

Non activated protein C supplementation in septic pediatric hematological patients

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Abstract

The purpose of the study was to examine safety and efficacy of non-activated Protein C (PC) supplementation in our cohort of septic pediatric hematological patients. We conducted a retrospective study of 22 septic patients receiving human plasma-derived PC concentrate from 2008 to 2015 at our Pediatric Oncology Center (Bari, Italy). The *Surviving sepsis campaign* definitions for sepsis, severe sepsis and septic shock were used to define the patients' septic status. For each patient, we calculated Lansky performance status scale (LPSS) and a risk score defined the *Hematologic risk score* (HRS) that we created in 2007. Patients were defined as *High risk for severe sepsis/septic shock* in case of $HRS > 3$. $HRS < 3$ identified low risk patients. Baseline serum PC levels, PC administration dosage and duration and days until a 20% improvement in LPSS. Observed baseline serum PC levels (bPC) blood concentrations ranged from 31 to 80%. Patients received PC supplementation in case of low age-related bPC levels or $> 10\%$ PC concentration decrease within 12 hours from the first evaluation. All patients received 80 U/kg/day PC, intravenously, every twenty-four hours. No drug-related adverse event was observed. The observed sepsis-related mortality rate in our cohort was 9%. PC supplementation in our cohort appeared to be safe, and, probably due to prompt PC administration, we observed an overall mortality that was much lower than expected mortality in cancer severe septic patients.

Introduction

Protein C (PC) is a vitamin-k dependent serine protease produced by the liver. It circulates as a proenzyme and is activated on endothelium by the thrombin-thrombomodulin-endothelial protein C receptor complex. The function of activated protein C (APC) as an anticoagulant is primarily exerted through its ability to

inactivate two important cofactors of the coagulation cascade, namely factors (F) V/Va and FVIII/VIIIa, thereby downregulating the coagulation system activity.

These events are enhanced by the presence of Ca²⁺, phospholipids, and cofactor protein S.¹

Moreover, APC indirectly contributes to fibrinolysis by virtue of its ability to inhibit the plasminogen activator inhibitor-1 (PAI-1) and promote thrombin down-regulation, thus suppressing the thrombin activatable fibrinolytic inhibitor (TAFI).

Given its central anticoagulant role, both a congenital (heterozygous or homozygous) and an acquired PC deficiency induces an increased risk of thrombosis. Besides this, the APC pathway plays an active anti-inflammatory and anti-apoptotic role, mainly through the direct downregulation of cytokine production. APC also decreases leukocyte adhesion and extravasation, and most of these functions are carried out via APC binding to the endothelial protein C receptor (EPCR), allowing pseudoautosomal region 1 (PAR1) activation. In addition to EPCR orchestration of these changes, CD11b is also capable of supporting APC signaling, although not all the molecular mechanisms underlying these activities are yet fully understood.² Sepsis is a life-threatening condition in which hyperactive and dysregulated inflammatory responses lead to the activation and migration of leucocytes, to clotting and the secretion of pro and anti-inflammatory cytokines, the inhibition of fibrinolysis, and an increased apoptosis. Severe sepsis, defined as sepsis associated with acute organ dysfunction resulting from a generalized procoagulant and inflammatory response, is associated with 17/70% of deaths in Pediatric Intensive Care Units (PICU) around the world. Differences in mortality rates mainly depend on patients' need for both mechanical ventilation and inotropic support (Piastra M, unpublished data).³ Decreased APC plasma levels in severe sepsis, resulting from an increased consumption, degradation, and/or decreased synthesis, are correlated with a high risk of macro or microvascular thrombosis and mortality, regardless of the patient's age, the presence of disseminated intravascular coagulation or shock, the degree of hypercoagulation or severity of the illness.⁴ To our knowledge, no clinical evidence has been published supporting APC administration in septic pediatric cancer patients. In this study, we retrospectively examined data on 22 pediatric septic cancer patients who received PC concentrates in our Pediatric Oncohematology Unit.

Materials and Methods

We conducted a retrospective study of 22

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patients (aged 18 months- 16 years/mean age 6 years 3 months) receiving human plasma-derived PC concentrate (Ceprotin, Baxter AG, Vienna, Austria) from 2008 to 2015 at our Pediatric Hematology/Oncology Center. Patients' data are reported in Table 1.

