Factors that influence heparin levels in patients with venous thromboembolism treated with subcutaneous weight-adjusted unfractionated heparin and low-molecular weight heparin, and whether heparin levels are associated with bleeding and recurrent venous thromboembolic events

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1. Abstract

1.1. Background It is uncertain whether 1) patient's characteristics (e.g., age, weight, height, and sex) influence anti-Xa heparin levels (hereafter referred to as "heparin levels"), or 2) if heparin levels influence recurrent venous thromboembolism (VTE) or bleeding events, in patients with acute VTE treated with weight-adjusted therapeutic-dose subcutaneous (SC) unfractionated heparin (UFH) or SC low-molecular weight heparin (LMWH). To determine if either association exist, we analyzed data from the Fixed-Dose Heparin (FIDO) study, in which patients were randomized to either SC UFH or SC LMWH, each given in fixed weight-adjusted doses and overlapped with 3 month of warfarin therapy for treatment of acute VTE.

1.2. Methods During the original study, 708 patients were asked to participate in a sub-study that would measure peak heparin levels while they were treated with heparin. 408 patients provided blood samples and met the eligibility criteria for the analyses in this thesis. Linear regression was used to examine the influence of patients' baseline characteristics (e.g., age, weight, height, body mass index [BMI], sex) on heparin levels. The influence of other factors (e.g., type of heparin [UFH or LMWH]) on heparin levels was also assessed. Logistic regression was used to examine the association of heparin levels with the

outcomes of 1) recurrent VTE during 3 months of follow up, and 2) bleeding events in the first 10 days of follow up.

1.3. Results: Mean heparin levels were 0.695 in patients treated with UFH, 0.698 in those treated with dalteparin and 1.034 in those treated with enoxaparin ($p<0.001$; $R^2=0.08$ for variability accounted for by type of heparin). In a univariable analysis, heparin levels increase by 0.04 IU/ml (95% CI 0.02-0.07; $p=0.001$; $R^2=0.03$) for every 10-kg increment in weight, by 0.02 IU/ml (95% CI 0.01-0.03; $p<0.001$; $R^2=0.04$) for each unit of BMI, and by 0.03 IU/ml (95% CI 0.01-0.05; $p=0.001$; $R^2=0.03$) for every 10 μmol/l increment in creatinine. In a multivariable analysis, weight, BMI, and creatinine still influenced heparin levels, after adjusting for type of heparin and timing of blood sample withdrawal. Although heparin levels increased with weight, the magnitude was not large enough to suggest altering the current weight-based dosing method for LMWH. Other baseline factors such as age, height, type of VTE, creatinine clearance and hospitalization status did not influence heparin levels in patients treated with UFH or LMWH. In a univariable analysis, when heparin levels were treated as a continuous variable, higher heparin levels were associated with a lower risk of recurrent VTE at 90-days in patients treated with LMWH (OR 0.04, 95% CI 0.003-0.550, for each 1.0 IU/ml increase in heparin levels), but not in patients

treated with UFH (OR 1.46, 95% CI 0.37-5.58, for each 1.0 IU/ml increase in heparin levels). In addition, higher heparin levels were associated with a higher risk of bleeding at 10-days in patients treated with UFH (OR 3.32, 95% CI 1.30-8.46 for each 1 IU/ml increase in heparin levels) but not in patients treated with LMWH (OR 3.77, 95% CI 0.42-33.92, for each 1.0 IU/ml increase in heparin levels). In a multivariable analysis, the association of heparin levels with VTE at 90-days in patients receiving LMWH (lower VTE events) and with bleeding events at 10-days in patients receiving UFH (higher bleeding events) persisted after adjusting for antiplatelet use at baseline and diagnosis of cancer at baseline. When heparin levels were treated as a dichotomous variable (subtherapeutic vs. non-subtherapeutic levels and supratherapeutic vs. non-supratherapeutic levels), the proportion of patient with recurrent VTE was significantly higher in patients with subtherapeutic levels compared with non-subtherapeutic levels in patients receiving LMWH (8.6% vs. 1.3%, p = 0.01). No significant difference was found in the proportion of patients with subtherapeutic levels and non-subtherapeutic levels in patients receiving UFH (0% vs. 3.4%, χ^2=0.15, p= 0.70). The test of interaction supported the decision to analyze LMWH and UFH groups separately (p=0.02). Finally, the proportion of patient with bleeding was higher in patients with supratherapeutic compared with non-supratherapeutic

heparin levels (6.5% vs. 1.5%, χ^2=7.65, p=0.01). The test of interaction did not support the decision to analyze LMWH and UFH groups separately (p=0.13).

1.4. Conclusions Although it was possible to identify factors that were associated with heparin levels in patients who had been treated with weight-adjusted UFH or LMWH, none of these associations were strong enough to suggest that variables other than weight should influence SC heparin dosing. Subtherapeutic heparin levels were associated with a higher risk of recurrent VTE in patients treated with LWMH but not UFH, and supratherapeutic heparin levels were associated with a higher risk of bleeding in patients treated with UFH but not LMWH. Indirectly, these findings suggest that adjusting UFH or LMWH dose in response to heparin levels might improve clinical outcomes.

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children, Haider and Hana, the two joys of my life, thank you for your understanding and patience.

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4. List of Common Abbreviations

AC	Anticoagulant
AD	Absolute Difference
aPTT	Activated Partial Thromboplastin Time
AT	Antithrombin
BID	Twice-Daily
BMI	Body Mass Index
CI	Confident Interval
CrCl	Creatinine Clearance
CTPA	Computed Tomography Pulmonary Angiography
DVT	Deep Vein Thrombosis
EM	Expectation Maximization
FIDO	FIxed DOse Heparin Study
GoF	Goodness of Fit

HL	Hosmer–Lemeshow
INR	International Normalized Ratio
IV	Intravenously
LMWH	Low-Molecular-Weight-Heparin
MCAR	Missing Completely at Random
MAR	Missing at Random
MNAR	Missing Not at Random
OD	Once-Daily
OLS	Ordinary Least-Squares
OR	Odds Ratio
P-P	Probability–Probability
PE	Pulmonary Embolism
PTP	Pretest Probability
PF4	Platelet Factor 4

R^2	Coefficient of Determination
RCT	Randomized Controlled Trial
RR	Relative Risk
SC	Subcutaneous
US	Ultrasound
UFH	Unfractionated Heparin
VKA	Vitamin K Antagonist
V/Q	Ventilation Perfusion
VIF	Variance Inflation Factor
VTE	Venous Thromboembolism

5. Declaration of Academic Achievement

The content of this thesis was completed by Mansoor Rashidhaider Radwi.

It is recognized that Dr. Clive Kearon, Dr. Susan Solymoss, Dr. James Douketis, Dr. Paul Ockelford, Dr. Sharon Jackson, Dr. Alexander G. Turpie, Ms. Betsy MacKinnon, Dr. Jack Hirsh, Dr. Michael Gent, Dr. Jeffrey S. Ginsberg and Prof. Jim Julian, who were FIDO investigators and authors of the original study publication, contributed data for analysis. Their contributions are much appreciated.

6. AIM OF THESIS

6.1. Objectives

6.1.1. Primary Objectives

- First part: To determine whether patient's baseline characteristics (e.g., age, weight, height, sex) influence heparin levels in patients treated with weight-adjusted SC UFH and SC LMWH.
- Second part: To determine whether heparin levels influence the risk of recurrent VTE and bleeding events.

6.1.2. Secondary Objectives

- To explore if the relationship between (1) baseline characteristics and heparin levels, and (2) heparin levels and clinical outcomes (bleeding and recurrent VTE) are consistent across the types of heparin (i.e., UFH, enoxaparin and dalteparin).

6.2. Importance

The study will answer two important questions. The first is if it is appropriate to dose SC UFH and LMWH directly in proportion to patient's weight (i.e., weight-adjusted dosing); perhaps the dose of UFH and LMWH should be influenced by

patient characteristics other than weight (e.g., sex, adiposity or differences in renal function).

The second question is if, in patients who are treated with SC UFH and LMWH, low heparin levels are associated with increased thrombotic outcomes and if high levels are associated with increased bleeding outcomes. The answers to these two questions may suggest that dosing of SC UFH and LMWH should be changed from current purely weight-based dosing to improve efficacy and safety.

6.3. Novelty

There is limited knowledge about the heparin levels achieved with therapeutic-dose SC UFH, and uncertainty about the appropriateness of dosing SC LMWH based on body weight alone. Also, there has been no comparison of heparin levels attained with SC UFH and with SC LMWH, when each is given in therapeutic (as opposed to "prophylactic") weight-based doses.

7. Study Background

7.1. VTE

7.1.1. Pathophysiology of VTE disease

Blood clots are physiologically formed when blood vessels are injured. The purpose of the clot is to close the gap in the blood vessel so as to prevent bleeding. VTE is a condition where an abnormal clot or "thrombus" is formed within the venous system. When a clot occurs in the deep venous system, it is called deep vein thrombosis (DVT). DVT most commonly occurs in legs and if these thrombi break free and are carried by the blood supply to the lungs, they are called pulmonary emboli (PE).

The mechanisms for VTE, referred to as Virchow's triad, include three dominant factors: stasis, endothelial damage, and a hypercoagulable state. Stasis of blood in the venous system occurs in immobile patients or legs such as after a stroke. Endothelial damage can be a result of many forms of trauma (e.g., aa central venous catheter insertion). Lastly, a hypercoagulable state or thrombophilia can occur due to an underlying genetic trait (e.g., factor V Leiden), or due to an acquired condition such as pregnancy, cancer, antiphospholipid syndrome, or estrogen therapy. Patients who develop VTE often have more than one component of Virchow's triad. For

example, patients undergoing surgery for hip fracture have endothelial damage, stasis, and an acquired hypercoagulable state.

VTE events can be classified as either unprovoked (also referred to as idiopathic), or provoked.[1] Provoked VTE can be further categorized as provoked by a reversible risk factor (e.g., recent surgery), or provoked by a non-reversible risk factor (e.g., active cancer). Whether VTE is provoked by a reversible risk factor, unprovoked, or provoked by a non-reversible risk factor, is clinically important because the risk of recurrence is lowest when there is a reversible risk factor and highest when there is a non-reversible risk factor.

7.1.2. Epidemiology of VTE disease

The annual incidence of VTE in adults is 1-2 cases per 1000 persons.[2,3] VTE incidence increases with age, with about a 2-fold increase every decade.[2] Overall incidence is slightly higher in males compared to females (about 1.2-fold); however, younger females have a higher incidence than younger males because pregnancy and estrogen-containing oral contraceptives increase the risk of VTE.[2]

7.1.3. Diagnosis of VTE

Diagnosis of DVT and PE, generally involves combinations of clinical pretest probability assessment for DVT/PE, laboratory testing for D-dimer level, and imaging of the deep veins or pulmonary arteries.[1] The clinical pretest probability assessment is aided by use of the Well's score (a clinical prediction rule for either DVT or PE), which combines symptoms and signs of VTE, the presence or absence of risk factors for VTE, and a physician's subjective assessment of whether DVT or PE is the most likely diagnosis.[4]

D-dimer is produced when thrombus (or a blood clot) is broken down by the endogenous fibrinolytic system, which occurs when there is an acute thrombotic state.[5] Because D-dimer also increases in other conditions such as cancer and inflammation, it is a sensitive but nonspecific test for diagnosing VTE.[6] Therefore, a normal D-dimer level has high negative predictive value and is very helpful for exclusion of VTE, but an abnormal D-dimer level does not have a high positive predictive value and is of little help for confirming VTE. The combination of a low or moderate (pretest probability) PTP and a negative D-dimer test excludes VTE. If D-dimers testing is positive, then imaging is performed to determine if VTE is present or absent. Patients with high PTP proceed to imaging directly because D-dimer testing is of little value in this group of patients because a negative D-dimer

test is rarely obtained and, if the test is negative, it is associated with a lower negative predictive value.

