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Fulminant meningococcal septic shock in a boy with combined inherited properdin and protein C deficiency

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SUMMARY

A 7-year-old patient with fulminant septic shock due to *Neisseria meningitidis* of the uncommon serogroup Y developed extensive gangrene of the limbs. Multiple amputations were necessary and a pulmonary embolism occurred within 2 days post-operatively. Complement and haemostatic system studies, done after recovery, showed a complete absence of properdin antigen and a low protein C antigen and activity level in plasma. Defective haemolytic activity in gel by the alternative pathway of complement activation could be restored with purified properdin, indicating a properdin deficiency type 1. Protein C antigen level as well as activity were in agreement with a protein C deficiency type I. The polymerase chain reaction (PCR) product of exon five of the protein C gene showed a substitution of ^{72}Gly by Arg. Both deficiencies were traced among relatives of the patient. Serum of the father of the patient's mother was also properdin-deficient. Microsatellite haplotyping of the X-chromosome of the patient and his relatives showed that a distinct haplotype cosegregated with the properdin deficiency (Lodscore 2·25; four informative meioses). The protein C type I deficiency was present in the patient's mother and her mother and cosegregated with the mutation found. So far as is known, this is the first patient described with combined inherited properdin deficiency and protein C deficiency.

Keywords Neisseria meningitidis meningococci properdin complement protein C

INTRODUCTION

Meningococcal disease has a wide spectrum of severity, ranging from intermittent fever alone to fulminant and often fatal septic shock. The varieties of meningococcal disease seen, once the meningococcus has entered the systemic circulation of the susceptible host, are meningococcaemia, fulminant meningococcal septic shock, and meningitis in the presence or absence of sepsis [1,2]. In about 50% of patients, but predominantly in those patients with fulminant meningococcal septic shock (80–90%), petechial or larger haemorrhagic skin lesions, purpura and ecchymoses, occur [1,2]. The pathogenesis of these skin lesions involves disseminated intravascular clotting, thrombocytopenia, thrombosis and the release of cytokines and acute-phase proteins in response to circulating meningococcal endotoxin [1,3]. In addition, a strong correlation has been found between endotoxin levels and activation of the complement system, suggesting that endotoxin is an important activator of the complement system [4]. As prognostic indicators for the outcome of meningococcal sepsis the evolution of haemorrhagic skin lesions [2], levels of endotoxin [5], cytokines [6] and protein C, as a key component in that part of the anticoagulant pathway that cleaves and inactivates the prothrombotic factors Va and VIIIa [7], have been used [3,8,9]. Low protein C levels, induced by meningococcal disease, have been associated with fatal outcome [3].

Deficiencies of the complement system have been identified as a risk factor for the development of meningococcal disease [10,11], indicating that activation of the complement system is an essential defence mechanism against *Neisseria meningitidis*. Predominantly, deficiencies of a late component of the complement system, C5–9, are found [10,12]. Patients with such deficiencies have mild and often (40–50%) recurrent meningococcal disease [10]. Classical and alternative pathway of complement activation deficiencies are less frequently found [10–12]. In patients with these deficiencies the severity of meningococcal disease varies [11,13,14]. Although the most frequent

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alternative pathway deficiency is a deficiency of properdin [10,11], only ≈ 24 patients with properdin deficiency and meningococcal disease, commonly due to the uncommon serogroups such as Y and W135, have been reported [11,12]. Three properdin deficiency variants, X-linked inherited, are recognized. Type 1 represents a total deficiency; type 2, a state with less than 10% of the normal level of properdin; and type 3, a deficiency with normal properdin antigen levels, without functioning properdin [11]. The coding gene for properdin has been localized on the short arm of the X-chromosome at Xp11.23-Xp11.3 [15]. The defects on DNA level resulting in the various deficiencies are not yet elucidated. So far as is known, no patient with an inherited protein C deficiency and meningococcal disease has been described, although the frequency of protein C deficiency is rather high. Autosomal inherited protein C deficiency occurs either homozygous with an estimated prevalence of 1 in 15 000, or heterozygous with an estimated prevalence of 1 in 200–300 [16]. Two types of heterozygous protein C deficiency have been recognized. Type I represents an antigenic as well as a functional protein C deficiency, and type II only a functional deficiency [7]. Both types are associated with an increased risk for deep venous thrombosis and pulmonary embolism [17]. Another frequent functional deficiency of the protein C anticoagulant pathway, increasing the risk of deep venous thrombosis 5–10-fold, is resistance to activated protein C [18]. Recently, the mutation that affects the cleavage of factor Va for protein C (FV Leiden) has been identified [19]. This mutant allele is carrried by about 2% of the normal population [19].