Patients' admission diagnosis ranged from sepsis to severe septic shock. The *Surviving sepsis campaign* definitions for sepsis, severe sepsis and septic shock were used to define the patients' septic status at admission, whereby sepsis was defined as the presence of infection together with systemic manifestations of infection; severe sepsis was defined as sepsis plus sepsis-induced organ dysfunction, and septic shock as severe sepsis plus hypotension not reversed with fluid resuscitation.⁵

Age at admission and disease were reported. Lansky performance status scale (LPSS) was calculated within the first 24 hours from the beginning of sepsis in order to evaluate patients' performance status.⁶

Any organ dysfunction and positive microbiological isolations were recorded.

For each patient, who matched the Multinational Association of supportive care in Cancer (MASCC) risk score and the *Neutropenic risk Score*,^{7,8} we calculated a risk score defined the *Hematologic risk score* (HRS), that we created in 2007, in order to evaluate patients risk for severe sepsis/septic shock. According to this specific score,

patients were defined as *High risk for severe sepsis/septic shock* (HRS>3) if they had at least three of the following features: Acute Myeloid Leukemia (AML)/Relapsed Acute Lymphoblastic Leukemia (R-ALL), expected neutropenia >7 days, dexamethasone treatment during Induction therapy for ALL, a central venous line, fever >38.5°C for more than 48 hours. HRS<3 identified low risk patients.

Serum PC levels were recorded every 12 hours from the beginning of the septic episode until sepsis resolution.

Baseline serum PC levels (bPC), PC administration dosage and duration and days until a 20% improvement in LPSS are reported in Table 2. Patients received 80 U/kg/day PC, intravenously, every twenty-four hours. Descriptive statistics were used for categorical and continuous variables. Comparisons of relevant parameters were performed between survivors and non-survivors.

Results

Eight patients were affected by ALL, four by relapsed ALL, one by biphenotypic leukemia, seven by AML, one by relapsed AML, one by Non-Hodgkin Lymphoma (NHL), one by nasopharyngeal carcinoma. At admission, 8/22 patients were septic, whereas severe sepsis occurred in 10/22 patients, due to different organ dysfunction. Septic shock was observed in 4/22 children; all of them required PICU admission, two of them did not survive the acute sepsis episode and died of septic shock after a mean duration of 2 days, two received Extra Corporeal Membrane Oxygenation and invasive ventilation for three and four days, respectively. Of 22 children, 8 had 30% LPSS, 12/22 20% LPSS, 2/22 were moribund (10% LPSS). Only 8/22

patients had positive blood cultures, one had Rotavirus isolated in stool, and one had positive stool and pharyngeal swab tests. All patients had HRS>3 and so they were all considered at high risk for septic shock. Observed bPC blood concentrations ranged from 31% to 80% (median 53%). mLow bPC levels (age-related normal PC levels ranges according to Nathan Oski) or >10% PC concentration decrease within 12 hours from the first evaluation were the two reasons for PC supplementation in our cohort. All patients received 80 U/kg/day PC, intravenously, every twenty-four hours. Mean PC supplementation duration was three days. No drug-related adverse event was observed. As regards survival, 2/22 patients died during the first 48 hours following the onset of severe sepsis. The mortality rate was 18% among patients with severe sepsis/septic shock (2/14) and the overall mortality rate was 9%.

Table 1. Data of the 22 patients of our retrospective study, who received human plasma-derived protein C concentrate from 2008 to 2015 at our Pediatric Hematology/Oncology Center (Bari, Italy).

