

# Sepsis and Organ(s) Dysfunction

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## **Terminology and risk factors**

Sepsis is one of the main problems in medicine due to its complexity from pathophysiology, clinical and therapeutic standpoints. Although several definitions have been proposed for this syndrome, it can in general be assumed that it represents the clinical manifestation of a system response of the body to infection or to an inflammatory-associated acute disease (1-4)

Despite advances in medical practice, sepsis, severe sepsis and septic shock, associated with different grading of organ(s) dysfunction/failure, are conditions limiting significantly quality of life and ultimately survival in the ICU patients; in any case health economic implications remain exorbitant (5).

Mortality rate in consequence of sepsis is associated with a pattern characterized by progressive dysfunction/failure of non-pulmonary organ systems and, in particular, worsening neurologic, coagulation and renal dysfunction over the first 3 days. Although initial pulmonary dysfunction is common in patients with sepsis syndrome, it is not associated with an increased mortality rate (6)

In five recent clinical trials that enrolled a total of 5,661 patients with severe sepsis (criteria = evidence of infection, SIRS, and at least one organ dysfunction/hypoperfusion), the incidence of septic shock ranged from 52% to 71% of patients with severe sepsis, with a mean of 58% (7-11). A recent study used the International Classification of Diseases (ICD)-9 hospital diagnostic codes for infection and acute organ dysfunction to estimate 751.000 cases of severe sepsis per annum in USA (3). Starting from these data septic shock would, therefore, be predicted to occur annually in 435,580 patients in USA. Mortality rate is a consequence of one or more factors, such as: age, immunodepression, presence of diseases and/or chronic failure of one or multiple organ system dysfunction/failure (12, 13). Pathophysiologic mechanisms are basically related to Gram-negative bacteria endotoxin (14), but also Gram-positive micro-organisms, viruses and mycetes, which are supposedly responsible for the local and systemic release of several mediators that, in turn, might be responsible for the organic response to infection, characterized by cardiovascular instability, hyperthermia, hypothermia, leukocytes and coagulation alterations as well as by involvement of one or multiple organs (15). The term sepsis is related with the concept of multiple organ dysfunction syndrome (MODS), which is frequently identified with the final event of infection, although it has been showed that the

septic syndrome is not specific to infection and can also be originated as a result of a variety of non infectious stimuli such as pancreatitis, burns and trauma (16).

The American College of Chest Physicians proposed new definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis (17). Indeed, although remarkable progress has been achieved on defining the pathophysiology of sepsis, the terminology associated with research in this field has remained confusing. The term of SIRS, until now very controversial, was developed to imply a clinical response arising from a non-specific insult; it includes two or more non-specific variables. Sepsis is defined as SIRS with documented infection. The sequela of SIRS/sepsis is multiple organ dysfunction syndrome (MODS) which can be defined as the failure to maintain homeostasis without intervention. Primary MODS is a direct result of a well-defined insult, while secondary MODS develops not as a direct response to an insult, but as the consequence of a host response. Roger Bone confirmed the above problems by reporting his personal experience with SIRS (18).

Several studies have examined the risk factors leading sepsis (Tab.1) able to predict patients outcome; the age > 65 years, the coexistence of chronic diseases, the presence of surgical sepsis (19). The CDC evidenced the risk factors for developing septic shock such as the presence of a central catheter, parenteral nutrition, antibiotic use, the presence of an arterial catheter or an endotracheal tube; other risk factors found to predict development of gram- negative bacteremia included the following : admission to an ICU, use of broad-spectrum antibiotics, immunosuppressive treatments, invasive procedures and devices, burns, trauma, advanced age, cancer, acquired immunodeficiency syndrome (AIDS), fever, low systolic blood pressure, and low platelet counts (19,20)

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#### Sepsis Predisposition

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

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#### Tab.1

### **Immune inflammatory response and biomarkers**

The immune-inflammatory process - is a normal response to infection and is essential not only for the resolution of infection but also for the initiation of other adaptive stress responses required host survival. The profound redundancy of action of many cytokines means that there are many overlapping pathways for cellular activation and further mediator release. In addition, the synergism of actions and effects of many cytokines suggests that imbalance in the process of the immune response may be adversely affected by inhibition of a single agent. Mediators of immunity and inflammation are part of an intercellular signalling language which allows cells/tissue/organs to take in new information and, based on past experience, decide what to do next. There are essentially two components to the immune response-innate (non specific) and acquired (antibody mediated) immunity. The complement system is a multi-component triggered enzyme cascade which attracts phagocytes to micro-organisms increasing capillary permeability and neutrophil chemotaxis and adhesion. Specific acquired immunity in the form of antibodies inactivates micro-organisms which are not destroyed by the innate immune system. Such micro-organisms either fail to activate the complement pathway or prevent activation of phagocytes. The cells involved in innate immunity include "professional" phagocytes (polymorphonuclear neutrophils, mastcells and macrophages) and "non-professional" phagocytes (endothelial cells and hepatocytes). Cells infected with viruses and parasites are

