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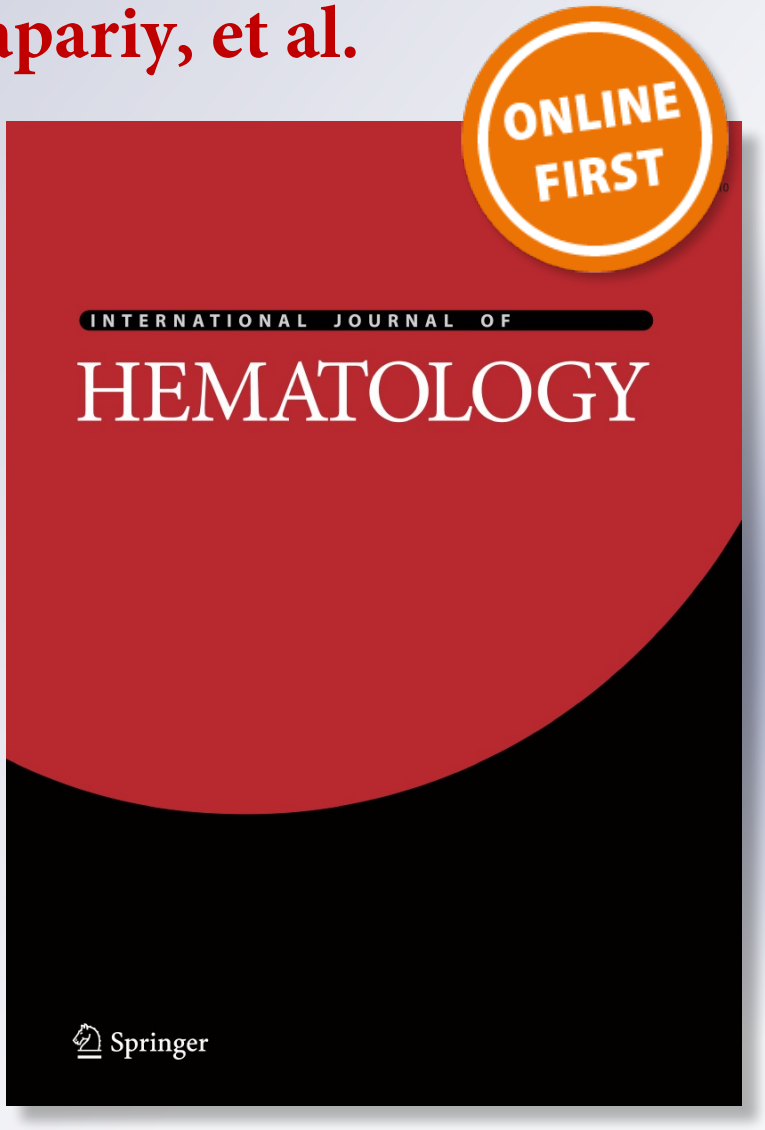

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ORIGINAL ARTICLE

Laboratory tests for coagulation system monitoring in a patient with β-thalassemia

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Abstract Sensitive methods for assessment of the hemostatic state are essential for providing adequate therapy to patients with β-thalassemia. The present study was designed to monitor the changes in the hemostatic state of a patient with β-thalassemia at the primary stage and under heparin treatment following splenectomy. The hemostatic state of the patient was assessed using conventional tests (activated partial thromboplastin time, prothrombin index, thrombin time), fibrinogen and D-dimer assays, thromboelastography (TEG), thrombin generation test, and a novel thrombodynamics clot growth assay. Thrombodynamics parameters indicated the hypercoagulation state on the primary evaluation which progressed after splenectomy: stationary clot growth velocity increased from 32 to 38 μm/min (normal range 20–30 μm/min). Hypercoagulation state was confirmed by Doppler echocardiography, which detected portal vein thrombosis on day 23 after surgery. The results of the other tests' parameters were in the normal ranges before splenectomy. The TEG parameters were sensitive to low molecular weight heparin (LMWH) injections; but the values were close to the normal ranges before and after injections. The thrombodynamics assay demonstrated a high sensitivity to LMWH injections, and registered a decrease of the hypercoagulability in the course of therapy ($P < 0.05$). TGT was not performed during LMWH therapy. This clinical case demonstrates the potential of the thrombodynamics assay to serve as a sensitive method for coagulation system monitoring and prediction of prothrombotic tendencies in patients with hemolytic anemias.

