems and cefoperazone/sulbactam in cases of *K. pneumoniae* infection, 100% sensitivity of *P. aeruginosa* to colimycin and piperacillin/tazobactam but low activity of aminoglycosides and third generation cephalosporins. Only 1 case (out of 3) of vancomycin-resistant *Enterococcus* and no cases of methicillin-resistant *S. aureus* were detected.

**Conclusions** Our study shows the emerging role of Gram-negative bacteria with increasing rates of multiresistant strains in the etiology of sepsis in patients receiving chemotherapy, HSCT, or immunosuppressive treatment. Analysis of antibiotic sensitivity shows that the principles of empiric antimicrobial agents use in hematological ICU should not be the same as in hematology or transplantology wards.

## P72

**Assessment of physiotherapy on ICU and effects of physiotherapeutic interventions on outcome of critically ill patients**

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**Background** Physiotherapists are members of the interdisciplinary team providing care for critically ill patients. So far, there are only very few studies indicating a significant benefit of physiotherapy on clinical outcome of ICU patients. In this study we investigated (i) the implementation of physiotherapy on ICU and (ii) the effect of physiotherapeutic interventions on outcome of patients with severe sepsis or septic shock.

**Methods** Retrospective statistical analysis of critically ill patients admitted to Jena University Hospital from January 2006 to December 2009. Data were extracted from the patient data management system (COPRA) and analyzed for various clinical variables and the frequency of physiotherapeutic measures. Frequency of physiotherapy is given as relative measures calculated as the number of treatments per day of ICU stay and is given in percentages. Cox proportional hazards regression analysis was performed to analyze effects of physiotherapeutic treatment on outcome of septic patients (in-hospital mortality). Hazard ratio was adjusted to established risk factors such as age, gender, disease severity scores, mechanical ventilation, comorbidity, etc.

**Results** In total 16,042 critically ill patients admitted to the ICU were analyzed. A total of 999 patients (mean age  $64.1 \pm 14.2$  years; mean length of ICU stay  $17.2 \pm 17.5$  days) developed severe sepsis or septic shock (mean APACHE II score at admission  $16.8 \pm 11$ ). Only 77% of these patients received physiotherapy with a relative therapeutic treatment of 42%. Cox regression analysis indicated a highly significant benefit of physiotherapeutic interventions on survival of septic patients (Table 1;  $P < 0.0001$ ).

**Conclusions** The data indicate that intensive physiotherapy may significantly increase survival of patients with severe sepsis or septic shock. Furthermore it was found that physiotherapy is not regularly applied to all ICU patients, physiotherapists rarely treat sedated patients, and there is a lack in communication among physicians, nurses, and physical therapists. Prospective studies are urgently needed to confirm the benefit of physiotherapy on critically ill patients.

Table 1

Quartiles of relative physiotherapeutic interventions	Hazard ratio (risk of death)	95%-Confidence interval
< 9%	-	-
9–39%	9.85	5.53–17.55
39–67%	3.67	3.67–10.04
> 67%	1.45	1.45–4.06

## P73

**Clinical peculiarities of Gram-positive sepsis**

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**Introduction** Sepsis as a disease with various clinical manifestations is an actual problem of modern medicine, especially for specialists of infectious diseases like surgeons, gynecologists, and stomatologists. Its polymorphism depends on various casual agents and the character of microbes. The aim of our research was to study clinical parameters in cases with Gram-positive bacterial sepsis.

**Materials and Methods** The investigation included 79 patients, 29 female (26.7%) and 50 male (63.3%), aged 16–65. All diagnoses were confirmed by: 1. positive hemoculture (*Staphylococcus aureus*, 41 [51.9%], *S.epidermidis*, 12 [15%], other Gram-positive cocci, 7 [8.9%], serologically, 19 [24%]); and 2. echoscopy.

