

Isolated antiplasmin deficiency presenting as a spontaneous bleeding disorder in a 63-year-old man

Vallathucherry C. Harish, Lin Zhang, Jason D. Huff, Heather Lawson and John Owen

Spontaneous bleeding in adults is a major problem, and in a significant number of these patients no cause is found. A 63-year-old Caucasian man presented to our hematology clinic with a large hematoma of his left thigh. Initial investigations did not show any conclusive abnormalities of primary or secondary hemostasis. Subsequent tests demonstrated a type 1 deficiency of antiplasmin. Treatment with low doses of ϵ -aminocaproic acid resulted in resolution of the hematoma and control of bleeding. We sought to determine the cause of the patient's isolated antiplasmin deficiency but no explanation was found. Three heterozygous polymorphisms were identified in his antiplasmin gene, ruling out major gene deletions. Each of these three polymorphisms has been previously reported in healthy blood donors. Finally, since response to antifibrinolytics can be dramatic, deficiencies of antiplasmin

must be considered in patients presenting at any age with a spontaneous bleeding disorder. *Blood Coagul Fibrinolysis* 17:673–675 © 2006 Lippincott Williams & Wilkins.

Blood Coagulation and Fibrinolysis 2006, 17:673–675

Keywords: antifibrinolytics, antiplasmin, bleeding disorder, polymorphism

Section on Hematology and Oncology, Department of Medicine, Wake Forest University School of Medicine, Winston-Salem, North Carolina, USA

Correspondence and requests for reprints to Dr Vallathucherry Harish, MD, Section on Hematology and Oncology, Medical Center Boulevard, Winston-Salem, NC 27157, USA
Tel: +1 336 7166777; fax: +1 336 8022501; e-mail: vharish@triad.rr.com

Sponsorship: This work was funded in part by the MacKay Foundation for Cancer Research.

Received 19 April 2006 Accepted 28 August 2006

Introduction

Spontaneous bleeding in adults is not an uncommon clinical presentation. In a majority of patients, an underlying cause for the bleeding is found. These include disorders of primary and secondary hemostasis such as inhibitors to coagulation factors, chronic liver disease and disseminated intravascular coagulation. There still is a minority of patients, however, in whom, despite extensive evaluation, no clear cause for the bleeding is identified.

We were faced with such a situation when a 63-year-old white man presented with a 1 week history of spontaneous bruising of his right flank and left thigh. Initial investigations did not reveal any platelet abnormalities. The prothrombin time and activated partial thromboplastin time were prolonged but there were no consistent coagulation factor abnormalities. We therefore went on to investigate the fibrinolytic system, which revealed antiplasmin deficiency [1]. The following presentation details the series of investigations undertaken to determine the cause of the patient's bleeding disorder.

Case report

Our patient, a 63-year-old white man, presented to our hematology clinic with complaints of spontaneous bruising for 1 week. He had no history of trauma, previous bleeding episodes or a family history of bleeding disorders. Physical examination revealed bruises in his right flank and a $0.15 \times 0.2 \text{ m}^2$ left inner-thigh hematoma. Initial laboratory studies, presented in Table 1, demon-

strated a hemoglobin level of 98 g/l and a normal platelet count. The prothrombin time and activated partial thromboplastin time were prolonged at 17.1 and 38.9 s, respectively. A mixing study failed to fully correct, but did not give the typical pattern of a coagulation factor inhibitor. A lupus inhibitor screen was negative. Coagulation factor assays did not reveal any conclusive abnormalities. The patient's antiplasmin activity, however, was at 35% of normal. A type-1 defect was identified when a Laurell immunoassay showed that his antigen was proportionately decreased at 30%.

The patient was treated with low doses of ϵ -aminocaproic acid for his antiplasmin deficiency. This resulted in resolution of the hematomas, normalization of his hemoglobin and no further bleeding episodes. To better characterize his type-1 antiplasmin deficiency we proceeded to sequence his antiplasmin gene.

