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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 or endothelial cells derangement, increased numbers of platelets as well as abnormal plasmatic 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 was taken as an index of the viscoelastic properties of whole blood after activation of coagulation and is characterized by the clotting time (CT) that may be considered as a conventional coagulation time; clot formation time (CFT), defined as the time needed for the clot to reach a fixed firmness and the maximum clot firmness (MCF), defined as the maximal amplitude of the tracing.

Results
All the thromboelastometry parameters determined in whole blood (including shortened CT and CFT, and increased MCF), 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 and might have important diagnostic and therapeutic implications.

Key words: ETP, 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 the 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 such global conventional coagulation tests as the prothrombin or activated partial thromboplastin times (PT, APTT). 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 such triggers 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 evaluation is an important prerequisite to the organization of prospective studies aimed at evaluating the risk of thrombosis in this category of patients. The study also aims at elucidating 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-sixty-nine patients with beta-thalassemia (71males and 98 females with an age spanning 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 check up volunteered to give an additional blood sample for the study. One-hundred-sixteen had thalassemia major and 53 intermedia. Splenectomized patients were 76/116 (65.6%) and 34/53 (64.2%) for thalassemia major and intermedia, respectively. 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 with an age spanning from 23 to 75 years) have been randomly selected among the population of medical students, the staff of our institution and other volunteers. Exclusion criteria were the presence of splenectomy, known hemorrhagic/thrombotic diseases or other conditions known to alter the hemostatic balance, the use of oral anticoagulants or other antithrombotic drugs, and the use of oral contraceptives. The values obtained for this population were used to establish reference intervals for thromboelastometry. Another group of 154 healthy individuals (71 males, 83 females, ageing from 17 to 64 years) comparable for age and gender to the patient population 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 anticoagulant at a proportion of 9:1 (blood:anticoagulant). One portion of the blood was used as such for thromboelastometry testing that was performed within 2 hours from blood collection and the other was centrifuged at 2880 g for 15 minutes at room temperature. Supernatant plasma was harvested, aliquoted in capped plastic tubes, quick 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 from blood collection. Blood samples from patients were collected at least three and four weeks after the last blood transfusion for thalassemia major and intermedia, respectively.

Thromboelastometry

Rotation thromboelastometry was performed by means of the 4 channel ROTEM® Gamma equipment according to manufacturer instructions and with type and concentration of reagents (undisclosed) as 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 (seconds) from start of the measurement until initiation of clotting7; (ii) The Clot Formation Time (CFT), defined as the time (seconds) from initiation of clotting until a clot firmness of 20 mm is recorded7; (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 upon triggering hemostasis with reagents containing partial thromboplastin from 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 reflect the intrinsic or extrinsic activation of hemostasis. All the measurements were taken on citrated blood according to 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 from blood collection. To minimize analytical variability equal numbers of plasmas from patients and controls were included at each test occasion. Thrombin generation was assessed as Endogenous Thrombin Potential (ETP) according to Hemker and coworkers[8] as described in details 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 mM, respectively. Testing for ETP was performed in the presence of soluble rabbit thrombomodulin (ICN Biomedicals, Aurora, Ohio) added to the reaction mixture at a final concentration of 4 nM. Continuous registration of the generated thrombin was achieved with a fluorogenic synthetic substrate (Z-Gly-Gly-Arg-AMC HCl, Bachem, Switzerland) added to the test system at a final concentration of 417 μM. The procedure was carried out with an automated fluorometer (Fluoroskan Ascent, ThermoLabsystem, 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 plasmatic balance between the action of pro-coagulants and anti-coagulants.

