Antithrombin replacement in patients with sepsis and septic shock

DANIELA GIUDICI,* FRANCESCO BAUDO,# GUAM TRO PALARETI,° ADRIANO RAVIZZA,* LORENZA RIDOLFI,° ARMANDO D'ANGELO§

*Unità di Terapia Intensiva e 1Servizio di Coagulazione, IRCCS H S.Raffaele, Milan; °Unità di Emotosi e Trombosi e 1Dipartimento di Terapia Intensiva, Ospedale Niguarda, Milan; #Dipartimento di Angiologia e 1Unità di Terapia Intensiva, Ospedale S. Orsola, Bologna, Italy

ABSTRACT

Sepsis is a frequent complication of critically ill patients and its incidence is increasing. Currently, septic shock is the most common cause of death in non-coronary intensive care units. Over the last 10 to 15 years, new antibiotics and increasingly sophisticated critical care have had little impact on the mortality rate of septic shock. The Italian SESPIS Study, carried out in 99 intensive care units in 1994, reported mortality rates of 52% and 82% for severe sepsis and septic shock respectively. New therapeutic approaches aimed at neutralizing microbial toxins and modulating host mediators have shown some efficacy in large clinical trials and/or in animal models, but to date, no therapy of sepsis aimed at reversing the effects of bacterial toxins or of harmful endogenous mediators of inflammation has gained widespread clinical acceptance. Because of the strong association of severe sepsis with a state of activation of blood coagulation and the potential role of capillary thrombosis in the development of the multiple organ dysfunction syndrome, anticoagulant agents have been tested in the setting of septic shock. However, neither administration of heparin nor of active site-blocked factor Xa or of anti-tissue factor antibodies has proven effective in preventing deaths due to septic shock in animal models. In contrast, infusion of antithrombin, protein C, or tissue factor pathway inhibitor all resulted in a significant survival advantage in animals receiving lethal doses of E. Coli. Antithrombin concentrates have been used in a significant number of critically ill patients. A double-blind, placebo controlled study carried out in 3 Italian intensive care units has recently shown that the administration of antithrombin aimed at normalizing plasma antithrombin activity had a net beneficial effect on 30-day survival of patients requiring respiratory and/or hemodynamic support because of severe sepsis and/or post-surgery complications.

Key words: sepsis, septic shock, diffuse intravascular fibrin formation, antithrombin replacement therapy, protein C

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Correspondence: Armando D’Angelo, M.D., Servizio di Coagulazione, IRCCS H S. Raffaele, via Olgettina 60, 20132 Milan, Italy. Phone: international +39-02-26432228 Fax international +39-02-26432640 E-mail: armando.dangelo@hsr.it

Infection is a common cause of admission into intensive care units (ICUs) and a frequent complication of critically ill patients. A series of factors have contributed to the increasing incidence of sepsis and of septic shock. Immunosuppressive therapy for malignancy, organ transplantation, or inflammatory disease places patients at increased risk of infectious complications. Patients predisposed by underlying diseases such as diabetes mellitus, renal failure, and cancer are more likely to suffer an increased rate of infections because they have now a longer life-expectation. Invasive life support procedures (hemodynamic and respiratory support) and broad-spectrum antibiotics have created a large hospital-based population at risk of nosocomial infection by resistant micro-organisms.

Septic shock is currently the most common cause of death in non-coronary ICU.1,2 Mortality is related to the severity of sepsis and of the underlying disorder that is nearly always present. Agreement about the definition of a septic syndrome has been only recently achieved. In 1992, a Consensus Conference of the American College of Chest Physicians and Society of Critical Care Medicine established a set of definitions that could be applied to patients with sepsis and its sequelae.3 The term sepsis implies a clinical response arising from infection, but a similar, or even identical, response may also develop in the absence of infection. This systemic inflammatory response syndrome (SIRS) can occur following a wide variety of insults, infectious or non-infectious, the latter including pancreatitis, ischemia, multiple trauma and tissue injury, etc. SIRS is defined by the occurrence of two or more of the following conditions: temperature >38°C or <36°C, heart rate >90 beat/min, respiratory rate >20 breath/min or PaCO2 < 32 torr, white blood cell count >12,000/µL or >10% immature forms. When the systemic inflammatory response syndrome is the result of a confirmed infectious process, it is termed sepsis.