Ultrasound (U/S) is the preferred imaging modality to diagnose DVT, because it's noninvasive and has high sensitivity and specificity.[7,8] Inability to fully compress a deep vein with application of U/S probe pressure is diagnostic of DVT in patients who have not had a previous DVT.

Computed tomography with pulmonary angiography (CTPA) or ventilation perfusion scan (V/Q scan) are two imaging modalities that are used for the diagnosis of PE. CTPA is generally preferred over V/Q scan for two reasons: 1) V/Q scans are more likely to produce inconclusive results compared to CTPA scans [9], and 2) CTPA has the advantage of identifying an alternative diagnosis that can explain the patient's symptoms.[10] A CTPA, however, is associated with greater risks of contrast induced nephropathy and radiation exposure.

7.1.4. Treatment of VTE

The goals of treating VTE with anticoagulant (AC) therapy is to prevent extension and embolization of the newly formed clot, and to prevent formation of additional clots in the future. There is strong evidence from randomized clinical

trials that AC therapy reduces recurrent VTE in patients with acute VTE, albeit with an increased risk of bleeding.[1]

Prior to the introduction of direct oral anticoagulants in 2003, AC therapy consisted of LMWH or UFH (I will refer to them as collectively as heparins in the remaining sections of the thesis) overlapped with and followed by vitamin K antagonist (VKA) drugs (e.g., warfarin). Heparin is administered parenterally while VKA drugs are administered orally. The anticoagulant effect of heparins is immediate when given as an IV bolus, and occurs within hours when given SC. Although VKA drugs antagonize vitamin K within hours of intake, it usually takes between 3 and 5 days for the level of active coagulation factors to decrease enough for the anticoagulant effect to become established. Because VTE needs prompts AC therapy, heparins must be administered with a VKA drug, and continued for a minimum of 5 days and until the AC effect is in therapeutic range (i.e., as reflected by the prothrombin time expressed as an International Normalized Ratio [INR] of >2.0 on two occasions 24 hours apart). Furthermore, if VTE is treated with a VKA without initial heparin therapy, or with inadequate heparin therapy, there is a high risk of recurrent VTE both initially (i.e., when heparin is meant to be given) and during the next 3 months of VKA therapy (after heparin has been stopped).[1] Heparin, therefore, is required to rapidly anticoagulated patients while a VKA is taking effect,

and to "turn off" thrombosis so that long term VKA therapy can keep thrombosis turned off.

7.2. Anticoagulation with heparin

7.2.1. Types of heparin preparation

The earliest form of heparin had a large mean molecular weight. In the 1970s, techniques were developed to split or fractionate heparin into smaller "low molecular weight" molecules. Hence, heparin preparations fall into two categories; UFH, which has large mean molecular weight (e.g., 15 kDa), and LWMH which has a much lower mean molecular weight (e.g., 5 kDa). UFH and LMWH also differ somewhat in their mechanism of action. Both bind to and activate antithrombin (AT). However, whereas the UFH-AT complex inactivates coagulation factor X and II, the LMWH-AT complex more selectively inactivates coagulation factor X. Because LMWH is make up of smaller molecules that UFH, it is also less likely to bind unselectively to plasma proteins (see Section 8.2.3).[11]

7.2.2. Dosage and Route of administration of UFH and LMWH

For treatment of VTE, UFH is most commonly administered intravenously (IV), but it can also be given SC. When given IV, a bolus dose is administered (e.g., 80 units/kg) followed by a continuous infusion (e.g., 18 units/kg per hour). Weight-

adjusted dosing of IV UFH has been shown to get patients into the therapeutic range more quickly that using the same (i.e., non-weight adjusted) starting does of UFH in all patients (e.g., 1,000 units or 1,250 units per hour).[12] Traditionally, if UFH is given SC in therapeutic doses, patients are usually given either a fixed dose of ~17,500 twice a day (BID) or 250 units/kg BID and then have doses adjusted in response to the activated partial thromboplastin time (aPTT).

LMWH is (essentially) only given SC. The total daily dose of LMWH used to treat a patient with VTE is usually directly proportional to his/her weight (e.g., 200 anti-Xa units/kg per day), with this dose being given as a single daily dose, or divided into twice-daily doses. This means a patient weighing 60 kg will receive 12,000 units of LMWH per day, whereas as patient of 90 kg will receive 18,000 units per day.

7.2.3. Monitoring of anticoagulation effect

Because LMWH has less non-selective binding to plasma proteins that UFH, it is believed to have predictable pharmacokinetics and, therefore, not to require adjustment of LMWH dose in response to laboratory measurement of coagulation. Use of direct weight-adjusted dosing assumes that treatment of a 90-kg male or female with 18,000 units once-daily (OD) (or 9,000 units BID) will achieve the same

AC effect as treatment of a 60-kg male or female with 12,000 units OD (or 6,000 units BID). This hypothesis, however, has not been well tested. Because LMWHs are largely eliminated through the kidneys, LMWH are considered contraindicated in patients with severe renal failure.

The pharmacokinetics of UFH are unpredictable; hence, despite treatment with the same dose, the AC intensity of UFH (at least as reflected by aPTT results) vary between patients, and within the same patient over time. Consequently, it is generally believed that UFH therapy needs to be monitored via laboratory tests of coagulation with UFH dose adjusted according to the test results. (see Section 8.3)

7.2.4. Efficacy of UFH vs. LMWH in preventing recurrent VTE

Many meta-analyses have compared the efficacy of SC LMWH to IV UFH for the treatment of acute VTE.[1,13] Although the evidence suggests that recurrent VTE (relative risk (RR) 0.72 [95% CI 0.58-0.89]), major bleeding [RR 0.67 (95% CI 0.45-1.0)] and mortality (RR 0.79 [95% CI 0.66-0.95]) are less with LMWH, the quality of evidence is low because 1) studies were open labeled and 2) there is a high risk of publication bias in favor of LMWH. [1,6] A meta-analyses found no difference between SC LMWH and SC UFH, when used for treatment of acute VTE, in terms of recurrent VTE (RR 0.87 [95% CI 0.52-1.45]), major bleeding (RR 1.27 [95% CI

0.56-2.9]) and mortality (RR 1.1 [95% CI 0.68-1.76]). Because of the imprecision, the quality of evidence in this meta-analysis is moderate.

7.3. Laboratory measurement of AC intensity of heparin

The anticoagulation effect of a medication can be measured either by a clot-based test or a chromogenic test. A clot-based test measures the time required to form a clot in a blood sample under specific conditions in the laboratory. The relationship between the time required to form a clot ex vivo (i.e., in the test tube) and the intensity of AC effect in vivo (i.e., in the body) is assumed to be proportional; that is, the more intense the AC effect is, the longer it will take to form a clot and vice versa. The aPTT is an example of a clot-based test. The aPTT test measures the time, in seconds, for a clot to form when phospholipid, a contact activator, and calcium are added to a plasma sample that contains citrate. For many aPTT assays, a normal aPTT is between 27 – 35 seconds. The therapeutic range for patients treated with IV UFH is generally an aPTT of 1.5 to 2.5 times normal (e.g., 60 – 80 seconds). LMWH cannot be monitored with the aPTT test, as aPTT reagents are sensitive to inhibition of factor II (i.e., thrombin) and are not sensitive to inhibition of factor X, which is the predominant target for the LMWH-AT complex.[14]

A chromogenic assay is a colorimetric assay that uses light absorption to measure the concentration of a substance in a fluid (in our case, concentration of heparin). The substance has to result in a color change when it interacts with an added indicator agent. When these conditions are met, there will be a fixed relationship between 1) concentration of the substance of interest, 2) the color of the fluid, and 3) the amount of light that is absorbed when a light source is shone through the sample. The analyzer can then convert the light measured (after it has passed through the sample) into a concentration (of the substance of interest) using a calibration curve. For patients who are taking heparin, the greater the concentration of heparin in their plasma, the greater its ability to inhibit activated factor X (i.e., anti-Xa activity). To measure ability of a patient's plasma sample to inhibit Xa, exogenous activate Xa (e.g., purified bovine Xa) is added to the patient's plasma sample. Factor Xa specific chromogenic substrate is also added to the sample. The enzymatic interaction between exogenous Xa and factor Xa specific chromogenic substrate results in production of paranitroanaline (pNA) chromophore. pNA chromophore is colored, and its concentration can be measured by an analyzer called spectrophotometer. Because heparin prevents enzymatic interaction between exogenous Xa and factor Xa specific chromogenic substrate, the amount of pNA chromophore (measured by spectrophotometer) reflects heparin concentration

(obtained from a calibration curve that describes the relationship between heparin levels and light absorption).

The anti-Xa therapeutic range for an IV infusion of UFH is generally considered to be 0.3 to 0.7 IU/ml.[15] Although a therapeutic anti-Xa range has not been established for SC UFH, it is usually assumed that the peak anti-Xa level about 4 to 6 hours after a SC injection of UFH should also be in this range When blood is drawn about 4 to 6 hours after injection (peak level), the anti-Xa therapeutic range for SC LMWH is usually assumed to 0.6-1.0 IU/ml when LMWH is given BID, and 1.0-2.0 when LMWH is given OD.[16,17]

7.3.1. Variables known to influence heparin levels

There are two types of factors that affect heparin levels in patients treated with UFH or LMWH therapy: in-vivo and ex-vivo factors. Ex-vivo factors are non-patient variables that effect the measurement of heparin levels after the sample has been drawn and, therefore, ex-vivo factors can result in falsely increased or decreased heparin levels. An example of an ex-vivo factor is binding of heparin to platelet factor 4 that is released from platelets after the blood sample has been collected; this leads to a false decreased heparin level. An increase in UFH or

LMWH dose in response to artificially lowered heparin levels will result in excessive anticoagulation in-vivo and an increase in bleeding.

In-vivo factors are patient variables that effect the anticoagulation intensity of heparin in the body before the sample is withdrawn. Thus, they are considered real and important. An example of an in-vivo factor that effects heparin levels is markedly reduced renal function in patients who are treated with LMWH. Because LMWH is excreted by the kidneys, patients with markedly reduced renal function will have higher in-vivo heparin levels. In such circumstances, it is appropriate to reduce LMWH dose.

Few studies have evaluated associations between heparin levels and in-vivo factors, other than renal function, in patients treated with weight-adjusted LMWH or UFH. Because heparin is mainly distributed in the blood volume compartment, dosing per weight alone might not be enough, as blood volume differs by sex and height in addition to weight.[18] For example, in a study that compared weight-based IV UFH with IV heparin whose dose was adjusted to sex, height, age, and weight, the weight-adjusted dose method was less successful at achieving therapeutic heparin levels in the first 6 hours (37% vs. 62, p=0.0001).[19] However, a second small study (n=32) did not find that adjusting IV UFH dose according to sex, height, age, and weight achieved a therapeutic heparin levels more rapidly compared to dosing

according to weight alone.[20] Another example of an in-vivo factor thought to effect heparin levels is the diagnosis of acute pulmonary embolism. Compared to DVT, it has been suggested that PE accelerates heparin elimination from the plasma, although whether this difference between DVT and PE is real and the mechanism behind this phenomenon is uncertain[21,22].

7.4. Relationship between heparin's anticoagulant effect and clinical outcomes

7.4.1. Recurrent VTE and heparin's anticoagulant effect (measured as aPTT or heparin levels)

Two meta-analysis compared rates of recurrent VTE in patients with a sub-therapeutic aPTT level compared to a therapeutic aPTT level in the first 48 hours of IV UFH therapy.[23,24] Only patients treated with at least 30,000 units/24 hours of UFH were included in these analyses. Both meta-analysis found that recurrent VTE rates were not different between the subtherapeutic and therapeutic groups.