Keywords Thalassemia · β-Thalassemia · Hemolytic anemia · Thrombodynamics · Hypercoagulation · Hypercoagulability · Endogenous thrombin potential

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Introduction

Thalassemia is defined as an increased destruction of red blood cells (RBCs). The β-thalassemia syndromes encompass a group of inherited disorders of hemoglobin synthesis characterized by various degrees of defective β-chain production, imbalance in α/β-globin chain synthesis, ineffective erythropoiesis, and anemia [1–3]. β-Thalassemia is characterized by severe anemia that requires regular blood transfusions and chelation therapy for patient survival. There is increasing evidence that thalassemia is characterized by a hypercoagulable state [1–11]. However, the underlying mechanisms for the development of the hypercoagulability in thalassemia

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remain largely unclear [1–7]. In addition to an increased thrombin and fibrin generation, increased tissue factor activity, and increased platelet activation, patients with thalassemia manifest thrombotic complications [5–8]. Furthermore, the risk of thromboembolic complications appears to be higher following splenectomy [3, 7]. The mechanism of the coagulation system activation in hemolytic anemias is likely multifactorial [2, 3, 5].

The mechanisms contributing to hypercoagulability in thalassemia are diverse and include chronic platelet activation, abnormality of red blood cells, abnormal expression of adhesion molecules on vascular endothelial cells, liver dysfunction, cardiac dysfunction, and dysregulation of hemostasis. Regular transfusions decrease the risk of thrombosis, whereas post-splenectomy thrombocytosis significantly increases the risk [3–14].

Due to a wide range of thrombotic risk factors, patients with thalassemia should be monitored by laboratory tests such as clotting tests, fibrinogen concentration, D-dimer assay, thrombin generation test (TGT), thromboelastography (TEG), and thrombodynamics. From the therapeutic standpoint, assessment of the hemostatic system is especially important to allow for adequate anticoagulant therapy and prophylaxis.

Previous studies have shown that conventional diagnostic methods (clotting tests, fibrinogen concentration) are often unable to detect the systemic activation of the coagulation system [15]. Therefore, there is a need to expand the arsenal of tests for the hemostatic monitoring of patients with natural hypercoagulability and a high risk for thrombotic complications. At present, global tests of hemostasis, such as TGT and TEG, are being increasingly used to detect prothrombotic tendencies in patients [16]. Recently, a novel global test, thrombodynamics, has been developed that is based on the recording of the dynamics of spatial clot growth initiated by the surface with immobilized tissue factor (TF) [17].

In the present study, in addition to the conventional tests (clotting tests, Fibrinogen concentration, D-dimer assay, TEG, and TGT), we used the new thrombodynamics assay to monitor the state of the coagulation system in a patient with β-thalassemia. This clinical case supports the previously reported tendency for hypercoagulation in patients with thalassemia, and demonstrates that the thrombodynamics assay and TEG can be used jointly to monitor and/or correct therapy during treatment of such patients.

Materials and methods

Patient

A 45-year-old male patient with β-thalassemia from the Department of Rare Diseases, National Research Center for Hematology, was enrolled in the study, and was monitored before and after splenectomy. The clinical protocol was approved by the National Research Center for Hematology Ethics Committee.

The schedule of coagulation system monitoring for this patient by date and treatment type is shown in Fig. 1. The first four assessment points of coagulation monitoring were without therapy. The splenectomy was performed on May 3rd, 2012, between the second and third assessment points of coagulation monitoring. Treatment with an antiplatelet agent, Acetylsalicylic acid at a dose of 100 mg/day, was initiated on the 6th assessment point. Anticoagulant therapy with Bemiparin sodium at a daily dose of 3500 U was started on the 8th assessment point. Therapy with Enoxaparin sodium was started on May 20th, 2012 (the day between assessment points 10 and 11) at a dose of 0.3 mL/day, and the dose was increased to 0.6 mL/day on assessment point 14. The patient was discharged in a satisfactory condition the day after the 17th point of coagulation monitoring.

Reagents

Thromboplastin was obtained from Renam, Moscow, Russia; Thromborel S, Test Thrombin Reagent, and D-dimer PLUS were obtained from Dade Behring, Germany; and thrombodynamics assay reagents were from LLC HemaCore, Russia.

Blood collection and plasma preparation

Blood samples were drawn into 9 mL vacuum tubes with 3.8 % sodium citrate buffer at a blood: anticoagulant

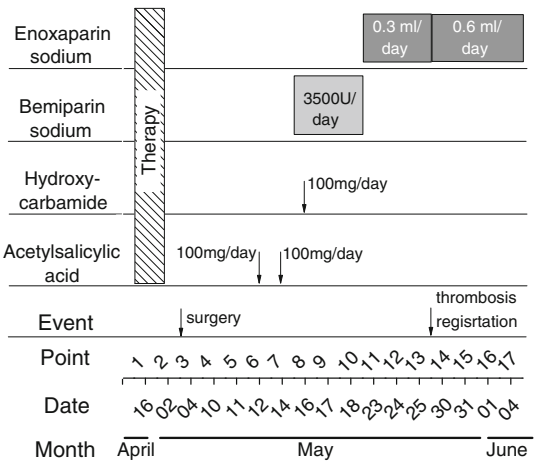

Fig. 1 Schedule of coagulation monitoring and LMWH therapy. The splenectomy was performed on May 3rd, 2012 (the day between assessment points 2 and 3). Treatment with acetylsalicylic acid was initiated on assessment point 6. Therapy with bemiparin sodium was started on assessment point 8. Therapy with enoxaparin sodium was started on May 20th, 2012 (the day between assessment points 10 and 11), and the dose was increased to 0.6 mL/day on assessment point 14. The patient was discharged the day after assessment point 17