We report here a 7-year-old boy with a combined inherited properdin deficiency and protein C deficiency, who suffered from fulminant meningococcal septic shock. Analysis showed that a type 1 properdin deficiency was present, in conjunction with a heterozygous protein C type I deficiency due to a not yet described mutation.

PATIENT AND METHODS

Case history

In 1992 a so far healthy 7-year-old boy (Fig. 1, IV:2) was admitted to the Paediatric Intensive Care Unit of the Academic Medical Centre (Amsterdam, The Netherlands) with fulminant septic shock, widespread petechiae and extending ecchymoses on his limbs, 12 h after the onset of fever. There were no signs of meningeal irritability. From the blood *N. meningitidis* serogroup Y:nt:P1.2,5 was cultured. On the day of admission protein C activity level was 2% (reference value 70–120%), protein C antigen level < 1% (reference value 65–110%), protein S total 30% (reference value 65–108%), free protein S 13% (reference value 26–71%), factor VII < 10% (reference value 80–140%), and fibrinogen 1·3 g/*l* (reference value 1·5–4·0 g/*l*). The patient received antibiotic (penicillin) and intensive supportive treatment: artificial ventilation, low-dose heparin, inotropic medication, transfusion with erythrocytes, fresh frozen plasma and platelets. When the patient was haemodynamically stable epoprostenol was also infused. Despite this treatment, extensive gangrene of the limbs developed. The boy recovered but both his feet, as well as his left hand and part of the right ring finger, had to be amputated 31 days after disease onset. Post-operatively, the patient developed a pulmonary embolus, as diagnosed by perfusion/ventilation scanning. Protein C activity level was then 50%, protein C antigen 40%, protein S total 71%, free protein S 26%, factor VII 60% and fibrinogen 5·9 g/*l*. Subsequently, treatment was with heparin and coumarin for 3 months. Although no family history of Neisserial infections was reported, the culture of a meningococcus of an unusual serogroup from the blood of the boy prompted us to study his complement system [12]. Because of the patient's low protein C levels, the occurrence of post-operative pulmonary embolism and his family history, protein C studies were also done.

Family history reported the death of a dysmature born brother (Fig. 1, IV:1) on postnatal day 22. On review of the medical chart and the autopsy report the cause of death was respiratory failure, probably because of a congenital hypoplasia of the right brain stem and a left-sided infarction of the arteria cerebri posterior. Coagulation studies were not performed. An uncle (Fig. 1, III:1) of the patient had a son with an unknown congenital neurologic disorder. Patient's grandfather (Fig. 1, II:1) used an anticoagulant (Acenocoumarol) because of a myocardial infarction at the age of 41 years.

Complement assays

Blood samples taken from the antecubital vein were collected from the patient after recovery and from 11 relatives. Sera were flash frozen, transported on dry ice and stored at −70°C. Serum samples were screened for a deficiency in the alternative, classical and late pathway of complement activation in the haemolytic assay in gel, using guinea pig erythrocytes and sensitized sheep erythrocytes [20], as well as in free solution, using rabbit erythrocytes and sensitized sheep erythrocytes [21]. Alternative pathway of complement components in the patient's serum were measured by ELISA [22], C1q, C4 and C3 by radial immunodiffusion with monospecific antisera as described [23]. Properdin used for reconstitution experiments was purified as reported [24]. Serum C3 depletion was performed by incubation of equal amounts (v/w) of serum and zymosan in the presence of MgEGTA at 37°C [25]. As a control, serum of an unrelated properdin-deficient person was included in each C3 depletion experiment. Purified chromosomal DNA from blood lymphocytes was used for properdin deficiency carrier detection by microsatellite haplotyping of CA/GT repeats close to the properdin coding gene [26]. Polymerase chain reaction (PCR) of the microsatellite loci was performed in the presence of a ^{32}P-labelled nucleotide. The microsatellite loci analysed were PFC1, PFC2 (both < 15 kb downstream of the properdin structural gene) [26], DXS426 (localization Xp11.23) [27], DXS228.1 and 2 (localization Xp11.4) [27], DXS7.pcr (localization Xp11.3) [27], DXS1003 (localization Xp11.23) [27], DXS983 (localization Xq13.3) [27] and Kallman (localization Xp22.3) [28].

