Abstract

Objective and Importance: We have evaluated retrospectively in splenectomized hematologic patients by 10 year follow-up the incidence of thromboembolic events (VTE) in relationship not only with thrombocytosis but also with other possible risk factors, particularly anemia and some genetic mutations.

Clinical presentation: Fifty children of both sexes with Hereditary Spherocytosis (HS), Pirvurate Kinase Deficit (PK), Congenital Dyserythropoiesis II (CDII), Chronic Idiopathic Thrombocytopenic Purpura (cITP), β thalassemia intermedia (TI), splenectomized at 12.8 ± 6.2 years and untransfused or not transfused in the last 5 years were followed. Four patients with iron deficiency anemia had, on follow-up, deep venous thrombosis (DVT) during pregnancy (1 HS and 1 cITP) or after blood losses (2 PK deficits). Two patients with mild chronic anemia affected by CDII and TI respectively showed cerebrovascular thrombosis (CVT). None patient with VTE had genetic mutations.

Intervention (& technique): Platelet count (PLT), Hb levels, clotting tests were performed once a year and compared with healthy controls. Genetic mutations of Methyleneptehydrofolate reductase (MTHFR) C677T, Factor V Leiden G1691A and Prothrombin G20210A were studied.

VTE was diagnosed by Doppler Ultrasonography and Magnetic Resonance.

Conclusion: CDII, TI and PK deficit subjects had altered Anti-thrombin III, Protein S, Protein C, activated partial thromboplastin time, thrombocytosis and lower Hb levels than other patients (p<0.05). Longer persistence of altered red cells and/or restricted erythropoiesis may increase the risk of VTE.

Keywords: Splenectomy; Thrombocytosis;Venous Thromboembolism (VTE); Deep Venous Thrombosis (DVT); Cerebrovascular Thrombosis (CVT)

Introduction

Hypercoagulability, characterized by activation of the coagulation system, plays an important role in the pathogenesis of venous thromboembolism (VTE) [1-3].

In recent years, studies on the central role of hypercoagulability in the pathogenesis of thrombosis have led to an earlier diagnosis with more appropriate therapies [1-3].

Moreover, hypercoagulability could play a role in the loss of thromboresistant properties of vascular endothelium [4,5].

Both inherited and acquired hypercoagulable states have been recognized [1,6].

Different acquired conditions such as infections, inflammation, liver disease, pregnancy and splenectomy have all been associated with degrees of VTE risk even in patients without other thrombophilic risk factors [1,5].

The increased risk of VTE in some splenectomized hematologic patients with reactive thrombocytosis has been reported in about 80% of cases [1,3,5].

In most cases this is a self-limiting event, even if extreme thrombocytosis with platelet count ≥ 1000 x 10^9/l may result in thromboembolic events such as vascular accidents, deep venous thrombosis (DVT), cerebrovascular thrombosis (CVT), acute myocardial infarction, mesenteric vein thrombosis and pulmonary embolism [2,7,8].

Splenectomy is usually performed for treatment of a variety of hematologic disorders such as chronic Idiopathic Thrombocytopenic purpura , hereditary spherocytic and non-spherocytic hemolytic anaemia, sideroblastic anaemia and particularly thalassemia and hemoglobinopathies [5].

The aim of this study is to evaluate the symptomatic or asymptomatic thromboembolic complications in splenectomized patients for hematologic diseases other than thalassemia major and not transfused at last 5 years in relationship not only with thrombocytosis but also with other possible risk factors, particularly anemia and some genetic mutations.

Materials and Methods

Over the last 20 years we followed 169 splenectomized adolescents mean age 12.8 ± 6.2 years of both sexes with hematologic disorders.

Patients were retrospectively selected on the basis of regular follow-up 10 years after splenectomy. Exclusion criteria were severe liver disease, bone marrow transplantation, transfusions in the last 5 years and other conditions influencing hemostatic functions.