killed by large granular lymphocytes termed natural killer (NK) cells, and eosinophils. Acquired immune defences against specific micro-organisms (antigens) form the second component of the immune response. Antibodies activate the complement system, stimulate phagocytic cells and specifically inactivate micro-organisms. Lymphocytes, the basis of the acquired immune defence system, consist of antibody-producing plasma cells derived from B-lymphocytes, and T-lymphocytes which control intracellular infections. Binding of micro-organisms to antibodies on the cell surface of B-cells leads to preferential selection of these antibody-producing cells. This is termed priming, and subsequent responses are faster and amplified, and provide the basis of vaccination. T-cells exploit two main strategies to combat intracellular infections: secretion of soluble mediators which activate other cells to enhance microbial defence mechanisms, and production of cytolytic T-cells which kill the target organism. Adaptive selection of specific T-cell subsets occurs in response to local balance of cytokine concentrations (21). Hollenberg et al (22) reported the existence of a circulating vasodilating substance that may play a role in the pathogenesis of septic states. As the sepsis condition progresses, there is evidence of a complex disturbance in vasomotor tone (peripheral vasculopathy), characterized by non-specific systemic vasodilation, pulmonary vasoconstriction and increased vascular permeability. Pinsky and Matuschak (23) showed that endotoxaemia in experimental model in animals produced a marked increase of peripheral vascular capacitance without changing in compliance. Vasodilation was unrelated to the level of endogenous autonomic tone. Peripheral vascular paralysis leads to the inability to regulate the distribution of blood flow to the peripheral circulation. The ominous importance of this vasculopathy was shown in a clinical study by Parker et al (24).

Experimental and clinical studies indicate that the excess release of pro-inflammatory cytokines and other host-derived inflammatory mediators contributes to the basic pathophysiology of human septic shock (25). Cytokines are the primary communicators of the innate immune system; they serve the body as chemical messengers between cells, and are involved in such processes as cell growth and differentiation, tissue repair and remodelling, and the regulation of immune response (26). Large quantities of TNF- $\alpha$ , IL-1 or many other inflammatory mediators, which are good for the host in localized infections, are detrimental when released in systemic circulation. Cytokines are a group of small signalling proteins produced by a large variety of cells that are thought to be important for host defence, wound healing and other essential host functions.

While cytokines are generally viewed as a destructive development in the patient that generally leads to multiple organ dysfunction, cytokines also protect the body when localized. Cytokines are highly pleiotropic, and they appear capable of producing markedly different effects depending on the nearby hormonal milieu. Furthermore, the body has a highly complex, tightly regulated network of receptor antagonists and other regulatory agents that continuously modulate the effects of cytokine release; this fact may explain why the trials of various anti-cytokine agents have produced disappointing results (25).

Although cytokines are important for these homeostatic functions, excessive production and release of cytokines initiate widespread tissue injury which can result in organ dysfunction. Four cytokines, TNF- $\alpha$ , IL-1, IL-6 and IL-8 have been most strongly associated with sepsis syndrome. Cytokines are not stored in intracellular compartments and are freshly synthesized and released in response to inflammatory stimuli. This regulation occurs predominantly at the level of gene transcription with the new expression of cytokine mRNA. Cytokines have synergistic, overlapping and antagonist effects. Anti-inflammatory cytokines as well as pro-inflammatory cytokines are produced following upon the activation of the cytokine cascade.

The sequela of SIRS/sepsis is multiple organ dysfunction failure. This condition has been defined as the swinging of a pendulum across the whole spectrum of SIRS, compensatory anti-inflammatory response syndrome (CARS) and mixed antagonist response syndrome (MARS). Tissue insult/injury triggers a triad of systems encompassing the macrophages, cytokines and endothelial cells. This results in SIRS/CARS/MARS which result in terminal organ dysfunction. This condition can progress to MODS, particularly when aggravated by a second hit (another tissue insult/injury), or can move towards resolution, particularly when second hits are avoided (27). The interaction between pro-inflammatory and anti-inflammatory mediators can be viewed as a battle between opposing forces, which are often unbalanced. Initially, these mediators interact in the microenvironments. If the mediators balance each other and the initial response is overcome, homeostasis is restored. If not, pro-inflammatory and anti-inflammatory mediators may be found in systemic circulation. If balance cannot be established there and homeostasis is not restored, a massive pro-inflammatory reaction (SIRS) or an anti-inflammatory reaction (CARS) will ensue. A range of clinical sequelae may then follow, in accordance with the acronym CHAOS (Tab 2)

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CHAOS induced by SIRS/CARS/MARS

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- C** – Cardiovascular compromise (usually manifesting as shock; in this setting SIRS predominates)  
**H** – Homeostasis (return to health; this represents a balance between SIRS and CARS)  
**A** – Apoptosis (programmed cell death; SIRS predominates)  
**O** – Organ dysfunction, single or multiple organ or increased susceptibility to infection; CARS predominates  
**S** – Suppression of the immune system; here again, SIRS predominates
- 

Tab.2

Inflammatory response is a highly orchestrated system of cellular activation and local release of pro-inflammatory and anti-inflammatory response. The released cytokines and inflammatory mediators activate and modulate the responses of other immunocytes to attack and destroy the infecting microbes (28).

