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The laboratory control of anticoagulant thromboprophylaxis during the early postpartum period after cesarean delivery

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Abstract

Introduction: The incidence of venous thromboembolism (VTE) after cesarean section is up to 0.6%, and the widespread use of cesarean section draws attention to this group. The dosage and duration of low-molecular-weight heparin (LMWH) prophylaxis after delivery is estimated by anamnestic risk-scales; however, the predictive potency for an individual patient's risk can be low. Laboratory hemostasis assays are expected to solve this problem. The aim of this study was to estimate the potency of tests to reflect the coagulation state of patients receiving LMWH in the early postpartum period.

Materials and methods: We conducted an observational study on 97 women undergoing cesarean section. Standard coagulation tests (Fg, APTT, prothrombin, D-dimer), an anti-Xa assay, rotation thromboelastometry and thrombodynamics/thrombodynamics-4D were performed. Coagulation assay parameters were compared in groups formed in the presence or absence of LMWH to estimate the laboratory assays' sensitivity to anticoagulation.

Results: Coagulation assays revealed hypercoagulation after delivery and a tendency toward normalization of coagulation during early postpartum. The thromboprophylaxis results revealed a higher percentage of coagulation parameters within the normal range in the LMWH group.

Conclusion: This research is potentially beneficial for the application of thrombodynamics and thrombodynamics-4D in monitoring coagulation among patients with high VTE risk who receive thromboprophylaxis with heparin.

Keywords: Cesarean section; coagulation; LMWH; postpartum; pregnancy; spatial thrombin generation; thrombodynamics.

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Introduction

During pregnancy, the coagulation balance is shifted towards a state of hypercoagulability, which is most marked around term and early postpartum [1]. This change does not lead to thromboembolic episodes [venous thromboembolism (VTE)] in uncomplicated pregnancies. However, in the presence of additional VTE risk factors, including previous VTE episodes, thrombophilia and cesarean section, the incidence of VTE increases up to 10% [2, 3]. The widespread use of cesarean sections draws attention to this risk group [4]. The absolute incidence rate of VTE after cesarean section varies from 5.8 to 60 VTEs per 10,000 cesarean sections (vaginal delivery VTE rates: 2.1 per 10,000 deliveries [5]) and depends on the

population studied and obstetric practices. The highest rate of thrombosis is observed in the early postpartum period [6, 7]; thus, the control of hemostasis after cesarean delivery is essential.

Conventionally, the dosage and duration of anticoagulant prophylaxis is estimated by guidelines based on anamnestic data. After the introduction of guidelines, the risk of deep venous thrombosis after cesarean deliveries decreased by approximately 1.6 times [4, 5]; however, thrombosis still remains a serious problem. Moreover, there is no assay with a proven ability to predict VTE during pregnancy and the early postpartum that is also approved for thromboprophylaxis control. Standard coagulation assays, such as prothrombin time (PT) and activated partial thromboplastin time (APTT) are not capable of identifying hypercoagulation during pregnancy [8]. In contrast, global hemostasis assays can identify pregnancy hypercoagulation [9, 10], but the data are scarce and conflicting. D-dimer concentration studies have shown an increased correlation with the gestational age [10–13], but as D-dimers are fibrin derivatives, the increase indicates clotting that has already occurred and is the result of hypercoagulation rather than the reverse. We found no evidence that D-dimer is an effective predictor of VTE in pregnant women. The adequacy of low-molecular-weight heparin (LMWH) thromboprophylaxis is conventionally assessed by an anti-Xa assay that monitors the LMWH concentration in plasma. It is important to note that the anti-Xa assay is an indirect assay used to measure the amount of LMWH in plasma and is in no way suitable for assessing the overall anticoagulant effect (limitations of heparin monitoring reviewed elsewhere [14–16]). Finding an instrument for the direct assessment of plasma coagulability in the presence of LMWH is an area of great interest. Global hemostasis assays are promising for monitoring LMWH therapy and prophylaxis [17–19].

The objective of this study was to compare the results of standard coagulation assays and global hemostasis assays, such as rotation thromboelastometry (ROTEM®), thrombodynamics and thrombodynamics-4D during early postpartum and to investigate the sensitivity of these assays to patient condition during heparin prophylaxis.

Methods

Patients

An observational study was conducted in women undergoing cesarean section at the Research Center for Obstetrics, Gynecology and Perinatology (Moscow, Russian Federation). All participants met the following inclusion/exclusion criteria: (1) age over 18 years old; (2) elective cesarean section; (3) absence of a history of psychiatric diseases (including alcohol- and drug-induced); and (4) absence of trauma or surgical treatment in the 90 days before the cesarean section. Recruitment occurred at the date of hospitalization and not less than 24 h before the cesarean section. Blood samples were obtained during routine venipunctures after the cesarean delivery due to the center's follow-up protocol so that no additive venipunctures were performed. The study was approved by the Ethical Committee of the center.

