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Spectrum of sepsis, mediators, source control and management of bundles

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1. ABSTRACT

Sepsis is a modern medicine icon and the onset of organ dysfunction is one of the worst scenario. More than 100 distinct molecules have been proposed as useful biological markers of sepsis. TNF-alpha, IL-6, chemokines and cytokines are considered the first line factors able to drive the dynamic process of sepsis. The PIRO scheme is a new classification of different aspects, used to stage sepsis. Resuscitation bundles must be started within 6 hours of presentation (serum lactate measured; blood cultures obtained before antibiotic therapy; broad-spectrum antibiotics within 3 hours from emergency admission and 1 hour from ICU admission; in case of hypotension and/or lactate higher than 4 mmol/L deliver an initial 20 ml/kg of crystalloid or colloid solution or apply vasopressors for hypotension not responding to initial fluid resuscitation to maintain mean arterial pressure above 65 mmHg). A management bundle should be implemented within 24 hour (low-dose steroids administered for septic shock; recombinant human activated protein C; glucose control maintained at less than 8.3 mmol/L; inspiratory plateau pressures maintained at less than 30 cm H_2O).

2. INTRODUCTION

Every year several millions of people in the world suffer from pandemic infections caused by Gram negative bacteria, Gram positive microorganisms, fungi and viruses (1,2). Sepsis is a modern medicine icon and the onset of organ dysfunction is one of the worst-case scenario. Bacteremia and endotoxemia, sometimes openly but most of the times treacherously, are the critical points in the process of sepsis; moreover, the degree of host response can elicit different grades of severity of the syndrome, which are conventionally defined as sepsis, severe sepsis and septic shock; besides, each grade involves increasing morbidity, mortality and cost of care (3,4). The patient's clinical state can change rapidly in relation to the intrinsic strength of the micro-organisms and the condition of the host; therefore, the patient needs a timely intensive care management and supportive measures with the aim of preventing or treating the slippery slope of sepsis leading ultimately to a condition of organ dysfunction/failure (5).

Sepsis is defined as a systemic inflammatory response syndrome (SIRS) associated with a suspected or

Table1. The sepsis predisposition

Age
Infection
Site of infections
Co-morbidity
Severity
Gender
Genotype
Mediator/marker

proven infection. Sepsis is one of the main problems of modern medicine, due to its pathophysiological, clinical and therapeutic complexity (6). Sepsis is a consequence of the activation of an innate immune response, with changes in the expression and activity of several endogenous mediators of inflammation, coagulation, and intermediary metabolism. In fact, more than 100 distinct molecules have been proposed as useful biological markers of sepsis (7).

In the USA there are 750000 new cases per year (6), and prospectively the incidence of this syndrome in western countries might increase by 1.5% every year (2). According to the severity of host response, the septic syndrome can be conventionally defined as: sepsis, severe sepsis, or septic shock (8); the immune system works by recognition of the microorganisms and their products by immunocompetent cells, leading to a stereotyped host response that consists in the activation of monocytes and macrophages and in the burst of the primary mediators that initiate the synthesis of several cytokines (9). The metabolic explosion of mediators determines a loss of physiological modulation leading towards a derangement of the body's homeostasis. Procalcitonin (PCT) levels, for example, have proved to be higher in patients with infection, and to drop in response to adequate antibiotic therapy (10). Scoring systems evaluate the seriousness of clinical conditions. The SOFA (sequential organ failure assessment) score (11) has been developed by a Working Group of the European Society of Intensive Care Medicine. In contrast to other scoring systems (e.g. APACHE II), the aim of the SOFA score is not to predict mortality but to describe organ dysfunction/failure. It analyzes six systems by grading them 0 to 4 (worst function), using routinely measured and easy to obtain parameters. A daily calculation of scores can provide an objective assessment of the evolution of the disease and the response to treatment. They should be employed to facilitate stratification of patients and comparison of results in clinical trials (11). PIRO is a new classification scheme that takes into consideration different aspects to stage sepsis: Predisposition, Infection, host Response and Organ dysfunction. Predisposition factors (P) include age, infection, site of infection, genetic features, chronic underlying disease, mediators (Table 1). The type of Infection (I) drives host response and decision making in therapy, and depends on the site of infection, type of organism and extent of the process. Response (R) means the host's capacity to react to sepsis and it is related to physiologic factors, specific mediators and generic markers. Organ (O) dysfunction, single or multiple, is the fourth element in the PIRO model (12).