Patient	Age (yr)	Setting	Sepsis/severe sepsis/septic shock	Organ disfunction	LPSS%	Microb isol	HRS	Outcome
1	5	R-ALL	Severe sepsis	GI	30	None	4	CR
2	15	ALL	Septic shock	MOF	20	None	3	CR
3	2.6	R-AML	sepsis	None	30	None	3	CR
4	5	ALL	Severe sepsis	Renal	20	None	3	CR
5	3	ALL	Sepsis	none	30	Candida in blood culture	3	CR
6	9	ALL	Sepsis	None	30	None	4	CR
7	12	NHL	Sepsis	None	30	None	3	CR
8	1.6	ALL	Severe sepsis	pulmonary	20	<i>Klebsiella pneumoniae</i> in blood culture	4	CR
9	6	AML	Septic shock	MOF	10	Rotavirus in stool	4	+
10	5	ALL	Severe sepsis	Circulatory	20	None	4	CR
11	4	Rhinopharyngeal sarcoma	Sepsis	None	30	None	3	CR
12	16	R-ALL	Sepsis	None	30	None	3	CR
13	1.6	AML	Septic shock	MOF	30	Candida in blood culture	4	CR
14	1.6	AML	Severe sepsis	GI and pulmonary	20	<i>Pseudomonas aeruginosa</i> in blood culture	4	CR
15	5	ALL	Severe sepsis	Pulmonary	20	Candida in blood culture	4	CR
16	7	AML	Severe sepsis	Circulatory	20	<i>Klebsiella pneumoniae</i> in blood culture	3	CR
17	13	ALL	Sepsis	None	30	None	4	CR
18	12	AML	Severe sepsis	GI, liver	20	None	3	CR
19	2	AML	Septic shock	MOF	10	<i>Klebsiella pneumoniae</i> in blood culture	4	+
20	3	R-ALL	Severe sepsis	None	30	Candida in blood culture	4	CR
21	12	AML	Severe sepsis	GI, liver	30	<i>Stenotrophomonas</i> <i>Maltophilia</i> in faringeal swab, <i>candida lusitanae</i> in stool	4	CR
22	3.8	R-ALL	Sepsis	GI	30	None	3	CR

yr, years; LPSS, Lansky performance status scale; Microb isol, Microbiological isolation; HRS, Hematologic risk score; R-ALL, Relapsed Acute Lymphoblastic Leukemia; GI, Gastrointestinal; CR, Complete remission from sepsis; ALL, Acute Lymphoblastic Leukemia; MOF, Multiple organ failure; R-AML, Relapsed Acute Myeloid Leukemia; NHL, Non-Hodgkin Lymphoma; AML, Acute Myeloid Leukemia; +, death.

Discussion and Conclusions

Thanks to recent advances in supportive care and chemotherapy, the prognosis of children with cancer has improved considerably. This has, of course, entailed an increasing need for intensive care admission and management, which affects about 35% of patients during the disease course.⁹

Pediatric cancer patients account for approximately 3% of all PICU admissions, and mortality due to severe sepsis among these patients remains similar to the figure in the general pediatric ICU population, except in the case of bone marrow transplant, since bone marrow transplantation patients have an increased mortality rate.^{10,11}

At the end of the 1990s, trials on APC concentrates administration in septic patients demonstrated that PC plasma levels at baseline are inversely correlated with morbidity and mortality. Moreover, early directional changes in APC levels seemed also to be correlated with outcome.

Given these considerations, since 2000, numerous trials (in particular, the PROWESS study) have been focused on recombinant human activated APC (rhAPC) supplementation, aimed at reducing severe sepsis-related mortality.¹² Finally, given its demonstrated

ability to improve survival in adult patients with sepsis-induced organ dysfunction, the drug received approval by the US Food and Drug Agency and the European Agency with specific limitations. Bleeding was the most serious adverse event observed and no data were available among pediatric septic patients. Therefore, its use was contraindicated in children. Long before rhAPC was considered for use in pediatrics, case reports appeared on the administration of APC zymogen among children affected by severe sepsis. APC use appeared to be safe and not associated with bleeding.

Reviewing all manuscripts describing APC supplementation in pediatric patients (updated to November 2014), we identified 17 publications; two randomized studies and 15 case reports or case series.¹³⁻²⁸

Veldman's national retrospective multi-center study aimed at demonstrating that PC supplementation in purpura fulminans (PF) correlates with a PF improvement and less need of dermatoplasty and amputations.¹³ PC supplementation did not cause any bleeding event among the 94 pediatric patients enrolled. In the same way, De Kleijn and colleagues performed a phase 2, dose-finding study in 30 children affected by PF receiving PC treatment.¹⁴ The authors concluded that PC supplementation had a positive effect on sep-

sis-induced coagulation disorders and that expected mortality was higher than the actual mortality in the group receiving PC treatment at 200 IU/Kg/day.

