Hypercoagulability in splenectomized thalassemic patients detected by whole-blood thromboelastometry, but not by thrombin generation in platelet-poor plasma

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ABSTRACT

Background
The mechanisms responsible for the increased thrombotic risk associated with thalassemia are still unclear. They might be related to the effects of red blood cell or endothelial cell derangements, increased numbers of platelets as well as abnormal plasma coagulation.

Design and Methods
To evaluate the relative role played by cells and plasma we investigated 169 patients with thalassemia by means of thromboelastometry and thrombin generation tests. Thromboelastometry measures indices of the viscoelastic properties of whole blood after activation of coagulation and is characterized by the clotting time, which may be considered as a conventional coagulation time, clot formation time, defined as the time needed for the clot to reach a fixed firmness, and the maximum clot firmness, defined as the maximal amplitude of the tracing.

Results
All the thromboelastometry parameters determined in whole blood (including shortened clotting time and clot formation time, and increased maximum clot firmness), were consistent with hypercoagulability, especially in splenectomized patients. Conversely, thrombin generation as determined in platelet-poor plasma was not.

Conclusions
These findings point to blood cells and/or platelets rather than to plasma abnormalities as the most important determinants of the thrombotic risk observed in thalassemic patients who had been splenectomized. These results might have important diagnostic and therapeutic implications.

Key words: thromboelastometry, thrombosis, hypercoagulability.

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Introduction

Thalassemia is a congenital hemolytic anemia characterized by reduced synthesis of globin chains.[1,2] From the hemostatic stand point it is characterized by hypercoagulability and an increased risk of venous and/or arterial thromboembolism.[3-6] However, not all patients with thalassemia present with the same risk and the identification of those at higher risk is not possible on the basis of the measurements of the individual components of the hemostatic system nor on the basis of global conventional coagulation tests such as the prothrombin time (PT) or the activated partial thromboplastin time (APTT). The failure of these tests to identify hypercoagulability in thalassemia might be due to the absence of blood cells and platelets which may play a key role in the mechanisms responsible for thrombosis in this setting. Thromboelastometry, which measures the viscoelastic properties of clotting whole blood after activation of coagulation by triggers such as tissue factor or partial thromboplastin in combination with calcium chloride, is in principle a better candidate than the PT and APTT to assess hypercoagulability in patients with thalassemia. Such an assessment is an important prerequisite to the organization of prospective studies aimed at evaluating the risk of thrombosis in this category of patients. In this study we investigated these issues and also tried to elucidate the pathogenesis of hypercoagulability in thalassemia by comparing paired measurements for the same patients performed by means of thromboelastometry in whole blood versus thrombin generation in plasma.

Design and Methods

Patients

One-hundred and sixty-nine patients with β-thalassemia (71 males and 98 females aged from 19 to 62 years) were enrolled in this study which was approved by the institutional review board of our institution. They were consecutive patients referred to the Thalassemia Unit for regular clinical visits who, on the occasion of phlebotomy for their check-ups, voluntarily gave an additional blood sample for the study. One-hundred and sixteen had thalassemia major, of whom 76 (65.6%) were splenectomized, and 53 had thalassemia intermedia, of whom 34 (64.2%) were splenectomized. None of the patients was on vitamin K antagonists at the time of blood sampling.

Healthy individuals

Eighty-six healthy individuals (33 males and 53 females aged from 23 to 75 years) were randomly selected from among medical students, the staff of our institution and other volunteers. Subjects who had had a splenectomy, had known hemorrhagic/thrombotic diseases or other conditions known to alter the hemostatic balance, used oral anticoagulants other antithrombotic drugs, or oral contraceptives were not included. The values obtained in this population were used to establish reference intervals for thromboelastometry. Another group of 154 healthy individuals (71 males, 83 females, aged from 17 to 64 years) comparable for age and gender to the population of patients were used as controls for the thrombin generation assay.