3. FAILURE OF SEPSIS TRIALS AND MEDIATORS

The spectrum of infection-sepsis-organ dysfunction definitions, the controversies over SIRS and the inclusion criteria for patient enrolment are considered critical points. In the past decades, the most important gap to be filled in the clinical trials on infections and sepsis consisted in the mistake of lumping together all kinds of patients, without an appropriate use of the scoring systems, which led to a delay in medical and surgical interventions. The Acute PHysiology And Chronic Health Evaluation (APACHE II) and the Sequential Organ Failure Assessment (SOFA) scores are useful indices to evaluate the seriousness of the clinical conditions (13,14).

Cytokines are physiologic factors useful to maintain homeostasis (15,16). TNF-alpha, IL-6, chemokines and cytokines are considered the first line factors able to drive the dynamic process of infection, bacteremia, sepsis or non-infectious conditions such as trauma, pancreatitis, burns, etc. These molecules interact with specific receptors of different organs inducing immune system depression, which facilitates the onset of secondary sepsis (17,18).

Randomized and controlled studies conducted on the so-called immunomodulating agents did not entail advantages in terms of improvement in survival rates. Why have clinical trials failed? There are many explanations, such as unsuitable laboratory data or ineffective experimental agents (for instance, antiendotoxin HA-1 and E5); as a matter of fact, researchers thought that these factors could bind to the lipid A portion of endotoxins and, hence, that they could neutralize endotoxin activity, whereas *in vitro* tests proved that none of these compounds could limit endotoxin activity or reduce interleukin (IL-1) or TNF-alpha release (19-21).

Instead, a combination of therapies directed to the many arms of the septic process (22) resembling the strategy used for cancer and HIV infection is required; the five potential intervention points are towards microbial mediators, pathogen-associated molecular patterns, signal transduction mechanisms of immune effector cells, host response mediator networks and antiapoptotic pathways.

4. IMPORTANCE OF THE SURVIVING SEPSIS CAMPAIGN

Prevention of infections in Intensive Care Unit (ICU) patients represents a gold standard. Sepsis management remains largely supportive, with an emphasis on several prevention and management strategies such as: fluid resuscitation, vasopressors/inotropes, adequate antibiotic treatment, discovering and eradicating the source, avoiding lung injury with low tidal volume, and adopting adjunctive therapies such as glycemic control combined with nutritional protocols (23-25).

The interactions between the injury and repair cascades most likely determine the outcome of the injurious process. Rivers *et al*. (26) reported that early goal-directed therapy before admission to intensive care units in order to treat patients affected by severe sepsis and septic shock significantly reduces mortality. The same study also proved that decreases in morbidity and mortality rates depend on early identification and treatment of at-risk patients. Protein C plays an important role in maintaining coagulation homeostasis; as a matter of fact, in the course of sepsis, protein C levels decrease, whereas endothelial injuries weaken protein C functions, since they reduce its activation (27).

Moreover, low protein C levels are quite frequently reported in patients affected by sepsis and septic shock; this factor plays a decisive role in the coagulation process: it modulates endogenous fibrinolytic activity and inflammatory response, including the ability to stop nuclear translocation NF-kB factor which is a key mechanism for cytokine formation from mononucleate cells and the endothelium. In this context, activated protein C is likely to modulate an anti-apoptosis action and to limit endothelial injuries (28). Predisposition represents an increased risk for developing sepsis. Genetic predisposition can be considered in terms of high-risk and low-risk exposure, and independent or dependent exposure. High risk often involves dependence on single genes, so that a single mutation produces the disease; and lower risk often reflects dependence on multiple genes. Sepsis is probably a multiple gene problem (29). Acquired factors are complex and difficult to separate from heritable factors. Age, gender, chronic health or disease, acute illness, exposures, and interventions are all acquired factors. However, traditional genetic studies are not possible in sepsis because family members usually do not become septic at the same time and because treatments have changed over time. The study of injury in critical illness is now occurring "upstream", at the genetic and cellular levels, to understand how the damaging effects of acute inflammation caused by injury can be prevented or modulated. Genomic and proteomic studies suggest evidence that repair processes begin shortly after injury (30).