No study has looked at the association between sub-therapeutic heparin levels (rather than aPTT results) and recurrent VTE in patients receiving IV UFH or SC LMWH for the treatment of acute VTE. However, there is indirect evidence from the Pitié-Salpêtrière Registry on Ischemic Coronary Syndromes (PARIS Registry),

a cohort study that evaluated patients with acute coronary syndromes who were treated with weight adjusted SC enoxaparin (i.e. 100 units/kg every 12-hour). In this study, multivariable analysis found that sub-therapeutic heparin levels (<0.5 IU/ml) measured 4 to 6 hours after injection were associated with the composite outcome of re-infarction and death at 30 days (odds ratio [OR]= 3.45 [95% CI 1.34-8.86]).[25]

7.4.2. Bleeding and heparin's anticoagulant effect (measured as aPTT or heparin levels)

The evidence from studies examining the association between aPTT and bleeding is conflicting for patient treated with IV UFH. For example, in a study of 199 patients with acute VTE who were randomized to receive IV UFH for either 5 or 10 days, bleeding rates were no higher in patients with supratherapeutic aPTT (defined as an aPTT >85 seconds on or before day 4, and not followed by an aPTT value of ≤85 seconds measured within the next 24 hours) compared to non-supratherapeutic aPTT (defined as aPTT value < 55 seconds measured on or before day 4, and not followed by an aPTT value ≥ 55 seconds measured within the next 24 hours) (8.6% vs. 12%, p=0.49).[26] On the other hand, two studies found an association between aPTT and bleeding events.[27,28] In the OASIS-II study, where 10,141 patients with acute coronary syndromes were randomized to receive IV UFH (5000 U bolus followed by an initial infusion at 15 units/kg/h) or IV hirudin, the probability of

bleeding in the heparin group increased by 7% (95% CI 3%-11%; p=0.0004) for every 10 second increase in the mean aPTT. In the GUSTO-IIb study, 12,142 patients with ACS were randomized to either IV UFH or IV hirudin. aPTT was measured 6, 12 and 24 hours after the start of the infusion. When the relationship between aPTT and bleeding events (moderate or severe) was evaluated, the authors concluded that longer aPTT time at 6 hours was associated with increased bleeding events (p value not provided).

One study examined the relationship between heparin levels and bleeding.[29] In this study of 194 patients with acute VTE who were randomized to receive either a bolus of 2,500 units of UFH followed by a continuous infusion of 30,000 units/24 hours, or a bolus of 2,500 units of LMWH followed by a continuous infusion 15,000 units/24 hours, major bleeding risk increased from 11% to 40% (p=0.05) for patients who had a mean heparin level above 0.8 IU/ml, whether they were treated with UFH or LMWH.

8. Methodology

8.1. Outline of the FIDO (Fixed Dose Heparin) Study

The FIDO (FIxed DOse heparin) study was a non-inferiority randomized clinical trial that compared fixed-dose weight-adjusted SC UFH with fixed-dose weight-adjusted LMWH for the acute treatment of VTE.[30] The study was unique in that it used fixed-doses of weight-adjusted SC UFH that were not subsequently adjusted according to aPTT values or heparin levels.

8.1.1. Population

The study population consisted of 708 adult patients with acute VTE. The inclusion criteria were any patients aged 18 years or older with acute VTE (i.e., DVT of the legs or PE). Patients were excluded if they had any of the following:

1. a contraindication to SC delivery of UFH or LMWH.
2. active bleeding.
3. life expectancy < 3 months.
4. received AC therapy for > 48 hours prior to enrollment.
5. a creatinine level > 200 μmol/L.
6. already on a long-term AC therapy.
7. pregnancy.

8. unable to have follow-up assessment.

8.1.2. Interventions

Patients were randomized to either SC UFH (first dose of 333 units/kg, followed by 250 units/kg BID) or SC LMWH (100 units/kg BID), that was overlapped with warfarin and given for at least 5 days and until the INR was ≥2.0 on two occasions 24 hours apart. Warfarin was continued for at least 3 months. Neither UFH nor LMWH doses were adjusted in response to laboratory measures of coagulation.

8.1.3. Outcomes

The study had two main outcomes: it compared efficacy in terms of rates of VTE at 3 months; and compared safety in terms of rates of bleeding at 10 days.

8.1.4. Result of the FIDO study

A total of 708 patients were randomized in the study. The rates of recurrent VTE and major bleeding in the two treatment groups were similar. Recurrent VTE was 3.8% in the UFH arm compared with 3.4% in the LMWH arm (absolute difference [AD] 0.4%; 95% CI -2.6% to 3.3%). Major bleeding during the first 10 days of treatment was 1.1% in the UFH arm and 1.4% in the LMWH arm (AD, -0.3%; 95%

CI -2.3% to 1.7%). The study concluded that UFH is as safe and effective as LMWH when both are given SC in fixed, weight-adjusted, doses for the treatment of VTE.

8.2. A sub-study in the FIDO study

A sub-study to evaluate heparin levels was included in the original study. Patients were asked to provide a single blood sample on the 3rd day of the study (referred to as Day 3), 6 hours after their UFH or LMWH injection. The blood samples were analyzed in a central laboratory in Hamilton, Ontario, Canada, after the study was completed. Therefore, measurements on these samples, including heparin levels, were not communicated to the treating physicians, and these levels have yet to be analyzed. This data will be used to answer the objectives of the thesis.

8.2.1. Eligibility Criteria for analysis

For FIDO patient to be included in the current analysis, all the following criteria had to be satisfied:

A. Heparin level were measured and available for analysis.
B. A blood sample had to have been drawn within an acceptable window:
    a. Between 3.0 and 9.9 hours after the injection of UFH or LMWH
    b. On the 2nd to 6th day (inclusive) after starting UFH or LMWH

8.3. Data analysis

8.3.1. Methods of analysis

Descriptive analyses: Data for continuous variables will usually be presented as means and standard deviations (medians, 1st and 3rd quartile values when data are clearly not parametrically distributed), while categorical variables will be presented as frequencies and percentages.

Comparative analyses: Linear regression analysis will be used to determine whether patient's baseline characteristics influence heparin levels on Day 3 (or within the acceptable time window), and logistic regression analysis will be used to determine whether heparin levels on Day 3 influence bleeding and VTE during follow-up. When needed, chi-squared test will be used to compare dichotomous data.

8.3.2. Overview of regression analysis

Regression is a statistical technique used to explore relationship between two or more variables. For example, we can use regression to explore the effect of age on the annual incidence of VTE in a population. In this example, age is the independent variable, and incidence of VTE is the dependent variable (i.e., the change in the incidence of VTE depends on changes in the age but not vice versa). The number of independent variables in a regression model can range from 1 to an

unlimited number. For both logistic and linear regression analysis, if the effect of an independent variable on a dependent variable differs among categories of patients (e.g., males and females), then that characteristic (e.g., sex) is an “effect modifier”. The following example will further illustrate this point: the RR for developing VTE in males compared to females is greater than 1.0 in the general population, however, as mentioned in section 8.1.2 (*Epidemiology of VTE*), this relationship is reversed (RR less than 1.0) in those less than 40 years. Sex, therefore, is an "effect modifier" of the relationship between age and the risk of developing VTE. To detect and accommodate for such an effect, a product of variables (i.e., an interaction term), in this case "Sex X Age", should be incorporated in the model.

8.3.3. Reasons for choosing regression analysis

Simpler analytical methods to explore associations between two variables, such as a comparison of mean heparin levels in males vs. females (e.g., using a t-test), or rates of VTE in patients with supra-therapeutic vs. non-supra-therapeutic heparin levels (e.g., using a chi-squared test or Fisher’s exact tests) have the limitation that these tests cannot be adjusted for other factors that could influence the association of interest, and have lower power to detect associations between two continuous variables (e.g., body weight and heparin levels).[31,32]

8.3.4. Linear vs. logistic regression

The choice between linear or logistic regression analysis depends on the type of outcome i.e. the dependent variable. If the outcome is a continuous measurement such as heparin levels then linear regression is used, whereas if the outcome is a binary measurement such as a VTE or no VTE during follow-up then logistic regression is used.[33,34]

8.3.5. Difference between univariable vs. multivariable analysis

There are two ways for estimating the effect of an independent variable on a dependent variable (e.g., effect of weight on heparin levels). A univariable analysis examines the association between **one** independent variable and a dependent variable (e.g., weight and heparin levels). However, such a comparison may be misleading as it does not consider other risk factors, or "confounders" (i.e., other independent variables, such as patient sex) that may influence heparin levels and may be associated with the independent variable of primary interest (in this case weight). To estimate the effect of weight on heparin levels independently of differences in another factor such patient sex, a multivariable analysis should be performed that includes patient sex as **an additional** independent variable (or covariate) in the regression model.[35]

8.4. Linear regression

The relationship between a continuous dependent variable and an independent variable (continuous or binary) can be plotted on a two-dimensional y/x Figure where the y (vertical) axis is the dependent variable and x (horizontal) axis is the independent variable (e.g., heparin levels on the y axis and weight of patients on the x axis).

8.4.1. Ordinary least-squares (OLS) method

When the OLS technique is used to fit a line that describes the relationship between two variables, the best fitting straight line minimizes the "sum of squares" of the difference (vertical distance) between each data point in the scatter plot and the fitted line (also referred to as “sum of squares of residuals”). Some features of the OLS straight line include: 1) it passes through the point that corresponds to the mean of x and the mean of y; and 2) the sum of the vertical distances, or residuals, above and below the line is zero.

8.4.2. Linear assumptions

There are 4 key assumptions that should be met for it to be appropriate to use OSL regression and for its results to be valid:

1. *Independence:* The measurements of the x values must be independent of each other (i.e., x values are not a series of measurement [e.g., weight] in the same person over time).
2. *Linearity:* The relationship between the independent variable and the dependent variable should be a straight line. This can be assessed by examining either the x/y scatter plot or the residual plot (described in the next section, *Homoscedasticity)*. If a nonlinear relationship is detected, transformation of the x values or the y values should be performed to try and achieve a linear relationship between x and y before linear regression analysis is carried out. The method of transformation depends on the pattern of non-linearity seen in on the scatter plot.
3. *Homoscedasticity*: This assumption requires that the distribution of residuals should be similar for any value x takes. This can be tested by plotting the residual values around the predicted values of the dependent variable y. The plot should show a random pattern. Violation of this assumption is called "heteroscedasticity", where plotted residual values take a specific or systematic pattern (e.g., a funnel appearance, with widening of residuals as the value of y increases). Only severe heteroscedasticity invalidates use of OLS analysis.

4. *Normality:* This implied that the distribution of residuals for any x value should be conform to a “normal” or Gaussian distribution. This can be tested by examining the probability–probability (p-p) plot, where a plot of observed cumulative distribution function of residuals against expected cumulative distribution function should show little or no deviation from a straight line. If skewness of the line in a p-p plot occurs because of a few residuals, then such residuals should be evaluated and corrected if they are due to errors that may have occurred during data collection or entry.

8.5. Logistic regression

Like linear regression, the relationship between a dependent and independent variable can be plotted on a two-dimensional y/x axis where y is the probability of a binary dependent variable (e.g., probability of VTE) and x is the independent variable (either continuous [e.g., heparin levels) or binary [e.g., sex]). However, unlike linear regression, where values on the y axis can vary from 0 to infinity, values on y axis in logistic regression must vary between 0 (outcome absent in all persons) and 1 (outcome present in all persons).

