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The safety and efficacy of adjusted-dose enoxaparin (Clexane®) in pregnant patients with increased risk for venous thrombo-embolic disease: A retrospective analysis of clinical and laboratory data

Dr Jenique Bailly

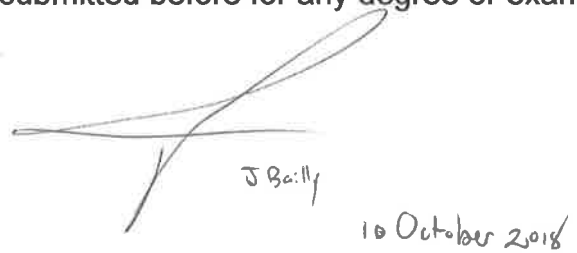


A research report (in the format of a "submissible" paper) submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in the branch of Haematology

Johannesburg, 2018

Declaration

I, Jenique Bailly, declare that this research report (in the format of a "submissible" paper) is my own, unaided work. It is being submitted for the Degree Master of Medicine (MMed), at the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



(signature of candidate)

Contribution of the candidate to the paper

Declaration: Student's contribution to the article and agreement of co-authors

I, Jenique Bailly, student number 1481330, declare that this Research Report is my own work and that I contributed significantly towards research findings presented in the paper intended for the publication below.

Signature of student:
Date: 28 May 2018

Primary Supervisor: Professor Barry Jacobson
Signature:
Date: 28 May 2018

Co-supervisor: Dr Susan Louw
Signature:

Date: 28 May 2018

Agreement by co-authors: By signing this declaration, the co-authors listed below agree to the use of the article(s) by the student as part of his Research Report.

Article Title: The safety and efficacy of adjusted-dose enoxaparin (Clexane®) in pregnant patients with increased risk for venous thrombo-embolic disease: A retrospective analysis of clinical and laboratory data.

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Presentations arising from this research project

1. SASTH Congress 2017, 29th October, Johannesburg, Gauteng (poster presentation).
2. 11th Annual Haematology Oncology symposium, 11th to12th May 2018, Johannesburg, Gauteng (oral presentation).

Abstract

Low molecular weight heparin (LMWH) is the preferred drug to prevent venous thromboembolic disease (VTED) in pregnancy. Physiological changes in pregnancy however affect the pharmacokinetics of LMWH, potentially resulting in ineffective thromboprophylaxis. Anti-Xa guided dose adjustments will theoretically improve outcomes. This study assessed the efficacy and safety of anti-Xa guided dose adjusted LMWH thromboprophylaxis in at risk pregnant women. The data of 151 pregnant patients at risk for VTED treated with adjusted-dose LMWH thromboprophylaxis were analysed. The prevalence of pregnancy-related VTED was 1.3% (95% CI, 0.1-4.7) with one antenatal event, despite adequate prophylaxis according to anti-Xa levels, and the second occurring in the postpartum period related to prolonged protracted labour. The prevalence of pregnancy related bleeding was 2% (95% CI, 0.4-5.7) excluding 3 miscarriages (2.6%; 95% CI, 0.7-6.6). The bleeding events were considered unrelated to LMWH prophylaxis. This study demonstrated the efficacy and safety of anti-Xa dose-adjusted LMWH thromboprophylaxis in at risk pregnant women.

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List of abbreviations

CI	Confidence interval
C/S	Caesarean section
CTPA	Computed Tomography Pulmonary Artery
DVT	Deep vein thrombosis
FVL	Factor V Leiden
LMWH	Low molecular weight heparin
VTE	Venous thrombo-embolism
VTED	Venous thromboembolic disease

Research article in the format of a 'submissible' paper

Title page

The safety and efficacy of adjusted-dose enoxaparin (Clexane®) in pregnant patients with increased risk for venous thrombo-embolic disease: A retrospective analysis of clinical and laboratory data

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Short title: Anti-Xa dose adjusted Enoxaparin in pregnant women at risk of venous thrombosis.

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Author contributions: J Bailly and B Jacobson conceived the study topic. J Bailly wrote the study protocol, collected and analysed the data, wrote the article and approved the final version. B Jacobson and S Louw supervised the study conduct and data collection, analysed the data, revised the article and approved the final version.

Abstract

The study objective was to assess the efficacy and safety of anti-Xa guided dose adjusted low molecular weight heparin (LMWH) thromboprophylaxis in pregnant women at an increased risk of venous thromboembolic disease (VTED). The clinical and laboratory data of 151 at risk pregnant women treated with dose adjusted LMWH thromboprophylaxis were analysed. The prevalence of VTED was 1.3% (95% CI, 0.1-4.7) and that of pregnancy-related bleeding was 2% (95% CI, 0.4-5.7). The bleeding events were considered to be unrelated to LMWH thromboprophylaxis. This study demonstrated that anti-Xa dose-adjusted LMWH is effective and safe in preventing VTED in at risk pregnant women.

Key words: pregnancy, thromboprophylaxis, anti-Xa dose adjusted enoxaparin

Introduction

Venous thrombo-embolic disease (VTED) is a major cause of maternal morbidity and mortality, contributing to approximately 3.2% of all-cause maternal mortality worldwide.[1,2] Normal pregnancy and the puerperium increases the risk of developing VTED by 10- to 20-fold compared to a matched non-pregnant population. This increased risk is largely due to physiological changes in the coagulation system in preparation for the major haemostatic challenge of delivery. Additional risk for VTED during pregnancy relates to relative stasis of venous blood flow due to the pressure-effects of the gravid uterus as well as vascular injury during delivery with release of large amounts of tissue factor from the placenta.[3,4] The risk of pregnancy related VTED is further increased in patients who have had a previous unprovoked VTED, VTED during a previous pregnancy or related to hormone exposure such as the oral contraceptive pill, positive family history of VTED and/or underlying inherited or acquired thrombophilia.[3,5] In addition to an increased risk of VTED, certain thrombophilic conditions are also associated with significant risk to the foetus such as placenta-mediated complications with resultant foetal loss or still births.[4]

Low molecular weight heparin (LMWH) is the preferred agent for thromboprophylaxis during pregnancy and the postpartum.[6] LMWH is also used to prevent recurrent pregnancy loss in patients with underlying thrombophilia or previous placental thrombosis because of its apparent beneficial effects on placental function and acceptable safety-profile during pregnancy.[4]

The use of LMWH during pregnancy, either for thromboprophylaxis or to treat VTED, is included in the American College of Chest Physicians (ACCP) and South African guidelines for current best practice.[6-8] These guidelines recommend the use of LMWH for thromboprophylaxis as opposed to only vigilant clinical monitoring during pregnancy and the puerperium in different patient groups (Table 1).[6,8] The use of LMWH in pregnancy currently remains an off-label recommendation largely due to difficult registration processes and ethical dilemmas of clinical studies in pregnancy.[4] However, the use of a LMWH in combination with low dose aspirin in pregnant patients with antiphospholipid syndrome is currently a grade 1A recommendation and proven to increase the rate of live births in this patient group.[6] Other anticoagulants such as warfarin and the Direct Oral Anticoagulants (DOACs) are contraindicated in pregnancy

since these drugs cross the blood-placenta barrier posing unacceptable risks to the foetus.[9]

