SIGNA VITAE 2010; 5(1): 13 - 19

REVIEW

Human protein C concentrate in pediatric septic patients

GIOVANNI LANDONI • GIACOMO MONTI • ALBERTO FACCHINI • FRANCESCO CAMA • ELENA BIGNAMI • LUCA CABRINI• FEDERICO PAPPALARDO • ALBERTO ZANGRILLO

GIOVANNI LANDONI (✉) •
GIACOMO MONTI •
ALBERTO FACCHINI •
FRANCESCO CAMA •
ELENA BIGNAMI•
LUCA CABRINI•
FEDERICO PAPPALARDO •
ALBERTO ZANGRILLO
Department of Anesthesia
and Intensive Care
Università Vita-Salute San Raffaele Milano
V. Olgettina 60, Milano
Phone: +39 0228433737
Fax: +39 0226432200
E-mail: landoni.giovanni@hsr.it

ABSTRACT

Severe sepsis and septic shock are leading causes of morbidity and mortality in the pediatric population. Unlike what is suggested for the adult population, recombinant human activated protein C (rhAPC) is contraindicated in children. Long before rhAPC was considered for use in pediatric patients, case reports appeared on the safe administration of protein C zymogen. Therefore, we conducted a systemic review of currently available data on protein C zymogen (PC) use among children affected by severe sepsis or septic shock.

A total number of 13 case series or case reports and a dose-finding study were found on the use of PC in the pediatric intensive care unit, reporting on 118 treated children, with an overall survival of 84%. There was no bleeding complication, the only reported complication being a single mild allergic reaction.

These studies show that PC is safe, not associated with bleeding and possibly useful for improving coagulation abnormalities of sepsis.

Key words: sepsis, pediatric, protein C, drug therapy, review

Severe sepsis and septic shock are leading causes of morbidity and mortality in the pediatric population. A large epidemiological study estimated 0.56 cases per 1000 children, or 42,364 cases per year in the United States. (1) Infants have the highest incidence (5.16 per 1,000) and sepsis is the 4th cause of death; children are less affected (5.16 vs 0.20 per 1000), but sepsis is the 2nd cause of death in this age group. The overall hospital mortality rate in this study was 10.3%. (2)

In Italy, sepsis, severe sepsis and septic shock represent 7.9%, 1.6% and 2.1% of pediatric intensive care unit (PICU) admissions. Mortality for septic shock is 62.5%. (3) Sepsis in the pediatric population is a major challenge and an area of paramount importance. The Surviving Sepsis Campaign guidelines of 2004 and 2008 includes a section named "pediatric consideration". (4) Unlike what is suggested for the adult population, recombinant human activated protein C (rhAPC) is contraindicated in children with a moderate evidence level.

Given the beneficial effects of rhAPC in adult septic patients, although much controversy exists on this subject, (5) a multicentric phase III trial was undertaken to assess rhAPC effectiveness in pediatric patients, the RESOLVE study. (6) That trial was stopped prematurely for futility: 477 patients were randomized to receive either rhAPC or placebo. There was no difference in the primary endpoint, Composite Time to Complete Organ Failure Resolution (CTCOFR) and in the secondary endpoints, i.e., 28-day mortality and major amputations. There were numerically more (although not statistically significant) central nervous system (CNS) bleeding events in the rhAPC group during infusion (5 vs 1, p=0.10) and the 28-day period (11 vs 5, p=0.13). There were significantly more study-drug-related serious adverse events and serious bleeding events during the infusion and 28-day periods in the rhAPC group.

Long before rhAPC was considered for use in pediatric patients, case reports

appeared on the safe administration of protein C zymogen (PC) in septic children with congenital or acquired PC deficiency. (7)

In our paper, we conducted a systemic review of currently available data on PC use in the literature, updated to January 2009.

Protein C and synonymous terms were used to search the literature using different Medical Databases (PubMed, Embase, Google Scholar) and the 'snowballing' technique was applied. Experts in this field were also contacted to retrieve further published papers on PC. No language restriction was applied. Papers were included in this review if at least one patient less than 18 years of age was treated with PC.

A total number of 13 case reports (7-19) and a dose-finding study (20) were found on the use of PC in the PICU, as shown in table 1. In 118 treated children, the only complication reported was a mild allergic reaction (11) and no bleeding complications occurred.