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Fifty patients of both sexes (28F, 22M) were selected for the study: 18 (10 F- 8 M) with Hereditary Spherocytosis (HS); 5 females with Piruvate Kinase deficit (PK deficit); 4 males with Congenital Type II Dyserthropoiesis (CD II); 20 (12F- 8M) with Chronic Immunologic Thrombocytopenic Purpura (cITP); 3 (1F-2M) with β- thalassemia intermedia (TI).

Postsplenectomy all patients received regular prophylactic therapy with acetyl salicylic acid.

Patients were evaluated every year for 10 years.

The following tests were assessed to evaluate the possible relationship between thrombocytosis and anemia with thrombophilic alterations: Hb, Platelet count (PLT), Activated Partial Thromboplastin Time (aPTT), free Protein C activity(PC), Protein S (PS), Antithrombin III (AT III), D-Dimer.

Mean values were evaluated.

A Comparison was made with 20 healthy controls (HC) of both sexes (10 males, 10 females) matched for age.

The following genetic risk factors were also investigated: Factor V gene G1691A mutation (FVL) as described by Ridker et al. [9], Prothrombin G20210A mutation (F2) as described by Iwahana et al. [10] and Methylentetrahydrofolate reductase (MTHFR) C677T polymorphism as described by Frost et al. [11].

Patients who had thromboembolic events were clinically evaluated. Hematological tests and appropriate diagnostic imaging (Doppler ultrasonography and Magnetic Resonance (MR) in patients with DVT, MR in patients with CVT), were performed during the disease.

Statistical analysis was carried out with ANOVA. A p value ≤ 0.05 was considered statistically significant.

Informed consent was obtained from patients or guardians. Principles outlined in the Declaration of Helsinki were followed.

Results

Our data show that splenectomized hematologic patients affected by CDII and TI have PLT counts significantly higher than other subjects (p<0.05), as well as patients with PK deficit show higher PLT count than cITP and HC patients (p<0.05) (Table 1).

Hematologic diseases aPTT* b (24-36) free PS ** b (F 58-113%) PC** b (70-140%) AT III** b (70-120%) D-Dimer (<0.5 μg/ml) HS* 28.6 ± 3.1 87.2 ± 15.3 89.4 ± 8.8 93.1 ± 7.8 <0.5 PK deficit* 38.6 ± 4.7 55.6 ± 13.1 64.8 ± 12.1 62.5 ± 5.5 <0.5 CD II* 37.3 ± 5.2 47.4 ± 6 58.7 ± 5.4 59.2 ± 7.8 <0.5 cITP* 29.7 ± 3.3 91.4 ± 14.3 94.1 ± 7 93.1 ± 7.2 <0.5 TP* 36.2 ± 2.8 50.7 ± 5.2 58.4 ± 7 60.2 ± 5 <0.5 HC* 27.9 ± 2.9 96 ± 11 98 ± 28 103 ± 9 <0.5

aPTT* p<0.05 PK deficit, CDII, TI vs HS, cITP, HC free PS ** p<0.05 vs HS, PK deficit, cITP, HC PC** b p<0.05: PK deficit vs HS, cITP, HC Hb** p<0.05: CDII and TI vs HS, PK deficit, cITP, HC

Table 1: Mean values of Platelet count (PLT) and Hb in splenectomized hematologic patients and controls.

Patients with PK deficit, CDII and TI show alteration of aPTT and significantly lower Hb levels than other subjects (p<0.05) while Hb levels of PK deficit subjects are significantly lower than HS, cITP, and HC ones (p<0.05) (Table 1).

Patients with PK deficit, CDII and TI show alteration of aPTT and significantly lower levels of free PS,PC and AT III than other patients (p<0.05) with D-dimer >0.5 μg/ml (Table 2).

Thromboembolic events have been observed in six patients (Table 3).

One patient with HS aged 22 years showed DVT diagnosed by Doppler Ultrasonography and MR during pregnancy (Table 3). This girl had an Hb level of 8.5 g/dl due to iron deficiency and increase of Platelet count. She had higher D-dimer (>0.5 μg/ml) and lower levels of PS, PC, and ATIII with aPTT 45° (Table 4).

