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SAFETY AND EFFICACY OF LOW-MOLECULAR-WEIGHT HEPARIN RELATED TO PREGNANCY AND RISK OF THROMBOEMBOLISM IN THE POSTPARTUM-PERIOD

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ACADEMIC DISSERTATION

To be presented by the permission of the Medical Faculty of the University of Helsinki for public discussion in the Seth Wichmann Auditorium of the Department of Obstetrics and Gynecology, Helsinki University Hospital on May 5th 2017 at 12 o'clock noon.

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To my family

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This thesis is based on the following original publications, referred to in the text by their Roman numerals:

List of original publications

- I. Galambosi P, Kaaja R, Stefanovic V and Ulander V-M. The safety of low molecular weight heparin during pregnancy: a retrospective controlled cohort study. *EJOGRB* 2012; 163:154-9
- II. Galambosi P, Ulander V-M and Kaaja R. The incidence and risk factors of recurrent venous thromboembolism during pregnancy. *Thromb Res.* 2014; 134:240-5
- III. Galambosi P, Hiilesmaa V, Ulander V-M, Laitinen L, Tiitinen A and Kaaja R. Prolonged low-molecular-weight heparin use during pregnancy and subsequent bone mineral density. *Thromb Res.* 2016; 143:122-6
- IV. Galambosi P, Gissler M, Kaaja R and Ulander V-M. Incidence and risk factors of venous thromboembolism during postpartum-period: a population-based cohort-study. *Acta Obstet Gynecol Scand.* 2017;accepted, DOI:10.1111/aogs.13137. Copyright Wiley.

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Abbreviations

APC	activated protein C
aPL	antiphospholipid antibodies
APS	antiphospholipid syndrome
ART	assisted reproductive techniques
AT	antithrombin
BMD	bone mineral density
BMI	body mass index
CI	confidence interval
CS	Cesarean section
CTPA	computed tomography pulmonary angiogram
CUS	compression ultrasonography
CVT	cerebral vein thrombosis
DOAC	direct oral anticoagulant
DVT	deep venous thrombosis
FGR	fetal growth restriction
HIT	heparin-induced thrombocytopenia
LMWH	low-molecular-weight heparin
OHSS	ovarian hyperstimulation syndrome
OR	odds ratio
PE	pulmonary embolism
UFH	unfractionated heparin
V/Q	ventilation/perfusion
VKA	vitamin K antagonist
VTE	venous thromboembolic event

Abstract

Despite widespread thromboprophylaxis, venous thromboembolic event (VTE) remains one of the leading causes of maternal deaths in the Western world. In Finland, VTE is the number one cause of maternal deaths. The highest VTE risk occurs during the postpartum period. Since the 1980s-1990s, low-molecular-weight heparin (LMWH) has replaced unfractionated heparin (UFH) for the management of acute VTE and for VTE prophylaxis during pregnancy due its reduced risk of bleeding, more predictable anticoagulant response, and longer half-life.

The aim of the study was to evaluate the maternal and neonatal safety and the efficacy of the use of LMWH during pregnancy. Moreover, we studied the incidence, risk factors, and mortality of postpartum VTE in Finland through 180 days after delivery.

We conducted retrospective observational studies (I-III) at the Department of Obstetrics and Gynecology, University Hospital of Helsinki to evaluate the safety and efficacy of LMWH during pregnancy. Women with singleton pregnancies treated with LMWH at any stage of pregnancy (n=475) in 1994-2007, were included in Study I to evaluate maternal and neonatal safety and the efficacy of long-term use of LMWH during pregnancy. Study II was a sub-study of Study I concerning recurrent VTEs limited to those with a history of previous VTE (n=271). In Study III, which was carried out during 2008-2012, we evaluated whether there is a subsequent decrease in bone mineral density (BMD) after long-term use of LMWH during pregnancy. Women with (n=92) and without LMWH exposure (n=60) during pregnancy were recruited for measurement of BMD by dual-energy X-ray absorptiometry (DEXA). Additionally a population-based, controlled cohort study was conducted using the combined data of five large registers in Finland (Study IV). In this study we evaluated the incidence and risk factors for postpartum VTE in 2001-2011. The study population consists of all women with deliveries (N=634292) and a total fertile-aged women population without deliveries or medical abortions (N=1232841) in the last calendar year. Those women with an inpatient or outpatient admission because of VTE after the date of delivery (n=1169) were collected from the Care Register for Health Care (HILMO).

The incidences of thrombocytopenia, bleeding during pregnancy and delivery, preterm delivery, stillbirth, pre-eclampsia, or fetal growth restriction did not differ between LMWH-exposed women and healthy controls. The incidence of allergic skin reactions for LMWH was low. No HIT or osteoporotic fractures were observed. The incidence of VTE despite ongoing LMWH prophylaxis was high, 2.5%. The risk for recurrent VTE despite ongoing LMWH was high in women with a history of two or more previous VTEs, prior VTE related to hormonal risk factors, prior VTE in connection with antiphospholipid antibody syndrome (APS), and use of long-term anticoagulation before pregnancy. The risk for recurrent VTE before the intended initiation of LMWH was associated with a history of two or more prior VTEs and a history of VTE related to earlier pregnancy. No association between decreased BMD and LMWH exposure, when adjusted for potential confounding factors, was found. The risk of postpartum VTE was highest during the first week after delivery: 37-fold compared with non-pregnant women, declining rapidly thereafter to 2-fold through 175 postpartum days. The risk remained elevated for 180 days in women with thrombophilia, Cesarean section, multiple birth, varicose veins, and cardiac disease. Three VTE-related deaths occurred.

In conclusion, we showed that the use of LMWH during pregnancy was safe for both the mother and the fetus. The high VTE recurrence rate in our department reflected insufficient dosing in high-risk women due to lack of consistent recommendations concerning LMWH prophylaxis in high-risk groups. Individual risk assessment and studies on the optimal dosing of LMWH to prevent recurrent VTE during pregnancy in high-risk groups are needed. Because the VTE risk remained elevated for six months after delivery, it is possible that in the high-risk groups LMWH prophylaxis for six weeks might be too short, necessitating more studies to investigate the optimal duration of LMWH prophylaxis.

Introduction

The term venous thromboembolic event (VTE) encompasses both deep venous thrombosis (DVT) and pulmonary embolism (PE). Despite widespread thromboprophylaxis, VTE remains a leading cause of maternal deaths in the Western world, causing 9.3% of all maternal deaths in the United States (1). The risk of VTE is 5- to 10-fold higher in pregnant women than in non-pregnant fertile women (2-5). The postpartum period is the time of the highest risk, 15- to 35-fold that of age-matched non-pregnant non-puerperal women (2,3,6,7).

Because of hazardous adverse effects of earlier used unfractionated heparin (UFH), low-molecular-weight heparin (LMWH) is today recommended in prevention and treatment of acute VTE during pregnancy and prevention of recurrent pregnancy loss in women with antiphospholipid antibodies (aPL) (8-10). The knowledge about the safety and efficacy of LMWH increases continuously along with the use of LMWH in gestational settings. Previous studies of comparatively large sample sizes have demonstrated the safety and efficacy of LMWH during pregnancy (11-14).

The experience of LMWH use in rare cases of women with a high risk of VTE (e.g. prior VTE related to high-risk thrombophilias) is, however, increasing slowly. While recommendations for pregnancy-related LMWH therapy in common situations are rather similar worldwide, the recommendations concerning high-risk cases are mainly based on earlier case reports, case series, or studies conducted in non-pregnant patients (10,15). Moreover, only a few reports on recurrent VTEs during pregnancy exist (16-18). Because of limited cohort sizes in high-risk groups, the optimal dose and duration of LMWH prophylaxis to prevent recurrent VTE during pregnancy in these patients are unknown (10,19).

Bone loss is a well-recognized hazardous adverse effect of UFH, with observed symptomatic osteoporotic fractures in 2-9% of women after long-term use of UFH during pregnancy (20,21). Several case series and animal studies have suggested that the decrease

in bone mineral density (BMD) with LMWH is less than that seen with UFH (22-24). It is recognized that a decrease in BMD occurs in normal pregnancy and is escalated by lactation; this decrease has been, however, demonstrated to be reversible (25,26). Duration of lactation can vary considerably and a longer time between delivery and BMD measurement may disclose this reversible confounding factor. It is not yet known whether long-term LMWH use during pregnancy can lead to a long-term decrease in BMD, i.e. several years after delivery.

Because re-admissions due to postpartum VTE occur at internal medicine departments in Finland, the burden of this entity is not visible to obstetricians, and this might lead to an underestimation of the actual VTE incidence and a false sense of security. Only a few previously published studies with a long (>3 months) follow-up time after delivery exist (27,28). One previous study on efficacy of LMWH prophylaxis demonstrated that the incidence of postpartum recurrent VTE was 7.0%, and 40% of these events occurred after the cessation of the 6-week LMWH prophylaxis – all in high-risk women. This finding suggests that postpartum prophylaxis for 6 weeks might be too short in high-risk cases (17).

We conducted a large controlled cohort study on the safety and efficacy of long-term LMWH use during pregnancy and a population-based register study on the incidence, risk factors, and mortality of postpartum VTEs.

The use of LMWH in gestational settings is increasing constantly because sicker, older, and heavier women around the world are currently becoming pregnant. Our aims were to investigate the following important issues in order to help clinicians to properly identify pregnant women at risk of VTE: 1. The safety of long-term use of LMWH during pregnancy for the mother and the fetus; 2. The incidence and associated risk factors of recurrent antepartum VTE in women with prior VTE; 3. The possibility of subsequent decrease in BMD after a pregnancy-related long-term use of LMWH; and 4. The incidence and mortality of VTE through 180 postpartum days in order to identify associated risk factors during three different postpartum periods and to compare the incidence of postpartum VTE with that of non-pregnant fertile women.

Review of the literature

Venous thromboembolism during pregnancy and during the postpartum-period

Pathophysiology of venous thromboembolic event (VTE) during pregnancy

Pregnancy is associated with several physiological and anatomic changes, which increase the risk of VTE. Venous stasis caused by progesterone-induced venous distension, pelvic venous compression by the enlarging uterus (29,30) and decreased mobility (31) occur during pregnancy. During late pregnancy gravid uterus compresses the right common iliac artery, which further compresses the left iliac vein at the point where they intersect, leading to the propensity for left leg DVT (>80%) (May-Thurner syndrome) (32,33).

The increasing amount of estrogen as pregnancy progresses alters the levels of coagulation factors responsible for hemostasis (34,35). Substantial rises in plasma levels of fibrinogen, von Willebrand factor (vWF), factor (F)VII and FVIII (36) and modest rises in FIX and FX (34,35,37) lead to increased thrombin production, measured by increased soluble fibrin (38). The increased amount of coagulation factors is not balanced by the increased amount of anticoagulants; a decrease in the level of protein S and increased activated protein C resistance in turn reduce the anticoagulant activity during pregnancy (34). Maternal plasma fibrinolytic activity decreases in pregnancy due to increased plasminogen activator inhibitor (plasminogen activator inhibitor -1 and 2) activity and decreased tissue plasminogen activator (39). Vascular injury to the pelvic vessels, which can occur after normal or instrumental vaginal delivery and especially by Cesarean section (CS), explains the pathophysiology of VTE in the postpartum period compounded by postpartum immobility (40). Figure 1 depicts the coagulation cascade on the cell membrane of platelet.

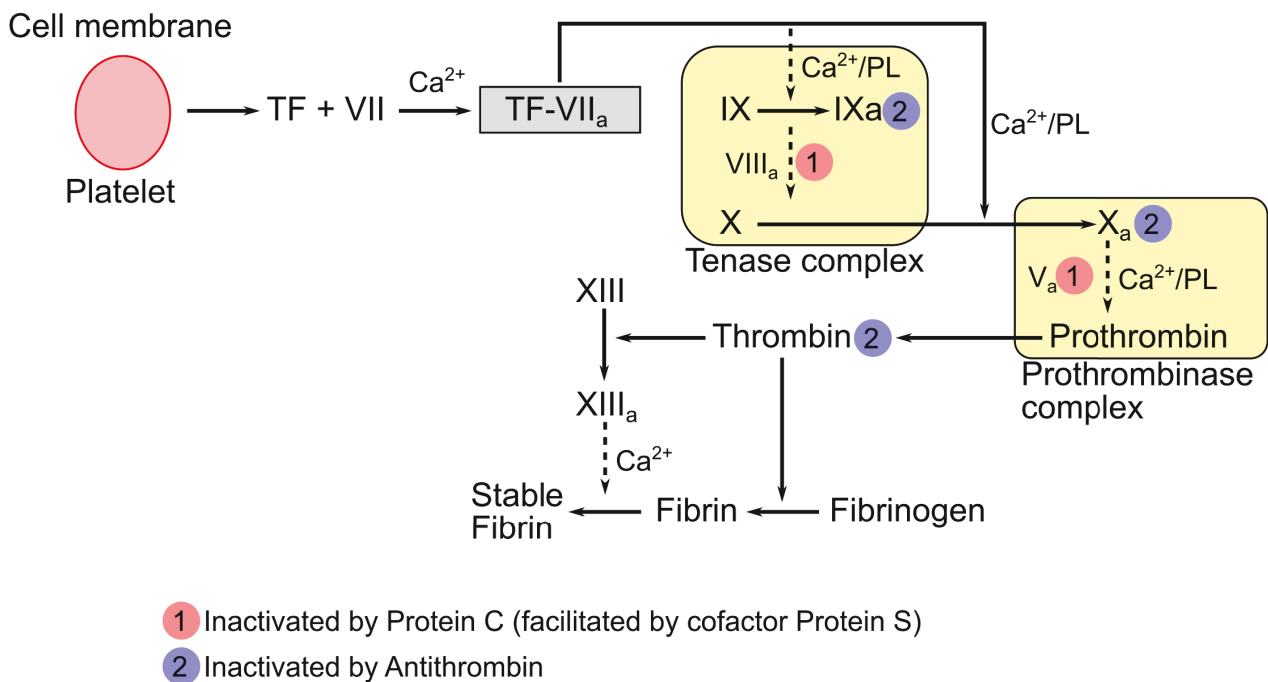


Figure 1: Coagulation cascade on the phospholipid cell membrane of platelet. Tenase complex: Factor (F) IXa adheres to FVIIIa, which together activate FX. FXa adheres with FVa=Prothrombinase complex. Prothrombinase complex in turn converts Prothrombin to Thrombin, which in turn converts soluble Fibrinogen to Fibrin.

a=activated coagulation factor, TF=tissue factor, Ca^{2+} =Calcium, PL=Phospholipids (Phosphatidylserine and Phosphatidylethanolamine)

The overall effect of these above-mentioned changes results in a thrombogenic state in pregnancy, which protects women from hemorrhage during implantation and placental separation at delivery, but in turn predisposes to VTE (34). The hypercoagulability of pregnancy is its highest in the early postpartum period and gradually returns to normal (41,42).

Epidemiology of VTE during pregnancy

VTE is a complication of 0.5-2.2 per 1000 pregnancies (3,6,7,43-47). The incidence of VTE during pregnancy has been estimated to be 5- to 10-fold that of non-pregnant fertile women (2-4). VTE can occur at any stage of pregnancy, but the majority of studies have reported the postpartum period as being the time of highest risk: 15- to 35-fold that of age-matched non-pregnant non-puerperal women (2,3,6,7). The risk of VTE is greatest during the first week after delivery, declining rapidly in the following 3 weeks, although a small

residual risk persists for 12 weeks after delivery (48).