Information regarding age, body mass index (BMI), family history of VTE, presence of diabetes, autoimmune disorders, history of previous deliveries, and current pregnancy complications was obtained for all patients. All patients were grouped by the anamnestic data-based Royal College of Obstetricians and Gynaecologists (RCOG) scale (obtained from RCOG Green-top Guidelines No. 37a [20]; please see Table S1 in the Supplementary Materials). The following VTE-risk groups were formed according to the score: low-risk group (score 0–1), medium-risk group (score 2) and high-risk group (score 3 and greater). For the low-risk group, an elastic compression without LMWH thromboprophylaxis for 6–7 days after delivery was prescribed. For the medium-risk group an elastic compression with/without LMWH thromboprophylaxis for 6–7 days after delivery, starting from 8 h after the surgery, was prescribed. For this group, the prescription of LMWH by RCOG recommendations was more advisory than mandatory, so it was based on the physician's discretion. For the high-risk group, an elastic compression with LMWH prophylaxis for 6 weeks after delivery was prescribed. Nevertheless, the physician had control over administration or termination of LMWH prophylaxis and relied on clinical observation and laboratory test results to make the decision. LMWH thromboprophylaxis during early postpartum was performed with 4000–6000 IU anti-Xa of enoxaparin once a day or 2850–3800 IU anti-Xa of nadroparin once a day.

Blood sampling

Blood samples for coagulation checking were collected at the following two time points: 3–5 h after the cesarean section (before the first LMWH injection, if performed) (Point 1, P1) and 2 days after the cesarean section (just before the regular LMWH injection, if performed) (Point 2, P2).

Blood was drawn into vacuum tubes (Monovette, Sarstedt, Germany) with 106 mM sodium citrate buffer (pH 5.5) at a 9:1 blood:anticoagulant volume ratio. The blood was obtained during fasting, and the analyses were performed within 30 min. Whole blood was used for rotation thromboelastometry (ROTEM®). The remaining blood was processed by centrifugation at $1600 \times g$ for 16 min to obtain platelet-poor plasma (PPP). This plasma was analyzed for APTT, prothrombin, fibrinogen concentration and D-dimer. The remaining PPP was repeatedly processed by centrifugation at $10{,}000 \times g$ for 5 min to obtain platelet-free plasma (PFP), which was used for a thrombodynamics assay. The remaining platelet-free plasma was frozen in liquid nitrogen and stored at –80°C for the anti-Xa and thrombodynamics-4D assays. As anti-Xa activity is conventionally measured in PPP, we performed an *in vitro* assay to check if the PFP was acceptable. The difference between anti-Xa activity measured in PPP and PFP prepared from the same blood sample was not significant, so PFP was considered to be acceptable for this assay.

Laboratory methods

Standard tests (APTT, prothrombin, fibrinogen concentration and D-dimer) were performed using Sysmex CA-1500 (Sysmex Corporation, Japan) with Pathromtin®SL, Thromborel®S and INNOVANCE®D-Dimer (Siemens Healthcare Diagnostics Products GmbH, Germany) reagents.

Rotation thromboelastometry was performed on whole blood with the ROTEM® (Pentapharm, Germany) and reagents. For Ca level restoration and to start clotting, 0.2 M calcium chloride was used (NATEM assay). The clotting time (CT), clot formation time (CFT), angle (α) and maximum clot firmness (MCF) parameters were used for further data analysis.

The assay for quantitative determination of LMWH activity (anti-Xa assay) was performed with an automatic coagulometer ACL TOP 700 and HemosIL® reagents (Instrumentation Laboratory, USA).

A thrombodynamics assay was performed with a thrombodynamics analyzer and Thrombodynamics kit (HemaCore LLC, Russia). This method is based on registering spatial fibrin clot growth after activation of clotting in a thin layer of plasma after contact with an immobilized tissue factor bearing surface. The process of clot growth was registered by serial photography during the test. Based on the photos, a plot of clot growth versus time was obtained (please see Figure S1 in Supplementary Materials). In some cases, spontaneous clotting (clot formation in the cuvette space not associated with the main clot growth) occurred and was described with a spontaneous clotting plot. The following standard parameters were used to characterize clot growth in the assay: lag time (Tlag) – the delay between the test start and the onset of clot formation; the initial velocity of clot growth (Vi) – calculated as the mean clot growth velocity over a beginning period of growth; the stationary velocity of clot growth (Vst) – calculated as the mean clot growth velocity over a 10-min interval of the stationary growth period; the velocity of clot growth (V) – the parameter, calculated as the mean clot growth velocity over the 10-min interval before spontaneous clotting occurs and equal to Vst in cases without spontaneous clot formation (for an image of spontaneous clotting see Figure S1); the density of the clot (D) – the light scattering intensity of the clot; and spontaneous clotting time (Tsp) – the time required to fill 5% of the analyzed cuvette area with spontaneous clots.