Protein C antigen, activity and DNA analysis

Blood samples from the patient and six relatives were collected in 1/10 volume of 0·11 mmol/*l* trisodium citrate. Protein C activity was measured by a chromogenic assay (Coatest; Chromogenix, Mölndal, Sweden) [29]. Protein C antigen was quantified by ELISA (Asserachrom Diagnostica Stago, Asnieres-sur-Seine, France) [30]. Purification and activation

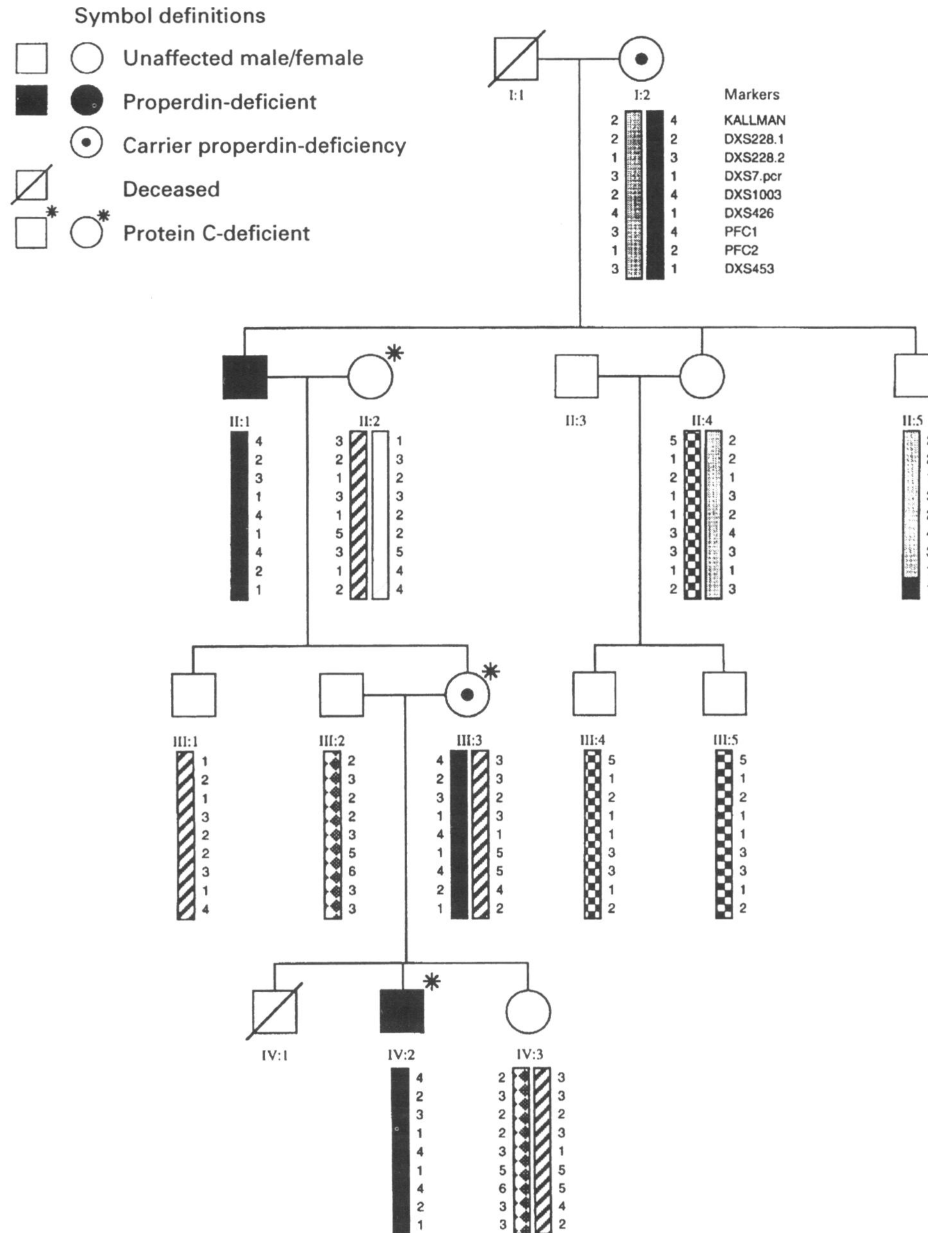

Fig. 1. Pedigree of the patient (IV : 2) shows the cosegregation of the properdin deficiency with a distinct microsatellite haplotype of the X-chromosome and the inheritance of the protein C deficiency. Microsatellite haplotyping (see Patient and Methods) was performed with the indicated markers.

of protein C were performed by standard procedures [31]. DNA was isolated from blood lymphocytes according to established procedures [32]. The strategy for mutation detection was limited to finding abnormalities in the promoter region, the coding region, and all splice functions of the protein C gene [33]. PCR amplification conditions for the protein C gene [33] and the techniques employed for direct sequence analysis of each amplified fragment are as described elsewhere [33,34]. On basis of the sequence of the exon 5 PCR product found, restriction enzyme Bsi EI (New England BioLabs Inc., Beverly, MA) was chosen to obtain the specific digestion pattern of this fragment [35].

RESULTS

Patient

Complement studies. Assessment of complement components showed normal levels of C1q (12 mg%, reference value 10–14 mg%), C4 (23 mg%, reference value 17–30 mg%) and C3 (81 mg%, reference value 68–104 mg%), whereas properdin was completely absent (Table 1, IV : 2). The classical and late pathway of complement activation were normal as tested with sensitized sheep erythrocytes. The alternative pathway of complement-mediated haemolysis of guinea pig erythrocytes in gel was defective. This defect was completely restored by reconstitution of the serum with purified properdin. A delayed