Enoxaparin, a LMWH, is widely used in South Africa and laboratory monitoring of anticoagulant activity with anti-Xa levels is available to guide dose adjustment to achieve either a prophylactic or therapeutic target level. The target anti-Xa reference range for prophylactic dose LMWH at our centre in Johannesburg, South Africa, is 0.2-0.6 IU/mL which is in line with published recommendations.[10] Enoxaparin has a bio-availability of 91% and is linearly absorbed after subcutaneous administration. It has a predictable non-saturable, dose-independent renal elimination rate.[10,11] The dose is further adjusted according to body weight and renal function. Renal glomerular filtration rate and body weight increases throughout pregnancy, which potentially could affect the pharmacokinetics of LMWH. Anti-Xa adjusted dose LMWH is indicated for anticoagulation in pregnant patients with mechanical heart valves (grade 1A recommendation). There are however no clear guidelines for LMWH dose adjustment according to anti-Xa levels for VTED prophylaxis in the pregnant population without mechanical valves.[1,9]

Roeters van Lennep et al. retrospectively assessed the safety and efficacy of fixed low-dose LMWH during pregnancy and the postpartum in patients at high risk of VTED. Although this analysis demonstrated favourable outcomes in terms of safety, it highlighted the fact that fixed low-dose LMWH is not sufficiently effective in preventing VTED in these pregnant patients.[9]

Objectives

The objective of this study was to determine if adjusted-dose LMWH, based on anti-Xa levels, during pregnancy and the postpartum period, provided effective protection against VTED in at risk pregnant patients without a concomitant increase in bleeding.

Methods

Study population and data collection

This was a single-centre, retrospective cohort study performed at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), a quaternary healthcare facility in South Africa. The study was approved by the University of the Witwatersrand Human Research Ethics Committee (ref. no. M170527). The medical records of 151 consecutive pregnant patients at risk of VTED and treated with dose-adjusted thromboprophylactic LMWH during pregnancy and the postpartum were analysed. The patients were referred by obstetricians to the Haematology Department for thromboprophylaxis due to underlying VTED risk related to previous occurrences, underlying hypercoagulable test results or family history.

Demographic data collected on the cohort included age, ethnicity and weight. Obstetric history (previous pregnancy loss and outcomes), personal and family history of VTED and the results of thrombophilia tests were recorded. Current and previous anti-platelet and LMWH therapy were recorded with details of the gestational age at initiation of these agents. Laboratory results that were extracted from the laboratory information system included platelet counts, D-dimer and anti-Xa levels during pregnancy and the postpartum. The LMWH dose adjustments, gestational age at delivery, mode of delivery, complications during pregnancy focussing on bleeding and thrombotic events, pregnancy loss and complications during delivery and postpartum were recorded.

End points

The efficacy endpoint was pregnancy-related VTED and/or pregnancy loss despite anti-Xa guided dose-adjusted LMWH thromboprophylaxis. VTED was defined as a non-compressible venous segment on compression ultrasound of the deep or superficial venous system, and/or a diagnosis of pulmonary embolism on computed tomography scan of the pulmonary arteries (CTPA) or ventilation-perfusion scan. Pregnancy loss was defined as a miscarriage in any trimester including stillbirth. The safety endpoint was clinically significant antenatal or postpartum haemorrhage (>500ml) or development of a wound haematoma whilst on LMWH. The World Health

Organization (WHO) definition for postpartum haemorrhage and International Society on Thrombosis and Haemostasis (ISTH) definition for major bleeding and clinically relevant non-major bleeding were used to assess the safety end point (Table 2). Post-partum haemorrhage secondary to retained products of conception was excluded from the sub-cohort with bleeding complications.

Statistical analysis

Statistical analysis was performed on GraphPad Prism® version 7.04 software. Descriptive statistics of the study participants were tabulated and presented as either means with standard deviations if normally distributed or as medians with interquartile ranges (IQR) if not normally distributed. Percentages were presented for discrete variables. The absolute risk with 95% confidence interval (CI) was calculated for the following: 1) The prevalence of VTE despite adequate LMWH prophylaxis according to anti-Xa levels during pregnancy; 2) The prevalence of VTE despite adequate LMWH prophylaxis according to anti-Xa levels during the postpartum period; 3) The prevalence of pregnancy loss despite adequate LMWH prophylaxis according to anti-Xa levels; 4) The prevalence of significant bleeding during pregnancy or the postpartum period on dose adjusted LMWH. The Chi squared test of independence was used to examine the relationship between the prevalence of VTE in our study cohort versus that reported for fixed, low dose LMWH published in the literature. A p-value of <0.05 was considered as statistically significant.

Results

Study population demographics and baseline characteristics

The files of 113 consecutive pregnant patients on prophylactic LMWH were reviewed and 151 pregnancies were recorded for these patients. Enoxaparin (0.5mg/kg) was initiated in 150 of the 151 pregnant patients at various gestational ages depending on underlying VTE risk factors and previous pregnancy outcomes. One patient suffered a first trimester miscarriage just prior to starting enoxaparin. Three patients (2.0% of the cohort) defaulted postpartum follow-up (Figure 1). All the patients had normal body mass indices (BMI) and normal renal function tests as per estimated glomerular

filtration rate (eGFR). One patient was HIV positive and virally suppressed on anti-retroviral therapy. All patients were followed-up within 1 week of commencing enoxaparin to measure the anti-Xa level (target prophylactic range of 0.2-0.6 IU/mL). Seventy-three (48.3%) patients required LMWH dose adjustments due to sub-prophylactic anti-Xa levels and the majority (64.3%) of these dose adjustments were required in the 3rd trimester. A further 32.8% and 2.7% of patients required LMWH dose adjustment in the 2nd and 1st trimesters respectively. Adjustment in the enoxaparin dose was followed-up with repeat anti-Xa testing. Sub-prophylactic anti-Xa levels recorded in 2 patients during the 3rd trimester were not corrected with enoxaparin dose increases due to perceived increased bleeding risk related to placenta previa. The dose of enoxaparin was also not adjusted despite sub-prophylactic anti-Xa levels in a patient with heterozygous Prothrombin G20210A mutation with concomitant Von Willebrand's disease due to the perceived increased bleeding risk. These 3 pregnancies were not complicated by thrombosis or significant blood loss.
Patients previously diagnosed with an inherited or acquired thrombophilia comprised 62.8% of the study population (Figure 2). A previous VTE episode was recorded for 28% of these patients.

One patient with underlying Systemic Lupus Erythematosus (SLE) developed significant thrombocytopenia (platelet count < 80 x 10^9/L). Her pregnancy was complicated by an SLE exacerbation and subsequent infection requiring antibiotic treatment. The thrombocytopenia persisted throughout pregnancy and resolved postpartum. None of the patients developed heparin induced thrombocytopenia (HIT).

