Haematologica 1999; 84:452-460 trends in hematology

Antithrombin replacement in patients with sepsis and septic shock

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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 spetic 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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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, 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.[4,5] 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.[6]

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.[7,8] The decreased artero-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 hyperdynamic 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.[9] 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.[10] 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,[11,12] 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*[13] (ARDS). From 40% to 60% of patients with Gram-negative septic shock develop ARDS.[14] 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 hypoalbuminemia. 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 aminoacids frequently accompany this *obtundation of sepsis*.[15] 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 supports, the eradication of micro-organisms, the neutralization of microbial toxins, and the modulation of host mediators.[16]

Intensive life supports to maintain vital functions involve careful monitoring of patients in a critical care unit setting. Metabolic derangements (electrolyte disturbances, acidosis) should be aggressively corrected, as they can worsen the hemodynamic abnormalities of septic shock. The hematocrit should be maintained above 30% to improve the oxygen-carrying capacity. Respiratory failure requires mechanical ventilation.

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 with a Swan-Ganz catheter, and laboratory monitoring of the metabolic profile.

All patients with severe sepsis and septic shock have moderate to profound intra-vascular hypovolemia due to vasodilatation and loss of fluids in the extravascular spaces. The type and amount of fluid (crystalloids, colloids, and albumin) are highly controversial. When the mean arterial pressure is less than 60 mmHg, volume resuscitation is the initial treatment of choice, to avoid limiting coronary and cerebral artery autoregulation and to prevent inadequate tissue perfusion. Fluids should be infused rapidly to maximize ventricular performance. In general, this can be obtained at a pulmonary capillary wedge pressure of 12 to 15 mm Hg. Patients with higher wedge pressures carry a substantial risk of developing pulmonary edema. If, in spite of volume resuscitation, the mean arterial pressure remains below 60 mmHg when the pulmonary capillary wedge pressure is above 15 mmHg, inotropic agents used singly or in combination may offset the myocardial dysfunction and augment cardiac output. No universal agreement exists as to how these agents should be utilized, in view of their different effects on cardiac stimulation, vasoconstriction and vasodilatation (Table 1). Dopamine is commonly employed in this setting because of the β-adrenergic effects enhancing cardiac performance and the α-adrenergic effects supporting arterial blood pressure. The potent vasoconstrictor effects of norepinephrine are advantageous in septic shock patients who are unresponsive to high doses of dopamine. Dobutamine may be used alone or in combination with other catecholamines to improve cardiac performance.

Eradication of micro-organisms requires early antibiotic administration. This is initially empirical, using broad-spectrum antibiotics against Gram-positive and Gram-negative bacteria and sometimes against fungi. Cultures of body fluids are helpful in the identification of the micro-organisms involved, but radiological investigations may be required to discover the site of infection. Specific foci of infection should be drained and necrotic tissue surgically removed when appropriate.

Septic shock may, however, present with no identifiable source of infection and with negative blood

Table 1. Vasopressor therapy in septic shock.

Inotropic agent	*Cardiac stimulation (β-1)*	*Vaso-constriction (α-1)*	*Vaso-dilatation (β-2)*
Dopamine 5-10 µg/kg/min	++	+	++
↓			
Dopamine 10-20 µg/kg/min	+++	+++	+
↓			
Norepinephrine 0.02-0.2 µg/kg/min	+++	++++	0
+ Dopamine 2-4 µg/kg/min	++	+	++
↓			
Norepinephrine 0.02-0.2 µg/kg/min			
+ Dopamine 2-4 µg/kg/min	++	+	++
+ Dobutamine 5-10 µg/kg/min	++++	+	++

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.[17] 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.[3] 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,[18-19]), others only in animal models (monoclonal anti-TNF antibodies,[20-22]). 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[23] nor of active site-blocked factor Xa[24] 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.[25] In contrast, infusion of natural inhibitors of blood coagulation (antithrombin, protein C, tissue factor pathway inhibitor), all

Table 2. Mortality rate of consecutive patients admitted to Italian intensive care units: results of the Italian SEPSIS Study.

ACCP/SCCM diagnosis on admission:		*Nil*	*SIRS*	*Sepsis*	*Severe sepsis*	*Septic shock*
Patients	n.	421	573	50	23	33
	(%)	38.3	52.1	4.5	2.1	3.0
Mortality rate	(%)	24.0	26.5	36.0	52.2	81.8

Table 3. Novel therapeutic approaches in septic shock.

