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# How can Secondary Thromboprophylaxis in High-Risk Pregnant Patients be Improved?

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**Abstract**

Low-molecular-weight heparin (LMWH) is suggested for thromboprophylaxis in pregnant women with previous venous thromboembolism (VTE**)**. Anyway, there is only limited amount of studies evaluating the effect of LMWH on hemostatic parameters during pregnancy of patients with previous VTE and the need of secondary thromboprophylaxis. We therefore provide results of prospective and longitudinal assessment of changes in hemostasis in high-risk pregnant women at four times during pregnancy (T1–T4) and one time after the postpartum period (T5) used for individualized modification of thromboprophylaxis. In this study, the results of coagulation factor VIII (FVIII) and protein S (PS) activity, ProC Global ratio and anti-Xa activity were evaluated. Despite the thromboprophylaxis, an increased predisposition to thromboembolic complications was detected (significant increase in FVIII activity and decrease in PS function, ProC Global ratio not normalized even after the postpartum period – $p < .0001$ between controls and T5 for PS and ProC Global). These results indicate that hemostasis may not be restored even 6 to 8 weeks after delivery and pose the question when is it safe to withdraw the anticoagulant thromboprophylaxis in high-risk patients with prior VTE.

**Keywords**

at-risk pregnancy, venous thromboembolism, thromboprophylaxis, hemostasis

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## Introduction

Pregnancy is a prothrombotic clinical condition that is developed with the aim to prevent an excessive bleeding during delivery. In hemostasis, the activity of coagulation factor II, VII, VIII, IX, X, XII, von Willebrand factor (vWF), prothrombin fragments (F1 + 2) and fibrin increases, function of protein S (PS) and protein C decreases and an increased resistance to activated protein C (APCR) can also be detected. Moreover, decreased velocity of venous flow, venous dilation and restriction of venous return by a gravid uterus contribute to stasis of blood flow. Considered together, all these factors account for 6% to 11% of pregnancy-associated deep venous thrombosis (DVT).[1,2]

Venous thromboembolism (VTE) that includes DVT and pulmonary embolism (PE) is diagnosed in approximately 1 per 1000 humans and leads to 60,000 to 100,000 deaths annually.[3,4] Pregnancy-associated VTE is therefore a major cause of maternal morbidity and mortality worldwide.[5] The risk of

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thrombosis is highest at 1 to 3 weeks postpartum and remains increased up to 12 weeks after delivery.[6–14] Moreover, generally, in the comparison with subjects with no history of VTE, patients with previous episodes are at increased risk of future events of DVT and PE. Thus, women with a history of VTE have a three- to fourfold higher risk of VTE in the course of subsequent pregnancies than outside pregnancy.[15,16]

Low-molecular-weight heparin (LMWH) is preferred to unfractionated heparin for thromboprophylaxis in pregnancy due to a decreased risk of development of heparin-induced thrombocytopenia and heparin-associated osteoporosis.[6,17] According to the current American Society of Hematology (ASH) guidelines for the management of VTE in the context of pregnancy, there is a strong recommendation for antepartum anticoagulant thromboprophylaxis with a history of unprovoked or hormonally associated VTE.[18] Similarly, according to the recommendations of the American College of Chest Physicians (ACCP), for all pregnant women with previous VTE, postpartum thromboprophylaxis lasting for 6 weeks with prophylactic- or intermediate-dose of LMWH is suggested (Grade 2B) and for pregnant patients at moderate to high risk of recurrent VTE (single unprovoked VTE, pregnancy- or estrogen-related VTE or multiple unprovoked VTE without long-term anticoagulation), antepartum thromboprophylaxis with prophylactic or intermediate dose of LMWH is recommended (Grade 2C). Intermediate dose of LMWH is for example represented by dalteparin used in the dose 5000 IU administered subcutaneously every 12 h or enoxaparin in the dose 40 mg given subcutaneously every 12 h.[15] However, the optimal management of individuals with incidentally detected thrombophilia is less apparent and recommendations vary depending on the underlying conditions.[6]

Therefore, in this article, we share with the results of prospective and longitudinal monitoring of acquired changes in hemostasis in the population of high-risk pregnant patients receiving secondary anticoagulant thromboprophylaxis with LMWH due to a previous thromboembolic event. According to the results obtained in this study, the dose of LMWH could be modified with the aim to increase the effectiveness of the thromboprophylaxis. Subsequently, we compared the results of this single-center study with the healthy non-pregnant control group and with similar studies published in the available literature.

## Materials and Methods

### Patients and Controls

46 pregnant women of Caucasian origin with a history of unprovoked or estrogen-related thromboembolic complications, with or without detected inherited thrombophilia receiving anticoagulant thromboprophylaxis in the form of LMWH were included in the study.

The study compared the results of the patients with the control group composed of 54 healthy women without personal or family history of thromboembolism, without history of pregnancy complications, such as repeated pregnancy loss, placental abruption, intrauterine growth restriction (IUGR), fetal demise or VTE during pregnancy. These individuals did not take any drugs that could influence hemostasis – anticoagulants, antiplatelet drugs, oral contraceptives etc. Thus, healthy individuals included in the study were healthy non-pregnant women.

### Study Design

Before the clinical examination, nurse at the outpatient department performed atraumatic blood sampling of fasting pregnant woman into Vacutainer® blood collection tube with anticoagulation reagent (3.2% sodium citrate) for the analysis of hemostatic parameters. To be more exact, advanced tests of special hemostatic parameters (coagulation factor VIII (FVIII) activity, function of PS, ProC Global test (measured by an automated coagulometer BCS XP, Siemens®, Erlangen, Germany) and anti-Xa activity (Liquid Anti-Xa, HemosIL®, Bedford, USA) (measured by an automated coagulometer ACL TOP 550CTS, Werfen®, Bedford, USA) were evaluated. Because of the need of the monitoring of the peak anti-Xa activity, pregnant woman was instructed to administer LMWH 3-4 h prior taking of blood samples in the morning.

Blood samples were collected at five time points: T1 was scheduled in the 10th–12th week of gestation, T2 in the 16th-18th week of gestation, T3 in the 26th-28th week of gestation, T4 in the 35th-36th week of gestation and T5 during sixth to eighth week postpartum. At the T1 visit, the data about patients' history of medical illnesses, family history, information about allergies and drug intolerance, drugs indicated by other specialists, gynecological history and further demographics were collected (if this was the first visit of the patient at our department) and modified at each of the visits. After the processing of the results, obtained values were compared between particular time points T1–T5, but preferentially between T1 and T5, T2 and T5, T3 and T5, and between T4 and T5 (time point during pregnancy compared with the postpartum period, when it is presumed that the levels of particular parameters should be relatively normalized). Besides the comparisons of results of high-risk pregnant patients at each of the time points in the pregnancy (T1–T4) and after the postpartum period (T5), the results of at-risk pregnant women obtained from T1–T5 were compared with the results of the control group.

### Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava (approved on 11 December 2013 with the protocol code EK 1422/2013).

Written informed consent was obtained from all subjects involved in the study.

## PS Function

For the detection of the PS function, functional PS assay was used. After blood sampling in the tube with 0.11 M sodium citrate in the ratio 1:10, platelet-poor plasma (PPP) of pregnant patients was obtained by centrifugation for 10 min at 1 500 g. Activated protein C proteolytically cleaved FVa that was formed during the activation of coagulation cascade with the reaction with Russell's viper venom (RVV). PS (reagent Protein S Ac®, Siemens®, Erlangen, Germany) acted as a cofactor accelerating this reaction and the consequence was the prolongation of the coagulation time directly correlating with PS activity in the measured sample.

BCS® XP (Siemens®, Erlangen, Germany) blood coagulometer was used for the measurements and reagents needed for the analysis were Protein S Ac®, PS-deficient plasma, Standard Human Plasma, Control Plasma P, Control Plasma N (manufactured by Siemens®, Erlangen, Germany), starting reagent (RVV) and water for injections.