Table 1. Age, serum haemolytic activity and properdin level, and plasma protein C levels of the patient and his relatives

Pedigree no. of the relatives	Age (years)	Alternative pathway haemolysis		Properdin	Protein C	
		In free suspension (ref. values: 60–192%)	In gel	(ref. values: 17·1–27·7 μg/ml)	Activity (ref. values: 70–120%)	Antigen (ref. values: 65–110%)
I : 2	91	112	+	14·8	ND	ND
II : 1	66	41	−	<0·01	32*	33*
II : 2	66	113	+	34	68	61
II : 4	59	107	+	21	ND	ND
II : 5	65	97	+	23·8	ND	ND
III : 1	40	88	+	32·9	118	ND
III : 2	39	137	+	37·2	96	88
III : 3	37	148	+	20·6	61	53
III : 4	37	112	+	20·4	ND	ND
III : 5	40	92	+	20·5	ND	ND
IV : 2 (proband)	9	110†	−	<0·01	58	52
IV : 3	7	87	+	23·9	75	75

* IL-1 treatment with coumarin; ND, not determined.
† See Results.
+ and − indicate lysis and no lysis, respectively.

haemolytic activity in the alternative pathway for rabbit erythrocytes in free suspension was found in the patient's serum. Haemolytic activity was only 38%, 58% and 63% of that of normal pooled human serum after 2, 5 and 10 min of incubation, respectively. Haemolytic activity within the normal range was achieved after 30 min of incubation (Table 1). This haemolytic activity was probably not complement-mediated. Using a control serum of an unrelated properdin-deficient (type 1) patient, reconstituted with properdin, the haemolytic activity in free suspension in the presence of MgEGTA completely disappeared by zymosan-induced C3 depletion. Depletion of C3 by zymosan in the presence of MgEGTA in properdin reconstituted patient's serum, did not decrease haemolytic activity in free suspension (data not shown). The haemolytic activity of patient's serum was heat-labile, since heating the serum at 56°C for 20 min resulted in complete disappearance of the alternative pathway haemolytic activity in free suspension.