Neutralization of microbial toxins	*Modulation of host mediators*
Anti-endotoxin antibodies:	Anti-TNF antibodies (TNF MoAb)
Polyclonal antibodies (*E.coli* J5 antiserum, antibodies to Lipid A)	Interleukin-1 receptor antagonists
Monoclonal antibodies (HA-1A, E5)	Anti-C5a antibodies
Lipid A analogs (lipid X, monophosphoryl lipid A)	Eicosanoid inhibitors
	Antioxidants
Cationic polypeptide antibiotics	Corticosteroids
	PAF antagonists
Plasma detoxification (plasmapheresis, continuous artero-venous hemofiltration)	Inhibitors of coagulation (antithrombin, protein C)

resulted in a significant survival advantage in animals receiving lethal doses of *E. coli*.[26-29]

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).[30] 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.[31-34] 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.[35-39] 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.[34] 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.[40] However, in an Italian study of patients with an established diagnosis of DIFF, the administration of AT concentrates did not result in any significant survival advantage.[41] 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.[42] 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.[43] Similar results were obtained by Lamy *et al.*[44] (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.[47] 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 1 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/10 kg body weight if the platelet count was $< 50 \times 10^9$/L.

The simplified acute physiologic score (SAPS)[48] was recorded in each patient at admission; a modified multi-organ failure (MOF) score,[47,49] 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.[48] 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.

Author (ref.)	*No. of patients*	*Mortality rate*		*Odds Ratio*	*95% C.I.*
		Standard treatment	*AT replacement*		
Blauhut *et al.*, 1985[34]	51	12%	15%	1.29	0.18-42.9
GISACID, 1990[41]	41	23%	29%	1.33	0.24-∞
Harper *et al.*,1991[45]	50	32%	32%	1.00	0.26-3.85
Albert *et al.*, 1992[46]	33	31%	25%	0.73	0.11-4.40
Fourrier *et al.*, 1993[43]*	32	50%	28%	0.40	0.06-2.19
Lamy *et al.*, 1996[44]*	42	41%	25%	0.48	0.10-2.16

**Double blind studies.*

MOF score (AT arm: 5.6±2.5; placebo arm 4.8±2.3, p = 0.08). As a result, more patients in the AT group required hemodynamic support (53 in the AT arm versus 42 in the placebo arm, p = 0.04). Forty-nine patients in the AT group and 51 patients in the placebo group had sepsis. The infectious agents were identified in 93 patients by blood, urine and bronchoaspirate cultures and were similarly distributed in the two treatment arms (46 Gram-positive: *S. Aureus, S. Epidermidis*; 44 Gram-negative, *P. Aeruginosa, Serratia, Actinobacter, Enterobacter, K. Pneumoniae, E. Coli*; 34 fungi: *C. Albicans, Aspergillus*; *cytomegalovirus* 1). In patients 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 an 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.[50] 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).

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

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.
**For unitary increase in % antithrombin activity.*

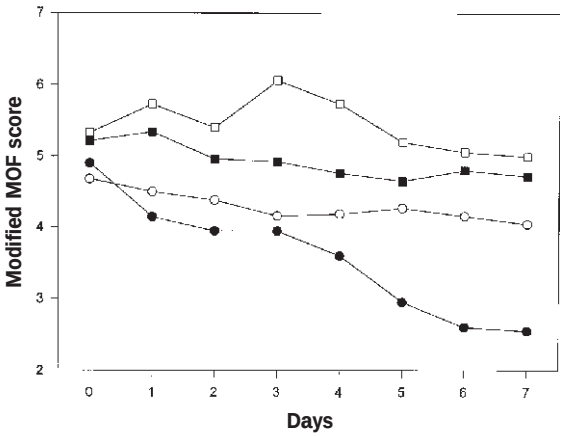

Figure 1. Adjusted changes in the modified MOF score after stratification of patients for the presence (squares) or absence (circles) of septic shock. Time (p=0.0001), presence of septic shock (p=0.002) and treatment allocation (AT replacement, closed symbols, versus placebo, open symbols, p=0.05) exerted independent significant effects on the modified MOF score.

