Factor V Leiden mutation, prothrombin gene mutation, and deficiencies in coagulation inhibitors associated with Budd-Chiari syndrome and portal vein thrombosis: results of a case-control study

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In a collaborative multicenter case-control study, we investigated the effect of factor V Leiden mutation, prothrombin gene mutation, and inherited deficiencies of protein C, protein S, and antithrombin on the risk of Budd-Chiari syndrome (BCS) and portal vein thrombosis (PVT). We compared 43 BCS patients and 92 PVT patients with 474 population-based controls. The relative risk of BCS was 11.3 (95% CI 4.8-26.5) for individuals with factor V Leiden mutation, 2.1(95% CI 0.4-9.6) for those with prothrombin gene mutation, and 6.8 (95% CI 1.9-24.4) for those with protein C deficiency. The relative risk of PVT was 2.7 (95% CI 1.1-6.9) for individuals with factor V Leiden mutation, 1.4 (95% CI 0.4-5.2) for those with prothrombin gene mutation, and 4.6 (95% CI 1.5-14.1) for those with protein C deficiency. The relative risk of BCS or PVT was not increased in the presence of inherited protein S or antithrombin deficiency. Concurrence of either acquired or inherited thrombotic risk factors was observed in 26% of the BCS patients and 37% of the PVT patients. We conclude that factor V Leiden mutation and hereditary protein C deficiency appear to be important risk factors for BCS and PVT. Although the prevalence of the prothrombin gene mutation was increased, it was not found to be a significant risk factor for BCS and PVT. The coexistence of thrombogenic risk factors in many patients indicates that BCS and PVT can be the result of a combined effect of different pathogenetic mechanisms. (Blood. 2000;96:2364-2368)

Introduction

Budd-Chiari syndrome (BCS) represents occlusion of hepatic outflow either at the level of the hepatic veins or inferior vena cava.[1] Clinically, the disease is characterized by hepatomegaly, manifestations of portal hypertension, and sometimes rapidly deteriorating liver function.[2] Portal vein thrombosis (PVT) often occurs in conditions leading to decreased portal flow and also becomes manifest by symptoms of portal hypertension.[3] Both BCS and PVT have been linked to several hypercoagulable states, primarily myeloproliferative disorders.[4] However, most studies on the pathogenesis of BCS and PVT contain few patients or are hampered by the lack of complete testing for thrombophilia and absence of a well-documented control group.

Resistance to activated protein C due to factor V Leiden mutation is to date the most frequent cause of hereditary thrombophilia.[5,6] Its prevalence in the general white population is approximately 5%, but the relative risk of BCS and PVT for subjects carrying factor V Leiden mutation is uncertain. In a recent study, factor V Leiden appeared to be present in about one fourth of patients with BCS, whereas its occurrence in patients with PVT was negligible.[7]

Carriers of the prothrombin gene mutation, which results from guanine to adenine transition at nucleotide position 20210 in the 3′ untranslated region of the gene, exhibit increased plasma levels of prothrombin and may therefore be at increased risk for venous thromboembolisms.[8] This mutation, also referred to as prothrombin 20210 A variant, has recently been recognized as a risk factor for a selected group of patients with idiopathic PVT.[9] With regard to BCS, the impact of the prothrombin gene mutation is uncertain.[10] For both BCS and PVT, the importance of several other hypercoagulable states, such as inherited deficiencies of the coagulation inhibitors protein C, protein S, and antithrombin, is also unknown because their association with BCS and PVT is based solely on anecdotal reports.[11,12]

We initiated a large multicenter population-based case-control study to establish the influence of factor V Leiden mutation, prothrombin gene mutation, protein C deficiency, protein S deficiency, or antithrombin deficiency on the risk of BCS and PVT.

Patients and methods

Patients were selected by means of a search in the computerized hospital registration systems (ZIS) of 7 academic hospitals in The Netherlands. The hospitals were located in Leiden, Groningen, Rotterdam, Utrecht, Nijmegen, Amsterdam, and Maastricht. All