The thrombodynamics-4D assay was performed with the Thrombodynamics Analyzer System T-2T and a Thrombodynamics-4D PLS kit (HemaCore Labs LLC, Russia). The method combines the features of thrombodynamics and the thrombin generation test, and the analyzer is equipped with an additional fluorescence detection system for thrombin generation and to register thrombin spatial distribution. Thrombin activity was measured by the rate of fluorogenic substrate cleavage. The principle of clotting activation resembled thrombodynamics; however, before the initiation of clotting via the tissue factor bearing surface, PFP was pre-incubated with a fluorogenic substrate for thrombin (400 μM) and artificial phospholipid microvesicles (4 μM), which mimicked the activated platelet surface. A previous study demonstrated [21] that thrombin's spatial distribution is a wave-shaped peak, which propagates from the clotting activation surface with a constant rate and amplitude (please see Figure S1 in Supplementary Materials). From this assay, we obtained the stationary amplitude of the thrombin peak (Ast), which reflected the amount of thrombin formed.

Some of the hemostasis parameters during pregnancy differ from their values during the non-pregnant state and between the trimesters. A variety of reference intervals for coagulation tests among pregnant women can be obtained from the literature [12, 22–24] or laboratory assays. We decided to use reference intervals for non-pregnant women for all tests because during the investigation, women proceeded from delivery to postpartum, so the reference intervals for some tests must change between investigation points. Furthermore, we could not obtain approved postpartum reference intervals for some of the tests. The reference ranges for non-pregnant women for APTT, prothrombin, fibrinogen concentration, D-dimer assay in standard coagulation assays and CT, CFT, α, and MCF in ROTEM® were obtained from the clinical hemostasis laboratory of the Research Center for Obstetrics, Gynecology and Perinatology. Reference ranges for thrombodynamics were obtained from [25] and reference ranges for thrombodynamics-4D were obtained from the Bakulev Scientific Center for Cardiovascular Surgery. For all tests results we distinguished "mild" hypercoagulation, which includes parameter values in the interval 100%–120% of the normal upper limit (for D-dimers, α and MCF in ROTEM®, Vi, Vs, V, CS, D, Tsp and Ast for thrombodynamics) or lower limit (for APTT, CT and CTF in ROTEM®) and "marked" hypercoagulation, which was defined as values 120% and higher than the upper limit (or less than lower limit, respectively) of the normal range alongside spontaneous clotting for thrombodynamics.

Statistical analyses were performed using Origin Pro 8 (OriginLab Corp., USA) software. The median and 5–95 percentile values were used to estimate the assay results. The nonparametric pair-sample Wilcoxon signed rank test, Mann-Whitney *U*-test and Fisher's exact test were used for analysis due to the non-normal distribution of data, and the significance level was set at $P < 0.05$.

To estimate the coagulation test's sensitivity during heparin prophylaxis, a receiving operating curve (ROC) curve analysis was used. The presence of anticoagulant prophylaxis was selected as a positive test value. A ROC analysis was assessed as positive vs low values for D-dimer, fibrinogen, prothrombin, α angle, MCF, V, Vs, and Ast and as positive vs. longer values for APTT, CT and CFT. Sensitivity was reported as the ratio of the amount of patients with a low value of D-dimer, fibrinogen, prothrombin, α angle, MCF, V, Vs, and Ast or a high value of APTT, CT and CFT to the total amount of patients receiving anticoagulant prophylaxis. Specificity was counted as the ratio of the amount of patients with high values in D-dimer, fibrinogen, prothrombin, α angle, MCF, V, Vs, and Ast or low values in APTT, CT and CFT not receiving the anticoagulant prophylaxis to the total amount of patients not receiving anticoagulant prophylaxis.

Results

A total of 102 participants were enrolled in the study. Ninety-seven patients were included in the analysis, and five were excluded because a blood sample was not collected at P2. All participants underwent a successful cesarean delivery. For all 97 participants, the VTE-risk was estimated before delivery using the RCOG scale. According to the estimation, 55 women had a low risk of VTE, 21 had a medium risk of VTE, and 21 had a high risk of VTE. Patient characteristics are shown in Table 1. There was no correlation between any laboratory test parameter and score

Table 1: Patient characteristics. Statistical analysis of difference between not receiving LMWH and receiving LMWH in the P2 groups was performed using the Mann-Whitney *U*-test (p_{mw}).