In contrast with many acute and severe diseases such as acute myocardial infarction, pancreatitis, renal and liver failure, adrenal dysfunction, etc, sepsis lacks a specific marker. Several biomarkers of sepsis have been proposed such as circulating non-segmented neutrophils, acute phase proteins such as C-reactive protein and neopterin, cytokines (TNF- $\alpha$  and IL-6) and chemokine (IL-8) (29). Practically the above mentioned markers have shown some utility in detecting a septic condition, but all of them lack specificity for severe infection or for infection inducing organ dysfunction and/or shock. Procalcitonin blood level was reported firstly in 1993 in pediatric patients suffering sepsis (30). The monitoring of procalcitonin blood level is useful in identifying patients with severe sepsis and septic shock (31). Besides procalcitonin represents an important marker able to differentiate SIRS patients from those with sepsis (32).

However, sepsis diagnosis represents a true challenge considering the prevalence of aspecific clinical signs such as tachycardia, tachypnea, leukocytosis and fever. On the other side blood culture may be positive in patients suffering sepsis in 30-40% only. The exact cellular and organ source for the pro-hormone, its regulation and its relationship with bacteria and bacterial product remain largely unknown (33). The prognostic value of daily measurements of procalcitonin appears superior to that of C-reactive protein (34)

### **Microcirculation dysfunction**

Microcirculatory dysfunction during sepsis is the consequence not of one single metabolic or other defect, or of one single mediator – even though TNF- $\alpha$  and IL-1 $\beta$  have long been explored as central mediators in sepsis – but of a rather complex, still incompletely understood cascade of mediators. The coagulation cascade and the complement system become activated, and arachidonic acid is metabolised to form leukotrienes, thromboxane, and prostaglandins. T-cells are activated to release cytokines and growth stimulatory factors. Most of these mediators systems affect the microcirculation in one or many ways and there is a striking redundancy in their modes of action. For instance, endotoxin, TNF- $\alpha$ , PAF, leukotrienes have all been shown to affect the endothelial barrier function individually and it can be speculated how they might act in concert to damage circulation during sepsis (35). Systemic inflammation which occurs during sepsis leading to a complex biological interaction with profound changes in endothelial function (36).

The endothelium itself may release nitric oxide or endothelin, with antagonistic effects on vascular tone. Many mediators, including complement fragments, may either prime or/and directly stimulate neutrophils to release inflammatory mediators, reactive oxygen species, or hydrolytic enzymes, to aggregate with each other and or platelets, to adhere to endothelial cells, and finally to obstruct capillary lumina. Further players in the above mediator cascade include kallikrein, kinins, thrombin, endorphins, and heat shock proteins.

Maintenance of optimal tissue perfusion is important to minimize ischaemia-induced cellular injury and to decrease ischaemia resulting from stress and the inflammatory response. Inadequate oxygen supply to tissues relative to cellular consumption results in an oxygen debt that impairs cellular function. The vasopressor and inotropic agents in particular epinephrine and dobutamine, have been shown to exert vasodilatory effects on

the gastric microcirculation as assessed by laser Doppler flowmetry and thereby to improve oxygen delivery to splanchnic organs (37).

Nitric oxide is a potent vasodilator at the microcirculatory level (38) and altered microvascular permeability. Inhibition of nitric oxide in clinical trials in septic patients yielded increases in blood pressure, but at the same time increases in liver enzymes and increased pulmonary resistance as evidence for the jeopardized microvascular blood flow and tissue oxygenation (39).

Experimental and clinical trials have demonstrated positive effects of hypertonic fluids in patients with sepsis-related ARDS and has found significant increase in systemic and pulmonary arterial pressure, increased cardiac output, increased stroke volumes and, at the same time, significant improvements in tissue oxygen delivery and consumption.

One of the novel approaches to treating sepsis is to intervene at specific key events within the signal transduction cascade. It becomes increasingly evident that the transcription factor NF- $\kappa$ B is one of the principal final common pathways in regulating genes participating in immune and inflammatory responses, including numerous genes encoding cytokines, growth factors, ICAM and acute phase proteins (40). How NF- $\kappa$ B activation could be limited within the context of MODS and sepsis is being considered at this time as a possible target for therapeutic intervention (41)

To date, therapeutic approaches, such as anticytokine and anti-oxidant regimens, which have been highly successful in experimental models, have failed to demonstrate clinical efficacy

### **Coagulation pathways**

Activation of the coagulation system assumes a key role in patient in septic patients. The key role of the inflammatory response of microvascular endothelium on the progress of organ dysfunction is a well known process; in fact, it is the case that Factor Xa induces the expression of a range of inflammatory cytokines such as interleukin 6 (IL-6) and IL-8 as well as adhesion molecules in endothelial cells in culture (42).

Clinical experience evidenced an activation of clotting factors and endogenous anticoagulants such as anti-thrombin III and protein C; in spite of this a condition of disseminated intravascular coagulation (DIC) in sepsis is very seldom except in some specific situation such as meningococcal septicaemia. In experimental and clinical studies of sepsis has been demonstrated fibrin deposition in several organs and the subsequent activation of fibrinolysis may be an important protective mechanism preventing MODS in patients presenting DIC (43,44). The inflammatory response following exposure to lipopolysaccharide (LPS) or tumour necrosis factor (TNF) is one example. It is known that F expression is regulated by activator protein 1 (AP 1) and nuclear factor kappa (NF $\kappa$ B) (two transcription factors known to regulate other mediators of inflammation) (45). The inhibition of the TF/Factor VIIa pathways, in animal experiments by administration of monoclonal antibodies showed no evidence of DIC following infusion of endotoxin or live *Escherichia coli* (46).