Blood sampling and plasma preparation

After informed consent blood samples from patients and healthy individuals were collected into vacuum tubes (BD, Meylan, France) containing 0.109 M sodium citrate as an anticoagulant at a proportion of 9:1 (blood:anticoagulant). One portion of the blood was used as such for thromboelastometry testing which was performed within 2 h of blood collection, and the other was centrifuged at 2880 g for 15 min at room temperature. Supernatant plasma was harvested, aliquoted in capped plastic tubes, quickly frozen in liquid nitrogen and stored at –70°C for later testing of conventional coagulation parameters and thrombin generation, performed in batch analyses within 6 months of blood collection. Blood samples from patients were collected at least 3 and 4 weeks after the last blood transfusion for thalassemia major and intermedia, respectively.

Thromboelastometry

Rotation thromboelastometry was performed using four channel ROTEM® Gamma equipment according to the manufacturer's instructions and with the type and concentration of reagents (undisclosed) provided by the manufacturer (Pentapharm, Munich, Germany). Among the parameters that were recorded we report on the following: (i) the clotting time (CT), defined as the time (in seconds) from the start of the measurement until initiation of clotting;[7] (ii) the clot formation time (CFT), defined as the time (in seconds) from the initiation of clotting until a clot firmness of 20 mm was recorded;[7] (iii) maximum clot firmness (MCF), defined as the maximal amplitude (mm) of the tracing obtained after addition of the hemostatic trigger.[7] CT, CFT and MCF were measured after triggering hemostasis with reagents containing partial thromboplastin of rabbit origin, ellagic acid and calcium chloride (INTEM®, Pentapharm) or with reagents containing tissue factor and calcium chloride (EXTEM®, Pentapharm). INTEM and EXTEM are considered to trigger intrinsic and extrinsic activation of hemostasis, respectively. All the measurements were taken on citrated blood according to the manufacturer's instructions. Samples from patients and healthy individuals were handled in the same manner and within the same time frame.

Thrombin generation

Thrombin generation was assessed on thawed plasma in batch analyses within 6 months of blood collection. To minimize analytical variability equal numbers of plasma samples from patients and controls were included on each test occasion. Thrombin generation was assessed as endogenous thrombin potential (ETP) as proposed by Hemker *et al.*[8] and described in detail

by Chantarangkul *et al.*[9] Briefly, the test is based on the activation of coagulation in platelet-poor plasma after addition of human relipidated recombinant tissue factor (Recombiplastin, Instrumentation Laboratory) in the presence of the synthetic phospholipids 1,2-dioleoyl-sn-glycero-3-phosphoserine (DOPS), 1,2-dioleoyl-sn-glycero-3-phosphoetanolamine (DOPE) and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) (Avanti Polar Lipids Inc., Alabaster, AL, USA) in the proportion of 20/20/60 (M/M). The concentrations of tissue factor and phospholipids in the test system were 1 pM and 1.0 μM, respectively. Testing for ETP was performed in the presence of soluble rabbit thrombomodulin (ICN Biomedicals, Aurora, OH, USA) added to the reaction mixture at a final concentration of 4 nM. The generated thrombin was measured continuously by using a fluorogenic synthetic substrate (Z-Gly-Gly-Arg-AMC HCl, Bachem, Switzerland) added to the test system at a final concentration of 417 μM, and an automated fluorometer (Fluoroskan Ascent®, Thermo-Labsystem, Helsinki, Finland). Readings from the fluorometer were automatically recorded and calculated by a dedicated software (Thrombinoscope™, Thrombinoscope BV, Maastricht, The Netherlands), which displays thrombin generation curves [nM thrombin versus time (minute)] and calculates the area under the curve, defined as ETP and expressed as nM thrombin times minutes (nM*min). Thrombin generation is measured as function of an internal calibrator for thrombin (Thrombin Calibrator, Thrombinoscope BV). ETP represents the balance between the action of pro-coagulants and anti-coagulants in the plasma.