The first study was published by Gerson et al. in 1993 (7) and reported on a case of purpura fulminans in a 13-year-old boy. The patient was administered PC in addition to fresh frozen plasma, to avoid fluid overload and to restore plasma PC activity that was markedly reduced at baseline (20-50%), to an arbitrary level >100% (100-200%). The child eventually survived, though he required amputation of 3 toes and surgical debridement of pelvic osteonecrosis. No complications were reported during or after PC use.

In 1995 Rivard et al. (8) described 4 cases of meningococcal purpura fulminans treated with PC (age 3 months-15 years). All patients survived. They obtained normalization of PC plasma activity, increase of fibrinogen and decrease of D-dimer levels, data implying activation of PC to activated PC. Two patients, who were treated at a relatively later stage than the other two, required amputations, leading the authors to speculate in favor of prompt administration of the drug, when needed. Again, no adverse effects were observed. Patients received 100 units/kg body weight infused over 15-20 minutes, every 6-8 hours. This was continued until skin lesions ceased to develop and/or coagulation tests were stable.

Smith et al. in 1997 (9) reported observed and expected mortality in 12 patients with meningococcal purpura fulminans. No patient died, in spite of an expected mortality from the Glasgow meningococcal septicaemia prognostic score (21) and the pediatric risk of mortality score of 80% and 57%, respectively. PC was administered within 18 hours of admission in 10 out of 12 patients. No one experienced adverse reactions to the drug. Two children required amputations, of whom one had a thrombotic cerebrovascular accident with complete recovery. Unfractionated heparin was used in 11 patients and antithrombin in one.

Ettingshausen et al. in 1999 (10) treated 8 patients with meningococcal purpura fulminans who had severe acquired PC deficiency on hospital admission. Improvement or even normalization of global hemostatic parameters was achieved in all patients. Markedly elevated plasminogen activator inhibitor - 1 (PAI-1) levels prior to treatment, reflecting a reduced fibrinolytic potential, decreased rapidly under PC substitution. Concomitantly, improving signs of purpura fulminans reflected by decreasing size of skin lesions, demonstrated a restoring microcirculation. Six of the eight patients survived. One patient required limb amputation; two patients, with severly diminished PC levels at admission, died because of multiorgan failure. No adverse effects were observed with the PC concentrate administration.

In a case described by Clarke et al. in 2000, (11) the authors observed normalization of prothrombin time within 3 days from the first dose of PC, reduction of activated partial thromboplastin time to slightly supranormal levels and reduction of fibrinogen to normal values. The child received 100 units/kg body weight, intravenously, eight-hourly in addition to fresh frozen plasma 300 mL every 8-12 hours. His recovery was surprisingly rapid and without sequelae. At the second administration of PC, the patients experienced a blotchy, transient erythematous rash without associated cardiovascular instability. To the best of our knowledge, this is the only side effect reported and associated with PC use in children or adults, among all the available published literature.

In 2003, the only randomized controlled trial on PC use among the pediatric population was published by de Klein. (20) That was a well designed, phase 2, dose finding study in meningococcal septic shock. Children were randomized to receive 0, 200, 400 or 600 IU/kg/day PC in a 4 times-a-day schedule. As alredy noted by other authors, activation of PC to activated PC (APC) was achieved. That result was significantly faster with increasing dosages of PC concentrate. The study was not adequately powered to detect a difference in mortality as an outcome. Nonetheless, expected mortality (from PRISM score) was higher than actual mortality (40 vs 23%) in the treatment (all dosage) group. De Kleijn et al. demonstrated also that PC concentration in blood is dependent on the zymogen dose administered, that supplementation in a 4-shots-a-day schedule rose and maintained increased concentrations between doses. Importantly, authors showed that with a 200 IU/Kg/day, PC concentrations were restored to normal value, higher doses raising them to supra-normal levels. De Kleijn demonstrated also that PC is effectively activated after administration, and that also activation is dose-dependent.

In 2003 Fourrier et al. (14) examined the pharmacokinetic properties of combined PC and antithrombin (AT) supplementation in severe purpura fulminans, to find substantial correction of both values in the patients' plasma, that peaked at 18 and 48 hours after drug administration, respectively. They used 100 IU/kg AT as a starting bolus and 100-150 IU/kg/day for 4 days, and 100 IU/kg PC concentrate as a starting bolus plus 100 IU/kg/day in adults and 100 IU/kg every 6 hours in children. AT and PC activities at 18 hours and 24 hours,

Table 1. Summary of published papers on the use of protein C concentrate in children affected by severe sepsis or septic shock, updated January 2009.