The physiological regulatory mechanisms of coagulation impaired by activation of coagulation cascade may contribute to fibrin formation. Plasma levels of the most important inhibitor of thrombin, ATIII, are usually markedly reduced in sepsis. This is a consequence of a combination of increased consumption, degradation by elastase released from activated neutrophils, and also impaired synthesis. In addition, there is a decrease in the protein C/protein S system which also enhances the pro-coagulation state (47). Protein C is a circulating protein which is an inactive precursor of protease and is converted to APC in the presence of the thrombin/thrombomodulin complex.

There is a protein C receptor, called endothelial protein C receptor, which facilitates activation of protein C; however it is not an absolute requirement for activation. APC inactivated Factors Va and VIIIa and so limit thrombin generation (48) and also promotes fibrinolysis by inhibiting activity of plasminogen activator inhibitor 1 (49). It is also suggested that APC may reduce inflammation by inhibiting cytokine production and white cell activation (50). APC was shown to have a protective role against MODS and mortality in experimental models of sepsis (51) and has subsequently been shown to decrease mortality in humans suffering severe sepsis (9).

### Early source control

The early localisation of infection is crucial for the clinical evolution of sepsis; abdominal sepsis means synonymous of peritonitis. Abdominal sepsis is classified as primary, secondary and tertiary. Primary peritonitis is characterized by spontaneous bacterial peritonitis or catheter infections. Secondary peritonitis is from perforations and anastomotic leaks, pancreatitis, cholecystitis, and similar conditions. Tertiary peritonitis, there might be a marked inflammatory response in the abdomen without pathogenesis or with only low-grade pathogens that usually occurs after control of the secondary or primary insult. Frequently the patient suffering for an an occult abdomen-borne sepsis, a condition that can be accompanied by an alteration in mental function and disorders affecting one or more organs. In some circumstances priority issue is the difficulty to determine whether the abdominal situation is a factor secondary to the septic process.

Necrotic tissue identification can be difficult, especially in the event of deep infections (52). Moreover, antibiotic treatments are often administered in an empirical manner, even though this approach can turn out to be decisive, given that patients who are administered antibiotic coverage record a better survival rate (53) compared with non-treated patients. At any rate, when bacteriological data are available, the choice of antibiotic treatments should be goal-directed. Under these circumstances, single antibiotic treatments can be as effective as combined antibiotic treatments (54,55)

However, most of comparative studies on the choice of antibiotic treatments were performed on neutropenic patients; therefore, the comparison between these findings and those relative to a population of patients affected by severe sepsis is not justified (56). Improved diagnostic imaging, sonography and CT scanning in particular, have paved the way to more accurate and timely diagnosis and prognosis has consequently improved; besides, frequently, percutaneous drainage is the method of choice in the majority of abdominal abscesses in high risk patients while surgery is to be preferred for deep abscess which are difficult to remove with lower invasive techniques.

### Scoring systems and PIRO model

Scoring systems as a means of mortality risk/severity of illness prediction have evolved from simple identification of risk factors in an attempt to summarize and quantify these individual findings. Several scores, such as the APACHE II (age, physiology, chronic health evaluation) and the SAPS II (simplified acute physiology score) are not satisfactory to define organ failure.

Several scores recently developed, e.g. MODS (multiple organ dysfunction score), SOFA (sepsis-related organ failure assessment), choose to study the following six organs: respiratory, renal, haematologic, liver, cardiovascular, neurologic. It is accepted that the degree of gastrointestinal dysfunction would be useful to quantitate particularly with recent experimental and clinical data in its role in the pathogenesis of MOF. Unfortunately, accurate assessment of gastrointestinal function is still virtually impossible (Tab.3).

Organ System	Measurable parameters
Respiratory	PaO <sub>2</sub> /FiO <sub>2</sub>
Renal	Serum creatinine
Hematologic	Platelet count
Central nervous	Glasgow coma score
Hepatic	Serum bilirubine
Cardiovascular	Blood pressure
Grastrointestinal	No appropriate one

Tab. 3

A working group of the European Society of Intensive Care Medicine recently developed the SOFA score. In contrast to older scores, the aim of the SOFA score is not to predict outcome but to describe organ dysfunction. SOFA studies six organs with a scale for each from 0 (normal) to 4 (worse situation), using parameters readily available and routinely measured in most ICU's (Tab.4).

The use of such scoring systems alone cannot guide acute changes in therapy but regular, daily calculation of scores can provide an objective assessment of the evolution of the disease process and the response to therapy. They can also be employed to facilitate stratification of patients and comparison of results in clinical trials of new therapies.