Properdin DNA analysis. Microsatellite haplotyping of the X-chromosome showed a distinct haplotype (Fig. 1, IV : 2).

Protein C, activity, antigen and DNA analysis. Plasma protein C activity and antigen levels were markedly reduced (Table 1), whereas levels of total (88%) and free (30%) protein S were within the normal range, substantiating the diagnosis of a hereditary protein C deficiency type I. Analysis of all targeted regions of the protein C gene showed only a single abnormality in exon 5. This abnormality was at nucleotide 3157 in the gene, a G for C transversion in codon 72 of the first epidermal growth factor domain of mature protein C. This nucleotide change predicts the replacement of Glycine by Arginine. The transversion at 3157 creating a recognition sequence for the restriction enzyme Bsi EI, provided us with the opportunity to screen easily the DNA obtained from the lymphocytes of the relatives of the patient for the presence of the mutation in the exon 5 PCR fragment.

Relatives

Complement studies. Sera of the 11 relatives (Table 1) tested all had normal haemolytic activity in the late and classical pathway. In serum of the mother's father (Fig. 1, II : 1) defective haemolytic activity of the alternative pathway both for guinea pig erythrocytes in gel and for rabbit erythrocytes in free suspension was present (Table 1). Properdin was not detectable in this serum. The mother (Fig. 1, III : 3), although being a obligate carrier, had a normal properdin level. The serum from her grandmother (Fig. 1, I : 2) had a reduced properdin level, indicating a carrier state. Zymosan-induced complement depletion was complete, as measured in haemolytic assay, in the serum of the mother (Fig. 1, III : 3) and in serum of the grandfather, reconstituted with purified properdin (Fig. 1, II : 1).

Properdin DNA analysis. The microsatellite haplotype of the X-chromosome present in the patient (Fig. 1, IV : 2) was also found in the properdin-deficient grandfather (Fig. 1, II : 1), as well as in the mother (Fig. 1, III : 3) and great-grandmother (Fig. 1, I : 2). This finding confirms the carrierships of properdin deficiency of the mother (Fig. 1, III : 3) and great-grandmother (Fig. 1, I : 2). No recombination between the deficiency and the microsatellite haplotype was observed, yielding a three points Lodscore of about 2·25 using one marker on each side of the gene.

Protein C, activity, antigen and DNA analysis. The mother (Fig. 1, III : 3) and the grandmother (Fig. 1, II : 2) had reduced protein C levels (Table 1), whereas the level of the vitamin K-dependent coagulation factor VII was within the normal range. Protein C antigen and activity levels in plasma of the grandfather (Fig. 1, II : 1) were low due to treatment with coumarin (Table 1). Analysing DNA by PCR, gel electrophoresis and digestion with restriction enzyme Bsi EI showed that the mutation present in the patient was also present in the mother (Fig. 2, III : 3) and grandmother (Fig. 2, II : 2).