8.5.1. Logistic function

To ensure that values on the y axis are between 0 and 1, a modification called the “logit function” is incorporated into the linear equation (as linear equations otherwise allows values below 0 and above 1).

8.6. Dealing with multicollinearity

Multicollinearity implies that two or more variables are conveying the same information in the model. For example, weight and dose of heparin are very highly (or rigidly) correlated in patients with acute VTE when the dose of heparin is weight-adjusted.

Multicollinearity between independent variables can affect the results of a regression model in a few ways, such as altering the direction of the regression coefficient, or widening the CIs of the regression coefficient, when the second variable is added to the regression model.

Multicollinearity can be quantified by variance inflation factor (VIF). As the name implies, VIF examines how much the variance of an estimate have increase (inflated) due to multicollinearity. The more severe the multicollinearity is, the larger the increase in the variance will be. An apriori threshold level is defined for VIF before testing the model for multicollinearity (usually>5), and any variable that exceeds that threshold indicates the presence of important multicollinearity.

8.7. Dealing with missing data

There are three types of missing data: 1) “Missing Completely at Random” (MCAR), means that the data is missing due to a random factor, unrelated to other variables, whether observed or unobserved (e.g., part of a questionnaire lost in the mail). 2) “Missing at Random” (MAR), means that the data that is missing is related to an observed variable in the data (e.g., heparin levels is measured twice from a blood sample of patients taking heparin). If the two values differed considerably due to an error in measurement, then a third test will be conducted. Hence, values of third test will be missing for the samples that did not differ during initial testing. 3) “Missing Not at Random” (MNAR), means that the data that is missing is linked to unobserved variable in a way that is not known.[36]

The “Little's MCAR test” is a statistical method to help determine whether the missing data is of MCAR type or non-MCAR type (i.e., MAR or MNAR).[37] The null hypothesis tested in Little’s test is that missing data is of the MCAR type. To reject the null hypothesis, the p-value should be less than 0.05.

Analysis of data without dealing with missing data can lead to biased results and conclusions. A method to deal with missing data of the MCAR or MAR type is

multiple imputation of plausible values of attained by expectation maximization (EM) method.[38]

8.8. Evaluating *"goodness-of-fit"* of a model

For a linear regression model, goodness-of-fit (GOF) can be assessed by using the "*coefficient of determination"* (R^2). R^2 indicates how well a model can explain variability in the dependent variable. It can take on a value that range from 0 to 1. For example, if the R^2 value is 0.75 for a model that contains heparin levels as the dependent variable and weight as the independent variable, then weight explains 75% of the variability observed in heparin levels.

For logistic regression, the Hosmer–Lemeshow (HL) test is a statistical test that can be used to assess GOF. The null hypothesis in this test is that the model is appropriate (i.e., the predicted values in the model are similar to observed values in the data set).

8.9. Overview of the regression strategies used in this thesis

Because the aim of thesis is to answer predefined questions such as 1) if a particular patient characteristic influenced heparin levels, and 2) if heparin levels influenced recurrent VTE and bleeding, a "hierarchal strategy" will be used. In this strategy, co-variates which are chosen because of background knowledge about their biological influence on the dependent variable are forced into the regression model. Therefore, the decision to include variables in the regression model is independent of whether they are statistically significantly related to the dependent variable (unlike the variables that are selected by a "stepwise" regression analysis strategy).

In multivariable analysis, a partial F-test is used to identify if variables statistically significantly influenced the dependent variable after adjusting for co-variates in the model. The result of the partial F-test is converted to a p-value; in my thesis, I will refer to such a p-value as a "partial p-value".

The objectives of this thesis are divided into a series of questions. When possible and appropriate, each new question builds on the findings of the analyses that I did to address a preceding question. Usually, as previously described, regression is the method of analysis that I have used to answer each question.

In the section that examined the influence of baseline characteristics on heparin levels, I assumed that mean heparin levels, and the influence of baseline characteristics on heparin levels (i.e., slope of the relationship as assessed by regression analyses), might differ between patients who were treated with UFH and LMWH. In relationship to mean heparin levels, my expectation was that levels would be higher in patients on LMWH compared to UFH and that, within the LMWH and UFH groups, heparin levels would not differ according to the type (or brand) of LMWH and UFH. However, I unexpectedly found that there was a marked difference in mean heparin levels in patients treated with the two LMWH preparations (i.e., dalteparin and enoxaparin). Among patients who were treated with UFH, there was no apparent difference in heparin levels according to the type (or brand) of UFH (Leo heparin versus non-Leo heparin; r^2=0.015, p=0.24). Consequently, I decided that it was appropriate to analyze the effect of heparin type on heparin levels as three categorical variables (UFH, dalteparin, enoxaparin) rather than two (UFH, LMWH).

The decision to include effect modifiers (i.e., interaction terms) in a model will also adhere to the "hierarchal strategy" of selection, whereby addition of the interaction term will address a predefined question, such as "Is the influence of

creatinine clearance (CrCl) on heparin levels the same in patients who are treated with UFH, dalteparin, and enoxaparin?"

In the section that examined the association between heparin levels and clinical outcomes, the effect of heparin levels was examined first as a dichotomous variable, and then as a continuous variable. As a dichotomous variable, heparin levels were divided into: (1) subtherapeutic level and non-subtherapeutic level (i.e., therapeutic or supratherapeutic levels) when the outcome was recurrent VTE at 90 days; and (2) supratherapeutic level and non-supratherapeutic level (i.e., therapeutic or subtherapeutic levels) when the outcome was bleeding events at 10 days. Patients were considered supratherapeutic if their heparin levels were above 0.7 IU/ml if treated with UFH, and above 1.0 IU/ml if treated with LMWH.[16,17] Patients were considered subtherapeutic if their heparin levels were below 0.3 IU/ml if treated with UFH and below 0.6 IU/ml if treated with LMWH. The associations between heparin levels and clinical outcomes were examined in a 2 x 2 Table, and a chi-square test was used to determine if the associations were statistically significance. As a continuous variable, the influence of heparin levels on outcomes was first examined in a univariable logistic regression analysis, followed by a multivariable analysis.

Qualitative variables that are dichotomous in nature were coded as yes=1, and no=0 (e.g., female sex: yes=1, no=0; presence of cancer at baseline: yes=1, no=0;

inpatient status at time of randomization: yes=1, no=0). Because type of heparin includes UFH, dalteparin and enoxaparin, dummy variables were created for dalteparin and enoxaparin.

8.10. Some Additional Methodological Issues

First, examination of associations can lead to statistically significant findings that have occurred by chance. To reduce this risk, I pre-specify (i) the associations that will be present (and absent), (ii) the direction of those associations, (iii) the method of analysis, and (iv) adjustment for multiple testing. Second, I anticipate having to deal with missing data before carrying out any regression analysis (see Section 8.7). Finally, limited sample size and low clinical event rate may limit ability to detect the influence of patient characteristics on heparin levels, and ability to detect an association between heparin levels and clinical outcomes. Acknowledging that I will be working with a modestly sized database, particularly when considering the relationship between heparin levels and clinical outcomes, I will consider the possibility of a "small sample bias", and how it can be detected and minimized.

8.11. Safety and Ethical Considerations

This is a retrospective analysis using data collected from the FIDO study that was conducted from 1998-2004. There are no plans to collect new data either by interviewing patients or reviewing their medical charts. Investigators for the FIDO study secured Research Ethics Board approval prior to the start of study. To ensure confidentiality, patients' identifiers were removed from the data prior to being analyzed for the thesis. To ensure autonomy, patients provided informed consent before enrolling in the study. At the time of original study consent, patients who are included in this analysis also consented to analysis of the samples that were collected during the study, and were informed that the results could be published.

8.12. Software

Statistical analyses were completed using SPSS software v20.0 (IBM Corp., Armonk, NY, USA).

9. Results

9.1. Descriptive analysis

Of the 708 patients in the FIDO study, 450 (65%) had heparin levels drawn. Of these, 408 patients had their blood samples drawn between 3 and 9.9 hours after injection on days 2 to 6 after starting heparin and, therefore, were eligible to be included in this analysis (Figure 1).

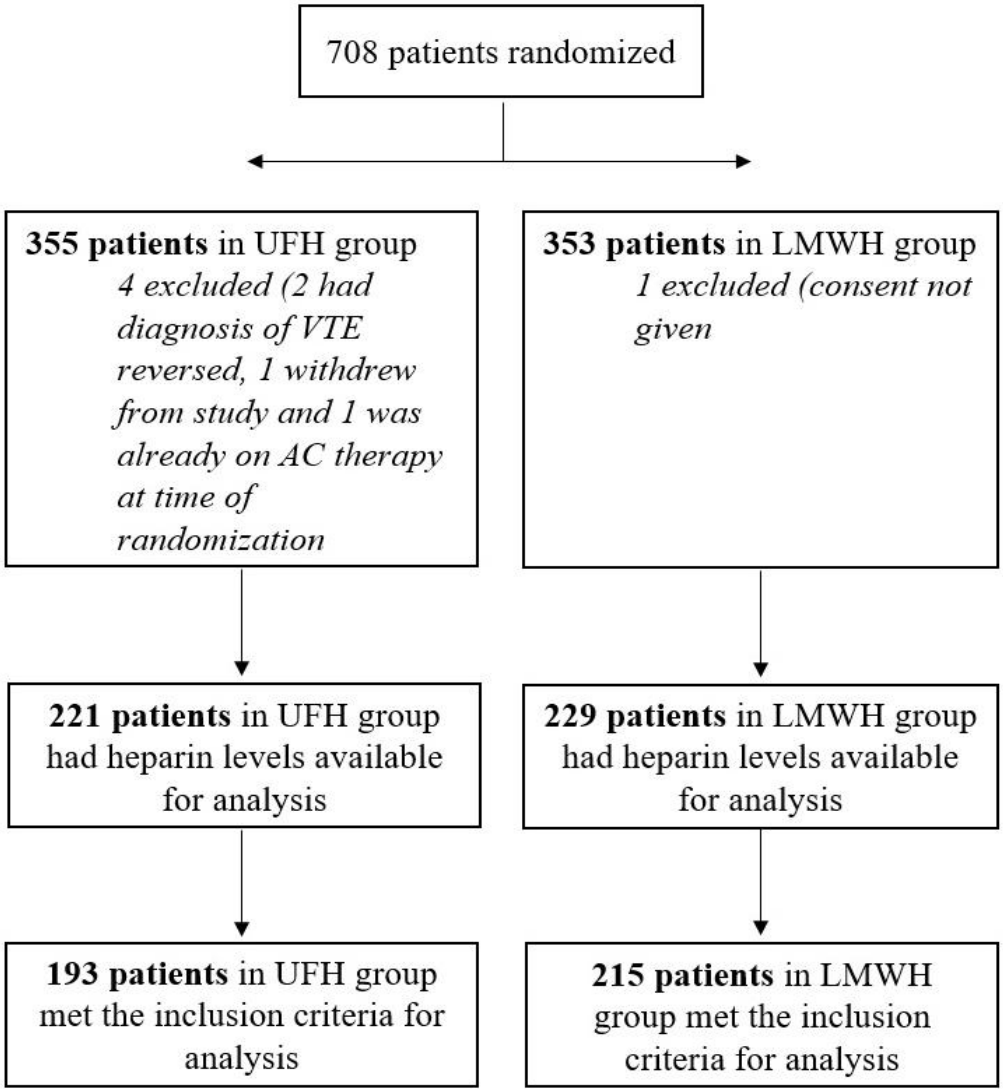


Figure 1: Patient flow

Table 1 shows the baseline characteristics for the 450 patients who had heparin levels measured, separated into the 408 patients of these patients who are included and the 42 patients who were excluded from my analysis. The upcoming section describes the baseline characteristics for the 408 patients.