Fifteen months from original presentation, the patient was admitted to our institution with anasarca and new-onset nephrotic range proteinuria. Treatment with ϵ -aminocaproic acid was continued but his hospitalization was complicated by a sepsis syndrome, multi-organ failure and death. At the request of his family no autopsy was performed.

Materials and methods

Platelet aggregation studies and coagulation factor assays were performed by standard techniques. Activatable

Table 1 The levels of coagulation, hemostatic factors and other variables at the time of presentation

	Patient	Normal range
Hemoglobin (g/l)	98	120–160
Platelets ($\times 10^9/l$)	300	160–360
Prothrombin time (s)	17.1	10.8–13.9
Activated partial thromboplastin time (s)	38.9	<34
Fibrinogen (g/l)	3.47	1.80–3.63
D-dimer ($\mu\text{g/ml}$)	2.0	<2.30
Lupus inhibitor	Absent	Absent
Factor II activity (%)	98	75–135
Factor V activity (%)	56	50–150
Factor VII activity (%)	101	65–135
Factor VIII activity (%)	78	50–200
Factor IX activity (%)	76	50–200
Factor X activity (%)	46	45–155
Factor XI activity (%)	80	65–135
Factor XII activity (%)	29	50–150
Factor XIII activity	Normal	
Platelet aggregation	Normal	
Ristocetin-induced platelet aggregation	Normal	
Euglobin clot lysis time (min)	>200	>180
Plasminogen activity (%)	70	70–120
Plasmin-antiplasmin complex (ng/ml)	78	0–514
Tissue plasminogen activator (ng/ml)	3.12	2–8
Antiplasmin activity (%)	35%	85–135

plasminogen was measured by a chromogenic assay utilizing streptokinase as the activator (Technoclone, Vienna, Austria). Tissue plasminogen activator levels and plasmin–antiplasmin levels were measured by sandwich enzyme-linked immunosorbent assay technique (Technoclone). A chromogenic assay was used to determine antiplasmin function (Quest Labs, San Juan, California, USA).

Antiplasmin was measured by electroimmunodiffusion (Laurell technique) using polyclonal rabbit anti-human antiplasmin (Nordic Labs, Tillburg, The Netherlands). Pooled normal plasma obtained from 20 normal donors was used as the 100% standard. The coding sequence of the patient's antiplasmin gene was determined using genomic DNA extracted from whole blood using a QIAmp mini kit (Qiagen, Valencia, California, USA). Polymerase chain reaction primers were designed from the published genomic sequence [2], using the Primer-3 program [3]. Primers for exons 1–10, inclusive of the promoter region and splice junctions, are detailed in Table 2.

Exons 1–10, splice junctions and the promoter region of the antiplasmin gene were amplified by the polymerization chain reaction method in a minicycler using a touchdown program [4]. In this program denaturation occurs at 92°C for 1 min and amplification proceeds for 1 min at 72°C during each cycle. During initial amplification, annealing temperatures decrease from 70 to 55°C over 14 cycles. Subsequent amplification proceeds after the products have been annealed at 55°C for 25 cycles. Exon 10 was amplified in four overlapping sections due to its large size (1143 base pairs). Polymerase chain reaction products were purified using the Promega Wizard DNA

Table 2 Polymerase chain reaction primer sequences used in the amplification of 10 exons of the antiplasmin gene