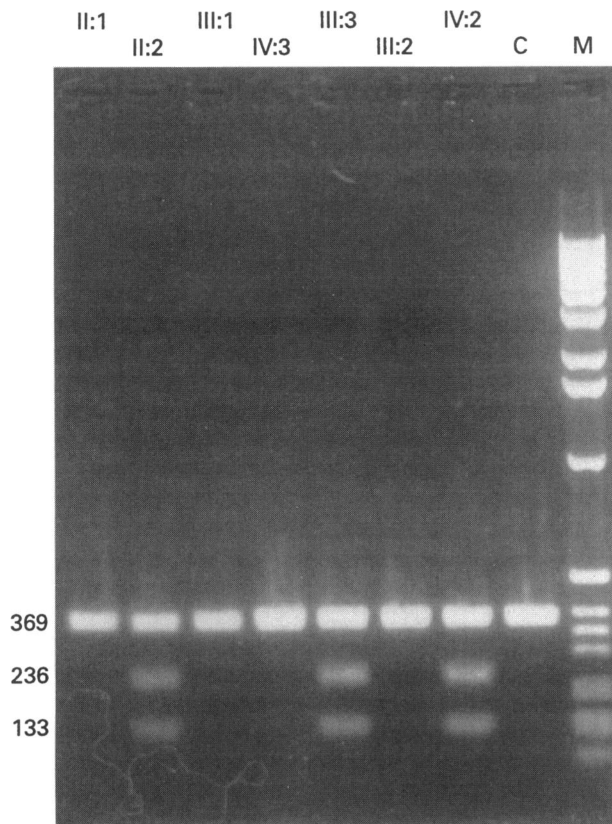

Fig. 2. Agarose gel electrophoresis shows polymerase chain reaction (PCR) products of the protein C gene exon five after digestion with restriction enzyme Bsi EI. In lanes 1–7 the PCR products of the patient (IV : 2) and six relatives, in lane 8 of a control (C) and in lane 9 a molecular weight marker (M) are shown. The normal PCR product is 369 bp. In lane 2 (II : 2), lane 5 (III : 3) and lane 7 (IV : 2) the defect PCR product shows as 236 bp + 133 bp, because the ^{72}GGC for ^{72}CGC transversion introduces a restriction site for Bsi EI.

DISCUSSION

The combined defect in the complement and haemostatic system in our patient, a 7-year-old boy with fulminant meningococcal septic shock and a pulmonary embolus after surgical intervention, consists of a properdin deficiency type 1 and a heterozygous protein C deficiency type I. Combined inherited defects of these systems are rarely described. Only two haemophilia A patients with either complement C2 or C6 deficiency have been reported [23,36]. Among 11 relatives of the proband the properdin deficiency was present in the grandfather, whilst the protein C deficiency was present in the grandmother. The defects passed in combination by the mother to the proband. Since the coding gene for protein C is on chromosome 2 [7] and for properdin on the short arm of the X-chromosome [15], a linkage between the coding genes for these deficiencies is impossible. The properdin deficiency cosegregated with a distinct microsatellite haplotype of the X-chromosome (three points Lodscore 2·25). The crossing over on the X-chromosome with the marker DXS893 in a non-affected grand uncle (Fig. 1, II : 5) of the proband may be due to the localization of this marker on the long arm of the X-chromosome, as recently reported [27]. The cosegregation of the protein C gene mutation with a low protein C antigen level as well as a reduced activity indicate that the ^{72}Gly for Arg replacement is the causative mutation. The novel mutation reported here is in agreement with the finding that protein C type I deficiency is often associated with missense mutations of the protein C gene [34].

Consequences of the inherited properdin and protein C deficiencies with regard to meningococcal disease are different. Properdin deficiency is associated with an enhanced risk of meningococcal disease [11], whilst protein C deficiency type I is associated with the risk of deep venous thrombosis and coumarin-induced skin necrosis [7], but not yet reported in association with meningococcal disease.

The high susceptibility for meningococcal disease in properdin deficiency may be due to low C3b generation on the surface of the meningococcus by the alternative pathway and classical pathway-induced amplification loop, resulting in inefficient serum-mediated lysis and phagocytosis of meningococci [37,38]. Meningococcal infection in properdin-deficient patients occurs usually at a later age (mean 14 years) than in the general population (3 years) [11,39] and is often due to uncommon serogroups such as W135, Y and X [11,12,40]. Our patient also developed his meningococcal disease at the relatively late age of 7 years, and serogroup Y was isolated from the blood. Generally, meningococcal disease due to uncommon serogroups is associated with more fulminant disease [41] and the fatality rate of properdin-deficient patients with meningococcal disease is 28% [11]. However, our experience is that in nine families, encompassing 21 properdin-deficient relatives, none of them died from a meningococcal disease [13], and recently Schlesinger *et al.* reported also only mild meningococcal disease in three properdin-deficient families [14]. We hypothesize that the fulminant course of meningococcal disease in the properdin proband is due to the concomitant presence of protein C deficiency, since protein C plays a central role in modulating endotoxin-mediated coagulopathies [42].

