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Non-activated plasma-derived PC improves amputation rate of children undergoing sepsis

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ABSTRACT

Low circulating protein C (PC) levels have been observed in sepsis, especially in patients with *Neisseriae Meningitides* infections. Poor clinical outcome and high limb amputation rates have been associated in infected patients with low circulating PC levels. Published studies using activated PC replacement therapy patients with sepsis have shown reduced mortality rates, however, its use has been associated with severe bleeding events. Paediatric sepsis studies using non-activated plasma-derived PC (Ceprotin®) are lacking. We present a retrospective study in children with sepsis who were treated with Ceprotin® focusing on amputation rate post treatment. Thirty subjects were identified. Median age at diagnosis was 2 years. Twenty-one (70%) were treated for *Nesseria Meningitides* and one (3%) for *Streptococcus-A β-haemolyticus*, another 8 (26%) patients with malignancies were treated for neutopenic sepsis. Following Ceprotin® administration, a significant increase in leukocyte count ($p = 0.004$), neutrophil count ($p = 0.001$) and PC (pretreatment = 13%, posttreatment = 88.5%; $p = 0.0001$) was seen. Prothrombin time (pretreatment = 30.3 seconds, posttreatment = 16.5; $p = 0.000$) and activated partial thromboplastin time (pretreatment = 61.8 sec, postreatment = 42.6 sec; $p = 0.000$) were significantly reduced, while fibrinogen levels were significantly elevated (pretreatment = 1.9 g/dL, posttreatment = 4.4 g/dL; $p = 0.000$). The median time between admission to intensive care and Ceprotin® administration was 10 hrs. Limb amputation rate was reduced (16-23% versus 30-50% from previous studies) and there were no haemorrhagic events observed. This study demonstrates the safe administration of non-activated plasma-derived PC concentrate in patients with sepsis who are coagulopathic and it associated with a reduction in amputation rates.

Introduction

Severe congenital protein C (PC) deficiency due to homozygous or compound heterozygous mutations is characterised by neonatal *purpura fulminans* and large-vessel venous-thrombosis Heterozygous PC deficiency is associated with a life-long increased risk for thromboembolic events or coumarin-induced skin necrosis [1–3]. Acquired PC deficiency, reported in approximately 20% of cases of *Neisseriae Meningitides* infections is associated with cutaneous haemorrhages, skin bullae, tissue necrosis and microvascular thrombosis which may lead to limb amputation and even death.

The mortality rate for *Neisseriae Meningitides* infection before the advent of PC replacement was in excess of 50% [4,5] with a limb amputation rate of 30-50% [6,7]. Recently, PC substitutes have become available and include; recombinant activated protein C (APC) (drotecogin alfa or Xigris®) and non-activated plasma-derived PC (Ceprotin®), both have been shown to be effective for the treatment of either inherited or acquired PC deficits [8–10]. Most of the studies on PC replacement therapies have used Xigris® [11,12]. A previous paediatric study on plasma derived PC concentrates to identify PC dose finding has also been carried out [13]. The use of Xigris® has been approved in the treatment of adults with severe sepsis with a high risk of death or systemic inflammatory response syndrome (SIRS). However, Xigris® has been associated with an increased incidence of serious bleeding if

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predisposing risk factors are present. This risk was reported to be as high as 4.8% in a large paediatric study (n = 83) [12]. According to this study an elegant and exhaustive Cochrane Review article conducted by Martí-Carvajal AJ et al. in adults and children, showed that in sepsis and septic shock, APC seems to be associated with a higher risk of bleeding [14]. Moreover in 2011, the European Medicines Agency (EMA) was informed by the drug company Eli Lilly of the results of the PROWESS-SHOCK study [15]. This study failed to meet the primary endpoint of a statistically significant reduction in 28-day all-cause mortality in patients treated with Xigris® compared with placebo [16]. For this reason Xigris® was withdrawn from the market and therefore to date comparative study with Ceprotin® are not possible.

The study by White B et al. involving a large group of adults and children treated with Ceprotin® [9] and indeed, other studies [15,17–19] have shown a reduction in morbidity and mortality rate when Ceprotin® was administered. However, due to an understandable reluctance to start critically ill children on a treatment potentially associated with severe haemorrhage, no randomized paediatric study comparing the two forms of PC replacement (Xigris® and Ceprotin® h) has been carried out thus far. Also, as reported by the EMEA in 2011, due to the lack of efficacy, Xigris® was been withdrawn from the market. Although most studies to date using PC replacement have shown an improvement in mortality rate, the amputation rate in survivors and non-survivors has not been fully highlighted. Given the clinical and psychosocial impact of limb amputation we felt it important to measure the effect of PC therapy on this specific endpoint. We report the first study on children with septicaemia treated with plasma derived PC concentrate (Ceprotin©) and is impact on amputation rate.

Patients Materials and Methods

The medical records for all consecutive paediatric patients treated with Ceprotin©, at Our Lady's Children Hospital Dublin and Temple Street University Hospital, Dublin, Ireland between January 2001 and January 2009 were retrospectively reviewed.