Conventional coagulation parameters and blood cells counts

The following parameters were measured on thawed plasmas at the end of the study and no later than 10 months from the beginning of the enrolment. PT and APTT with results expressed as ratio (patient-to-normal coagulation time) by means of a human recombinant relipidated thromboplastin (Recombiplastin, Instrumentation Laboratory, Orangeburg, NY) 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); factors II, VIII and V by one-stage coagulation assays. Results for antithrombin, factors II, VIII and V were expressed as % of a reference frozen plasma prepared by mixing equal volumes of plasmas 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 cells counts were performed with an automated device (ABX Micros 60, ABX International, Montpellier, France).

Data analyses

Results are presented as medians and ranges (min-max values). The Mann-Whitney, Kruskal Wallis and the Spearman rho correlation tests were used as appropriate. *p* values of less than 0.05 were considered as 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 percentage of patients whose results fell outside the relevant cut-off values for the various categories investigated were compared by the Pearson Chi square test. All analyses were performed with the 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. Platelets and leucocytes numbers and factor VIII activity for patients who had been splenectomized were significantly greater than 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, the comparison of thalassemia major vs. intermedia revealed a significant difference only for protein C activity (mean 63%, range 16-124% *vs.* 72%, 35-129%) ($p<0.05$).

Thromboelastometry

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

Clotting time (CT)

Overall, CT values were not significantly different between thalassemia major (median 159 sec, range 110-220) and intermedia (163 sec, 116-213) ($p=0.34$). However, within both groups values for splenectomized patients were significantly shorter than those recorded for non-splenectomized patients [major, 157 sec (110-220) vs. 176 sec (135-200), $p=0.005$; 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 intermedia. Within both types of thalassemia, the percentage of abnormal CT values was relatively greater for those patients who had been splenectomized than those who had not [major=28.9% *vs.* 17.5%, $p=0.18$; intermedia=32.4% *vs.* 0%, $p=0.005$) (Figures 1A and 2).

Clot formation time (CFT)

Overall, CFT values were not significantly different between thalassemia major (61 sec, 33-236) and intermedia (66 sec, 30-204), ($p=0.11$). However, within both

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

Parameter	Thalassemia Major			Thalassemia Intermedia		
	Splenectomy		Total	Splenectomy		Total
	Yes	No		Yes	No	
PT*	0.98	0.98	0.98	0.98	0.97	0.97
(Reference	(0.74-1.28)	(0.73-1.18)	(0.73-1.28)	(0.78-1.11)	(0.74-1.06)	(0.74-1.11)
range: 0.90-1.15)						
APTT*	1.07[a]	1.16[a]	1.10	1.06	1.11	1.07
(Reference	(0.68-1.59)	(0.87-1.39)	(0.68-1.59)	(0.79-1.29)	(0.93-1.32)	(0.79-1.32)
range: 0.90-1.15)						
Platelets x10^9/L	574[a]	289[a]	440	651b	159b	543
	(202-1,169)	(76-421)	(76-1,169)	(339-1,121)	(72-376)	(72-1,121)
Erythrocytes x10^{12}/L	3.5	3.4	3.5[c]	3.6[b]	4.5[b]	3.9[c]
	(2.6-4.3)	(3.0-4.3)	(2.6-4.4)	(2.8-5.0)	(2.5-6.4)	(2.5-6.4)
Leucocytes x10^9/L	13.2[a]	6.8[a]	10.5	13.5[b]	6.8[b]	10.2
	(6.0-28.4)	(2.7-11.0)	(2.7-28.4)	(5.5-18.0)	(3.4-9.8)	(3.4-18.0)
Factor II#	77	77	77	75	81	79
(Reference	(29-100)	(48-104)	(29-104)	(49-103)	(22-121)	(22-121)
range: 60-120)						
Factor V#	97a	80a	91	97	92	95
(Reference	(56-138)	(38-128)	(38-138)	(42-149)	(56-147)	(42-149)
range: 60-120)						
Factor VIII#	124a	107a	118	121b	95b	117
(Reference	(58-248)	(69-199)	(58-248)	(86-274)	(59-200)	(59-274)
range: 60-130)						
Fibrinogen	256	230	248	240	281	251
mg/dL	(143-410)	(136-417)	(136-417)	(139-653)	(166-691)	(139-691)
(Reference						
range: 100-350)						
Antithrombin#	85	88	85	81b	94b	85
(Reference	(36-115)	(52-119)	(36-119)	(68-116)	(69-123)	(68-123)
range: 80-120)						
Protein C#	63	63	63c	72	77	72c
(Reference	(16-124)	(33-95)	(16-124)	(42-93)	(35-129)	(35-129)
range: 60-120)						