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patients registered with the diagnosis of BCS or PVT between January 1984 and July 1997 were identified. Diagnostic criteria for BCS and PVT were partial or complete obstruction of hepatic outflow or the portal vein, respectively, as documented by appropriate radiographic abdominal imaging (ie, Doppler ultrasonography, computed tomography, magnetic resonance imaging, venography) or laparotomy. We excluded patients with veno-occlusive disease, patients with hepatic outflow obstruction caused by congestive heart failure, and patients who were younger than 15 years of age in July 1997. For all identified patients (BCS, n = 76; PVT, n = 214) a standardized questionnaire, asking for specific clinical information to confirm the diagnosis of PVT and BCS, was completed with data obtained from the medical charts by local investigators under close supervision of one member of our team (H.L.A.J.). Twenty-five patients with BCS and 100 with PVT had died before July 1997. Patients alive were asked to visit the hospital in which they were registered for blood sampling, at the same time enabling investigators to complete the questionnaire with information on previous thrombotic events, familial thrombosis, acquired risk factors of thrombosis, and the use of anticoagulants at the time of venipuncture. Eight patients could not be traced, 14 were unwilling to participate, and 5 were registered in 2 hospitals; in 3 cases insufficient material for DNA extraction was obtained. Forty-three patients with BCS and 92 patients with PVT were enrolled in the study and underwent a full screening for thrombogenic disorders. Five patients had both BCS and PVT. These patients were included in the analyses of both diseases. The population-based control group consisted of 474 healthy individuals who had no history of venous thromboembolism, had not used coumarin derivatives for at least 3 months before blood sampling, and had no myeloproliferative or malignant disease (LETS study control population[6]). Age, sex, and ethnic descent of patients and the controls were similar. More than 95% of patients and controls were of Caucasian origin. The study was approved by the ethical committee of each participating hospital and the participants gave their informed consent before entering the study.

Blood was collected in tubes containing 3.2% trisodium citrate. After centrifugation for 10 minutes at 2000*g*, plasma and white blood cells were separated and stored at −20°C until analysis. Material was collected and transported to Leiden by the study coordinators. All laboratory assessments were performed at the Haemostasis and Thrombosis Research Centre of the Academic Hospital Leiden. High molecular weight DNA was isolated from the white blood cell fraction by standard methods. The presence of mutations in the *factor V* gene (1691, G→A) and the prothrombin gene (20 210, G→A) was determined as previously described.[5,8] Other coagulation assays were done according to established procedures: protein C activity and antithrombin activity were measured by Coamate (Chromogenix, Mölndal, Sweden) on an ACL-200 (Instrumentation Laboratory, Milan, Italy), total protein S antigen by a polyclonal enzyme-linked immunosorbent assay, factor II and factor X antigen by immunoelectrophoresis, and factor V activity by a one-stage clotting assay on an Electra 1000C coagulometer (MLA, Pleasantville, New York). In view of potential liver failure and anticoagulant treatment of patients with BCS and PVT, the presence of hereditary deficiencies of protein C, protein S, and antithrombin could only be estimated by modified criteria as compared to controls. For controls the criteria for diagnosis of protein deficiencies were plasma levels under the lower limit of normal, combined with normal factor II and prothrombin time values.[13] Patients with BCS and PVT who satisfied these protein deficiency criteria and those with a ratio of protein C antigen, protein S antigen, or antithrombin value to (factor II + factor X)/2 below 0.7[14] were selected for individual evaluation of the presence of hereditary protein deficiencies by 2 hemostasis physicians (F.R.R. and F.J.M.v.d.M.). These investigators, who were not involved in patient management and were unaware of the hepatologic diagnosis, based their decision on the results of protein C, protein S, and antithrombin testing in relation to the patient's use of anticoagulants, the use of oral contraceptives, and the degree of liver failure as indicated by results of a panel of coagulation tests (protein C, protein S, antithrombin, fibrinogen, prothrombin time, partial thromboplastin time, factor II, factor V, and factor X). A clear isolated deficiency of either coagulation inhibitor in comparison to other coagulation tests was considered as an inherited deficiency. In all cases, the presence of myeloproliferative disease was confirmed by bone marrow examination. Latent primary myeloproliferative disorder, as diagnosed by erythroid colony formation, was not tested in all patients and was not considered to be a myeloproliferative disease. Six BCS patients and 4 PVT patients had undergone liver transplantation before the date of blood sampling. For 7 of these 10 patients plasma taken before liver transplantation could be obtained and investigated for protein C, protein S, and antithrombin deficiency. Separate analysis of the pretransplantation samples did not alter the decision on inherited deficiency of these coagulation inhibitors. The frequencies of factor V Leiden mutation, prothrombin mutation, and deficiency of protein C, protein S, and antithrombin among cases and controls were compared by simple cross-tabulation. Patients who, except for these investigated inherited thrombogenic conditions, did not exhibit risk factors for thrombosis were referred to as idiopathic cases. The relative risks of BCS or PVT among individuals with thrombogenic conditions were estimated as the odds ratio (OR) and the 95% confidence interval (CI) according to Woolf.[15]