Sepsis and its sequelae represent a continuum of clinical and pathophysiological severity. Sepsis is defined as severe when it is associated with organ dysfunction, hypoperfusion abnormalities or sepsis-
induced hypotension. Hypoperfusion abnormalities include lactic acidosis, oliguria, or an acute alteration of mental status. Sepsis-induced hypotension is defined by the presence of a systolic blood pressure of <90 mm Hg or its reduction by more than 40 mm Hg from the baseline, in absence of other causes for hypotension (cardiogenic shock etc.). Septic shock is a subset of severe sepsis and is defined as sepsis-induced hypotension, persisting despite adequate fluid resuscitation, along with the presence of hypoperfusion abnormalities or organ dysfunction. Patients receiving inotropic or vasopressor agents might no longer be hypotensive by the time they manifest hypoperfusion abnormalities or organ dysfunction. Patients receiving inotropic or vasopressor agents might no longer be hypotensive by the time they manifest hypoperfusion abnormalities or organ dysfunction, yet they are still considered affected by septic shock.

A frequent complication of SIRS is the development of organ system dysfunction, as a process of progressive failure of several interdependent organ systems. The detection of altered organ function in acutely ill patients constitutes a syndrome that should be termed multiple organ system dysfunction syndrome (MODS), in which organ function is not capable of maintaining homeostasis. The term dysfunction emphasizes the dynamic nature of the process, although specific descriptions of this continuous process are not currently available. Multiple organ dysfunction syndrome is subject to modulation by numerous factors, both interventional and host-related, at varying time periods. MODS may be primary when it occurs early in response to a well-defined insult such as trauma, pulmonary contusion, rhabdomyolysis, massive transfusions, or it may be secondary to the host response to the insult when it is characterized by a generalized activation of the inflammatory reaction in organs remote from the initial insult. When due to infection, secondary MODS usually evolves after a latent period after the provoking injury or event, and is a frequent complication of severe infection.

Pathogenesis

The pathogenesis of sepsis and septic shock are not completely understood. Gram-positive organisms releasing exotoxins, Gram-negative organisms containing endotoxins and fungi can initiate this pathogenic cascade. The process begins with the proliferation of micro-organisms at a nidus of infection. The organisms may invade the bloodstream directly (leading to a positive blood culture) or may proliferate locally and release various substances into the bloodstream. These events trigger host cells (neutrophils, monocyte-macrophages) to release a variety of interacting cytokines (tumor necrosis factor [TNF], interleukins, interferons). This results in the activation of several pathways (complement, coagulation, fibrinolytic, and hormonal) and in the increased production of numerous endogenous mediators (C5a, eicosanoids, endorphins, toxic oxygen radicals, nitric oxide, and platelet-activating factor), with profound physiologic effects on the cardiovascular system and on the function of other organs.

Early in severe sepsis, systemic vascular resistance decreases – primarily mediated by the release of bradykinin and histamine – and cardiac output increases. In this hyperdynamic phase, septic shock is a classic form of distributive shock, resulting from abnormal distribution of blood flow. Despite an often elevated cardiac output, tissue oxygen utilization is reduced. The decreased arterio-venous oxygen difference suggests that oxygen is not reaching or not being used by tissues. The exact mechanisms responsible for decreased tissue perfusion are poorly understood. In septic shock, many vascular beds are dilated, but some are constricted, and some are occluded by microthrombi. The aggregation of neutrophils and platelets may lead to impairment of blood flow. Neutrophil migration occurs along the vascular endothelium, resulting in the release of many mediators and the migration of neutrophils into tissues. Neutrophils can release active oxygen species, such as superoxide radicals, that can directly damage cells. Components of the complement system, such as C5a, are activated. Inflammatory mediators, such as prostaglandins and leukotrienes are released from many types of cells and can cause either vasoconstriction or vasodilatation, with increased permeability of the vascular endothelium and passage of fluid from the intravascular to the interstitial fluid space. Endothelial damage may per se decrease oxygen and substrate utilization by the tissues.

In the hypodynamic phase blood pressure is normal or slightly reduced, the skin is warm and dry, there is tachycardia, urine output is satisfactory, and the patient hyperventilates and is pyretic. Fever results from the direct effects of endotoxins and interleukin-1 on the hypothalamus. The release of inflammatory mediators and endothelial damage also lead to the development of diffuse intravascular fibrin formation (DIFF) and deposition, followed by a secondary bleeding tendency. DIFF decreases organ blood flow, causing hypoxia, lactic acidosis, organ dysfunction and failure. This occurs especially in the circulation of the lungs, liver, kidneys, and gastric mucosa with the manifestations of secondary MODS.

