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CASE REPORT

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Hereditary protein S deficiency associated with rheumatoid arthritis: a case report of pulmonary embolism after total hip arthroplasty

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Abstract A 57-year-old woman with RA of 10 years' duration presented with a history of right coxalgia. Three weeks after total hip arthroplasty, the patient developed an acute pulmonary embolism. The results of screening for hypercoagulable states revealed a protein S (PS) deficiency, and all PS values, i.e., total PS antigen, free PS antigen, and PS cofactor, were lower than the normal ranges, showing that the patient had type I PS deficiency. She had no past history of embolism or deep venous thrombosis. The values of three PS-related parameters were also lower than normal in her daughter. The responsible mutation may be located on exon 15 of genomic PS DNA, as indicated by polymerase chain reaction. We therefore diagnosed hereditary PS deficiency.

Key words Hereditary protein S deficiency · Pulmonary embolism · Rheumatoid arthritis (RA)

Introduction

Pulmonary embolism (PE) is a well-known complication associated with total hip or knee arthroplasty.[1,2] Several factors predisposing to deep venous thrombosis (DVT), such as aging, obesity, prolonged immobilization, and congenital or secondary thrombotic states and thrombophlebitis, have been reported.[3,4]

The protein S (PS) locus has been mapped to chromosome 3, and PS deficiency is inherited in an autosomal-dominant fashion, while a more severe form of PS deficiency is inherited in an autosomal-recessive fashion. Hereditary PS deficiency is a recognized risk factor for thrombotic disease and appears to account for approximately 5%–8% of cases of hereditary thrombophilia.[5] This is the report of a case of PE caused by hereditary PS deficiency genetically diagnosed after total hip arthroplasty (THA) for rheumatoid arthritis (RA). The patient had no past history of DVT or embolism.

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Case report

A 57-year-old uniparous woman who fulfilled the diagnostic criteria of the American College of Rheumatology was diagnosed with RA (stage III) of more than 10 years' duration.

Present illness

Severe right coxalgia developed in February 1997. She was admitted to this hospital for THA on November 30, 1998. She was then receiving oral prednisolone (2.5 mg), dicrofenac sodium (75 mg), and bucillamine (a disease-modifying antirheumatic drug) (100 mg) daily. She had no history of any other significant disease in the past and did not smoke or drink alcohol.

Physical status

Blood tests revealed a moderate increase in serum C-reactive protein level (0.6 mg/dl) and an erythrocyte sedimentation ratio of 37 mm/h. Her coagulation profile revealed a platelet count of $29.3 \times 10^4/\mu l$ (normal range 20–40 $\times 10^4/\mu l$), bleeding time of 2 min (normal range 1–3 min), prothrombin time (PT) of 12.5 s, prothrombin activity of 108.6% (normal range 70%–130%), and activated partial thromboplastin time (APTT) of 29.0 s (normal range 24–37 s). Right THA was performed on December 21, 1998, using a cup without cement and a stem with cement, i.e., the hybrid technique. She was able to be transferred to a wheel-

chair on December 23 after the removal of a drainage tube, and partial weight-bearing was planned to start 3 weeks after the surgery.

At the time weight-bearing began, on January 10, 1999, at 14:30, she developed sudden chest pain, breathing difficulty, and tachycardia. Arterial blood gas measurement revealed PaO_2 and $PaCO_2$ of 78.3 mmHg and 22.5 mmHg, respectively, and SaO_2 of 97.6%, which suggested hyperventilation. Her symptoms momentarily resolved, but at 16:20 her conciousness level decreased, although she recovered within 5 min. At 16:45, after 25 min, vomiting and tachypnea were noted. Her conciousness level and respiratory function again deteriorated. At that time her blood pressure could not be recorded, she was resuscitated, and assisted ventilation was instituted. Brain computed tomography (CT) did not reveal any abnormal findings. PE or acute myocardial infarction were suspected, and heparin sodium (5000 IU) was administered immediately.