Two female patients affected by PK deficit aged 14 and 16 years had respectively DVT, diagnosed by Doppler Ultrasonography and MR, and CVT diagnosed by MR. In the first case DVT relapsed after 2 years (Table 3). These patients were affected by hypermenorrhea and showed iron deficiency with Hb values ≤ 7.5 g/dl and PLT ≥600.000/ mmc. They have higher D-dimer (>0.5 μg/ml) and lower levels of PS, PC, and ATIII with aPTT 42° and 39° respectively (Table 4).

One splenectomized patient with cITP, aged 24 years showed DVT during pregnancy (Table 3). She presented higher D-dimer (>0.5 μg/ml) and lower levels of PS , PC, and ATIII with aPTT 40°, iron deficiency anemia with Hb 8.2 g/dl and increase of platelet count (Table 4).

One male patient with CDII and another one male with TI aged respectively 13 and 18 years had CVT clinically characterized by severe thrombocytosis and anemia with thrombophilic alterations: Hb, Platelet count (PLT), Activated Partial Thromboplastin Time (aPTT), free Protein C activity(PC), Protein S (PS), Antithrombin III (AT III), D-Dimer.

Table 2: Mean values of clotting tests in splenectomized hematologic patients and controls.

Hematologic diseases VTE b n°cases gender interval from splenectomy/ years Relapses HS DVT 1 F 9 - yes PK deficit DVT 1 F 10 - no cITP DVT 1 F 7 - no CD II DVT 1 M 10 - no TP CVT 1 M 10 - no

Table 3: Hematologic values in splenectomized patients with venous thromboembolism (VTE).

Furthermore, splenectomized patients with CD II and TI show significantly lower Hb levels than other subjects (p<0.05) while Hb levels of PK deficit subjects are significantly lower than HS, cITP, and HC ones (p<0.05) (Table 1).

Patients with PK deficit, CDII and TI show alteration of aPTT and significantly lower levels of free PS,PC and AT III than other subjects (p<0.05) with D-dimer >0.5 μg/ml (Table 2).

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Table 1: Mean values of Platelet count (PLT) and Hb in splenectomized hematologic patients and controls.
migraine and diagnosed by cerebral RM. CVT relapsed in patients with TI (Table 3). They had Hb values 9 g/dl and 9.2 g/dl with platelet counts of 750,000 mmc and 780,000mmc respectively and show higher D-dimer (>0.5 μg/ml) and lower levels of PS, PC, and ATIII with aPTT 45'' (Table 4).

Analysis of genetic risk factors shows that 2 patients with previous cITP had heterozygous MTHFR and FV polymorphisms respectively, without clinical or hematologic alterations (data not shown).

**Discussion**

None of the family members had thrombotic events.

Thromboembolic complications following splenectomy for hematologic disorders occur in about 10% of patients and have been frequently reported in β-thalassemia major and particularly in non transfused or occasionally transfused patients with β-th intermedia before and after splenectomy [5,12].

Although the reactive thrombocytosis after splenectomy could represent a predisposing factor of thromboembolism, it is uncertain whether it is the only risk factor because many patients have comparable high platelet count without VTE complications [7,13,14].

Recent reports showed that the risk of developing VTE related to reactive thrombocytosis presents a variability of about 34% with linear relationship with a platelet count >400 x 10^9/L [5,14].

Moreover, in thalassemic patients it has been reported that various and different alterations such as severe liver diseases or other conditions influencing hemostatic functions may lead to thromboembolic events even in unsplenectomized subjects [15].

In not regularly transfused or not transfused patients affected by TI or CDII with mild anaemia, thromboembolic complications, particularly transient ischemic attacks and cerebrovascular events, are frequent.

Therefore, the pathogenesis of thrombotic disorders is less clearly defined and interactions of various mechanisms have been suggested. Both hemostatic changes and activation of the coagulation cascade are involved in thrombophilic events. The loss of the thromboresistant properties of vascular endothelium likely plays an important role. Endothelial damage has been reported as the main cause of vascular occlusion by interacting with hemostatic alterations [4,5,16-18]. Furthermore, reduced levels of Nitric oxide linked to intensity of hemolysis increase vasoconstriction and could contribute to hypercoagulability [15].