Results

Characteristics of patients and controls are shown in Table 1. Myeloproliferative disease was present in 12 (28%) BCS patients and 16 (17%) PVT patients. Thirty-two (74%) BCS patients and 24 (26%) PVT patients were treated with coumarin derivatives. This difference may be attributable not only to the lack of evidence that

Table 1. Characteristics of patients with Budd-Chiari syndrome (BCS) and portal vein thrombosis (PVT) and controls

	BCS n = 43	PVT n = 92	Controls n = 474
Age* (y)	40 (19-60)	51 (19-83)	47 (16-73)
Male (%)	16 (37)	48 (52)	202 (43)
Oral anticoagulation (%)	32 (74)	24 (26)	—
Duration of disease* (y)	6.4 (0.3-25)	7.0 (0.5-45)	—
Myeloproliferative disorders (%)	12 (28)	16 (17)	—
Polycythemia vera (%)	10 (23)	8 (9)	—
Essential thrombocythemia (%)	1 (2)	3 (3)	—
Myelofibrosis (%)	—	4 (4)	—
Unclassified (%)	1 (2)	1 (1)	—
PNH (%)	—	2 (2)	—
Lupus anticoagulant (%)	2 (5)	—	—
Biopsy documented cirrhosis (%)	6 (14)	16 (17)	—
History of pancreatitis (%)	—	10 (11)	—
Previous abdominal surgery (%)	10 (23)	28 (30)	NA
Oral contraceptives (%)	12 (28)	12 (13)	65 (14)

PNH indicates paroxysmal nocturnal hemoglobinuria; NA, not available.
*Median (range).

Table 2. Differences in prevalences of factor V Leiden mutation, prothrombin gene mutation, protein C deficiency, protein S deficiency, and antithrombin deficiency in patients with Budd-Chiari syndrome (BCS) and controls

	BCS (%) n = 43	Controls (%) n = 474	OR	95% CI
Factor V Leiden mutation	11 (25.6)	14 (2.9)	11.3	4.8-26.5
Prothrombin gene mutation	2 (4.7)	11 (2.3)	2.1	0.4-9.6
Protein C deficiency	4 (9.3)	7 (1.5)	6.8	1.9-24.4
Protein S deficiency	0 (0)	11 (2.3)	—	—
Antithrombin deficiency	0 (0)	9 (1.9)	—	—

CI indicates confidence interval; OR, odds ratio.

anticoagulation is beneficial in PVT but also to the predominance of variceal bleeding in PVT patients (36%) compared to BCS patients (7%).

Among the 43 BCS patients factor V Leiden mutation (n = 11, 25.6%, OR 11.3, 95% CI 4.8-26.5), hereditary protein C deficiency (n = 4, 9.3%, OR 6.8, 95% CI 1.9-24.4) and prothrombin gene mutation (n = 2, 4.7%, OR 2.1, 95% CI 0.4-9.6) were more prevalent than in controls (Table 2). In particular, factor V Leiden mutation and protein C deficiency were predominant risk factors for BCS. One patient exhibited homozygous carriership for factor V Leiden mutation; the combined presence of factor V Leiden mutation and hereditary protein C deficiency was found in 2 patients. Protein S deficiency and antithrombin deficiency could not be demonstrated for any of the BCS patients. Among the 19 patients with idiopathic BCS, factor V Leiden mutation was found in 7 (36.8%, OR 19.1, 95% CI 6.5-56.0) protein C deficiency in 2 (10.5%, OR 7.8, 95% CI 1.5-40.6), and prothrombin gene mutation in none.

Among the 92 PVT patients, factor V Leiden mutation was observed in 7 patients (7.6%, OR 2.7, 95% CI 1.1-6.9), hereditary protein C deficiency in 6 patients (6.5%, OR 4.6, 95% CI 1.5-14.1), and prothrombin gene mutation in 3 patients (3.2%, OR 1.4, 95% CI 0.4-5.2) (Table 3). Although less pronounced than in the BCS group, the relative risk of PVT was increased in the presence of these thrombogenic factors. In one patient with primary biliary cirrhosis factor V Leiden mutation and protein C deficiency were present simultaneously. The prevalence of both protein S deficiency (2.2%, OR 0.9, 95% CI 0.2-4.3) and antithrombin deficiency (1.1%, OR 0.6, 95% CI 0.1-4.5) was low, being comparable to that found for controls. In the group of 21 patients with idiopathic PVT factor V Leiden mutation was observed in 3 (14.2%, OR 5.5, 95% CI 1.4-20.7), prothrombin gene mutation in 1 (4.8%, OR 2.1, 95% CI 0.3-17.1), and protein C deficiency in none.