5. SOURCE CONTROL

Source control is defined as the physical measures aimed at eradicating a focus of infection to eliminate ongoing microbial contamination and prevent microbial growth and tissue invasion (31). Source control should be considered an integral component of therapy and includes: 1) drainage; 2) debridement, 3) device removal; 4) devitalized infected tissue removal**.**

Many surgical diseases may cause intra-abdominal sepsis. In critically ill patients, physical examination is not always reliable, particularly in mechanically ventilated patients. Improved diagnostic imaging, sonography and computed tomography (CT) have paved the way for more accurate and timely diagnosis. Abdominal ultrasonography can be performed in the ICU, but it depends on operator interpretation and cannot diagnose pathologies in the presence of abdominal gas. Abdominal CT has a high sensitivity and high specificity in the diagnosis of intra-abdominal sites of infection. It is useful for the diagnostic evaluation of the retroperitoneal space. Ultrasonography represents the modality of choice. The Surviving Sepsis Campaign (23-25) remarks that when ultrasonography is not diagnostic, CT scanning should be performed (grade E). Appropriate imaging studies are important not only in the diagnosis but also for therapy. Ultrasonographically or CT-guided surgical procedures (e.g., percutaneous abscess drainage) can minimize the need for more invasive surgical interventions. Abdominal fluid collections should be identified by imaging and aspirated as a matter of routine (grade E). Collections identified by imaging studies should, if possible, be aspirated and drained for rapid Gram staining and for both aerobic and anaerobic cultures (grade E). In order to better clarify this issue, we briefly present a case report of a patient suffering from intra-abdominal sepsis. Besides, we compared the outcome in two groups of patients suffering from infections vs. sepsis (personal unpublished data).

5.1. Case Report

We evaluated two small groups of patients (5 patients for each group) treated during a period of four months. In the first group we enrolled 5 patients presenting episodes of bacteremia and urinary tract infections; in the second group we enrolled 5 patients who developed ventilator associated pneumonia (VAP) and septic shock with multiple organ dysfunction/failure. In the first group, IL-6 values remained within a normal range and all patients had survived on day 28. In the second group of patients suffering from septic shock, IL-6 levels evidenced a peak value of 4,000 pg/mL ($p>0.05$); mortality rates in this group of patients reached 80% (personal unpublished data) (Figure 1).

6. SURGICAL STRATEGY

Open drainage may be necessary when there are no well-defined fluid collections or necrotic tissues requiring debridement or when percutaneous drainage failed. Diagnostic laparoscopy or relaparotomy may also be necessary and useful when other techniques fail in the early detection of the source of infection. Identification of patients at risk for development of tertiary peritonitis on a surgical intensive care unit are reported by Chromik *et al.*, showing that the Mannheim Peritonitis Index (MPI), CRP and the Simplified Acute Physiology Score II (SAPS II) on the second postoperative day help to identify patients at risk for tertiary peritonitis (32). Open surgery is required in patients with infected pancreatic necrosis or intra-abdominal infection caused by hollow organ rupture. An approach to the management of patients with severe acute necrotizing pancreatitis is sewing a piece of surgical, polypropylene mesh with a centrally located nylon zipper to the fascia. This allows for repeated accesses to the peritoneal cavity for further explorations.

Relaparotomy is indicated when the general condition of the patient does not improve. Surgical