If the clinical state is not recognized and treated within a few hours the patient enters the hypotensive phase of septic shock, in which the combination of decreased systemic vascular resistance and myocardial depression induces hypotension which is independent from adequate fluid resuscitation. A reversible depression of myocardial function, with decreased ejection fraction and left ventricular dilation, is common in septic shock. Circulating antiinotropic substances, termed myocardial depressant substances, may play an important role in the pathogenesis of myocardial depression. In the hypotensive phase the patient is oliguric with cold, pale skin, and is cyanotic, features which are typical of an established shock syndrome. As a consequence of the arteriolar dilation and of the increased capillary and
post-capillary venule permeability, especially in the infected tissues, there is increased fluid transfer from capillaries to the interstitial fluid. The hypovolemia decreases venous return, cardiac output and blood pressure. Baroreceptor compensation increases sympathetic activity so causing vasoconstriction in the skin, the splanchnic areas, kidney and muscles.

Bronchoconstriction is an early finding in many patients with severe sepsis. This is probably due to endotoxin or to release of inflammatory mediators. At this time the chest radiograph is often normal, but gas exchange may be mildly abnormal. Later, if septic shock occurs many patients develop diffuse alveolar damage consistent with the adult respiratory distress syndrome (ARDS). From 40% to 60% of patients with Gram-negative septic shock develop ARDS. Alveolar-capillary membrane damage allows for leakage of fluid and proteins into the pulmonary interstitium. Alveoli are subsequently flooded, causing a marked increase in intrapulmonary shunting and severe arterial hypoxia. At this stage the chest radiograph demonstrates diffuse bilateral alveolar infiltrates. Hypoxic pulmonary vasoconstriction, in situ thrombosis, and aggregation of neutrophils and platelets in the pulmonary microvascular system increase pulmonary artery pressure and right ventricular afterload, leading to a worsening of right ventricular performance.

In addition to the cardiopulmonary systems, other systems may sequentially become dysfunctional in septic shock because of the role of inflammatory mediators. Visceral hypoperfusion and decreased intestinal peristalsis may lead to alterations of the barrier function of the gastrointestinal tract; gastrointestinal bleeding may follow stress ulceration of the gastric mucosa. Liver dysfunction may manifest as hyperbilirubinemia, elevated aminotransferase levels, cholestasis, progressive and intractable hypoglycemia and hypoaalbuminemia. As kidney function declines, urine output falls and blood urea and creatinine levels rise. Renal failure is mainly due to acute tubular necrosis induced by hypotension or capillary injury, but drug-induced renal damage may also occur. Alterations of the mental status can occur, ranging from mild confusion and lethargy, to stupor and coma; abnormalities in the blood brain barrier and changes in the concentrations of circulating amino acids frequently accompany this obtundation of sepsis. Abnormalities of the clotting system, ranging from mild prolongation of the prothrombin time and of the partial thromboplastin time, to profound thrombocytopenia and frank disseminated intravascular coagulation are common in patients with septic shock.

**Treatment**

The treatment strategy for severe sepsis and septic shock is based on the provision of intensive life support, the eradication of micro-organisms, the neutralization of microbial toxins, and the modulation of host mediators.

The treatment strategy for severe sepsis and septic shock should include the following interventions:

- **Intensive life support**: Maintain vital functions, including cardiovascular support and respiratory support.
- **Monitoring**: Continuous monitoring of vital signs and organ function is essential.
- **Vasopressors**: Dopamine, norepinephrine, and vasopressin are commonly used to maintain blood pressure.
- **Fluid replacement**: Intravenous fluids are used to correct hypovolemia.
- **Antimicrobial therapy**: Early and effective antimicrobial therapy is crucial.
- **Nutritional support**: Provision of adequate nutrition is necessary.
- **Integrative therapies**: Consideration of integrative therapies, such as probiotics, may be beneficial.

**Monitoring**

Patient monitoring is essential to the choice of the cardiovascular support and includes cardiac rhythm monitoring, intra-arterial invasive blood pressure monitoring, right-sided heart catheterization, and laboratory monitoring of the metabolic profile.