Protein S Ac® (APC reagent composed of the activated human protein C and calcium chloride) was diluted in 2 mL of distilled water. The content of the vial was gently mixed and stored at 15 to 25°C for 60 min before the analysis.

Lyophilized PS-deficient plasma was diluted in 1 mL of distilled water. Before the analysis, it was gently mixed and stored for 60 min at 15 to 25°C. Starting reagent was diluted in 5 mL of distilled water, gently mixed and stored in thermostat at 37°C for 60 min.

Calibration was performed using Standard Human Plasma (reagent Standard Plasma®, Siemens®, Erlangen, Germany) with the table of analytical values specific for the particular batch. Calibration data were provided against the World Health Organization (WHO)-Standard or Fresh Normal Plasma pool. Quality control reagents used in our study were Control Plasma N (reagent Control N®, Siemens®, Erlangen, Germany) and Control P® (Siemens®, Erlangen, Germany). Lyophilized reagents Standard Human Plasma, Control Plasma N and Control Plasma P were reconstituted in 1 mL of distilled water, gently mixed and before the use stored for 15 min at 15 to 25°C.

The result was determined as the percentage of the measured value derived from the calibration curve. The curve was linear in the range 2.5 to 130%. Reference range in our laboratory is 60 to 130%.

## ProC Global Assay

For the determination of ProC Global ratio, the test based on the principle of activated partial thromboplastin time (aPTT) was used. PPP obtained by centrifugation as described above was incubated with the activator of protein C (venom of Agkistrodon contortrix) and contact phase of the activator led to the activation of endogenous protein C of the intrinsic cascade. By the activation of protein C during the common pathway with intrinsic PS, procoagulant cofactors – activated coagulation factor V and VIII (FVa and FVIIIa) were inactivated. This way, the formation of blood clot was prolonged. The time of such clot formation was determined by the protein C activity depending clotting time (PCAT). In plasma sample with decreased capacity of protein C, PCAT was significantly prolonged. Such prolongation of coagulation time could be caused by the deficiency of coagulation factors or excess of heparin in the sample. Therefore (as the control), PCAT/0 (prepared with the addition of buffer to plasma of protein C activator) that should be $\leq$ 60 s was determined. The results were expressed as the normalized ratio that was obtained by dividing the patient's ProC Global ratio by the PCAT ratio of a standard plasma. Standard reference plasma was calibrated against an internal reference plasma pool with a sensitivity value assigned to it of 1.0.[19]

BCS® XP (Siemens®, Erlangen, Germany) blood coagulometer was used for the measurements and reagents needed for the analysis were the diagnostic kit for ProC Global composed of aPTT reagent, activator (venom of Agkistrodon contortrix) and buffer, Standard Human Plasma with the sensitivity value (reagent Standard Plasma®, Siemens®, Erlangen, Germany), ProC® Control Plasma (Siemens®, Erlangen, Germany), Control Plasma N (reagent Control N®, Siemens®, Erlangen, Germany), water for injections and calcium chloride.

Before the analysis, aPTT reagent was gently mixed, activator was diluted in 2 mL of water for injections, buffer was heated to room temperature and calcium chloride was prepared in the concentration 0.025 mol/l, heated to 37°C and mixed.

For the calibration with Standard Human Plasma with known sensitivity value (SV), according to the particular batch, the analyzer automatically calculated the calibration factor (CF). Formula used for the calculation was following:

$$CF = SV / (PCAT{:}PCAT / 0)_{\text{standard human plasma}}$$

For the internal quality control, PCAT and PCAT/0 were determined according to the pipetting protocol for the analysis (Table 1).

For the comparability of the results between the laboratories, the result was expressed as the normalized ratio (NR) calculated from the formula:

$$NR = (PCAT{:}PCAT / 0) \times C$$

**Table 1.** Pipetting protocol for the analysis of ProC Global used in our laboratory

| | PCAT (µl) | PCAT/0 (µl) | Incubation (seconds) |
|---|---|---|---|
| Analyzed sample, standard human plasma | 50 | 50 | |
| Activated protein C activator | 50 | — | 20 |
| Buffer | — | 50 | 20 |
| APTT reagent | 50 | 50 | 180 |
| Calcium chloride | 50 | 50 | measurement of time |

Abbreviations: APTT, activated partial thromboplastin time; PCAT, protein C activity depending clotting time; PCAT/0, protein C activity depending clotting time used as the control.

**Table 2.** Reference interval for for the calculation of ProC Global

| | Median | Interval |
|---|---|---|
| PCAT (seconds) | 132 | 85 to 200 |
| PCAT/0 (seconds) | 47.5 | 35 to 60 |
| NR | 0.94 | 0.69 to 1.56 |

Abbreviations: NR, normalized ratio; PCAT, protein C activity depending clotting time; PCAT/0, protein C activity depending clotting time used as the control.

Detection limit of the analyzer for PCAT/0 was less than 60 s and for PCAT less than 300 s. Reference interval for particular parameters is specified in the Table 2.

## Determination of FVIII Activity

For determination of FVIII activity in the plasma of pregnant patients, we used one-stage FVIII assay. FVIII activity was assessed by measuring the capability of the sample to correct the prolonged aPTT of factor-deficient plasma using a standard curve established with a known calibrator.[20] At first, PPP was prepared by processing of citrated plasma as described in the paragraph about the detection of PS function. BCS® XP (Siemens®, Erlangen, Germany) blood coagulometer was used for the measurements and reagents needed for the analysis were Actin® FS, FVIII-deficient plasma, Standard Human Plasma, Control Plasma P, Control Plasma N (produced by Siemens®, Erlangen, Germany), water for injections, sodium chloride and calcium chloride.

Actin® FS as the ready-to-use reagent was mixed before the particular analysis. For the use of FVIII-deficient plasma, lyophilized plasma was diluted in 1 mL of distilled water. Before the analysis, this reagent was stored 15 min at 15 to 25°C and subsequently it was gently mixed.

Calibration was performed using Standard Human Plasma with the table of analytical values specific for the particular batch. Calibration data were provided against the World Health Organization (WHO)-Standard or Fresh Normal Plasma pool. Quality control reagents used in our study were Control Plasma N (reagent Control N®, Siemens®, Erlangen, Germany) and Control P® (Siemens®, Erlangen, Germany). Lyophilized reagents Standard Human Plasma, Control Plasma N and Control Plasma P were reconstituted as described in the paragraph about the detection of PS function.

The result was determined as the percentage of the measured value derived from the calibration curve. Reference range in our laboratory of the National Center of Hemostasis and Thrombosis is 0.6 to 1.5 IU/ml.

## Anti-Xa Activity

For the determination of anti-Xa activity of administered LMWH, chromogenic anti-Xa method using calibration curve (reagent Liquid Anti-Xa, HemosIL®, Bedford, USA) was used. It is a kinetic method based on the inhibition of the constant amount of activated coagulation factor X (FXa) by the tested heparin in the presence of endogenous antithrombin and hydrolysis of FXa synthetic chromogenic substrate (S-2732) by the remaining FXa. This way, chromophore para-nitroaniline (pNA) was removed from the substrate. The amount of the released pNA correlated with the remaining FXa activity.[21]

Three levels of lyophilized calibrators (reagent Heparin Calibrators, HemosIL®, Bedford, USA) were prepared from human citrated plasma at three different concentrations: calibrator 1 with human lyophilized plasma containing buffer and stabilizers (heparin is absent, so heparin concentration is 0 IU/ml), calibrator 2 with human lyophilized plasma containing heparin in the concentration 0.8 IU/ml, buffer and stabilizers and calibrator 3 with human lyophilized plasma containing heparin in the concentration 2 IU/ml, buffer and stabilizers. Before the analysis, the contents of the vials with calibrators were dissolved with 1 mL of CLSI Type CLR water, stored at 15 to 25°C for 30 min, gently swirled and inverted to mix.