The mean age of the patients included in the analysis was 59 years (minimum 20 years, maximum 95 years). There were 178 female patients (44%) and 230 male patients (56%). Mean weight was 83.8 kg (minimum 43 kg, maximum 169 kg), and mean BMI was 28.5 (minimum 18, maximum 53). The index VTE at enrollment was DVT alone in 332 patients (81%) and PE (with or without symptomatic DVT) in 76 patients (19%). Of those who presented with DVT alone, 310 (96%) were proximal DVT and 12 (4%) were isolated distal DVT. The index episode of VTE was a first episode in 327 patients (81%) while 81 patients (19%) had a previous VTE. VTE was provoked in 208 patients (51%) and unprovoked in 200 patients (49%). Cancer (16% of all patients) and recent surgery (10% of all patients) were the leading provoking risk factors. Most patients (69%) had no bleeding risk factors. Among the patients with bleeding risk factors, a majority had only 1 risk factor (67%).

There were no apparent differences between the 408 included and the 42 excluded patients except for a marginally higher hemoglobin (131.0 g/l vs. 124.5 g/l,

p= 0.04) and that a higher proportion were randomized to LMWH (67%% vs. 47%, p= 0.02). From hereafter, analysis will be restricted to the 408 patients included in the analysis.

Table 1: Baseline characteristics for the 450 patients with heparin levels

Quantitative variable	**Missing**	**Included (N=408)**		**Missing**	**Excluded (N=42)**		***P value***
		Mean	***SD***		***Mean***	***SD***	
Age (years)	-	**59.1**	16	-	**59.1**	18	0.98
Weight (kg)	-	**83.8**	18	-	**82.9**	22	0.52
Height (cm)	8	**171.6**	10	5	**170.5**	11	0.55
BMI	8	**28.5**	5	5	**28.5**	6	0.95
Hb (g/l)	20	**124.5**	18	10	**131.0**	18	**0.04**
Platelets (x 10^9/L)	20	**263**	97	10	**287**	126	0.20
Creatinine (µmol/l)	39	**89.4**	25	13	**91.9**	29	0.61
CrCl (ml/min)	47	**56.1**	21	16	**53.6**	25	0.55
Qualitative variable	**Missing**	***N***	***(%)***	**Missing**	***N***	***(%)***	***P value***
Sex (male)	-	**230**	56	-	**22**	52	0.63
Diagnosis of DVT	-	**332**	81	-	**37**	88	0.40
Outpatient status	-	**282**	69	-	**32**	76	0.38
Randomized to UFH	-	**215**	53	-	**14**	33	**0.02**
Cancer	-	**70**	17	-	**6**	14	0.83
VTE at 90 days	-	**12**	3	-	**1**	2	0.83
Bleeding at 10 days	-	**13**	3	-	**2**	5	0.59

9.1.1. Description of anticoagulation treatment and AC intensity measurement

Of the 408 patients included in the analysis, 193 patients received UFH while 215 received LMWH (139 received dalteparin and 76 received enoxaparin). The mean 1st dose of UFH was 326.2 units/kg (SD 21.4) and the subsequent mean twice-daily dose was 248.9 units/kg (SD 7.6). For the LMWH group, the mean dose (1st and twice-daily) was 99.8 units/kg (SD 4.7). The mean heparin level in all 408 patients was 0.76 (SD 0.47). Two thirds of the patients were treated with heparins entirely as an outpatient.

9.1.2. Description of VTE and bleeding outcomes

There were 12 (2.9%) VTE events within 90 days of randomization. Recurrent VTE presented as DVT in 8 patients (67%) and as PE (with or without DVT) in 4 patients (37%). Only two VTE events occurred in the first 10 days (both in the LMWH arm). The other 10 recurrent episodes of VTE occurred between 20 and 45 days (5 events in each of the UFH and LWMH arms).

Thirteen patients (3.3%) had bleeding events in the first 10 days of randomization (7 events in the UFH arm and 5 events in the LMWH arm). Of these, 2 were major bleeding events (both in the LMWH arm) and 11 were minor bleeding events (7 events in the UFH arm and 3 events in the LMWH arm).

9.2. Handling of missing values

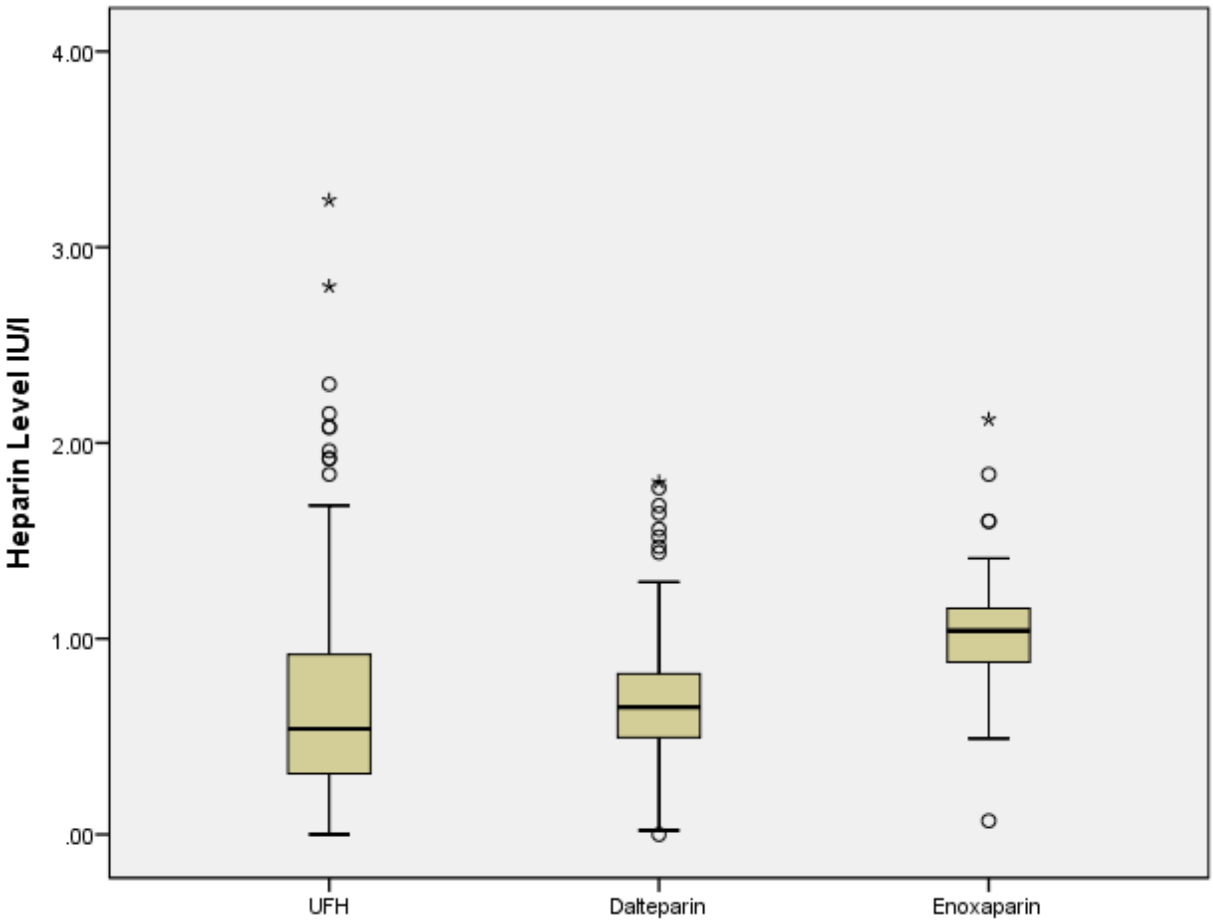
Of the independent variables used in the regression models, 5 had missing values (Table 1). Using Little's MCAR test, I could not reject the null hypothesis that the missing values were of MCAR type (Chi-Square = 432, DF = 426, p value =0.40). Imputation of 20 sets was performed using the EM method to fill in missing data prior to model building.

9.3. Influence of baseline characteristics on heparin levels

9.3.1. Influence of type of heparin on heparin levels

Did heparin levels differ between patients treated with UFH, dalteparin and enoxaparin?

Figure 2 below shows box-plots that describe heparin levels in patients treated with each of the three types of heparin. The box plots show: the median (line in middle of center box); 1st quartile (Q1) to 3rd quartile (Q3) (vertical limits of center box); "1.5 x interquartile range (IQR) minus Q1" and "1.5 x interquartile range (IQR) plus Q3" (whiskers); "*outliers"* indicated by circles, which represent values lying outside of the whiskers and < 3 time the height of the box; and "*extreme outliers"* indicated by stars, which represent values lying outside of the whiskers and > 3 times the height of the box.



Heparin level = 0.695 **+ 0.003 Dalteparin** **+ 0.340 Enoxaparin**

Partial p **0.95** **<0.001**

N=408; R^2=0.08; F (2, 405)=17.1; p<0.001

Figure 2: Box-plot of Heparin level against types of heparin

Interpretation and answer

Heparin levels were 0.695 units in the UFH group, 0.698 in the dalteparin group, and 1.034 in the enoxaparin group and differed among the three heparin

groups ($p<0.001$) (Figure 2). Therefore, as shown in the regression equation, relative to heparin levels in the UFH group (referent group, and UFH will generally be used as the referent group in the rest of these analyses), heparin levels were 0.003 (95% CI –0.1-0.1; p=0.95) higher in the dalteparin group, and 0.34 (95% CI 0.22-0.46; $p<0.001$) higher in the enoxaparin group. Heparin levels were 0.34 (95% CI 0.22-0.46; $p<0.001$) lower in the dalteparin group compared to the enoxaparin group. Type of heparin (UFH, enoxaparin, dalteparin) accounted for 8% of the variability in heparin levels among all patients (Table 2), and accounted for 19% of the variability in heparin levels among the LMWH patients (N= 215; $R^2 = 0.19$; F [1, 213] = 50.6; $p<0.001$).