Pregnancy-associated VTE manifests usually as deep venous thrombosis (DVT) or pulmonary embolism (PE). DVT in the lower limb is the most common type of pregnancy-associated VTE, accounting for 75-80% of cases, with the remaining 20-25% caused by PE (7,46,49). The most common site for DVT has been reported to be in the proximal veins (femoral or iliac veins), in 71% of cases, without simultaneously thrombosis in calf vein (50,51). Isolated DVT of a calf vein is reported in only 6% of cases (50), whereas the majority of non-pregnant patients (58-87%) have proximal thrombosis with involvement of the calf veins, suggesting that perhaps the pathophysiology of the VTE during pregnancy differs from that in the general population. Even though upper extremity DVT is uncommon, it has been increasingly reported during pregnancy, particularly with the use of assisted reproductive techniques (ARTs) and with a history of ovarian hyperstimulation syndrome (OHSS) (52,53). The incidence of upper extremity DVT is estimated to be 0.08-0.11% of ART treatment cycles, and the jugular vein appears to be involved more often than the subclavian vein (53). Upper extremity VTE associated with ART and OHSS has recently been proposed to be induced by drainage of estradiol-containing ascites to the jugular and subclavian veins via the thoracic and lymphatic ducts (54). Cerebral vein thrombosis (CVT) is rare, occurring in 1.32/100000/year in high-income countries; the incidence is higher in middle- and low-income countries (55). Risk for CVT is increased during childhood and pregnancy. It may account for a substantial portion of all pregnancy strokes (56). CVTs are now being diagnosed with rising frequency due to the increased use of magnetic resonance imaging (MRI) for investigating patients with acute and subacute headaches and new onset seizures (55).

As a result of better recognition of women at risk of VTE and widespread thromboprophylaxis, VTE is no longer the leading cause of maternal mortality worldwide, as it was a few years earlier (57). However, it remains one of the most important causes of direct pregnancy-associated deaths in Western countries (57). Eighteen deaths due to VTE (16 due to PE and 2 due to CVT) occurred in the UK during 2006-2008, which is notably fewer than the 41 deaths in 2003-2005 (57). In Finland, however, VTE seems to be the leading cause of maternal deaths; 17 of 52 maternal deaths (33%) occurred due to VTE or amniotic fluid embolism in 1996-2014 (58). Because pregnancy-associated VTE is often massive (59), it is an important cause of long-term maternal morbidity in the form of post-

thrombotic syndrome, comprising swelling, pain, discomfort, eczema, varices and ulcer(s) of the affected limb (60).

Diagnosis of VTE during pregnancy

The clinical diagnosis of VTE is difficult because symptoms such as leg swelling, muscular pain of the lower limbs, and shortness of breath are common during pregnancy and are usually not associated with VTE (61). Typical symptoms or signs of VTE are presented in table 1. Measurement of D-dimer (a lytic product of crosslinked fibrin) is currently not recommended in the investigation of suspected VTE since D-dimer levels increase as pregnancy progresses, peaking on the first postpartum day (62). The first test in pregnant women suspected to have DVT is compression ultrasonography (CUS) of the lower extremities and/or iliac veins, unless symptoms in the upper extremity exist (63). When suspecting a calf vein DVT, CUS is recommended to be repeated in 7 days if negative because it is less accurate below the knee, with a sensitivity of 11–100% and a specificity of 90–100% (64,65). Since approximately 10% of iliac DVTs are missed on CUS, in case of high suspicion, CUS should be repeated and, if negative, magnetic resonance imaging (MRI) should be considered (66).

When suspecting PE, a chest X-ray can rule out other pathologies, such as pneumonia or pneumothorax, which may mimic the symptoms of PE (49). It should be performed first, although it is normal in >50% of patients with diagnosed PE (49). Bilateral CUS could also be performed when suspecting PE. If this confirms a DVT, there is no need for further investigations because therapy is often the same for both conditions and unnecessary radiation to the mother and fetus must be avoided (67). However, CUS in this indication is not very useful because in a study with 67 pregnant women with suspected PE no cases of DVT were found (68). If suspicion of PE remains and chest X-ray and CUS are normal a computed tomography pulmonary angiogram (CTPA) or ventilation/perfusion (V/Q) lung scan is recommended (67). Both CTPA and V/Q lung scan have equivalent clinical negative predictive value: 99% for CTPA and 100% for V/Q (69). CTPA is, however, better than V/Q lung scan if chest X-ray is abnormal since it may also identify alternate diagnoses such as aortic dissection (70,71).

Table 1: Symptoms and signs of VTE during pregnancy

Deep vein thrombosis (DVT)	Pulmonary embolism (PE)
Painful and warm leg	Pleuritic chest pain
Swelling	Dyspnea
Erythema	Tachypnea
Tenderness	Cough
Lower abdominal or back pain	Hemoptysis
	Tachycardia
	Raised jugular venous pressure
Cerebral vein thrombosis (CVT)	Focal chest signs
Headache	Collapse
Vomiting	Shock
Photophobia	
Seizures	Upper extremity DVT
Impaired consciousness	Unilateral swelling
Focal neurological signs	Pain
	Dilated collateral circulation

Modified table from Book: Cohen H, O'Brien P. Disorders of Thrombosis and Hemostasis in Pregnancy A Guide to Management. 2nd.ed. Springer-Verlag London 2015; p. 85. and Stone et al. 2016

The adverse effect of lung imaging is the risk of ionizing radiation to the fetus and mother. However, the fetal radiation dose caused by chest X-ray at any gestational age is negligible (<0.1 mGy) (72). The estimated fetal radiation exposure from CTPA (0.1 mGy) is similar to that from V/Q lung scan (0.5 mGy). These exposures are well below the thresholds associated with teratogenesis and risk of childhood cancer (73). The radiation dose to the breast tissue with CTPA (up to 20 mGy) may be 20- to 100-fold that with V/Q lung scan, raising a concern of a risk of breast cancer (74). Breast tissue during pregnancy is very sensitive to radiation (73,75), but radiation doses can be halved by using a lower peak kilovoltage (120 kVp), automatic tube current modulation, and bismuth anterior chest wall shields (75). In Finland, CTPA has been the modality of choice nowadays, but the use of V/Q lung scan is increasing worldwide when chest X-ray is normal. V/Q lung scan can be recommended over CTPA in pregnant women due to concern of breast radiation (personal communication with Risto Kaaja). Magnetic resonance pulmonary angiography is being investigated in detecting PE in pregnancy (76).

In cases with suspected CVT, MRI and magnetic resonance venography are more valuable

and sensitive to the diagnosis of CVT than computed tomography (77)

Risk factors of VTE during pregnancy

Previous VTE

The risk of VTE is reported to be higher in the third trimester than in the second or first trimester, being particular high in puerperium (4). The increased risk of VTE is already present from the first trimester often before the anatomic changes of pregnancy occur (78). In recent years, attention has been given to stratifying risk factors of VTE in order to reduce the rate of these events (79). The most important risk factor for VTE in pregnancy is a personal history of VTE, with the increased recurrence risk being 3- to 4-fold, and 15-25% of all VTE cases in pregnancy are recurrent events (80). Circumstances of previous VTE are essential to stratify because they considerably affect the risk of recurrent event (81). A woman with a temporary risk factor associated with prior VTE (trauma, surgery, long-haul flight, or intravenous drug use) has a very low risk of antenatal VTE (82). If previous VTE is estrogen-provoked (during previous pregnancy or with the use of estrogen-containing contraception pill), the recurrence risk is notable higher, approximately 10% in subsequent pregnancies (80,83,84). If prior VTE was unprovoked, it has been shown to be associated with an increased risk of recurrence relative to those provoked by a temporary risk factor in non-pregnant populations (85). A positive family history increased the risk of pregnancy-associated VTE also regardless of the risk factors precipitating the thrombosis (86).

Thrombophilia

The next strongest risk factor for pregnancy-associated VTE is the presence of a thrombophilia, which means alterations of acquired or inherited coagulation factors predisposing to thrombosis (87). Risk and prevalence of VTE during pregnancy in relation to inherited thrombophilia are presented in table 2.

Table 2: Risk and prevalence of VTE during pregnancy according to inherited thrombophilia

Thrombophilia	Pregnancy-associated VTE OR (95% CI)	Prevalence in women with VTE in pregnancy (%)
Antithrombin deficiency	4.7 (1.3-17.0)	7-12 %
Protein C deficiency	4.8 (2.2-10.6)	10
Protein S deficiency	3.2 (1.5-6.9)	8
Factor V Leiden (homozygous)	34.4 (9.9-120.1)	9-17
Factor V Leiden (heterozygous)	8.3 (5.4-12.7)	8-44
Prothrombin (homozygous)	26.3 (1.2-559.3)	..
Prothrombin (heterozygous)	6.8 (2.5-18.8)	3-10

Data from (Robertson et al. 2006, Lim et al. 2007)

Thrombophilia can increase VTE risk of almost any risk factor, and together with pregnancy, it has important harmful effects for both mother and fetus (87). Different thrombophilias possess different levels of VTE risk (81).

Hereditary thrombophilias

The prevalence of inherited thrombophilia is 20-50% in women with VTE events during pregnancy or during the postpartum period (81). Inherited thrombophilia can be homozygous or heterozygous (88).

Activated Factor V (FVa) is a cofactor required for thrombin generation. FVa is inactivated by Activated Protein C (APC). In Caucasian populations, inherited APC resistance is most often caused by FV Leiden gene point mutation (R506Q) (89), which is the most common thrombophilia. The prevalence of heterozygous FV Leiden mutation is dependent on the population observed. It has been reported to be 5% in white individuals, the highest figure of 7% observed in Southern Europe and the lowest figure of 0-0.6% in Africa (90,91). Its prevalence in Finland is estimated to be 2-3% (92). Acquired APC resistance may occur in connection with pregnancy or antiphospholipid antibodies (aPLs) (89)

The prothrombin gene mutation is due to a single G20210A nucleotide change in the prothrombin gene, which increases thrombin generation (93,94). The prothrombin gene

mutation is inherited dominantly in autosomes and is found in 2% of white individuals in the general population (95).

Antithrombin inactivates multiple enzymes generated by the coagulation cascade: mainly thrombin, FX, IX, XI, XII, and proteins S and C, and hence, regulates generation of thrombin and inhibits already generated thrombin (96). AT deficiency is a rare autosomal dominant disorder, caused by mutations in the AT gene. AT deficiency is considered one of the most severe thrombophilias. Over 250 different mutations have been reported thus far (97). Type 1 AT deficiency is characterized by a quantitative reduction of antithrombin and is a stronger risk factor for VTE than type 2 AT deficiency, which is characterized by the production of qualitatively abnormal antithrombin (96). However in Finland, type 2 AT deficiency is caused predominantly by a single point mutation Pro73Leu, which is also associated with a significant thrombotic risk, up to 100-fold in some families (97). The estimated prevalence of AT deficiency has been found to be 5-17 per 10000 individuals (98). Thrombosis has been reported to complicate approximately 9% of pregnancies in women with AT deficiency who are ≥ 35 years and 6% in those < 35 years (99), which is 30-fold (5- to 50-fold) higher than in those without AT deficiency (97).

APC inactivates FVIIIa and Va, thus inhibiting thrombin generation (100). Protein C deficiency is associated with 270 different mutations and results in impairment of the ability to control coagulation through destruction of FVa and FVIIIa. The prevalence of mild (heterozygous) protein C deficiency in the general population is estimated at 1 per 200-500 individuals. Severe protein C deficiency is rare, 14–50 per 10000 individuals, and is usually associated with severe prothrombotic diseases (101).

Protein S is a vitamin K–dependent anticoagulant protein that acts as a cofactor for APC in the inactivation of coagulation factors Va and VIIIa (102). Sixty percent of protein S binds to C4b binding protein, and the remaining 40% is responsible for the anticoagulant activity. Inherited protein S deficiency is uncommon (less than 1% of the general population) and may be either quantitative (type I, more common, approximately 2/3 of cases) or qualitative (types II and III, less common). Protein S values vary with age and gender. In addition, acquired PS deficiency is common and known to occur with acute VTE, nephrotic syndrome, inflammatory syndromes, disseminated intravascular coagulation, liver disease, malnutrition, pregnancy, estrogen therapy, vitamin K deficiency, and VKAs (103).

Factor VIII (FVIII) plays an essential role in normal hemostasis by acting as a critical cofactor for activated factor IX. An increasing amount of case-control studies support the hypothesis that increased FVIII levels are associated with VTE and recurrent VTEs (104). An odds ratio (OR) for VTE of 4.8 (95% CI 2.3-10.0) was determined for individuals with high FVIII levels (105).

Acquired thrombophilias

Antiphospholipid syndrome (APS) is an acquired thrombophilia with the presence of persistently positive antiphospholipid antibodies (aPL): lupus anticoagulant and/or anticardiolipin and/or anti- β 2-glycoprotein 1 antibodies on two consecutive occasions at least 12 weeks apart in association with a history of arterial or venous thrombosis or adverse pregnancy outcomes. The risk of arterial or venous thrombosis is estimated to be 0.5-30% (106). It is widely accepted that lupus anticoagulant is a more important predictor for VTE than anticardiolipin and anti- β 2-glycoprotein 1 antibodies (107). Triple positivity correlates better with thrombosis and pregnancy morbidity than any other aPL profile (107). Antiphospholipid antibodies have been reported to activate endothelium and complement (108). They also inhibit the anticoagulant properties of APC (109). Up-regulation of the tissue factor pathway caused by aPLs, impairs fibrinolysis by reducing tissue factor pathway inhibitor activity (110).

It is not known why APS in some patients develops into VTEs, while others present with morbidity in pregnancy. While a minority of patients may also develop a life-threatening “catastrophic” form of APS with multiple organ involvement and a high death rate, others never develop any aPL- related manifestation (111).

The risk for VTE in pregnant women with APS is highest among those with a history of thrombosis and in those with all above mentioned three test positive (87). The risk of recurrent thrombosis during pregnancy, despite thromboprophylaxis, was 5% in women with APS (112).

Thrombophilias and pregnancy complications

APLs are also associated with other adverse pregnancy outcomes besides VTE. A diagnosis of obstetric APS requires at least one of these criteria together with persistently positive tests for one or more aPLs: 1. Three or more consecutive unexplained miscarriages before the 10th week of gestation, 2. One or more unexplained deaths of a morphologically normal fetus at 10 weeks' gestation or older, 3. One or more premature births of a morphologically normal fetus at 34 weeks' gestation or younger associated with severe pre-eclampsia or placental insufficiency (113). Fifteen percent of women with recurrent miscarriage have obstetric APS (114,115). In a prospective study, with recruitment prior to pregnancy, the miscarriage rate was shown to be 90% with no pharmacological treatment (114).

The mechanism of pregnancy losses has been demonstrated to be the harmful effect of aPLs on implantation by affecting the function of both the uterine decidua and the trophoblasts (116,117). Additionally, the role of complement activation with focal necrosis, apoptosis, and neutrophil infiltration in aPL-related pregnancy loss has been shown (118). Annexin V is a protein, which normally serves as an anticoagulant on trophoblast membranes in placenta by blocking FXa and prothrombin. Thrombosis during the development of the normal materno-placental circulation may arise via interference with this function. Moreover, women with APS have disrupted and reduced annexin V binding to the surface of trophoblastic cells (119).

Preterm delivery is common in women with obstetric APS. The premature delivery rate (before 37 gestational weeks) was 32%, predisposing offspring to the consequences of premature delivery such as low birth weight and behavioral abnormalities (120,121). Almost 30% of these offspring have been shown to passively acquire aPL, predisposing them to the risk of hemorrhagic and thrombotic complications (122).