For the quality control of the Liquid Anti-Xa assay (HemosIL®, Bedford, USA), when testing for LMWH on ACL TOP 550CTS Coagulation System (Werfen®, Bedford, USA), LMW Heparin Controls (HemosIL®, Bedford, USA) were used. They were prepared from lyophilized human citrated plasma at two different LMWH concentrations: Low LMWH assayed control intended for the assessment of precision and accuracy of the assay at the low concentration of LMWH containing also buffer and stabilizers and High LMWH assayed control intended for the assessment of precision and accuracy of the assay at the high concentration of LMWH containing also buffer and stabilizers. The Low and High LMW Heparin Controls activity ranges were determined over multiple runs on IL Coagulation Systems using a specific lot of Liquid Anti-Xa reagents. The mean of the control range determined in each laboratory may vary due to the lot of reagent used for the particular measurement.

Before the analysis, the contents of the vials with controls were dissolved with 1 mL of CLSI Type CLR water, stored at 15 to 25°C for 30 min, gently swirled and inverted to mix.

Reference range in our laboratory is 0.2 to 0.4 IU/mL in the case of the prophylactic dose of LMWH and 0.5 to 1.2 in the case of therapeutical dosage of LMWH.

## Statistical Analysis

The data were explored and analyzed in R (R), version 4.0.3. Boxplot, overlaid with swarmplot, was used to visualize the data and explore the distribution.[22] To test the null hypothesis of the equality of the population means, ANOVA test with Welch correction for unequal variances was used. It was followed by the Tukey HSD post-hoc test. Findings with $p$ value $< .05$ were considered statistically significant.

Moreover, we analyzed the results of anti-Xa activity versus PS, anti-Xa activity versus ProC Global and anti-Xa activity versus FVIII separately for time points of blood sampling T1–T5 with line fitted using robust regression. Subsequently,

p value for slope (the rise of regression line) and adjusted R2 (adjusted coefficient of determination) measuring effect size (tightness of the association – close to 1 is tighter) were calculated.

Few patients contributed data for to the assays at T1 and T2 points. Indeed, this is an imbalanced design, where different groups have different number of observations. ANOVA test takes this into account, as do also the post-hoc tests, hence the p values are the correct ones.

## Results

The average age of the patients was 30.24 years (age range 19-40 years). Family history was positive in 50% of included patients. Inherited prothrombotic mutations, such as Factor V Leiden mutation and prothrombin variant G20210A were present in 11.7% and 0.9% of all studied individuals. The most common acquired thrombophilia was PS deficiency developed in 26.1% of at-risk pregnant patients. The second most frequent acquired thrombophilic state was increased activity of FVIII present in 18.9% and the third one was APCR occurring in 15.3% of cases. Sticky platelet syndrome was diagnosed in 18%, antiphospholipid syndrome was detected in only 1.8% of the cases and hyperhomocysteinemia in 0.9% of the patients. Controls were healthy women with average age 28.91 years (age range 18-45 years).

Average timepoint of the beginning of the anticoagulant thromboprophylaxis with LMWH was the 19.fourth week of gestation. This initiation of thromboprophylaxis with LMWH was delayed due to later commencement of management by the hematologist, because of taking further antithrombotic drugs (eg acetylsalicylic acid) or due to the accompanying complications, such as gynecological bleeding etc

Pregnant patients took LMWH in dosages from 0.2 to 0.8 mL once daily. Average initial dose was mostly 0.3 mL of nadroparin administered subcutaneously once a day (35.2% of the patients), the second most frequent starting dose of LMWH was 0.4 mL of nadroparin once daily (23.1% of the patients included in the study). Average dose of LMWH recommended in the 35th week of gestation was 0.4 mL of nadroparin once daily prescribed in 28.2% and the second most common dose of LMWH in the 35th week of gestation (in T4) was 0.6 mL of nadroparin administered once daily recommended in 18%.

Development of prothrombotic changes increasing the risk of the recurrence of thrombotic event could be observed in several studied parameters.

### PS Function

Function of PS decreased in the course of pregnancy and increased after the postpartum period but did not achieve reference range values or values measured in the control group (Table 3, Figure 1). Therefore, we obtained statistically significant data in the comparisons between control group and all blood sampling time points in the studied patients T1–T5. P value for the comparison between T1 and the control group was .0028. For the particular comparisons between T2–T5 and the control group, p value was repeatedly < .0001.

### ProC Global Ratio

Values of ProC Global ratio were decreased during pregnancy without normalization after the postpartum period (in T5) (Table 3, Figure 2). Again, we obtained statistically significant difference expressed as $p$ value < .05 in the comparisons between the control group and blood samplings in studied patients – $p$ value between controls and T3 was < .0001, between controls and T4 it was .0049 and $p$ value in the comparison between the control group and T5 was < .0001.

### FVIII Activity

FVIII activity was increased in the course of pregnancy peaking in T4 and decreasing after the postpartum period achieving reference range values in T5 (Table 3, Figure 3). Statistically significant results in the at-risk patients were obtained in the comparisons between T1 and T5 ($p$ value .0003), T2 and T4

**Table 3.** Dynamics of function of PS, FVIII activity, ProC Global ratio and anti-Xa activity in at-risk patients in the course of pregnancy

| Time point of blood sampling (T1-T5) | T1 (n, number of the patients) | T2 (n, number of the patients) | T3 (n, number of the patients) | T4 (n, number of the patients) | T5 (n, number of the patients) | Controls (n, number of the controls) |
|---|---|---|---|---|---|---|
| PS – arithmetic mean (%) | 42.2 (8) | 38.73 (12) | 41.84 (26) | 39.77 (20) | 56.01 (24) | 84.59 (53) |
| ProC Global – arithmetic mean | 0.62 (10) | 0.61 (10) | 0.55 (22) | 0.59 (15) | 0.55 (18) | 0.78 (52) |
| FVIII activity – arithmetic mean (%) | 2.44 (11) | 1.81 (9) | 2.21 (22) | 2.6 (17) | 1.39 (11) | 1.27 (53) |
| Anti-Xa activity – arithmetic mean (IU/ml) | 0.39 (8) | 0.35 (17) | 0.36 (31) | 0.39 (36) | 0.47 (31) | |

Abbreviations: FVIII, coagulation factor VIII; IU, international unit; PS, protein S; T1, time point 1; T2, time point 2; T3, time point 3; T4, time point 4; T5, time point 5.