The majority (95.1%) of the patients underwent an elective Caesarean section (C/S) after cessation of LMWH 24 hours prior to spinal anaesthesia and surgery. Three patients had to undergo an emergency C/S for either ruptured membranes (1 patient) or preterm labour (2 patients). In all 3 instances, enoxaparin could be stopped more than 12 hours prior to C/S with no excessive peri- or postpartum bleeding. Four patients (2.6%) underwent normal vaginal delivery. Although 3 of these cases were uneventful, the 4th patient suffered prolonged protracted labour complicated by a left proximal deep vein thrombosis (DVT). The characteristics of the study population are further summarized in Table 3.

Prevalence of VTE during pregnancy and the postpartum

Two patients (1.3%; 95% CI, 0.1-4.7) developed proximal left leg DVT confirmed on compression ultrasound but without any clinical evidence of pulmonary embolism. Both patients had previous VTE events unrelated to pregnancy.

One patient (0.6%; 95% CI, 0.02-3.6) developed a DVT at 8 weeks gestation despite adequate anti-Xa levels on a dose of 40mg enoxaparin once daily (0.5 mg per kilogram) started at 6 weeks gestation. The enoxaparin dose was increased to 40mg twice daily for 3 months when the DVT was diagnosed with subsequent dose reduction to 60mg daily for the duration of the pregnancy and for 4 weeks postpartum at which time the D-dimer level returned to normal. This patient had an underlying Factor V Leiden (FVL) heterozygous mutation and a positive family history of VTED.

The second patient (0.6%; 95% CI, 0.02-3.6) developed a DVT during the postpartum period after prolonged protracted labour necessitating prolonged interruption of thromboprophylaxis. This patient had a previous history of VTED but normal thrombotic screen.

Prevalence of pregnancy loss

Four (2.6%; 95% CI, 0.7-6.6) of the 151 pregnancies analysed in this study were complicated by pregnancy loss. One patient suffered a miscarriage at 14 weeks gestation prior to starting LMWH. This patient was on warfarin therapy at the time of conception for a previous sagittal sinus thrombosis and was therefore at an increased risk of pregnancy loss. A subsequent pregnancy was uneventful after commencing prophylactic enoxaparin at 8 weeks gestation with discontinuation of warfarin pre-conception. The second pregnancy loss occurred in the second trimester despite adequate anti-Xa levels in a patient with a strong family history of VTED and recurrent pregnancy losses but with normal laboratory investigations for hypercoagulability. The cause for the pregnancy loss could not be determined. In the third case, pregnancy loss occurred in a patient with a history of recurrent pregnancy losses, underlying heterozygous prothrombin G20210A mutation and family history of VTED. This miscarriage occurred in the first trimester despite adequate LMWH prophylaxis as per anti-Xa levels and concomitant anti-platelet therapy (aspirin). The cause for this

pregnancy loss was also not clear. The fourth miscarriage occurred in a patient with Protein S deficiency who suffered 2 previous pregnancy losses due to placental infarctions. She miscarried at 10 weeks gestation despite adequate anti-Xa levels. The cause of the pregnancy loss could also not be determined.

Prevalence of significant bleeding episodes

Significant bleeding was observed in 3 (2%; 95%, CI, 0.4-5.7) of the 147 pregnancies not complicated by pregnancy loss. Antenatal bleeding complicated 2 (1.3%, 95% CI, 0.1-4.7) pregnancies. One bleeding episode was due to placenta previa requiring an emergency C/S at 31 weeks gestation. The other was related to recurrent haemorrhoidal bleeding necessitating blood transfusion in the third trimester for iron deficiency anaemia. Postpartum haemorrhage occurred in 1 (0.6%; 95% CI, 0.02-3.6) patient who required a uterine compression suture to achieve haemostasis. This patient did not require a blood transfusion. No post-surgery wound haematomas were recorded in the cohort. None of the patients were on anti-platelet agents.

Discussion

This retrospective cohort study evaluated the clinical and laboratory information of pregnant patients with an increased risk of VTED who received thromboprophylaxis with LMWH (enoxaparin) during pregnancy and in the postpartum period. The enoxaparin dose was adjusted antenatally and during the postpartum period according to anti-Xa levels. LMWH was administered during the postpartum period for a minimum of 4 weeks which was extended to 6 weeks if the D-dimer levels remained elevated. This study aimed to provide evidence for the clinical efficacy and safety of enoxaparin dose-adjustment to achieve adequate anti-Xa levels during pregnancy and the postpartum period in patients at risk of VTED.

The mean patient age in the current cohort was 32.3 years (range 22 - 45) and reflects the age distribution of obstetric patients in South Africa.[12] The racial distribution (82.3% Caucasian patients) in the cohort did not mirror that of the general population in South Africa and probably reflects the fact that the prothrombotic mutations, FVL and the Prothrombin G20210A, do not occur in the African black population.[13,14] These

mutations were present in 32% of the current cohort. Furthermore, only 1 patient was infected with human immunodeficiency virus (HIV) despite the high HIV prevalence in South Africa.[15] HIV infection increases the risk of VTED[13], however, there is a paucity of data pertaining to the increased VTED risk in pregnant HIV infected women. The under-representation of African black patients and HIV infection in this cohort is further discussed under limitations of this study.

We observed a prevalence of pregnancy-related VTED of 0.6% (95% CI, 0.02-3.6) in the antenatal, and 0.6% (95% CI, 0.02-3.6) in the postpartum period. The antenatal event occurred despite adequate thromboprophylaxis and was recorded for a patient with a previous history of VTED and heterozygosity for FVL. The postpartum event was related to prolonged protracted labour which resulted in prolonged thromboprophylaxis interruption in a patient with a previous DVT after a long-haul flight but with no family history of VTED and a normal thrombotic screen. No VTE events were recorded in patients with adequate anti-Xa levels in the postpartum period.

In a retrospective cohort study, Roeters van Lennep et al. assessed the efficacy of fixed, low dose LMWH for the prevention of VTED during pregnancy and the postpartum period in women at high risk of VTED. High risk patients included those with antithrombin deficiency, homozygosity for FVL or Prothrombin G20210A mutation or compound heterozygous states, women with a single episode of previous idiopathic VTED or previous VTED with an underlying thrombophilia and any women with recurrent VTED. Of the 91 women included in their study, 57 were categorised as high risk with a total of 82 pregnancies observed in the cohort during the study period. The high-risk group received prophylactic dose LMWH during pregnancy and the postpartum and those in the intermediate risk group (thrombophilia other than those mentioned in the high-risk group with a positive family history of VTED, those without thrombophilia but history of VTED in a first-degree family member and those with previous VTED due to a temporary risk factor) received only postpartum thromboprophylaxis. All 8 VTED events recorded in their study occurred in high-risk patients despite fixed, low dose prophylactic LMWH. Two of the 82 pregnancies were complicated by VTED in the antenatal and 5 in the postpartum period of which 2 occurred after LMWH was stopped. All 8 patients in this study who suffered from VTED had previous events of which 3 were pulmonary emboli and 5 of the previous events were hormone-related. Four patients had an underlying thrombophilia (1 patient was

heterozygous for FVL, 2 homozygous for FVL and 1 heterozygous for Prothrombin G20210A mutation).