Tab. 4 **SOFA Score**

<b>SOFA score</b>	1	2	3	4
<b>Lung</b>				
PaO <sub>2</sub> /FiO <sub>2</sub> (mmHg)	? 400	? 300	? 200	? 100
<b>Coagulation</b>				
Platelets X10/mm <sup>3</sup>	? 150	? 100	? 50	? 20
<b>Liver</b>				
Bilirubine,mg/dl	1.2-1.9	2.0-5.9	6.0-11.9	12
<b>Cardiovascular</b>				
Ipotension*	MAP	Dopamine ? 5 µKg/min. Dobutamine ? 2-4µKg/min.	Dopamine ? 5µKg/min. Epinefrine ? 0.1µKg/min. Norep.efrine ? 0.1µKg/min.	Dopamine ? 1.5µ/Kg/min. Epinefrine ? 0.1µKg/min. Norep.efrine ? 0.1µKg/min.
<b>Central Nervous System</b>				
GCS	13-14	10-12	6-9	? 6
<b>Kidney</b>				
Creatinine, mg/dl	1.2-1.9	2.0-3.4	3.5-4.9	
Mmol/L	(110-170)	(171-299)	(300-440)	o 440 o
Diuresis			(500ml/die)	220ml/die

\*adrenergic agents infusion more than 1 hour with the dosage of µKg/min.

Considering the complexity of the septic process and to facilitate patients enrollment in the clinical trials, several researchers have suggested to introduce in the clinical practice a sort of cancer scoring system considering that this situation is assimilable to a sepsis condition.

PIRO model has been introduced to define septic patients (57, 58) starting from the concept of several likeness between sepsis and cancer : intricated pathophysiolgy; high indices of mortality; organs and system involvement; different medical and surgical management strategies; high cost of combined pharmacological treatment. PIRO definition describes different aspects related to sepsis : Predisposition, Infection, the host Response, Organ(s) dysfunction/failure.

Predisposition (**P**) factors assume a key role of individual genetic characterization during health conditions or during of a disease process such as ageing, chronic illness, immunosuppression related to a chronic pharmacological management, etc.

Recent advances on the genetic characterization techniques evidenced the importance on the possibility to know the several factors able to increase the risk of infection and the mortality index as consequence of sepsis. TNF $\alpha$  genetic polymorphism and TNF-2 allele polymorphism, induce an high blood level of TNF with increased mortality during septic shock (59).

Infection (**I**) is a second key point able to cause host response and decision making for the treatment. This condition include several elements such as : bacteria type. Infection localization; for example, urinary tract infection vs pulmonary infection; seriousness of the infection such as lobar pneumonia vs bilateral pneumonia etc.

Response (**R**) is the third element; this term means the host capacity to react to the septic; this condition may validated in presence of other important signs such as the number of white cells, protein C, procalcitonin blood level etc.

Organ (**O**) dysfunction is the last element of PIRO model. The SOFA score is a good index (tab 3), proposed by the European Society of Intensive Care (60), to establish the severity of organ dysfunction; although the final score express the level of morbidity than the mortality index; it represent an important sequential index to evaluate entity of organ dysfunction (61) or improvement of the clinical conditions.

### **Standard of care and early-goal directed therapy (EGDT)**

Lundberg *et al.* (62) reported that, with reference to hospitalised patients affected by septic shock, significant delays in the decision to transfer these patients to intensive care units are recorded; delaying the administration of fluid therapies and inotropic drugs has a huge impact on the increase in mortality rates.

Rivers *et al* (11) reported that early aggressive 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. Therefore, it is crucial to prefer an early approach when patients are admitted to emergency departments (63) this approach is facilitated by the presence of experienced teams, as well as by the possibility to pose early diagnosis and to assess the patients' disease severity by means of commonly known severity indicators used for patients in ICUs (APACHE II, SAPS II, SOFA). In particular, the early use of procalcitonin (64) and C-reactive protein values, as well as the dosage of inflammation mediators, can be very useful to pose an early diagnosis of sepsis. These simple diagnostic tests can be combined with non-invasive haemodynamic methods and mixed venous blood saturation monitoring, whereas sublingual capnography can show the impairment degree of haemodynamic values (65) and early control of glycemia value represents an important standard of care (66). However, several supportive measures are necessary to optimize the standards of care such as : patients posture during artificial ventilation, central venous catheterization aseptic maneuvers, hand washing to manage each patient, timing and selection of antibiotics, prevention of nosocomial infection for patients at risk, stress ulcer prevention prevention, thoracic drainages when indicated for infection control, support ventilation techniques using low tidal volume and protective maneuvers, protocol of sedation and analgesia.

Preserving tissue oxygenation and function is a priority when treating sepsis. Septic patients treatment optimisation is based on some key elements: suppression of the focus of infection and goal-directed antibiotic treatment (67,68) and aggressive shock treatment (69-76). Taking into account that the old dispute on crystalloids-colloids did not lead to positive results on the prevalence of first approach on the second one on the contrary, with reference to vasopressing agents, dopamine remains the preferred drug, although epinephrine, norepinephrine, phenylephrine and vasopressine are effective in order to improve arterial pressure and haemodynamics in patients affected by septic shock (73, 74). Aggressive treatments can be decisive. In this context, it is very interesting to read the recent contribution by Rivers (11), who reported that early optimisation of haemodynamics can significantly reduce mortality in the event of sepsis.

In particular, this study was aimed at assessing whether early circulation support before admission to intensive care units of patients affected by sepsis could lead to decreases in morbidity and mortality rates. The author enrolled 263 patients with suspected infection and SIRS signs, lactacidemia levels higher than

4mM/L or systolic pressure lower than 90 mmHg after proper resuscitation manoeuvres. Patients were recruited within 1 hour after their arrival to the hospital. The intensive care unit team that received the patients was not aware of the random assignment of patients to the various groups. Central venous pressure, average arterial pressure and hourly diuresis were measured every hour.

