Management of Neonatal Purpura Fulminans with Severe Protein C Deficiency

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Neonatal purpura fulminans is a life-threatening clinical entity characterized by extensive subcutaneous thrombosis and disseminated intravascular coagulation usually manifesting shortly after birth. We report an autosomal recessive form of the disease in a neonate who was diagnosed with compound heterozygosity for mutations in his protein C gene as the molecular basis of his disorder.

Keywords: *Protein C, Purpura fulminans.*

Protein C (PC) is a vitamin K-dependent serine protease anticoagulant that plays an essential role in regulating coagulation by degrading activated factors V and VIII in plasma. Mutations affecting proteins in the PC pathway (proteins C and S, factors V and VIII) are the most common genetic predisposing factors for venous thromboembolism in adults. Whereas, heterozygous mutations in the PC gene (PROC; OMIM: 176860) confer an ~five-fold increased risk for venous thrombosis, a point mutation in the factor V gene producing resistance of factor V (factor V Leiden) towards inhibition by activated PC is the most common genetic risk factor for hereditary thrombophilia[1].

Homozygosity or compound heterozygosity for PC mutations, producing an absolute deficiency of PC anticoagulant activity are exceedingly rare, found in only ~1/2,50,000 newborns[2]. However, the complete lack of plasma PC activity in these patients results in neonatal purpura fulminans, a devastating thrombotic disorder of the newborn, characterized by sudden onset of widespread purpuric lesions progressing to gangrenous necrosis, accompanied with disseminated intravascular coagulation (DIC) and shock. An acquired form, usually recognized in older infants as a post-meningococcal sepsis syndrome is also caused by decreased levels and activity of PC[3].

Here, we describe a neonate with purpura fulminans who was diagnosed with compound heterozygosity for PC mutations as the cause of his hereditary thrombophilia.

Case Report

The patient is a male newborn who was delivered at term by cesarean section and referred to our Center on the second day of his life with a single gangrenous lesion on his scrotal area. The neonate weighed 2435g at birth and had no apparent congenital malformations; he is the first offspring of non-consanguineous parents who were childless for 11 years. Maternal history for recurrent miscarriages or antenatal TORCH infections and family history for thromboembolic events were negative. The newborn had received vitamin K prophylaxis at birth.

Within the next 72 hours, purpuric lesions appeared over the perineal region, the flexor surface of the right thigh and the right lower quadrant of his abdomen. The lesions soon

enlarged and vesiculated, forming hemorrhagic bullae with subsequent necrosis and black eschar formation. The margins of the lesions became erythematous and indurated. A complete blood count examination (CBC) revealed moderate thrombocytopenia with normal values for hematocrit and total and differential leukocyte counts (*Table I*). The patient received 2 units of fresh frozen plasma (FFP) immediately and was started on a course of intravenous vancomycin and ceftazidime.

On the twelfth day of life, the patient developed hematuria and marked oliguria and started to ooze blood from the umbilical stump and the skin lesions. A repeat CBC was significant for severe thrombocytopenia (~16,000/mL) which, along with grossly elevated fibrin degradation product (FDP) levels, high levels of D-dimers (>10, 000 ng/mL) and markedly decreased plasma fibrinogen levels (*Table I*) confirmed a laboratory diagnosis of DIC.

The patient was immediately treated with 2 units of platelet concentrate that reversed the thrombocytopenia (>1,60,000/mL) and the bleeding manifestations. We performed an urgent color doppler study of the renal vessels

that excluded renal vein thrombosis as the cause of his acute renal failure. Over the next 2 days, the urine output increased and the hematuria subsided.

Clinically, the cutaneous thrombotic manifestations culminating in DIC with sudden onset in a newborn was strikingly similar to neonatal purpura fulminans. In the absence of signs of generalized septicemia in this patient, a deficiency of the anticoagulant factors, protein C and protein S remained the leading differential diagnoses in the etiopathogenesis of the disorder. We assessed endogenous plasma PC activity in this patient using a chromogenic assay (Berichrom® protein C; Dade-Behring GmbH, Marburg, Germany); whereas normal levels vary between 0.8-1.3 IU/mL, PC activity in the patient was in the undetectable range (*Table I*).

With a provisional diagnosis of purpura fulminans due to PC deficiency, we began fresh frozen plasma (FFP) transfusion at 15 mL/kg every 12 hours. The skin lesions and general condition of the patient started to improve rapidly. After three weeks of FFP transfusion, we shifted to low molecular weight heparin at 1 mg/kg for three days

TABLE I–*Laboratory Test Values in the Patient for Evaluation of Coagulopathy.*

	72 h	12th day	16th day	14 days after FFP transfusion	Reference values
Platelets ($\times \mu$L)	90	16	–	524	100-300*
APTT, sec	–	NC	–	28.3	35-52**
PT (INR)	–	–	–	1.06	0.9-2.7**
Fibrinogen (mg/dL)	–	50	–	–	95-245**
D-Dimer (μg/mL)	–	10	–	–	<0.5***
Protein C					
Clotting activity (%) (96 h after FFP treatment)	–	–	ND	–	80-130

– not measured; NC: not coagulable; ND: not detectable, *(11) ** (13) ***(14).

following which, oral anticoagulation with warfarin was instituted, gradually titrating the dose to 0.3 mg/kg so as to maintain the international normalized ratio (INR) at ~ 2.5 following the recommendations of the International Committee on Thrombosis and Hemostasis(4).