Patients	Total	Not receiving anticoagulants	Receiving anticoagulants	p_{mw}
n	97	58	39	
Age (year)				
Median (min–max)	33 (21–45)	31 (21–41)	36 (23–45)	<0.001
Gestational age (week)				
Median (min–max)	38 (28–41)	39 (28–41)	38 (28–40)	<0.05
BMI				
Median (min–max)	27.0 (21.3–38.4)	26.4 (21.2–36.8)	27.3 (22.1–36.8)	<0.05
VTE risk score				
Low risk (0–1 points)	55	44	11	
Medium risk (2 points)	21	11	10	
High risk (≥3 points)	21	3	18	

counted with an anamnestic scale. Thus, the laboratory coagulation assays provided the additional information and could be used in combination with the anamnestic scales (see Table S2 in Supplementary Materials).

At P1 before the first LMWH injection, fibrinogen and D-dimer concentrations, CFT and α in thromboelastometry, and Vi, V, Vst in thrombodynamics and Ast in thrombodynamics-4D were shifted towards hypercoagulation compared to the reference ranges. From P1 to P2, the fibrinogen concentration and prothrombin increased slightly, APTT, α and MCF in thromboelastometry and Tlag and D in thrombodynamics did not change, and all the remaining parameters had a tendency towards normal coagulation (see Table 2).

A total of 39 women received thromboprophylaxis with LMWH (AC group). Among all of the parameters, only Vi, V, Vs and Ast demonstrated a difference between the AC and no-AC groups (see Table 2) and were significantly closer to the normal range in the AC group. No differences between groups were revealed with other assays. Because the null hypothesis was not rejected for any of the other laboratory tests, a power calculation was performed for thrombodynamics only. For the thrombodynamics data, in which the null hypothesis was rejected by the Mann-Whitney test, we performed the power calculation. The primary endpoint was considered dichotomous and was represented by the percentage of patients with V values within the normal range in the AC group (33% of women) and no-AC group (4% of women). We calculated a power value of 98%, which confirmed that the difference between the groups was significant.

We estimated the sensitivity of laboratory assays to the additional LMWH effect on hemostasis stabilization after cesarean delivery using a ROC analysis. APTT, D-dimer, CT in thromboelastometry, V, Vs in thrombodynamics and Ast in thrombodynamics-4D were selected as representative parameters (Figure 1). The area under the ROC curve (AUC) quantified the overall ability of the test to discriminate between patients receiving and not receiving the anticoagulant prophylaxis with a cut-off value of AUC = 0.5, representing a non-discriminative test. Vs had a maximum of AUC = 0.76 and Ast had a maximum of AUC = 0.75. For all standard coagulation tests and thromboelastometry, the AUC range varied from 0.45 to 0.55.

At P2, 12 women (31% of AC group) had marked hypercoagulation, as demonstrated by the thrombodynamics assay (please see Figure 2A, D, G, J). Despite anticoagulation, they had an increased clotting rate (represented by parameter V) of higher than 34 μm/min (20% higher than upper limit of normal) and marked spontaneous clotting.

For all women receiving anticoagulant thromboprophylaxis, an anti-Xa assay was performed. Women were grouped according to the presence (Figure 2A, D, G, J, M) or absence of anti-Xa activity (Figure 2B, E, H, K, N). The group with zero anti-Xa activity (anti-Xa = 0) had significantly higher V and Ast values than the group with traces of heparin (anti-Xa > 0). No other tests demonstrated differences between these groups. Moreover, most of the V and Ast values in groups with zero anti-Xa activity were above the reference range (see Figure 2K, N). For the correlation between the V of thrombodynamics and anti-Xa activity, please see Figure S2 in the Supplementary Material. Based on the thrombodynamics analysis, the VTE-risk did not differ in groups with "effective" and "ineffective" anticoagulation (Table S3).

To support the correlation between coagulation tests and anti-Xa activity values, an independent analysis was performed. Subgroups of "positive" or "negative" dynamics among patients receiving LMWH were formed based on assay value dynamics from P1 to P2 for each coagulation test. If the parameter showed the expected normalization of the value from P1 to P2, the dynamics were considered

Table 2: Coagulation assay parameters measured during peripartum and the early postpartum period (No AC – patients, not receiving anticoagulants at P2; AC – patients, receiving anticoagulants at P2) ($N_{total} = 97$; $N_{no\ AC} = 58$; $N_{AC} = 39$).