Because of the assumption that aPL induces thrombosis causing pregnancy loss, it has been assumed that any prothrombotic state may also increase the change of pregnancy

loss by causing placental insufficiency due to placental vascular thrombosis (123). It is, however, controversial whether hereditary thrombophilia is associated with obstetric complications (124,125). Evidence from a meta-analysis suggests that hereditary thrombophilia might be a contributory factor rather than a primary cause of pregnancy complications (124). According to a systematic review, FV Leiden mutation and Prothrombin gene mutation had a weak association with late pregnancy complications (second trimester pregnancy loss, pre-eclampsia, intrauterine fetal growth restriction (FGR), and placental abruption) (88). However, a meta-analysis of prospective cohort studies concluded that those with FV Leiden mutation have a small absolute increased risk of late pregnancy loss (OR 1.52, 95% CI 1.06-2.19). Women with FV Leiden mutation or Prothrombin mutation appeared not to be at increased risk of pre-eclampsia or FGR (126). In a population-based study in Finland, Factor V Leiden mutation was not observed to be a significant risk factor for pre-eclampsia (127); it was, however, associated with stillbirth (128).

Other risk factors for pregnancy-associated VTE

Clinical risk factors for VTE are presented in table 3. Obesity is common in non-pregnant as well as pregnant populations in Western countries. Body mass index (BMI) is over 30 kg/m² (obesity) in 27% of women and between 25 and 30 kg/m² (overweight) in 31%. The proportion of morbidly obese (BMI over 40 kg/m²) women is 4% (129). Obesity is reported to be associated with a higher risk of PE (OR 14.9, 95% CI: 3.0-74.8) than of DVT (OR 4.4, 95% CI: 1.6-11.9) as compared with normal-weight controls (130). The risk of VTE increased with age from 1.64/1000 deliveries in those < 35 years to 2.27/1000 deliveries in those > 35 years (46).

Maternal smoking during pregnancy has been shown to be a risk factor for VTE during pregnancy, but no clear difference in the risk of DVT versus the risk of PE in relation to smoking was found (130).

Cesarean section increases the risk of postpartum VTE 4-fold compared with vaginal delivery (131). In a recent meta-analysis with over 50 reports, the pooled OR was 3.7 (95% CI 3.0-4.6) compared with vaginal deliveries. The incidence of VTE was 2.6/1000 CS. The

higher risk of VTE was associated with an emergency CS compared with an elective CS, with incidences of 1.6/1000 pregnancies (95% CI 1.2-2.2) and 2.4/1000 pregnancies (95% CI 0.8- 4.5), respectively (131).

Table 3: Clinical risk factors for VTE determined from case-control or cross-sectional studies

Risk factor	Adjusted OR/HR (95% CI)
Antepartum risk	
Immobility (strict bedrest for ≥ 1 week with BMI ≥ 25 kg/m ²)	62.3 (11.5-337.0)
Immobility (strict bedrest for ≥ 1 week with BMI < 25 kg/m ²)	7.7 (3.2-19.0)
Assisted reproductive techniques - first trimester only	4.6 (2.9-7.2)
Spontaneous twins	2.6 (1.1-6.2)
Antepartum hemorrhage	2.3 (1.8-2.8)
Smoking (10-30 cigarettes/day prior to or during pregnancy)	2.1 (1.3-3.4)
Pre-pregnancy BMI ≥ 25 kg/m ² - no immobilization	1.8 (1.3-2.4)
Weight gain < 7 kg	1.7 (1.1-2.6)
Postpartum	
Immobility (strict bedrest antepartally for ≥ 1 week with BMI ≥ 25 kg/m ²)	40.1 (8.0-201.5)
Immobility (strict bedrest antepartally for ≥ 1 week with BMI < 25 kg/m ²)	10.8 (4.0-28.8)
Postpartum infection following vaginal delivery	20.2 (6.4-63.5)
Postpartum infection following cesarean section	6.2 (2.4-16.2)
Postpartum hemorrhage ≥ 1 litre with surgery	12.0 (3.9-36.9)
Postpartum hemorrhage ≥ 1 litre with no surgery	4.1 (2.3-7.3)
Pre-eclampsia with fetal growth restriction	5.8 (2.1-16.0)
Fetal growth restriction (birth weight < 2.5 percentile)	3.8 (1.4-10.2)
Smoking (10-30 cigarettes/day prior to or during pregnancy)	3.4 (2.0-4.4)
Smoking (5-9 cigarettes/day prior to or during pregnancy)	2.0 (1.1-3.7)
Pre-eclampsia	3.1 (1.8-5.3)
Hyperemesis	2.5 (2.0-3.2)
Pre-pregnancy BMI < 25 kg/m ² - no immobilization	2.4 (1.7-3.3)
Pre-pregnancy BMI ≥ 25 kg/m ² - no immobilization	1.8 (1.3-2.4)
Risk period not specified	
Systemic lupus erythematosus	8.7 (5.8-13.0)
Blood transfusion	7.6 (6.2-9.4)
Heart disease	7.1 (6.2-8.3)
Sickle cell disease	6.7 (4.4-10.1)
Multiple pregnancy	4.2 (1.8-9.7)
BMI ≥ 30 kg/m ²	5.3 (2.1-13.5)
Assisted reproductive techniques	1.8 (1.4-2.3)
Anemia	2.6 (2.2-2.9)
Diabetes	2.0 (1.4-2.7)
Hypertension	1.8 (1.4-2.3)
Weight gain > 21 kg (vs. 7-21 kg)	1.6 (1.1-2.6)
Parity > 1	1.5 (1.1-1.9)

OR, odds ratio; HR, hazard ratio; BMI, body mass index

Data from Lindqvist et al. 1999, Simpson et al. 2001, James et al. 2006, Jacobsen et al. 2008, Knight et al. 2008, Henriksson 2013, Jacobsen 2008

Immobilization during pregnancy has been shown to be associated with the risk of VTE, especially in conjunction with higher BMI (132). Travel duration in excess of 4 h (not just by air) has also been proved to increase the risk of VTE due to immobility during the travel

(133).

Assisted reproductive techniques increases the risk of VTE, mainly due to OHSS, both antenatally (OR 4.4, 95% CI 2.6-7.5) and postnatally (OR 2.2, 95% CI 1.1-4.3) (132). Surgery during pregnancy is associated with increased risk of VTE (including termination of pregnancy and operations performed due to ectopic pregnancy) because of damage from surgery to vessel walls and platelet aggregation at the site of injury, engaging the clotting cascade and releasing clotting factors into the circulatory system (134). Hyperemesis may increase the risk of VTE due to dehydration (46).

Ethnicity affects the risk of VTE. The risk of VTE is observed to be lower in white women (1.75/1000 deliveries) than in black women (2.64/1000 deliveries). The rate for black women was 64% higher than that for women of other races at all ages. The risk of VTE is lowest in Asian women (1.07/1000 deliveries) (46).

Risk factors for pregnancy-associated VTE are partially different during the antepartum and postpartum periods. Multiple pregnancy (often conceived after ART and related to OHSS) (7,45,135) and gestational diabetes (135) were significant risk factors for antepartum VTE. Strong postpartum risk factors were found to be CS and pre-eclampsia (135). Postpartum hemorrhage (often requires re-operation or transfusion) and infection were also associated with the risk of postpartum VTE (46).

Recommendations for treatment and prophylaxis of pregnancy-associated VTE

Although evidence-based recommendations for the use of anticoagulants during pregnancy exist, they are based mainly on observational studies and data extrapolated from non-pregnant patients. There are a few important questions that should be addressed when planning optimal care: risks of anticoagulants during pregnancy and breastfeeding, best treatment strategy of acute VTE during pregnancy, prevention of pregnancy-associated VTE, and peripartum anticoagulation. The current guidelines are listed in table 4.

Because high-quality data in pregnancy are lacking (randomized prospective studies), the published recommendations differ (79). An example of such a difference would be in preventing VTE in situations with asymptomatic thrombophilias (especially milder); while some guidelines recommend clinical surveillance, others suggest considering antepartum LMWH. There are also differences in preferred LMWH dosages (prophylactic, intermediate, weight-adjusted treatment doses) for preventing recurrent VTE in situations with different thrombophilias.

Table 4. Current guidelines for the treatment and prophylaxis of obstetrics-associated VTE

ACOG	American College of Obstetricians and Gynecologists; www.acog.org
SOGC	Society of Obstetricians and Gynaecologists of Canada; www.sogc.org
RCOG	Royal College of Obstetricians and Gynaecologists; www.rcog.org.uk
ACCP	American College of Chest Physicians; www.chestnet.org
	Clinicians from Australia and New Zealand; www.nationalwomenshealth.adhb.govt.nz

Since the 1980s-1990s, LMWH has been widely used for prophylaxis and treatment of VTE during pregnancy (8,9,136). It replaced UFH after large trials in non-pregnant patients showed that LMWHs are at least as safe and effective as UFH (137,138). Nowadays, all guidelines recommend LMWH over UFH because it is considered safer in terms of the risks of osteoporosis, HIT, allergy, and bleeding complications (19). It is also easier to use thanks to pre-filled syringes, dosing once or twice a day, and little or no need to monitor the effect of anticoagulation (139).

It must be noted, however, that inconsistency exists between authors in their risk thresholds for recommending LMWH prophylaxis, and thus, the prophylaxis thresholds were determined by the majority result of an anonymous vote of the authors. The risk thresholds chosen by authors ranged from 1% to 5% for antepartum prophylaxis and from 1% to 3% for postpartum prophylaxis. The panel ultimately assessed the need for thromboprophylaxis by using a risk threshold $\geq 3\%$ for both antepartum and postpartum periods. This means that for risk factors for which only case-control data are available, a relative risk of at least 30-fold antepartum and 60-fold postpartum are required to reach these thresholds (assuming antepartum and postpartum baseline risks of 0.1% and 0.05% respectively) (140).

Management of acute VTE

Once VTE is confirmed, anticoagulation should be initiated. LMWH is recommended to be used twice daily as weight-adjusted treatment doses calculated by early pregnancy weight. Table 5 shows the recommended doses for common LMWHs for both initial and maintenance therapies (79). In pregnant women receiving a treatment dose of LMWH, anti-Xa monitoring is controversial and not evidence-based (141). Periodic testing of anti-Xa levels may be indicated in women at extremes of weight (<50 kg or >90kg), women who are bleeding or at bleeding risk, women with recurrent VTE despite treatment, and women with renal failure. Guidelines recommend that a treatment dose of LMWH should be continued throughout the pregnancy and for at least 6 weeks in the postpartum period, until at least 3 months has been given in total(10,67). After 3 months' initial treatment, dosing intensity could be decreased to an intermediate (75% of full-treatment dose) or prophylactic dose for the remainder of the pregnancy for at least 6 weeks' postpartum (79). Women with acute VTE should be hospitalized (especially in cases with PE or of hemodynamic instability) or followed closely as outpatients for at least the first 2 weeks (9).

Table 5: Treatment-doses of acute VTE

Initial dose	Early pregnancy weight (kg)			
	<50	50-69	70-89	90-109
Enoxaparin (mg bd)	40	60	80	100
Dalteparin (iu bd)	5000	6000	8000	10000
Tinzaparin	175 units/kg once daily (all weights)			

>110 kg: haematologist should advise on LMWH dose
bd, twice daily

Table extracted from Royal College of Obstetricians and Gynaecologists
Green Top guideline 2015b

A recent placebo-controlled multicenter randomized trial of active versus placebo elastic compression stockings used for 2 years to prevent post-thrombotic syndrome or leg pain found no benefit of elastic compression stockings (142). Royal College of Obstetricians and Gynaecologists guidelines still (67), nevertheless, advise wearing elastic compression stockings on the affected leg to reduce pain and swelling, but clinicians should be aware

that the benefit in the prevention of PTS is unclear. Early mobilization has not been demonstrated to increase the risk of PE once the patient is stable and anticoagulated in non-pregnant patients. The same management seems to apply in pregnancy (143), page 92.

Prevention of recurrent VTE in women with prior VTE(s)

The management of pregnant women starts with accurate assessment of their risks of VTE. Accurate and sensitive counseling of the risks of VTE, signs and symptoms of VTE, and benefit of thromboprophylaxis of women is required.

Women with previous VTE should be stratified into intermediate-risk, high-risk, and very high-risk groups by taking into account the circumstances of previous VTE and possible thrombophilia status (10). Table 6 lists the VTE risk by type of thrombophilia and circumstances of possible prior VTE.

Table 6. Stratification and management of VTE risk by type of thrombophilia and circumstances of possible prior VTE

Very high risk	Treatment recommendation
Prior VTE on long-term warfarin	Antenatal weight-adjusted treatment/
Antithrombin deficiency	intermediate LMWH dose and
APS+previous VTE	6 weeks' postnatal LMWH or VKA
High risk	Treatment recommendation
Prior recurrent or unprovoked VTE	Antenatal and 6 weeks' postnatal
Prior estrogen-related VTE	LMWH with prophylactic doses
Prior VTE + thrombophilia	
Prior VTE+ family history of VTE	
Thrombophilia(combined defects	
or homozygous FVL or prothrom-	
bin gene mutation) without prior VTE	
Intermediate risk	Treatment recommendation
Single previous VTE associated	7 days postnatal prophylactic LMWH
with transient risk factor,	6 weeks if other risk factors present
no family history or thrombophilia	
Thrombophilia without prior VTE	
(other than above mentioned)	

VTE, venous thromboembolic event; LMWH, low-molecular weight heparin ;VKA, vitamin K antagonist

APS, antiphospholipid syndrome, Low-dose aspirin is recommended for all women with APS

Extracted from Book: Cohen H, O'Brien P. Disorders of Thrombosis and Hemostasis in Pregnancy A Guide to Management. 2nd.ed. Springer-Verlag London 2015; p. 85.

Table 7 presents the suggested LMWH dosing regimens for prophylaxis against pregnancy-related VTE. The risk factors for bleeding and contraindications for LMWH should also be assessed (79). Table 8 shows the risk factors for bleeding.

Table 7: Suggested LMWH-doses for prophylaxis against pregnancy-related VTE

Prophylactic LMWH^a
Dalteparin 5000 units once daily
Tinzaparin 4500 units or 75 units/kg once daily
Enoxaparin 40 mg once daily
Nadroparin 2850 units once daily
Intermediate-dose LMWH^a
Dalteparin 5000 units twice daily or 10.000 units once daily
Tinzaparin 10.000 units once daily
Enoxaparin 40 mg twice daily or 80 mg twice daily
LMWH adjusted to a peak anti-Xa level 0,2-0,6 units/ml

^aHigher doses might be used in with increased maternal weight
LMWH, low-molecular-weight heparin

Extracted from the Guidance for the treatment and prevention of obstetric-associated venous thromboembolism (Bates et al. 2016)

Table 8: Risk factors for bleeding

Active antenatal or postpartum bleeding
Increased risk of major hemorrhage (e.g. placenta previa)
Bleeding diathesis (von Willebrand's disease, hemophilia, coagulopathy)
Thrombocytopenia (platelet count $<75 \cdot 10^9/l$)
Acute stroke in the last 4 weeks (ischemic or hemorrhagic)
Uncontrolled hypertension (systolic BP >200 mmHg mmHg or diastolic BP >120 mmHg)
Severe liver or renal disease

BP, blood pressure

Extracted from Book: Cohen H, O'Brien P. Disorders of
Thrombosis and Hemostasis in Pregnancy

A Guide to Management. 2nd.ed. Springer-Verlag London 2015; p.
85.

Patients with AT deficiency and a history of VTE, particularly type I, have a very high risk of recurrent thrombosis. Before pregnancy, these patients are likely to be on long-term warfarin, and therefore, weight-adjusted treatment dose or intermediate dose of LMWH may be indicated throughout pregnancy until 6 weeks' postpartum, when long-term warfarin is again initiated. The efficacy of heparins in AT deficiency is unclear (mode of action is antithrombin-dependent), thus monitoring of anti-Xa levels would be reasonable and antithrombin substitution during delivery is often needed (144).