Another risk of VTE in not regularly transfused or non transfused patients affected by TI as well as CDII with mild anaemia could be the longer persistence of altered erythrocytes that form reactive oxygen species and enhance cohesiveness and aggregation [15].

In our cohort with TI and CDII, we found persistently reactive thrombocytosis, mildly low Hb levels and altered clotting tests. Ischemic cerebrovascular events occurred in 2 patients with TI and CDII respectively.

Therefore, the pathogenesis of VTE after splenectomy in hematologic diseases unlike multitransfused thalassemic syndromes is poorly understood [19].

Furthermore, it has been reported that in oncologic patients with chemotherapy induced anaemia, increased risk to develop thrombocytosis and VTE may be related to the use of Erythropoiesis Stimulating Agents (ESA) that determine iron restricted erythropoiesis. These patients treated with IV iron were significantly less likely to develop thrombocytosis and had a decreased incidence of VTE [20].

In our cohort we found that 3 splenectomized patients (2 PK deficiencies, 1 cITP) with low Hb values and iron deficiency during pregnancy had thromboembolic events. In these cases the platelet count, previously normal, was increased probably related to iron restricted erythropoiesis.

However, the risk of thrombotic events could be related to pregnancy itself, because about 1/2000 women develop thrombosis during pregnancy with a 10-fold greater risk than non pregnant women of the same age [21,22].

In conclusion the occurrence of VTE in our splenectomized patients appears in relationship with chronic mild anaemia in untransfused CDII and TI patients showing persistent thrombocytosis after splenectomy. In splenectomized patients with other hematologic diseases we observed VTE in relationship with iron deficiency anaemia and increase of platelet count.

Moreover we report a little cohort of hematologic splenectomized patients. Multicenter studies on larger cohorts of patients need to understand the pathogenesis and evaluate the frequency of VTE, which is often clinically silent. More knowledge of risk factors and their interactions will enable us to identify early patients at risk with a closer follow-up.

<table>
<thead>
<tr>
<th>Hematological disease</th>
<th>Hb (g/dl)</th>
<th>serum ferritin (μg/ml)</th>
<th>PLT c count/mm3</th>
<th>ATIII</th>
<th>PS free</th>
<th>PC</th>
<th>aPTT</th>
<th>D-dimer (μg/ml)</th>
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</thead>
<tbody>
<tr>
<td>HS* DVT*</td>
<td>8.5</td>
<td>8.2</td>
<td>650,000</td>
<td>58%</td>
<td>50%</td>
<td>58%</td>
<td>45''</td>
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</tr>
<tr>
<td>PK defici* DVT* CVT*</td>
<td>7.5</td>
<td>8.5</td>
<td>600,000</td>
<td>44%</td>
<td>52%</td>
<td>56.2%</td>
<td>42''</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>cITP* DVT* CVT*</td>
<td>8.2</td>
<td>7.2</td>
<td>620,000</td>
<td>50%</td>
<td>48%</td>
<td>50%</td>
<td>40''</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>CDII* CVT*</td>
<td>9</td>
<td>200</td>
<td>750,000</td>
<td>45%</td>
<td>45%</td>
<td>53%</td>
<td>45''</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>TI* CVT*</td>
<td>9.2</td>
<td>480</td>
<td>780,000</td>
<td>42%</td>
<td>40%</td>
<td>49%</td>
<td>45''</td>
<td>&gt;0.5</td>
</tr>
</tbody>
</table>

*Hereditary Spherocytosis: HS; Piruvate Kynase Deficit: PK; Congenital Dyserithropoiesis II: CDII; Chronic Idiopathic Thrombocytopenic Purpura: cITP; β thalassemia intermedia: TI

**DVT: Deep Venous Thrombosis; CVT: Cerebrovascular Thrombosis**

**Activated Partial Thromboplastin Time; aPTT: Free Protein C activity; PC: Protein S; PS: Antithrombin III; AT III: Platelet count: PLT**

**Table 4: Hematologic alterations in splenectomized patients with venous thromboembolism (VTE).**
References