Parameter median (5–95 percentile)	Reference intervals for non-pregnant women	P1 total	P2 total	p_w	P1 No AC	P1 AC	p_{mw}	P2 No AC	P2 AC	p_{mw}
Standard coagulation tests										
Fg (g/L)	2.0–4.7	5.7 (3.3–7.3)	6.5 (4.8–8.3)	<0.001	5.5 (3.1–7.4)	5.9 (3.3–7.1)	NS	6.6 (4.9–9.8)	6.5 (4.5–8.0)	NS
APTT (s)	28–40	28 (24–34)	29 (24–34)	<0.001	28 (23–33)	28 (24–37)	NS	30 (24–34)	29 (24–34)	NS
Prothrombin (%)	80–119	113 (87–137)	119 (92–145)	<0.001	113 (93–134)	112 (81–142)	NS	121 (92–145)	117 (92–139)	NS
D-dimer (μg/L)	<550	5118 (1532–9999)	1705 (789–8677)	<0.001	5299 (1709–9999)	4459 (1209–9999)	NS	1718 (789–9999)	1761 (727–8969)	NS
Thromboelastometry (NATEM) test parameters										
CT (s)	575–891	582 (388–832)	623 (341–816)	<0.05	584 (388–835)	581 (212–832)	NS	601 (341–931)	631 (323–916)	NS
CFT (s)	164–430	156 (93–292)	173 (90–427)	<0.05	153 (97–304)	168 (86–290)	NS	169 (90–427)	177 (86–488)	NS
α (degree)	32–60	61 (46–73)	60 (36–73)	NS	61 (44–72)	60 (46–73)	NS	60 (40–73)	60 (34–74)	NS
MCF (mm)	39–65	57 (45–69)	59 (39–73)	NS	58 (45–69)	57 (43–66)	NS	59 (43–76)	59 (34–70)	NS
Thrombodynamics test parameters										
Tlag (min)	0.6–1.5	0.75 (0.65–1.15)	0.8 (0.6–1.25)	NS	0.75 (0.65–1.15)	0.75 (0.55–1.25)	NS	0.85 (0.65–1.25)	0.75 (0.55–1.35)	NS
Vi (μm/min)	38–56	63 (54–70)	60 (50–68)	<0.001	63 (53–70)	63 (54–69)	NS	60.6 (52.5–67.9)	57.6 (46.1–70.5)	<0.05
V (μm/min)	20–29	39 (32–57)	35 (25–53)	<0.001	39 (32–58)	40 (31–55)	NS	35.4 (29.2–48.51)	31.6 (23.5–64.3)	<0.05
Vs (μm/min)	20–29	38 (32–46)	34 (25–45)	<0.001	37 (32–46)	39 (31–45)	NS	35.1 (29.2–45.4)	30.7 (23.5–43.1)	<0.01
D (i.e.)	15,000–32,000	31,367 (27,421–33,736)	31,894 (26,848–34,295)	<0.05	31,108 (27,021–33,155)	31,697 (28,223–34,720)	NS	31,683 (28,449–35,021)	32,118 (21,530–34,212)	NS
Thrombodynamics–4D test parameters										
Ast (AU/L)	40–100	206 (97–344)	138 (60–297)	<0.001	193 (97–299)	208 (122–346)	NS	169 (87–304)	94 (54–258)	<0.001

The table presents the median and 5–95 percentile range. Statistical analyses were performed using the pair-sample Wilcoxon signed rank test (p_w) and Mann-Whitney *U*-test (p_{mw}). NS represents not significant difference.