Compared to the Roeters van Lennep et al. study, our study cohort had a higher frequency of inherited thrombophilia (62.8% versus 41.8%, p value 0.043) as well as a higher prevalence of high-risk markers i.e. antithrombin deficiency, single episode of previous idiopathic VTE or previous VTE with an underlying thrombophilia and recurrent VTE (57.5% versus 36.2%, p value 0.043). Additionally, there was no significant difference in the frequency of previous VTED among patients with underlying thrombophilia (28% versus 35%, p value 0.65). Our patient cohort had a significantly higher total number of high risk patients as set out above (80% versus 62%, p value 0.007). The favourable clinical outcome result (lower prevalence of VTED) in our study, compared to the Roeters van Lennep et al. study, was therefore achieved in a larger cohort of patients with a higher risk of VTED.

The prevalence of pregnancy loss observed in our study was 2.6% (95% CI, 0.7-6.6). This was distributed equally between the first and early second trimesters. Warfarin exposure occurred in 1 patient on lifelong Warfarin therapy for previous sagittal sinus thrombosis. Warfarin exposure is associated with a high rate of pregnancy loss and this was probably the underlying precipitating cause.[16] The other 3 patients each had a history of 2 or more previous miscarriages. Two of these patients had an underlying inherited thrombophilia (Protein S deficiency and Prothrombin G20210A mutation respectively). Obstetric causes, such as placental infarction or abruptio placenta, could unfortunately not be excluded in the patients who suffered miscarriages.

Significant pregnancy-related bleeding unrelated to pregnancy loss occurred in 2 patients (1.3%;95% CI, 0.1-4.7). One patient had placenta previa which is associated with an increased bleeding risk in the second half of pregnancy.[17] The second patient required a uterine compression suture. The indication for this was not clear but the patient did not require a blood transfusion. Sirico et al. reported a higher risk of blood loss postpartum in patients receiving thromboprophylaxis with LMWH compared to controls.[18] None of the patients in our cohort suffered from immediate or delayed postpartum haemorrhage. We therefore consider the use of LMWH maintaining prophylactic anti-Xa levels safe during pregnancy and in the postpartum period. The risk of pregnancy and non-pregnancy related bleeding should however always be weighed up against the benefit of VTED prevention.

In conclusion, maintaining adequate anti-Xa levels during pregnancy and the postpartum in patients on thromboprophylactic LMWH appears efficacious and safe. We recommend routine anti-Xa monitoring and dose adjustment in all pregnant women on prophylactic LMWH during pregnancy and the postpartum.

Limitations

Limitations of this study include the retrospective design, absence of a control group receiving fixed low dose LMWH during pregnancy and lack of data pertaining to the cause of pregnancy losses as discussed above.

Furthermore, the racial distribution of the patient cohort does not mirror that of the South African population. It was previously mentioned that 32% of our study cohort had underlying FVL and/or Prothrombin G20210A mutations. These mutations do not occur in the black African population which partially explains the racial bias. However, as a racial predilection for VTED is present in black patients resulting in an increased risk of postpartum VTED compared to Caucasian patients, an adequate representation of black African patients would have been meaningful.[19]

Lastly, HIV infection is endemic in South Africa and people living with HIV infection are at an increased risk of developing VTED.[13] Studies are needed to assess the risk of VTED in the HIV positive pregnant patients to guide prophylactic anticoagulation practices in this group of patients. Only 1 patient in the current study was HIV positive, therefore, our study could not make recommendations in this regard.

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Table 1. ACCP (2012) and SASTH (2013) guidelines for risk stratification and thromboprophylaxis during pregnancy and the puerperium

1) Patients at moderate or high risk for recurrent VTE: Risk factors include previous unprovoked VTE, VTE during a previous pregnancy and oestrogen-related VTE (Grade 2C).[6]

2) Patients with hereditary thrombophilia considered high risk for VTE (homozygous factor V Leiden or prothrombin 20210A mutations) with no previous VTE but with a positive family history of VTED (Grade 2B).[6]

3) Patients with any other hereditary thrombophilia and no previous VTE but with a positive family history of VTED. In these patients, ante-partum clinical surveillance and post-partum thromboprophylaxis with LMWH is suggested (Grade 2C).[6]

4) Patients with anti-phospholipid syndrome. LMWH combined with low-dose aspirin is recommended (Grade 1B).[6]

5) These guidelines further recommend the discontinuation of LMWH at least 24 hours prior to caesarean section (Grade 1B).[6] Thromboprophylaxis should be recommenced or initiated 12 hours after the caesarean section if there is no active significant haemorrhage and continued for 4 to 6 weeks post-partum at a prophylactic dose of 0.5 mg/kg i.e. 40mg subcutaneous (SC) administration in normal weight individuals or 60mg SC in obese patients (body mass index >35).[8]

ACCP, American College of Chest Physicians; SASTH, Southern African Society of Thrombosis and Haemostasis.

Table 2. The WHO and ISTH definitions of major bleeding, clinical-relevant non-major bleeds and post-partum haemorrhage [9,20]	
Major bleeding in the non-surgical patient	• Symptomatic blood loss leading to demise of the patient or with extravasation into an anatomical site considered critical (e.g. central nervous system, intra-ocular, retro-peritoneal, intra-articular, pericardial or within a muscular compartment) • Blood loss causing a drop-in haemoglobin by at least 2 g/dl or requiring blood transfusion of at least 2 units of blood.
Clinically relevant non-major bleeding in the non-surgical patient	Blood loss that does not fit the definition for major bleeding, however, meets one more of the following criteria: • Requiring medical intervention • Leads to hospitalization • Prompts seeking consultation (face-to-face)
Post-partum haemorrhage (WHO criteria)	Blood loss of more than 500ml from the genital tract during the first 24 hours post-delivery

WHO, World Health Organisation; ISTH, International Society of Thrombosis and Haemostasis.