A chest x-ray revealed dilation of the pulmonary arteries in the hilar region, while peripheral vascular markings were diminished bilaterally in the middle and lower lung fields. Echocardiography revealed pressure overload of the right ventricle and regurgitation of the tricuspid valve. Pulmonary arterial thromboembolisms in the right middle and lower pulmonary arteries and left lower pulmonary artery were diagnosed by emergent pulmonary angiography (Fig. 1). Selective thrombolysis was performed using 420×10^4 IU tissue plasminogen activator to the right middle and lower arteries, and 300×10^4 IU to the left lower artery, and successful recanalization of the thrombolyzed pulmonary arteries was achieved. Additional warfarinization was also performed.

Cause of the PE

To investigate the cause of the thrombosis or embolism, we examined the presence of inherited thrombotic disorders, including antithrombin III deficiency, anticardiolipin IgG/IgM antibodies, and protein C (PC) and PS deficiency. Except for PS levels, all other factors were within normal limits. At the time of the occurrence of the PE, the patient's total PS antigen level was 49% (normal range 65%–135%) and her PS cofactor activity level was 24% (normal range 60%–150%) using enzyme-linked immunosorbent assay (ELISA) (ELISA PS, Boehringer, Mannheim, Germany). To rule out the influence of the anticoagulants, PS levels were again measured on May 14. The total levels of PS antigen, free PS antigen, and PS cofactor activity were 58%, 27% (normal range 60%–150%), and below 10%, respectively. We measured the PS levels to determine whether the PS deficiency had been transmitted from this patient to her daughter in an autosomal-dominant fashion, or whether she had acquired secondary deficiency. After obtaining informed consent, measurement of the PS levels of the daughter, showed a total PS antigen level, free PS antigen level, and PS cofactor activity of 50%, 30%, and 31%, respectively. Thus, all the PS levels had decreased to a similar extent in the daughter as in the mother (Table 1).

These results show that the PS deficiency in this patient had been inherited in an autosomal-dominant fashion and was not acquired, and that this deficiency could be classified as type I according to the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis (München, July 1992). This classification divides PS deficiency into three types. Type I deficiency is defined as a quantitative defect, type II deficiency as a functional defect caused by a dysfunctional PS molecule, and type III deficiency is characterized by decreased levels of functionally active free PS antigen with normal levels of total PS antigen. We then examined peripheral blood samples to investigate the genomic DNA responsible for the hereditary PS deficiency.

PCR amplification of PS genomic DNA

Total genomic DNA was extracted from peripheral blood cells using a rapid DNA Extractor WB-Rapid Kit (Wako Pure Chemical, Osaka, Japan). The primers used for the