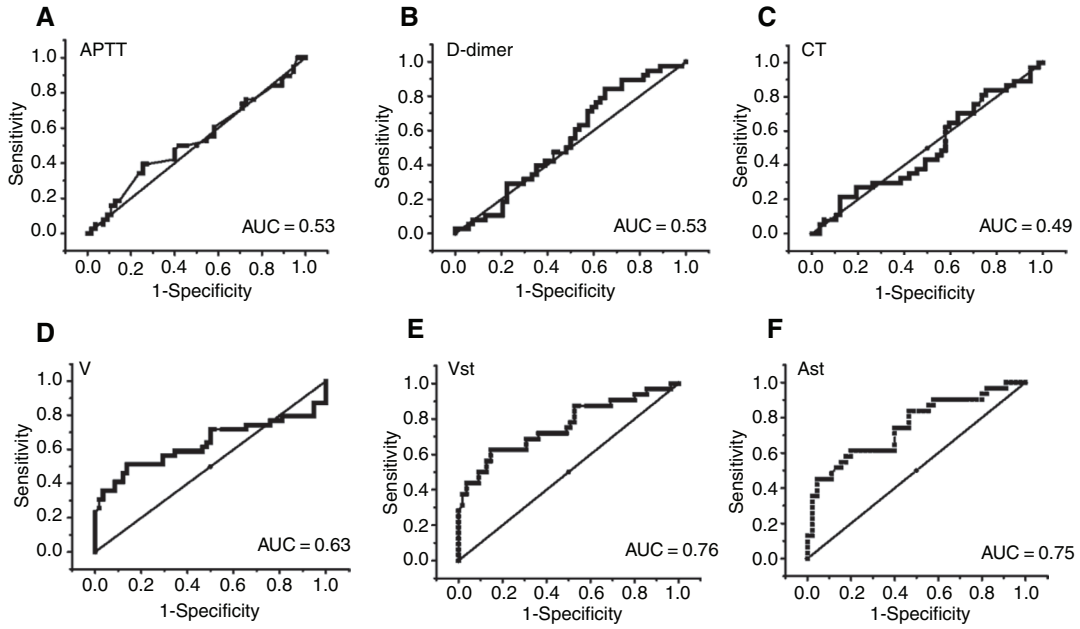

Figure 1: ROC-analysis of laboratory test results among patients, receiving and not receiving anticoagulant prophylaxis in early postpartum (P2, 2 days after cesarean section): A – APTT, B – D-dimer, C – CT in ROTEM, D – V in TD, E – Vst in TD, and F – Ast in TD-4D. The area under the ROC curve (AUC) quantifies the overall ability of the test to discriminate between the patients receiving and not receiving the anticoagulant prophylaxis with a cut-off value of AUC = 0.5, representing a non-discriminative test.

"positive". If the parameter did not change or was shifted towards hypercoagulation from P1 to P2, the anticoagulation effect was considered ineffective and the dynamics were considered "negative". With the exception of clot growth rate in thrombodynamics, none of the parameters revealed a significant correlation with anti-Xa activity presence (Figure 2C, F, I, L, O).

Discussion

This study was conducted to compare the results of coagulation assays in early postpartum and to provide a comprehensive assessment of the capabilities of these assays to assess the LMWH effect in early postpartum during thromboprophylaxis. Considering the maximal thrombosis rate around delivery and during the first week postpartum [4], the study was set during the first days after delivery. Application of laboratory assays to estimate the immediate state of the coagulation system as an addendum to an anamnestic VTE-risk scale score in the LMWH prophylaxis decision may help to improve prophylaxis individualization. This mechanism could potentially lead to further decreases in maternal thromboembolism during early postpartum, on the one hand, and rationalization of LMWH usage, on the other hand. Thrombodynamics was approved to be sensitive to hypercoagulation [26–28] and to anticoagulation with heparin [29, 30]; however, no evidence exists to justify using this test during the early postpartum period. A successful application of a global coagulation tests for LMWH prophylaxis monitoring in pregnant women was performed earlier by Chowdary et al. [19]

Our study is consistent with earlier study results regarding hemostasis during peripartum and early postpartum [1, 9, 13, 22, 31]. D-dimers and global hemostasis assays reveal hypercoagulation several hours after delivery and a trend towards normalization in early postpartum. However, most assays did not show differences between the AC and no-AC groups at P2 and did not reflect an additional effect of LMWH on hemostasis stabilization after cesarean section, except for parameter V in thrombodynamics. The ROC analysis confirmed a better sensitivity of clot growth rate V and stationary amplitude of thrombin Ast to LMWH in plasma compared to other tests (Figure 1).

Coagulation changes from P1 to P2 in the AC-group may be caused by the following two phenomena: (1) normalization of hemostasis within 2 days after resolved pregnancy and surgery, and (2) residual LMWH effect after previous injection. Approximately 26% (nine of 39 patients) of the patients had a detectable LMWH concentration, and no heparin was found in 74% (30 from