Region amplified	Size (base pairs)	Primer sequence
Exon 1	232	5'-agccatcaccctgctta-3' 5'-agaacatcgccatgagcaac-3'
Exon 2	234	5'-gggatgtgagatgggaacag-3' 5'-caggggagaactgtggagaa-3'
Exons 3 and 4	293	5'-gggacctctatcctcatcc-3' 5'-ctagccccgccactctt-3'
Exon 5	286	5'-gagctgacccttgacctc-3' 5'-ctcccagctcttggctg-3'
Exon 6	243	5'-cagtgggggtgagaaaggac-3' 5'-accaggggcaggactgag-3'
Exon 7	294	5'-ctggagccctgggaacag-3' 5'-acgtccagctcagcctac-3'
Exon 8	299	5'-aagctgtgcccatcgac-3' 5'-ctccacagcctgtccactc-3'
Exon 9	373	5'-acttagctcggggcttct-3' 5'-ggaaaagagcaggacacag-3'
Exon 10A	395	5'-gcagctctgaccagccatc-3' 5'-ttaaagtcaggccgaaaag-3'
Exon 10B	399	5'-gcaacaaggactctccag-3' 5'-ctagaagcacctccctctg-3'
Exon 10C	389	5'-cttccaacaggctcagagg-3' 5'-gaggaaggaaggaaatgct-3'
Exon 10D	294	5'-cttgtcacgccagactcc-3' 5'-ggttctcagcagctccac-3'

purification system. Nucleotide sequencing was performed by the Dye-deoxy method (ABI 377; PE Biosystems, Foster City, California, USA).

Results

Hemostatic factors did not show any consistent defects and are detailed in Table 1.

Antiplasmin levels in our patient were consistently decreased at 30–35% of normal. Both the antigen and activity of antiplasmin were proportionately decreased, thus identifying a type-1 defect. The sequence of his antiplasmin gene showed no candidate mutations that would explain his deficiency. All splice junctions and 70 base pairs upstream of exon 1 were normal. Three heterozygous polymorphisms were found. The first polymorphism, C to T, in exon 2 causes substitution of alanine by valine at position –26 in the signal peptide [5]. The next polymorphism, C to T, in exon 3 causes substitution of arginine by tryptophan at position 6 and, finally, G to A in exon 10 causes substitution of arginine by lysine at position 407. These three polymorphisms have been previously found in blood donors [6]. Their presence in our patient ruled out major gene deletions.

Discussion

In this report we describe an older patient who presented with a new spontaneous bleeding disorder. Investigations demonstrated an isolated type-1 deficiency of antiplasmin that was successfully treated with ϵ -aminocaproic acid.

Antiplasmin, a serine protease inhibitor [7], is the most important physiological inhibitor of plasmin [8]. It is synthesized primarily in the liver [9] with a plasma

concentration of 0.7 mg/l. The human gene for antiplasmin was sequenced in 1988 [2] and has been mapped to human chromosome 17 [10]. The gene contains 10 exons and nine introns spanning approximately 16 kbases DNA [2].

The amino acid sequence of antiplasmin has been deduced and the full-length mature protein is 464 amino acids long [5] and contains three functional regions. The 464-amino-acid form (Met form) is converted by the antiplasmin-cleaving enzyme [11] to the 452-amino-acid form (Asn form) [5]. The Asn form is present in plasma as 60% of the total antiplasmin [5] and is physiologically more active than the Met form [12].

Congenital deficiencies of antiplasmin leading to bleeding are very rare and the real prevalence of these disorders is not known [13]. Congenital antiplasmin deficiency is inherited in an autosomal recessive fashion. Homozygotes in general have a severe bleeding disorder, while heterozygotes have a milder version [14]. Some patients with heterozygous mutations in the antiplasmin gene have presented with clinically significant bleeding late in life [15]. Based on these reports, we investigated the possibility of a heterozygous mutation in the antiplasmin gene causing a bleeding disorder in our patient. On sequencing, his antiplasmin gene was found to have three heterozygous polymorphisms that have been identified in blood donors [6]. The polymorphisms are widely distributed in the gene sequence, effectively ruling out a major gene deletion. Small deletions were ruled out by the sequence results.

This raises important questions about the patient's antiplasmin deficiency. Having ruled out a major gene deletion, how might the lack of antiplasmin be explained? Either epigenetic factors involved in the regulation of gene transcription or translational factors could be responsible for his deficiency. Although non-neutralizing antibodies [16] to antiplasmin causing increased clearance have not been reported in the literature, this could explain the patient's type-1 deficiency.