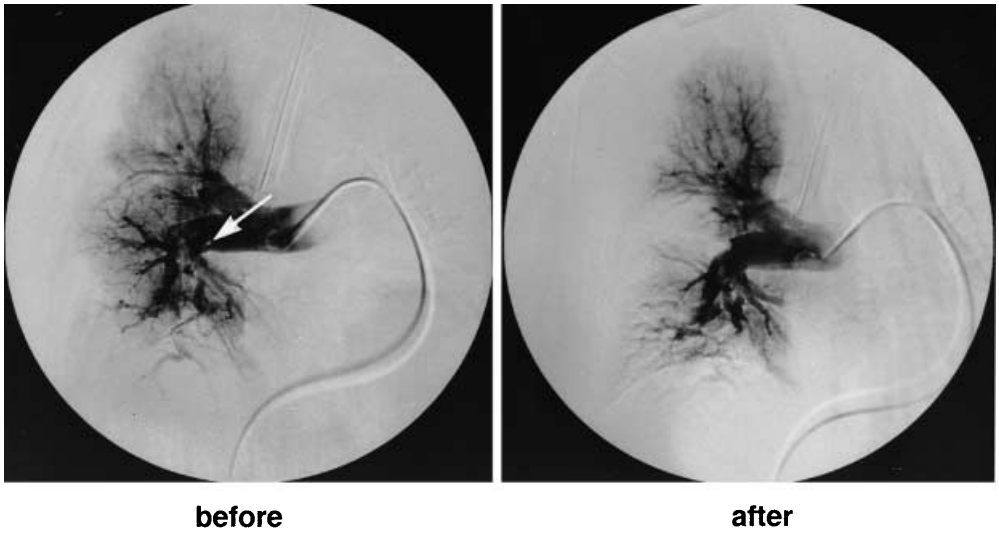

Fig. 1. Right pulmonary angiography. Selective thrombolysis was performed using a tissue plasminogen activator, 420×10^4 IU, to the right middle and lower arteries, and successful recanalization of the thrombolized pulmonary arteries was achieved. The *arrow* indicates the thrombus

Table 1. Protein S (PS) values of the patient and her daughter

Subject	Total PS antigen (%) (65%–135%)	Free PS antigen (%) (60%–150%)	PS cofactor (%) (60%–150%)	Initial phenotypic diagnosis
Patient				
1st (January 11)	49↓		24↓	Type I
2nd (May 14)	58↓	27↓	10>↓	Type I
Daughter	50↓	30↓	31↓	Type I

All the PS, total PS antigen, free PS antigen, and PS cofactor values of the patient and her daughter were lower than the normal limit, and this PS deficiency was classified as Type I. The data within parentheses show the normal ranges

DNA amplification were from PS gene regions that differed from the pseudogene sequences[5]: primer 1 was 5′-CATAATGATATTAGAGCTCAC-3′ and primer 2 was 5′-TTCTTAGATAGCAAGAGAAGT-3′(exon 15) from the complementary strand. The polymerase chain reaction (PCR) conditions used were as follows: 1-min denaturation at 95°C, 1-min annealing at 50°C, and 2-min extension at 72°C; PCR amplification was performed for 40 cycles in a volume of 50 μl of a mixture containing 2.5 units of Taq polymerase, 0.5 μg human genomic DNA, 100 p moles of each primer in 800 μM dNTPs, 2.5 mM $MgCl_2$, and 1 × PCR reaction buffer (Takara Biomedicals, Tokyo, Japan). PCR with primers resulted in the amplification of a fragment of 271 bp.

Restriction analysis of the amplified fragments

The PCR products (30 μl) were digested with the restriction enzyme BstXl (Boehringer, Mannheim, Germany) and two polymorphic alleles were detectable: allele a of 271 bp and allele b of 235 bp from genomic DNA. The a allele corresponds to the codon CCG, and the b to the codon CCA. The two polymorphic alleles were electrophoresed on 12% polyacrylamide gel. Only the b band was detected in the sample from a healthy individual, but the sample from the patient exhibited two bands (Fig. 2).

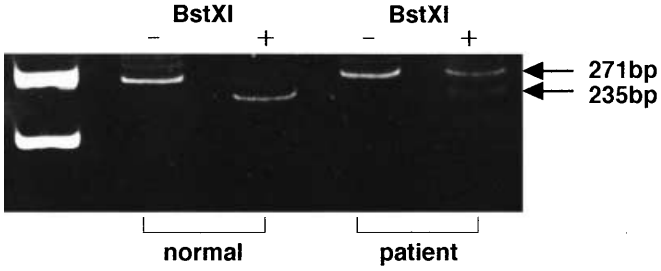

Fig. 2. Polymerase chain reaction products were digested with the restriction enzyme BstXl and two polymorphic alleles were detected, i.e., 271 bp and 235 bp from genomic DNA. After restriction by BstXl, only the 235 bp band was detected from a sample from a healthy individual, but the sample from the patient showed two bands (271 bp and 235 bp)