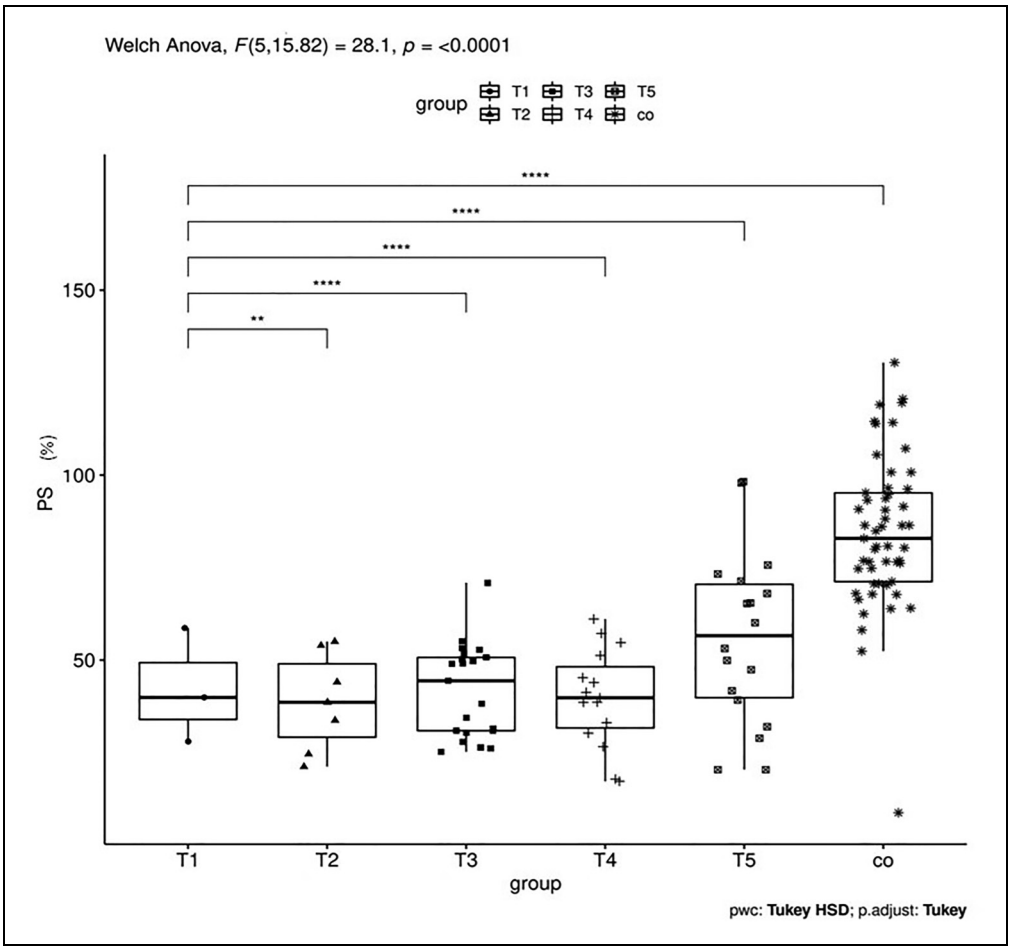


**Figure 1.** Dynamics of PS function.

($p$ .0144), T3 and T5 ($p$ .0007) and between T4 and T5 ($p <$ .0001). When comparing controls with the values detected during pregnancy and postpartum period, significant differences can be seen in the comparisons between the control group and T1, between controls and T3 and in the comparison between controls and T4 ($p$ value in all of these three comparisons was < .0001).

## *Anti-Xa Activity*

Values of anti-Xa activity were in the upper range for prophylactic dosing and below the range for the therapeutic dosing (Table 3). We divided them into the group of prophylactic (P, blue line), adjusted (A, red line) or intermediate dose of LMWH (I, green line) (Figure 4). There was no significant difference in the comparison between particular time intervals of blood sampling during pregnancy and postpartum period and even between the above-mentioned groups (P value for the group of adjusted dose of LMWH was .39 and for the prophylactic dose it was .31).

Using the results of the robust regression analysis calculated as p values and adjusted R2 coefficients of the associations of anti-Xa activity versus PS, anti-Xa activity versus ProC Global and anti-Xa activity versus FVIII separately for T1–T5, we found statistically significant positive association between ProC Global ratio and anti-Xa activity in T4 ($p$ value = .044, AdjR2 = 0.43) (Figure 5). This result, however, should be taken into account with caution, as it was calculated using only 6 values.

When evaluating the results of anti-Xa activity and ProC Global ratio, at T1, T2, T4 and T5, there are limited numbers of pregnant patients included in the study. Limited numbers were caused by technical factors, or due to the timing of visits to the haematology outpatient department or in some cases to personal or health problems.

## *Clinical Data*

Based on the results outlined above, change in the dose of LMWH according to the dynamics of acquired changes of hemostasis (decrease in the function of PS, ProC Global ratio or increase in FVIII activity) occurred most commonly at T3 (in 35.6% of the cases). The second most frequent point when a change in LMWH dose was recommended was T4 (change was suggested in 30.5% of patients).

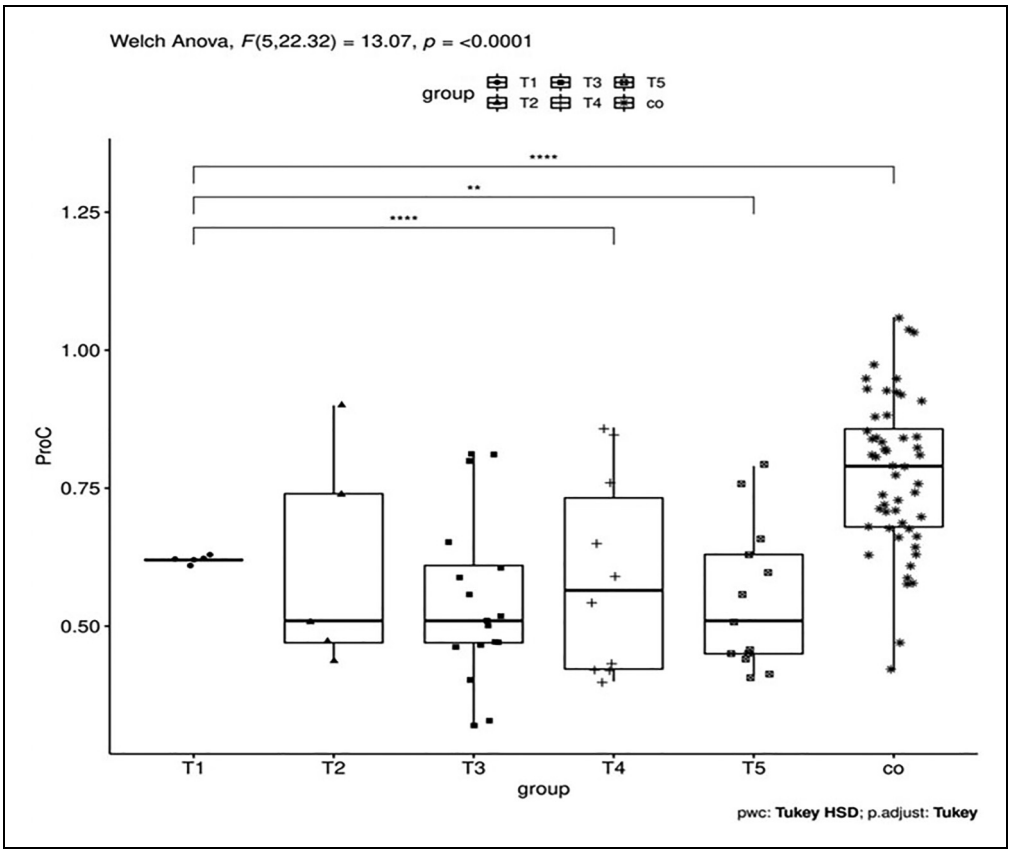


**Figure 2.** Development of changes in values of ProC Global ratio in at-risk pregnant patients.

In all cases, a healthy newborn was delivered, average week of gestation at the time of delivery was 39.24th week. In 61% of cases, the delivery was spontaneous vaginal and in the remaining percentage (39%) of pregnant women, cesarean section was needed to be performed either due to the anatomic variations and size of the baby, previous cesarean section in the woman, preterm prelabor rupture of the membranes or hypoxia of the fetus because of various other reasons. Average parameters of the newborns were weight 3377.71 g and length 51.21 cm. Recurrence of VTE despite thromboprophylaxis with LMWH was observed in one patient included in our study (this patient was then excluded from our study, as she was managed with therapeutic dose of LMWH). The use of LMWH in our study group was well tolerated without the occurrence of any serious side effects. An allergic reaction with a subsequent need to switch to another brand of the drug (either nadroparin, enoxaparin or dalteparin) was observed in 47.8%.

This frequency may seem to be relatively high. All these reactions were only in the form of cutaneous adverse drug reactions with clinical manifestations such as hives and itching (without serious systemic reactions) and affected patients were mainly patients with allergic reactions to further substances in food and drugs. Based on this clinical finding, we changed the drug in 95.5%, the product was changed from nadroparin to enoxaparin in 77.3%, from nadroparin to dalteparin in 13.7% and from nadroparin and enoxaparin to fondaparinux in 4.5%. In 4.5%, the allergic reaction was identified in T5 (after the postpartum period), when LMWH was routinely discontinued.