Author	Journal	N	Setting	Survival (n, %)	Predicted survival (%)	Bolus dose	Following doses, length of treatment
Gerson	Pediatrics	1	Meningococcal purpura fulminans	1 (100%)	Not reported	70 IU/kg	70 IU/kg every 6 h than 10 IU/kg/h perfusion
Rivard	J Pediatr	4	Meningococcal septic shock	4 (100%)	Not reported	100 IU/kg	100 IU/kg every 6 h
Smith	Lancet	12 (2 adults)	Meningococcal septic shock	12 (100%)	GMSPS: 20% PRISM: 43%	100 IU/kg	15 IU/kg/h adjusted according plasma levels
Ettingshausen	Semin Thromb Hemost	8	Meningococcal septic shock	6 (75%)		80-120 IU/kg	50 IU/kg every 6 h
Clarke	Intensive Care Med	1	Meningoccal severe sepsis	1 (100%)	Not reported	100 IU/kg	100 IU/kg every 8 h
Leclerc	Crit Care Med	1	Meningococcal septic shock	1 (100%)	Not reported	100 IU/kg/day	Not reported
White	Blood	36	Septic shock and presumptive meningococcemia	33 (92%)	GMSPS: 50%	100 IU/kg	10 IU/kg/h according to plasma levels
De Kleijn	Crit Care Med	30	Severe sepsis or meningococcal disease	23 (76%)	PRISM: 60%	50-150 IU/kg	50-150 IU/kg every 6 hours
Fourrier	Intensive Care Med	≥10	Meningococcal purpura fulminans	6 (60%)	PRISM: 31%	100 IU/kg	100 IU/kg every 6 h
Pettenazzo	Minerva Anestesiol	8	Septic shock	6 (75%)	PRISM: 56%	100 IU/kg	80-100 IU/Kg every 6-8 h, according to plasma levels
Silvani	Minerva Anestesiol	11	Severe sepsis or septic shock	8 (73%)	Not reported	Mean dose: 324 IU/kg/day	
De Carolis	Turk J Pediatr	1	Septic shock	1 (100%)	Not reported	100 IU/kg	50 IU/kg every 6 h
Schellongowski	Vox Sang	≥1	Severe sepsis or septic shock	6 (75%)	SAPS II: 79%	10 IU/Kg test dose; than 100 IU/kg	10 IU/kg/h or 100 IU/kg every 6 h
Ma kris	J Thromb Haemost	1	Severe sepsis	1 (100%)	Not reported	80-90 IU/kg	80-90 IU/kg every 6 h
TOTAL		>118		84%			

GMSPS, Glasgow Meningococcal Septicaemia Prognostic Score; PRISM, Pediatric Risk of Mortality Score; SAPS II, Simplified Acute Physiology Score II.

respectively, were significantly higher in survivors than in non-survivors. They estimated an apparent half-life of clearance from plasma of 16 hours for AT and 6 hours for PC. According to these observations, they hypothesized that, in order to correct AT and PC plasma concentrations, regimens of 200 IU/kg loading dose plus 150 IU/kg/day, and 250 IU/kg loading dose plus 200 IU/kg/day are to be reached, respectively. The mortality rate in that study was 60%, mainly due to refractory shock.

In 2004 Pettenazzo et al. (15) published their experience in managing 8 pediatric patients (aged 1 month-7 years) with acquired PC deficiency. PC concentrate was administered as a 100 IU/kg loading dose plus 80-100 IU/kg every 6-12 hours depending on the plasma levels they achieved. They reported a 75% survival rate with prompt response to therapeutic efforts. Two patients died of Enterovirus myocarditis and acute myeloblastic leukemia; blood cultures yielded P. aeruginosa in both cases. No drug-related adverse events were reported.

In 2005 Silvani et al. (16) retrospectively examined data from 29 patients from 3 PICUs during one year. 11 (38%) patients received PC concentrate and experienced improvement in plasma PC activity, prothrombin time (PT), partial thromboplastin time (PTT) and fibrinogen, with a favourable, although nonsignificant, trend toward normalization of D-dimer, AT and platelet counts. Mean dose was 324 IU/kg/day (range 66-400). No difference in mortality was evident between treated and untreated patients. Again, no drug-related adverse event was seen. Notably, in this paper, just 4 of 11 treated patients were affected by N meningitidis, whereas, the other 5 were affected by other pathogens. In 2 cases the pathogen was unknown.