Table 2: Proportion of heparin levels variability accounted for by different baseline factors

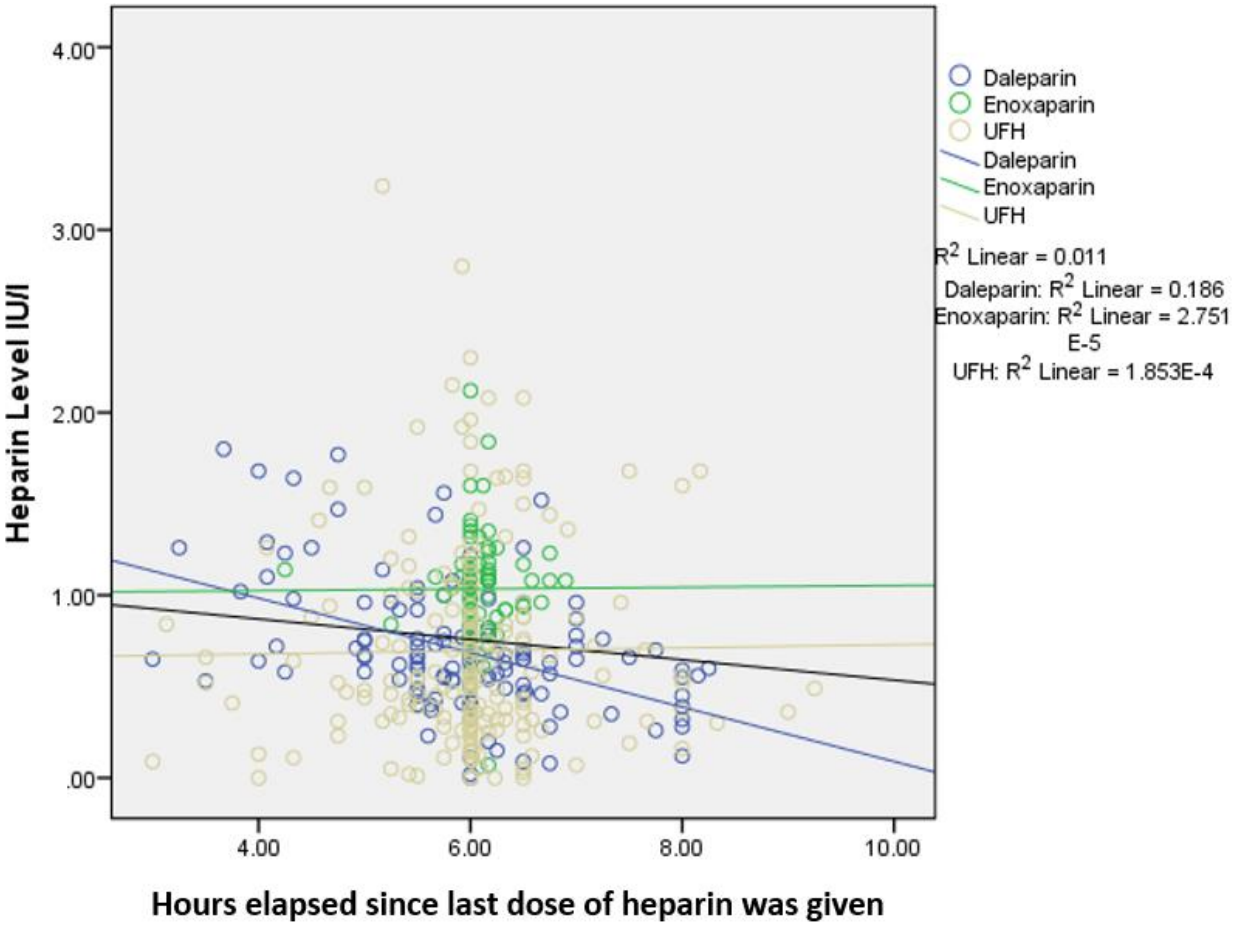
Variables in the model	R^2
Type of heparin	0.08
Weight	0.03
Weight and type of heparin	0.11
Weight and BMI	0.04
BMI	0.04
BMI and type of heparin	0.14
Sex	0.01
Sex and BMI	0.06
Sex and type of heparin	0.11
Age	0.00*
Height	0.00*
CrCl	0.00*
Creatinine	0.03
Creatinine and CrCl	0.03
Creatinine and type of heparin	0.13
Cancer	0.00*
Hospitalization status	0.00*

***(Continued)* Proportion of heparin levels variability accounted for by different baseline factors**	
Variables in the model	***R^2***
Type of VTE	0.00*
Platelet count	0.02
Platelet count, and type of heparin	0.13

Asterisk indicating p value <0.01

9.3.2. Influence of timing of blood sample withdrawal on heparin levels

Did heparin levels differ according to time of blood sample withdrawal (after the last dose of heparin) in patients treated with heparins?



Heparin level = 1.09 – **0.06 (per hour)**

N=408; R^2=0.01; F (1, 406)=4.4; p=0.04

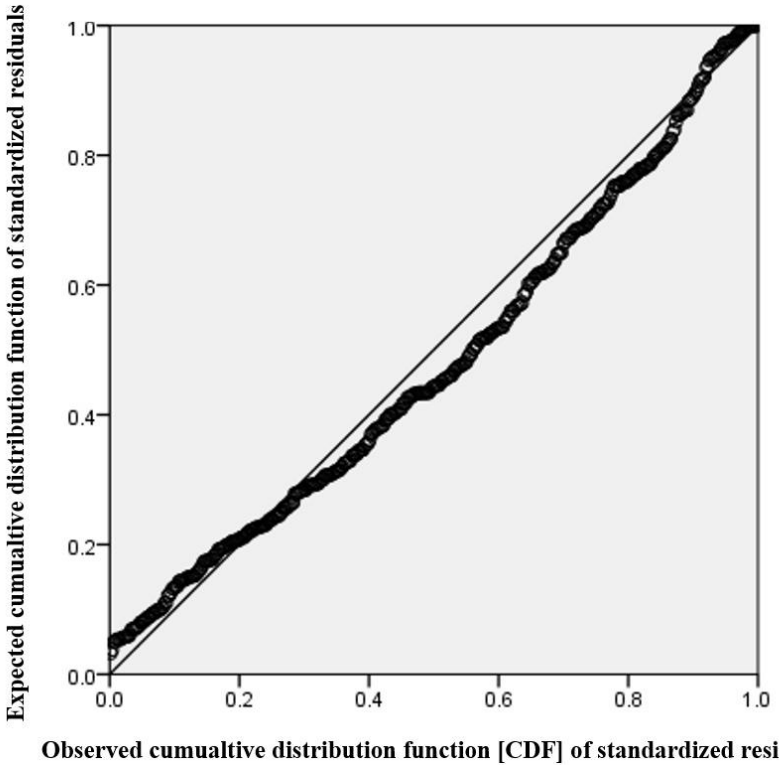
Figure 3: Heparin level against hours elapsed since last dose of heparin in patients was given

Interpretation and answer

In an analysis of all patients, when type of heparin was not controlled for (Figure 3), heparin levels decreased by 0.06 IU/ml (95% CI –0.1 to –0.003, p=0.04) for each 1-hour that elapsed between when heparin was last given and when the blood sample was obtained. The time of blood withdrawal for heparin levels measurement only explained 1% of variability in heparin levels (Table 2).

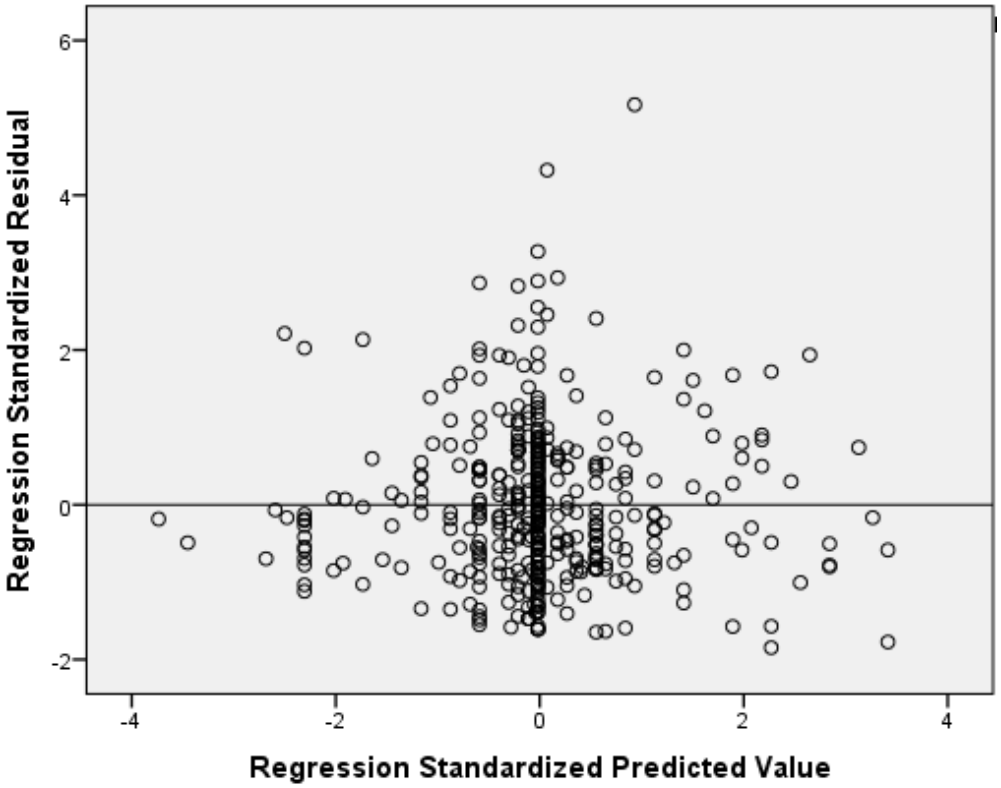
Summary of the diagnostic testing

The linear assumptions were not violated (Figure 4 and 5). **In future sections, figures relating to the linear assumptions will only be shown when there is concern about the assumption.**



If this assumption is satisfied, the plot should show minimal or no deviation from the straight line of identity, which is the case with this plot

Figure 4: P-P plot evaluating the normality assumption for linear model examining influence of timing of blood withdrawal on heparin levels



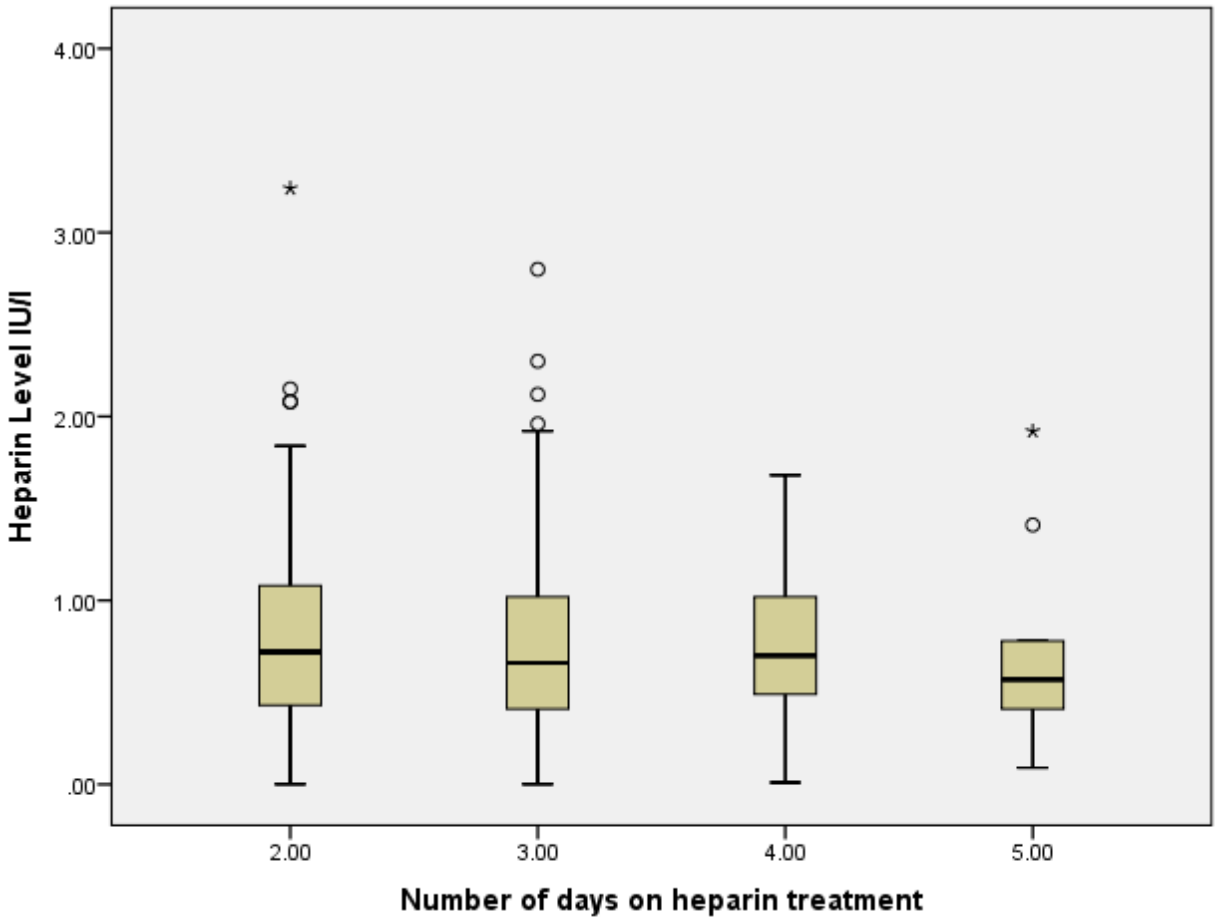
If this assumption is satisfied, the plot should show a similar pattern of residuals above and below the zero line for the regression standard residuals. In this plot, the distribution of standardized residuals are not grossly different accross the predicted values

Figure 5: Evaluation of Homoscedasticity for linear model examining influence of timing of blood withdrawal on heparin levels

9.3.3. Influence of number of days on heparin treatment at time of blood sample withdrawal on heparin levels

Did heparin levels differ by number of days on heparin treatment in patients treated with heparins?