Successful PC administration in pediatric patients with PF was described in two case series as well, and again PC therapy seemed to be correlated with high survival and a low rate of disabilities.^{15,16}

Pettenazzo, Silvani, De Carolis and colleagues reported cases of acquired PC deficiency in severe sepsis/septic shock.¹⁷⁻¹⁹ PC supplementation did not improve survival in Silvani's retrospective study, whereas De Carolis and Petterazzo observed a prompt improvement and low mortality rate in patients receiving PC treatment. Acquired severe PC deficiency in meningococemia has been examined by many authors. Almost all reports describe a prompt clinical improvement and normalization of hemostatic parameters after use of PC concentrates. No adverse effects were observed after PC administration.

As for immunocompromized patients, Panwar and colleagues demonstrated that PC levels at baseline are lower in immunocompromized than immunocompetent severe sepsis patients, whereas Mesters and colleagues observed that low PC concentrations at the onset of fever can predict an unfavorable outcome far before the onset of clinical symptoms in neutropenic patients.²⁰ To our knowledge, this is the first study on PC supplementation in pediatric cancer patients.

Our experience primarily shows that PC administration is safe and not associated with bleeding or severe allergic complications in such patients. We believe this is important because hematological patients often suffer from disease- or chemotherapy-induced coagulation disorders. Therefore, during septic episodes, their already impaired coagulation balance becomes more fragile, and decisions to adopt any medical supplementations such as antithrombotic and/or profibrinolytic agents become harder.

We did not observe any statistically significant correlation between low bPC and mortality, although both patients who did not survive had very low bPC levels.

Even though both non-survivors and some survivors had very low bPC levels, all survivors received prompt administration of PC concentrate (within 6 hours from measurement). On the contrary, the non-survivors, both admitted in 2008, had started PC treatment later (12 and 15 hours from the PC deficit detection). This was because both PC laboratory measurement and PC concentrate supply took longer at that time. Therefore, a rapid start of treatment after patient presentation represents an important goal for a favorable outcome.

Expected mortality in our cohort (as mentioned above) was much higher than the actu-

Table 2. Baseline serum protein C levels (bPC), protein C administration dosage and duration and days until a 20% improvement in Lansky performance status scale (LPSS).

Patient	bPC	Dosage (U/Kg/die)	gg>LPSS
1	74	80 U for 2 days	3
2	37	80 U for 2 days	6
3	58	80 U for 3 days	2
4	31	80 U for 4 days	3
5	44	80 U for 1 day	1
6	75	80 U for 3 days	2
7	71	80 U for 3 days	2
8	67	80 U for 3 days	3
9	45	80 U for 5 days	-
10	63	80 U for 2 days	1
11	67	80 U for 2 days	2
12	52	80 U for 2 days	2
13	80	80 U for 2 days	1
14	54	80 U for 4 days	4
15	39	80 U for 3 days	2
16	41	80 U for 2 days	3
17	36	80 U for 5 days	2
18	48	80 U for 3 days	4
19	43	80 U for 5 days	-
20	47	80 U for 3 days	2
21	60	80 U for 2 days	4
22	50	80 U for 3 days	1

al mortality. We believe that PC supplementation played an important role in preventing/treating disseminated intravascular coagulation and shock. In fact, only four out of 14 patients with severe sepsis/septic shock needed PICU admission; all of them had septic shock at admission. Moreover, none of the patients with sepsis receiving PC supplementation developed any organ dysfunction and their clinical conditions did not progress to severe sepsis.

Given the results of our study, we propose the HRS calculation in all hematological children with initial signs of sepsis or systemic inflammatory response syndrome.

Our proposal is to promptly measure bPC levels in all high-risk patients (HRS>3). If bPC levels are initially low, we support a rapid PC deficiency correction. In cases of normal age-related results, we recommend a repetition of PC level estimation every 24 or 12 hours in cases of stable or deteriorating clinical conditions, respectively. We then advise prompt PC administration in cases with $a > 10\%$ PC concentration decrease within 12 hours from the previous evaluation.

Being a retrospective analysis, our study has obvious limitations: the lack of a control group and a prospective design makes it difficult to comment on the effects of PC on survival. Moreover, ours is a single-center analysis with a small number of patients.

However, our results, together with studies demonstrating a correlation between bPC levels and outcome in febrile neutropenic patients, strongly encourage the prompt measurement and subsequent PC administration in such cohorts of children. We are currently carrying out a prospective study aimed at demonstrating that bPC levels can actually predict risk for severe infectious complications in neutropenic patients with fever or initial signs of an impairment of their general conditions.

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