**Results** Among the investigation patients, 15% (12) were drug abusers. In 27 (34.2%) cases endocarditis was revealed. In 56 (70.9%) cases clinical manifestation of the disease was acute with high fever 39–40 °C, chills, tachypnoea (26–34 min.), and respiratory insufficiency. Vegetation like bacterial damage of the tricuspid valves was revealed in every drug abuser patient. The rest of the patients revealed mitral and aortic valve. Onset of the disease with severe destructive pneumonia confirmed by radiological investigations was revealed in 59 (74.7%) cases; 14 (17.7%) patients were placed in the department of intensive therapy because of worsened hemodynamic and respiratory failure. The mechanical ventilation of lungs was required in 4 (5%) cases. All patients had hepatomegaly, but 36 (45.6%) had splenomegaly. At the onset of the disease 7 (8.9%) patients with septic endocarditis had low-grade fever. Three to 4 weeks later they developed hectic temperature and chills. Four patients died because of thromboembolic complications (Machabelli syndrome) and acute heart failure. Three patients with septic endocarditis recovered by conservative treatment (6–8 months): antibacterial and heparinotherapy. Every patient with acute sepsis was treated with etiotropic treatment (10 days of apyrexia period), and a lower dose of heparin; 10,000 units because of underlying disease (gastric ulcer) was administered to the 4 patients that died. Complete blood count in 3 patients (3.8%) revealed leucopenia, the rest have leucocytosis and high ECR; left side shifting of leukocyte formula. According to the coagulogram all patients revealed hypercoagulation and a high level of fibrinogen; 72 (91%) had moderately increased prothrombin and sharply positive ethanol test, 7, slightly positive.

**Conclusions** Thus, common clinical manifestation of sepsis and septic endocarditis is destructive pneumonia with hepatolinar syndrome, high fever with chills, leucocytosis in blood count (in severe and fulminant forms, leucopenia) increased ECR, disturbance of coagulation (increased fibrinogen). The outcome of the disease depends on an etiotropic treatment as one of the important components of therapy such as the indirect anticoagulant, heparin.

## P74

**Detection of Epstein-Barr Virus in Iranian patients with multiple sclerosis**

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**Background** Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS), the etiology of which is believed to have both genetic and environmental components. Although no single virus has proven to cause MS, several different viruses, including retroviruses and herpesviruses, have been implicated in MS pathogenesis.

**Aim** This study was conducted to evaluate the presence of Epstein-Barr virus (EBV) infection in Iranian patients with MS.

**Material and Method** In a prospective case-control study, we studied 78 patients with MS and 123 healthy controls for viral DNA detection and antibody assay. DNA extracted from serum and real-time PCR was employed to the detection of the EBV genome. The levels of anti EBV IgG were measured in samples by enzyme-linked immunosorbent assays (ELISA) in Dr Ahmadi's medical laboratory ( $P < 0.05$ ).

**Results** We found EBV DNA in the 58% and 31% of patients and healthy controls, respectively. Furthermore, higher levels of anti EBV IgG were detected in patients in contrast with healthy controls. Moreover, genome copy number of EBV was significantly increased in patients.

**Conclusion** Results showed significant correlation between EBV DNA and development of MS in selected patients. These results support the hypothesis that EBV may contribute to the MS disease thought to establish active infection process and induce immune response. The role of EBV in the modulation of MS requires further study.

## P75

**Comparison of different modes of CVVHF in therapy of septic shock**

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**Background** Sepsis is developed mainly during hospital stays (eg, in intensive care patients) and remains a therapeutic dilemma despite advances in drug therapy and intensive care. Sepsis is a major cause of morbidity and mortality in intensive care clinics and the incidence is continuously increasing. In recent years, a component of intensive therapy of severe sepsis has used methods of hemofiltration. However, there remains the unresolved question of the mode and extent of substitution therapy. The aim of our study was to determine the effect of normal and high volume hemofiltration on central hemodynamics and gas exchange in patients with septic shock.

**Materials and Methods** The study included 41 patients randomized into 2 groups. The 1st group included 21 patients, age  $60.1 \pm 6.2$  years. CVVHF was conducted at a volume of substitution of 35 mL / kg / hours. The 2nd group included 22 patients, ages  $58.4 \pm 4.8$  years. CVVHF was conducted at a volume of substitution of 70 mL / kg / hours. within 12 hours, then at a rate of substitution of 35 mL / kg / hours.