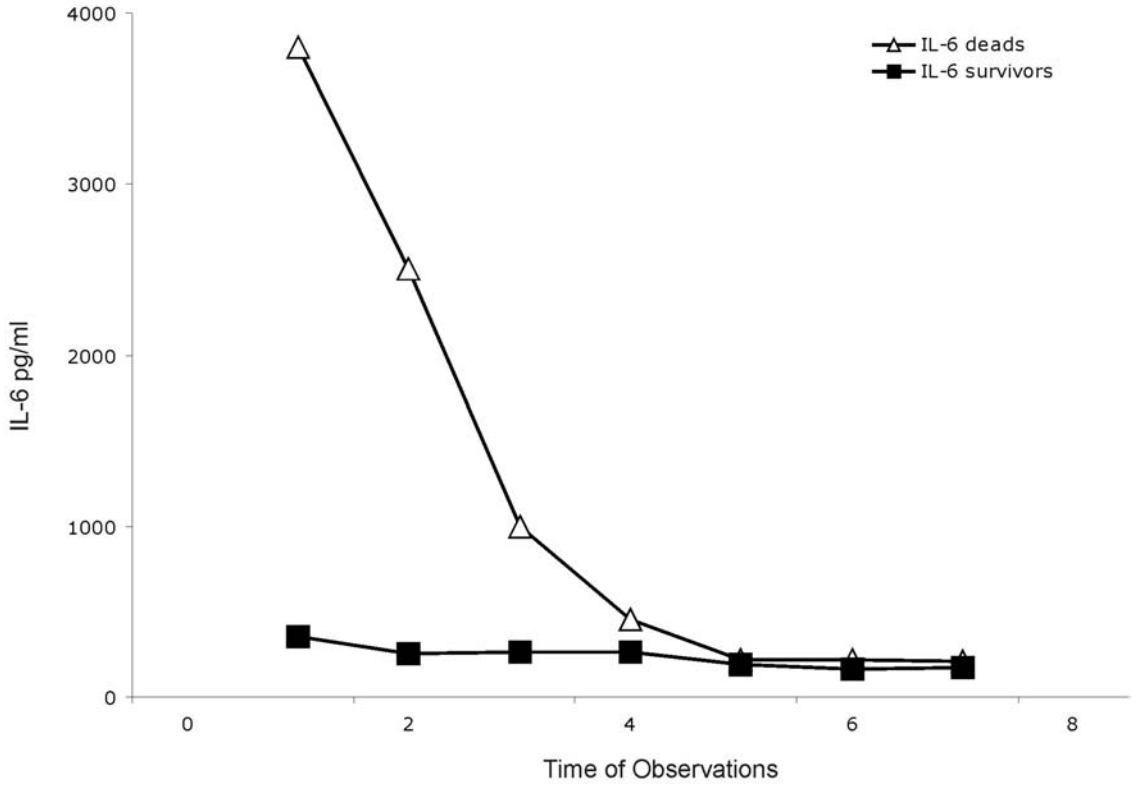

Figure 1. IL-6 levels into two small groups of patients (5 patients for each group) treated during a period of four months.

procedures are indicated when intestinal viability is problematic, when the discrimination between viable and nonviable tissues is not possible during the first surgical intervention, and when intraoperative bleeding impedes complete surgical debridement. The right timing of surgery is also important: early debridement of necrotizing fascitis improves outcomes but, conversely, delayed surgery for pancreatic abscesses and necrotizing pancreatitis is more beneficial than early surgery.

7. RESUSCITATION AND MANAGEMENT BUNDLES

An early approach to septic patients, before their admission to the ICU, is mandatory and represents a gold standard before patients are admitted to the ICU; it is facilitated by an experienced team, performing an early diagnosis and assessing the disease severity by using scoring systems.

The SSC (23-25) includes 45 recommendations for septic patients. They are based on an expert assessment of clinical trials. Evidence from the trials was graded (levels I–V) according to the quality and reliability of data. The evidence assessment was then used to grade the level of each recommendation (grade A: best evidence; grade E: weakest evidence). The SSC (23-25) guidelines stress the importance of a timely and aggressive therapy and the concept of the "golden hour" should be applied in sepsis. To allow implementation of these guidelines in daily practice, therapeutic bundles have been defined. Resuscitation bundles (33,34) must be started within 6 hours of presentation to the ICU (serum lactate measured; blood cultures before antibiotic therapy; broad-spectrum antibiotics within 3 hours from emergency admission and 1 hour from ICU admission; in case of hypotension and/or lactate higher than 4 mmol/L deliver an initial 20 ml/kg of crystalloid or colloid solution or apply vasopressors for hypotension not responding to initial fluid resuscitation to maintain mean arterial pressure above 65 mmHg; in case of persistent hypotension and/or lactate higher than 4 mmol/L: achieve a central venous pressure of more than 8 mmHg and a central venous oxygen saturation of more than 70%).

A management bundle should be implemented within 24 hour (low-dose steroids administered for septic shock; recombinant human activated protein C; glucose control maintained at less than 8.3 mmol/L; inspiratory plateau pressures maintained at less than 30 cm H_2O for mechanically ventilated patients; Tables 2 and 3).

8. RECOMMENDATIONS AND CONCLUSION

The triad infections-sepsis-organ dysfunction seems to represent a dangerous package, as a sea-storm causing rupture of the body's homeostasis. To improve outcome in septic patients, it is crucial to perform an early diagnostic procedure to detect the focus of infection and to provide, as soon as possible, an effective therapeutic

Table 2. Sepsis resuscitation bundle (6-h bundle)

1. Serum lactate
2. Blood culture
3. From the time of presentation, broad spectrum antibiotics administered within 3 h for ED admission and 1 h for non-ED ICU admission
4. In the event of hypotension and/or lactate > 4 mmol/L (36mg/dL) • deliver an initial minimum of 20 mL/Kg of crystalloids or colloid equivalent • Apply vasopressor for hypotension non responding to initial fluid resuscitation to maintain MAP > 65 mmHg
5. In the event of persistent hypotension despite fluid resuscitation • achieve CVP of > 8 mmHg • achieve $ScvO_2$ of > 70%

Table 3. Sepsis resuscitation bundle (24-h bundle)

1. Low-dose steroids administered for septic shock in accordance with a standardized ICU policy
2.Drotrecogin alpha (activated) administered in accordance with a standardized ICU policy
3.Glucose control
4.Inspiratory plateau pressures

pproach, first by applying resuscitation and management bundles, and then by source control itself.