Prevention of VTE in women with asymptomatic thrombophilia

There are limited data on benefits of antenatal LMWH prophylaxis of asymptomatic women with a known heritable thrombophilia, other than those with antithrombin deficiency or combined/homozygous defects, for whom antenatal LMWH prophylaxis is usually necessary (79,144,145). VTE risk should be assessed individually and considered along with possible family history and other risk factors before reaching a conclusion. When other risk factors, such as age >35 years, obesity, or immobility, are present, the use of antenatal LMWH prophylaxis could be beneficial (79). Postpartum LMWH prophylaxis (10 days to six weeks) in these situations is, however, recommended by the majority of guidelines, especially if other risk factors exist (79).

Prevention of VTE in women with another clinical risk factors

Recent publications have used large databases to provide population-level absolute and relative risks for VTE (146,147). Most established risk factors have only a modest effect on VTE risk, with a few increasing the absolute risk by about 1%. It is unclear how combinations of independent risk factors affect the overall VTE risk and whether they result in additive or multiplicative risks. Further research in this field is required (79). The clinical risk factors and the estimated risk shown as adjusted OR/HR were earlier listed in Table 3. Clinical risk factors for VTE antepartum and postpartum and an example of a risk assessment tool combining all of the clinical risk factors demonstrated to increase VTE risk are shown in Table 9.

Table 9. Ante-and postpartum risk assessment and management

Antepartum LMWH-prophylaxis	Postpartum LMWH-prophylaxis
Initiate from beginning of the pregnancy if following:	At least 6 week
Prior VTE (not related to major surgery)	Any previous VTE Anyone requiring antenatal LMWH High-risk thrombophilia
Consider if following:	Low-risk thrombophilia + family history of VTE
Prior VTE related to major surgery	
High risk thrombophilia (no prior VTE)	At least 10 days
Hospital admission	If persisting or >3 risk factors consider extending
Medical comorbidities (SLE, IBD, IV drug user) ^a	
Surgery during pregnancy	
OHSS ^b (first trimester only)	Cesarean section in labour
≥4 risk factors: from the first trimester	BMI ≥ 40 kg/m ²
3 risk factors: from 28th gestational week	Hospitalization ≥ 3 days
<3 risk factors : mobilization + avoidance of dehydration if following:	Surgery (except repair of the perineum)
	Medical comorbidities (SLE, IBD, IVDU) ^a
Low-risk thrombophilia	
BMI > 30 kg/m ²	At least 10 days
Parity ≥ 3	≥ 2 risk factors
Age > 35 years	< 2 risk factors: mobilization + avoidance of dehydration if following:
Current smoking	
Gross varicose veins	
Current pre-eclampsia	Age > 35 years
Immobility (paraplegia)	BMI ≥ 30 kg/m ²
Family history of VTE	Parity ≥ 3
Multiple pregnancy	Current smoking
IVF/ART ^c	Elective Cesarean section
	Family-history of VTE
	Low-risk thrombophilia
	Gross varicose veins
	Prolonged delivery (>24 hours)
	Postpartum hemorrhage (>1 l)
	Preterm delivery (<37 gestational weeks)
	Stillbirth in this pregnancy
	Multiple pregnancy
LMWH, low-molecular weight heparin; VTE, venous thromboembolic event	
^a SLE, systemic lupus erythematosus, IBD, inflammatory bowel disease, IV, intravenous	
^b ovarian hyperstimulation syndrome	
^c in vitro fertilization/assisted reproductive technology	

Modified table extracted from two different tables
Royal College of Obstetricians and Gynaecologists Green-top Guideline No. 37b

The use of elastic compression stockings is recommended in pregnancy and in puerperium for women who are hospitalized and have a contraindication to LMWH. Elastic compression stockings are also recommended to reduce venous stasis for women who are hospitalized after Cesarean section (possible combined with LMWH) and for those considered to be at particularly high risk of VTE (e.g. previous VTE, more than four risk factors antenatally, or more than two risk factors postnatally) (133). However, there are no trials to support the use of elastic compression stockings in pregnancy and in puerperium, with existing recommendations largely derived from extrapolation of results for the hospitalized non-pregnant population (148)

Management before delivery

Timing of delivery could be considered in women who receive a weight-adjusted treatment dose of LMWH. Guidelines recommend discontinuation of LMWH at least 24 hours before the expected time of neuroaxial anesthesia. If spontaneous labor occurs, neuroaxial anesthesia should not be used due to a risk of paraparesis caused by a dorsolumbar epidural hematoma (149). Women with DVT within 2-4 weeks before delivery may be candidates for placement of a retrievable inferior vena cava filter. Guidelines recommend discontinuing prophylactic or intermediate-dose LMWH upon onset of spontaneous labor or a planned induction of labor or Cesarean section. Neuroaxial anesthesia can be administered 10-12 hours after the last dose of LMWH. LMWH may be started 4-6 hours after neuroaxial catheter removal upon occurrence of full neurologic recovery and no evidence of bleeding (79,149).

Prevention of adverse obstetric outcomes in women with thrombophilia

Pregnancy outcome could be improved significantly in women with obstetric APS. Today, low-dose aspirin combined with LMWH is recommended for women with obstetric APS (150). This treatment combination has been shown to lead to a live birth rate of over 70% (151-153). The live birth rate with low-dose aspirin alone was 42% compared with 71% for

the combination of low-dose aspirin and heparin (151). Low-dose aspirin predominantly inhibits cyclo-oxygenase-1, which leads to a reduction in the synthesis of thromboxane A₂ without affecting the synthesis of prostacyclin, restoring the ratio of the two substances to a more normal value in women with a history of obstetric complications (154). Low-dose aspirin appears to promote successful embryonic implantation in the early stages of pregnancy by protecting the trophoblast from attack by aPL. Later in pregnancy, the combination treatment helps to protect against subsequent thrombosis of the uteroplacental vasculature (155,156). The American College of Chest Physicians guideline recommends prophylactic dose LMWH in combination of low-dose aspirin to prevent pregnancy loss (10), suggesting that prophylaxis be initiated as soon as pregnancy is confirmed and continuing until 6 weeks' postpartum (157).

The thromboprophylactic therapy to prevent obstetric adverse outcomes in hereditary thrombophilias has been investigated, but no benefit has been found. In a recent meta-analysis with eight trials, LMWH in women with hereditary thrombophilia and prior late (≥ 10 gestational weeks) or recurrent early (< 10 gestational weeks) pregnancy loss did not improve live birth rates relative to those without LMWH (RR 0.81, 95% CI 0.55-1.19, $p=0.28$) (158).

Another meta-analysis with eight trials showed that LMWH did not significantly reduce the risk of recurrent placenta-mediated pregnancy complications (early or severe pre-eclampsia, FGR, placental abruption, pregnancy loss ≥ 20 gestational weeks) in women with hereditary thrombophilia (absolute difference -2.3% (95% CI -17.6-13.0, $p=0.77$) for those with heterozygous FV Leiden or prothrombin gene mutation and -2.0% (95% CI -13.8-9.9, $p=0.74$) for those with stronger thrombophilias) (159). The EAGeR (multicenter randomized trial), in which one group received preconceptionally initiated low-dose aspirin plus folic acid and the second group received placebo plus folic acid, revealed no difference in live birth rates and pregnancy losses between the groups (160).

Anticoagulants in pregnancy

Unfractionated heparin (UFH)

Pharmacokinetics of UFH

Heparin is a sulphated glycosaminoglycan that activates antithrombin, leading to rapid inhibition of the procoagulant activity of thrombin and factors IXa, Xa, XIa, and XIIa (161). Commercial preparations of heparin are heterogeneous. They consist of saccharide units with molecular weights ranging from 3000 to 30000 daltons (mean, 15000). The interaction with antithrombin is mediated by a unique pentasaccharide sequence. Any pentasaccharide-containing heparin chain can inhibit the action of factor Xa, but to inactivate thrombin, heparin must bind to both antithrombin and thrombin by forming a complex, which can be formed only by pentasaccharide-containing heparin chains composed of at least 18 saccharide units (162). These longer pentasaccharide sequences are distributed randomly to only one-third of the chains of UFH, which is why only about one-third of the heparin binds to antithrombin (163). UFH affects hemostasis also in many other ways, e.g. by inhibiting platelet function (164) and increasing the permeability of vessel walls (165).

UFH in clinical use

UFH is poorly absorbed from the gastrointestinal tract so it must be administered parenterally or subcutaneously (166). It has complex pharmacokinetics with nonlinear anticoagulant response and wide inter-patient variation (167). UFH does not cause fetal bleeding or teratogenicity because it does not cross the placenta(168). Anticoagulation with UFH as a continuous intravenous infusion requires regular activated thromboplastin time monitoring (APTT) and dose changes according to results. Twice daily injections are required if administered subcutaneously due to the short half-life (169). The intensity and duration of the anticoagulant effect rise disproportionately with increasing doses, predisposing women to bleeding complications (170,171).

The most important complication of long-term use of UFH is osteoporotic fractures (172). The decrease in BMD is due to decreased bone formation and increased resorption since UFH reduces the number and activity of osteoblasts and increases the number and activity of osteoclasts (173,174). The incidence of symptomatic bone fractures after long-term UFH use during pregnancy is reported to be 2-9% (20,21).

Another disadvantage of UFH is the risk of heparin-induced thrombocytopenia (HIT), which is a life-threatening antibody-mediated effect of heparin that leads to arterial and venous thrombosis, typically occurring 5-10 days after the first UFH dose. The pathogenic IgG antibodies recognize complexes of platelet factor 4 and heparin, leading to intravascular platelet activation. Clinical features of HIT are thrombocytopenia (more than 50% platelet count fall rapidly but not lower than 20×10^9 /litre) and venous and arterial thrombotic events (175). The risk of HIT for non-pregnant patients receiving therapeutic doses of UFH is estimated to be 3% (176). For pregnant women on a prophylactic dose of UFH, the incidence of HIT is variable: between 0.1% and 1% (177).

Low-molecular-weight heparin (LMWH)

Pharmacokinetics of LMWH

LMWHs are fragments of UFH produced by controlled enzymatic or chemical depolymerization processes, the end-products of which are chains with a mean molecular weight of about 5000 Da. Like UFH, LMWH acts by activating antithrombin. However, while almost one-third of UFH chains contain the pentasaccharide sequence of at least 18 saccharide units, only 15-25% of LMWH chains contain this sequence (178). Relative to UFH, which has activity against factor Xa and thrombin, fewer LMWHs are of sufficient length to bind to both antithrombin and thrombin, so LMWHs have a greater activity against factor Xa (179). The inhibitory activity of LMWHs against factor Xa persists longer than their inhibitory activity against thrombin, reflecting the more rapid clearance of longer heparin chains (169).

Coagulation mechanisms and targets of action of UFH and LMWH are shown in Figure 2. Antithrombin-mediated inactivation of thrombin and/or factor Xa by UFH and LMWH is presented in Figure 3.

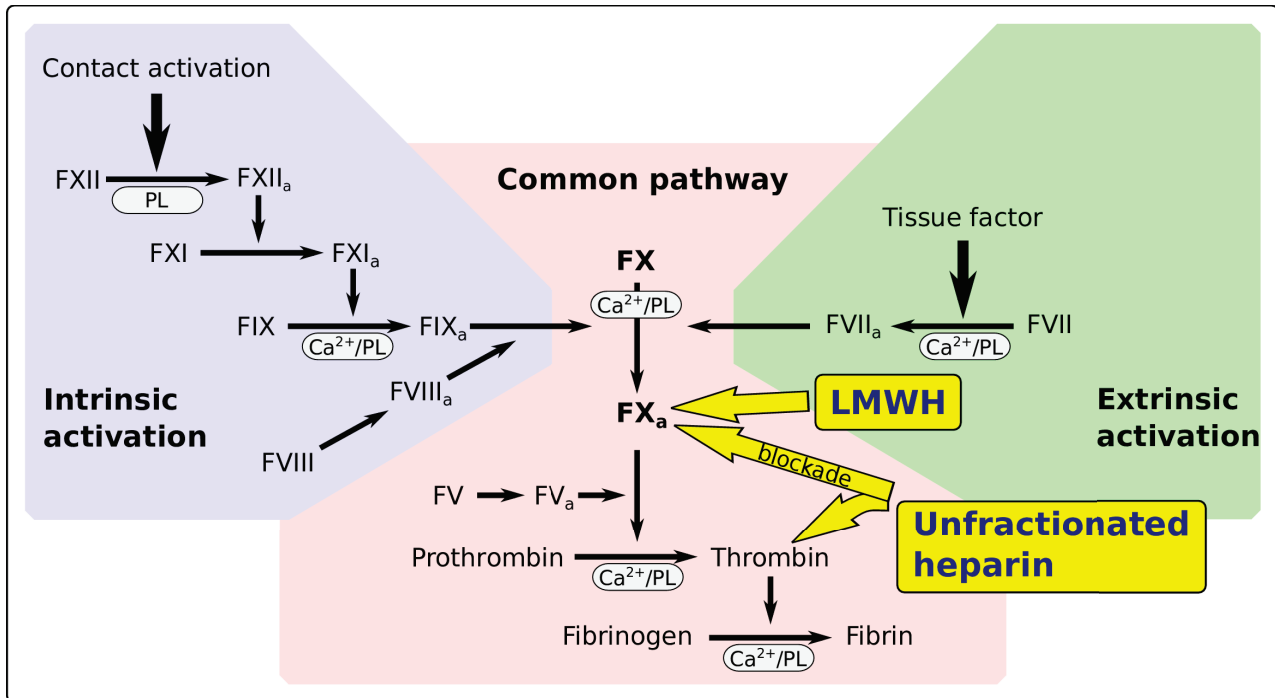
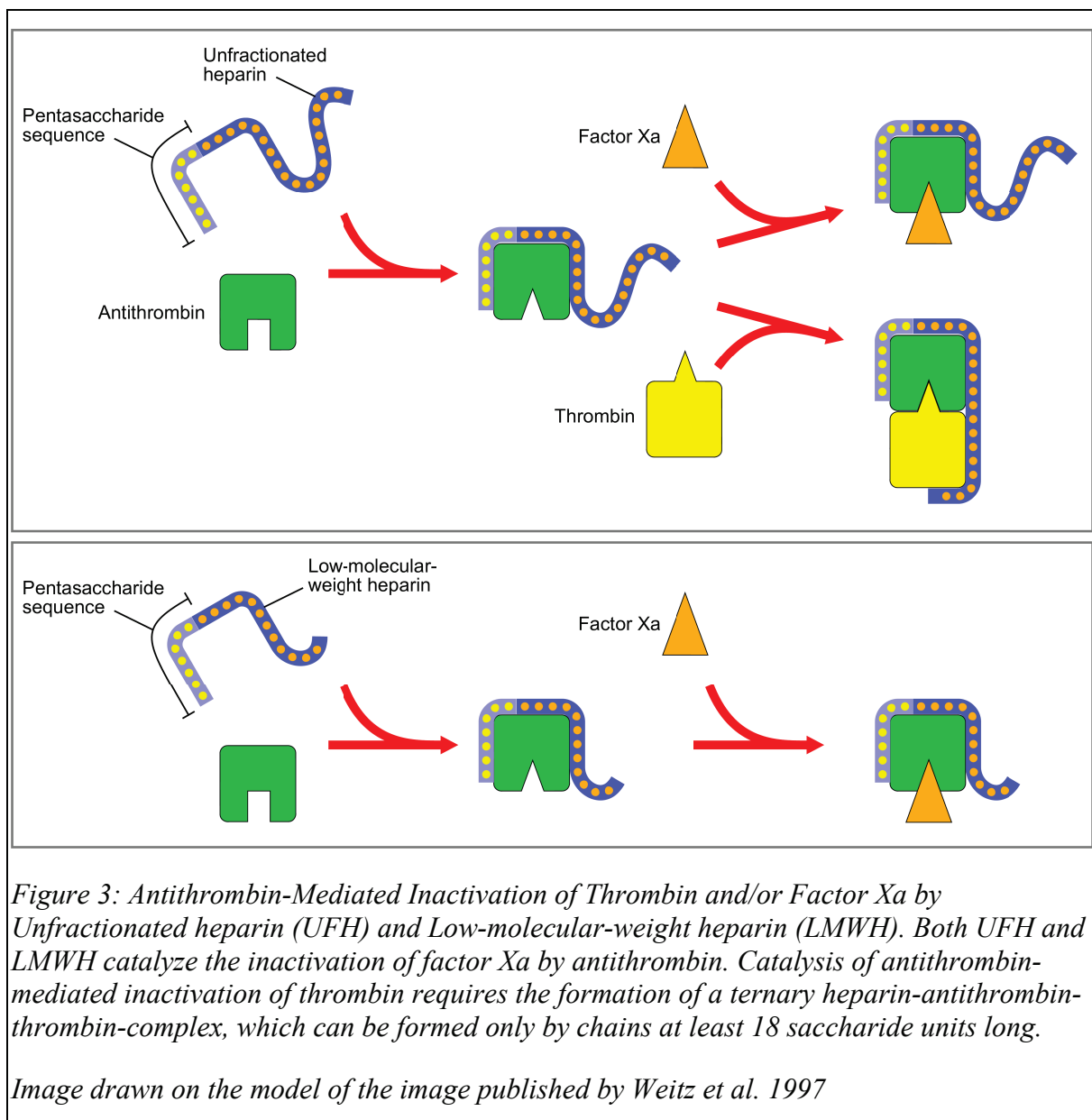