Conventional coagulation parameters and blood cells counts

The following coagulation parameters were measured on thawed plasma samples at the end of the study and no more than 10 months after the beginning of the enrollment: PT and APTT, with results expressed as ratios (patient-to-normal coagulation time), by means of a human recombinant relipidated thromboplastin (Recombiplastin, Instrumentation Laboratory, Orangeburg, NY, USA) or the automated APTT (bioMerieux, Durham, NC); antithrombin, as heparin co-factor activity with Electrachrome Antithrombin (Instrumentation Laboratory); protein C, as anticoagulant activity (PC clot, Instrumentation Laboratory); and factors II, VIII and V by one-stage coagulation assays. Results for antithrombin, factors II, VIII and V are expressed as percentages of values from a reference frozen plasma, prepared by mixing equal volumes of plasma obtained from blood of 30 healthy individuals and arbitrarily set at 100% activity. Fibrinogen (mg/dL) was measured by means of a functional thrombin-based coagulation assay as clottable protein (Q.F.A. Thrombin, Instrumentation Laboratory). Complete blood cell counts were performed with an automated device (ABX Micros 60, ABX International, Montpellier, France).

Data analyses

Results are presented as medians and ranges (minimun-maximum values). The Mann-Whitney, Kruskal Wallis and the Spearman's rho correlation tests were used as appropriate. p values of less than 0.05 were considered statistically significant. For the purpose of this study reference intervals were determined as the values below the 5th (CT and CFT) or above the 95th (MCF) percentiles of distribution of results for healthy individuals. The percentages of patients whose results fell outside the relevant cut-off values for the various categories investigated were compared using Pearson's χ^2 test. All analyses were performed with SPSS version 17.0 software (Chicago, IL, USA).

Results

Conventional coagulation parameters and blood counts for the population of thalassemic patients are reported in Table 1. Platelet and leukocyte numbers and factor VIII activity were significantly greater for patients who had been splenectomized than for those who had not, both for thalassemia major and intermedia. Factor V activity was significantly greater for splenectomized than for non-splenectomized patients with thalassemia major. Antithrombin activity was significantly lower for splenectomized than for non-splenectomized patients with thalassemia intermedia. Finally, only protein C activity differed significantly between patients with thalassemia major and those with thalassemia intermedia (mean 63%, range 16-124% *vs.* 72%, 35-129%) (p<0.05).

Thromboelastometry

The distributions of results for thromboelastometry parameters are shown in Figure 1 (panels A-C) and Table 2, and the percentages of patients with abnormal values are shown in Figures 1A-1C and 2). In general, there were negligible differences within each parameter regardless of whether it was determined for INTEM or EXTEM; therefore, results for each parameter are shown for INTEM.

Clotting time

Overall, CT values were not significantly different between patients with thalassemia major (median 159 sec, range 110-220) and those with thalassemia intermedia (163 sec, 116-213) (p=0.34). However, within both groups the CT values for splenectomized patients were significantly shorter than those recorded for non-splenectomized patients [thalassemia major, 157 sec (110-220) *vs.* 176 sec (135-200), p=0.005; thalassemia intermedia 159 sec (117-210) *vs.* 178 sec (149-213), p<0.001] (Figure 1A and Table 2). Overall, the percentage of patients with abnormally shortened CT values (i.e., below the 5th percentile of the healthy population) was 25% for thalassemia major and 20.8% for thalassemia intermedia. Within both types of thalassemia, the percentage of abnormal CT values was relatively greater for those patients who had been splenectomized than for those who had not [thalassemia major=28.9% *vs.* 17.5%, p=0.18; thalassemia intermedia=32.4% *vs.* 0%, p=0.005) (Figures 1A and 2).

Table 1. Values [median (range, minimum-maximum)] of conventional coagulation parameters and blood counts for patients with thalassemia.