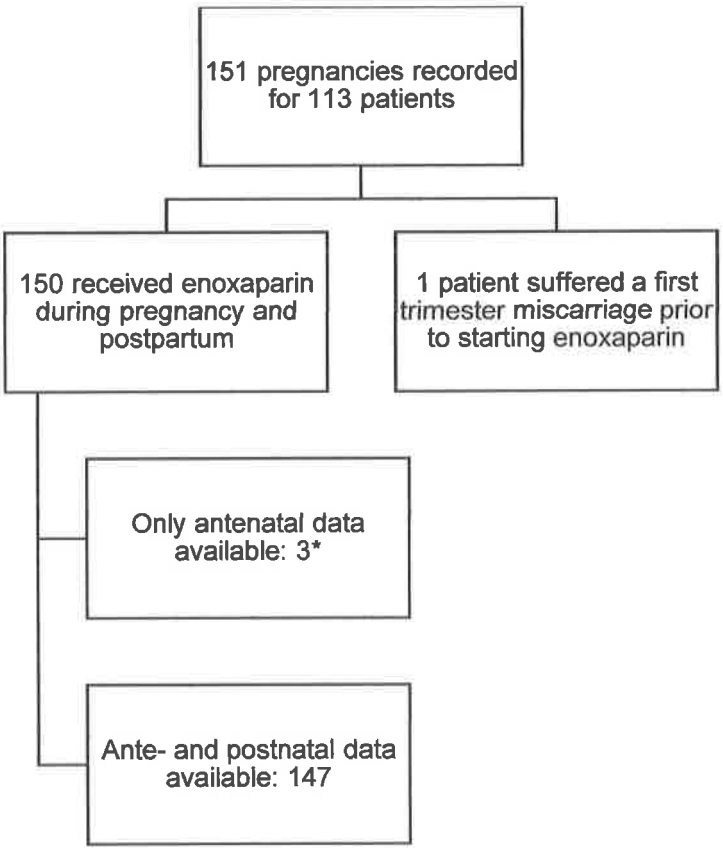


Figure 1. Flow chart for the study population

*These 3 patients defaulted postpartum follow-up

Table 3. Demographic data and baseline characteristics of the study population	
Characteristics	All women (n = 113)
Number of pregnancies	151
Age of patients (years) mean ± SD	32.3 ± 4.0
Ethnicity, n (%)	
Caucasian	93 (82.3)
African Black	5 (4.4)
Coloured	9 (7.9)
Indian	5 (4.4)
Asiatic	1 (0.8)
HIV infection, n (%)	1 (0.8)
Parity, median of all pregnancies (IQR range)	0 (0-2)
Gravidity, median of all pregnancies (IQR range)	2 (1-3)
Primigravida, n (%)	77 (50.9)
Previous miscarriages, n (%)	29 (25.6)
≥ 3 miscarriages, n (%)	7 (6.2)
Previous VTE, n (%)	72 (63.7)
Previous idiopathic VTE, n (%)	10 (8.8)
Recurrent, n (%)	12 (10.6)
Pregnancy-related, n (%)	10 (8.8)
Related to oestrogen therapy, n (%)	31 (27.4)
Other acquired causes, n (%)*	9 (7.9)
Previous Pulmonary embolism, n (%)	25 (22.1)
Thrombophilia diagnosis, n (%)	72 (62.8)
With previous VTE, n (% of all women)	32 (28.0)
With ≥ 3 miscarriages, n (% of thrombophilia)	5 (6.9)
Family history of VTE, n (%)	37 (32.7)

Gestational age at which LMWH was commenced (weeks) mean ± SD**	13.9 ± 9.0
Twin pregnancy, n (%)	8 (5.3)
IVF, n (% of all pregnancies)	7 (4.6)
Pregnancy outcome	
Live births (% of all pregnancies)	147 (97.3)
Delivery route	
Caesarean section, n (% of all live births)	140 (97.2)
Emergency, n (% of Caesarean section)	3 (2.1)
Vaginal delivery, n (% of all live births)	4 (2.7)
Required enoxaparin dose adjustment, n (% of all pregnancies)	73 (48.3)
First trimester, n (%)	2 (2.7)
Second trimester, n (%)	24 (32.8)
Third trimester, n (%)	47 (64.3)
Pre-term deliveries, n (%)***	8 (5.5)
Concomitant Aspirin use, n (%)	20 (13.2)
With significant bleeding episode, n (%)	0 (0)
Thrombocytopenia while on LMWH, n (%)	1 (0.66)
Considered LMWH-related, n (%)	0 (0)

IVF, in vitro fertilisation; HIV, Human Immunodeficiency Virus.

*Includes five cases related to surgery, two to long haul flights and a single case related to HIV infection.

**Data for 147 patients available. ***Data for 141 patients available.

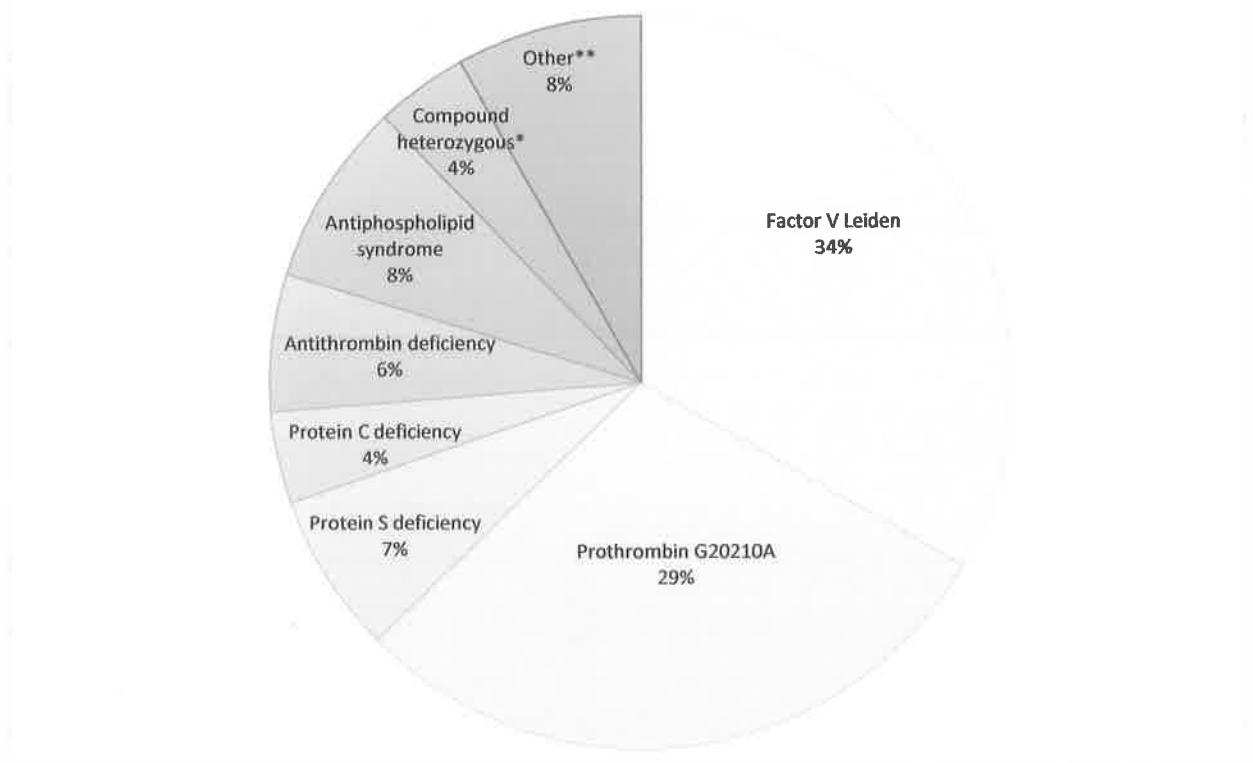


Figure 2. Thrombophilia diagnosis and acquired thrombophilic conditions

*Compound heterozygous states included FVL/Prothrombin G20210A, FVL/Protein S deficiency and Prothrombin G20210A/Antithrombin deficiency.