Figure 2: Coagulation mechanism and targets for action of UFH and LMWH. F, factor; PL, phospholipid surface; Ca²⁺, calcium-ion

LMWH in clinical use

LMWHs have many advantages relative to UFH. They have lower affinity to plasma proteins and cells, leading to a more predictable anticoagulant response, and thus, better bioavailability, 2-4-times longer half-life, and dose-independent clearance (180). After subcutaneous injection, the bioavailability is approximately 90% compared with 20-30% for UFH. This is why subcutaneous dosing once or twice daily is sufficient to achieve therapeutic levels and no continuous intravenous infusion is required, unlike with UFH (181). Peak anti-Xa levels are achieved 3-5 h after subcutaneous administration and



elimination half-life is 3-6 h after the injection independent of dose (181). LMWHs can

however accumulate in patients with renal failure (creatinine clearance of less than 30 ml/min) leading to increased bleeding risk especially when therapeutic doses are administered (171). The most commonly used LMWHs are dalteparin, enoxaparin and tinzaparin.

Vitamin K antagonists

Vitamin K antagonist inhibits vitamin K oxide reductase, which is the enzyme responsible for the cyclical conversion of oxidized vitamin K to its reduced form, and then participates in the carboxylation of the coagulation factor precursors. This, in turn, inhibits the K-vitamin-dependent process to change the coagulation factors II, VII, IX, and X to procoagulants (182). Warfarin sodium is the most used VKA in clinical use outside pregnancy. It has many disadvantages, including slow onset of action, narrow therapeutic window, and necessitation of frequent monitoring (183). It crosses the placenta and can lead to miscarriage, stillbirth, preterm birth, embryopathy, and hemorrhagic complications to the fetus. Manifestations of the warfarin embryopathy include nasal hypoplasia, epiphyseal stippling, ocular abnormalities, and hypoplasia of the extremities. The critical period of exposure for embryopathy appears between gestational weeks 6 and 12 (183). During pregnancy its use is limited to rare occasions in women with mechanical heart valves. It is then, however, recommended to substitute with heparin between 6 and 12 weeks to reduce the risk of harmful effects to the fetus. However, this increases the risk of thromboembolic complications in those with mechanical heart valves (184).

Direct oral anticoagulants (DOACs)

Direct oral anticoagulants (DOACs) are increasingly used also in fertile-aged women for prevention and treatment of arterial and venous thrombotic diseases. Dabigatran is a direct thrombin inhibitor. Rivaroxaban, apixaban, and edoxaban are direct factor Xa inhibitors (185). These preparations are contraindicated during pregnancy since published experience with them is scarce (186). Animal studies of dabigatran and rivaroxaban have demonstrated fetal loss and harm (186). Dabigatran crosses the human placenta (187).

Case series of 37 women using rivaroxaban at the beginning of pregnancy exist. All of these individuals discontinued rivaroxaban upon discovery of a positive pregnancy test (188). One major malformation (conotruncal cardiac defect) occurred in a woman with a previous fetus with cardiac malformation (without exposure of rivaroxaban in this pregnancy). Although results of the case series might be reassuring, the limited cohort size does not rule out increased malformation risk and does not support the use of rivaroxaban during pregnancy (188). It is also unknown whether DOACs are excreted in breast milk (186).

Safety and efficacy of LMWH during pregnancy

Previous studies focusing on safety and efficacy of LMWH

Because of disadvantages of UFH, such as osteoporotic fractures, HIT, bleeding, and impracticality, LMWH has replaced UFH since the 1980s (19). Prospective trials regarding the safety and efficacy of LMWH are scarce mainly due to ethical reasons. Earlier data and recommendations regarding these issues in pregnancy were based on extrapolation of data from non-pregnant patients (137,189). Currently, several retrospective case series and one large prospective study also exist with pregnant patients (11-14,18). Table 10 summarizes the results of the largest studies with 100+ patients who received prolonged LMWH for various indications during pregnancy. Knowledge about the safety and efficacy of LMWH has increased with the wide use of this medication in pregnant women. A systematic review (19) of 64 different studies reporting the safety and efficacy of LMWH in 2777 patients demonstrated LMWH to be safe and effective. VTE was reported in 0.86% and arterial thrombosis in 0.50% of pregnancies. Significant bleeding (>500 ml), mainly associated with obstetric causes, occurred in 1.98%, allergic reactions in 1.80%, thrombocytopenia in 0.11%, and osteoporotic fractures in 0.04% of patients. No HIT or maternal deaths occurred. The total live birth rate was 94.7%. LMWH was found to be effective also for those patients with recurrent pregnancy loss; 85.4% of these pregnancies resulted in live birth.

Table 10: Summary of studies investigating the maternal safety and efficacy of LMWH during pregnancy

Study	Pregnancies Indication (T/P)		LMWH-type used	Major bleeding (≥1 litre) n(%)	VTE n(%)	Thrombocytopenia n (%)	Allergic reactions
	n	n/n					
Lepercq et. al. 2001	624	49/574	Enoxaparin	20 (3.2)	8 (1.3)	10 (1.6)	0
Deruelle et al. 2005	111	0/111	4 types of LMWH ^a	3 (2.7)	6 (5.4)	5 (4.5)	?
Bauersachs et al. 2007	810	0/810	Dalteparin	24 (3.0)	5 (0.6)	18 (2.2)	?
Nelson-Piercy et al. 2011	1267	254/1013	Tinzaparin	10 (0.9)	15 (1.2)	35 (2.8)	6 (0.5)
Andersen et al. 2010	166	13/153	Tinzaparin/Dalteparin	11(7.2)	0	0	2 (1.2)
Lindqvist et al. 2011	326	0/326	Dalteparin	20 (6.2)	4 (1.2)	0	?
Santoro et al. 2009	130	2/128	3 types of LMWH ^b	2 (1.5)	1 (0.8) ^d	0	1 (0.8)
Khalifeh et al. 2014	149	21/128	Tinzaparin	27 (18%) ^c	3 (3.6)	2 (1.3)	0

T, treatment; P, prophylaxis

^a Dalteparin, Tinzaparin, Nadroparin and Enoxaparin

^b Nadroparin, Enoxaparin and Dalteparin

^c >500 ml ml

^d superficial VTE

(Khalifeh et al. 2014, Lepercq et al. 2001, Deruelle et al. 2007, Bauersachs et al. 2007, Andersen et al. 2010, Lindqvist et al. 2011, Nelson-Piercy et al. 2011, Santoro et al. 2009)

Decrease in bone mineral density

Osteoporosis is a systemic skeletal disease characterized by low bone mass, increased bone fragility, and risk of fractures (190). Bone mineral density is expressed either as an absolute value (g/cm^2) or as a T- or Z-score. T-score measures the difference by number of standard deviations of an individual BMD from the mean of a young female reference population. Z-score is the number of standard deviations by which a patient's BMD differs from the average BMD of a population of the same age, sex, and ethnicity (190). In osteoporosis, the T-score is -2.5 or less and in osteopenia between -2.5 and -1 (190). During normal pregnancy maternal bone loss of 1-4% may occur in the last months of pregnancy, when the fetal skeleton is rapidly mineralizing (26). During lactation there is an additional mobilization of calcium from maternal bone, which can lead to a transient loss of 5-10% in BMD, but this is restored 6-12 months after cessation of breastfeeding (47).

UFH decreases bone formation and increases bone resorption by reducing the number and activity of osteoblasts and increasing the number and activity of osteoclasts (173). LMWH might have a lesser effect on BMD by acting only on bone formation, and it has been found to cause 6- to 8-fold less inhibitory effect on bone nodule formation than UFH (191,192). Most studies (although with small sample sizes) investigating the association of long-term LMWH use and decrease in BMD have found no or only a minor effect on BMD (21,193-195). Prolonged UFH use during pregnancy has been reported to be associated with 2-9% of symptomatic osteoporotic vertebral fractures (20,21). However, some case reports of LMWH-associated osteoporotic fractures exist (196). In a review of 11 cases of LMWH-induced osteoporotic fractures, eight occurred during pregnancy (197).

The combined data of these studies suggest that the risk for developing an osteoporotic fracture after prolonged use of a prophylactic dose of LMWH during pregnancy is low, although data on therapeutic doses and long-term BMD are limited. Previous literature suggest, however, that some negative effect on BMD after long-term prophylactic LMWH exposure during pregnancy may exist, but because of the small sample sizes in the studies this is only speculative. The effects of LMWH on BMD require further investigation.

Recurrent VTE during pregnancy despite LMWH-prophylaxis

As earlier mentioned, the most important risk factor for VTE in pregnancy is a personal history of VTE, with the increased recurrence risk being 3- to 4-fold (80). However, only a few studies limited to women with a history of VTE concerning recurrent VTE during subsequent pregnancy exist. Moreover, studies evaluating recurrent VTEs despite LMWH prophylaxis are very scarce.

The risk of recurrent VTE antepartum if LMWH prophylaxis is withheld is 2.4-7.4% according to four previous studies (82-84,198). The subjects participating in these studies are heterogeneous in terms of circumstances of previous VTE. The study with the lowest incidence, 2.4% (82), was limited to low-risk women without thrombophilia enrolled in mid-pregnancy (early VTEs could have been missed). The risk of recurrent VTE during the postpartum period without thromboprophylaxis was 6.7-8.3% according to three previous studies (83,84,198).

In a prospective trial (12) with 810 pregnant women, 5 recurrent VTE events (0.6%) occurred in the high-risk and very high-risk groups that received weight-adjusted LMWH (50–100 IU and 100–150 IU/kg/day, respectively) initiated in early pregnancy until 6 weeks' postpartum. The authors concluded that risk-stratified LMWH prophylaxis was associated with a low incidence of symptomatic VTE.

A large prospective follow-up study with 326 pregnant women on prophylactic LMWH initiated in early pregnancy found that the incidence of recurrent VTE was 0.6% antepartum and 0.6% postpartum (0-42 days). All women had a history of one prior VTE (antithrombin deficiency and APS excluded). The authors concluded that LMWH was effective (relative risk reduction 88%). The postpartum VTE risk when LMWH was stopped (43-100 days) was 0.9%, and it was 28-fold relative to the control group of delivered women without prior VTE (18).

A retrospective cohort (17) with 44 pregnancies of intermediate risk (LMWH for 6 weeks' postpartum) and 82 pregnancies of high risk (LMWH antepartum and for 6 weeks'

postpartum) found that the incidence of pregnancy-related VTE was 5.5% (n=7) . All VTE episodes occurred in women at high risk (5 events postpartum, 2 events antepartum). All episodes occurred in women with a previous VTE: five were hormone-related, four had underlying thrombophilia. Of the postpartum events, 40% occurred after 6 weeks' LMWH prophylaxis, suggesting that in the high-risk group postpartum prophylaxis for 6 weeks might be too short.

Allergic skin reactions

Cutaneous allergic reactions have been reported to be associated with the use of LMWH, UFH, and warfarin. These skin reactions vary from local allergic manifestations to skin necrosis and can be confirmed by intracutaneous testing (199). Generally, they occur 3-10 days after the initiation of treatment, but may also occur later. The incidence of LMWH-associated allergic skin reactions has been found to be 0.5-1.2% (13,14,200).

In patients with heparin-induced skin reactions, danaparoid, a "low-molecular-weight heparinoid" that differs in chemical structure and is free of heparin fragments, may be used after negative intracutaneous testing in some patients (201). Because danaparoid is not always available, fondaparinux (a synthetic analog of the heparin pentasaccharide sequence) is commonly used as alternative in patients with allergic skin reactions or HIT associated with LMWH. Although transplacental passage of fondaparinux can occur, no complications in the mother and child have been shown in case reports (202).

Aims of the study

The aims of the study were to investigate

- I. The maternal and fetal safety of long-term LMWH exposure during pregnancy and its efficacy in preventing VTE during pregnancy

- II. The incidence and risk factors of recurrent antepartum VTE in women with a history of one or more prior VTEs

- III. The possible subsequent decrease of BMD in lumbar spine and/or femoral neck after long-term LMWH exposure during pregnancy and/or during the postpartum period

- IV. To calculate the cumulative and weekly incidence of VTE and mortality through 180 postpartum days, to identify the associated risk factors during three different postpartum periods, and to compare the incidence of postpartum VTE with that of the non-pregnant fertile-aged female population to determine when the risk of VTE decreases to baseline levels.

Materials and Methods

Study design

A description of the studies is presented in Table 11. The study design is a retrospective observational cohort (Studies I-III) undertaken at the Department of Obstetrics and Gynecology, Helsinki University Hospital, Finland. Selection bias in Studies I-III was avoided by checking the data of all participants from the electronic hospital database.

Table 11: Materials and Methods in studies I-IV

	Study I	Study II	Study III	Study IV
Study group	Women with LMWH-exposure for different indications during pregnancy and untreated controls	LMWH-exposed women with ≥one prior VTE (substudy of study I)	Women with LMWH-exposure for different indications during pregnancy and untreated controls	Women aged 15-49 years with registered delivery
Time frame	February 1994-January 2007	February 1994-January 2007	2008-2012	2001-2011
Design	Retrospective observational cohort	Retrospective observational cohort	Retrospective observational cohort	A population-based controlled cohort
Outcome	Maternal or fetal complications during pregnancy and pregnancy outcome	Recurrent VTE despite LMWH-prophylaxis or before the planned LMWH-initiation	Subsequent decrease of BMD in lumbar spine/femoral neck 4-7 years after the delivery	Incidence, risk and mortality of VTE during six months postpartum-period
Number of subjects (pregnancies)	475 (648)	25 (28)	92 (107)	1169 deliveries
Number of controls (pregnancies)	622 (626)	245 (341)	60	633.123 deliveries

Study IV is a population-based controlled cohort based on the data of five large registers in Finland in 2001-2011 (The Care Register for Health Care (HILMO), The Medical Birth Register, the Cause of Death Register, Population Register Centre and The Register of Induced Abortions). HILMO is one of the oldest individual-level hospital discharge registers covering the entire country. It has been intensively used for research purposes. Completeness and accuracy in the register seem to vary from satisfactory to very good. The accuracy of registry-based diagnoses has been shown to be good; more than 95% of discharges could be identified from the HILMO (203). Moreover, multiple data sources and register linkages used increase the completeness of data.