Parameter	Thalassemia Major			Thalassemia Intermedia		
	Splenectomy		Total	Splenectomy		Total
	Yes	No		Yes	No	
Prothrombin time* (Reference range: 0.90-1.15)	0.98 (0.74-1.28)	0.98 (0.73-1.18)	0.98 (0.73-1.28)	0.98 (0.78-1.11)	0.97 (0.74-1.06)	0.97 (0.74-1.11)
APTT* (Reference range: 0.90-1.15)	1.07[a] (0.68-1.59)	1.16[a] (0.87-1.39)	1.10 (0.68-1.59)	1.06 (0.79-1.29)	1.11 (0.93-1.32)	1.07 (0.79-1.32)
Platelets $\times 10^9$/L	574[a] (202-1,169)	289[a] (76-421)	440 (76-1,169)	651b (339-1,121)	159[b] (72-376)	543 (72-1,121)
Erythrocytes $\times 10^{12}$/L	3.5 (2.6-4.3)	3.4 (3.0-4.3)	3.5[c] (2.6-4.4)	3.6[b] (2.8-5.0)	4.5[b] (2.5-6.4)	3.9[c] (2.5-6.4)
Leukocytes $\times 10^9$/L	13.2[a] (6.0-28.4)	6.8[a] (2.7-11.0)	10.5 (2.7-28.4)	13.5[b] (5.5-18.0)	6.8[b] (3.4-9.8)	10.2 (3.4-18.0)
Factor II# (Reference range: 60-120)	77 (29-100)	77 (48-104)	77 (29-104)	75 (49-103)	81 (22-121)	79 (22-121)
Factor V# (Reference range: 60-120)	97[a] (56-138)	80[a] (38-128)	91 (38-138)	97 (42-149)	92 (56-147)	95 (42-149)
Factor VIII# (Reference range: 60-130)	124[a] (58-248)	107[a] (69-199)	118 (58-248)	121[b] (86-274)	95[b] (59-200)	117 (59-274)
Fibrinogen mg/dL (Reference range: 100-350)	256 (143-410)	230 (136-417)	248 (136-417)	240 (139-653)	281 (166-691)	251 (139-691)
Antithrombin# (Reference range: 80-120)	85 (36-115)	88 (52-119)	85 (36-119)	81[b] (68-116)	94[b] (69-123)	85 (68-123)
Protein C# (Reference range: 60-120)	63 (16-124)	63 (33-95)	63[c] (16-124)	72 (42-93)	77 (35-129)	72[c] (35-129)

**ratio of patient-to-normal coagulation times; #% of the pooled normal plasma; [a]p<0.05 splenectomy yes vs. no (thalassemia major); [b]p<0.05 splenectomy yes vs. no (thalassemia intermedia); [c]p<0.05 thalassemia major vs. intermedia.*

Clot formation time

Overall, CFT values were not significantly different between patients with thalassemia major (61 sec, 33-236) and those with thalassemia intermedia (66 sec, 30-204), (p=0.11). However, within both groups, values for splenectomized patients were significantly shorter than those for non-splenectomized patients [thalassemia major, 52 sec (33-88) *vs.* 85 sec (56-236), p<0.001; thalassemia intermedia 60 sec (30-87) *vs.* 116 sec (50-204), p<0.001] (Figure 1B and Table 2). Overall, the percentage of patients with abnormally shortened CFT values (i.e., below the 5th percentile of the healthy population) was 29.3% for thalassemia major and 24.5% for thalassemia intermedia. Within both types of thalassemia, the percentage of abnormal CFT values was significantly greater for those patients who had been splenectomized than for those who had not [thalassemia major=44.7% *vs.* 0%, p<0.001; thalassemia intermedia = 35.3% *vs.* 5.3%, p=0.01) (Figures 1B and 2).

Maximum clot firmness

Overall, MCF values were not significantly different between patients with thalassemia major (64 mm, 43-81) and those with thalassemia intermedia (66 mm, 45-80) (p=0.75). However, within both groups values for splenectomized patients were significantly greater than those recorded for non-splenectomized patients [thalassemia major, 66 mm (55-81) *vs.* 59 mm (43-70), p<0.001; thalassemia intermedia 68 mm (60-80) *vs.* 56 mm (45-71), p<0.001] (Figure 1C and Table 2). Overall, the percentage of patients with an abnormally increased MCF value (i.e., above the 95th percentile for the healthy population) was 25.9% for thalassemia major and 32.1% for thalassemia intermedia. Within both types of thalassemia, the percentage of abnormal MCF values was significantly greater for those patients who had been splenectomized than for those who had not [thalassemia major=38.2% *vs.* 2.5%, p<0.001; thalassemia intermedia=47.1% *vs.* 5.3%, p=0.002) (Figures 1C and 2).