**ratio of patient-to-normal coagulation times; #% of the pooled normal plasma; ap<0.05 splenectomy yes vs. no (thalassemia major); bp<0.05 splenectomy yes vs. no (thalassemia intermedia); cp<0.05 thalassemia major vs. intermedia.*

groups, values for splenectomized patients were significantly shorter than those for non-splenectomized patients [major, 52 sec (33-88) *vs.* 85 sec (56-236), $p<0.001$; 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 intermedia. Within both types of thalassemia, the percentage of abnormal CFT values was significantly greater for those patients who had been splenectomized than those who had not [major=44.7% *vs.* 0%, $p<0.001$; intermedia = 35.3% vs. 5.3%, $p=0.01$) (Figures 1B and 2).

Maximum clot firmness (MCF)

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

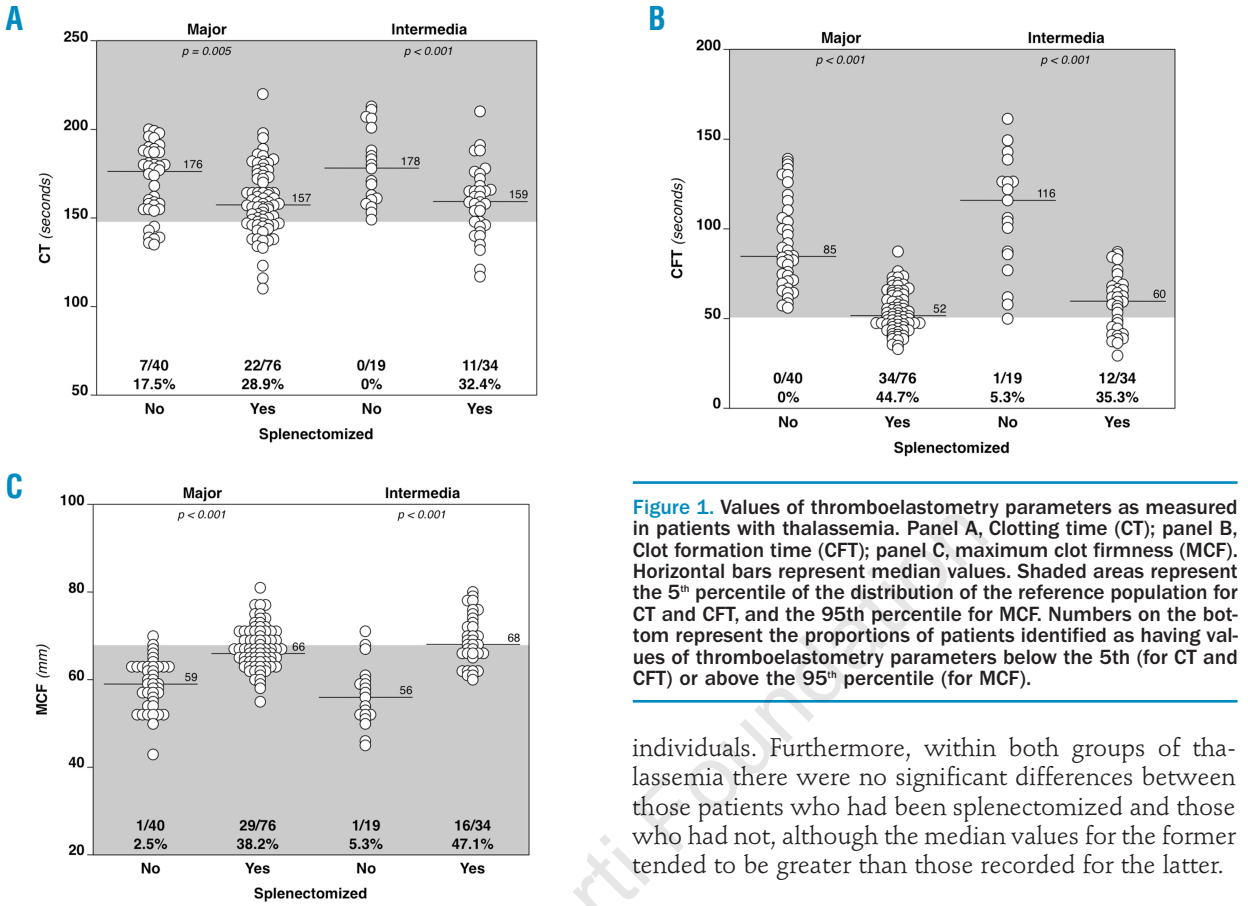

Figure 1. Values of thromboelastometry parameters as measured in patients with thalassemia. Panel A, Clotting time (CT); panel B, Clot formation time (CFT); panel C, maximum clot firmness (MCF). Horizontal bars represent median values. Shaded areas represent the 5th percentile of the distribution of 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).