In 2008, De Carolis et al. (17) reported a case of severe sepsis in a 28-day premature neonate with grade II intraventricular hemorrhage, caused by Serratia marcescens, successfully treated with PC concentrate (100 IU/kg plus 50 IU/kg every 6 hours for 4 days). Twenty-four hours after drug administration the baby's condition promptly improved, with rapid (48 hours) weaning from dopamine, rapid resolution of acute renal failure, and extubation by day 7. Skin lesions slowly improved during PC use. No evidence of bleeding or worsening of intraventricular hemorrhage, as assessed by transcranial doppler ultrasound, was reported.

Initial reports on adult patients receiving PC concentrate recently appeared in the literature. (22, 23)

In infection, the inflammatory mediators released by the immune system cells upon pathogen recognition induce endothelial cell expression of procoagulant and anti-fibrinolytic molecules, such as tissue factor and plasminogen activator inhibitor (PAI)-1. (24) This is aimed at preventing pathogen spread beyond the infectious focus through the systemic circulation by means of microvascular occlusion by thrombosis, and inhibition of clot lysis by hampering plasmin generation. This local response is profoundly deranged in sepsis, where an overwhelming immune response to the pathogen(s) leads to a massive systemic release of cytokines such as interleukin (IL)-1b, IL-6, and tumor necrosis factor (TNF)-a. (25) These, in turn, induce endothelial recruitment to a proinflammatory and procoagulant state on a systemic scale. (26) Thus, thrombus formation occurs as a widespread process all over the microcirculation, potentially resulting in disseminated intravascular coagulation (DIC), with ensuing bleeding due to consumptive coagulopathy. This process is key to organ damage, in addition to systemic hypoperfusion due to refractory vasodilation and decreased cardiac contractility.

Thrombin generation by the procoagulant endothelium effectively enhances inflammation by cleavage of protease-activated receptor (PAR)-1 and induction of downstream gene expression through NF-kB (nuclear factor – kappaB). Thereby, the procoagulant and proinflammatory responses intertwine and promote each other in a self-perpetuating vicious circle. (27) The PC pathway has emerged as an intriguing and effective target of therapy, in that it directly acts as a natural anticoagulant and profibrinolytic by preventing and reversing microvascular thrombus formation, and as an anti-inflammatory and cytoprotective mediator, through interaction with membrane-bound PAR-1. Recently, also a relashionship of PC pathway and strict glicemic control has been supposed. (28, 29)

PC is a vitamin K-dependent plasma protein that circulates in its inactive (zymogen) form and is readily converted to APC by thrombin upon binding to thrombomodulin, a transmembrane protein. Thrombin:thrombomodulin complex cleaves endothelial PC receptor (EPCR)-bound PC to its active form. APC, a serine protease, inactivates coagulation factors VIIIa and Va, thereby preventing assembly of the tenase and prothrombinase complexes, respectively, and thus blocks the coagulation cascade. On the other hand, APC cleaves PAR-1 (in a different site than thrombin) so as to convey endothelial and immune system cells anti-inflammatory and cytoprotective signals. (30) Moreover, the protein C pathway attenuates the neutrophil chemotactic response to proinflammatory cytokines. (31)

Generation of APC does not seem to be the only mechanism involved in the PC pathway: in vitro studies have shown that EPCR-bound zymogen PC induces PAR-1 cleavage by thrombin so as to generate an anti-inflammatory signal. Therefore, EPCR appeared to switch the signaling specificity of thrombin from a cytotoxic and proinflammatory to a protective effect in cultured endothelial cells. (32) This seems to agree with animal studies that show reduced mortality from lipopolysaccharide (LPS) induced endotoxemia and enhanced survival in experimental models of peritoneal sepsis by means of a recombinant APC variant with intact signalling properties on PAR-1, but $< 10\%$ anticoagulant activity. This might allow use of higher doses of those APC variants, with beneficial effects on inflammation and markedly reduced hemorrhagic risk. (33, 34)

Such observations would suggest that PC/APC anticoagulant and anti-inflammatory activities are distinct, at least in vitro and in animal models of sepsis. The extent to which this is true in clinical practice is unknown. However, it seems reasonable that some anticoagulant and profibrinolytic activity is useful to promote microvascular clot lysis and organ reperfusion.