**Intensive Care Unit (ICU)**

ICU monitoring helps in identifying the severity of the sepsis and guides the therapeutic interventions. The ICU monitoring includes continuous monitoring of vital signs, laboratory tests, and imaging studies.

**Critical Care Management**

- **Vascular access**: Secure adequate vascular access for fluid resuscitation.
- **Fluid resuscitation**: Rapid fluid resuscitation is crucial to stabilize hemodynamics.
- **Vasopressors**: Use vasopressors to maintain mean arterial pressure above 70 mmHg.
- **Oxygen therapy**: Provide high-flow oxygen therapy to maintain arterial oxygen saturation.
- **Invasive monitoring**: Use Swan-Ganz catheter for central venous pressure measurement.
- **Antimicrobial therapy**: Start empiric antimicrobial therapy and adjust based on culture results.
- **Nutritional support**: Initiate parenteral nutrition and enteral feeding.
- **Integrative therapies**: Consider integrative therapies such as probiotics and antioxidants.

**Conclusion**

Septic shock is a serious and lifethreatening condition that requires prompt and effective management. A multidisciplinary approach involving intensive care physicians, microbiologists, and critical care nurses is essential for optimal outcomes.
cultures especially in neutropenic patients.

Over the last 10 to 15 years, new antibiotics and increasingly sophisticated critical care have had little impact on the mortality rate of septic shock, which remains extremely high as demonstrated by the results of the Italian SEPSIS Study. The aim of this prospective, multicenter investigation was to evaluate the clinical outcome of consecutive patients admitted to intensive care units on the basis of the diagnostic criteria of the ACCP/SCCM Consensus Conference. The study was carried out in 99 ICUs in Italy from April 1993 to March 1994. In a preliminary analysis of 1100 patients, severe sepsis and septic shock had mortality rates of 52.2% and 81.8% respectively (Table 2). As a result, new therapeutic approaches have been tested, aimed at neutralizing microbial toxins and modulating host mediators (Table 3). Some of the agents have shown some efficacy in large multicenter clinical trials (anti-endotoxin monoclonal antibodies, anti-TNF antibodies, monoclonal anti-TNF antibodies), others only in animal models (monoclonal anti-TNF antibodies, anti-C5a antibodies, anti-C5a antibodies). To date however, no therapy of sepsis aimed at reversing the effects of bacterial toxins or of harmful endogenous mediators has gained widespread clinical acceptance.

Antithrombin concentrates in sepsis and septic shock

In view of the strong association of severe sepsis with a state of activation of blood coagulation and the potential role of capillary thrombosis in the development of MODS, anticoagulant agents have been tested in the setting of septic shock. However, neither administration of heparin nor of active site-blocked factor Xa have proven effective in preventing deaths due to septic shock in animal models. Even the administration of anti-tissue factor antibodies did not prevent severe manifestations of septic shock in animal models, although resulting in effective blockade of the clotting system. In contrast, infusion of natural inhibitors of blood coagulation (antithrombin, protein C, tissue factor pathway inhibitor), all resulted in a significant survival advantage in animals receiving lethal doses of E. coli.

Because of their commercial availability, antithrombin concentrates have been used in a significant number of critically ill patients. Antithrombin (AT), a glycoprotein synthesized by the liver and the kidney, is a main physiologic inhibitor of serine proteases generated during blood coagulation (FIIa, FIXa, FXa, FXIa, FXIIa). The rate of neutralization of the above-mentioned proteases is increased by 3 orders of magnitude in the presence of heparin and heparin-sulphate. The concentration of AT in plasma is decreased in conditions associated with diffuse intravascular fibrin formation, particularly in sepsis and shock. The decreased plasma concentration of AT may be an indication of the role of DIFF in the pathogenesis of multi-organ failure; it is a poor prognostic factor and correlates with survival. Two randomized studies addressed the use of AT concentrate supplementation in the treatment of severe sepsis and shock, but they did not include a placebo-control group. Blauhut et al. randomized 51 patients with shock of different etiology (sepsis, trauma, hepatic coma) to receive AT, heparin or AT + heparin. The time to normalization of the platelet count and of the fibrinogen concentration was shorter in patients receiving AT, but no difference in survival was observed. In a subsequent study, including only
patients with traumatic shock, Vinazzer reported a significant reduction in the mortality of patients treated with AT concentrate. However, in an Italian study of patients with a clinical diagnosis of liver disease, the administration of AT concentrates did not result in any significant survival advantage. These results are difficult to interpret. In critically ill patients, the evaluation of the efficacy of therapeutic agents requires a double-blind design, to avoid the bias of the attending clinician who is confronted with patients with a potentially fatal outcome. Fourrier et al. published the first randomized double blind, placebo-controlled study in septic shock. Patients treated with AT tended to have a survival advantage, but the difference from the placebo group did not reach conventional statistical significance. Similar results were obtained by Lamy et al. (Table 4).