volume ratio of 9:1. The blood samples were processed by centrifugation at 1500 × g for 15 min to obtain platelet-poor plasma, part of the plasma was subsequently subjected to centrifugation at 10000 × g for 5 min to obtain platelet-free plasma [24].

Clotting time tests, fibrinogen and D-dimer assays

The following tests were performed using fresh platelet-poor plasma samples and the aforementioned reagents: activated partial thromboplastin time (APTT), prothrombin index (PI), thrombin time (TT), Fibrinogen and D-dimer concentrations. All tests were performed in the Coagulation Laboratory of the National Research Center for Hematology, using a Sysmex CA-1500 (Sysmex Corporation, Japan) automated analyzer, according to respective manufacturer's instructions.

Thromboelastography

Thromboelastography was performed using a TEG 5000 Hemostasis Analyzer System and disposable cups (Haemonetics Corporation, USA). The assays were performed 10–30 min after blood collection using citrated blood samples (340 µL) recalcified with 20 µL of 0.2 M $CaCl_2$. The reaction time (R), clot formation time (K), angle alpha (α), and maximum amplitude (MA) were calculated.

Thrombin generation test

The kinetics of thrombin generation in plasma was monitored by measuring the rate of thrombin-induced hydrolysis of a fluorogenic substrate Z–Gly–Gly–Arg-AMC as described by Hemker et al. [18]. The kinetics of accumulation of the fluorescent product 7-amino-4-methylcoumarin (AMC) was recorded for 60 min with a fluorimetric reader (Appliskan; Thermo Fisher Scientific, Finland) ($\lambda_{ex.} = 355$ nm; $\lambda_{em.} = 460$ nm). For all calculations, the program OriginPro 8.0 (OriginLab Corporation, USA) was used. The area under the thrombin generation time curve for a sample was calculated to determine the total amount of thrombin generated in that sample over the period of 50 min. This parameter was defined as endogenous thrombin potential (ETP).

Thrombodynamics

The Thrombodynamics (TD) spatial clot growth assay was performed using an experimental device. The plasma clotting was activated by a surface with immobilized TF, and the clotting process subsequently propagated into the bulk of the plasma. Images were captured every 15 s for 45 min (Fig. 2a). Based on the clot size dynamics, the following parameters were calculated (Fig. 2b): lag time Tlag, the initial clot growth velocity Vi, and the stationary velocity Vs as described in [17, 19–26].

Reference values for laboratory tests

Reference values for conventional laboratory tests were taken from the respective testing reagents' instructions. Conventional tests were conducted in the clinical diagnostic laboratory of the National Research Center for Hematology. To define the normal ranges of the spatial clot growth parameters, the thrombodynamics test was performed with plasma samples obtained from 112 healthy donors (age 19–65 years, median 33; 34 female and 78 male donors). For each parameter, the ranges were established as mean values ± standard deviation (S.D.).

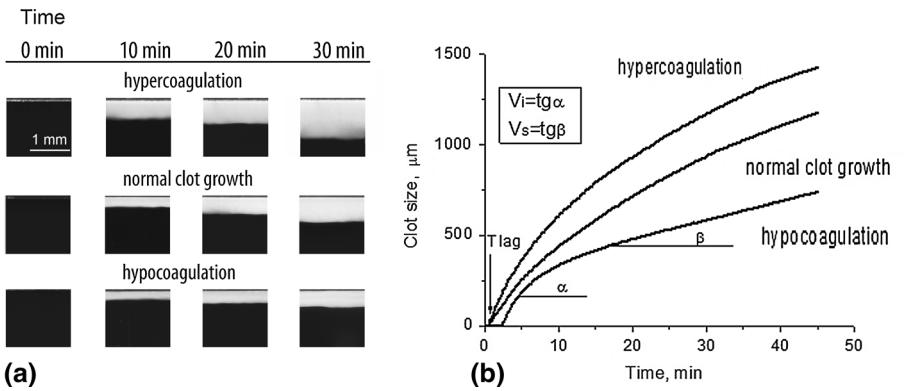

Fig. 2 Thrombodynamics test: sensitivity to different states of the hemostatic system. **a** Typical light-scattering time-lapse images of clot growth initiated by immobilized TF in healthy donor plasma (*middle panel*) and in plasma from patients with the hypocoagulation (*lower panel*) or hypercoagulation (*upper panel*) state. The TF-coated activator surface is seen as a *horizontal black strip* at the top of each image. **b** Plot of clot size versus time for the experiments shown in (**a**). The *curves* also indicate the parameters used for analysis throughout the study: lag time, time to clot growth initiation, Vi (tg α), the initial clot growth velocity, Vs (tg β), the stationary clot growth velocity

Statistical analysis

The statistical analyses of the differences between the data sets were performed using the Mann–Whitney test for statistical significance (p) at a 0.05 level. For all calculations, the program OriginPro 8.0 (OriginLab Corporation, USA) was used.