PC levels reach the lower limit of normal adult levels (60-70%) around 6-12 months of age, (35) thereby increasing the risk of coagulopathy in the pediatric septic population. Moreover, in some studies involving adults, deficiency of PC caused by consumptive coagulopathy is more marked than deficiency of other hemostatic factors, such as antithrombin. According to many recent studies, protein C levels are frequently decreased in sepsis, probably due to increased conversion to APC, whose short plasma half-live (≈20 minutes in vivo) induces depletion of its precursor levels in the attempt to achieve sustained plasma activities; decreased liver synthesis; degradation by neutrophil elastases; and formation of EPCR:APC complexes. Protein C levels inversely correlate with morbidity and mortality outcome of septic patients, regardless of age, infecting microorganisms, presence of shock, DIC, degree of hypercoagulation, or severity of illness. (36)

Taken together, these considerations suggest a strong correlation between PC pathway and survival from severe sepsis/septic shock, and reinforce the rationale for the attempts to normalize plasma activity of PC to improve survival, hamper coagulopathy, and modulate inflammation.

One of the limitations to PC use both in pediatric and adult septic patients comes from a study by Faust et al. (37) who collected skin biopsies from patients with meningococcal purpura fulminans and found, within areas of intact endothelium, reduced immunohistochemical staining for EPCR and thrombomodulin, thus suggesting reduced ability of the septic endothelium to activate PC to APC. Some considerations, however, should be made: 1) immunohistochemistry might not be the most suitable method for assessing membrane expression of surface proteins, since oxygen radicals and elastases produced by immune system cells damage those moluceles and induce conformational changes that can prevent their binding to the stains; 2) APC levels in the plasma were not measured, so no definitive demonstration of impaired activation of PC in sepsis was provided.

On the contrary, a more recent study by Liaw et al. (38) showed significant variability in their capacity to activate PC among adult septic patients, as reflected by different baseline plasma APC values. Upon hospital admission, APC was measured in the plasma of 32 septic patients by means of a monoclonal antibody specifically binding APC and not PC. APC levels were associated with the outcome: survivors had significantly higher APC values than nonsurvivors, although overlap exists between the two groups, indicating that other pathways beside PC may be involved in influencing survival from the sepsis syndrome. APC, however, is generated on demand where required, in sites of major thrombin activation. APC could be produced where and when needed, exert its protective effects on inflammation and coagulation, and then be rapidly consumed in this process. It is unlikely that undetectable plasma levels reflect the inappropriateness of our measurement techniques to explore what actually happens in the microcirculation. In fact, Liaw et al. showed that the monoclonal antibody deployed for such scope can detect both therapeutic (i.e., following administration of rhAPC) and physiologic (i.e., endogenous) concentrations of APC within 1.5 to 19 hours, inversely depending on plasma APC levels.

If the distinct existence of two groups of septic patients were to be validated on a larger scale, they could indicate that patient having endothelial dysfunction, as appears from impaired ability to generate APC, are at higher risk of death. Baseline plasma APC activity, beside PC levels, as previously reported, might be inversely related to survival. Moreover, identification of an "activator" versus "non-activator" phenotype in relation to the ability to generate APC might indicate the best treatment option for the patient: "activators" might benefit from PCz administration, whereas "non-activators" should perhaps be administered rhAPC.

Still another aspect may be taken into account: in the already cited study by Bae et al. (32) a complementary mechanism of PAR-1 recruitment by PC to an antiapoptotic, anti-inflammatory, and cytoprotective signalling is described. It appears that, at least in vitro, the protective effects of PAR-1 are mediated by EPCR binding by either APC or PC zymogen, thus suggesting that the anti-inflammatory properties of the PC pathway may not necessarily require APC formation.

Conclusion

Evidence has accumulated on the clinical relevance of the PC pathway in modulating overwhelming inflammation and preventing coagulation derangements, two key mediators of organ damage, and thus of mortality and morbidity, in sepsis.

The RESOLVE trial (6) on the use of rhAPC in septic children has yielded negative results, indicating that rhAPC is of no benefit in the pediatric population and is indeed associated with increased serious adverse events and bleeding complications.