Ethical considerations

The study protocol (Studies I-III) was approved by the local ethics committee (Studies I and II: 17 March 2010, Dnro 45/13/03/03/2010, and Study III: 16 September 2009, Dnro 351/E9/2007). The Population Register Center gave permission for address data on 13 March 2015 (Dnro THL/1374/5.05.00/2014). No ethics statement was required for Study IV since the study was performed with anonymous register data and none of these individuals were contacted.

Study population

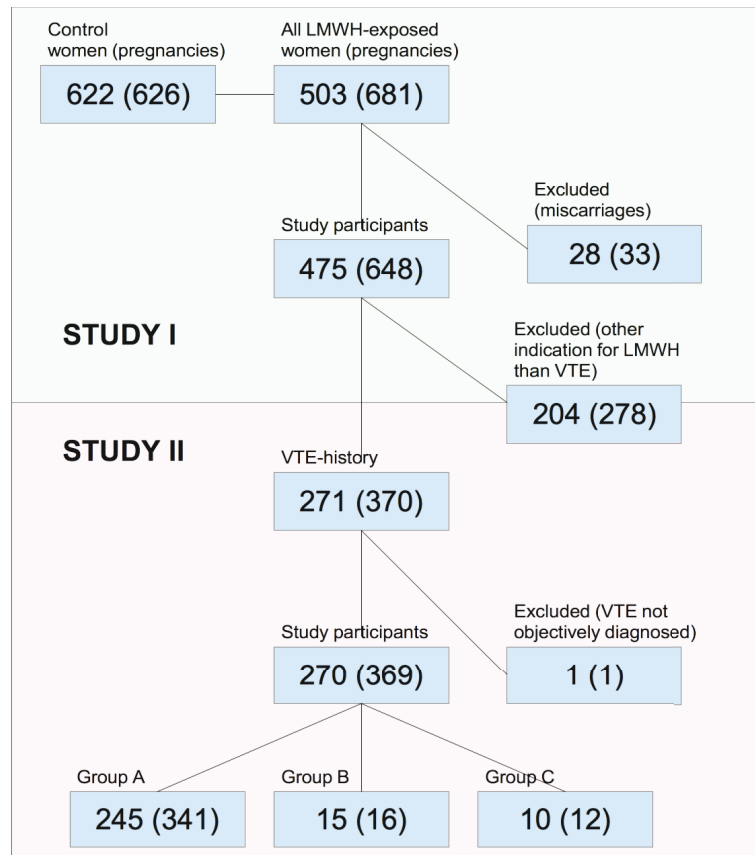


Figure 4: Distribution of study participants in studies I and II. Number of women (number. of pregnancies) are shown. Participants were retrieved from electronic medical records by using ICD-10 codes: I73-74, I80-83, D68 and M32 between February 1994 and January 2007. Group A: history of VTE with successful LMWH-prophylaxis during the index pregnancy, Group B: VTE in the index pregnancy despite ongoing LMWH-prophylaxis and Group C: recurrent VTE in the index pregnancy before the initiation of planned LMWH-prophylaxis. One woman (two different pregnancies) was analysed in groups A and C and one woman (two different pregnancies) in groups B and C.

Figure 4 outlines the recruitment of cases and controls in Studies I and II. Women with singleton pregnancies treated with LMWH at any stage of pregnancy, who delivered at ≥ 22 gestational weeks between February 1994 and January 2007, were included in Study I. We identified the cases from the electronic hospital database by using the following ICD-10 codes: I73, I74, I80, I81, I82, I83, D68, and M32. These studies include also some patients

based on determined anti-FXa values, as a part of routine follow-up of LMWH-treated women in the 1990s to improve data reliability. The next woman to deliver, untreated with LMWH, and matched for age and delivery route was selected as a control. Those LMWH-treated women who experienced miscarriages in early pregnancy were excluded. Study II is a sub-study of Study I limited to those with a history of previous VTE.

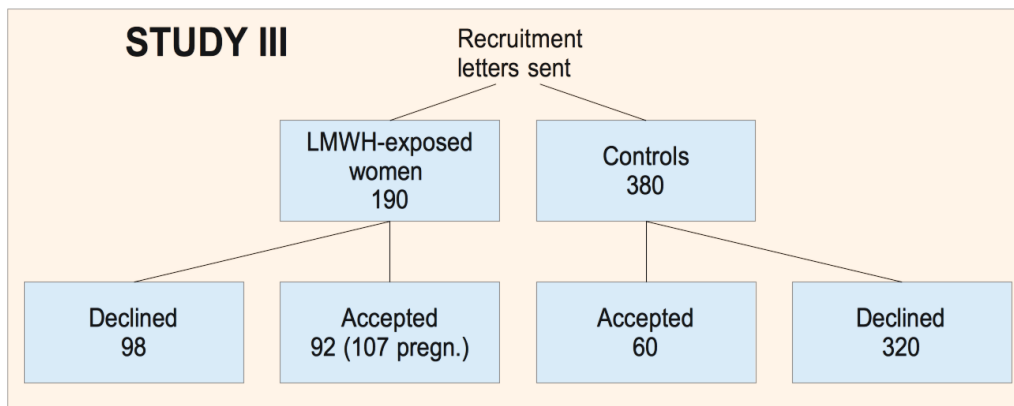


Figure 5: Recruitment of study participants in study III, years 2008-2012

Figure 5 outlines the recruitment of cases and controls in Study III. This study was carried out in 2008-2012. Cases were identified from the electronic hospital database by using the following ICD-10 codes: I73, I74, I80, I81, I82, I83, D68, and M32. After the index case, the next parity-matched woman to deliver without LMWH exposure was selected as a control. Altogether 190 recruitment letters were sent to the women who received LMWH during the last pregnancy and 380 letter to potential controls.

Study IV consists of all women with a registered delivery in Finland in 2001-2011. The date of delivery was obtained from the Medical Birth Register for all women with deliveries. Those women with an inpatient or outpatient admission because of VTE after the date of delivery were collected from the Care Register for Health Care (HILMO). Cases were identified from HILMO with the following ICD-10 codes: I80.1-I80.9, I82.0-I82.9, I26.0, I26.9, O87.1, and O88.2. Women were identified by using their personal identification number to avoid double recognition. All women aged 15-49 years with a registered delivery without the diagnosis of postpartum VTE served as a control group for the women with

postpartum VTE to calculate the cumulative and weekly incidence of VTE through 180 postpartum days and to identify the associated risk factors during three different postpartum periods. The non-pregnant fertile-aged population comprised all women aged 15-49 years without registered delivery or induced abortion in the same calendar year in order to compare the incidence of postpartum VTE with that in non-pregnant fertile-aged women. The mortality for postpartum VTE was assessed from the Cause of Death Register.

Cases and controls

Study I

After excluding 28 LMWH-exposed patients with 33 pregnancies that ended in miscarriages, 475 pregnant women with 648 LMWH-exposed pregnancies remained. Six hundred and twenty-two untreated controls with 626 singleton pregnancies were selected as controls. Patients used LMWH for different indications with varying duration of therapy. Pregnancy, delivery, and baseline data on the participants were retrieved from the electronic hospital database. Data on age, gravidity, parity, BMI, obstetric history, primary diseases, history of VTE, thrombophilias, platelet count, and alanine aminotransferase (ALAT) values were collected. The type, dosing and duration of the LMWH therapy as well as any other medications used were reported. During the study period the treatment protocol was as follows: prophylactic dose of enoxaparin 40 mg/day or dalteparin 5000 IU/day, intermediate dose (50% of weight-adjusted treatment dose) of enoxaparin 1 mg/kg/day or dalteparin 100 IU/kg/day, and weight-adjusted treatment dose of enoxaparin 1 mg/kg twice daily and dalteparin 100 IU/kg twice daily.

Study II

Because the proportion of those with VTE, despite ongoing LMWH medication, was so high in Study I, we decided to conduct a sub-study limited to those LMWH-exposed women with a history of at least one previous VTE. After excluding one women with non-

accurately diagnosed VTE in the index pregnancy, 270 women with 369 pregnancies remained. The following data concerning the previous VTE were collected from the electronic hospital database: amount and type(s) of previous VTE(s), risk factors, such as thrombophilia, and circumstances of previous VTE. Age, gravidity, parity, BMI in connection with the first prenatal care visit, type, dose, and time of initiation and duration of LMWH therapy were also documented. Women with a history of idiopathic, estrogen-related, or multiple prior VTE(s) were advised to initiate LMWH prophylaxis as soon as the pregnancy test confirmed pregnancy. Women with a history of VTE in combination with thrombophilia initiated LMWH prophylaxis as soon as possible during the first trimester. Special attention to initiate LMWH prophylaxis as soon as possible was particularly targeted to those women with antithrombin deficiency or combined thrombophilias. VKAs were replaced with LMWH immediately after a positive pregnancy test. In cases with a history of VTE related to some causal factor, such as immobility or operation, which was no longer present during pregnancy, LMWH prophylaxis was initiated at gestational weeks 34-36.

Women were divided into three groups: women with a history of VTE with successful LMWH prophylaxis during the index pregnancy (Group A), women with recurrent VTE in the index pregnancy despite ongoing LMWH prophylaxis (Group B), and women with recurrent VTE in the index pregnancy before the initiation of planned LMWH prophylaxis (Group C). Group A was used as a control group for Groups B and C when comparing risk factors in connection with the recurrent VTEs.

Study III

This observational cohort study included 92 women who had received LMWH therapy (as prophylaxis or treatment of VTE) during their previous pregnancy/pregnancies. Fifteen women had a history of two LMWH-exposed pregnancies. Sixty women with no LMWH exposure ever were recruited as controls. A recruitment letter with information about DEXA and the associated marginal radiation was sent to invitees. Our primary aim was to select two controls for every case matched for age and parity. We finally succeeded in

recruiting only 60 controls because many potential controls refused DEXA. ICD-10 codes: I73, I74, I80, I81, I83, D68, and M32 were used to collect potential LMWH-exposed subjects. The parity-matched next delivered women after the index case was selected as a control.

In LMWH-exposed women, DEXA measurement was performed between the years 2008 and 2009. Due to delayed funding, DEXA in the control women was carried out between 2011 and 2012, causing an age difference between LMWH-exposed woman and controls at the time of DEXA analysis. Controls were also significantly heavier than cases.

The prophylactic LMWH dose was enoxaparin 40 mg/day or dalteparin 5000 IU/day. The weight-adjusted treatment dose was enoxaparin 1 mg/kg or dalteparin 100 IU/kg twice daily. The intermediate dose was enoxaparin 1 mg/kg/day or dalteparin 100 IU/kg/day. The total LMWH dose was also calculated by taking into account all LMWH-exposed pregnancies. All participants filled in a questionnaire regarding lifestyle factors (smoking, physical exercise, dietary calcium intake, and alcohol use) and medical history (menstrual cycle, contraception, underlying diseases, and duration of breastfeeding, etc.). Baseline characteristics and data on the LMWH therapy were retrieved from the electronic hospital database.

Study IV

In 2001-2011, a total of 634292 deliveries were registered. Cases comprised 1169 deliveries with a diagnosis of postpartum VTE, and the remaining 633123 deliveries without a diagnosis of postpartum VTE served as controls. For baseline VTE incidence, 1232841 non-pregnant non-puerperal fertile-aged women followed up for 13.56 million woman-years without registered delivery or induced abortion in the same calendar year served as the control group.

The women with deliveries were divided into six different age groups: 15-19, 20-24, 25-29, 30-34, 35-39, and 40 years or more. The cumulative incidence of total VTE, DVT, and PE

was calculated separately in each age group. We identified VTE-associated risk factors during three different postpartum periods: 0-21 days, 22-42 days, 43-180 days.

Definitions and Outcomes

Primiparity was defined as no deliveries ≥ 22 gestational weeks before the index pregnancy, in accordance with the International Statistical Classification of Diseases and Related Health Problems of the World Health Organization.

FGR was defined according to the criteria of the American College of Obstetricians and Gynecologists as estimated fetal weight below the 10th percentile for gestational age (204).

Pre-eclampsia was defined as severe requiring delivery ≤ 37 gestational weeks when these criteria were fulfilled: systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 110 mmHg and proteinuria ≥ 5 g/day (205).

HIT was suspected if platelet count halved after initiation of LMWH with simultaneously existing heparin-dependent IgG antibodies.

The criteria of the antiphospholipid antibodies carrier were lupus anticoagulants and anticardiolipin antibody or anti- β glycoprotein-1 antibody on two or more occasions at least 12 weeks apart without clinical manifestations. Antiphospholipid syndrome was defined as 1) a clinical entity with one or more clinical episodes of arterial or venous thrombosis in any tissue or organ with no alternative cause of thrombosis or fetal loss and 2) the presence of antiphospholipid antibodies.

T-score is the difference, by number of standard deviations, of an individual's BMD from the mean of a young female reference population. Osteoporosis is diagnosed when T-score

is -2.5 or less. Osteopenia is diagnosed (decreased bone density) when T-score is between -2.5 and -1. Z-score is the number of standard deviations by which a patient's BMD differs from the average BMD of a population of the same age, sex, and ethnicity.

Daily calcium intake was determined based on declared calcium-containing food consumption by using the Fineli[®] nutrient composition database.

Calibrations of the DEXA equipment were as follows: daily calibrations by using a standard calibration block, which includes 3 cavities that simulate BMD values 0.5, 1.0, and 1.5 g/cm². The BMD of every cavity must be within 0.03 g/cm² of the expected value. Repeatability over the long term was adjusted through weekly measurements by using a skeleton phantom (Accuracy Phantom, SN 21979, BMD 1.265 g/cm²). The day-to-day variation of the equipment used was 0.01 g/cm² and the coefficient of variation 1.0% (%CV= standard deviation/percentage of average BMD) for the lumbar spine and femoral neck.

Study I: The principal outcome variables were adverse effects of LMWH exposure, including bleeding, HIT, increase in ALAT values, allergic skin reactions, and osteoporotic fractures. Recurrent VTEs were reported. Adverse pregnancy outcomes documented were FGR, pre-eclampsia, preterm delivery, and stillbirth. Delivery route, blood loss at delivery, birth weight, 1-min and 5-min Apgar scores, and umbilical cord pH were also documented.

Study II: The main outcome variable was recurrent antepartum VTE despite prophylactic LMWH or before the aimed initiation of LMWH, which was objectively diagnosed by CUS or CTPA. We also retrospectively classified all women according to the risk of recurrent VTE based on the ACCP guidelines (James, Committee on Practice Bulletins-Obstetrics 2011) and compared the realized LMWH initiation times with the ones recommended in the ACCP guidelines.

Study III: The principal outcome variables were BMDs, T-scores, and Z-scores in the lumbar spine and femoral neck, which were measured by using DEXA (Lunar Prodigy advance Full Size, encore software version 15, GE Medical Systems-Lunar Madison, WI,

USA). Secondary outcome variables were established osteoporosis, osteopenia, and clinically evident osteoporotic fractures.

Study IV: Primary outcomes were to identify the cumulative and weekly incidence and mortality of postpartum VTE through 180 postpartum days. Secondary outcomes were to identify the associated risk factors for postpartum VTE during three different postpartum periods and to calculate the baseline incidence of VTE from the non-pregnant fertile-aged population and compare it with the postpartum VTE incidence.