Case report

Part 1: patient's medical history and admission

A 45-year-old male patient was admitted to the hospital for weakness and pallor. Since childhood, he suffered from hereditary hemolytic anemia as diagnosed by his pediatrician. The thalassemia was confirmed in 2012 by DNA diagnostics.

The patient never smoked, did not abuse alcohol intake, and did not take any prescription or recreational drugs. The patient received his last blood transfusion in February 2012. One of patient's siblings (brother) also had thalassemia.

On admission, the patient was conscious and rational. No neurological deficits were detected. Test results for Hepatitis B surface antigen and Hepatitis C antibody were negative. Test results for anti-nuclear antibody and HIV serology were also negative. Venereal Disease Research Laboratory (VDRL) test was non-reactive. May-Grunwald staining of blood smears revealed nucleated RBC and red cell clumps suggestive of acute hemolysis. Active hemolysis was also suggested by the results of the laboratory analyses that revealed a decreased hemoglobin level, low platelet count, and increased levels of bilirubin and lactate dehydrogenase (LDH), Table 1.

On admission, the patient's pulse rate was 92 bpm. Blood pressure was 110/70 mmHg. On auscultation, the heart was in sinus rhythm but muffled. The results of the respiratory system examination were normal. On abdominal examination, the liver was enlarged by 2–4 cm, and the spleen took up the entire left side of the abdomen. The ultrasound investigation and computer-assisted tomography of the abdomen showed the enlarged spleen (240 × 150 × 275 mm) that pushed left kidney posterior. Based on the low hemoglobin level, low platelet and leukocyte counts, and splenomegaly, a decision was made to perform a splenectomy.

Part 2: splenectomy

Before splenectomy, on the initial and second assessment points of coagulation monitoring without therapy, most of the tests reflected normal coagulation, except for APTT which indicated hypocoagulation (Table 2). The patient had thrombocytopenia due to the low platelet count. Unlike conventional tests, the Thrombodynamics test showed the increased velocities (Vi and Vs) of clot growth. Sometimes we can observe spontaneous activator-independent clotting in plasma of patients with hypercoagulation, but there was no spontaneous clotting observed on the first and all following assessment points of coagulation monitoring in this patient with β-thalassemia.

On the first day after the surgery (3rd assessment point), the patient started complaining of abdominal pain. The conventional coagulation tests (APPT, PI) indicated hypocoagulation; ETP was slightly below the normal range; TT and TEG parameters were in the normal ranges; while Thrombodynamics and Fibrinogen tests revealed hypercoagulability. Notably, the D-dimer level was significantly (>100-fold) higher than the normal values (Table 2).

Table 1 Laboratory tests

Test	Reference range	Patient data		
		On admission	After surgery	On discharge
Hb (g/L)	120–140	**51**	**76**	**70**
Reticulocyte count (%)	2–10	45	–	14
Erythrocytes ((10^{12})cells/L)	3.90–4.70	3.04	3.63	4.23
Leukocyte count ((10^9) cells/L)	4.0–9.0	5.2	36.0	16.5
MCV (fl)	80–100	58.0	71.1	60.0
MCH (pg)	30.0–35.0	16.0	20.9	17.0
Platelets ((10^9) cells/L)	180–320	60	25	**1031**
Total bilirubin (μmol/L)	3.4–17.1	**35.8**	**40.7**	14.5
Indirect bilirubin (μmol/L)	3.4–13.7	**30.7**	**33.2**	–
Direct bilirubin (μmol/L)	0.0–3.4	**5.1**	**7.5**	–
AST (u/L)	5–40	16	23	20
ALT (u/L)	5–40	7	15	13
LDH (u/L)	208–378	**648**	**749**	**450**
Serum creatinine (μmol/L)	40–110	35.8	71	57
Serum sodium (mmol/L)	130–145	139	134	140
Serum potassium (mmol/L)	3.4–5.1	4.8	4.2	5.0

Numbers in bold indicate parameter values out of the reference ranges

MCV mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *LDH* lactate dehydrogenase

Table 2 Results of coagulation monitoring without therapy (assessment points 1–7)