Patients

Thirty patients were identified, M/F ratio 1.5 (18 male, 12 female). Median age at diagnosis was 2 years (range 3 months to 15 years). Twenty-one patients (70%) had a diagnosis of *Neisseriae Meningitides* sepsis, one (3%) *Streptococcus Pneumoniae* sepsis, four (13%) had acute lymphoblastic leukaemia (ALL), two patients (6%) rhabdomyosarcoma, one patient (3%) Langerhans cells histiocytosis (LCH) and one patient (3%) neuroblastoma. All patients eligible to receive Ceprotin® replacement therapy were in septic shock and had documented low circulating levels of PC. Septic shock criteria were defined as previously reported [9]: systolic blood pressure <75 mmHg for patients <4 years of age and <80 mmHg for patients ≥4 years of age, requiring inotropic support and assisted ventilation. Patient demographics are shown in Table 1.

Clinical information

For all patients, relevant clinical information regarding outcome and complications was recorded. Special relevance was given to limb and/or digit amputations). Other clinical information included type of antibiotic given, presence of diffuse skin necrosis, major and minor bleeding following Ceprotin® administration, cardiovascular complications, administration of pressors (noradrenaline and dobutamine) and renal failure impairment.

PC measurement

Plasma PC levels was measured using the Hemo IL Test TM PC kit based on a synthetic chromogenic substrate assay. Protein C levels in patient plasma are measured automatically on HemosIL Coagulation Systems in two stages: (i) Incubation of plasma with protein C activator; (ii) Quantification of the APC with a synthetic chromogenic substrate. Paranitroaniline released is monitored kinetically at 405 nm and is directly proportional to the Protein C level in the test sample.

Table 1
Principal patients demografical and clinical characteristics.

Patients	gender	age at diagnosis	DIAGNOSIS	status	cause of †	tim e frame from ICU admissionto	Am putation yes/no	Renal failure
#1	**M**	**2 y 4 Mo**	**Meningitis**	**alive**	**na**	1 hr	**no**	yes/no
#2	**M**	**2 y 6 Mo**	**ALL**	**alive**	**na**	**na**	**no**	**yes**
#3	**M**	**4 y 2 Mo**	**LHL**	**†**	**asystolic arrest**	**na**	**no**	**no**
#4	**M**	**5 Mo**	**Meningitis**	**alive**	**na**	**14 hrs**	**no**	**yes**
#5	**M**	**6 y 7 Mo**	**ALL**	**†**	**cellulitis/neutropenia**	**8**	**no**	**no**
#6	**F**	**1 y 10 Mo**	**Meningitis**	**alive**	**na**	**1 hr 30 m**	**no**	**yes**
#7	**M**	**2 y**	**5**	**†**	**sepsis**	**na**	**no**	**no**
#8	**M**	**1 y 3 Mo**	**Meningitis**	**†**	**renal failure myoglobinuria**	**2 hrs 30 m**	**no**	**yes**
#9	**M**	**1 y 2 Mo**	**Meningitis**	**alive**	**na**	**3 hrs**	**no**	**yes**
#10	**F**	**11 y 7 Mo**	**ALL**	**†**	**septic shock**	**5 hrs**	**no**	**yes**
#11	**M**	**1 y 9 Mo**	**Meningitis**	**alive**	**na**	**5 hrs**	**no**	**no**
#12	**F**	**7 y 4 Mo**	**Meningitis**	**alive**	**na**	**19 hrs**	**no**	**yes**
#13	**F**	**3 y 5 Mo**	**Meningitis**	**alive**	**na**	**40 hrs**	**bilateral below knee**	**yes**
#14	**F**	**7 y 8 Mo**	**Rhabdomyosarcoma**	**alive**	**na**	**na**	**no**	**no**
#15	**F**	**2 y 6 Mo**	**Meningitis**	**alive**	**na**	**1 hr**	**no**	**yes**
#16	**M**	**2 y 1 Mo**	**Meningitis**	**alive**	**na**	**8 hrs**	**no**	**no**
#17	**F**	**8 y 9 Mo**	**ALL**	**†**	**desaturation/lungs infiltrates**	**na**	**no**	**yes**
#18	**M**	**3 y 8 Mo**	**Meningitis**	**alive**	**na**	**3 hrs**	**digits gangrene**	**no**
#19	**M**	**2 y 2 Mo**	**Meningitis**	**alive**	**na**	**20 hrs**	**no**	**yes**
#20	**F**	**15 y 10 Mo**	**Meningitis**	**alive**	**na**	**3 hrs**	**no**	**yes**
#21	**M**	**8 Mo**	**Meningitis**	**alive**	**na**	**6 hrs**	**no**	**yes**
#22	**M**	**1 y 1 Mo**	**Meningitis**	**†**	**septic shock**	**6 hrs**	**no**	**yes**
#23	**F**	**7 y 4 Mo**	**Rhabdomyosarcoma**	**alive**	**na**	**21 hrs**	**R leg below knee**	**no**
#24	**F**	**5 Mo**	**Meningitis**	**alive**	**na**	**19 hrs**	**no**	**no**
#25	**M**	**2 y 2 Mo**	**Meningitis**	**alive**	**na**	**5 hrs**	**no**	**no**
#26	**M**	**8 Mo**	**Meningitis**	**alive**	**na**	**140 hrs**	**no**	**yes**
#27	**F**	**5 Mo**	**Meningitis**	**alive**	**na**	**10 hrs**	**digits gangrene**	**no**
#28	**M**	**1 y 8 Mo**	**Meningitis**	**alive**	**na**	**36 hrs**	**no**	**yes**
#29	**M**	**1 y 9 Mo**	**Meningitis**	**alive**	**na**	**10 hrs**	**no**	**yes**
#30	**F**	**3 y 7 Mo**	**Str β haemolytic**	**alive**	**na**	**10 hrs**	**digits gangrene**	**yes**

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