The patients assigned to early treatment protocol were also controlled for SvO<sub>2</sub>. When the SvO<sub>2</sub> value was below 70mmHg, blood transfusions were performed in order to obtain a haemoglobin concentration of 10 g/dl. For patients who reached this value, but whose SvO<sub>2</sub> value remained low, an infusion of 20 mcg/kg/min of dobutamine was started, and this treatment was not discontinued during intensive care. The patients divided into two groups were uniformly distributed by sex and age. All patients were compulsorily followed up by the emergency department for at least 6 hours, whereas the patients belonging to the control group were admitted to the intensive care unit when the first bed became available.

The analysis of the results obtained from both groups of patients showed that they achieved the objective of average arterial pressure and central venous pressure value optimisation within 6 hours. However, 40% of the patients belonging to the control group failed to reach SvO<sub>2</sub> values of 70%. The check of resuscitation endpoints after 72 hours showed a lasting positive effect for patients who were administered haemodynamic support with reference to both their haemodynamic indicator and organ dysfunction level. Intra-hospital mortality was 16% lower and statistically significant ( $P = 0.009$ ) in patients submitted to the early-goal directed therapy in comparison with the control group (46,5% vs 30%). This difference was unchanged at day 28 and, although it was statistically low, it remained significantly better after 60 days ( $P = 0.03$ ).

### **Clinical trials and Human Recombinant Activated Protein C (APC)**

Despite the progress made in the knowledge of inflammatory process feeding mechanisms in the course of sepsis, the findings of many clinical trials performed during the last decade reported negative results in terms of survival. In particular, randomised and controlled studies performed with the so-called “immunomodulating” agents did not entail advantages in the improvement of survival rates. Why did these studies fail?

There are many explanations such as unsuitable laboratory data or ineffective experimental agents (for instance, anti-endotoxin, HA-1 and E5); as a matter of fact, researchers thought that these factors could bind to the lipid A portion of endotoxin and, hence, neutralise endotoxin activity, whereas *in vitro* tests proved that none of these compounds could limit endotoxin activity or reduce interleukin (IL-1) or tumour necrosis factor (TNF- $\alpha$ ) releases (77).

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. Moreover, low protein C levels are quite frequently reported for patients affected by sepsis and septic shock; this factor plays a decisive role in coagulation process and has important anti-inflammatory functions, including the ability to stop nuclear translocation NF- $\kappa$ B factor (78), which is a key mechanism for cytokine formation from mononucleate cells and endothelium. In this context, activated protein C is likely to modulate an anti-apoptosis action and to limit endothelial injuries. Protein C is a Vit K-dependant protease; protein C is converted into activated protein C when thrombin combines with thrombomodulin, a trans-membrane glycoprotein factor contained in endothelium. Activated protein C inhibits Va and VIIa factors, thus actually reducing thrombin production. Moreover, it modulates endogenous fibrinolytic activity and inflammatory response. The fast protein C depletion in the event of sepsis induces, together with other factors, coagulopathy, which leads to a severe prognosis.

The decrease in thrombomodulin levels in the blood of patients suffering from meningococemia suggests that the ability to convert protein C into activated protein C is impaired; on the contrary, soluble thrombomodulin can stop the formation of clots and cell activation; moreover, protein C activation and the ability to inhibit thrombin in various experimental models suggests that soluble thrombomodulin can be useful for treating sepsis. Only activated protein C, and not protein C, showed a low decrease in mortality at both the experimental and clinical levels (79).

The safety and effectiveness of human recombinant activated protein C (Drotrecogin- $\alpha$ ) for severe sepsis treatment was demonstrated by a recent multicentre stage III trial for which 1690 patients affected by severe

sepsis were enrolled (9); as to patients treated with APC, a decrease in mortality down to 24.7% was reported compared with the placebo group, which recorded a mortality of 30.8%.

Drotrecogin  $\alpha$  was administered at a dosage of 24 $\mu$ g/kg/hour x 96 hours. In this study, the incidence of severe bleeding was higher for the group treated with APC than for the control group (3.5% vs. 2.0%).

## **Steroids**

In 1980, Roger Bone *et al.* performed a study aimed at demonstrating that corticosteroid administration could suppress inflammatory response thanks to its ability to modulate signal transmission at the cell level (78).

In particular, the anti-inflammatory role of corticosteroids was demonstrated with reference to the following aspects: ability to prevent inflammatory cascade activation by the complement, possibility to inhibit endotoxin-induced leukocyte adhesion, assessment of endotoxemia-induced platelet factor activation level, assessment of tumour necrosis factor and interleukin-1 releases by monocytes, and prevention of prostaglandin production through phospholipase A2 inhibitor induction. Owing to these elements, corticosteroids were regarded as useful to treat sepsis and able to reduce morbidity and mortality rates. However, this issue still presents many controversial points, since some maintain that corticosteroids can also be dangerous for a patient's outcome (79).

Annane *et al.* (80) started from the assumption that absolute adrenal failure is present in about 1-2% of patients admitted to intensive care units, whereas adrenal dysfunction occurs for 30% of patients hospitalised in the same structure. 297 patients with septic shock and depending on vasopressors agents were enrolled. They underwent ACTH stimulation at the onset of shock and randomly assigned to hydrocortisone or fludrocortisone vs. placebo administration for 7 days; the intravenous cortisone dosage was 50 mg 4 times a day; a significant decrease in mortality (10%) was reported for treated patients (80,81) compared with controls. These findings, although promising, are not conclusive. The stratification of at-risk patients under intensive care is a key point in daily clinical practice.