On the contrary, PC use has long been attempted in sepsis-induced coagulopathy, especially in the context of purpura fulminans, and an improvement of coagulation parameters and of survival has been described in case reports. Its use was sometimes reported to be associated with improved survival compared to expected figures extrapolated from validated indices. To date, no serious adverse event related to PC use was described, except for a mild, transient cutaneous allergic reaction in a 17-year-old male. (11)

The experience collected through these studies shows that PC is safe, in that it is

not associated with bleeding or severe allergic complications, and possibly useful, at least to improve the coagulation abnormalities brought about by sepsis. Unfortunately, however, all we know comes from case series or case reports or an underpowered randomized controlled study. A randomized clinical trial, adequatly powered for mortality or clinically relevant outcome, is necessary to confirm PC efficacy in the pediatric population.

REFERENCES

1. Watson RS, Carcillo JA. Scope and epidemiology of pediatric sepsis. Pediatr Crit Care Med 2005;6:3-5.
2. Watson RS, Carcillo JA, Linde-Zwirble WT, Clermont G, Lidicker J, Angus DC. The epidemiology of severe sepsis in children in the United States. Am J Respir Crit Care Med 2003; 167:695-701.
3. Wolfler A, Silvani P, Musicco M, Antonelli M, Salvo I. (Italian Pediatric Sepsis Study (SISPe) group) Incidence of and mortality due to sepsis, severe sepsis and septic shock in Italian Pediatric Intensive Care Units: a prospective national survey . Intensive Care Med 2008;34:1690-7.
4. Dellinger R, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, et al. For the International Surviving Sepsis Campaign Guidelines Committee Surviving Sepsis Campaign: International guidelines for management of severe sepsis and septic shock: 2008. Crit Care Med 2008;36:296-327.
5. Finfer S, Ranieri VM, Thompson BT, Barie PS, Dhainaut JF, Douglas IS, et al. Design, conduct, analysis and reporting of a multi-national placebo-controlled trial of activated protein C for persistent septic shock. Intensive Care Med 2008;34:1935-47.
6. Nadel S, Goldstein B, Williams MD, Dalton H, Peters M, Macias WL, et al. REsearching severe Sepsis and Organ dysfunction in children: a gLobal perspective (RESOLVE) study group. Drotrecogin alfa (activated) in children with severe sepsis: a multicentre phase III randomised controlled trial. Lancet 2007;369:836–43.
7. Gerson WT, Dickerman JD, Bovill EG, Golden E. Severe acquired protein C deficiency in purpura fulminans associated with disseminated intravascular coagulation: treatment with protein C concentrate. Pediatrics 1993;91:418-22.
8. Rivard GE, David M, Farrell C, Schwarz HP. Treatment of purpura fulminans in meningococcemia with protein C concentrate. J Pediatr 1995;126:646-52.
9. Smith OP, White B, Vaughan D, Rafferty M, Claffey L, Lyons B, et al. Use of protein-C concentrate, heparin, and haemodiafiltration in meningococcus-induced purpura fulminans. Lancet 1997;29:1590-3.
10. Ettingshausen CE, Veldmann A, Beeg T, Schneider W, Jäger G, Kreuz W. Replacement therapy with protein C concentrate in infants and adolescents with meningococcal sepsis and purpura fulminans. Semin Thromb Hemost 1999;25:537-41.
11. Clarke RC, Johnston JR, Mayne EE. Meningococcal septicaemia: treatment with protein C concentrate. Intensive Care Med 2000;26:471-3.
12. Leclerc F, Cremer R, Leteurtre S, Martinot A, Fourier C. Protein C concentrate and recombinant tissue plasminogen activator in meningococcal septic shock. Crit Care Med 2000;28:1694-7.
13. White B, Livingstone W, Murphy C, Hodgson A, Rafferty M, Smith OP. An open-label study of the role of adjuvant hemostatic support with protein C replacement therapy in purpura fulminans-associated meningococcemia. Blood 2000;96:3719-24.
14. Fourrier F, Leclerc F, Aidan K, Sadik A, Jourdain M, Tournoys A, et al. Combined antithrombin and protein C supplementation in meningococcal purpura fulminans: a pharmacokinetic study. Intensive Care Med 2003;29:1081-7.
15. Pettenazzo A, Malusa T. Use of protein C concentrate in critical conditions: clinical experience in pediatric patients with sepsis. Minerva Anestesiol 2004;70:357-63.
16. Silvani P, Camporesi A, Licari E, Wolfler A. Use of protein C concentrate in pediatric patients with sepsis. Minerva Anestesiol 2005;71:373-8.
17. De Carolis MP, Polimeni V, Papacci P, Lacerenza S, Romagnoli C. Severe sepsis in a premature neonate: protein C replacement therapy. Turk J Pediatr 2008;50:405-8.
18. Makris PE, Girtovitis F, Papadopoulos A, Tamioulaki A. Treatment of DIC: the role of PC. J Thromb Haemost 2003;1S1:P0600.
19. Schellongowski P, Bauer E, Holzinger U, Staudinger T, Frass M, Laczika K, et al. Treatment of adult patients with sepsis-induced coagulopathy and purpura fulminans using a plasma-derived protein C concentrate (Ceprotin®). Vox Sanguinis 2006;90:294-301.
20. de Kleijn ED, de Groot R, Hack CE, Mulder PG, Engl W, Moritz B, Joosten KF, et al. Activation of protein C following infusion of protein C concentrate in children with severe meningococcal sepsis and purpura fulminans: a randomized, double-blinded, placebo-controlled, dose-finding study. Crit Care Med 2003;31:1839-47.
21. Evans SF, Johnson P, Garewal DS, Khan ZP. Glasgow meningococcal septicaemia prognostic score. Crit Care Med 1992;20:439.
22. Landoni G, Crivellari M, Monti G, Gerli C. Human protein C concentrates in adult septic patients. Signa Vitae 2008;3:12-16,301.