p<0.001; 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, alpha 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. Patients with either type of thalassemia obtained ETP values not significantly different from those of healthy individuals. Furthermore, within both groups of thalassemia there were no significant differences between those patients who had been splenectomized and those 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 major.[10] Interestingly, venous thromboembolism has been recorded more frequently than arterial thromboembolism (stroke) both for patients with thalassemia major (48% *vs.* 28%) or intermedia (66% *vs.* 9%).10 Furthermore, the risk was greater for patients who had

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

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

ap: N.S.; bp: 0.005; cp <0.001.

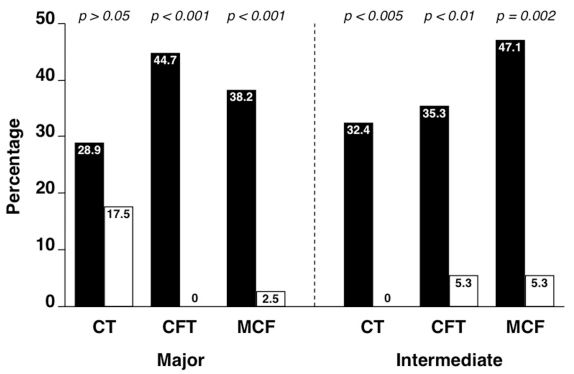

Figure 2. Percentage of patients identified as having values of thromboelastometry parameters below the 5th (for CT and CFT) or above the 95th percentile (for MCF). Solid and open columns represent splenectomized or non-splenectomized patients.

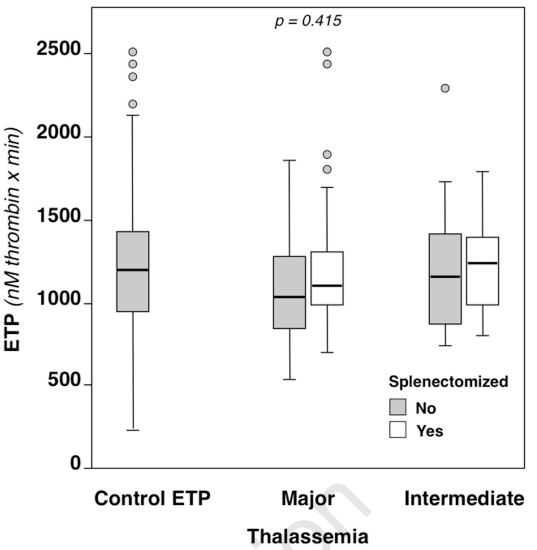

Figure 3. Box plots showing distribution (median, lower and upper quartiles) of ETP values for healthy individuals and patients with thalassemia.