## **Experimental therapies**

In this context, IgM immunoglobulins seem to play an important role. As a matter of fact, a decrease in mortality and the absence of adverse effects shown by this treatment were encouraging results; in particular, immunoglobulins have various important functions such as decrease in body temperature and inflammatory parameters (procalcitonin), reduction in FiO<sub>2</sub>, which is an indirect sign of better oxygen saturation, and stabilisation of average arterial pressure and heart rate (82, 83). Pentaglobin is likely to reduce morbidity and mortality in septic patients and plays an important role in immunity system modulation (84). Of course, there are no magic wands to treat sepsis; however, in the light of the results obtained from these experimental therapies, the rationale of associating different drugs should be supported. There is increasing evidence that adenoceptor modulation can prevent tissue injury through a variety of pathways (85).

Adenosine is a metabolite of adenosine triphosphate (ATP) with a short half-life (84) due to its rapid metabolism. It accumulates in areas where ATP is used but not reformed, for example, during ischemia (87) and possibly during sepsis (88). Adenosine acts on a variety of cells including myocytes (AV nodal block), mast cells, macrophages, and neutrophils. There are four adenoceptors, known as A1, A2a, A2B, and A3 receptors. Endogenously released adenosine was shown to protect human vascular endothelial cells from injury by stimulated neutrophils (89).

The use of adenosine modulation in ischaemia/reperfusion injury has been the subject of considerable investigation, although experience with its use in sepsis is limited; adenosine may attenuate I/R injury by a number of possible mechanisms (90), including purine salvaging, improved tissue perfusion, anti-inflammatory action, and a direct intracellular initiator/effector mechanism; experimental data in sepsis evidenced that adenosine strongly inhibit extracellular superoxide anion release (91); furthermore, adenosine has unwanted cardiovascular side effects, causing bradycardia and hypotension; alternatives to adenosine administration include modulation of its metabolism and the administration of specific antagonists/antagonists (92)

### **Injury and sepsis : Genomics and Proteomics perspectives**

A predisposition for sepsis represents an increased risk of developing sepsis. Genetic predisposition can be considered in terms of high risk and low-risk exposure and independent and dependent exposure. High risk often involves dependence on single genes, so single mutation produces the disease; and lower risk often reflects dependence on multiple genes. Sepsis probably is a multiple gene problem. Acquired factors are complex and difficult to separate from heritable factors. Age, gender, chronic health or disease, acute illness, exposures, and interventions all are acquired factors. Such acquired factors confound all studies of genetic predisposition of multifactorial diseases and multidimensional responses. However, traditional genetic studies are not possible in sepsis because family members usually do not become septic at the same time and because the treatment have changes over time. The study of injury in critical illness is now occurring “upstream”, at the genetic and cellular levels, to understand how damaging effects of acute inflammation from injury can be prevented or modulated. Genomic and proteomics evidence documents that repair processes begin shortly after injury (93).

The interactions between the injury and repair cascades most likely determine the outcome of the injurious process. With closer examination of the heterogeneity inherent in the human population, the different genotypic expressions also include differences in the kind of repair response mounted. These differences include varied Th1 vs Th2, or hyperinflammatory vs hypoinflammatory, helper T-cell responses to a septic or inflammatory insult. In addition, different degrees of apoptosis occur, with often deleterious sequela (94).

Definitions of injury and repair are important because they are somewhat arbitrary and may in fact be interchangeable in terms of body processes. Injury is defined as the disruption of molecular, cellular, or organ functions resulting from an external or internal stimulus. The external stimuli include infection, hypoxia, ischemia, chemical or thermal injury, toxins, and trauma. The internal stimuli include the acute inflammation cascade, shock, and reperfusion injury. An alternative organization groups the injurious stimuli as physical (radiation, extreme temperature, mechanical trauma), chemical (toxins), biological (infections, cell-mediated toxicity, cytokine mediated toxicity, enzymatic activity), and substrate deficiency (oxygen, glucose). Whatever the initial stimulus, once the injury occurs they manifest similar results once the body activates its repair response. Repair is defined as an adaptive process that occurs in response to injury and involves both local and systemic responses that serve to restore structure and regulation for the purpose of organ/tissue function. The repair responses to injury probably vary as a result of genetic factors; some people react with a more vigorous inflammatory response than others (95). They represent an organized effort to reestablish cellular and tissue integrity after injury and involve a complex order of cellular and biochemical events. The initial steps of the acute phase response include coagulation, leukocytes activation, edema formation from extravasation, and apoptosis (96). The apoptotic response correlates with worse outcome (97); because it can induce cell injury and death to an extent that exacerbates the morbidity of the injury (98).

Potential signals rush into and out of cells through plasma membrane disruptions. These might trigger cell or tissue level adaptive responses serving to facilitate future disruption repair or mechanically reinforce the cells environment. One well-characterized example of a signal that exists through a disruption is a fibroblast growth factor (FGF)-2. This polypeptide growth factor, like several others, lacks a signal peptide sequence and so cannot be secreted by the conventional exocytotic pathway (99).