**'Other: 1 patient with Essential Thrombocythemia (JAK2 V617F mutation positive), 1 patient with rheumatoid arthritis, 1 patient with Systemic Lupus Erythematosus (SLE), 1 patient with lower limb arterio-venous malformation, 2 patients with platelet hypersensitivity.

Appendix A

Approved research protocol

RESEARCH TITLE:

The safety and efficacy of adjusted-dose enoxaparin (Clexane®) in pregnant patients with increased risk for venous thrombo-embolic disease: A retrospective analysis of clinical and laboratory data.

STUDENT

NAME : Dr Jenique Bailly, MBChB, DipPEC(SA)

DEGREE : Master of Medicine in Haematology

STUDENT NO : 1481330

INSTITUTION : Department of Molecular Medicine and Haematology,

University of the Witwatersrand

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SUPERVISORS

1. Professor Barry F. Jacobson, MBChB, MMed, FRCS, PhD, FCPath (Haem)

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MMed Protocol

The safety and efficacy of adjusted-dose enoxaparin (Clexane®) in pregnant patients with increased risk for venous thrombo-embolic disease: A retrospective analysis of clinical and laboratory data

Background

Venous thrombo-embolism (VTE) is a major cause of maternal morbidity and mortality. Normal pregnancy and the puerperium puts the patient at a 10 to 20 times increased risk for developing thrombo-embolic disease compared to the normal non-pregnant population. This increased risk is largely due to physiological coagulation changes that occur during pregnancy to prepare the body for the major haemostatic challenge of delivery. This risk is even higher in patients with additional risk factors for VTE e.g. previous unprovoked VTE, VTE during previous pregnancy, positive family history of VTE and/or underlying thrombophilic conditions (1).

The use of anticoagulant drugs during pregnancy carries risks for both mother and foetus. This risk necessitates close clinical and laboratory monitoring (1-3). Low molecular weight heparin (LMWH) is currently the preferred agent for thromboprophylaxis during pregnancy and the puerperium. Currently, its use in pregnancy (either for thromboprophylaxis or to treat venous thrombo-embolic disease), forms part of the America College of Chest Physicians (ACCP) and South African guidelines for current best practice (2, 4, 5). Other anticoagulants such as Warfarin and the Direct Oral Anticoagulants (DOACs) are contraindicated in pregnancy (6).

The optimal approach to thromboprophylaxis using LMWH with regards to dosing has not been established in pregnancy (6). Clinical recommendations are mostly based on data obtained from studies conducted on non-pregnant participants and anecdotal experience (7). The use of LMWH in pregnancy remains an off-label recommendation largely due to difficult registration processes (8).

Numerous studies have evaluated the use of fixed-dose LMWH during pregnancy. Roeters et al retrospectively assessed the safety and efficacy of fixed low-dose LMWH during pregnancy and post-partum in patients at increased risk for developing VTE. Although this analysis demonstrated favourable outcomes in terms of safety, the study highlighted the fact

that fixed low-dose LMWH is not sufficiently effective in preventing thrombo-embolic events in pregnant patients at high-risk of VTE (6).

The latest ACCP (2012) and South African guidelines (2013) recommend the following patient groups for prophylaxis with LMWH as opposed to only vigilant clinical monitoring during pregnancy and the puerperium:

1) Patients at moderate or high risk for recurrent VTE: Risk factors mentioned include previous unprovoked VTE, VTE during a previous pregnancy and oestrogen-related VTE (Grade 2C) (2).

2) Patients with hereditary thrombophilia considered high risk for VTE (homozygous factor V Leiden or prothrombin 20210A mutations) with no previous VTE but with a positive family history of thrombo-embolic disease (Grade 2B) (2).

3) Patients with any other hereditary thrombophilia and no previous VTE but with a positive family history of thrombo-embolic disease. In these patients, ante-partum clinical surveillance and post-partum thromboprophylaxis with LMWH are suggested (Grade 2C) (2).

4) Patients with anti-phospholipid syndrome, LMWH combined with low-dose aspirin is recommended (Grade 1B) (2).

These guidelines further recommend the discontinuation of LMWH at least 24 hours prior to caesarean section (Grade 1B) (2). Thromboprophylaxis should be recommenced or initiated 12 hours after the caesarean section if there is no active significant haemorrhage and continued for 4 to 6 weeks post-partum at at a prophylactic dose of ½ mg/kg i.e. 40mg subcutaneous (sc) administration in normal weight individuals or 60mg sc in obese patients (body mass index >35) (5).

This study aims to assess the safety and efficacy of adjusted-dose enoxaparin[1] in pregnant patients with a perceived increased risk for VTE. Enoxaparin has a 91% bioavailability, is linearly absorbed after subcutaneous administration and has a predictable non-saturable, dose-independent elimination rate. Its clearance is almost entirely renal dependant (9, 10).

[1] Enoxaparin is commonly used in clinical practise in South Africa and adequate laboratory monitoring is readily available for this drug. Laboratory monitoring for enoxaparin is not interchangeable for other LMWHs.

LMWH activity is assessed with an anti-Xa quantitative assay. The anti-Xa assay guides dose adjustments to achieve a specific target range. Currently the targeted anti-Xa reference range for prophylactic dose LMWH is 0.2-0.6 IU/mL (10).

Due to the increased glomerular filtration rate resulting in increased drug clearance and changing body weight, enoxaparin activity monitoring with anti-Xa levels and dose adjustment to achieve the targeted reference range of 0.2-0.6 IU/mL is indicated during pregnancy (9, 10). Currently, LMWH dose adjustments guided by laboratory monitoring during pregnancy is a grade 1A recommendation in patients with mechanical heart valves. There is however no clear guideline for VTE prophylaxis and treatment in the rest of pregnant population (1). Prospective studies assessing pregnant patients with perceived increased risk for VTE on adjusted-dose LMWH based on desired anti-Xa levels versus fixed-dose LMWH are needed, but are difficult to perform given the reluctance of both medical practitioners and patients to participate in them. Retrospective case series will remain a cornerstone in obtaining data on the safety and efficacy of current practise.

Aim and study rationale

LMWH is widely used for thromboprophylaxis during pregnancy and the puerperium. Its use in certain at risk groups for VTE forms part of international and South African guidelines for best current practice (2, 5). Adequate dosing regimens to safely prevent VTE in pregnant patients at increased risk for VTE are currently not established (7). Numerous pregnancy-related physiological changes, such as weight gain and increased glomerular filtration rates, affect the pharmacokinetics of LMWH (9). Fixed, low-dose LMWH is not sufficiently effective in preventing VTE in patients at high-risk for developing VTE (6).

This study aims to retrospectively assess the safety and efficacy of adjusted-dose enoxaparin based on anti-Xa levels in pregnant patients with an increased risk for developing VTE. Outcomes of this retrospective data analysis will be compared against the findings of previous studies in the literature measuring similar endpoints using fixed-dose LMWH in this group of patients.

- Primary efficacy endpoint: VTE and/or pregnancy loss despite adjusted-dose LMWH aimed at maintaining adequate anti-Xa levels throughout pregnancy.