We planned a double blind study to evaluate the effect of AT administration on survival of a selected group of patients requiring hemodynamic and/or respiratory support because of severe sepsis and/or post-operative complications. A major assumption was that the observation of decreased AT levels – unrelated to evidence of impaired liver synthesis of the protein – may reflect uncontrolled activation of the clotting system in critically ill patients, with a potentially unfavorable role in their prognosis. In line with this hypothesis, we tested the possibility that the maintenance of normal AT levels by infusion of AT concentrate could have a beneficial effect on survival of critically ill patients irrespective of the causes leading to the requirement for hemodynamic and/or respiratory support.

The study was randomized and double blind, with the inclusion of a placebo control arm. Identification of the infused material by the attending physicians was prevented by the use of identical black bottles, syringes and infusion-sets. Patients were included in the study if they were 18 to 75 years old, were admitted to the intensive care unit (ICU) because of sepsis and/or post-operative complications requiring respiratory and/or hemodynamic support and had plasma AT activity <70% of normal. Septic shock was defined as sepsis-related hypotension requiring vasoactive drugs for more than 24 hours, persisting despite adequate fluid resuscitation, along with the presence of hypoperfusion abnormalities or organ failure. The respiratory support consisted of assisted or controlled ventilation for more than 24 hours. The hemodynamic support consisted of the administration of inotropic (dopamine or dobutamine, >5 µg/kg/min) and/or vasoactive amines (epinephrine or norepinephrine).

Patients were excluded if they had suffered multiple trauma, had liver cirrhosis or acute liver failure, cancer in terminal phase, immunodeficiency, or leukemia, if they were pregnant, or were being submitted to heparin therapy for hemodialysis, hemofiltration or other indications. Patients receiving heparin prophylaxis were not excluded.

The AT concentrate and the placebo (albumin solution, 50 g/L) were supplied by the manufacturer (Immuno) in identical black bottles containing either 2,000 U of AT or 2 g of albumin in lyophilized form. A fixed dose of 4,000 units of AT or 4 g of albumin were injected as a bolus in 30 min, followed by a bottle every 12 hours for 5 days by a pump-driven syringe. There was no limitation to standard medical care in each ICU; but for the infusion of fresh frozen plasma, indicated for patients with active bleeding and/or with PT ratios >2.0, or of platelet concentrates, which were administered at the dosage of 1 unit/kg body weight if the platelet count was <50 x 10⁹/L.

The simplified acute physiologic score (SAPS) was recorded in each patient at admission; a modified multi-organ failure (MOF) score, was recorded at admission and daily thereafter for 7 days. Baseline AT determinations for the enrolment of patients were carried out locally in each hospital. Thereafter, no local AT determinations were permitted. AT data reported were obtained after centralized measurement against an established calibrator (Immuno).

The main end-point of the study was survival at 30 days. The sample size was calculated to detect a 50% reduction of the expected mortality in the placebo group (60%) with an α error of 0.05 and a β error of 0.10. This mortality figure was anticipated based on the results of a previous study validating the SAPS score in consecutive patients referred to ICUs. No separate randomization blocks were applied for patients with or without septic shock.

One hundred and twenty consecutive patients were enrolled (60 in each treatment arm) from January 1991 to November 1994 in three ICUs: 92 patients because of post-operative complications, 12 patients because of bronchopneumonia with septic shock, and 16 patients with a miscellany of disorders. One hundred patients had sepsis and 56 had septic shock at admission. The distribution of patients in the two arms was well balanced except for the number of patients with septic shock (33 in the AT arm versus 23 in the placebo arm, p = 0.08) and for the baseline

Table 4. Controlled studies of antithrombin replacement therapy in septic shock and critically ill patients.