We obtained informed consent from the parents to analyze the PROC gene in the patient and his parents. Amplification of all nine exons and direct sequencing of the amplicons revealed that the patient was a compound heterozygote for two missense alleles: a 6245C>T mutation in exon 7 inherited from the father and an 8752C>T mutation in exon 9 from the mother, both alleles numbered according to Foster *et al.,*(5). The paternal allele is predicted to cause an R178W alteration within the activation peptide of PC. The maternal allele is predicted to cause an A346V change in the serine protease domain of the mature protein. Significantly, both the R178 and A346 residues are highly conserved amino acids in mammalian PC species; mutations in these residues have been previously reported to co-segregate with familial type I PC deficiency, in which, there is a quantitative reduction in plasma PC antigen levels secondary to defective production, folding or secretion of the protein(6,7). Our results therefore confirm that the purpura fulminans in our patient is due to a type I PC deficiency resulting from compound heterozygous mutations in his PROC gene.

The patient was discharged after the cutaneous lesions healed and his INR is currently being monitored fortnightly on an outpatient basis. Although warfarin-induced skin necrosis has been previously reported in patients undergoing oral coumarin therapy, our patient has tolerated the low-dose therapy very well. However, at ~3 months of age, the infant was diagnosed with diminished light perception and bilateral vitreous hemorrhage, as an ocular complication of the initial episode of DIC. Imaging studies (USG, CT) have ruled out any other intra-abdominal or cerebral thrombotic events.

Discussion

PC deficiency is predominantly inherited in an autosomal dominant manner with hetero-zygous carriers often remaining asympto-matic until later in life when they become highly susceptible to venous thrombo-embolism. Autosomal recessive PC deficiency is less common, caused by homozygous or compound heterozygous mutations in PROC, and usually leads to a more severe form of the disease with onset of thrombotic mani-festations at birth(4). The PC mutation data-base (PROC database: http://www.xs4all.nl/~reitsma/Prot_C_home.htm - version 1.2.1, updated 1998), an excellent repository of PC mutations identified worldwide, together with recent studies report only 65 symptomatic cases with recessive inheritance, 34 of them being compound heterozygotes(7-10).

This study reports a case with the classic manifestations of neonatal purpura fulminans caused by compound heterozygous mutations in the PROC gene. Since adults with hetero-zygous PC deficiency are often asymptomatic, our results emphasize that even in the absence of parental history of familial thromboembolic events, an autosomal recessive PC deficiency should always be sought as an explanation for thrombotic disorders in the newborn with manifestations of DIC. Genotyping of suspected patients for PROC mutations provides an unambiguous molecular confirmation of a clinical diagnosis. Detection of mutations is also useful in the management and prognosis of autosomal recessive PC deficiency. These patients require long-term oral anticoagulation which, if well tolerated,

can be adequate for them to remain free of coagulopathy throughout life(4). Finally, genetic analysis is essential in the risk assessment for affected couples planning to conceive and for prenatal testing of the fetus during a subsequent pregnancy.

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REFERENCES

1. Seligsohn U, Lubetsky A. Genetic suscepti-bility to venous thrombosis. N Engl J Med 2001; 344: 1222-1231.

2. Rosendaal FR. Venous thrombosis: A multi-causal disease. Lancet 1999; 353: 1167-1173.

3. Brandtzaeg P, Sandset PM, Joo GB, Ovstebo R, Abildgaard U, Kierulf P. The quantitative association of plasma endotoxin, anti-thrombin, protein C, extrinsic pathway inhibitor and fibrinopeptide A in systemic meningococcal disease. Thromb Res 1989; 55: 459-470.

4. Marlar RA, Montgomery RR, Broekmans AW. Diagnosis and treatment of homozygous protein C deficiency. Report of the Working Party on Homozygous Protein C Deficiency of the Subcommittee on Protein C and Protein S, International Committee on Thrombosis and Haemostasis. J Pediatr 1989; 114: 528-534.

5. Foster DC, Yoshitake S, Davie EW. The nucleotide sequence of the gene for human protein C. Proc Natl Acad Sci U S A 1985; 82: 4673-4677.

6. Poort SR, Pabinger-Fasching I, Mannhalter C, Reitsma PH, Bertina RM. Twelve novel and two recurrent mutations in 14 Austrian families with hereditary protein C deficiency. Blood Coagul Fibrinolysis 1993; 4: 273-280.

7. Alhenc-Gelas M, Gandrille S, Aubry ML, Aiach M. Thirty-three novel mutations in the protein C gene. French INSERM network on molecular abnormalities responsible for protein C and protein S. Thromb Haemost. 2000; 83: 86-92.

8. Millar DS, Johansen B, Berntorp E, Minford A, Bolton-Maggs P, Wensley R, *et al.* Molecular genetic analysis of severe protein C deficiency. Hum Genet 2000; 106: 646-653.

9. Ablin AR, Kushner JH, Murphy A, Zippin C. Platelet enumeration in the neonatal period. Pediatrics 1961; 28: 822-824.

10. Manco-Johnson MJ. Development of hemo-stasis in the fetus. Thromb Res 2005; 115 Suppl 1: 55-63.