We did not detect any abnormalities of the markers of renal function in our study. Average liver enzymes stayed in the reference range and none of the patients exhibited signs of HELLP syndrome or heparin induced thrombocytopenia.

## Discussion

Physiological changes in pregnancy contribute to a hypercoagulable state, thus increasing the risk of VTE.[23] This prothrombotic state resolves gradually after delivery, as coagulation factor levels normalize in 8 to 12 weeks postpartum.[24]

Protein S is a vitamin K-dependent protein circulating in plasma and playing a crucial role in the regulation of the processes of coagulation. It acts as a cofactor for activated protein C which inactivates FVa and FVIIIa. Moreover, it is also a cofactor for the tissue factor pathway, resulting in the inactivation of FXa and the complex of tissue factor and activated coagulation factor VII.[25–27]

There is a significant correlation between laboratory markers of hypercoagulability and estradiol and progesterone levels.[28] Pregnancy provokes a decrease in free tissue factor pathway inhibitor (TFPI) and free PS level that attenuates the function of the protein C system and results in elevated thrombin generation and increased occurrence of APCR. Moreover,

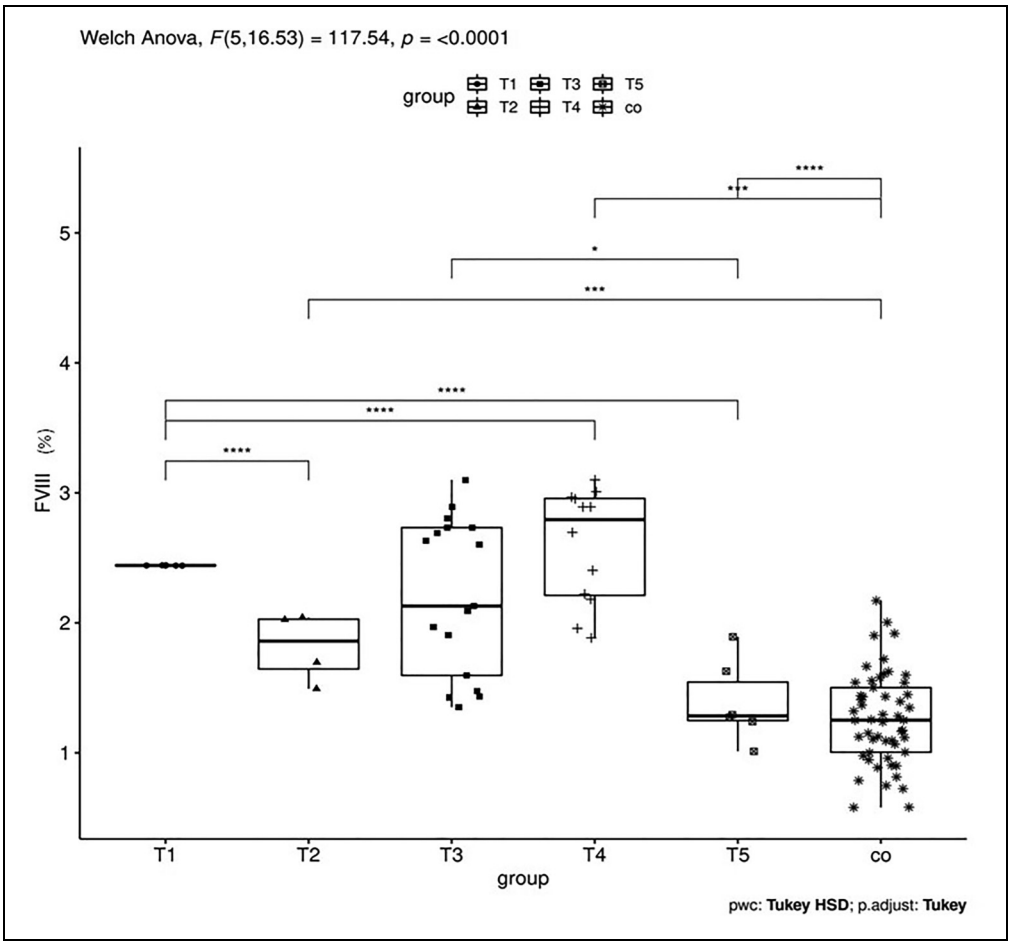


**Figure 3.** Dynamics of FVIII activity.

pregnancy-dependent hemodilution can contribute to the decreased peripheral PS level.[29] The level of PS is decreased to 60 to 70% in normal pregnancy.[2,30–32] Free PS significantly decreases from levels obtained before pregnancy by 32 days of gestation.[28] In futher studies, PS showed a statistically significant decrease from the first to the second trimester ($p < .0001$) and it remained stable thereafter.[33] Protein S deficiency can increase the risk of VTE.

However, there is no strict distinction between its physiological and pathological decline in pregnancy.[34–38] Moreover, in the study of Demir and Dilek, the authors investigating the status of natural coagulation inhibitors and APCR in preeclampsia showed even higher PS levels in the severe preeclamptic group in the comparison with the mild preeclamptic group.[39]

Even current ACCP guidelines in the document entitled "VTE, Thrombophilia, Antithrombotic Therapy, and Pregnancy Antithrombotic Therapy and Prevention of Thrombosis, ninth ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines", the authors repeatedly mentioned PS deficiency in the association with the risk of VTE in pregnant women or in the postpartum period without clarification of the borderline between its physiological and pathological decrease.[15] Thus, according to our knowledge and available literature, no exact definitions or studies characterizing the physiological decline of PS in pregnancy exist.

Anticoagulation therapy with LMWH is suggested in the patients with PS deficiency and was proven to be of benefit to pregnant women with recurrent pregnancy loss.[40,41] Similarly, pregnant patients with antithrombin, protein C or PS deficiency or with homozygous factor V Leiden mutation ought to be considered for ante- or postpartum thromboprophylaxis, or both.[42] Moreover, due to relatively frequent finding of PS and PC deficiency in patients with VTE during pregnancy, thrombophilia screening as the prevention of its recurrence is suggested in this population.[43]

In our study, similarly, the function of PS was decreased and increased after the postpartum period without achieving reference values in at-risk pregnancy and postpartum period when compared with healthy non-pregnant controls (Table 3, Figure 1). This indicates the fact that hemostasis may not be normalized even 6 to 8 weeks after the delivery with the need to individually evaluate the withdrawal of secondary anticoagulant thromboprophylaxis.

Changes in the levels of coagulation factors V, VIII and IX and anticoagulant factors PS and protein C may alter function of