<table>
<thead>
<tr>
<th>Author (ref.)</th>
<th>No. of patients</th>
<th>Mortality rate Standard treatment</th>
<th>AT replacement</th>
<th>Odds Ratio</th>
<th>95% CI.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blauhut et al., 1985</td>
<td>51</td>
<td>12%</td>
<td>15%</td>
<td>1.29</td>
<td>0.18-42.9</td>
</tr>
<tr>
<td>GISACID, 1990</td>
<td>41</td>
<td>23%</td>
<td>29%</td>
<td>1.33</td>
<td>0.24-10.0</td>
</tr>
<tr>
<td>Harper et al., 1991</td>
<td>50</td>
<td>32%</td>
<td>32%</td>
<td>1.00</td>
<td>0.26-3.85</td>
</tr>
<tr>
<td>Albert et al., 1992</td>
<td>33</td>
<td>31%</td>
<td>25%</td>
<td>0.73</td>
<td>0.11-4.40</td>
</tr>
<tr>
<td>Fourrier et al., 1993</td>
<td>32</td>
<td>50%</td>
<td>28%</td>
<td>0.40</td>
<td>0.06-2.19</td>
</tr>
<tr>
<td>Lamy et al., 1996</td>
<td>42</td>
<td>41%</td>
<td>25%</td>
<td>0.48</td>
<td>0.10-2.16</td>
</tr>
</tbody>
</table>

* Double blind studies.
were similarly abnormal in the two treatment arms. With septic shock, hemodynamic parameters at entry were similarly abnormal in the two treatment arms. Some degree of dyshomogeneity between the baseline characteristics of patients enrolled in the three centers participating in the study was observed, with statistically significant differences affecting SAPS (p=0.003), and AT levels (p=0.004), and probably resulted from poor standardization of AT measurements between the 3 laboratories.

Four patients received therapy for less than 24 hours: 1 patient, in the placebo group, was transferred to another hospital after the bolus infusion and 3 patients (2 in the ATIII group and 1 in the placebo group), included in the intention to treat analysis, died on the day of enrolment. The mean time interval from admission to the ICUs and enrolment into the study (5.0±6.5 days) was not different for patients allocated to AT or placebo. Significant bleeding, requiring transfusion of red blood cell packs and platelet concentrates occurred in 6 patients in the placebo group and 5 patients in the AT group. No differences were observed between the treatment arms with respect to transfusion requirements with fresh frozen plasma, platelet and red blood cell packs. No side effects possibly related to AT treatment were observed.

Changes in plasma AT concentrations were not observed either in patients receiving placebo or in patients receiving AT after the initial rise observed following the first bolus injection (range 98-101%). Survival curves were calculated for the 119 (intention to treat) and the 116 patients after the exclusion of the early deaths. By Kaplan-Meier analysis survival was not different in the two arms. At day 30, 30 patients in the AT arm (50%) and 27 patients in the placebo arm (46%) were alive. Because of the unbalanced randomization for baseline variables potentially affecting survival, we analyzed, by the Kaplan-Meier approach, the influence on survival of the requirement for hemodynamic support, the presence of septic shock and the MOF score at entry. The presence of septic shock (p<0.0001) and the requirement for hemodynamic support (p<0.0001) were negatively associated with survival; 30-day mortality was 75% in patients with septic shock and 32% in patients without shock. In addition, among patients with an unfavorable outcome, 75% of patients with septic shock died by day 5, whereas the same percentage of deaths was recorded by day 22 in patients without septic shock.

The significant influence on mortality rates of variables imperfectly balanced by the randomization process, led us to analyze the net effect of treatment on 30-day mortality after adjusting for the presence of covariates in a Cox regression model. In addition to the presence of sepsis, septic shock and the requirement for hemodynamic support, the baseline MOF score and plasma AT activity, the time to treatment, age and center were included as covariates in the model (Table 5). At multivariate analysis, AT replacement had a net beneficial effect on 30-day survival (OR =0.56, p<0.02). Of the covariates analyzed, the presence of septic shock (p=0.0002) and the baseline MOF score (p=0.02) were negatively associated with survival, while plasma AT activity levels (p=0.003) were positively and independently associated with survival, which also differed according to the center (p=0.006).