Use of oral contraceptives was an important acquired risk factor for BCS and PVT. Among women in the age group of 15 to 49 years, oral contraceptives had been used at the time of diagnosis in 12 of 20 patients (60.0%, OR 2.4, 95% CI 0.9-6.2) with BCS and in 12 of 25 patients (48.0%, OR 1.5, 95% C 0.6-3.4) with PVT compared to 65 of 169 (38%) controls. Concurrence of inherited thrombogenic factors (ie, factor V Leiden mutation, prothrombin gene mutation, protein C deficiency, protein S deficiency, and antithrombin deficiency) and acquired prothrombotic states for BCS and PVT is shown in Table 4. For 11 of the 43 BCS patients (26%) and 15 of the 92 PVT patients (16%) no risk factors for thrombosis could be demonstrated. At least one of the inherited prothrombotic risk factors was present in 14 patients with BCS (32.5%, OR 3.9, 95% CI 1.9-7.9) and 17 patients with PVT (18.5%, OR 1.8, 95% CI 1.0-3.3). Coexistence of acquired and inherited risk factors was found for 6 (14%) of those with BCS and 13 (14%) with PVT. Analysis of all thrombogenic risk factors, irrespective of whether they were acquired or inherited, revealed 11 (26%) patients with BCS and 34 (37%) patients with PVT who had more than one risk factor (Table 5). Three BCS patients and 12 PVT patients exhibited 3 or more factors considered to be a risk for development of thrombosis in hepatic and portal veins, respectively.

Table 3. Differences in prevalences of factor V Leiden mutation, prothrombin gene mutation, protein C deficiency, protein S deficiency, and antithrombin deficiency in patients with portal vein thrombosis (PVT) and controls

	PVT (%) n = 92	Controls (%) n = 474	OR	95% CI
Factor V Leiden mutation	7 (7.6)	14 (2.9)	2.7	1.1-6.9
Prothrombin gene mutation	3 (3.2)	11 (2.3)	1.4	0.4-5.2
Protein C deficiency	6 (6.5)	7 (1.5)	4.6	1.5-14.1
Protein S deficiency	2 (2.2)	11 (2.3)	0.9	0.2-4.3
Antithrombin deficiency	1 (1.1)	9 (1.9)	0.6	0.1-4.5

CI indicates confidence interval; OR, odds ratio.

Table 4. Prevalence of acquired and inherited risk factors for Budd-Chiari syndrome (BCS) and portal vein thrombosis (PVT)

Acquired risk factor	Inherited risk factor	BCS (%) n = 43	PVT (%) n = 92
–	–	11 (25.6)	15 (16.3)
+	–	18 (41.8)	60 (65.2)
–	+	8 (18.6)	4 (4.3)
+	+	6 (13.9)	13 (14.1)

BCS: Inherited risk factor: factor V Leiden mutation n = 11, prothrombin gene mutation n = 2, protein C deficiency n = 4. Acquired risk factor: oral contraceptives n = 12, myeloproliferative disease n = 12, lupus anticoagulant n = 2, inflammatory bowel disease n = 2, liver abscess n = 1.

PVT: Inherited risk factor: factor V Leiden mutation n = 7, prothrombin gene mutation n = 3, protein C deficiency n = 6, protein S deficiency n = 2, antithrombin deficiency n = 1. Acquired risk factor: oral contraceptives n = 12, myeloproliferative disease n = 16, cirrhosis n = 16, abdominal surgery n = 28, pancreatitis n = 10, umbilical vein infection n = 6, inflammatory bowel disease n = 4, liver abscess n = 4, abdominal trauma n = 2, paroxysmal nocturnal hemoglobinuria n = 2, pregnancy n = 2, nodular regenerative hyperplasia n = 2, portal sclerosis n = 1, hepatocellular carcinoma n = 1, seminoma n = 1.

Discussion

This study shows a high prevalence of factor V Leiden mutation and hereditary protein C deficiency in patients with BCS and PVT, indicating that individuals with these thrombogenic conditions have an increased relative risk for both manifestations of thrombosis. The prevalence of the prothrombin gene mutation was less than 5% for BCS and PVT, and individuals with this mutation only had an increased relative risk for BCS and PVT, which was not significant. In general, the prevalence of the investigated hereditary

Table 5. Prevalence of combined risk factors (acquired and inherited) among patients with Budd-Chiari syndrome (BCS) and portal vein thrombosis (PVT)

Number of risk factors*	BCS (%) n = 43	PVT (%) n = 92
0	11 (25.6)	15 (16.3)
1	21 (48.8)	43 (46.7)
2	8 (18.6)	22 (23.9)
3	3 (6.9)	9 (9.8)
4	—	3 (3.3)
> 4	—	—

*Risk factors as shown.

risk factors for thrombosis was especially high among patients with idiopathic BCS and PVT.

