

Symptomatic Onset of Severe Hemophilia A in Childhood is Dependent on the Presence of Prothrombotic Risk Factors

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Key words

Severe hemophilia A, pediatric PUP patients, factor V G1691A, prothrombin G20210A

Summary

It has been recently suggested that the clinical phenotype of severe hemophilia A (HA) is influenced by co-inheritance with the factor V G1691A mutation. We therefore investigated 124 pediatric PUP patients with hemophilia (A: n = 111) consecutively admitted to German pediatric hemophilia treatment centers. In addition to factor VIII activity, the factor V (FV) G1691A mutation, the prothrombin (PT) G20210A variant, antithrombin, protein C, protein S and antithrombin were investigated. 92 out of 111 HA patients (F VIII activity < 1%) were suffering from severe HA. The prevalence of prothrombotic risk factors in children with severe HA was no different from previously reported data: FV G1691A 6.5%, PT G20201A 3.2%, and protein C type I deficiency 1.1%. No deficiency states of antithrombin or protein S were found in this cohort of hemophilic patients. The first symptomatic bleeding leading to diagnosis of severe hemophilia (< 1%) occurred with a median (range) age of 1.6 years (0.5-7.1) in children carrying defects within the protein C pathway or the PT gene mutation compared with non-carriers of prothrombotic risk factors (0.9 years (0.1-4.0; p = 0.01). The cumulative event-free bleeding survival was significantly prolonged in children carrying additionally prothrombotic defects (log-rank/Mantel-Cox: p = 0.0098). In conclusion, data of this multicenter cohort study clearly demonstrate that the first symptomatic bleeding onset in children with severe HA carrying prothrombotic risk factors is significantly later in life than in non-carriers.

Introduction

Hemophilia A and B are X-linked genetic hemorrhagic disorders resulting from deficiencies of blood coagulation factor VIII or IX. Subjects suffering from plasma levels of factor VIII coagulant activity or factor IX below 1% of normal are classified as severe hemophiliacs (1). While mild or moderate hemophilia is not always diagnosed during childhood, severe hemophilia is generally diagnosed at an early age (2-6). Although bleeding symptoms correlate with the levels of the remaining factor activity, it is reported that not all hemophilic subjects with factor VIII levels < 1% bleed with the same severity (7, 8), and in rare cases of severe hemophilia A (HA), thrombotic episodes have been reported also in childhood (9-15).

So far, various molecular defects of different hemostatic components have been established as risk factors for venous thromboembolic diseases in children and adults: deficiencies of protein C, protein S, and antithrombin, resistance to activated protein C mostly due to the factor (F) V G1691A gene mutation, and the prothrombin (PT) G20210A genotype respectively (16-19).

Besides the possibility that the mutation type within the factor VIII gene may influence the clinical severity of hemophilia (20, 22), it has been recently suggested that the clinical phenotype of severe hemophilia A is influenced by co-inheritance with the factor V G1691A mutation (21). The present study was therefore conducted to unravel the role of prothrombotic risk factors, i.e. deficiencies within the protein C pathway, the FV G1691A mutation or the PT G20210A variant, coinherited with severe hemophilia A with respect to the first symptomatic onset of the disease in pediatric Caucasian patients.

Methods

Ethics: The present retrospective study on consecutively recruited pediatric patients with hemophilia was performed in accordance with the ethical standards laid down in a relevant version of the 1964 Declaration of Helsinki and approved by the medical ethics committee at the Westfälische Wilhelms-University, Münster, Germany. With special regard to the data presented here, the ethics committee has approved the investigation of established prothrombotic risk factors possibly coinherited in pediatric patients with hemophilia A and B.

Inclusion criteria: Untreated Caucasian infants and children with previously undiagnosed severe hemophilia A (F VIII activity < 1%) aged neonate to 16 years admitted to the university pediatric hospitals in Frankfurt, Halle, Hanover, Munich and Münster, Germany, on the first symptomatic and spontaneous onset of the disease. In the patients enrolled the classification of HA based on the F VIII activity was confirmed by using the same aPTT reagents and factor VIII-deficient plasma in the patients investigated.

Exclusion criteria: Pretreated pediatric patients, subjects in whom surgery- or major (birth-) trauma-induced bleeding had occurred (5, 6). In addition, patients with prenatal diagnosis of HA were excluded, as were children with a diagnosis of hemophilia before the first bleeding episode.