Statistical analysis

Statistical analyses were undertaken by using Statistical Package for the Social Sciences Versions 17, 21, and 23 (SPSS Inc., Chicago, IL, USA) and the statistical software package SAS 9.3 (SAS Institute Inc., 100 SAS Campus Drive Cary, USA) . Qualitative variables were reported by using frequencies and percentages. Continuous variables were reported by using means (when data were normally distributed) and medians and ranges (when data deviated from normal distribution). Frequencies were compared by using Chi-squared test or Fisher's exact test and means by using Student's t-test or Mann-Whitney U-test. Only valid percentages were calculated. All tests were two-tailed and p-values <0.05 were considered to be significant. Multivariate regression analysis was used to adjust outcome variables for potential confounding factors. Multivariable logistic regression and 95% confidence intervals (CIs) were used to evaluate the factors associated with postpartum VTE risk.

Results

Safety of LMWH during pregnancy (Study I)

Table 12 summarizes the adverse events of LMWH and the maternal/neonatal adverse outcomes. The use of LMWH during pregnancy was safe. No osteoporotic fractures or HIT were found. The incidence rates of bleeding and thrombocytopenia did not differ between the LMWH-exposed women and the controls. The incidence of allergic skin reaction was low. Birth weight (3.4 kg vs. 3.5 kg, $p=0.02$) was lower in the LMWH group than in the control group, otherwise pregnancy outcomes did not differ between the groups. When the 60 pregnancies with prior adverse pregnancy outcomes in the LMWH group were excluded, no difference in incidences of preterm delivery, stillbirth, severe pre-eclampsia, or FGR existed between the groups. The rate of recurrent VTEs despite ongoing LMWH was, however, high (2.5%, $n=16$).

Table 12: The incidence of obstetric adverse events in LMWH-group and in control-group

Adverse events of LMWH	LMWH n (%)	Control n (%)	p-value
Thrombocytopenia ^a	56 (8.7)	32 (8.7)	0.8
Antenatal bleeding	69 (10.7)	49 (7.8)	0.07
Allergic skin reactions	2 (0.3)	0	
Increase in ALAT U/l	53 (23.5)	16 (12.3)	0.01
Venous thrombosis despite LMWH	16 (2.5)	0	
Bleeding during delivery >1000 ml	41 (6.8)	27 (4.5)	0.1
Heparin-induced thrombocytopenia	0	0	
Osteoporotic fractures	0	0	
Adverse pregnancy outcomes^b			
Preterm delivery ^c	51 (8.7)	41 (6.5)	0.05
Stillbirth	4 (0.7)	2 (0.3)	0.4
Severe pre-eclampsia	9 (1.4)	6 (1.0)	0.4
Fetal growth restriction ^d	16 (2.7)	14 (2.2)	0.6

LMWH, low-molecular-weight heparin, ALAT alanine aminotransferase

^aplatelet count <150 E⁹/l

^b only those women (LMWH-group) without prior adverse pregnancy outcome, $n=588$

^c<37 gestational weeks

^d<10th percentile

The indications for LMWH were as follows: prior VTE (55.6%, n=370), acute VTE in current pregnancy (28.2%, n=183), previous adverse obstetric outcome (24.6%, n=159), asymptomatic thrombophilia (17.7%, n=115), and other reasons such as mechanical heart valve, prior stroke, or immobilization (8.3%, n=53). In 223 pregnancies, the women had more than one indication for LMWH use. Dalteparin (86.6%) and enoxaparin (15.7%) were the preparations used. Sixty-eight women received low-dose aspirin as an associated treatment, and nine women received UFH due to acute VTE before switching to LMWH.

Thrombophilias were diagnosed in 197 women (41.5%). LMWH-exposed women differed from controls in terms of parity (fewer were nulliparous), higher BMI, and a history of more spontaneous abortions. The median time-point of initiation of LMWH was 17 gestational weeks, and the mean duration of LMWH exposure was 22 weeks.

The proportion of Cesarean sections was 21% in the LMWH group and 19% in the control group, with no statistical difference ($p=0.3$). One massive antenatal bleeding (3000 ml) occurred at 28 gestational weeks due to marginal placenta previa in a woman receiving intravenous UFH. One massive postpartum hemorrhage occurred (11000 ml) due to uterine atony and placental retention. Nine bleeding events were potentially LMWH-related requiring a dose reduction or a few days' pause in LMWH treatment.

Incidence and risk of recurrent VTE during pregnancy '

(Study II)

The proportion of successful LMWH prophylaxis was 92.4% in 341 women (Group A). There were 16 recurrent VTEs in 15 women despite ongoing LMWH prophylaxis (Group B) and 12 recurrent VTEs in 10 women before the intended start of LMWH prophylaxis (Group C). The overall incidence of recurrent antepartum VTE was 7.6% when analysis was limited to those with a history of VTE.

All VTEs in Group B were DVTs in the lower limb, mainly left-sided (56%). The mean initiation of LMWH prophylaxis in Group B occurred on the 7th gestational week, and the mean duration of LMWH prophylaxis before the recurrent VTEs was 15 weeks. In Group C, 9 DVTs occurred in a lower limb, mainly left-sided (67%), others were one PE and two cases of subclavian vein thrombosis. The mean time-point of diagnosis was at 10 gestational weeks.

Table 13 shows the medical history and risk factors for VTE in Groups A, B, and C.

In Group B, almost half (47%) of the women had long-term VKA before the pregnancy, which was replaced with LMWH immediately after a positive pregnancy test. All of these women were at very high risk of thrombosis (two or multiple prior VTEs, VTE in combination with a high-risk thrombophilia and/or aPLs). Because of the high-risk profile in Group B, 25% of women received intermediate doses and 6% weight-adjusted doses of LMWH. All women in Group C received weight-adjusted treatment doses of LMWH due to acute VTE.

Table 13: The medical history and risk factors for VTE: comparison of Groups A, B and C

Demographics n(%)	Group A 341 (92.4)	Group B 16 (4.3)	Group C 12 (3.3)	p-value ^a	p-value ^b
Previous VTEs n(%)				<0.001	0.02
1	231 (94.3)	9 (60.0)	7 (70.0)		
≥2	14 (5.7)	6 (40.0)	3 (30.0)		
Long-term vitamin K antagonist	18 (7.3)	7 (46.7)	0	<0.001	
Thrombophilia	64 (27.2)	8 (53.3)	4 (40.0)	0.04	0.47
FV Leiden gene mutation ^c	34 (14.5)	2 (13.3)	2 (20.0)	1	0.64
Prothrombin gene mutation ^c	3 (1.3)	0	1		
Antithrombin deficiency	4 (1.7)	1 (6.7)	0.27		
Protein C or S deficiency	9 (3.8)	1 (6.7)	1 (10.0)	0.47	0.35
APLs ^d	6 (2.6)	3 (20.0)	0	0.012	
Risk factors at first VTE					
None or unknown	62 (25.3)	0	0	<0.001	
Transient risk factor	64 (26.1)	6 (40.0)	2 (20.0)	0.24	1
Hormonal risk factor	148 (60.4)	14 (93.3)	9 (90.0)	0.01	0.09

^a Group A. vs. Group B.

^b Group A. vs. Group C.

^c heterozygous, ^dAPL, antiphospholipid antibodies

Long-term LMWH-exposure and subsequent bone mineral density (Study III)

Table 14 demonstrates BMD (lumbar spine and femoral neck) in women who received LMWH and in controls. BMD in the spine was significantly lower in women receiving LMWH than in control women, but BMD in the femoral neck did not differ between the groups. No difference was found between enoxaparin users and dalteparin users in the lumbar spine ($p=0.28$) or in the femoral neck ($p=0.65$).

Table 14. BMD, T-and Z-scores in LMWH-group vs. control

	LMWH-group		Control group n=60	p-value ^b	p-value ^c
	Prophylactic dose n=75	Other doses ^a n=17			
Lumbar spine					
BMD g/cm ² (SD)	1.22 (0.09)	1.20 (0.14)	1.27 (0.14)	0.03	0.07
T-score	0.43 (0.79)	0.22 (1.19)	0.83 (1.15)	0.02	0.06
Z-score	0.44 (0.80)	0.23 (1.18)	0.93 (1.16)	0.007	0.03
Femoral neck					
BMD g/cm ² (SD)	0.99 (0.11)	1.0 (0.15)	1.01 (0.12)	0.39	1.0
T-score	0.09 (0.91)	0.18 (1.18)	0.24 (0.97)	0.39	0.85
Z-score	0.2 (0.91)	0.28 (1.20)	0.43 (0.96)	0.15	0.58

BMD, bone mineral density; SD, standard deviation

^a weight-adjusted - or intermediate LMWH-dose

^b Women, who received prophylactic LMWH-dose vs. controls

^c Women, who received weight-adjusted - or intermediate LMWH-dose vs. controls

The incidence of osteopenia was 13% in the LMWH group and 8.3% in the control group ($p=0.40$). No osteoporosis or osteoporotic fractures were found.

Although the absolute BMD value in the spine differed between LMWH users and the control group, after adjustment for potential confounding factors LMWH exposure was no longer associated with decreased BMD in the spine. Duration of contraception pill use was independently associated with greater BMD in the spine. Treatment doses in the LMWH group were as follows: prophylactic (81.5%), weight-adjusted (10.9%), and intermediate (7.6%). Mean duration of prophylactic doses of LMWH was 216 days, and that of other doses was 218 days. Enoxaparin was used in 58% of cases and dalteparin in 42% of cases. The duration of contraceptive pill use was longer in the control group than in the LMWH group. Otherwise, the groups did not differ in terms of lifestyle factors or medical history.

Incidence and risk factors of VTE during the postpartum-period (Study IV)

Between 2001 and 2011, postpartum VTE was diagnosed in 1169 women after 634292 deliveries between 0-180 postpartum days. Figure 6 shows the proportion of women with postpartum VTE.

Figure 6

The majority of the VTEs were DVTs (77%), and the rest were PEs or both (DVT+PE) (23%). The 180 days of cumulative incidence of postpartum VTEs was 4-fold that of non-pregnant non-puerperal women. The incidence was highest during the first postpartum week: 37-fold that of non-pregnant non-puerperal women, thereafter declining to 2-fold. Almost half (48%) of the observed VTEs occurred between 43 and 180 days postpartum. Three VTE-related deaths occurred; all were PEs. Besides older age, there are many risk factors associated with increased risk for postpartum VTE; these are presented in Table 15.

Diagnosis codes for thrombophilia were registered in HILMO in 54 (4.6%) women with postpartum VTE and in 1363 (0.2%) women without it.

Table 15: Incidence of VTE 0-180 days postpartum among women with selected risk factors and adjusted OR

The Care register for Social Welfare and Health Care (HILMO)/the Medical Birth register, deliveries n=634.292									
Risk factor	n	0-180 days postpartum		0-21 days postpartum		22-42 days postpartum		43-180 days postpartum	
		Incidence n/10000	OR (95% CI)	Incidence n/10000	OR (95% CI)	Incidence n/10000	OR (95% CI)	Incidence n/10000	OR (95% CI)
Multiple pregnancy ^a	9546	47.1	2.53 (1.87-3.41)	23.0	2.87 (1.88-4.40)	5.2	3.35 (1.36-8.24)	18.9	2.09 (1.30-3.34)
Primiparity ^a	265562	17.9	1.24 (1.10-1.41)	7.5	1.12 (0.93-1.35)	1.5	1.27 (0.83-1.94)	8.8	1.36 (1.14-1.63)
Prior miscarriage ^a	132219	22.6	1.10 (0.96-1.25)	10.1	1.10 (0.90-1.35)	1.7	0.93 (0.57-1.50)	10.9	1.12 (0.92-1.36)
Smoking during pregnancy ^a	94788	17.4	1.14 (0.97-1.35)	7.4	1.09 (0.84-1.40)	1.9	1.56 (0.92-2.63)	8.1	1.13 (0.88-1.45)
Cesarean section ^a	104249	26.2	1.36 (1.19-1.57)	11.2	1.30 (1.05-1.60)	2.9	1.95 (1.25-3.02)	12.1	1.33 (1.09-1.63)
IVF-pregnancy ^a	13326	30.8	1.32 (0.96-1.81)	12.0	1.18 (0.71-1.94)	2.3	1.18 (0.37-3.75)	16.5	1.47 (0.95-2.26)
Antepartum hemorrhage ^a	7186	29.2	1.51 (0.98-2.33)	6.8	1.51 (0.78-2.93)	1.5	1.18 (0.37-3.75)	7.5	1.48 (0.79-2.78)
Gestational hypertension ^a	19089	21.5	1.09 (0.79-1.49)	27.8	1.22 (0.78-1.93)	7.0	1.64 (0.40-6.70)	22.3	0.88 (0.54-1.45)
Threatening premature birth ^a	16523	27.2	1.47 (1.08-1.98)	13.3	1.65 (1.07-2.54)	2.4	1.54 (0.62-3.80)	11.5	1.30 (0.82-2.06)
Thrombophilia ^a	1417	381.1	20.9 (15.7-27.9)	247.0	30.8 (21.7-43.8)	21.7	14.1 (4.4-44.9)	116	13.1 (7.9-21.7)
IVF + Ovarian hyperstimulation	1942	56.6	2.94 (1.69-5.11)	30.9	3.03 (1.35-6.83)	10.3	5.59 (1.37-22.87)	25.9	2.37 (0.98-5.74)
Chronic hypertension ^a	2635	38.0	1.98 (1.11-3.52)	15.2	1.50 (0.56-4.04)	0.0	NA	30.4	2.78 (1.38-5.61)
Systemic lupus erythematosus ^a	442	67.9	1.94 (0.61-6.20)	45.2	2.50 (0.60-10.41)	0.0	NA	22.7	1.54 (0.21-11.14)
Prolonged delivery ^a	25938	13.5	0.96 (0.71-1.29)	6.9	0.86 (0.54-1.38)	2.3	1.56 (0.68-3.56)	8.1	0.95 (0.61-4.46)
Varicose veins ^a	1398	136.9	4.74 (2.87-7.82)	5.5	5.48 (2.70-11.12)	0.0	NA	57.6	4.84 (2.39-9.79)
Hyperemesis ^a	1122	8.6	0.47 (0.07-3.32)	0	NA	0.0	NA	8.9	0.98 (0.14-6.95)
Renal disease ^a	3050	136.9	1.39 (0.69-2.79)	19.7	2.30 (1.03-5.16)	0.0	NA	6.6	0.75 (0.19-3.00)
Cardiac disease ^a	416	102.7	12.65 (6.97-22.96)	144.2	12.95 (5.58-30.04)	0.0	NA	146.3	14.01 (6.16-31.83)

Medical Birth Register, deliveries n= 468.671 (2004-2011)									
BMI ≥25 ^a	145232	26.4	1.67 (1.46-1.91)	13.1	1.78 (1.46-2.15)	1.9	1.44 (0.89-2.33)	11.4	1.60 (1.30-1.96)
BMI ≥30 ^a	50372	31.8	1.78 (1.50-2.12)	18.3	2.24 (1.78-2.83)	2.8	2.02 (1.12-3.63)	10.7	1.30 (0.98-1.74)
Gestational diabetes ^b	46190	30.5	1.24 (1.01-1.5)	14.9	1.16 (0.87-1.55)	1.1	0.48 (0.18-1.25)	14.5	1.51 (1.13-2.03)
Pre-eclampsia ^d	20344	11.3	1.34 (0.89-2.03)	6.9	1.71 (1.00-2.92)	1.0	1.46 (0.36-5.97)	3.4	0.92 (0.43-1.94)
Anemia ^a	10451	30.6	1.69 (1.18-2.41)	15.3	1.75 (1.06-2.89)	1.9	1.33 (0.32-5.43)	13.4	1.69 (0.99-2.88)
Polyhydramnion ^a	2051	14.6	0.74 (0.24-2.31)	4.9	0.52 (0.07-3.73)	0.0	NA	9.8	1.11 (0.28-4.45)
Chorionamnionitis ^a	2061	58.2	3.12 (1.76-5.53)	29.1	3.25 (1.45-7.30)	9.7	6.46 (1.58-26.47)	19.5	2.34 (0.87-6.27)
Postpartum hemorrhage ^a	12987	22.3	1.16 (0.80-1.68)	13.9	1.53 (0.95-2.46)	0.8	0.48 (0.07-3.49)	7.7	0.48 (0.07-3.49)

VTE, venous thromboembolic event; OR, odds ratio, CI, confidence interval; BMI, body mass index; IVF, in vitro fertilization DVT, deep venous thrombosis; PE, pulmonary embolism
NA, not available

^a adjusted for age and the year of the delivery

^b adjusted for age, year of the delivery and number of fetuses

^c adjusted for age, thrombophilia, year of the delivery and the number of fetuses

^d OR 0-6 days postpartum 1.73 (95% CI 1.02-2.96)

Duration of the increased VTE rates varied depending on the type of risk factor. The risk remained elevated for 180 days in women with thrombophilia, Cesarean section, multiple pregnancy, varicose veins, and cardiac disease. In women with IVF pregnancy and OHSS, BMI ≥30 kg/m², or chorionamnionitis, the risk of VTE was increased up to 42 days postpartum. In women with threatening premature birth, renal disease, anemia, and BMI ≥25 kg/m², the risk of VTE was elevated only in the first 21 days postpartum. The VTE risk was elevated for the first 0-6 days after childbirth for those with pre-eclampsia with OR 1.73 (95% CI 1.02-2.96).