In the entire population of patients, the MOF score, adjusted for its baseline value showed a significant change with time (p<0.001), but not with treatment (p=0.26). The differences observed in mortality rates and in the effect of treatment in patients with or without septic shock led to a revaluation of the changes in the MOF score during the first week of patients' observation after stratification for the presence or the absence of septic shock. This analysis showed an independent effect of AT replacement in improving the MOF score both in patients with and without septic shock (p=0.05, Figure 1).

In spite of the observation of a favorable effect of AT replacement therapy on 30-day survival, this effect was no longer significant when considering the overall survival not truncated at day 30. The presence of septic shock (OR = 3.42, p<0.001) and the baseline AT levels (OR = 0.98, p<0.05) were the only variables associated with overall survival, which was, however, also significantly different in the three centers participating in the study.

Conclusions and future perspectives

Patients admitted to intensive care units because of the requirement for hemodynamic and/or respiratory support are a highly heterogeneous group, with underlying diseases of different etiology. It would therefore be expected that addition of a single drug to the therapeutic strategy would hardly have a major effect on mortality. In spite of this limitation, a similar trend for a beneficial effect of AT treatment has been reported in small double-blind studies of critically ill patients hospitalized in intensive care units. Our findings in septic shock patients are similar to those of Fourrier *et al.*,[43] who aimed to produce and 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*.[44] 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.

Contributions and Acknowledgments

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

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

Manuscript received November 6, 1998; accepted January 26, 1999.