Test	Reference range	1st point	2nd point	3rd point	4th point	5th point	6th point	7th point
APTT (s)	32–37	46	44	42	35	40	38	38
PI (%)	70–130	78	81	60	92	73	68	69
TT (s)	12–19	18	17	15	15	16	15	15
Fibrinogen (mg/mL)	1.8–3.5	2.6	2.8	**3.8**	**6.7**	**5.8**	**6.2**	**6**
D-dimer (μg/mL)	50–250	185	217	**26780**	**4490**	**2695**	–	**2471**
TD, Tlag (min)	0.3–1.5	0.5	0.7	1	1.4	1.3	0.6	0.6
TD, Vi (μm/min)	36–56	**59**	**57**	**59**	**64**	**65**	**61**	**66**
TD, Vs (μm/min)	20–30	**32**	**32**	**34**	**38**	**36**	31	**35**
TEG, *R* (min)	9–27	14.2	15.2	14.8	9.6	9.1	11	12.6
TEG, *K* (min)	2–9	4.4	9.1	4.5	**1.2**	2.4	2.2	2.2
TEG, α (°)	22–58	37.2	23.8	40.5	**72.3**	**59.8**	**63.3**	**63.9**
TEG, MA (mm)	44–64	53.5	50	52.8	**71.1**	**68.5**	**76.8**	**76.3**
ETP (nM × min)	950–1450	1035.5	1063.4	804.1	1219.2	963.1	617.5	970.8
Platelets ((10^9) cells/l)	180–320	60	55	24	176	–	**456**	**830**

Numbers in bold indicate parameter values out of the reference ranges

ETP endogenous thrombin potential

After the surgery, the laboratory tests continued to indicate thrombocytopenia and hemolysis (Table 1) suggesting that anemia persisted. To relieve the abdominal pain, the patient was given 1 mL of trimeperidine intramuscularly, in addition to the planned anesthesia with 1 g of Paracetamol three times a day after the surgery. Antiplatelet agents and anticoagulants were not prescribed in consideration of the low platelet count (average $121 \pm 52 \times 10^9$ cells/L) and prolonged APTT.

On the subsequent assessment points without therapy (4th–6th), the thrombodynamics parameters, Fibrinogen and D-dimer levels remained in the hypercoagulation area. Specifically, the values of the TD parameters, Vi and Vs, increased from the 1st–5th points. At that time, the TEG parameters, angle α and MA, were also out of the normal range and reflected hypercoagulation, likely caused by a progressive increase of the platelet count to 176×10^9 cells/L on the 4th assessment point (Table 2).

On the 6th point of monitoring, the platelet count further increased to 456×10^9 cells/L; at that time, the prophylactic desegregation therapy with acetylsalicylic acid at a daily dose of 100 mg was prescribed. Anticoagulant therapy was started on the 8th assessment point.

Part 3: low molecular weight heparin (LMWH) therapy

The subsequent assessment of the coagulation system (points 8 through 17) was aimed to monitor the effects of the anticoagulant therapy (the schedule of therapy is presented in Fig. 1). Therapy with Bemiparin sodium was started on assessment point 8, at a dose of 3500 U/day. Therapy with Hydroxycarbamide for high platelet count was started with a dose of 100 mg/day. Therapy with Enoxaparin sodium was started on May 20th, 2012 (between the 10th and 11th assessment points) with a dose 0.3 mL/day.

On May 26th, 2012 (the day after 14th assessment point during LMWH treatment), the patient started coughing and had elevated temperature (38.7 °C). Rales could be heard in the lower lobe of the lungs by auscultation, and the patient was diagnosed with pneumonia. The patient received antibacterial therapies with good effects. Control Doppler echocardiography of the abdominal vessels detected occlusive thrombi in the lumen of the main trunk of the portal vein and in the splenic vein. It was not possible to determine when thrombosis developed because there were no clinical symptoms. Due to thrombotic complication, the enoxaparin daily dose was increased to 0.6 mL.

All tests were performed before and 3 h after LMWH administration. The conventional clotting tests (APTT, TT, PI) were not sensitive to LMWH therapy, as there was no difference between the pre- and post-injection results at all assessment points (Table 3 shows the data during therapy with 0.6 mL/day enoxaparin sodium). TGT was not performed during LMWH therapy.