23. Crivellari M, Della Valle P, Landoni G, Pappalardo F, Gerli C, Bignami E, et al. Human protein C zymogen concentrate in patients with severe sepsis and multiple organ failure after adult cardiac surgery. Intensive Care Med 2009 Aug 1. ŠEpub ahead of printĆ
24. Russell JA. Management of sepsis. N Engl J Med 2006;355:1699-713.
25. Cohen J. The immunopathogenesis of sepsis. Nature 2002;420:885-91.
26. Esmon CT. Crosstalk between inflammation and thrombosis. Maturitas 2004; 47:305–14.
27. Bae JS, Yang L, Rezaie AR. Receptors of the protein C activation and activated protein C signaling pathways are colocalized in lipid rafts of endothelial cells. Proc Natl Acad Sci U S A 2007;104:2867-72.
28. Polli F, Savioli M, Cugno M, Taccone P, Bellani G, Spanu P, et al. Effects of recombinant human activated protein C on the fibrinolytic system of patients undergoing conventional or tight glycemic control. Minerva Anestesiol 2009;75:417-26.
29. Schultz MJ, Levi M. Recombinant human activated protein C and strict glycemic control in sepsis: mutually exclusive strategies? Minerva Anestesiol 2009;75:415-6.
30. Yan SB, Dhainaut JF. Activated protein C versus protein C in severe sepsis. Crit Care Med 2001;29:S69-74.
31. Esmon CT. Protein C anticoagulant pathway and its role in controlling microvascular thrombosis and inflammation. Crit Care Med 2001;29:S48-51.
32. Bae JS, Rezaie AR. Protease activated receptor 1 (PAR-1) activation by thrombin is protective in human pulmonary artery endothelial cells if endothelial protein C receptor is occupied by its natural ligand. Thromb Haemost 2008;100:101-9.
33. Weiler H, Ruf W. Activated protein C in sepsis: the promise of nonanticoagulant activated protein C. Curr Opin Hematol 2008;15:487-93.
34. Kerschen EJ, Fernandez JA, Cooley BC, Yang XV, Sood R, Mosnier LO, et al. Endotoxemia and sepsis mortality reduction by non-anticoagulant activated protein C. J Exp Med 2007;204:2439-48.
35. Andrew M, Paes B, Johnston M. Development of the hemostatic system in the neonate and young infant. Am J Pediatr Hematol Oncol 1990;12:95-104.
36. Fisher CJ Jr, Yan SB. Protein C levels as a prognostic indicator of outcome in sepsis and related diseases. Crit Care Med 2000;28:S49-56.
37. Faust SN, Levin M, Harrison OB, Goldin RD, Lockhart MS, Kondaveeti S, et al. Dysfunction of endothelial protein C activation in severe meningococcal sepsis. N Engl J Med 2001;345:408-16.
38. Liaw PC, Esmon CT, Kahnamoui K, Schmidt S, Kahnamoui S, Ferrell G, et al. Patients with severe sepsis vary markedly in their ability to generate activated protein C. Blood 2004;104:3958-64.