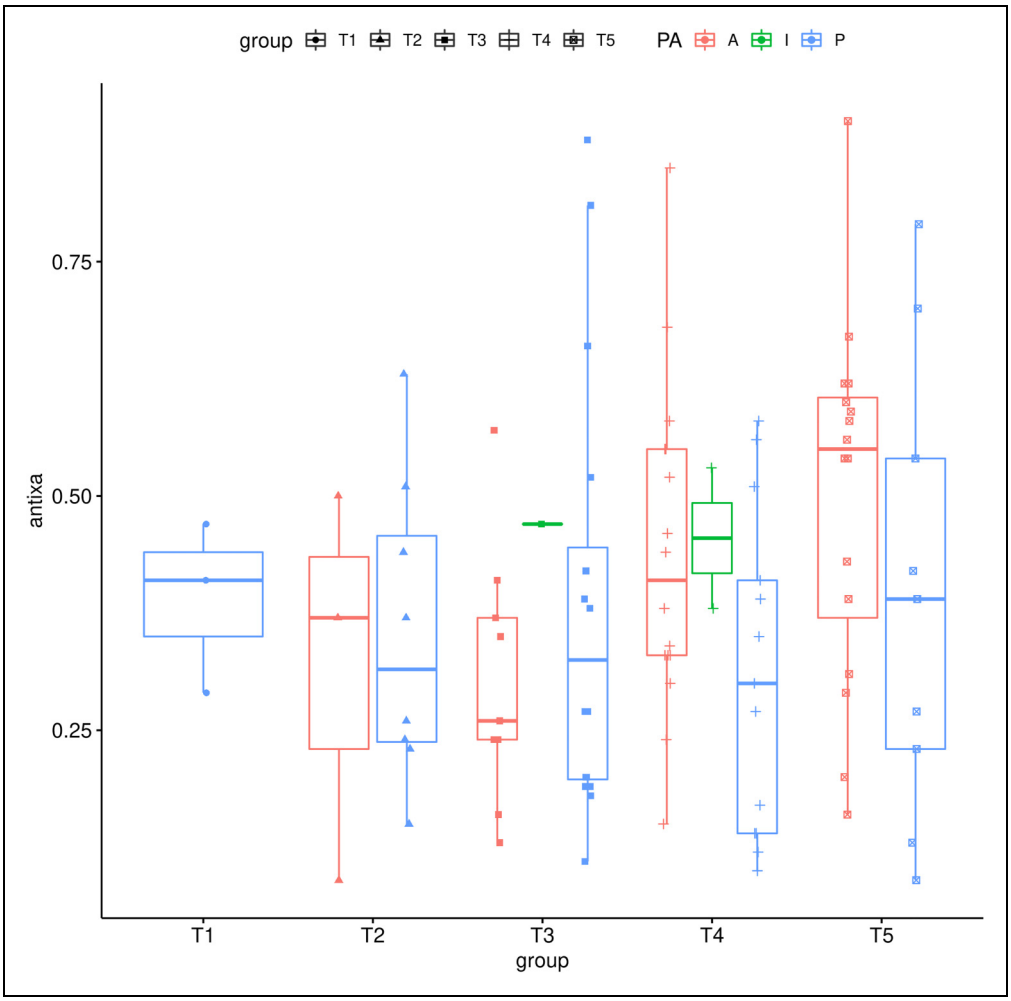


**Figure 4.** Comparison of anti-Xa activity in at-risk pregnant patients.

activated protein C and cause acquired APCR. Therefore, there is an association between an acquired APCR phenotype and increased levels of coagulation factors V, VIII and IX.[44] Interestingly, the frequency of antithrombin deficiency, increased occurrence of APCR and PS deficiency, and increased FVIII activity, was significantly higher in women with a history of perinatal mortality in the comparison with the control group.[45]

APCR is present in a significant number of pregnancies in the concomitant absence of the factor V Leiden mutation.[36] APC resistant phenotype is a risk factor for pregnancy-related VTE.[46,47] ProC Global test is used to quickly assess the presence of abnormalities in the Protein C-PS pathway including APCR.[19]

In our study, values of ProC Global were decreased in the course of pregnancy without normalization after the postpartum period (at T5) (Table 3, Figure 2). Along the above-discussed presence of the decrease in PS activity after the postpartum period, this fact may indicate the persistance of the prothrombotic risk for a longer time interval than up to the end of postpartum period. Therefore, the length of anticoagulant thromboprophylaxis should be evaluated strictly individually.

It would be correct to differentiate the results of APCR testing between the groups with and without FV Leiden mutation. However, after the distinction of the patients between those with and without this mutation, due to the limited number of the women included in our study, we obtained only limited data that are not usable for the statistical analysis. For instance, we processed the results of the dynamics of PS separatedly in patients with and without the FV Leiden mutation. Despite more frequent occurrence of PS deficiency in the studied patients in the comparison with the presence of APCR (26.1% vs 15.3%), even spaghettiplots for the analysis of the PS deficiency with and without FV Leiden mutation were not representative from the statistical point of view. Thus, we could not evaluate APCR this way.

Either in normal or high-risk pregnancy, levels of coagulation factors, especially vWF, coagulation factors V, VII, VIII, IX, X, XII, fibrinogen, alpha 1-antitrypsin and plasminogen activator inhibitor type I and 2 increase until delivery.[48–59] Elevated level of FVIII is associated with an increased risk of VTE and arterial thromboembolism.[60] The probability of thrombosis per pregnancy in patients with increased levels of FVIII:C (activity more than 172%) was reported as 1 in 385

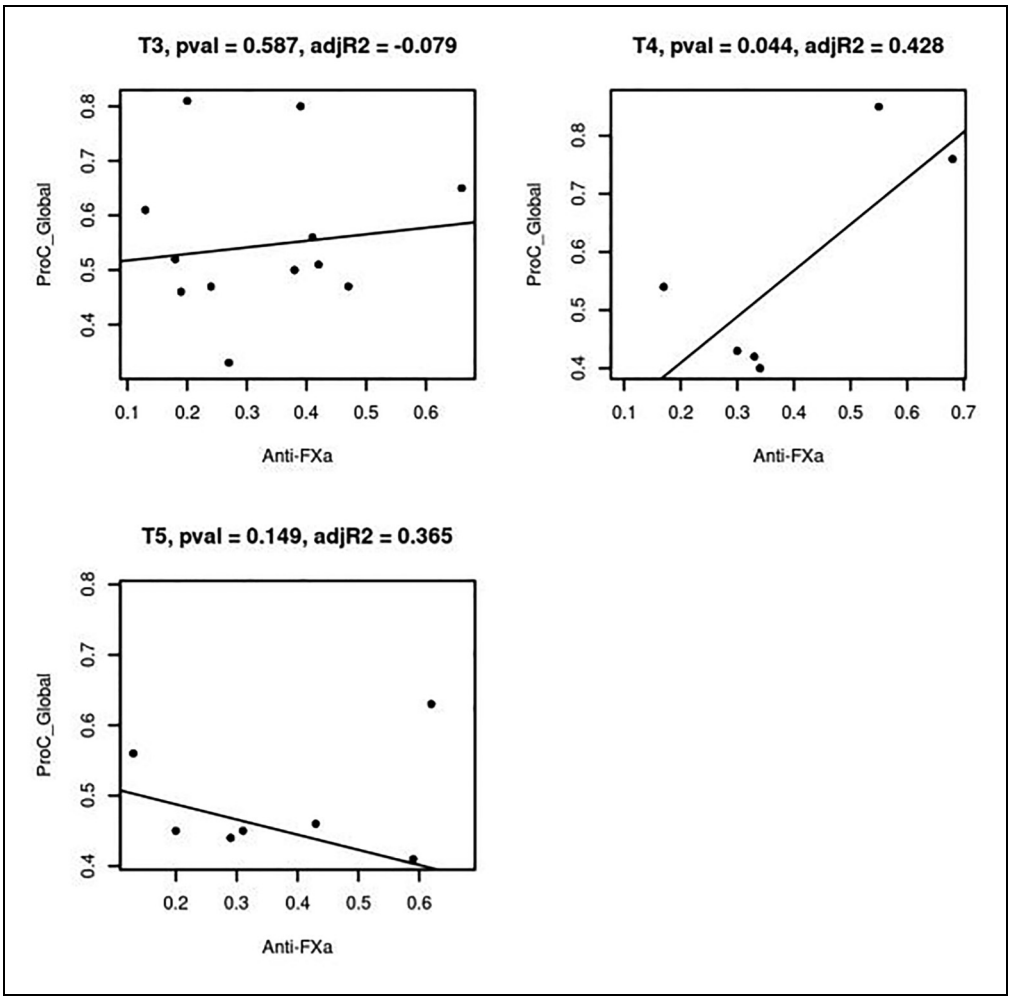


**Figure 5.** The associations of anti-Xa activity versus PS, anti-Xa activity versus ProC Global and anti-Xa activity versus FVIII.