Because the two treatment arms were unbalanced for septic shock, the interaction of treatment with septic shock was tested in the model and resulted to be significantly associated with survival (p<0.0001). After stratification of patients for the presence or absence of septic shock, a net effect of AT treatment on mortality was observed only in the septic shock group (OR =0.43, Table 5). In patients with septic shock, a significant effect of AT treatment on survival was also shown by Kaplan-Meier analysis (p =0.04). Septic shock patients receiving treatment had a 34% (95% CI: 19%-49%) probability of being alive at day 30, with a corresponding probability of 13% (95% CI: 0%-26%) for septic shock patients receiving placebo and a resulting 30% reduction in 30-day mortality. The reduction in mortality produced by AT replacement was even more apparent when excluding early deaths from the analysis (p=0.016).

Table 5. Variables independently affecting survival of critically ill patients by Cox hazard regression analysis (ref. #11c).

<table>
<thead>
<tr>
<th>Variables selected</th>
<th>Odds Ratio</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline AT % activity</td>
<td>0.97*</td>
<td>0.95-0.99</td>
</tr>
<tr>
<td>Center</td>
<td>1.61</td>
<td>1.15-2.24</td>
</tr>
<tr>
<td>Septic shock</td>
<td>3.97</td>
<td>1.77-6.25</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.56</td>
<td>0.31-0.91</td>
</tr>
<tr>
<td>Patients with septic shock</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline AT % activity</td>
<td>0.97*</td>
<td>0.95-0.99</td>
</tr>
<tr>
<td>Center</td>
<td>1.53</td>
<td>0.99-2.36</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.43</td>
<td>0.23-0.83</td>
</tr>
</tbody>
</table>

* For unitary increase in % antithrombin activity.

The model includes as variables: treatment, MOF, SAPS, and AT levels at baseline, requirement for hemodynamic support, centers, time from admission to ICU until enrolment in the study, sepsis, septic shock, age.
maintain very high levels of plasma AT activity for 5 days (175-200%). In their efficacy analysis treatment with AT resulted in normalization of laboratory parameters of DIFF in survivors within 10 days, and there was a 56% reduction in 30-day mortality in the active treatment arm. However, because of the low number of patients enrolled and of the relatively high overall survival rate (59%), this figure did not reach statistical significance. Lamy et al. infused a total amount of 18,000 units of AT over 5 days obtaining a 52% reduction in 30-day mortality for septic shock. This figure, too, did not reach statistical significance because of the low number of patients enrolled and the high survival rate (67%). In our series of 56 septic shock patients, the survival rate was 25%, similar to the 19% survival rate reported in Italian intensive care units.17

The beneficial effect of AT replacement in septic shock patients is indirectly further supported by the observation of the independent negative prognostic value of low plasma antithrombin activity at enrolment into the study. The predictive value on outcome of baseline plasma AT activity has been shown in DIC of different etiologies and in chemotherapy-induced neutropenia in acute leukemia and lymphoma. In one study,51 the development of septic shock in neutropenic patients was associated with early evidence of increased thrombin generation, and antithrombin levels lower than 70% at the onset of fever predicted a fatal outcome, with a sensitivity and specificity of 85%. Because the presence of AT levels <70% was a criterion for inclusion into our study, our findings point to the predictive value of AT in septic shock being independent of the severity of the disease (as also suggested by the non-influence of the baseline MOF score on survival) and they also indicate a causal relationship between the degree of activation of the coagulation mechanisms and the occurrence of death.

In spite of the beneficial effect of a 5-day course of AT replacement therapy, the overall mortality - not truncated at day 30 - was similar in patients receiving placebo or AT. The influence of AT replacement on laboratory markers of coagulation and fibrinolysis is currently being evaluated. It is possible that either the AT replacement protocol was insufficient to quench the activation of coagulation, or that approaches aimed at controlling inflammation may be required in addition to AT to obtain a prolonged effect on the survival of patients with septic shock. In animal models of septic shock, the administration of protein C had a clear-cut effect on survival.28 Protein C concentrate administration has proven highly effective in reducing mortality of patients with meningococcus-induced purpura fulminans,52 a syndrome characterized by very low levels of protein C activity and antigen.53 Because protein C has both anticoagulant and anti-inflammatory properties,54 future studies should evaluate the effect of the combination of AT and protein C administration on the survival of patients with septic shock.
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DG and ADA wrote the manuscript; FB, GP, AR and LR participated in the design of the study which is the major issue of the present review and they are listed in alphabetical order. All the authors read and approved the final version of this manuscript.

Disclosures
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Redundant publications: no substantial overlapping with previous papers.

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