Study endpoint: First symptomatic bleeding episode leading to the diagnosis of severe hemophilia A (2, 3).

Study period: From October 1985 to December 1999, 124 consecutive Caucasian pediatric patients with a first symptomatic onset of hemophilia A and B were recruited from different geographic areas of Germany.

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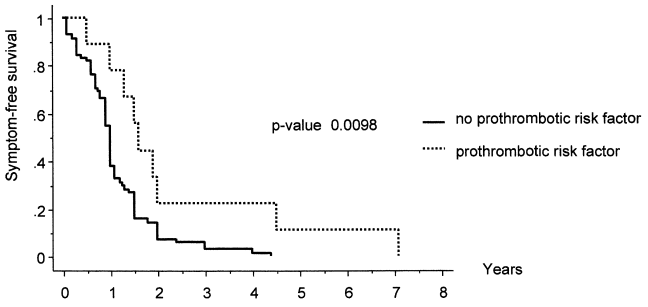

Fig. Symptom-free survival in children with severe hemophilia A with (dotted line) and without additional prothrombotic risk factor

Study population: Of the 124 consecutively recruited children, 111 were suffering from HA, and in 13 subjects hemophilia B was diagnosed. The entire study population presented here included 92 children with severe HA. Patients with hemophilia B were not included in the data presented here.

Blood samples for genotyping: For genetic analysis, which was retrospectively performed between 1998 and 1999 in all study patients, we obtained venous blood in EDTA-treated sample tubes (Sarstedt, Nümbrecht, Germany), from which cells were separated by centrifugation at 3000 g for 15 min. The buffy coat layer was then removed and stored at -70° C pending DNA extraction by standard techniques.

Blood plasma samples: With informed parental consent, fasting blood samples were collected by peripheral venipuncture into plastic tubes containing 1/10 by volume of 3.8% trisodium citrate (Sarstedt, Nümbrecht, Germany), and placed immediately on melting ice. Platelet poor plasma was prepared by centrifugation at 3000 g for 20 min at 4° C, aliquoted in polystyrene tubes, stored at -70° C and thawed immediately before the assay procedure.

Assays for genotyping: The G1691A polymorphism in the FV gene and the G20210A variant in the prothrombin gene were detected in patients with severe hemophilia A by PCR amplification as previously reported (17, 18).

Assays for quantification of plasma proteins: Activities of protein C and antithrombin were measured by means of the chromogenic substrates S-2366 and S-2765, respectively (Chromogenix, Mølndal, Sweden). Free protein S antigen and protein C antigen were quantified by ELISA (Asserachrom: free protein S, protein C; Diagnostica Stago, Asnières-sur-Seine, France) (19). The plasma levels of factor VIII were measured by one-stage clotting assays purchased from Behringwerke, Marburg, Germany using standard laboratory methods.

Statistics: The probability of the first bleeding onset as a function of time was determined with the method of Kaplan and Meier including the log-rank test to compare the bleeding-free survival in patients carrying prothrombotic risk factors with non-carriers. In addition, calculations of medians, ranges and nonparametric statistics (Mann-Whitney, log-rank) were performed with the Stat view 5.0 program. The significance level was set at 0.05.

Results

Clinical presentation at onset: In subjects with severe HA the majority of cases presented with mouth bleeding (28%), bleeding from soft tissues (19%), joint bleeding (18%), large hematomas (17%), or muscle bleeding (14%). In addition, gastrointestinal (3%) and intraventricular hemorrhage occurred as leading symptoms in 1% of patients respectively.

Prevalence of prothrombotic risk factors in German children with severe HA: The prevalence of prothrombotic risk factors in children with severe HA was no different from previously reported data: FV G1691A 6.5% (6/92), PT G20201A 3.2% (3/92), and protein C type I deficiency 1.1% (1/92). No deficiency states of antithrombin or protein S were found in this cohort of hemophilic patients.

Age at first symptomatic bleeding: The first symptomatic bleeding leading to diagnosis of severe hemophilia occurred with a median (range) age of 1.6 years (0.5-7.1) in children carrying defects within the protein C pathway or the PT gene mutation compared with 0.9 years (0.1-4.0) in non-carriers of prothrombotic risk factors ($p = 0.01$). As demonstrated in the figure, the cumulative event-free bleeding survival was significantly prolonged in children carrying a prothrombotic defect ($p = 0.0098$). However, at the age of 4 (non-carriers) and 7.1 years (carriers) all children with severe HA had already suffered at least one bleeding episode which led to the final diagnosis of the inherited hemorrhagic disorder.