Discussion

Well-planned and conducted observational cohort studies are needed to gather information on the association between different risk factors and diseases. In Finland, where the population is small, researchers can use unique nationwide registers, such as HILMO, whose completeness and accuracy seem to vary from satisfactory to very good (203). These registers are carefully maintained according to the precise instructions provided by medical authorities.

Long-term LMWH-use during pregnancy: safety and efficacy

Maternal and neonatal adverse events

In study I, LMWH was found to be safe for both the mother and fetus. No osteoporotic bone fractures or HIT were found. The incidences of thrombocytopenia (8.7%), allergic skin reactions (0.3%), and antenatal bleeding (10.7%) in the LMWH group were similar to those in controls and in agreement with the previously published literature (19). Only one antenatal bleeding event was several - not related to LMWH.

During delivery the incidences of major blood loss (>1000 ml) did not differ between LMWH-exposed women and controls. The rate of major blood loss during delivery was the same as that in a previously published Swedish study (6.8%) (18). However, there was a difference between our practices and theirs in LMWH use during delivery; we recommended temporary cessation of LMWH prior to delivery, whereas Lindqvist et al. continued with a half-dose of LMWH twice daily.

Although adverse events of LMWH were scarce, 16 women (2.5%) suffered a VTE despite ongoing LMWH medication. Every woman with recurrent VTE had received LMWH for at least one week before the diagnosis. This finding could arise from insufficient dosing of

LMWH for high-risk patients due to a lack of consistent recommendations for these patients.

In terms of neonates, birth weight was slightly lower in the group of LMWH-exposed women than in controls. The explanation for this could be the higher incidence of prior adverse pregnancy outcomes in the LMWH group. However, this finding may be clinically insignificant.

Strengths of this study were the larger sample size and the existence of controls relative to the majority of previously published studies with smaller sample sizes and no controls. All women were treated in the same hospital by a few specialists in accordance with the same guidelines. The available data were documented accurately in patients' electronic hospital records, and the medical data were checked for each patient separately to avoid selection bias.

Limitations of the study were imprecise data for some laboratory values (e.g. platelet count, ALAT) and bleeding events other than vaginal (e.g. epistaxis) in the control group. The controls were not screened for thrombophilias. Considering the size of the hospital and the duration of the study, the sample size was relatively small, probably due to the conservative approach in the 1990s during pregnancy, when indications for LMWH were current VTE or thromboprophylaxis for those with a history of VTE.

In conclusion, the use of LMWH in pregnant women is safe when anticoagulation is needed. The high recurrent VTE rate in our study may reflect insufficient dosing of LMWH in high-risk women. However, at present, reports on recurrent VTEs despite LMWH prophylaxis are scarce, and more studies investigating the risk factors and LMWH dosing in connection with high-risk situations are warranted.

Recurrent VTEs

In study II, the incidence of antepartum recurrent VTEs was 7.6% of those with a history of VTE. The risk factors for recurrent VTE despite ongoing LMWH were a history of multiple VTEs, use of long-term anticoagulation before pregnancy, a history of VTE in connection with antiphospholipid antibodies, and a history of VTE related to hormonal risk factor or of unknown etiology. The risk factors for recurrent VTE before initiation of LMWH were a history of multiple VTEs and a history of VTE related to prior pregnancy.

The incidence found here was significantly higher than that of previously published literature focusing on efficacy of LMWH during pregnancy (0.6-2.4%)(12,17,82). Comparison of these studies with our study is, however, challenging because risk profiles of these other populations were heterogeneous and not all limited to those with a history of VTE and anticoagulation treatment strategies were variable. The most comparable study to our work was undertaken by Roeters van Lennep et al. (2011), who reported an incidence of antepartum VTE of 1.6%. All of their VTEs occurred in high-risk women. These findings are in agreement with our results.

A possible explanation for the high incidence of recurrent VTEs in our hospital might be overly conservative dosing of LMWH in the 1990s and early 2000s due to lack of experience and inconsistent recommendations for LMWH treatment, particularly in high-risk groups.

The main strength of our study is the homogeneous population; all women had a history of VTE and were treated in the same hospital with unchanging guidelines. As far as we know, no previously published studies with this kind of design exist, i.e. women with treatment failure were compared with women with successful treatment.

A limitation of our study was its retrospective design. Data concerning confounding factors, such as smoking during pregnancy, were missing, which may bias the analysis.

Data on postpartum VTEs were not available. Data on the risk factors at the time of the first VTE in the control group were underreported. We did not find differences between the groups regarding such variables as BMI >30 kg/m² or age >35 years, probably because our study was underpowered for weaker risk factors. Due to the small number of women in Groups B and C, we were cautious in interpreting our results, merely considered them preliminary.

Based on these findings, we recommend individual risk assessment and even weight-adjusted treatment doses of LMWH in cases of high recurrence risk. LMWH should be initiated immediately after pregnancy is confirmed. More studies comparing different dosing regimens in high-risk groups are urgently needed.

Subsequent bone mineral density

In study III, long-term prophylactic LMWH use during pregnancy was not associated with a subsequent decrease of BMD in the lumbar spine after adjustment for potential confounders. The incidence of osteopenia was similar between the LMWH group and the control group. No osteoporosis or osteoporotic fractures were found in either group. a greater LMWH dose did not correlate with decreased BMD.

A recognized complication of prolonged UFH therapy is osteoporosis. In the 1990s, several case series and animal studies had suggested that the decrease in BMD with LMWH is less than that seen with UFH (22-24). It is under investigation if LMWH associates with the decreased BMD. Some marginal BMD decrease was reported in a small prospective cohort study (n=69) with LMWH-exposed pregnancies; the authors concluded that the effects of LMWH on bone demineralization require further investigation (206). In studies comparing BMD in LMWH-exposed pregnant women with that of healthy controls, no association between prolonged LMWH use and decreased BMD existed (194,207). Our findings are in line with these studies. There are, however, eight case reports of LMWH-induced osteoporotic fractures during pregnancy (197).

We found a slight positive association between combined oral contraceptive use and subsequent BMD. Because combined oral contraceptive use is contraindicated after VTE, the duration of the use of that was significantly shorter in the LMWH group than in the control group. Estrogens have been found to be important in the regulation of bone metabolism by suppressing osteoclastogenesis and inhibiting bone resorption by osteoclasts (208). In a review of 13 studies, nine studies indicated a favorable effect of COC use on BMD (209).

A strength of this study is the long follow-up time. No previous studies on BMD so many years after the LMWH exposure exist. Our sample size is larger than that of previously published studies on this same issue (21,172,193,194,207). The population was homogeneous; all women were Caucasians and the DEXA was carried out by the same equipment in the same hospital.

Some limitations of our study must be acknowledged. Many potential parity-matched controls declined the DEXA, and thus, our control group remained smaller than the LMWH group. Baseline BMD before the LMWH exposure was not available, so comparison of possible BMD alterations in the same subject was not possible. Because the control group was recruited later than the cases, differences in age, BMI, and timing of DEXA existed between the groups. Information on some potential confounders, such as vitamin D intake, calcium supplement, sun exposure, and family history of osteoporosis, was missing from the questionnaire. In addition, we were unable to determine whether higher doses of LMWH would cause more reduction in BMD because most women received prophylactic doses of LMWH. The population was homogeneous; all women were Caucasians and the DEXA was carried out by the same equipment in the same hospital.

Some limitations of our study must be acknowledged. Many potential parity-matched controls declined the DEXA, and thus, our control group remained smaller than the LMWH group. Baseline BMD before the LMWH exposure was not available, so comparison of possible BMD alterations in the same subject was not possible. Because the control group was recruited later than the cases, differences in age, BMI, and timing of DEXA existed between the groups. Information on some potential confounders, such as vitamin D intake, calcium supplement, sun exposure, and family history of osteoporosis, was missing from the questionnaire. In addition, we were unable to determine whether

higher doses of LMWH would cause more reduction in BMD because most women received prophylactic doses of LMWH.

Although these findings are promising, we conclude that indications for long-term LMWH during pregnancy should be based on guidelines, and lumbar spine DEXA could be considered after a LMWH-exposed pregnancy if other risk factors for osteoporosis exist. Prospective controlled studies with greater sample sizes comparing different LMWH dosages are required.

Incidence and risk factors of VTE during the postpartum-period

In study IV, the cumulative incidence of postpartum VTE during the six months after delivery was 18.4/10 000 deliveries. This is higher than in the majority of other studies reporting incidence rates of 4-10/10000 deliveries (5,7,46,210). The incidence was highest during the first week after delivery: 37-fold that of non-pregnant non-puerperal women. The incidence of postpartum VTE declined rapidly to 2-fold, but remained at that level for 175 days after delivery. This finding is in line with the study by Kamel et al. (2014) (28) who found that VTE incidence was 2-fold for 42-84 days after delivery.

Almost half (48%) of the postpartum VTEs occurred after the sixth postpartum week. This finding is in agreement with a previous study by Roeters van Lennep et al. (2011) (17) in which 40% of VTEs occurred later than six weeks after delivery in women at high risk of VTE, suggesting that postpartum prophylaxis for six weeks might be too short in the high-risk groups. However, comparison of these two studies is difficult because Roeters van Lennep et al. evaluated a selected group of women at high risk of VTE and we had a mixed population of all delivered women.

The risk of postpartum VTE increased with older age and higher BMI. Most of our findings concerning risk factors associated with postpartum VTE are similar to those published by others (7,46,135). Risk factors were thrombophilia, multiple pregnancy, gestational

diabetes, anemia, chorionamnionitis, threatening premature birth, IVF pregnancy with OHSS, primiparity, CS and pre-eclampsia. Postpartum haemorrhage was not associated with the risk of VTE, contrary to other studies (46,48,211). One explanation for this might be anticoagulation used by the women with postpartum hemorrhage. It should, however, be recognized that anemia, which is associated with postpartum VTE risk, might be due to postpartum hemorrhage.

Women who had thrombophilia, CS, multiple pregnancy, varicose veins, or cardiac disease had an elevated risk of VTE up to 180 days postpartum. The VTE risk was elevated up to 42 days for women with IVF pregnancy with OHSS, $BMI \geq 30 \text{ kg/m}^2$, or chorionamnionitis. For women with threatening premature birth, renal disease, anemia, and $BMI \geq 25 \text{ kg/m}^2$, the risk of VTE was elevated only in the first 21 days postpartum. The VTE risk was elevated for the first 0-6 days after childbirth for those with pre-eclampsia. Our findings are consistent with the results of Sultan et al. (211), who found the risk of VTE to remain elevated for six weeks in women with pre-eclampsia, $BMI > 30 \text{ kg/m}^2$, infection, and CS.

A strength of our study was the combined data of mandatory registers with almost 635000 deliveries. The completeness and validity of diagnosis coding of the National Medical Birth Register and HILMO have been shown to be good (203,212). The data includes the total population, which improves the reliability of the analysis. Only a few studies reporting postpartum VTE incidence with such a long follow-up exist (27,28). Studies comparing the incidences of VTE in puerperal women with those in women of reproductive age outside of pregnancy or puerperium are also scarce (3,27,28,213).

Some limitations must be noted. Data regarding whether VTEs are diagnosed accurately by imaging and whether there is a history of prior VTEs were not available due to the register-based design of this study. Moreover, restrictions prohibit Finnish health registers from gathering information on ethnicity. Data on patients treated in primary care were not available because data were retrieved only from specialized healthcare, and thus, the VTE incidence may have been underestimated. High-risk women with thrombophilia or other previously mentioned risk factors might have been anticoagulated, biasing the analysis. The proportion of thrombophilia in our study population was low (0.2%), even though the

prevalence of FV Leiden in Finland has been found to be 2-3% (92).

Only 4.6% of women with postpartum VTE had a thrombophilia diagnosis in HILMO, which is significantly less than in previous studies reporting a prevalence of inherited thrombophilia in 20-50% of women with VTEs during pregnancy or during the postpartum period (81). Data concerning thrombophilias are likely to be available only for those who have been tested due to previous VTE or VTE in their families. It is also possible that diagnosed thrombophilia (D68.8) three months after the delivery is forgotten to be entered into the electronic hospital records by the doctor, who usually discloses the diagnosis by phone.

Based on these findings, we conclude that the women with these observed risk factors require careful consideration in terms of VTE risk during the immediate postpartum period. Further studies on the optimal LMWH dose and duration of LMWH prophylaxis in association with the above-mentioned risk factors are needed.

Conclusions

- I. The use of LMWH is safe during pregnancy when anticoagulation is needed. The incidences of thrombocytopenia, bleeding, pre-eclampsia, preterm delivery, stillbirth, or FGR did not differ between the LMWH group and the control group. The incidence of allergic skin reactions related to LMWH was low. No HIT or osteoporotic fractures occurred. The incidence of recurrent VTEs was 2.5%, which is higher than reported by others, and thus, warrants further investigations.
- II. The risk of antepartum recurrent VTE is high in women with a history of two or more previous VTEs, VTE in connection with antiphospholipid antibody syndrome, or long-term anticoagulation. Antepartum LMWH prophylaxis with a prophylactic or even an intermediate dose might be insufficient in these high-risk women. For these women, individual risk assessment and weight-adjusted treatment dose of LMWH should be considered.
- III. Although BMD in the lumbar spine was observed to be lower in the LMWH group than in the control group, no significant association between long-term use of LMWH during pregnancy and decreased BMD, osteopenia, osteoporosis, or osteoporotic fractures was found. Indications for long-term LMWH during pregnancy should be based on current guidelines. Lumbar spine BMD measurement with DEXA could be considered after a pregnancy with LMWH exposure if there are other risk factors for osteoporosis.
- IV. The risk of postpartum VTE is highest during the first week after delivery, thereafter declining rapidly. A two-fold residual risk compared with non-pregnant non-puerperal fertile-aged women remains through 175 days. Thrombophilia, older age, and higher BMI besides other significant risk factors such as CS, multiple pregnancy, gestational diabetes, anemia, chorionamnionitis, threatening premature birth and IVF pregnancy with OHSS increased the risk of VTE. Careful consideration of VTE risk during the immediate postpartum period is required.

Optimal dosing and duration of LMWH prophylaxis in high-risk women are under debate and further studies are warranted.

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