Other thromboelastometry parameters

Other parameters of thromboelastometry such as the time to MCF, α angle, maximum velocity, time to maximum velocity and area under the tracing were all consistent with hypercoagulability with statistically significant differences for splenectomized versus non-splenectomized patients (*data not shown*).

Thrombin generation

The distribution of ETP values is shown in Figure 3. The ETP values of patients with either type of thalassemia were not significantly different from those of healthy individuals. Furthermore, within both groups of thalassemia there were no significant differences between values for patients who had been splenectomized and those for patients who had not, although the median values for the former tended to be greater than those recorded for the latter.

Discussion

It is widely recognized that patients with thalassemia are at increased risk of venous and/or arterial thrombosis. A recent survey, carried out in the Mediterranean area and Iran among 8,860 patients, estimated the cumulative prevalence of thromboembolic events at 1.65%, with thromboses occurring 4.38 times more frequently in patients with thalassemia intermedia than in those with thalassemia major.[10] Interestingly, venous thromboembolism was recorded more frequently than arterial thromboembolism (stroke) both for patients with thalassemia major (48% *vs.* 28%) and for those with thalassemia intermedia (66% *vs.* 9%).[10] Furthermore, the risk was greater for patients who had been splenectomized than for those who had not.[10] Other risk factors for thrombosis, especially in patients with thalassemia intermedia, may be older age (>20 years), previous thromboembolic events and family history. The mechanisms underlying hypercoagulability and the increased thrombotic risk associated with thalassemia are still unclear. They might be the result of combined effects of the endothelial and red blood cell derangements, the former occurring as a consequence of the ongoing inflammatory state associated with the disease and the latter as a consequence of oxidative stress and/or exposure of negatively-charged phospholipids (phosphatidylserine) on cell membranes which are able to accelerate the conversion of prothrombin to thrombin.[11,12] Although various studies identified abnormal coagulation (assessed by the measurement of individual components, both pro- and anticoagulant, of hemostasis), or increased numbers of platelets as an additional cause for thrombosis[6,13] other laboratory parameters, including genetic factors predisposing to thrombosis,[14] failed to identify patients at

