Severe thrombophilia in a paediatric patient with end-stage renal disease: detection of the prothrombin gene G20210A mutation

Christian Stier, Bernd Pötzsch, Gert Müller-Berghaus, Marianne Soergel and Günter Klaus

1Department of Pediatrics, Philipps University Marburg and 2Max-Planck-Institute for Physiological and Clinical Research, Bad Nauheim, Germany

Key words: end-stage renal disease; G20210A mutation; thrombophilia

Introduction

Thrombotic events are frequent among patients with end-stage renal disease (ESRD) [1]. In previous studies changes of the coagulation system induced by chronic haemodialysis treatment have been identified as acquired risk factors for increased thrombophilia in these patients [1,2]. The impact of genetic thrombophilic risk factors, however, remains unknown. In the present case, we report on a female paediatric patient suffering from severe thrombophilia on haemodialysis. In this patient the prothrombin mutation G20210A has been identified as a genetic risk factor for the development of recurrent fistula and needle thrombosis. Early graft thrombosis has been reported in abstract form for another patient presenting the same mutation [3].

Case

The patient was born in 1982 to caucasian parents. The family history was uneventful. In 1988, she had an unexplained hydrocephalus occlusus, possibly due to mumps. After placement of a ventriculoperitoneal shunt no neurological deficit was observed. The further history was uneventful until July 1993, when she presented with headache, arterial hypertension, and renal failure (creatinine 2 mg/dl). No further abnormalities were detected on physical examination. Urinalysis showed mild proteinuria and haematuria. Immunological work-up including complement system and antibodies (ANA, cANCA, pANCA, anti-DNA) was normal. No evidence for shunt nephritis was found. The patient had not taken any medication except occasional analgetics for headache. A kidney biopsy showed interstitial nephritis with marked interstitial fibrosis. More than 50% of the cortical tubulointerstitial compartment was involved and marked tubular atrophy was found. There were no signs of immune complex glomerulonephritis as found in shunt nephritis.

Renal function declined rapidly. In September 1994 the creation of a dialysis fistula was attempted. Within 3 weeks, four further interventions were required due to recurrent thrombosis despite giving low-dose heparin. Haemodialysis was initiated 1 month later and was complicated by frequent clotting of the canula immediately after puncture that could not be prevented by flushing the needles with heparin. Using systemic anticoagulation (heparin, 60 units/kg i.v.) via a peripheral vein at the contralateral arm prior to placing the needles, dialysis was possible without further complications.

In December 1994 the patient received her first renal transplant. After opening the anastomoses, a very variable organ appearance was noted, with the colour changing between rose and deep blue. Heparin was given within 30 min (150 units/kg bodyweight, i.v.), and was continued for 2 weeks. Immunosuppression included antithymocyte globulin for 2 days, methylprednisolone, azathioprine, and cyclosporin A. The postoperative course was uneventful except for two episodes of unexplained rise in serum creatinine and fever followed by spontaneous, almost complete recovery. A graft biopsy performed after the second episode showed inflammatory changes.

Five months later, steroid-resistant interstitial rejection occurred. Treatment with tacrolimus was partially successful, with a rise of the GFR from 28 to 47 ml/min/1.73 m² maintained for 7 months, followed by a rapid decline in renal function. Biopsy of the kidney transplant was done twice during this period and revealed marked interstitial fibrosis. Haemodialysis was reinstituted in August 1996 using a.v. fistula at the right upper arm. Again, thrombotic occlusion of the shunt occurred, cured by thrombectomy. The patient was given daily low-molecular-weight heparin subcutaneously yielding anti Xa-factor activity of 0.15

Correspondence and offprint requests to: Dr Christian Stier, MZ Kinderheilkunde, Deutschhausstr. 12, D-35033 Marburg, Germany.
Thrombophilia in end-stage renal disease: G20210A mutation

U/mL. Using this regimen, dialysis was performed without further complications. Prolonged menorrhagia was controlled by an oestrogen–gestagen combination.

The coagulation system was examined several times and revealed no evidence of thrombophilia. We excluded factor V Leiden mutation, deficiency of antithrombin (94%), protein S (99%) and protein C (100%), homocystinaemia, lupus anticoagulant (kaolin clotting time, 0.0), and grossly elevated Lp(a) (<12 mg%). Finally, the patient was screened for a new type of mutation described as thrombophilic index TTT-GGA-GAG-TAG-GGG-3 and mutagenic antisense: 5-AAT-AGC-ACT-GGG-AGC-ATT-GAA-GCT-3), 25 ng of factor [4]. Polymerase chain reaction (PCR) analysis of genomic DNA, and 0.1 U of Taq polymerase. The reaction involved 35 cycles of denaturation at 94°C, annealing at 65°C, and extension at 72°C each for 20 s. The PCR product was digested using 0.5 U of endonuclease HindIII at 37°C for 2 h. A fragment of 124 bp was observed in the presence of the mutated prothrombin gene after electrophoresis in 8% polyacrylamide gels. When the normal allele 20210G was present, there was no cleavage site for HindIII and the 142 bp fragment remained intact.