References

1. Parrillo JE, Parker MM, Natanson C, et al. Septic shock: advances in the understanding of pathogenesis, cardiovascular dysfunction, and therapy. Ann Intern Med 1990; 113:227-42.
2. Manship L, McMillin RD, Brown JJ. The influence of sepsis and multisystem and organ failure on mortality in the surgical intensive care unit. Am Surg 1984; 50:94-101.
3. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Chest 1992; 101: 1644-55.
4. Tilney NL, Bailey GL, Morgan AP, et al. Sequential system failure after rupture of abdominal aortic aneurysms. Ann Surg 1973; 178:117-21.
5. Baue AE. Multiple, progressive or sequential systems failure: a syndrome of the 1970s. Arch Surg 1975; 110:779-86.
6. Fahey III TJ, Tracey KJ. Cytokines, tumor necrosis factor and other mediators of sepsis. In: Carlson RW, Geheb MA, eds. Principles & Practice of Medical Intensive Care. Philadelphia, WB Saunders Company; 1993. p. 311-22.
7. Cunnion RE, Schaer GL, Parker MM, Natanson C, Parrillo JE. The coronary circulation in human septic shock. Circulation 1986; 73:637-44.
8. Dhainaut JF, Huyghebaert MF, Monsallier JF, et al. Coronary hemodynamics and myocardial metabolism of lactate, free fatty acids, glucose, and ketones in patients with septic shock. Circulation 1987; 75:533-41.
9. Marder VJ, Feinstein DI, Francis CW, Colman RW. Consumptive thrombohemorrhagic disorders. In: Colman RW, Hirsh J, Marder VJ, Salzman EW, eds. Hemostasis and thrombosis. Basic principles and clinical practice. Philadelphia, JB Lippincott Company, 1994. p. 1023-63.
10. Dixon AC, Parrillo JE. Cardiovascular dysfunction in septic shock. In: Carlson RW, Geheb MA, eds. Principles and Practice of Medical Intensive Care. Philadelphia: WB Saunders Co.; 1993. p. 323-40.
11. Lefer AM, Martin J. Origin of the myocardial depressant factor in shock. Am J Physiol 1970; 218:1423-9.
12. Parrillo JE, Burch C, Shelhamer JH, Parker MM, Natanson C, Schuette W. A circulating myocardial depressant substance in humans with septic shock. J Clin Invest 1985; 76:1539-53.
13. Hansen-Flaschen JH. Adult respiratory distress syndrome. In: Carlson RW, Geheb MA, eds. Principles and Practice of Medical Intensive Care. Philadelphia: WB Saunders Co.; 1993. p. 816-26.
14. Kaplan RL, Sahn SA, Petty TL. Incidence and outcome of the respiratory distress syndrome in gram-negative sepsis. Arch Intern Med 1979; 139:867-9.
15. Sibbald WJ, Darrah WC. Multiple organ system failure in sepsis. In: Carlson RW, Geheb MA, eds. Principles and Practice of Medical Intensive Care. Philadelphia: WB Saunders Co.; 1993. p. 340-52.
16. Solomon MA, Natanson C. Treatment of sepsis and septic shock. In: Carlson RW, Geheb MA, eds. Principles and Practice of Medical Intensive Care. Philadelphia: WB Saunders Co.; 1993. p. 365-80.
17. Salvo I, DeCian W, Musicco M, et al. The Italian SEPSIS study: preliminary results on incidence and evolution of SIRS, sepsis, severe sepsis and septic shock. Int Care Med 1995; 21:S244-9.
18. Ziegler EJ, McCutchan JA, Fierer J, et al. Treatment of gram-negative bacteremia and shock with human antiserum to a mutant *Escherichia Coli*. N Engl J Med 1982; 307:1225-30.
19. Ziegler EJ, Fisher CJ, Sprung CL, et al. Treatment of Gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin: a randomized, double blind, placebo-controlled trial. N Engl J Med 1991; 324:429-36.
20. Beutler B, Milsark IW, Cerami AC. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. Science 1985; 229:869-71.
21. Tracey KJ, Fong Y, Hesse DG, et al. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteremia. Nature 1987; 330:662-4.
22. Hinshaw LB, Tekamp-Olson P, Chang ACK, et al. Survival of primates in LD100 septic shock following therapy with antibody to tumor necrosis factor (TNFα). Circ Shock 1990; 30:279-88.
23. Coalson JJ, Benjamin B, Archer LT, et al. Prolonged shock in the baboon subjected to infusion of *E. Coli* endotoxin. Circ Shock 1978; 5:423-37.
24. Taylor FB Jr, Chang ACK, Peer GT, et al. DEGR-Factor Xa blocks disseminated intravascular coagulation initiated by *Escherichia Coli* without preventing shock or organ damage. Blood 1991; 78:364-8.
25. Warr TA, Rao VM, Rapaport SI. Disseminated intravascular coagulation in rabbits induced by administration of endotoxin or tissue factor: effect of anti-tissue factor antibodies and measurement of plasma extrinsic pathway inhibitor activity. Blood 1990; 75: 1481-9.
26. Triantaphyllopoulos DC. Effects of human antithrombin III on mortality and blood coagulation induced in rabbits by endotoxin. Thromb Haemost 1984; 51: 232-5.
27. Taylor FB Jr, Emerson TE Jr, Jordan R, Chang A, Blick KE. Antithrombin III prevents the lethal effects of *Escherichia Coli* infusion in baboons. Circ Shock 1988; 26:227-35.
28. Taylor FB Jr., Chang A, Esmon CT, D'Angelo A, Viganò D'Angelo S, Blick KE. Protein C prevents the coagulopathic and lethal effects of *E. Coli* infusion in the baboon. J Clin Invest 1987; 79:918-25.
29. Creasey AA, Chang AC, Feigen L, Wun TC, Taylor FB Jr., Hinshaw LB. Tissue factor pathway inhibitor reduces mortality from *Escherichia Coli* septic shock. J Clin Invest 1993; 91:2850-6.
30. Buller HR, Ten Cate JW. Acquired antithrombin III deficiency: laboratory diagnosis, incidence, clinical implications and treatment with antithrombin III concentrate. Am J Med 1989; 87(suppl 3B):44s-8s.
31. Blauhut.B, Necek S, Vinazzer H, Bergmann H. Substitution therapy with an antithrombin III concentrate