In contrast, the parameters Vi and Vs of the thrombodynamics test demonstrated a significant difference between the values before and 3 h after LMWH administration during the course of therapy (Fig. 3). At the start of the therapy, the Vi and Vs values were in the hypercoagulation area, were lowered by LMWH injections to the normal range, but tended to return to the hypercoagulation area within 24 h post-injection. Notably, at the later assessment points of LMWH therapy, the Vi values consistently remained in the normal range within 24 h post-LMWH injection (Fig. 3a). The Vs parameter was even more sensitive to LMWH, with the post-administration

Table 3 Results of coagulation monitoring during therapy with enoxaparin sodium, 0.6 mL/day (mean values for assessment points 14–17)

Test	Reference range	Before, mean ± SD	After, mean ± SD	Before, min–max	After, min–max	*p*
APTT (s)	32–37	41.8 ± 1.3	43.6 ± 3.4	40–43	43–46	0.05*
PI (%)	70–130	89.4 ± 10.7	87.8 ± 3.9	71–102	81–89	0.88*
TT (s)	12–19	17.8 ± 0.5	18.8 ± 2.2	18–18	18–22	0.07*
Fibrinogen (mg/mL)	1.8–3.5	3.9 ± 1	3.5 ± 1.1	3.0–4.1	2.8–3.1	0.14*
D-dimer (μg/mL)	50–250	1356 ± 561	812 ± 422	561–636	422–563	0.06*
TD, Tlag (min)	0.3–1.5	0.7 ± 0.2	0.8 ± 0.2	0.5–0.9	0.5–0.9	0.87*
TD, Vi (μm/min)	36–56	**56.2 ± 6.1**	46.8 ± 3.9	51–**65**	41–52	0.11*
TD, Vs (μm/min)	20–30	25.2 ± 3.6	12.2 ± 2.3	22–**31**	9–15	0.03**
TEG, *R* (min)	9–27	14.2 ± 1.6	21.3 ± 8.3	12.4–16.3	13.8–35.2	0.31*
TEG, *K* (min)	2–9	3.9 ± 0.9	7.6 ± 2.9	3.2–4.0	4.2–11.9	0.03**
TEG, α (°)	22–58	49.6 ± 4.1	30.8 ± 11.5	48.7–54.4	18.4–45.0	0.03**
TEG, MA (mm)	44–64	**65.7 ± 9.2**	**64.3 ± 8.6**	65.7–75.2	62.6–71.8	0.66*

Numbers in bold indicate parameter values out of the normal ranges

* The difference is not statistically significant

** The difference is statistically significant

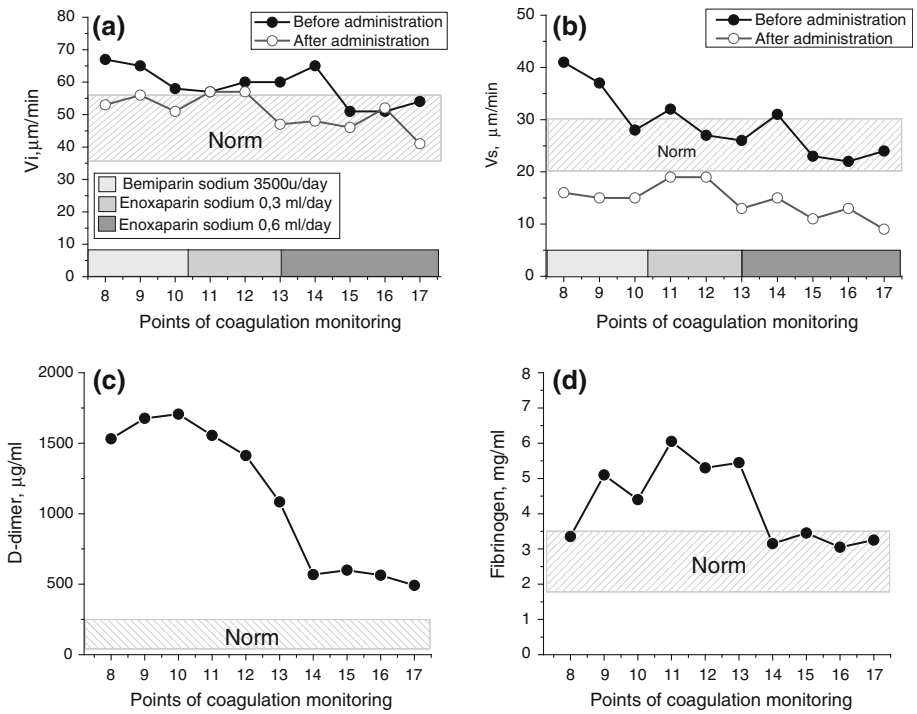

Fig. 3 The effects of LMWH therapy: assessed by the thrombodynamics parameters, the initial (Vi) and stationary (Vs) velocities before and 3 h after injection at the indicated points of the treatment course (**a**, **b**). Please note normalization of the Vi and Vs parameters "before administration" in the course of therapy. The D-dimer and fibrinogen levels gradually decreased during LMWH therapy (**c**, **d**)

values decreasing below the normal range to the hypocoagulation area (Fig. 3b).