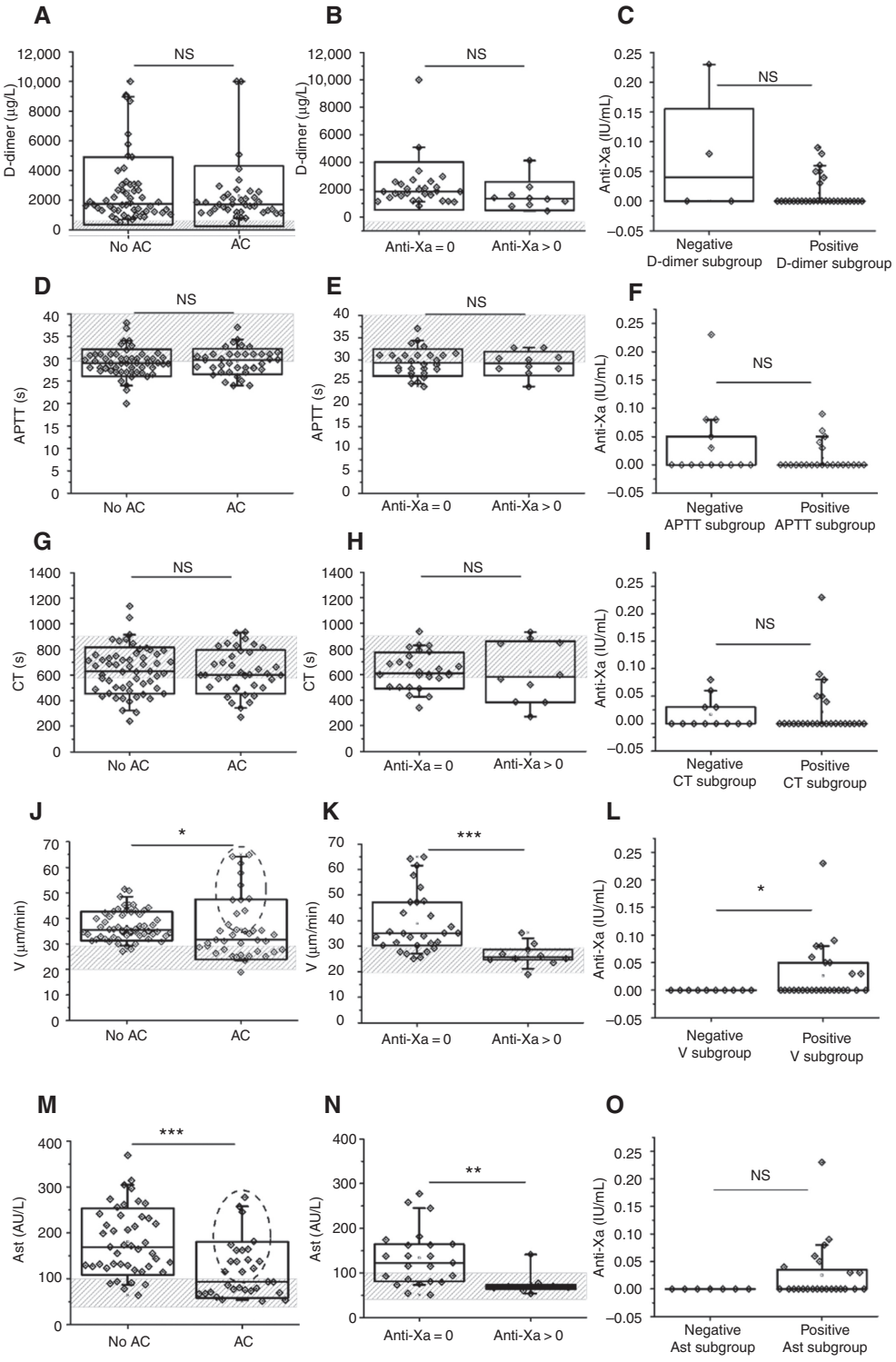

Figure 2: Laboratory coagulation test parameters of patients, receiving (AC) and not receiving anticoagulant prophylaxis (No AC) during the early postpartum period (measured at P2, 2 days after the delivery); laboratory coagulation test parameters in AC subgroup: patients receiving LMWH, divided into groups formed based on the presence (anti-Xa > 0 IU/mL, n = 10) or absence (anti-Xa = 0 IU/mL, n = 29) of anti-Xa activity; the results of anti-Xa assay for groups of negative (having a shift towards hypercoagulation in early postpartum) and positive (having the expected normalization of coagulation in early postpartum) dynamics: A–C – D-dimer (μg/L); D–F – APTT (sec); G–I – CT in ROTEM (s); J–L – V in thrombodynamics (μm/min), M–O – Ast in thrombodynamics (AU/L). The box plots indicate the following parameters: the mean value (the dot inside the box), the median (the horizontal line inside the box), the 25th and 75th percentiles (the bottom and top of the box, respectively) and the 10th and 90th percentiles (the ends of the whiskers) *P < 0.05, Mann-Whitney U-test; **P < 0.01, Mann-Whitney U-test; ***P < 0.001, Mann-Whitney *U*-test; NS represents no significant difference. The dashed circle represents patients with marked hypercoagulation.

39 patients) of the AC-group patients at P2. This observation can be explained by the fact that the coagulation assays at P2 were performed 24 h after the previous injection of heparin. With the exception of clot growth rate V ($P < 0.001$, Figure 2K) and the stationary amplitude of thrombin Ast ($P < 0.01$ Figure 2N), the tests did not detect any differences between patients with and without LMWH (Figure 2B, E, H).

A trend towards normalization of coagulation in most patients not receiving LMWH thromboprophylaxis was observed. However, since the percentage of women with coagulation parameters within the normal range for thrombodynamics was higher in the LMWH-receiving group, we believe that prophylactic LMWH doses have an effect (which is additional to the natural normalization of coagulation after the delivery), but most of the assays lack the appropriate sensitivity. Since both hemorrhage and thrombosis can complicate the early postpartum period and the rate of heparin metabolism is highly individual, we tried to find an instrument that would be able to control even prophylactic doses of LMWH and reflect the actual state of coagulation rather than the heparin concentration. The individualization of LMWH prophylaxis is necessary to protect the mother from hemorrhage in cases with an excess of LMWH and thrombosis in cases of insufficient LMWH.