Clinical efficacy was assessed by parameters: CI, SVRI, APmean, ELWI, PaO<sub>2</sub>/FiO<sub>2</sub>, IDO<sub>2</sub>, IERO<sub>2</sub>, PCT, SOFA, lactate, and vasopressor (% from baseline). Stages: I - before treatment (baseline); II - within 12 hours from the beginning; III - after 24 hours from the beginning. Hemodynamics were obtained by thermodilution (PICCO technology). Both groups in the study received standard therapy (Surviving Sepsis Campaign International Guidelines 2008).



Table (abstract P75).

Parameters	I stage		II stage		III stage	
	1-st group	2-nd group	1-st group	2-nd group	1-st group	2-nd group
CI (l/min/m <sup>2</sup> )	4.1 ± 0.5	4.5 ± 0.3	4.9 ± 0.2	4.2 ± 0.6	4.5 ± 0.4	3.4 ± 0.9
AP <sub>mean</sub> (mmHg)	49.3 ± 12.3	51.1 ± 9.8	61.2 ± 10.1	78.7 ± 5.3	69.5 ± 7.8	88.3 ± 5.9
SVRI (dyn*s*cm <sup>-5</sup> *m <sup>2</sup> )	780 ± 24	808 ± 26	910 ± 13	1620 ± 25	1460 ± 34	1980 ± 58
ELWI (ml/kg)	12.1 ± 0.8	12.8 ± 0.2	10.5 ± 1.3	8.3 ± 0.9	7.9 ± 1.7	6.28 ± 0.5
PaO <sub>2</sub> /FiO <sub>2</sub>	124 ± 14	130 ± 17	166 ± 10	184 ± 9	190 ± 12	210 ± 8
DO <sub>2</sub> I (ml*min/m <sup>2</sup> )	270 ± 24	282 ± 19	370 ± 20	450 ± 27	460 ± 29	580 ± 35
ERO <sub>2</sub> (%)	18 ± 1	17 ± 2	20 ± 2	24 ± 2	23 ± 1	31 ± 4
Lactate (mmol/l)	5.6 ± 1.2	5.9 ± 2.0	4.2 ± 1.1	3.7 ± 0.9	3.1 ± 1.1	2.1 ± 0.5
PCT (ng/ml)	18.2 ± 3.4	17.8 ± 2.8	13.3 ± 2.2	11.2 ± 0.5	5.7 ± 1.1	2.6 ± 0.2
Vasopressor (% from baseline)	0	0	32.1 ± 4.9	58.9 ± 5.1	53.2 ± 8.5	75.1 ± 7.6
SOFA	18 ± 1	18 ± 1.5	14 ± 1.8	11 ± 1.4	9 ± 2.1	7 ± 1.6

**Results** Hemodynamics, pulmonary oxygenation, oxygen transport, tissue perfusion, and vasopressor dose are presented in Table 1. In both groups a statistically significant reduction in vasopressor dose, improvement in lung oxygenation, increased IDO2, normalization IERO2, reducing ELWI, and lactate were revealed. The CI levels were not significantly changed.

**Conclusion** The regimen of high volume CVHF at the beginning of extracorporeal treatment is more effective in the treatment of septic shock resulting from rapid correction of violations in the system of oxygen transport and tissue perfusion.

## P76

## Withdrawn

## P77

#### Complement inhibition decreases the procoagulant response and provides organ protection in a baboon model of *E. coli* sepsis

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**Introduction** Severe sepsis leads to massive activation of coagulation and complement cascades that could contribute to multiple organ failure (MOF) and death.

**Materials and Methods** To investigate the role of the complement and its crosstalk with the hemostatic system in the pathophysiology and therapeutics of sepsis, we have used a potent inhibitor (compstatin) administered early (T0 to T+8 hrs) or late (T+5 to T+11 hrs) post *E. coli* challenge in a baboon model of sepsis-induced MOF.