($p < .001$).[61] Patients with and without preeclampsia differ significantly ($p < .05$) with respect to activated FVIII, homocysteine and free PS.[62] FVIII was even found in granular deposits mostly in areas of fibrinoid necrosis in normal term placentae. This indicates that coagulation and fibrinolysis are activated in these areas and FVIII can serve as their marker.[63] Moreover, it was suggested that the activation of the coagulation system with the increase in nearly all coagulation factor activities except lower increase in FXII, inhibitor of plasminogen activation and prothrombin complex can also be the risk factors for IUGR.[64]

FVIII activity significantly increases from the levels obtained before pregnancy even by 59 days of gestation.[28] Moreover, it was found that women with a history of pregnancy-associated VTE and also with preeclampsia had increased FVIII compared with results obtained from women with normal pregnancies indicating a slight thrombin generation.[65–67] The authors Tanaka et al. confirmed that increased FVIII contributes to activated protein C insensitivity.[68]

FVIII activity was increased also in the course of the pregnancies of the patients included in our study. Significant results were present in the comparisons between T1 and T5 ($p$ value .0003), T2 and T4 ($p$ .0144), T3 and T5 ($p$ .0007) and between T4 and T5 ($p < .0001$). When comparing controls with the values detected during pregnancy and postpartum period, significant differences were found between the control and patient groups at T1, controls and T3 and controls and T4 ($p$ value in all three comparisons was < .0001) As published by others, FVIII in our study peaked at T4 and decreased after the postpartum period achieving reference range values in T5 comparable with the controls (Table 3, Figure 3). The decrease we observed after the postpartum period was contrary to the results of Bonnar et al. where FVIII remained raised. Such changes in the dynamics of FVIII can explain the increased predisposition to thromboembolic events in the puerperium.[69]

Although identification of patients with an increased risk of pregnancy-related VTE is relatively well defined, controversy regarding the optimal duration and intensity of anticoagulant prophylaxis of this complication still remains.[70] Thus, pregnancy can be challenging due to the questionable safety of LMWH[71] and heterogeneity in guidance for the monitoring of the anticoagulant effect of LMWH that may be evaluated

according to the level of thrombin generation, overall hemostatic potential and anti-Xa activity.[72]

According to the global experience, sufficient anti-Xa activity with detection of peak anti-Xa activity 3 h after the administration of LMWH is achieved using a weight-based regimen. Regular testing of LMWH effect is not routinely required in individuals with VTE administering therapeutic doses of LMWH.[30] Based on the results of a single-center case study investigating two groups of pregnant women treated with LMWH for VTE with and without monitoring of anti-Xa levels, the authors McDonnell et al. concluded that there is no significant difference between these two groups in any clinical outcome thus providing the evidence to support the recommendation not to measure anti-Xa activity in the majority of patients using therapeutic dose of LMWH in the antenatal period.[73] Similarly, according to several guidelines, routine monitoring of anti-Xa activity to guide the dose of LMWH is not recommended.[74–77]

In contrast, there are several studies recommending the use of determination of anti-Xa activity in pregnancy. In the pregnant patients with recurrent fetal loss, it was proposed to perform adjustment of the dose of LMWH based on anti-Xa levels, monitored regularly in the course of pregnancy to keep anti-Xa activity in the required range.[78] Due to the relative lack of the prospective studies, some authors propose to measure anti-Xa activity after the initiation of the treatment and then every 1 to 3 months during pregnancy.[79,80]

The advantages of the monitoring of anti-Xa activity in pregnancy, obesity, renal insufficiency and in children are still a matter of debate. Further aspects remain also controversial, such as the question whether to measure trough anti-Xa activity during pregnancy and how to monitor the effectiveness of LMWH in patients with antithrombin deficiency.[81,82] Monitoring of LMWH may be helpful in the case of bleeding during the first trimester of pregnancy or in high-risk pregnancy with insufficient anticoagulation response.[83]

Non-compliance of the patient with the treatment or renal failure may represent further reasons for monitoring the effectiveness of LMWH.[84,85] Moreover, physiological changes occurring in the course of pregnancy, such as weight gain due to the edema, polyhydramnios, large fetus and diet, increased glomerular filtration and renal clearance of LMWH and higher plasma volume with simultaneous increase in the distribution volume increasing with advanced stages of pregnancy influence the pharmacokinetic properties of LMWH.[72,86]

However, even achieving target peak anti-Xa activity does not always ensure maintenance of minimal trough level.[84,85] It was demonstrated that dalteparin dosage based solely on the weight of pregnant patients administered every 12 h was inadequate to maintain the results of anti-Xa activity in most pregnant women in the therapeutic range throughout pregnancy. Trough levels were rarely in the therapeutic range, despite maintaining the therapeutic peak levels. These notable changes in LMWH activity could explain reported failures in pregnancy[87] and may be easily improved by the monitoring of anti-Xa activity. When using enoxaparin pharmacokinetic parameters to simulate anti-Xa time profiles, it was shown that the maintenance of the same doses during pregnancy resulted in a progressive decrease in the mean and peak anti-Xa activity. Therefore, it is recommended to administer doses normalized also for the changes in body weight to counteract enoxaparin pharmacokinetic changes accompanying different stages of pregnancy.[88]

Besides the discussed weight of pregnant patients, further variables that can influence the dosage of LMWH may be the age of the patient, gestational age, parity, present thrombophilia or antiphospholipid syndrome.[89,90] LMWH dose was also adjusted according to the levels of thrombin-antithrombin complex and D-dimers.[72] A study combining analyses of anti-Xa activity and F1 + 2 indicates that this combination can improve the adjustment of LMWH dose during pregnancy.[91]

In the study of Gibson et al. in 13 pregnant patients with acute VTE or those requiring high-risk thromboprophylaxis due to the recurrent VTE, weight-based dosage failed to maintain therapeutic anticoagulation in 92% of patients (higher tinzaparin doses than those recommended by the manufacturer were needed to keep therapeutic level of anticoagulation according to the peak anti-Xa activity).[92]

Moreover, when comparing anti-Xa peak levels in pregnant and non-pregnant women receiving a therapeutic dose of enoxaparin administered every 12 h, in the majority of measurements, anti-Xa activity within therapeutic range was achieved in a lower percentage of pregnant patients than in the control group ($p$ value was 0.028, 0.008 and 0.003 in the three selected measurements). Therefore, the authors of the study recommend further assessment of a strategy that will include more frequent monitoring of anti-Xa activity resulting in more effective anticoagulation.[93]

In the study of Shapiro et al. change in the dose of enoxaparin to achieve target anti-Xa activity was needed in 69% of pregnancies in the prophylactic and in 55% of pregnancies in the therapeutic group. The weight-based prophylactic dose was 0.6 mg/kg in all three trimesters with a mean ± SD target anti-Xa activity 0.39 ± 0.18 IU/mL and the therapeutic dose was 0.9 mg/kg to maintain anti-Xa activity 0.71 ± 0.22 IU/ml. Thus, similarly as in the previous study, such significant increase in the LMWH dose requirements in the prophylactic group indicates more frequent need of monitoring of anti-Xa activity to keep target anticoagulant level.[94,95]

Most commonly, target peak anti-Xa activity in the treatment group was in the range 0.6 to 1.0 IU/mL and between 0.2 to 0.4 IU/mL in the prophylatic group.[72,96,97] These target ranges are similar to those used as the reference ranges in our study (0.2-0.4 IU/mL for the prophylactic dose of LMWH and 0.5-1.2 IU/mL for the therapeutic dose).

According to the ideas of the Working Group in Women's Health of the Society of Thrombosis and Haemostasis (GTH), although recommendations for the treatment of pregnancy-related VTE are available, a need for prospective studies comparing different management strategies and defining the optimal duration and intensity of anticoagulant treatment still persists.[98] For all these reasons, we decided to take into

account all possible clinical and laboratory aspects leading to the potential change in LMWH dose including detection of anti-Xa activity.