Other repair processes include proliferation, regeneration, remodelling, revascularization, and scar formation. Also involved in the dynamics of injury and repair are heat shock proteins, which can be manipulated to alter the outcome of injury and repair mechanisms. The heat shock proteins have both positive and negative effects on cytokine expression, and they modulate the tendency towards apoptosis and necrosis in stressful conditions, such as ischemia (100)

The preliminary theories of the tissue repair process, then, are compatible with the biochemical events seen in vitro. However, when applying the principles to an in vivo situation, questions still remain. For example, with repair processes, the outcome of each organ differ; is that a result of different repair processes occurring in the different organ system? Is the process of repair the same given different underlying mechanisms of injury – sepsis, acute respiratory distress syndrome, blunt trauma, hemorrhagic shock? And do the differing mechanisms modify the repair process? What organ is capable of regeneration? What is the timeline of the injury and repair? Do factors such as genomics or nutritional status modulate the repair rate? These are some of the several questions which need answers from researchers and clinicians in the future.

## Critical points and conclusion

In spite of the advances on the knowledge of the basic phenomenon of inflammation and its continuum on development of sepsis and organ(s) dysfunction the hope of finding a “magic bullet” to treat sepsis has been frustrating. Negative clinical result for survival are substantial different from experimental data. Monoclonal antibody, anti-inflammatory drugs, immunoglobulins, anti-endotoxin and other aspecific therapy, all failed to improve patient’s outcome as defined by the traditional primary endpoint of mortality (101). Clinical trials remain the most effective for assessing efficacy and safety of new therapies of sepsis (102).

After two decades of flop is time do reconsider the target for treatment in human being and a more appropriate end-point in the treatment of sepsis. Several thousand of patient have been enrolled in the sepsis trials series managed in the last 15 years. These trials have been conducted in intensive care units in heterogeneous patient population with various entry criteria and endpoints of response (99). So, the history of the therapeutic trials in sepsis has been one of unfulfilled expectations and conflicting results until the last successful trial on Activated Protein C (9). Sepsis is a disease but more frequently became very difficult a correct definition so it remains a syndrome. The development of organ dysfunction, with the signs and symptoms of sepsis and an infection, defines severe sepsis. The development of arterial hypotension in addition to organ dysfunction and symptoms of sepsis is defined as septic shock (103).

Why all clinical trials have failed? This is a question of paramount importance to clear up; in fact, heterogeneity of studied population is a crucial point; sepsis often presents with various co-morbid disease states and septic patients often receive different treatment for these co-morbid disease. The admission diagnosis and the consistency of underlying disease remain the major determinants of outcome (104).

Scoring systems is useful to focus the relative risk of death, although the degree of organ dysfunction and even the quality of life are also important (105, 106).

Considering the poor knowledge of sepsis pathophysiology the criteria used for the patients enrolment in sepsis studies are crucial to permit the right treatment at the right time in the right group of patients. Therefore, it is important to consider the presence and the source of infection, the type of micro-organism, the severity of the underlying disease, and the appropriateness of the non-trial study therapy. Furthermore, mixing together septic patients with and without documented infections may obscure relevant therapeutic effects of the intervention tested (107). Several aspects remain to be elucidated for clarifying more the complexity of sepsis and related conditions. For example, considering the difficulties to have an appropriate standard of care between various institutions in multi-centre trials, it is not surprising that the outcome will differ between ICUs.

Sepsis is a condition with high consuming resources, but until now mortality rate is not a rare event. In the clinical trials of sepsis 28 or 30 day all cause mortality has the primary endpoint for efficacy. All causes mortality ranges between 20 and 60% and represent the over-all death rate of a cohort of patients who developed bacteremic sepsis during their ICU or hospital stay (104). In critically ill patients, the underlying disease and the functional health status are the most important determinants of outcome. Underlying disease during gram-negative bacteremia are the most important determinant of outcome; thus, one may expect any novel therapeutic intervention to have only a modest effect on outcome from severe sepsis (108).

Considering the significative cons on mortality as primary end-point in sepsis it has been suggested not to use all cause mortality (28-day window) or attributable mortality as the sole endpoints, but regard the reduction of reversal of organ failure as a valid efficacy endpoint such as quality of life is an important parameter (109). Starting from these observations the importance of surrogate as an alternative to mortality has been considered. Organ failure scores represent a surrogate outcome in phase II and phase II clinical trials. Although mortality as an endpoint is characterized by some advantages, it must not be forgotten that the goal of treatment of sepsis is to preserve or improve organ function. Thus, the assessment of reduction in morbidity rather than in mortality give some advantage; five organ failure descriptors have been shown to correlate with ICU mortality in a dose dependent fashion as does hypotension (110). Treatment of sepsis remains largely supportive with emphasis on adequate antibiotic treatment and source control. Nevertheless, improved study designs (statistics, single versus multi-centre study; study population based on pathophysiological rather than quantitative aspects) are considered mandatory if future progress is to be made in the care of these patients. In conclusion sepsis and organ(s) dysfunction represent an hot topic in the field of critical care which offer to

the researchers and clinicians bad and good news on prevention and management (111,112); for the future the goal for all will be mandatory : from chaos to rationale; I hope we accept this challenge (113, 114)

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