Discussion

PS is a single-strand glycoprotein with a MW of 76000 daltons, and is a vitamin K-dependent plasma protein. PS acts as a cofactor for activated PC which regulates blood coagulation by inactivating factors V(a) and VIII(a) in the coagulation cascade,[6] and stimulates fibrinolysis by decreasing the activity of plasminogen activator-inhibitors;[7] complete deletion or mutation of PS induces a hypercoagulable state. The active PS gene consists of 15 exons in a DNA region, but its analysis is complicated by the presence of a pseudogene.[5] A study of PS gene polymorphisms and lesions could help to define PS defects and their molecular basis.[8]

The physiological importance of PS has been established by the observation of families with PS deficiency presenting with recurrent thrombosis or recurrent DVT.[9] Pabinger et al.[10] reported a prospective cohort study which showed that asymptomatic relatives of symptomatic patients with a PC or PS deficiency are at increased risk of thrombosis compared with those with no relatives with a deficiency. In the genetic analysis of PS deficiency, PS mRNA studies in association with clinical laboratory findings are valuable to distinguish between inherited and acquired PS deficiency, to classify inherited deficiency into one of the three types, and to determine the most efficient strategies to avoid interference from the inactive pseudogene.

Several point mutations have been reported in type I PS deficiency. For example, a premature termination, caused by a nonsense or frame-shift mutation, has been found to reduce mutated mRNA accumulation.[11] An alternative splicing or a gross gene abnormality would also be expected to affect the metabolism of the mutated mRNA.[4] Thus, type I PS deficiency is characterized by a reduced PS level. Recently, measurement of the CCA/CCG allelic ratio at the mRNA level has also helped to distinguish type I from type III PS deficiency.[12] However, PS deficiency is based on several mutations from exon 1 to 15 in the genomic DNA. In our results, we confirmed that the PS deficiency in this patient was the result of heterozygosity for the polymorphism and based on CCA/CCG in exon 15.

On the other hand, PE is a well-known complication of THA or total knee arthroplasty, and recently the incidence has been 1.6%, i.e., lower than previously reported.[13] In a comparison between cemented and cementless THA, the

incidence of PE was 5.3% following cementless THA and 4.3% following cemented THA; the difference was not significant.[1] Haake and Berkman[14] emphasized the clinical importance of PE after total joint arthroplasty, and reported that 68% of all postoperative deaths were attributable to PE. The incidence of fetal PE has previously been reported to be as high as 2.3%–3.4%,[15,16] but currently, the incidence is reported to be 0.34% lower than before.[2]

With regard to the history of PE after total arthroplasty, 10% of cases occurred within the first week, and 64% occurred by the end of the second week. An additional 23%, however, had symptomatic PE for more than 3 weeks after THA surgery.[16] Several investigators suggest anticoagulant therapy using low molecular-weight heparin for about 1 month, because the risk of late DVT (3 weeks after surgery) remains high, and the occurrence of DVT after THA was reported to be lower with heparin than that in a placebo group.[17,18]

The relationship between PE and DVT is not well understood; In autopsy studies, Havig[19] showed that 75% of patients with DVT had pathological evidence of PE. The risk factors for DVT are thought to be increasing age (more than 40 years), a history of thrombosis, varicose veins, obesity, pre- and postoperative immobilization, pelvic and lumbar surgery, and prolonged immobilization, among others. In this patient, multiple DVT occurred during the perioperative to postoperative period; patients with a hypercoagulable state are predisposed to DVT and PE.

Patients with symptomatic PS or PC deficiency should receive long-term warfarin therapy, which decreases the concentrations of the procoagulant vitamin K-dependent proteins II, VII, IX, and X, as well as those of PS and PC. Warfarin therapy is effective in preventing thrombosis and thrombosis has been reported to recur when therapy is inadequate or has been stopped.[20] We suggest that prophylactic treatment should be mandatory in high-risk situations predisposing to DVT, such as hereditary or acquired hypercoagulable state as well as PS or PC deficiency. In summary, we diagnosed PS deficiency following THA for RA, and clarified that the patient's daughter also had PS deficiency.

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