According to ACCP guidelines, for the use of various regimens of LMWH, the following protocols are used: prophylactic LMWH is for instance dalteparin used at a dose of 5000 units every day, tinzaparin at 4500 units per day, nadroparin 2850 units every 24 h or enoxaparin at 40 mg a day. Intermediate dose of LMWH is represented by the use of dalteparin at 5000 units administered every 12 h or enoxaparin at 40 mg applied every 12 h. Adjusted dose of LMWH is based on the weight-adjusted regimen or in the full-treatment form (eg dalteparin 200 IU/kg once a day, tinzaparin 175 IU/kg every 24 h, dalteparin 100 IU/kg every 12 h or enoxaparin 1 mg/kg every 12 h).[15]

Based on this knowledge, in the Results section we described the stratification of the results of anti-Xa activity in the prophylactic, intermediate or adjusted group according to the dosing regimen of LMWH (Figure 4). When LMWH was used in the initial phases of pregnancy, or due to the concomitant need for antiplatelet therapy in advanced stages of pregnancy, the use of lower doses (0.3-0.4 mL of LMWH once daily) was regarded as a prophylactic dose and the intended reference range for anti-Xa activity was 0.2 to 0.4 IU/ml. After adjustment of the dose of LMWH due to various above-mentioned reasons to doses 0.6 to 1.0 mL once daily, the dose was considered as adjusted and the intended reference values of anti-Xa activity were between 0.5 to 1.2 IU/mL.

The criteria for exactly the intermediate dose of LMWH were fulfilled only in 3 cases – in T3 in one pregnant woman, in T4 in two patients. Therefore, it is not possible to include them in the statistical analysis.

Values of anti-Xa activity in our study were on the upper reference limit for the prophylactic dose and below the range for the therapeutic dose (Table 3, Figure 4). This correlates with the need to adjust the dose of LMWH during pregnancy in response to the observed increase in laboratory parameters indicating a risk for thromboembolic complications (increase in FVIII activity, decrease in PS activity or ProC global values) most commonly in T3 and in the second most common time interval in T4 due to the change in the weight of pregnant patients (average weight of the included patients in T1 was 65.17 kg, average maximal weight in T4 was 75.29 kg). However, because this adjustment happened strictly individually with adjustments made also in T1 and even in T2 and T5, we did not detect any significant difference in anti-Xa activity in the comparison between particular time intervals of blood sampling during pregnancy and the postpartum period (T1–T5). P value for the group of patients using the adjusted dose of LMWH was 0.39 and for the prophylactic group was 0.31.

The dose of LMWH administered at the beginning of the follow-up of our at-risk pregnant patients was 0.2 to 0.8 mL used once daily dependent on the initial weight of the patient. Change in the dose in the particular patient was suggested according to several indices. It was proposed due to the increase in the patient´s weight or in the correlation with anti-Xa activity for LWMH. Another reason to modify the dose of LMWH was the detection of a significant change in the majority of the studied parameters (FVIII, PS, ProC Global) in the comparison with the results of the same patient from the previous blood sampling. Last, but not least, the modification of the LMWH dose was based on the comparison of particular results of the patient with median results obtained at the same time point of blood sampling of majority of the patients. Between the particular time intervals of blood sampling, in the most of the cases, we recommended the increase of the dose of LMWH in 0.1 to 0.2 ml.

We decided to extend secondary thromboprophylaxis with LMWH when we observed significant differences between the results of the studied patient and the results of the control group and after the inclusion of a sufficient number of the at-risk pregnant women in T5, also with the results at T5. Such extension was performed in 28.6% of the patients included in this study that is relatively high proportion.

PS function and values of ProC Global ratio in the high-risk pregnant patients in our study did not achieve reference range values determined in a healthy population and were even lower than values obtained in the control group, thus not normalising after the postpartum period. Therefore, we recommend individualising the endpoint of thromboprophylaxis based on the above-mentioned arguments.

According to the ASH 2018 guidelines for the management of VTE, women having a history of unprovoked VTE or VTE associated with a hormonal risk factor, antepartum anticoagulant prophylaxis is strongly recommended, but with low certainty in evidence about effects. For women who require such thromboprophylaxis, the ASH guideline panel suggests standard- or intermediate-dose LMWH thromboprophylaxis also during the postpartum period (this represents a conditional recommendation with very low certainty in evidence about effects).[18] According to ACCP guidelines, in selected high-risk patients in whom significant risk factors persist after delivery, extended thromboprophylaxis up to 6 weeks after delivery is suggested (Grade 2C).[15] In the study of Dahlman et al. blood coagulation and fibrinolysis were significantly increased during the first two weeks of the postpartum period. Three weeks post partum, these processes were normalized, although the inhibitors remained increased in the comparison to the non-pregnant control group.[99]

In our study, based on the significant persisting decrease in PS activity and the values of ProC Global ratio that were not normalised even after the postpartum period, we point to the observation that hemostasis may not be restored even six-eight weeks after delivery.

IUGR is ~~a~~ an antepartum state in which a fetus is unable to achieve its genetically determined size, confirmed by a low growth rate and, or by the specific causes (eg an impaired blood flow in placenta, genetic abnormality, fetal infection or other toxicity).[100] In our study, no IUGR was observed. The average week of gestation at the time of delivery in our patients was at term (39.24th week of gestation).

**Table 4.** Table of concluding recommendations

| |
|---|
| • we suggest to control the hemostatic profile in pregnant patients with the history of thromboembolic complications during pregnancy at least in each trimester, or as individually required and especially after the postpartum period |
| • for the initiation of the secondary thromboprophylaxis, start with the dose of LMWH 0.2 to 0.8 ml once daily depending on the initial weight of the woman |
| • modification of the dose of LMWH should be considered according following indices: |
| • correlation with anti-Xa activity |
| • according to the significant change in FVIII, PS or ProC Global ratio in the comparison with the results of the patient from the previous blood sampling |
| • the increase in the dose of LMWH between the time points of blood samplings may be in 0.1 to 0.2 ml |
| • after the postpartum period, the individual extension of secondary thromboprophylaxis with LMWH should be performed after the detection of significant differences of particular parameters between the time points of blood sampling |

Using the results described above, we wish to contribute to achieving higher certainty in decision-making for the management of anticoagulant thromboprophylaxis in high-risk pregnant patients.

We suggest there is a need to control the hemostatic profile in high-risk pregnant patients during pregnancy by modifying the dose of LMWH if it is ineffective in achieving the intended level of anticoagulation and especially to control the changes in hemostasis after the postpartum period. Based on the results of these available tests, the clinicians can safely and individually withdraw the anticoagulant thromboprophylaxis in women after the delivery according to the actual parameters. The summary of the recommendations of the authors is provided in Table 4.

## Conclusion

In the literature, there are many reports about hemostatic changes in physiological pregnancy. However, for the clinicians (Hematologists, Gynecologists, Obstetricians, specialists in Internal Medicine), it is also important to know how to safely and effectively manage secondary thromboprophylaxis of pregnant patients with LMWH. As stated above, despite such prophylaxis, according to the acquired changes in hemostasis, we detected the persistance of the hypercoagulable state with marked decrease in PS activity and ProC Global ratio after the postpartum period. Therefore, individual consideration of the endpoint of secondary anticoagulant thromboprophylaxis should be recommended. We sincerely hope that this manuscript will contribute to the optimization of the monitoring and management of the anticoagulant thromboprophylaxis in the at-risk pregnant patients.

There are several limitations of our study – the use of non-pregnant women as the control group (the absence of the pregnant control group), limited number of high-risk patients included in the study – predominantly in T1 due to the later visit at our outpatient department of hematology due to personal or health problems. Moreover, cut-off values for PS, FVIII, ProC Global ratio and anti-Xa activity were used using the reference ranges obtained in non-pregnant population of women and men and should be modified by the results of a large prospective study of "at-risk" pregnant patients. Last but not least, we could not differentiate the development of the acquired APCR between the patients with and without factor V Leiden mutation, because we obtained only limited data that are not usable for the statistical analysis. Curently, we continue to include the patients with the perspective of obtaining more results useful for the clinical practice.

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### Ethical Approval

### Informed Consent

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### Trial Registration

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