Figure 6 below shows box-plots (see Section 8.16.1 for explanation of the box plot) according to the number of days that patients were on heparin treatment when heparin levels were measured.



Heparin level = 0.83 – **0.23 (per day)**

N=408; R^2=0.001; F (1, 406)=0.52; p=0.43

Figure 6: Box-plot of Heparin level according to number of days on heparin treatment

Interpretation and answer

In an analysis of all patients, when type of heparin was not controlled for (Figure 6), number of days on heparin treatment did not influence mean heparin levels. Therefore, number of days on heparin treatment did not explain any variability in heparin levels (Table 2).

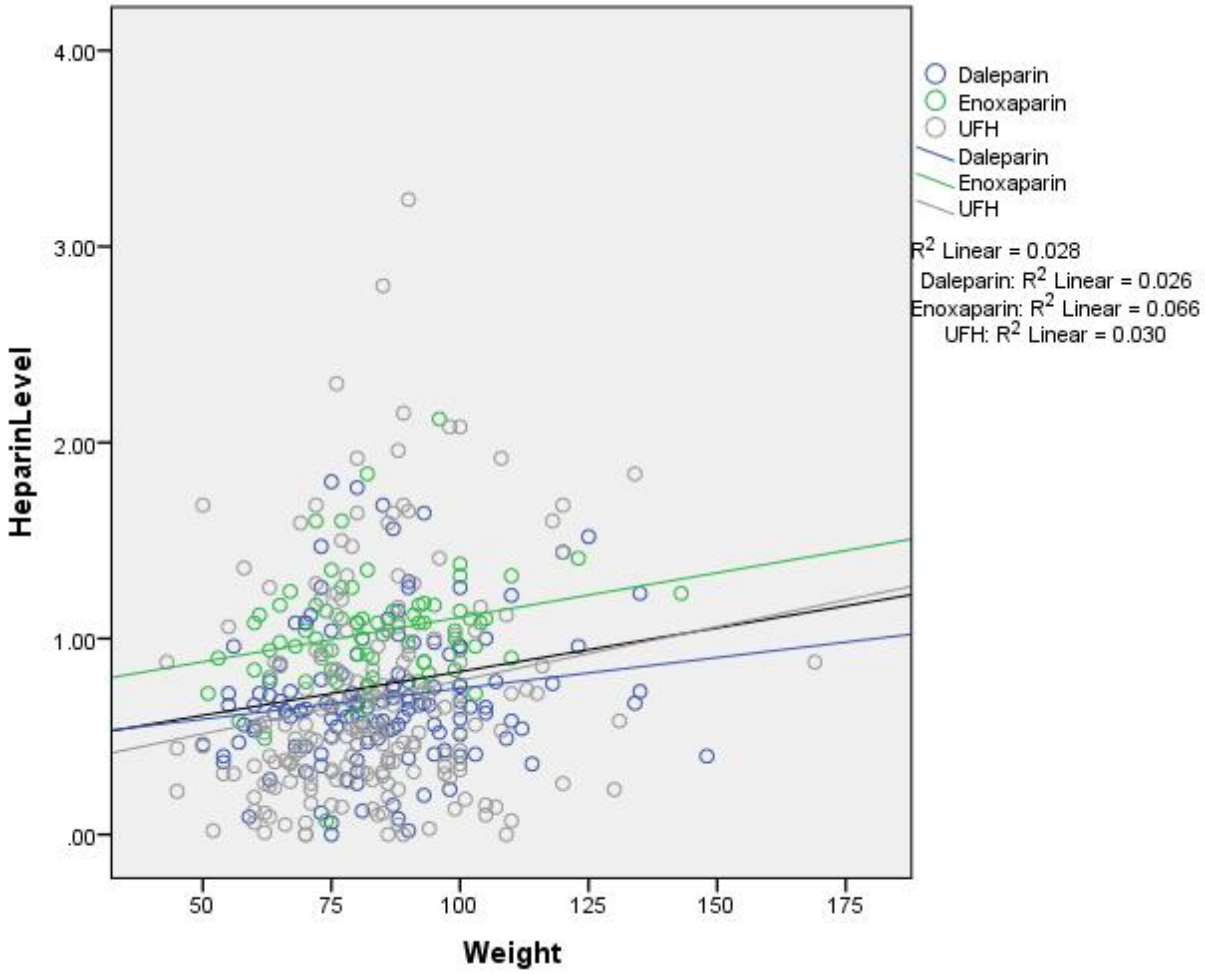
Summary of the diagnostic testing

The linear assumptions are not violated

NOTE: In subsequent analyses in this thesis, I have controlled (or adjusted) for the "timing of blood withdrawal" because differences in heparin levels due to differences in the timing of blood withdrawal are considered "noise" when the relationship between other baseline factors and heparin levels is being examined. Because heparin levels did not differ with the "number of days on heparin treatment", I have not routinely controlled for this factor.

9.3.4. Influence of weight on heparin levels

Did heparin levels differ by weight in patients treated with heparins?



Heparin level = 0.385 + **0.004 Weight (kg)**

N=408; R^2=0.03; F (1, 406)=11.52; p=0.001

Figure 14: Heparin level against weight

Interpretation and answer

In an analysis of all patients, when type of heparin was not controlled for (Figure 14), mean heparin levels increased with patient's weight. The increase in heparin levels was 0.004 IU/ml (95% CI 0.002-0.007, p=0.001) for each kg, and 0.04 per 10 kg increment in patient's weight. Weight explained 3% of variability in heparin levels (Table 2). The increase in heparin levels with body weight suggests that, relative to lighter patients, a weight-adjusted dose of heaprin results in a somewhat more intense anticoagulant effect in heavier patients.

Summary of the diagnostic testing

The linear assumptions are not violated.

Did the relationship between weight and heparin levels differ between patients treated with UFH, dalteparin and enoxaparin?

Heparin level =	0.62	**+ 0.006**	+ 0.23	+ 0.45	**– 0.003**	**– 0.001**
		Weight	Dalteparin	Enoxaparin	**Weight * Dalteparin**	**Weight * Enoxaparin**
Partial p		**0.003**	0.35	0.15	**0.32**	**0.72**

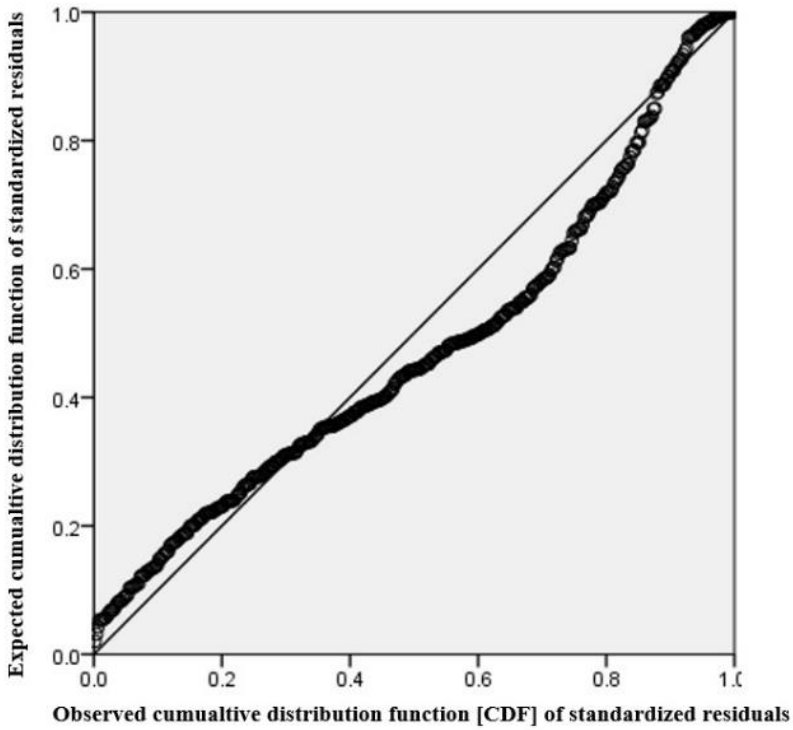
N=408; R^2=0.12; F (6, 401)=9.4; p<0.001

Interpretation and answer

To assess whether the relationship between weight and heparin levels differed between patients treated with UFH, dalteparin and enoxaparin, the multiple regression equation was extended to include UFH, dalteparin and enoxaparin, and an interaction term for "weight x dalteparin" and "weight x enoxaparin". If the interaction terms were statistically significant in this equation, this would indicate that the slope of the relationship between weight and heparin levels differed with the type of heparin. The partial P-value associated with the two “heparin type x weight” interaction terms were not significant indicating that the relationship between weight and heparin levels (i.e., slope of the linear regression line) did not differ among patients in the three heparin groups (UFH, enoxaparin, dalteparin). Because the slope did not differ, the analysis does not support a separate analysis of the relationship between heparin levels and weight for each of the UFH, dalteparin, and enoxaparin. Weight and type of heparin explained 11% of variability in heparin levels, and weight, type of heparin, and the “weight x heparin type” interaction terms also explained 11% of variability in heparin levels (Table 2).

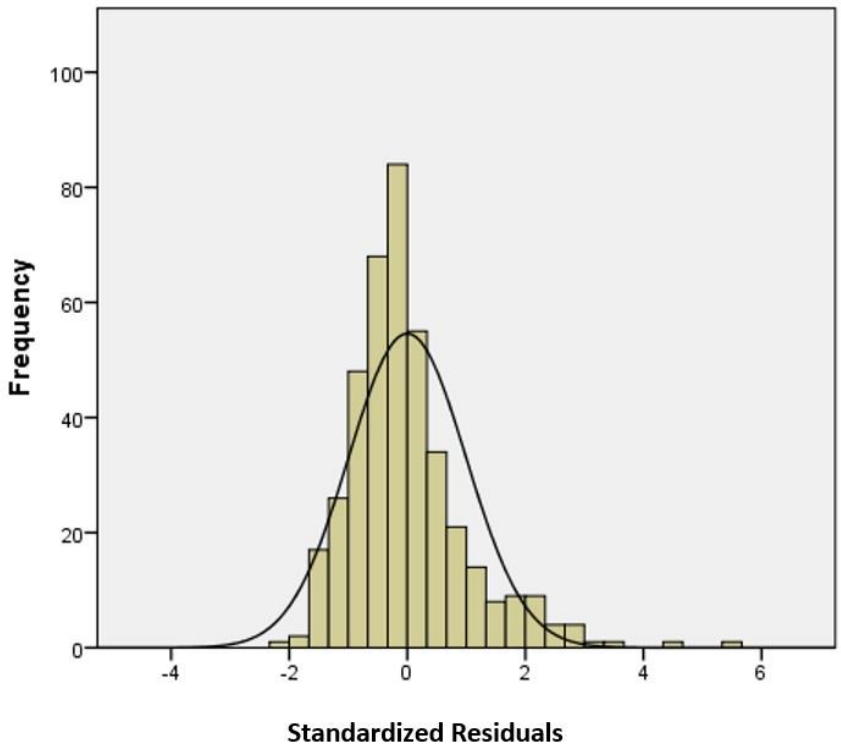
Summary of the diagnostic testing

The effect modifiers (products of weight and type of heparin) showed significant multicollinearity with type of heparin. The P-P plot showed some deviation from a straight line suggesting that the normality assumption was not fully satisfied (Figures 8 and 9).