in shock and DIC. Thromb Res 1982; 27:271-8.
32. Hesselvik JF, Malm J, Dahlbäck B, Blomback M. Protein C, protein S and C4b-binding protein in severe infection and septic shock. Thromb Haemost 1991; 62:126-9.
33. Fourrier F, Chopin C, Goudemand J, et al. Septic shock, multi organ failure and disseminated intravascular coagulation. Compared pattern of ATIII, protein C, protein S deficiencies. Chest 1992; 101:816-23.
34. Blauhut B, Kramar H, Vinazzer H, Bergmann H. Substitution of antithrombin III in shock and DIC: a randomized study. Thromb Res 1984; 39:81-9.
35. Wilson RF, Mammen EF, Robson MC, Heggers JP, Soullier G, DePoli PA. Antithrombin, prekallikrein and fibronectin levels in surgical patients. Arch Surg 1986; 121:635-43.
36. Sandset PM, Roise O, Aasen AO, Abildgaard U. Extrinsic pathway inhibitor in post operative/ post traumatic septicemia: increased levels in fatal cases. Haemostasis 1989; 19:189-95.
37. Hesselvik JF, Blomback M, Brodin B, Maller R. Coagulation, fibrinolysis and kallikrein systems in sepsis: relation to outcome. Crit Care Med 1989; 17:724-33.
38. Lammle B, Tran TM, Ritz R, Duckert F. Plasma prekallikrein, factor XII, ATIII, C1-inhibitor and α2 macroglobulin in critically ill patients with suspected disseminated intravascular coagulation (DIC). Am J Clin Pathol 1984; 82:396-404.
39. Maki M, Terao T, Ikenoue T, et al. Clinical evaluation of antithrombin concentrate (BI 6.013) for disseminated intravascular coagulation in obstetrics. Gynecol Obstet Invest 1987; 23:230-40.
40. Vinazzer H. Therapeutic use of antithrombin III in shock and disseminated intravascular coagulation. Semin Thromb Hemost 1989; 15:347-52.
41. Palareti G, Legnani C, Ludovici S, et al. Studio preliminare per la valutazione di fattibilità di uno studio clinico policentrico per la valutazione dell'efficacia terapeutica di un concentrato di antitrombina III (Immuno) nella sindrome da coagulazione intravascolare. Haematologica 1990; 75:21-8.
42. Lechner K, Kyrle PA. Antithrombin III concentrates - Are they clinically useful? Thromb Haemost 1995; 73: 340-8.
43. Fourrier F, Chopin C, Huart JJ, Runge I, Caron C, Goudemand J. Double-blind, placebo-controlled trial of antithrombin III concentrates in septic shock with disseminated intravascular coagulation. Chest 1993; 104:882-8.
44. Lamy M, Eisele B, KeineckeH-O, Delvos U, Thijs LG. Antithrombin III in patients with severe sepsis. A randomised, placebo-controlled trial of antithrombin III concentrates in septic shock with disseminated intravascular coagulation [abstract]. 9th European Congress on Intensive Care Medicine, Glasgow, 1996. p. 385-90.
45. Harper PL, Williamson L, Park G, Smith JK, Carrell RW. A pilot study of antithrombin replacement in intensive care management: the effects on mortality, coagulation and renal function. Transfusion Med 1991; 1:121-8.
46. Albert J, Blomqvist H, Gardlund B, Jakobsson J, Svensson J, Blomback M. Effect of antithrombin concentrate on haemostatic variables in critically ill patients. Acta Anaesthesiol Scand 1992; 36:745-52.
47. Baudo F, Caimi TM, de Cataldo F, et al. Antithrombin III (AT III) replacement therapy in patients with sepsis and/or postsurgical complications: a controlled double blind, randomized multicenter study. Int Care Med 1998; 24:336-42.
48. La Gall JR, Loirat P, Alperovith A, et al. A simplified acute physiology score for ICU patients. Crit Care Med 1984; 12:975-7.
49. Goris RJA, Boekhorst TP, Nuytinck JKS, Gimbrere JSF. Multiple organ failure. Arch Surg 1985; 120:1109-15.
50. Christensen E. Multivariate survival analysis using Cox's regression model. Hepatology 1987; 7:1346-58.
51. Mesters RM, Mannucci PM, Coppola R, Keller T, Ostermann H, Kienast J. Factor VIIa and antithrombin III activity during severe sepsis and septic shock in neutropenic patients. Blood 1996; 88:881-6.
52. Smtih OP, White B, Vaughan D, et al. Use of protein-C concentrate, heparin, and haemodiafiltration in meningococcus-induced purpura fulminans. Lancet 1997; 350:1590-3.
53. Fijnvandraat K, Derkx B, Peters M, et al. Coagulation activation and tissue necrosis in meningococcal septic shock: severely reduced protein C levels predict a high mortality. Thromb Haemost 1995; 73:15-20.
54. Esmon CT, Schwarz HP. An update on clinical and basic aspects of the protein C anticoagulant pathway. Trends Cardiovasc Med 1995; 5:141-8.