The application of the basic principles of source control, based on understanding of the biology of inflammation and of the natural history of the infectious processes, can generally provide the clinician with an appropriate set of early diagnosis, detecting the role of mediators, and lead to a correct decision about the timing of surgical management. Marshall and Reinhart published a smart review on the biomarkers of sepsis (7), in which they focused on useful principles to select more specific mediators to support an early diagnosis for a better and more appropriate management. Evaluation by a critical care surgeon with expertise in both critical illness and the range of surgical options is desirable, but in the absence of such a resource, close collaboration between intensivists and surgeons is the key to provide a successful outcome. Hence, in medicine, and particularly in critical care, it is necessary to do what the patient needs, according to evidence-based clinical decision-making and to the availability of resources for optimization of care.

9. REFERENCES

1. Le Gall JR, Alberti C, Brun Buisson C. Epidemiology of infection and sepsis in intensive care unit patients. *Bull Acad Nat Med,* 187(7)1115-1125 (2004)

2. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med*, 28:1303-1310 (2001)

3. Marshall JC, Foster D, Vincent JL, Cook DJ, Cohen J, Dellinger RP, Opal S, Abraham E, Brett SJ, Smith T, Mehta S, Derzko A, Romaschin A. Diagnostic and prognostic implications of endotoxemia in critical illness: result of MEDIC study. *J Infect Dis*, 190(3):527-34 (2004)

4. Tacconelli E, Smith G, Hieke K, Lafuma A, Bastide P. Epidemiology, medical outcomes and costs of catheter-related blood stream infections in intensive care unit of four European countries: literature and registry-based estimates. *J Hosp Infect*; 72(2):97-103 (2009)

5. Bihari D. Prevention of multiple organ failure in the critically ill. In Update in intensive care and emergency medicine. Ed. JL. Vincent; 3:26-39. Springer-Verlag (1986)

6. Bone RC. Immunologic dissonance: a continuing evolution in our understanding of the systemic inflammatory response syndrome (SIRS) and the multiple organ dysfunction syndrome (MODS). *Ann Intern Med*, Oct 15;125(8):680-687 (1996)

7. Marshall JC, Reinhart K. Biomarkers of sepsis. International Forum. *Crit Care Med*, 37(7):2290-2298 (2009)

8. Russel JA, Singer J, Bernard GR, Wheeler A, Fukerson W, Hudson L, Schein R. Summer W, Wright P, Walley KR. Changing pattern of organ dysfunction in early human sepsis is related to mortality. *Crit Care Med*, 28:3405-3411 (2000)

9. Kortgen A, Niederprum P, Bauer M. Implementation of an evidence-based "standard operating procedure" and outcome in septic shock. *Crit Care Med*, Apr; 34(4):943-949 (2006)

10. Charles PE, Tinel C, Barbar S, Aho S, Prin S, Doise JM, Olsson NO, Blettery B, Quenot JP. Procalcitonin kinetics within the first days of sepsis: relationship with the appropriateness of antibiotic therapy and outcome. *Crit Care*, 13(2):R3887-3892 (2009)

11. Vincent JL, de Mendonça A, Cantraine F, Moreno R, Takala J, Suter PM, Sprung CL, Colardyn F, Blecher S. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. *Crit Care Med*, 26:1793-1800 (1998)

12. Rubulotta F, Marshall JC, Ramsay G, Nelson D, Levy M, Williams M. Predisposition, insult/infection, response, and organ dysfunction. A new model for staging severe sepsis. *Crit Care Med,* 37(4):1329-1335 (2009)

13. Sakr Y, Krauss C, Amaral AC, Rèa-Neto A, Specht M, Reinhart K, Max G. Comparison of the performance of SAPS II, SAPS 3, APACHE II, and their customized prognostic models in a surgical intensive care unit. *Br J Anaesth*, 101(6):798-803 (2008)