Another important question is why do people with heterozygous deficiencies of antiplasmin present with bleeding late in life? Age-related vascular or connective tissue defects may unmask a dormant-inherited bleeding disorder, although none was identified in our patient. Unfortunately his demise due to the development of nephrotic syndrome and sepsis leaves us without answers to these questions. There is no documented case linking nephrotic syndrome with the use of ϵ -aminocaproic acid.

In conclusion, deficiency of antiplasmin must be considered in the work-up of any patient who presents with a spontaneous bleeding disorder [1], even in a older patient, since the response to antifibrinolytics can be dramatic.

Acknowledgements

The authors would like to thank Ms Martha Burke and Dr Mary Ann Knovich for their assistance.

References

- Griffin GC, Mammen EF, Sokol RJ, Perrotta AL, Stoyanovich A, Abildgaard CF. Alpha 2-antiplasmin deficiency. An overlooked cause of hemorrhage. *Am J Pediatr Hematol Oncol* 1993; **15**:328–330.
- Hirosawa S, Nakamura Y, Miura O, Sumi Y, Aoki N. Organization of the human alpha 2-plasmin inhibitor gene. *Proc Natl Acad Sci U S A* 1988; **85**:6836–6840.
- Rozen S, Skaletsky H. Primer3 on the WWW for general users and for biologist programmers. *Methods Mol Biol* 2000; **132**:365–386.
- Hecker KH, Roux KH. High and low annealing temperatures increase both specificity and yield in touchdown and stepdown PCR. *Biotechniques* 1996; **20**:478–485.
- Bangert K, Johnsen AH, Christensen U, Thorsen S. Different N-terminal forms of alpha 2-plasmin inhibitor in human plasma. *Biochem J* 1993; **291** (Pt 2):623–625.
- Lind B, Thorsen S. A novel missense mutation in the human plasmin inhibitor (alpha2-antiplasmin) gene associated with a bleeding tendency. *Br J Haematol* 1999; **107**:317–322.
- Holmes WE, Nelles L, Lijnen HR, Collen D. Primary structure of human alpha 2-antiplasmin, a serine protease inhibitor (serpin). *J Biol Chem* 1987; **262**:1659–1664.
- Collen D. Identification and some properties of a new fast-reacting plasmin inhibitor in human plasma. *Eur J Biochem* 1976; **69**:209–216.
- Aoki N, Yamanaka T. The alpha2-plasmin inhibitor levels in liver diseases. *Clin Chim Acta* 1978; **84**:99–105.
- Welch SK, Francke U. Assignment of the human alpha 2-plasmin inhibitor gene (PLI) to chromosome 17, region pter-p12, by PCR analysis of somatic cell hybrids. *Genomics* 1992; **13**:213–214.
- Lee KN, Jackson KW, Christiansen VJ, Lee CS, Chun JG, McKee PA. Antiplasmin-cleaving enzyme is a soluble form of fibroblast activation protein. *Blood* 2006; **107**:1397–1404.
- Sumi Y, Ichikawa Y, Nakamura Y, Miura O, Aoki N. Expression and characterization of pro alpha 2-plasmin inhibitor. *J Biochem (Tokyo)* 1989; **106**:703–707.
- Favier R, Aoki N, de Moerloose P. Congenital alpha(2)-plasmin inhibitor deficiencies: a review. *Br J Haematol* 2001; **114**:4–10.
- Aoki N. Genetic abnormalities of the fibrinolytic system. *Semin Thromb Hemost* 1984; **10**:42–50.
- Ikematsu S, Fukutake K, Aoki N. Heterozygote for plasmin inhibitor deficiency developing hemorrhagic tendency with advancing age. *Thromb Res* 1996; **82**:129–116.
- Bajaj SP, Rapaport SI, Barclay S, Herbst KD. Acquired hypoprothrombinemia due to non-neutralizing antibodies to prothrombin: mechanism and management. *Blood* 1985; **65**:1538–1543.