**Results** Both compstatin treatment regimens inhibited plasma complement activity and TCC levels and significantly inhibited MBL, C3b and C5b9 deposition in the kidney, suggesting decreased endothelial injury and IR induced nephrotoxicity. Moreover, compstatin treatment protected against shedding from the endothelial surface of two major negative regulators of complement function, CD55 and CD59, without significantly affecting the mRNA expression of these proteins. Compstatin treatment reduced sepsis-induced leucopenia and thrombocytopenia and lowered

the accumulation of macrophages and platelets in lungs and kidneys, as well as decreased C5b9 deposition on aggregated platelets detected in these organs. Compstatin treatment significantly reduced the coagulopathic response as reflected by decreased fibrinogen consumption, FDP and APTT values, downregulated tissue factor and PAI-1 expression and better preserved TFPI and thrombomodulin on the endothelial surface. In parallel, the plasma levels of sPselectin and sTM were decreased, suggesting diminished endothelial cell injury and platelet activation in compstatin treated animals. Compstatin infusion also improved cardiac function and the biochemical markers of kidney (creatinine) and liver (ALD, ALT and AST) injury. Inhibition of complement decreased the release of cytokine IL-6 and the chemotaxin eotaxin. Histological analysis of vital organs collected from animals euthanized after 24 hrs confirmed that compstatin provided substantial organ-protection. Differently from the non-treated group, compstatin treated animals showed no obvious signs of thrombosis or capillary leak in the lungs, no or less tubular necrosis and glomerular thrombosis in the kidneys, lack of hepatocyte vacuolization and liver degeneration, less leukocyte infiltration in the lung, liver adrenals and spleen, and decreased cell death in adrenals and spleen, all consistent with attenuated organ injury.

**Conclusion** We conclude that complement-coagulation interplay contributes to the progression of severe sepsis and blocking the harmful effects of complement activation products, especially during the organ failure stage of severe sepsis is a potentially important therapeutic strategy.

Complement  
Sepsis  
Non-human primates

## P78

#### A new role of the alternative complement activation pathway for innate host defense during sepsis

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**Introduction** It is well known that lack of complement activation leads to compromised survival during sepsis. The alternative pathway has been reported to exert an amplifying function for overall complement activation in other models of inflammation such as ischemia reperfusion but its contribution to the innate host response in sepsis is largely unknown. From a biological point of view, the concept of redundant/amplified complement activation appears to be not very convincing within the setting of innate host immune responses.

**Methods** We therefore investigated the contribution of the alternative pathway using factor D knockout mice (fD<sup>-/-</sup>) and compared crucial defense mechanisms in these mice during sepsis to mice compromised in classical complement pathway activation (C1q<sup>-/-</sup>).

**Results** Our results indeed demonstrated striking differences: Both knockout mice strains showed a significantly reduced survival when compared to control mice but death occurred for distinct different reasons: fD<sup>-/-</sup> mice demonstrated fully compensated bacterial clearance when compared to control mice, while C1q<sup>-/-</sup> mice rapidly died being overwhelmed by bacterial growth. Despite normal bacterial clearance,


fD<sup>-/-</sup> mice demonstrated uncontrolled inflammatory cytokine generation and neutrophil recruitment into lungs and the blood when compared to control mice and C1q<sup>-/-</sup> mice. As one possible mechanism, we identified a strongly increased NfκB activation capacity in neutrophils from fD<sup>-/-</sup> mice, suggesting a potential direct or indirect controlling function of factor D for crucial inflammatory host responses.

**Conclusion** Our results provide evidence for the new concept that the alternative complement activation pathway exerts a distinctly different contribution to the innate host response during sepsis when compared to the classical pathway.

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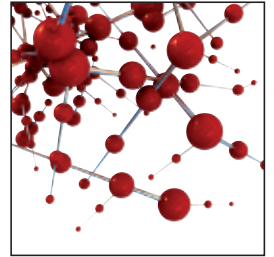
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