14. Minne L, Abu-Hanna A, de Jonge E. Evaluation of SOFA-based model for predicting mortality in the ICU: A systemic review. *Crit Care* 12(6). R161-165 (2008)
15. Gosain A, Gamelli RL. A primer in cytokines. *J Burn Care Rehabil,* 26(1):7-12 (2005)

16. Ulloa L, Tracey KJ. The "cytokine profile": a code for sepsis. *Trends Mol Med*, 11(2):56-63 (2005)

17.Cohen J. The immunopathogensis of sepsis. *Nature,* 19-26;420(6917):885-91 (2002)

18. Moine P, Abraham E. Immunomodulation and sepsis: impact of the pathogen. *Crit Care Med,* 22(4):297-308 (2004)

19. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, Cohen J, Opal SM, Vincent JL, Ramsay G. International sepsis definitions conference. 2001 SCCM/ESICM/ACCP/ATS/SIS International sepsis definitions conference. *Intensive Care Med,* 29(4):530-538 (2003)

20. Dellinger RP. Severe sepsis trials: why have they failed ? *Minerva Anestesiol,* 65(6):340-345 (1999)

21. Marshall J. Clinical trials of mediator-directed therapy in sepsis: what have we learned? *Intensive Care Med,* 26 Suppl 1:S75-83 (2000)

22. Cross AS, Opal SM. A New paradigm for the treatment of sepsis: is it time to consider combination therapy? *Ann Intern Med,* 18;138(6):502-505 (2003)

23. Dellinger RP, Carlet JM, Masur H, Gerlach H, Calandra T, Cohen J, Gea-Banacloche J, Keh D, Marshall JC, Parker MM, Ramsay G, Zimmerman JL, Vincent JL, Levy MM. Surviving sepsis campaign guidelines for management of severe sepsis and septic shock. *Crit Care Med,* 32(3):858-873 (2004)

24. Dellinger RP, Carlet JM, Masur H, Gerlach H, Calandra T, Cohen J, Gea-Banacloche J, Keh D, Marshall JC, Parker MM, Ramsay G, Zimmerman JL, Vincent JL, Levy MM. Surviving sepsis campaign guidelines for management of severe sepsis and septic shock. *Intensive Care Med,* 30(4):536-555 (2004)

25. Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, Reinhart K, Angus DC, Brun-Buisson C, Beale R, Calandra T, Dhainaut JF, Gerlach H, Harvey M, Marini JJ, Marshall J, Ranieri M, Ramsay G, Sevransky J, Thompson BT, Townsend S, Vender JS, Zimmerman JL, Vincent JL. Surviving Sepsis Campaign: International guidelines for management of severe sepsis and septic shock. *Intensive Care Med* 34:17-60 (2008)

26. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblick B, Paterson E, Tomlanovich M. Early goal-directed therapy collaborative group. Early goal directed therapy in the treatment of severe sepsis and septic shock. *N E J Med*, 345:1368-1377 (2001)

27. Mann HJ, Short MA, Schlichting DE. Protein C in critical illness. *Am J Health System Pharm,*668 (12):1089-1096 (2009)
28. Dhainaut JF, Yan SB, Cariou A, Mira JP. Soluble thrombomodulin, plasma-derived unactivated protein C, and recombinant human activated protein C in sepsis. *Crit Care Med,* 30(5 Suppl):S318-S324 (2002)

29. Mahara SD, O'Keefe GE. Genetic determinants of the inflammatory response. *Curr Opin Crit Care*, 10(5):318-324 (2004)

30. De Maio A, Torres MB, Reeves RH Shock. Genetic determinants influencing the response to injury, inflammation, and sepsis. 23(1):11-17 (2005)

31. Jimenenz MF, Marshall JC. Source control in the management of sepsis. *Intensive Care Med*, 27:S49-S52 (2001)

32. Chromik AM, Meiser A, Holling J, Sulberg D, Daigeler A, Meurer K, Vogelsang H, Seelig MH, Uhl W. Identification of patients at risk for development of tertiary peritonitis on a surgical intensive care unit. *J Gastroint Surg,* 13(7):1358-1367 (2009)

33. Machado FR, Freitas FG. Controversies for surviving sepsis campaign bundles: should we use them? *Shock*, 30(Suppl 1):34-40 (2008)

34. Zambon M, Ceola M, Almeida de Castro R, Gullo A, Vincent JL. Implementation of the Surviving sepsis campaign guidelines for severe sepsis and septic shock: we could go faster. *J Crit Care*, 23(4)455-460 (2008)

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