Protein C, activated upon binding of thrombin to the endothelial membrane receptor thrombomodulin [7], inactivates subsequently the procoagulant cofactors Va and VIIIa, and down-regulates the coagulation cascade, limiting the deposition of fibrin [7]. In addition, protein C is supposed to have a neutralizing effect on plasminogen activator inhibitor-1 (PAI-1), resulting in an increased fibrinolysis [7]. A mutation in coagulation factor V (FV Leiden) may result in resistance to inactivation by protein C [19], but factor V of our patient did not show the FV Leiden mutation. Inactivation of procoagulant factor Va by protein C is about 10-fold increased in the presence of free protein S [7]. The major part (60–65%) of plasma protein S is inactively present, being bound reversibly to the acute-phase protein C4 binding protein (C4 bp) [7,43]. Binding of protein S to C4 bp has no effect on the functional activity of C4 bp in the complement system [44]. C4 bp is a complex hetero-oligomeric protein composed of α and β polypeptide chains, of which the latter binds protein S [43]. In serum C4 bp is present in three molecular forms with different α, β chain ratios [43,47]. Although being an acute-phase protein, C4 bp levels are reported not to be high in fulminant meningococcal septic shock [45]. Our patient had at admission a C4 bp level of 45% (reference value 80–170%), probably reflecting the increased consumption due to the classical pathway of complement activation.

Our patient had at admission very low levels of protein C and low levels of both free and total protein S. The finding of

low total protein S indicates either enhanced consumption or reduced production. Because the function of the protein C anticoagulant pathway depends on the level of both protein C and free protein S, low levels of these proteins may explain the occurrence of severe haemorrhagic skin lesions. Declining levels of protein C in patients with meningococcal sepsis are reported to be commensurate with the rapidly progressing purpura fulminans due to dermal microvascular thrombosis and subsequent haemorrhagic necrosis induced by meningococcal endotoxin [3]. The proband had at admission the lowest protein C values and experienced the most severe sequelae among 35 patients who had been admitted to the Paediatric Intensive Care Unit of the Academic Medical Centre with fulminant meningococcal septic shock in the period 1990–93 [45].

Pulmonary embolism following the amputations may also be due to protein C deficiency, since protein C-deficient individuals experience their venous thrombosis in about 50% of cases as pulmonary embolus [46]. Serum level of C4 bp might increase up to 286% in response to surgery [43], resulting in a declined level of free protein S, enhancing the risk of embolism [7,43]. Whether the α and β chains increase equally is not yet clear [47].

The family history of the patient did not reveal an increased incidence of Neisserial infections despite the properdin deficiency among the relatives. In the family history, the finding of a fatal cerebral arterial thrombosis in the brother of the proband may point to heterozygous protein C deficiency type I. Central nervous system thrombotic events have been described in homozygous protein C-deficient infants [47]. In dysmature neonates the plasma level of both protein C antigen and protein C function is reduced [6,48]. In a dysmature neonate having a heterozygous protein C type I deficiency the levels of protein C may approximate the levels (lower than 5% of normal protein C levels) found in homozygous protein C-deficient patients.

Protein C-deficient patients with developing purpura fulminans may benefit from protein C infusion. The administered protein C would down-regulate excess generation of thrombin and simultaneously dissolve the excessively formed thrombi by inactivation of plasminogen activator inhibitor. Treatment with protein C concentrate has been reported. A 13-year-old boy with acquired protein C deficiency due to bacterial sepsis combined with a varicella infection [49], newborns with homozygous protein C deficiency and purpura fulminans [48] and an adult with coumarin-induced purpura fulminans [50] were treated successfully.

Prevention of meningococcal disease in properdin-deficient persons by vaccination with the currently available tetravalent (ACYW) meningococcal polysaccharide vaccine has been advocated [10,11]. The risk for meningococcal disease in protein C-deficient patients is unknown, so whether protein C-deficient individuals should also be vaccinated with this vaccine to prevent fulminant meningococcal disease, still remains to be investigated.

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