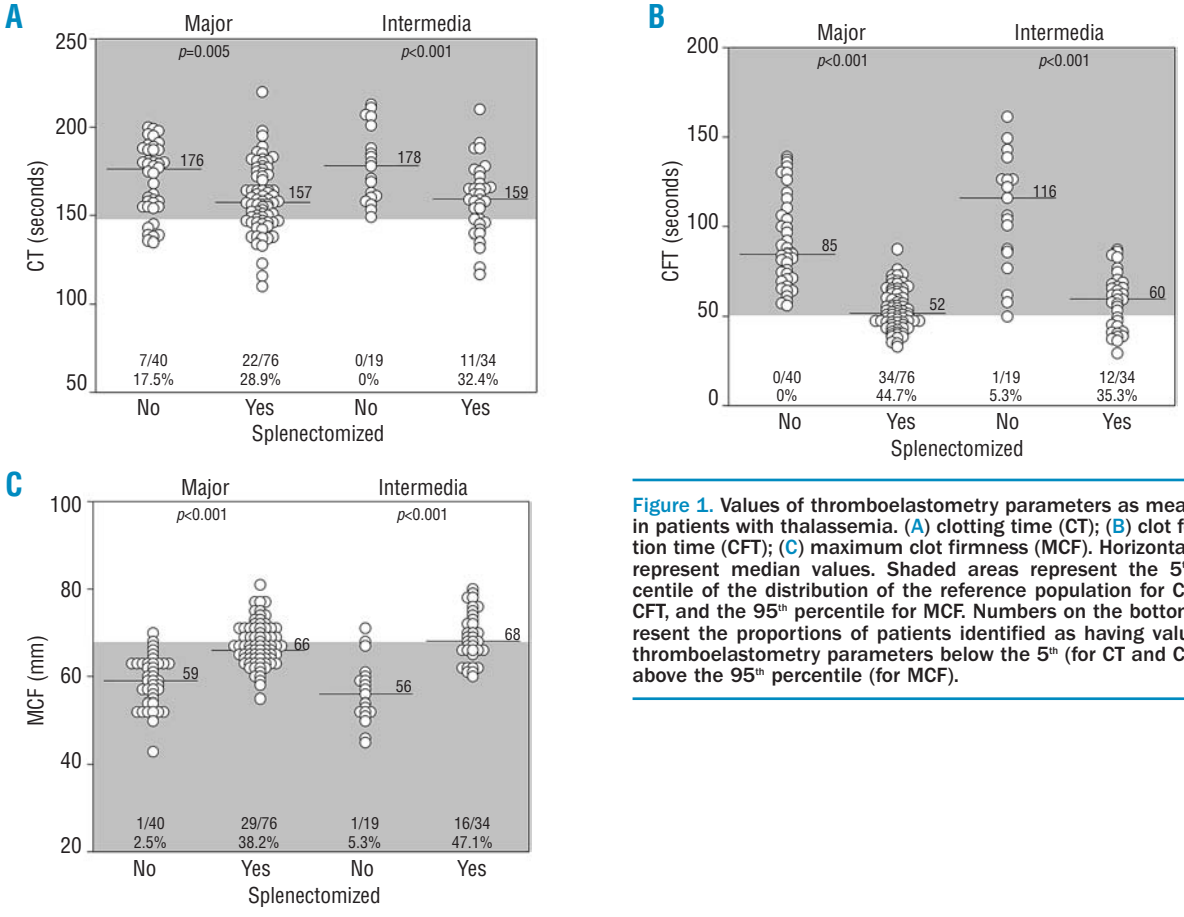

Figure 1. Values of thromboelastometry parameters as measured in patients with thalassemia. (A) clotting time (CT); (B) clot formation time (CFT); (C) maximum clot firmness (MCF). Horizontal bars represent median values. Shaded areas represent the 5th percentile of the distribution of the reference population for CT and CFT, and the 95th percentile for MCF. Numbers on the bottom represent the proportions of patients identified as having values of thromboelastometry parameters below the 5th (for CT and CFT) or above the 95th percentile (for MCF).

Table 2. Values [median (range, minimum-maximum)] of thromboelastometry parameters for patients with thalassemia.

Parameter	Thalassemia Major			Thalassemia Intermedia		
	Splenectomy yes	Splenectomy no	Total	Splenectomy Yes	Splenectomy no	Total
CT (cut-off value: <148 sec)	157[b] (110-220)	176[b] (135-200)	159[a] (110-220)	159[c] (117-210)	178[c] (149-213)	163[a] (117-213)
CFT (cut-off value: <50 sec)	52[c] (33-88)	85[c] (56-236)	61[a] (33-236)	60[c] (30-87)	116[c] (50-204)	66[a] (30-204)
MCF (cut-off value: >68 mm)	66[c] (55-81)	59[c] (43-70)	64[a] (43-81)	68[c] (60-80)	56[c] (45-71)	66[a] (45-80)

[a]*p: N.S.;* [b]*p: 0.005;* [c]*p <0.001.*

increased risk. We reasoned that such failure might be due to the fact that conventional tests do not truly represent the balance of coagulation as it occurs *in vivo* in these patients. Indeed, conventional tests for pro- and anti-coagulant factors were designed to be performed on plasma, thus missing the contributory effect that platelets, leukocytes and red blood cells may have on the hemostatic imbalance leading to thrombosis.