Mahmoud and associates, who investigated 30 BCS patients and 32 PVT patients, reported that factor V Leiden mutation was an important factor in the pathogenesis of BCS but not of PVT.[7] The present study, which included 92 PVT patients and therefore had more statistical power, demonstrates that factor V Leiden mutation is also a risk factor for PVT. Chamouard and colleagues recently found the prothrombin gene mutation in 4 of 10 patients with PVT.[9] Although they investigated a subgroup of patients with idiopathic PVT, the importance of the prothrombin gene mutation in the etiology of PVT could well be overestimated in that study due to the limited number of patients included. Large studies on venous thromboembolisms did not show a mutation frequency of more than 6.2%, and on pathophysiologic grounds there is no reason to believe patients with the prothrombin gene mutation would exhibit an additional risk for PVT.[8]

As previously documented for patients with deep venous thrombosis,[13] the prevalences of hereditary protein S deficiency and antithrombin deficiency were low in both BCS and PVT patients, and no differences with controls were observed. In view of the low prevalences of these thrombogenic states as well as the investigated diseases, we cannot exclude the association of protein S or antithrombin deficiency with BCS and PVT, as was suggested in several case reports.[12,16,17] Although the higher prevalence of protein S deficiency in our control group as compared to other estimates would not suggest so, the use of total protein S values instead of free protein S could have led to an underestimation of the prevalence of inherited protein S deficiency in our study.[13,18] In any case, our results indicate that inherited defects of both protein S and antithrombin are probably not major predisposing factors in the pathogenesis of BCS and PVT.

At least one of the inherited prothrombotic risk factors investigated (factor V Leiden mutation, prothrombin gene mutation, and protein C, protein S, or antithrombin deficiency) was present in approximately one third of the BCS population and in one fifth of the PVT population. The fact that the significance of these prothrombotic abnormalities was more pronounced in BCS than in PVT can be explained by the heterogeneous etiology of PVT in which many focal factors, such as abdominal surgery, pancreatitis, and preexistent cirrhosis, appear to be of importance.

The diagnosis of inherited deficiencies in protein C, protein S, and antithrombin in patients with BCS and PVT is difficult and should be interpreted as estimates primarily because acquired deficiencies can develop in the event of liver failure, acute thrombosis, and anticoagulant therapy. To minimize the number of incorrectly diagnosed inherited deficiencies we decided to (1) evaluate the levels of protein C, protein S, and antithrombin in relation to the levels of factor II and factor X, which are also liver- and vitamin K-dependent factors, and (2) subject all patients with an imbalance of these factors to review by a blinded expert panel. Furthermore, as in many previous studies of BCS and PVT patients, one might question whether selection is introduced by the inclusion of only patients who were alive. Patients with fulminant disease who died early in the course of their illness could bias the results. Nevertheless, a bias would only be present if one assumes that the investigated thrombogenic risk factors are directly related to mortality in BCS and PVT. This is unlikely, but has never been well investigated. For the present study, separate analysis of thrombophilia screening, as performed by local hematologists, indicated that 76% of the 25 registered patients with fatal BCS did not reveal a major difference in the prevalence of the investigated thrombogenic states. Most of the PVT patients who died were incompletely tested for thrombophilia because they either had abdominal malignancies or end-stage liver failure.

A remarkable finding of the present study is the coexistence of several thrombophilic states in about one fourth of patients with BCS and more than one third of those with PVT (Table 5). Several patients even had 4 thrombotic risk factors of either acquired or inherited origin. It is plausible that prothrombotic mutations in one or more genes create an inherited predisposition for BCS and PVT. Subsequent clinical thrombosis might then manifest in the presence of thrombotic stimuli such as use of oral contraceptives, pregnancy, myeloproliferative disease, and abdominal surgery.[19] Hence, for appropriate risk assessment even in the presence of an overt thrombotic risk factor, physicians should request complete thrombophilia screening for patients with life-threatening thrombotic manifestations such as BCS and PVT. With the continuing search for genetic molecular defects, the number of thrombogenic disorders will probably grow and we therefore might enter an era in which true idiopathic thrombotic disease will become uncommon.

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