Methods

Detection of the G20210A mutation of the prothrombin gene

Genomic DNA was extracted from peripheral blood by a standard method [5]. A 142 bp fragment of the 3’-untranslated region of the prothrombin gene was amplified by the PCR in a mixture of 54 μM Tris-HCl, pH 8.8, 1.5 mM MgCl2, 10 μM of each dNTP, 500 ng of each primer described by Makris et al. (6, sense: 5-ATT-GAT-CAG-TTT-GGA-GAG-TAG-GGG-3 and mutagenic antisense: 5-AAT-AGC-ACT-GGG-AGC-ATT-GAA-GCT-3), 50 ng of genomic DNA, and 0.1 U of Taq polymerase. The reaction yielded a heterozygotic mutation of the 3’-untranslated region of the prothrombin gene [4], prothrombin G20210A.

In September 1997, the second renal transplantation was performed. The patient was anticoagulated with heparin (500–700 IE/kg/bodyweight) starting intraoperatively (150 IE/kg bodyweight). On the seventh day, severe bleeding in the transplant region occurred, necessitating blood transfusions. Kidney function was not compromised. After discharge, daily subcutaneous low-molecular-weight heparin was continued. No further thromboembolic complications were observed. Immunosuppression included methylprednisolone, mycophenolate-mofetil, and cyclosporin A. Six months after kidney transplantation, the creatinine clearance is stable at 116 ml/min/1.73 m².

Discussion

Generally, a tendency towards thrombosis is induced by increased activation of the clotting cascade or by impaired anticoagulant or fibrinolytic mechanisms. Risk factors include hereditary and acquired conditions. The first genetic risk factors for thrombosis were identified in families in whom the thrombophilia segregated with an abnormal result in a plasma test (protein C and S, antithrombin, and APC resistance) [4]. None of these disorders, nor acquired risk factors except ESRD could be detected in our patient.

Recently, a single-point mutation of the prothrombin gene leading to the substitution of G by A at position 20210 has been described to be an independent risk factor for the development of venous thrombosis [4,7,8]. The G20210A mutation is located in the 3’-untranslated region of the prothrombin gene. An association was found between the presence of the G20210A allele and elevated prothrombin levels [4]. Elevated prothrombin itself is known to be a risk factor for venous thrombosis. The mechanism by which this mutation leads to increased plasma levels of prothrombin is unclear, but a higher stability of the transcribed mRNA has been suspected [4].

Homozygous G20210A mutation has been described in a patient with massive thrombosis leading to myocardial infarction, and subsequent ileofemoral venous thrombosis and massive saddle pulmonary embolus [7]. The common hemizygous state [4] is associated with mild thrombophilia restricted to the venous system. Thrombosis outside the veins in hemizygous subjects has only been described in two patients with ESRD, ours and the patient reported by Oh et al. [3]. We speculate that the association of the G20210A mutation and ESRD greatly increases the thrombotic risk.

The optimal strategy to prevent thrombosis in these patients is not yet defined. During the haemodialysis...
period, continuous treatment with s.c. low-molecular-weight heparin was effective in our patient, as was oral anticoagulation in the other patient [3]. After kidney transplantation, we gave 10 days of therapeutic anticoagulation with unfractioned heparin controlled by the activated PTT (twofold normal). Severe bleeding was observed. We judged the risk of bleeding to be justified by the risk of thromboembolic complications or even loss of the transplant as reported by Oh et al. [3]. The anticoagulant therapy with daily subcutaneous low-molecular-weight heparin has been continued in our patient until now, without further complications.

We suppose that the recurrent fistula and needle thrombosis on haemodialysis were associated with the G20210A mutation in the prothrombin gene. In unexplained thrombophilia in ESRD we recommend a search for this mutation. If it is present, intraoperative anticoagulation for renal transplantation may prevent thrombotic graft loss. Further investigations are needed to evaluate the incidence and the importance of this mutation in patients with ESRD.

References


Received for publication: 26.3.98
Accepted: 27.3.98