Statistical analysis of the overall TD data presented in Fig. 3a, b (points 8 through 17) confirmed that the difference in the Vi and Vs values was statistically significant ($p = 0.007$, $p < 0.001$, respectively). The results of the laboratory tests during therapy with 0.6 mL/day Enoxaparin sodium are presented in Table 3. Thus, the Thrombodynamics test demonstrated the sensitivity to the LMWH treatment and reflected a reduction of hypercoagulability in the patient during the course of therapy.

The TEG parameters seemed to also be sensitive to the LMWH injections during the treatment course (Fig. 4); the differences between pre- and post-administration values (points 8 through 17) were statistically significant, with $p(R) = 0.03$, $p(K) = 0.001$, $p(\text{angle } \alpha) = 0.008$, and $p(\text{MA}) = 0.03$. During treatment with high dose enoxaparin sodium (points 14 through 17), the angle α value decreased from 49.6 ± 4.1 before injection to $30.8° \pm 11.5°$ after injection ($p < 0.03$), and the *K* value increased from 3.9 ± 0.9 to 7.6 ± 2.9 min ($p < 0.03$) (Table 3). However, all TEG parameters were close to the normal ranges both before and after the LMWH administration (e.g., 22°–58° and 2–9 min for angle α and *K*, respectively) so that the change in the patient's hemostatic state could not be detected by this test. Only the MA

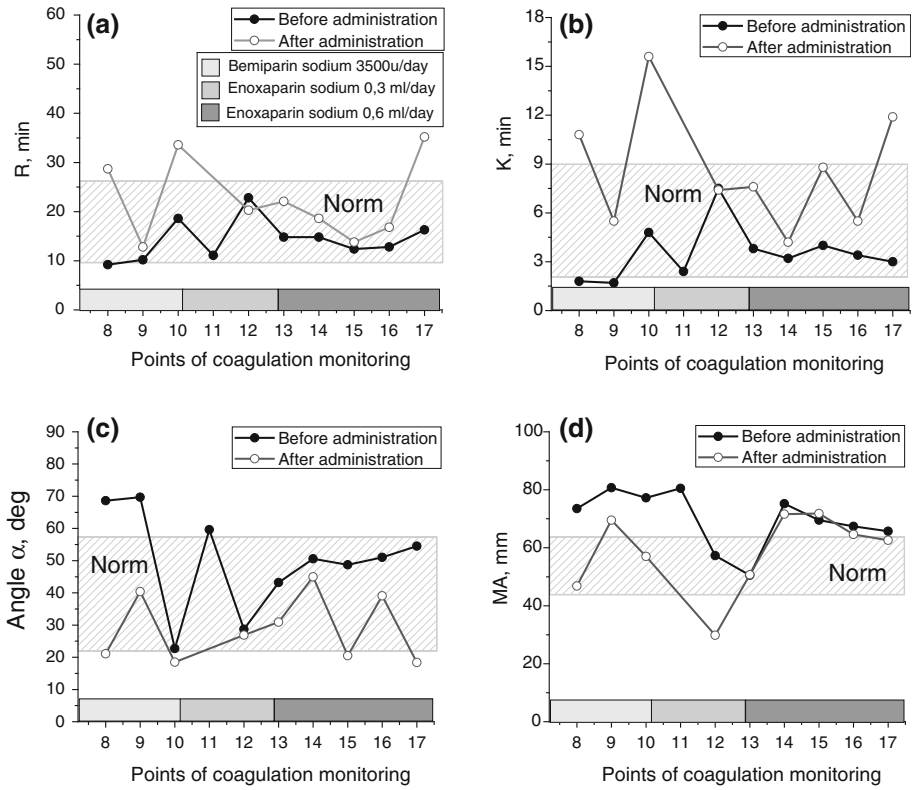

Fig. 4 The effects of LMWH therapy: assessed by the TEG parameters before and 3 h after injection at the indicated points of the treatment course (**a**–**d**)

parameter indicated the hypercoagulability state at the end of therapy course possibly due to thrombocytosis (platelet counts after the 1st and 5th administration of LMWH were 840×10^9 and 1692×10^9, respectively).

Control Doppler echocardiography on day 7 of enoxaparin therapy (point 17 of the coagulation monitoring) confirmed partial resolution of thrombus (thrombus was not occlusive but occupied 1/3 of the portal vein lumen). On patient's discharge from the hospital, the laboratory test results indicated a decrease of anemia. In particular, based on the reduced levels of total bilirubin, reticulocytes, LDH, and normalization of the erythrocyte count, it was concluded that hemolysis finally began to decrease in this patient (Table 1). The patient was discharged in satisfactory condition and continued anticoagulant and antiplatelet therapy at home with periodic check-up visits to a hematologist.