We divided AC patients into two subgroups based on coagulation test dynamics from P1 to P2 (See Figure S3) and the criteria are described above. Notably, V was the only parameter in which the results did not conflict with the anti-Xa activity results in the negative subgroup, i.e. no patients with a negative tendency on thrombodynamics had a detectable anti-Xa activity level (Figure 2L). For other assays in the negative subgroups, a significant number of patients had negative dynamics despite the presence of LMWH in plasma (Figure 2C, F, I).

Because effective LMWH thromboprophylaxis requires the constant protection of the patient from VTE between injections, we chose to perform the tests at the valley of the LMWH effect, when the concentration of heparin in blood is minimal, to estimate the real coagulation system state and risk of thrombosis in patients receiving prophylaxis rather than to estimate the heparin effect at the peak of its concentration. It is expected that some patients will have no detectable LMWH concentration in the blood 24 h from the previous LMWH injection time. Not all of these women are at high risk of VTE; however, a group of patients will have persistent hypercoagulation combined in the absence of heparin. Hypercoagulation indicates unsteady protection of the patient during the day, which may predispose the patient to VTE. Therefore, we can assume the insufficiency of once-a-day thromboprophylaxis for individual patients and correct the prophylactic strategy.

This is a longitudinal study with two time points and 97 participants for whom we can trace coagulation changes from delivery to puerperium. In this study, we simultaneously compared various coagulation tests, including global hemostasis assays. This is a strength of our study due to the lack of such comprehensive investigations regarding hemostasis. Thrombodynamics and thrombin generation tests have already been identified as sensitive to therapy and prophylaxis with LMWH in previous studies [17, 19, 29, 30]. Thrombodynamics and thrombodynamics-4D, which combines the features of thrombodynamics and thrombin generation tests, proved to be sensitive both to hypercoagulation and the effect of heparin, which means that both tests can be considered promising for the needs of anticoagulant monitoring in pregnancy and postpartum.

We used both thrombodynamics and thrombodynamics-4D during our study, with the expectation that thrombodynamics would provide a more "natural" clotting (due to the preservation of the patient's natural lipid content) and thrombodynamics-4D would estimate thrombin generation. However, both tests provided us with similar information, so we used only Ast (thrombin amplitude) for thrombodynamics-4D.

A limitation of the study is that the inclusion and exclusion criteria were not very strict. Due to the lack of data regarding laboratory hemostasis tests for women undergoing cesarean section, we decided to conduct a general study. Using data from our study, a more specialized study may be conducted. All clinical information from participants of this study was carefully collected.

A large proportion of the women were in the low and medium thrombosis risk categories, which can also be considered a weakness of the study. However, we performed our investigation on a representative sample in which the proportion of patients of different VTE risk categories was similar to the proportion among all obstetric patients in the hospital.

The short time range of the investigation and the absence of clinical outcomes may be interpreted as limitations of the study. We decided to conduct a general investigation and took into account that VTE in the early puerperium is maximal around the first week [4]. Observing the clinical outcomes was beyond the scope of the current study. As not all women showed clinical symptoms of VTE, the Doppler ultrasound was not conducted in the absence of clinical indications. Further studies are needed to compare laboratory designated risk-groups with clinical outcomes, such as VTE.

VTE during early puerperium is one of the most important complications in obstetrics. Establishing a screening test that can be used in combination with scores to estimate VTE-risk and monitor the heparin thromboprophylaxis effect is of great importance. Finding an assay that is sensitive both to hypercoagulation after cesarean section and the effect of LMWH would provide the ability to distinguish a group of patients for whom LMWH prophylaxis is ineffective. Using different coagulation assays, we estimated the sensitivity of laboratory assays to both hypercoagulation after cesarean section and the effect of LMWH. Furthermore, we identified a group of women with hypercoagulation despite receiving LMWH prophylaxis. This observation cannot be assumed to be heparin resistivity, but it may be a reason to discuss the LMWH dose and injection frequency.

However, further work is required to apply these findings to clinical practice. Further studies would include a larger number of participants and correlation with clinical outcomes.

Conclusion

In conclusion, we report a hypercoagulable state after cesarean delivery and a trend towards normalization during early postpartum. This research is potentially beneficial for the application of the thrombodynamics/thrombodynamics-4D assay to monitor coagulation in patients with medium and high VTE risk who receive LMWH thromboprophylaxis during early postpartum.

Author's statement

Conflict of interest: Authors state no conflict of interest.

Material and methods: Informed consent: Informed consent has been obtained from all individuals included in this study.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee. Approved by the Local Ethics Committee, Research Center for Obstetrics, Gynecology and Perinatology, Moscow, Russian Federation (29 January 2015, reference number: 1).

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