Thromboembolic events: Interestingly, two out of six carriers of the FV G1691A mutation (33.3%) developed symptomatic venous thrombosis: Portal vein occlusion occurred in a 14-year-old boy during continuous infusion of F VIII concentrate after intramural jejunal bleeding (14), and vena caval occlusion was diagnosed in a 13-month-old boy treated prophylactically thrice weekly via a Port catheter.

Discussion

From a small-scale study it has been suggested that coinheritance with the FV G1691A mutation, especially when sharing the identical factor VIII gene mutation (21), may influence the phenotype of severe HA. In contrast, data reported by Arbini et al. in hemophilic subjects, and very recently data shown by Lee et al. in 137 patients, failed to support these findings, showing that the proportion of severe hemophiliacs whose mild clinical course could be attributed to coinheritance with the FV G1691A mutation tended to be small (23, 24).

Pediatric patients with severe HA typically experience frequent bleeding episodes into joints or soft tissues (2, 3, 5), necessitating on-demand or prophylactic treatment twice or thrice weekly with individual amounts of factor VIII concentrates, either as a single dose administration, or now increasingly implemented by giving the factor concentrate by continuous infusion in case of surgical procedures (25, 26). On the one hand, the frequency of bleeding and the outcome with respect to joint damage are dependent on the severity of the disease, the corresponding factor F VIII gene mutation, or the development of inhibitors (8). On the other hand, the course of bleeding episodes is also influenced by the individual therapeutic regimen performed by each hemophilia center (25). Thus, a large number of patients in different treatment arms is required for an independently prospective study of these multiple variables potentially influencing the bleeding frequencies and the outcome with respect to the presence or absence of hemophilic arthropathy in previously untreated hemophilic children. The purpose of the present study, however, was to investigate whether the presence of prothrombotic risk factors influences the first symptomatic bleeding onset in children with previously undiagnosed severe hemophilia A.

Data of this multicenter cohort study clearly demonstrate that the first symptomatic bleeding onset leading in children with severe HA to diagnosis of the disease but not associated with surgery or further major trauma, occurs significantly later in life in hemophilic subjects additionally carrying prothrombotic risk factors than in non-carriers, thereby supporting the hypothesis of Nichols et al. (21) that the hemophilia phenotype is influenced by coinheritance with prothrombotic risk factors.

In this survey 10 of 92 subjects (10.9%) with severe hemophilia A carried prothrombotic risk factors, i.e. the factor V G1691A mutation

(n = 6), the PT G202010A variant (n = 3) and protein C type I deficiency (n = 1), which is in the reported range for healthy Caucasian individuals (18, 19). However, the development of symptomatic thromboembolism diagnosed in two out of 10 patients carrying additionally prothrombotic risk factors (20%) is a rare complication reported in sporadic cases of severe hemophilia. This complication has been previously reported in association with concomitant prothrombotic inherited or acquired risk factors, especially when the inherited hemorrhagic disease was corrected by factor replacement therapy in inhibitor patients, either as a single dose or, as recently shown, using continuous infusion therapy (9-15).

In conclusion, data of the cohort study presented here suggest that there is at least some evidence that the hemophilic phenotype is influenced by the presence or absence of prothrombotic risk factors. However, to obtain further insight into the possible putative effect of these prothrombotic risk factors on the severity of hemorrhagic disorders, especially of severe hemophilia A, which is supported also by the findings of Lindvist et al. showing that women carrying the FV G1691A mutation experienced less intrapartum blood loss (27), prospective large-scale studies in previously untreated hemophilic children are required. Such studies should not only focus on factor activity and factor VIII genotype but should also take into account the development of inhibitors and the different therapeutic treatment regiments applied.

Addendum

C. Escuriola Ettingshausen was responsible for acquisition and management of laboratory data, S. Halimeh collected and analyzed clinical and laboratory data and performed statistical analysis, K. Kurnik and R. Schobess collected clinical and laboratory data, C. Wermes analyzed clinical data, and R. Junker was responsible for management of laboratory data. In addition, W. Kreuz, H. Pollmann and U. Nowak-Göttl designed and coordinated the study and were responsible for study execution.

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