To test this hypothesis we investigated citrated whole blood from a large cohort of thalassemic patients by thromboelastometry, which can be considered as a global test for hemostasis. We also investigated platelet-poor plasma samples from the same patients by means of a thrombin generation assay in which coagulation activation is achieved by small amounts of tissue factor as a trigger, phospholipids as platelet substitutes and thrombomodulin as the activator of the endogenous protein C anticoagulant system. This is a global test defined by the area under the thrombin generation curve (i.e, thrombin concentration versus time) called ETP. The ETP can be considered as a reliable index of the amount of thrombin that any given plasma specimen may generate under the specified experimental conditions and represents the balance between the pro- and anti-coagulant proteins operating in plasma. The test as modified by the addition of thrombomodulin mimics what occurs *in vivo* more closely than any other plasma test. It can be useful to assess hypo- and hyper-coagulability[15,16] and the risk of the occurrence and recurrence of venous thromboembolism.[17,18]

This study shows that conventional parameters of blood coagulation in our cohort of thalassemia patients were near normal, except protein C and factor II which in patients with thalassemia major were close to the lower limits of their respective reference intervals (Table 1). The activity of factors V and VIII and the numbers of platelets and leukocytes were, however, significantly greater in patients who had been splenectomized than in those who had not (Table 1). On the one hand, these findings confirm previous information suggesting that elevated numbers of platelets and

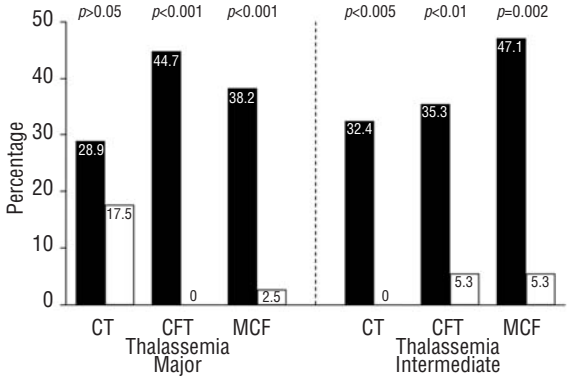

Figure 2. Percentage of patients identified as having values of thromboelastometry parameters below the 5th (for CT and CFT) or above the 95th percentile (for MCF). Solid and open columns represent splenectomized or non-splenectomized patients, respectively.

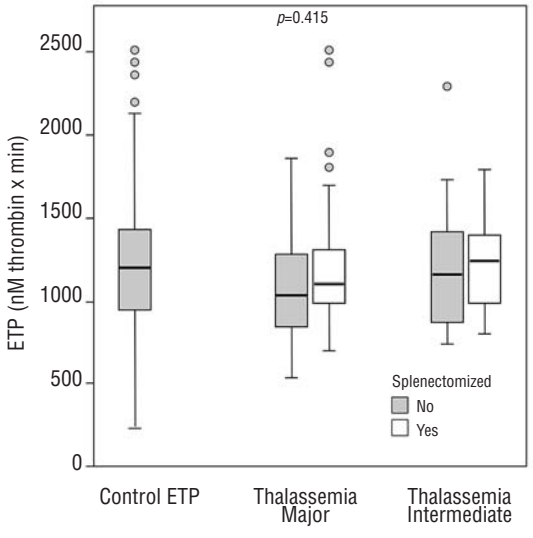

Figure 3. Box plots showing distribution (median, lower and upper quartiles) of ETP values for healthy individuals and patients with thalassemia.

leukocytes may be risk factors for thrombosis in splenectomized patients; numbers of platelets were, indeed, significantly correlated ($p<0.001$) with the three parameters of thromboelastometry (rho values -0.28; -0.65 and 0.63 for CT, CFT and MCF, respectively), but, on the other hand, they indicate that conventional coagulation parameters, with the possible exception of factor VIII, are of little value for assessing the risk of thrombosis in this category of patients. Elevated levels of factor VIII have, in fact, been associated with an increased risk of occurrence and recurrence of venous thromboembolism in thrombophilic patients[19] and might, therefore, play some role also in splenectomized thalassemic patients.