- Secondary efficacy endpoint: VTE during the post-partum period despite fixed-dose LMWH.
- Safety endpoints: Clinically significant antepartum bleeding, post-partum haemorrhage (>500ml) or wound haematoma up to 4 weeks post-partum[2].

Objectives

- To determine if adjusted-dose enoxaparin, based on anti-Xa levels during pregnancy safely provides effective protection against VTE in at risk pregnant patients.
- To determine if fixed-dose enoxaparin during the post-partum period safely provides effective protection against VTE in at risk patients.
- To compare the outcomes of this retrospective data analysis against previously published results in this patient population.

Methods

Study design

A single centre retrospective cohort study.

Study population and data analysis

The medical records of one hundred and fifty (150) consecutive pregnant patients at risk for VTE will be analysed (refer to inclusion and exclusion criteria below). The patient cohort will be enrolled from the practice of Prof. B.F. Jacobson based at Charlotte Maxeke Academic Hospital (CMJAH). Permission to use this data has been granted by Professor B.F. Jacobson and he is also a study supervisor (see annexure B).

[2] All patients in this cohort were followed-up until 4 weeks post-partum, unless prolonged thromboprophylaxis was indicated necessitating prolonged follow-up.

Inclusion criteria

- Pregnant patients with an increased VTE risk requiring VTE prophylaxis with dose adjusted enoxaparin during pregnancy until 4 weeks post-partum.

Exclusion criteria

- Renal failure with an estimated glomerular filtration rate (eGFR) below 30 mL/min per 1.73 m^2 in order to eliminate the influence of decreased renal excretion on D-dimer levels.
- Incomplete follow-up due to patient non-compliance.

The following demographics and clinical details will be recorded:

- Patient age and ethnicity
- Gestational age at presentation and outcomes of previous pregnancies
- Risk factor(s) for VTE (see annexure D)
- Relevant past medical history e.g. previous VTE, family history of VTE or pregnancy losses
- Concomitant drugs
- Weight at presentation and follow-up visits
- D-dimer levels at baseline and follow-up visits
- Doses of enoxaparin prescribed during the follow-up period
- Anti-Xa levels throughout the follow-up period
- Clinical and radiological assessments to exclude VTE
- Mode of delivery and details of any complications
- Occurrence of significant post-partum haemorrhage, bleeding episodes or wound haematoma

All data will be kept anonymous and a study number will be allocated prior to analysis. The link between patient identifying details and study number will only be available to the study doctor.

Safety of enoxaparin use will be defined as no clinically significant bleeding episodes during pregnancy, no major blood loss during the post-partum period, or wound haematoma formation up to 4 weeks post-partum.

The World Health Organization (WHO) definition for post-partum haemorrhage and International Society on Thrombosis and Haemostasis (ISTH) definition for major bleeding and clinically relevant non-major bleeding will be used to assess for the safety end points (see annexure A). The development of a wound haematoma whilst on Enoxaparin will be included. Post-partum haemorrhage secondary to retained products of conception will be excluded from the above.

Data collation and identification:

150 consecutive patients meeting the in- and exclusion criteria will be enrolled in this retrospective record review. The data collected from the patient files and the laboratory information system will be collated and transferred to a data collection sheet (see annexure C). All data will be kept anonymous with each case being assigned a study number.

Ethical considerations

Ethical clearance from the University of the Witwatersrand Human Research Ethics Committee will be requested prior to the initiation of the study. Individual informed consent will not be required for this retrospective data analysis. All data will be kept anonymous and a study number will be allocated prior to analysis.

Statistics

Data analysis will be performed under the guidance of a biostatistician.

Descriptive statistics of the study participants will be tabulated and presented using means /medians and ranges for continuous values and percentages for discrete variables.

- All normally distributed data will be recorded as a mean with +-SD.
- All data not normally distributed will be recorded as median with interquartile range.
- Absolute risk with 95% CI will be calculated for VTE and bleeding episodes.
- The above will be determined for:
    - The prevalence of VTE despite adjusted-dose enoxaparin maintaining adequate Anti-Xa levels
    - The prevalence of VTE despite fixed-dose enoxaparin until at least 4 weeks post-partum
    - The prevalence of significant bleeding episodes
        - Significant ante-partum haemorrhage
        - Post-partum haemorrhage
- Calculating the absolute risk:

  The ratio of people who had either VTE or a significant bleeding episode compared to the study population (who is at risk of developing the above events).
- Formula used to calculate prevalence:

  Prevalence rate = (n / total study population) x 10^n

  Where n is all new cases with either VTE or a significant bleeding episode
- Kaplan–Meier survival analysis to demonstrate the timing of VTE or bleeding events.
- The Chi squared test of independence will be used to examine the relationship between the prevalence of VTE and the dose of enoxaparin used by comparing the collected data to that previously recorded in the literature assessing the efficacy of fixed-dose enoxaparin.
- P-values will be given with a p-value of <0.05 considered as statistically significant.
- The above statistical parameters will be calculated using either Microsoft Excel, Stata or Medicalc with the assistance of a statistician where necessary.

Study timeline

	April/May 2017	August 2017	January 2018	February/March 2018	April 2018
PROTOCOL					
ETHICS					
EXECUTION					
ANALYSIS					
WRITE UP					
SUBMISSION					

Cost

No expenses are anticipated as this is a retrospective data analysis.

Conflict of interest

No conflict of interest is foreseen.

References

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Annexure A:

Definition of major bleeding, clinical-relevant non-major bleeds and post-partum haemorrhage as per the World Health Organisation (WHO) and International Society of Thrombosis and Haemostasis (ISTH) (11).

Major bleeding in the non-surgical patient	• Symptomatic blood loss with death as a result or with extravasation into an anatomical site considered to be critical (e.g. central nervous system including spinal, intra-ocular, retro-peritoneal, intra-articular, pericardial or within a muscular compartment causing significantly increased intra-compartmental pressure). • Blood loss causing a drop-in haemoglobin by at least 2 g/dl or requiring blood transfusion of at least 2 units of blood.
Clinically relevant non-major bleeding in the non-surgical patient	Blood loss that does not fit the definition for major bleeding and meets one more of the following criteria: • Requiring medical intervention • Leads to hospitalization • Prompts seeking consultation (face-to-face)
Post-partum haemorrhage (WHO criteria)	• Blood loss of more than 500ml from the genital tract during the first 24 hours post-delivery

Annexure B:

Professor Barry F. Jacobson

Department of Molecular Medicine and Haematology

Charlotte Maxeke Johannesburg Academic Hospital

Johannesburg

South Africa

Dear Professor Jacobson

RE: Request for permission to retrospectively analyse patient clinical records:

I am requesting permission to analyse patient clinical and laboratory data. The clinical and laboratory data were collected during the course of routine management of patients and will be anonymized. This data will be analysed and presented as part of an MMed project.

Kind regards,

Dr Jenique Bailly

Haematology Registrar

Department of Molecular Medicine and Haematology

University of the Witwatersrand, Johannesburg, South Africa

I, Professor Jacobson, give permission for access and analysis of all anonymized patient related data from the NHLS Laboratory Information System and my database for the purpose of MMed project.