Discussion

Patients with hemolytic anemia are known to have a tendency for natural hypercoagulability, and the risk of thrombosis further increases after splenectomy [3, 5, 7]. In this clinical case, investigation of the patient with β-thalassemia by various tests confirmed the previously reported complexity of the factors determining the state of hemostasis in patients with hemolysis. Despite the physicians' best efforts during patient's monitoring, such factors as splenectomy, thrombocytosis, hemolysis, and infection led to development of thrombosis in this patient that necessitated the anticoagulant therapy post-surgery.

The conventional laboratory tests failed to detect the hypercoagulability state in the patient before and after surgery as their values were within the normal ranges: on the initial assessment point, PI = 78 % (70–130 %), TT = 18 s (12–19 s), fibrinogen = 2.6 mg/mL (1.8–3.5 mg/mL), and D-dimer = 185 μg/mL (50–250 μg/mL). The TEG parameters (R = 14.2 min, K = 4.4 min, angle $\alpha = 37.2°$, MA = 53.5) and TGT parameter (ETP = 1035.5 nM × min) were also within the normal ranges (Table 2). The APPT value (46 s) even indicated the hypocoagulation state. Due to their specificity, the conventional laboratory tests cannot be sensitive to multifactorial mechanisms of the coagulation system activation in patients with hemolytic anemia, as reported previously [15].

In contrast, the Thrombodynamics test indicated the hypercoagulability state the day before splenectomy and the days following the surgery thus predicting thrombotic complications in the patient with β-thalassemia. Thrombodynamics revealed the hypercoagulability by the increased clot growth velocities: on the primary assessment (point 1), the initial velocity, Vi, was 59 μm/min with reference values of 36–56 μm/min, and the stationary velocity, Vs, was 32 μm/min with reference values of 20–30 μm/min. The velocity values continued to increase

after splenectomy: Vi = 66 μm/min and Vs = 35 μm/min on the 7th assessment point before the start of LMWH therapy.

After the surgery, on assessment point 3 and subsequent points, the results of the thrombodynamics test were consistent with the high D-dimer and fibrinogen levels, which are well-recognized indicators of thrombosis. At later stages (starting from point 4), the hypercoagulation state was also revealed by abnormal TEG parameters. Portal vein thrombosis was confirmed by control Doppler echocardiography which detected the presence of occlusive thrombus. Given the fact that the D-dimer levels were consistently elevated after the surgery, thrombosis could have developed during the period without therapy, or even after the start of the anticoagulant therapy.

After the start of the therapy with low molecular weight heparins, the patient continued to be monitored by the same panel of laboratory tests (Table 1). The conventional clotting tests (APTT, TT, PI) were not sensitive to LMWH therapy as there was no significant difference between the pre- and post-injection results at all assessment points (Table 3). TGT was not performed during LMWH therapy. While the TEG parameters appeared to be sensitive to LMWH injections, both the pre- and post-injection values were in the normal area except for the MA parameter. When the efficiency of the anticoagulant therapy was assessed by thrombodynamics, a statistically significant decrease in the initial ($p = 0.002$) and stationary ($p < 0.001$) velocities of clot growth was registered throughout the therapy course, and the TD parameter values were back in the normal ranges at the end of treatment.

The ability of thrombodynamics to predict thrombotic complications was reported earlier in a septic patient [17]. The new method indicated the hypercoagulability state one day before thrombosis occurred when the results of the conventional laboratory tests (APPT, PI, TT, TEG) remained within the normal ranges. In our study of the patient with β-thalassemia, thrombodynamics was the only test that revealed patient's natural hypercoagulability already on the primary point of coagulation monitoring, consistent with the reported tendency for hypercoagulability in patients with β-thalassemia. The thrombodynamic test was also an early indicator of an increased risk of thrombosis immediately after splenectomy, despite the APPT, PI and ETP results being in the normal or even hypocoagulation area, and despite thrombocytopenia in patient. The thrombotic complications predicted by the dynamics of clot growth were subsequently confirmed by dramatically increased D-dimer levels and ultrasound examination which detected thrombus in the portal vein lumen. Thrombodynamics proved to be the most sensitive test in monitoring the efficiency of LMWH therapy until the normal coagulation state was achieved. We cannot comment on the sensitivity of thrombin generation test to LMWH therapy as this test was not performed during this part of the study.

The clinical case described emphasizes the importance for physicians to use a variety of diagnostic methods for extended monitoring of patients with hemolytic anemias. The results of the study demonstrate that the recently developed thrombodynamics test is prospective in predicting prothrombotic tendencies, and may be a useful tool in developing adequate therapy for patients with β-thalassemia.

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Conflict of interest N.M.A. is an employee of the US Food and Drug Administration (FDA). Contributions of N.M.A. are an informal communication and represent her own best judgment. These comments do not bind or obligate FDA. E.A.S., A.P.Z., A.V.E., A.N.B., and F.I.A. are/were employees and/or founders of HemaCore LLC. All other authors declare that they have no conflict of interest.

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