been splenectomized than for those who had not.[10] Other risk factors of 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 explained by the combined effects of the endothelial and red blood cells derangement, the first occurring as a consequence of the ongoing inflammatory state consequent to the disease and the second as a consequence of the oxidative stress and/or exposure of negatively-charged phospholipids (phosphatidylserine) on cell membranes that are able to accelerate the conversion of prothrombin to thrombin.[11,12] Although a number of reports identified abnormal coagulation (assessed by the measurement of individual hemostatic components, either pro- or anticoagulants), 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 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. As a matter of fact, conventional tests for pro- and anticoagulant factors have been designed to be performed on plasma, thus missing the contributory effect that platelets, leucocytes and red blood cells may have on the hemostatic unbalance leading to thrombosis.

To test this hypothesis we investigated citrated whole blood from a large cohort of thalassemic patients by thromboelastometry that can be considered as a global test for hemostasis. We also sought to investigate platelet poor plasmas from the same patients by means of a thrombin generation assay where coagulation activation is attained by small amounts of tissue factor as 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 endogenous thrombin potential (ETP). The ETP can be considered as a reliable index of the amount of thrombin that any given plasma 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 more closely than any other plasma test what occurs *in vivo*. 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 were near normal, except protein C and factor II that in thalassemia major were close to the lower limits of their respective reference intervals (Table 1). There were, however, significantly greater activities of factors V and VIII and greater numbers of platelets and leucocytes for patients who had been splenectomized compared to those who had not (see Table 1). On one hand, these findings confirm previous information suggesting that elevated platelets and leucocytes 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 (ρ values -0.28; -0.65 and 0.63 for CT, CFT and MCF, respectively), but on the other indicate that conventional coagulation parameters are of little value to assess the risk of thrombosis in this category of patients with the possible exception of factor VIII. Elevated levels of factor VIII had, in fact, been associated with an increased risk of occurrence and recurrence of venous thromboembolism in thrombophilic patients19 and might, therefore, play some role also in splenectomized thalassemic patients.

This study also shows for the first time that patients with thalassemia had abnormalities for all the throm-

boelastometry 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 (see Fig.s 1A-C). Furthermore, the rate of abnormal values for the three parameters was significantly greater for those patients who had been splenectomized compared to those who had not (see 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 thalassemia patients. CFT is defined as the time needed for the clot to reach a fixed firmness (20 mm) and MCF as the maximal amplitude of the tracing after the addition of the trigger. Accordingly, shortened CFT and increased MCF can be considered as indexes 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 candidate to assess the risk of thrombosis in this category of patients. Although the retrospective nature of this study did not allow assessing the predictive value of thromboelastometry parameters for thrombosis, our findings pave the way to prospective studies based on CT, CFT and MCF that 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 is normal and there are no differences between values recorded for patients who had been splenectomized compared to those who had not (Figure 3). Thrombin generation has been evaluated in plasma without the addition of com trypsin inhibitor that 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 with or without 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, leucocytes, abnormal red blood 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 present with 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 shortage of samples. Therefore, we could not assess whether the increased numbers of platelets are more implicated in the thrombotic process than abnormal red blood or damaged endothelial cells. Second, 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 (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 this issue.

In conclusion, this study shows that all the thromboelastometry parameters determined in whole blood are compatible with the 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 plasmatic abnormalities as 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: conceived the study, helped interpreting results and revised the manuscript; VC: Set up methods, collected data and performed statistical analyses; LP: Set up methods and performed testing; MRF: Selected patients, managed enrollment and collected clinical data; AM: Selected patients, managed enrollment and collected clinical data; PMM: conceived the study, helped interpreting results and revised the manuscript

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