Professor Barry F. Jacobson

Annexure C:

DATA COLLECTION SHEET **STUDY NUMBER:**	
Form A: Patient demographics	
Date of baseline assessment	
Age of patient	
Ethnic origin	
Gravidity and Parity Previous pregnancy loss	
Form B: Clinical information	
Primary thrombophilia diagnosis and date of diagnosis	
Gestational age at first visit	
Gestational age when commencing Enoxaparin and starting dose	
Singleton or twin pregnancy	
Previous VTE defined as clinical deep vein thrombosis and/or pulmonary embolism	
Family history of VTE	
eGFR	
Weight, height and BMI at first consult	
Was enoxaparin stopped 24 hours before delivery?	
Was enoxaparin commenced 12 post-delivery? If not, reason?	
Was enoxaparin continued until 4 weeks post-partum?	
Any recorded complications during delivery	
Anti-Xa levels and possible dose adjustments	
D-dimer levels at baseline and subsequent measurements	
Clinical suspected DVT: • Gestational age • D-dimer levels • Radiological assessments	
Clinical significant bleeding • Ante-partum • Post-partum	

Annexure D:

Risk factors for VTE as set out by the ACCP guidelines for thromboprophylaxis in pregnancy (2).

Major risk factors
• Previous VTE (especially previous unprovoked VTE, VTE during previous pregnancies or oestrogen-related VTE) • Thrombophilia such as Factor V Leiden, Prothrombin gene mutation G20210A and antithrombin deficiency, especially if a positive family history of VTE • Antiphospholipid syndrome • Strict bed rest for more than 7 days during pregnancy
Minor risk factors
• Thrombophilia such as Protein S or Protein C deficiency, especially if a positive family history of VTE

Appendix B

Ethics clearance certificate

R14/49 Dr Jenique Bailly

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170527

NAME: (Principal Investigator)	Dr Jenique Bailly
DEPARTMENT:	Molecular Medicine and Haematology University of the Witwatersrand National Health Laboratory Services
PROJECT TITLE:	The Safety and Efficacy of Adjusted Enoxaparin (Clexane) in Pregnant Patients with Increased Risk for Venous Thrombo-Embolic Disease. A Retrospective Analysis of Clinical and Laboratory
DATE CONSIDERED:	26/05/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof BF Jacobson and Dr Susan Louw
APPROVED BY:	Professor P. Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	19/05/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004,10th floor, Senate House/3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially review May and will therefore be due in the month of May each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date 20/6/17.

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix C

Turn-it-in report

1481330:MMed_for_submission_Turnitin.docx

ORIGINALITY REPORT

14%	4%	14%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	J. E. ROETERS VAN LENNEP. "Prophylaxis with low-dose low-molecular-weight heparin during pregnancy and postpartum: is it effective? : Low-dose LMWH during pregnancy and postpartum", Journal of Thrombosis and Haemostasis, 03/2011 Publication	2%
2	"Anticoagulation Therapy", Springer Nature, 2018 Publication	2%
3	K E Hodkinson, J N Mahlangu. "Deep-vein thrombosis in the era of high HIV and tuberculosis prevalence: A prospective review of its diagnosis and treatment in a quaternary centre", South African Medical Journal, 2017 Publication	1%
4	Shannon M. Bates, Ian A. Greer, Saskia Middeldorp, David L. Veenstra, Anne-Marie Prabulos, Per Olav Vandvik. "VTE, Thrombophilia, Antithrombotic Therapy, and Pregnancy", Chest, 2012	1%

	Publication	
5	www.ajol.info Internet Source	1%
6	"Posters", Clinical Microbiology and Infection, 4/2007 Publication	1%
7	Calderwood, C.J.. "Thromboembolism and thrombophilia in pregnancy", Current Obstetrics & Gynaecology, 200612 Publication	<1%
8	"Principles of Critical Care in Obstetrics", Springer Nature, 2016 Publication	<1%
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10	link.springer.com Internet Source	<1%
11	Ana Boban, Samuel Paulus, Catherine Lambert, Cedric Hermans. "The value and impact of anti-Xa activity monitoring for prophylactic dose adjustment of low-molecular-weight heparin during pregnancy", Blood Coagulation & Fibrinolysis, 2017 Publication	<1%
12	Submitted to University of Aberdeen	

	Student Paper	<1%
13	Kate Bramham, Andrew Retter, Susan E. Robinson, Michael Mitchell, Gary W. Moore, Beverley J. Hunt. "How I treat heterozygous hereditary antithrombin deficiency in pregnancy", Thrombosis and Haemostasis, 2017 Publication	<1%
14	Lussana, F.. "Pregnancy-related venous thromboembolism: Risk and the effect of thromboprophylaxis", Thrombosis Research, 201206 Publication	<1%
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27	"Data on Aminopenicillins Reported by Researchers at University of L'Aquila.(Report)", Biotech Week, July 4 2012 Issue	<1%

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30	Kaatz, S., D. Ahmad, A.C. Spyropoulos, and S. Schulman. "Definition of clinically relevant non-major bleeding in studies of anticoagulants in atrial fibrillation and venous thromboembolic disease in non-surgical patients: communication from the SSC of the ISTH", Journal of Thrombosis and Haemostasis, 2015. Publication	<1%
31	"Abstracts", Journal of Thoracic Oncology, 2009 Publication	<1%
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Appendix D

Clinical Obstetrics and Gynecology journal author guidelines

Clinical Obstetrics and Gynecology

Instructions for Authors

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Journal article
1. Magrina JF, Walter A, Schild S, et al. Laparoscopic radical parametrectomy and pelvic and aortic lymphadenectomy for vaginal carcinoma: A case report. *Gynecol Oncol* 1999;75:514–516.

Book chapter
2. Mendelsohn ML. The somatic mutational component of human carcinogenesis. In: Moolgavkar SH, ed. *Scientific Issues in Quantitative Risk Assessment.* New York: Birkhaeuser Boston, 1990:22–31.

Entire book
3, Nielson TF, Sachs BP, Beard R, et al. *Reproductive Health Care for Women and Babies.* Oxford: Oxford University Press, 1995:279.

Software
4. Epi Info [computer program]. Version 6. Atlanta: Centers for Disease Control and Prevention; 1994.

Online journals
5. Friedman SA. Preeclampsia: a review of the role of prostaglandins. Obstet Gynecol [serial online]. January 1988;71:22–37. Available from: BRS Information Technologies, McLean, VA. Accessed December 15, 1990.

Database
6. CANCERNET-PDQ [database online]. Bethesda, MD: National Cancer Institute; 1996. Updated March 29, 1996.

World Wide Web
7. Gostin LO. Drug use and HIV/AIDS [*JAMA* HIV/AIDS web site]. June 1, 1996. http://www.ama-ssn.org/special/hiv/ethics. Accessed June 26, 1997.

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2. Create, Scan and Save your artwork and compare your final figure to the Digital Artwork Guideline Checklist (below).
3. Upload each figure to Editorial Manager in conjunction with your manuscript text and tables.

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