This study also shows for the first time that patients with thalassemia have abnormalities for all the thromboelastometry parameters suggestive of hypercoagulability. Median CT and CFT values were significantly smaller and median MCF values were greater for patients who had been splenectomized compared to those who had not (Figure 1A-C). Furthermore, the rate of abnormal values for the three parameters was significantly greater for those patients who had been splenectomized than for those who had not (Figures 1A-C and 2). CT may be considered as a conventional coagulation time and was, in fact, correlated with the APTT ratio (rho=0.52, $p<0.001$). Since shortened APTT has been associated with an increased risk of occurrence[20] and recurrence[21,22] of venous thromboembolism in thrombophilic patients, it is not surprising that shortened CT detects hypercoagulability in splenectomized thalassemic patients. CFT is defined as the time needed for the clot to reach a fixed firmness (20 mm) and MCF is defined as the maximal amplitude of the tracing after the addition of the trigger. Accordingly, shortened CFT and increased MCF can be considered as indices of hypercoagulability. These findings are in line with the clinical evidence that splenectomized thalassemic patients are at increased risk of thrombosis and suggest thromboelastometry as a potential method to assess the risk of thrombosis in this category of patients. Although the retrospective nature of this study did not allow an assessment of the predictive value of thromboelastometry parameters for thrombosis, our findings pave the way to prospective studies based on CT, CFT and MCF which may substantiate our hypothesis.

Another important and new finding of this study is that thrombin generation assessed as ETP in platelet-poor plasma from thalassemic patients was normal and there were no differences between values recorded for patients who had been splenectomized and those who had not (Figure 3). Thrombin generation was evaluated in plasma without the addition of corn trypsin inhibitor, which quenches undesirable contact activation. This may be regarded as a limitation of our study. However, it is unlikely that the effect of contact activation on thrombin generation was different in the two populations of patients who had or had not undergone splenectomy. The information on normal thrombin generation if compared to the thromboelastometry findings might have important implications. First, it demonstrates that the risk of thrombosis in thalassemic patients is mediated by platelets, leukocytes, abnormal red bloods and/or damaged endothelial cells, rather than by plasma abnormalities, thus substantiating and extending previous evidence from the literature.[10] It is well established that activated platelets play a crucial role in thrombin generation.[23] In addition, platelets from thalassemic patients show increased adhesion under flow conditions,[24] presumably due to oxidative stress with the generation of reactive oxygen species.[25] However, it is unknown whether this increased adhesiveness corresponds to an increased procoagulant activity. Unfortunately, ETP in platelet-rich plasma could not be measured due to the shortage of samples. We could not, therefore, assess whether the increased numbers of platelets are more implicated in the thrombotic process than are abnormal red blood cells or damaged endothelial cells. Secondly, if one assumes that plasma is not implicated in the thrombotic process, then vitamin K antagonists, which are the drugs of choice to prevent recurrence of venous thromboembolism,[26] might be inappropriate for patients with thalassemia. Aspirin, on the other hand, has not yet been investigated for its effectiveness in preventing the occurrence or recurrence of venous thromboembolism in the general population of thrombophilic patients and there is no evidence on its effectiveness in thalassemic patients. Perhaps, alternative approaches could be the reduction of the numbers of red blood cells exhibiting pro-coagulant activity in splenectomized patients by regular transfusions or the correction of the red blood cell abnormalities induced by reactive oxygen species by administration of antioxidants.[27] Clinical studies are warranted to investigate these issues.

In conclusion, this study shows that all the thromboelastometry parameters determined in whole blood are compatible with hypercoagulability in splenectomized thalassemic patients. Conversely, thrombin generation determined in platelet-poor plasma is not. These findings point to the blood, endothelial cells and/or platelets rather than to plasma abnormalities as being the most important determinants of the thrombotic risk observed in this category of patients and might have important diagnostic and therapeutic implications.

Authorship and Disclosures

AT: conceived the study, interpreted results and wrote the manuscript; MDC and PMM: conceived the study, helped to interpret results and revised the manuscript; VC: designed methods, collected data and performed statistical analyses; LP: designed methods and performed testing; MRF and AM: selected and managed the enrollment of patients and collected clinical data.

The authors reported no potentual conflicts of interest.

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