The plot is showing some deviation from straight line of identity, suggesting residuals are not normally distributed (i.e., skewed to right)

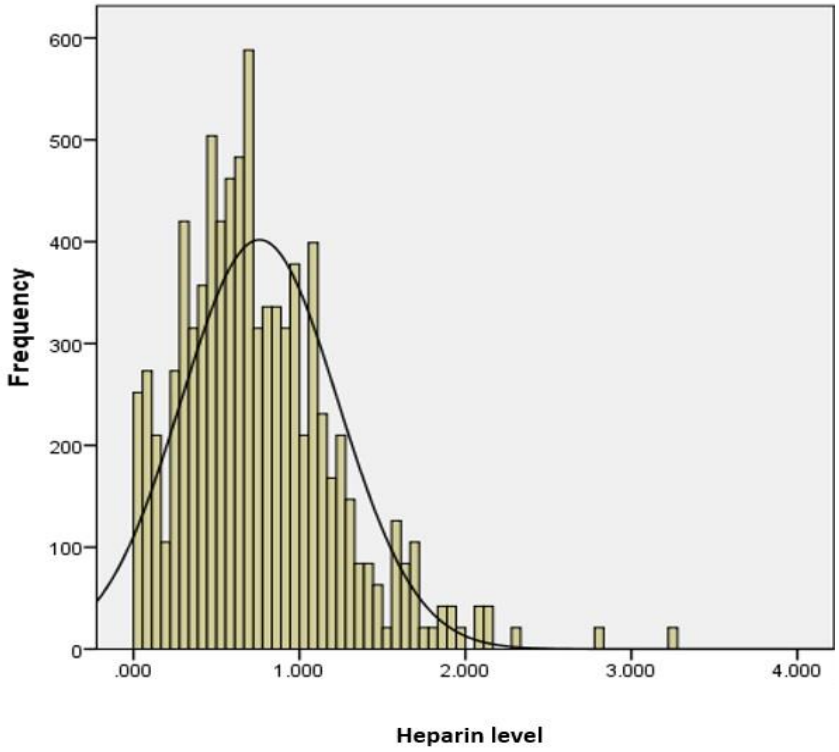
Figure 8: P-P plot evaluating normality assumption for linear model examining influence of weight on heparin levels



A left sided skewness is noted in the distribution of standardized residuals, rather than a normal distribution

Figure 9: Histogram of standardized residuals for linear model examining influence of weight on heparin levels

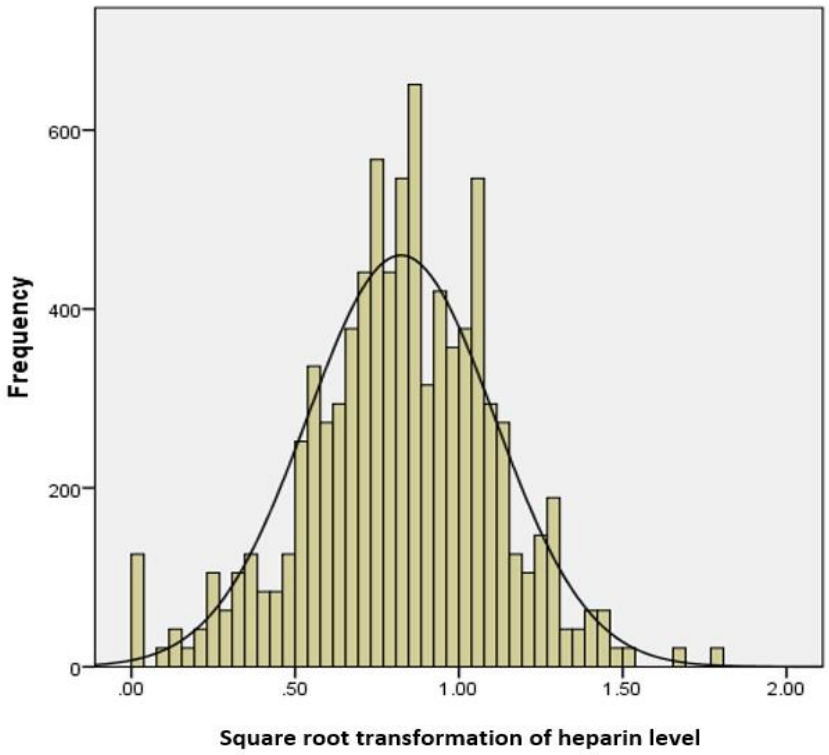
The violation was noticed to occur only when type of heparin was added as a covariate in the model; therefore, heparin levels (the dependent variable) may have contributed to skewness seen in the P-P plot of residuals. The histogram of the heparin levels confirmed that heparin levels were skewed (Figure 10) and, therefore, transformation of heparin levels was carried out.



The histogram shows a right sided skewness in the distribution of heparin levels

Figure 10: Histogram of heparin levels

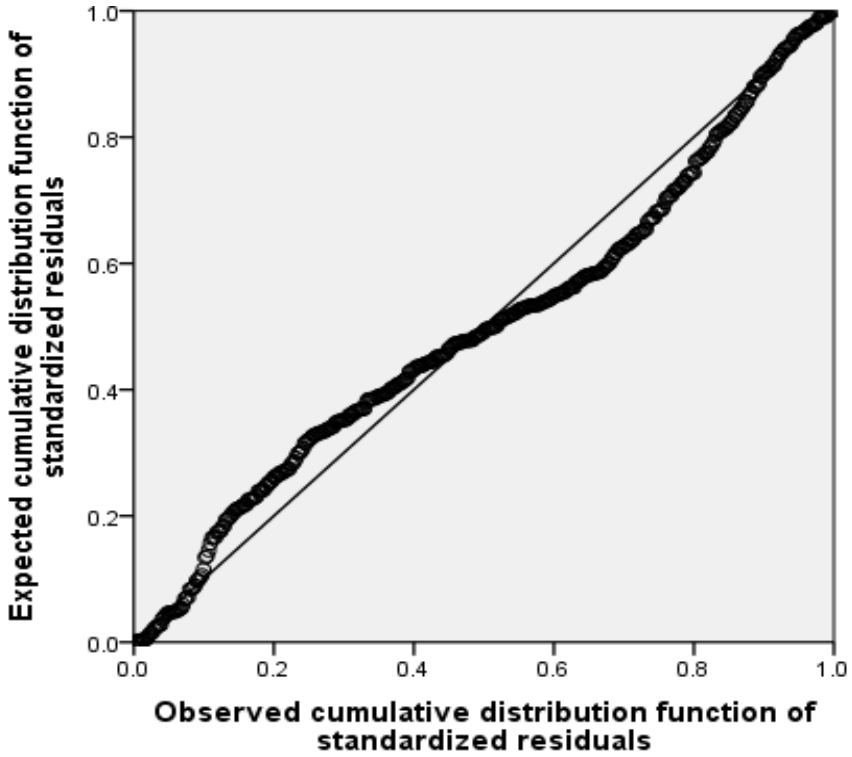
Of the three transformations attempted to normalize the distribution of heparin levels (square root vs. natural logarithm vs. common logarithm), the square root of heparin levels provided the best results (Figure 11).



The distribution of heparin levels approximating a normalized distribution after transformation

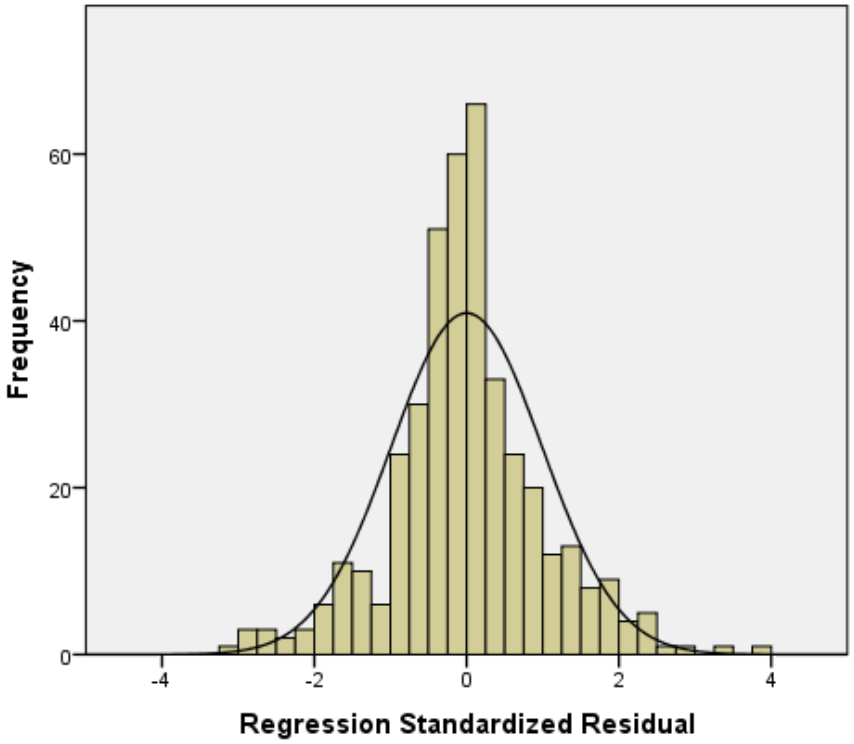
Figure 11: Histogram of heparin levels after square root transformation

Using the square root value of heparin levels as the dependent variable (instead of heparin levels), I re-ran the regression model using the same independent variables (weight, type of heparin, and products of weight and type of heparin) and it resulted in a better p-p plot and histogram distribution (Figures 12 and 13).



The plot is showing a correction toward the straight line of identity after transformation

Figure 12: P-P plot evaluating normality assumption for linear model examining influence of weight on heparin levels after square root transformation of heparin levels



The histogram shows a normal distribution of standardized residuals

Figure 13: Histogram of standardized residuals for linear model examining influence of weight on heparin levels after square root transformation of heparin levels

However, the results of significance (i.e., p values) did not change importantly for weight, type of heparin, and products of weight and type of heparin. Based on these findings, the original analyses can be used without square root transformation because: 1) essentially the same results were obtained when heparin levels were, and

were not, transformed; 2) the regression method can accommodate some deviation from normality; and 3) transforming the dependent variable would make it difficult to interpret the regression equations and, therefore, such transformation should be avoided unless there would otherwise be major violations of the regression assumptions. From this point forward in my thesis, the evaluation of linear assumptions is restricted to linearity, independence, and homoscedasticity.

Did weight and BMI independently influence heparin levels in patients treated with heparins?

Heparin level = 0.57 **+ 0.02 BMI – 0.001 Weight (kg)**

Partial p **0.01** **0.80**

N=400; R^2=0.05; F (3, 396)=7.4; p<0.001

Interpretation and answer

Heparin levels increased with BMI after adjusting for weight. Heparin levels increased by 0.02 IU/ml (95% CI 0.01-0.04, p=0.01) for each unit of BMI. However, weight had no effect on mean heparin levels after adjusting for BMI (partial p=0.75). BMI and weight in combination only explained 4% of variability in heparin levels (Table 2), which is the same proportion of variability that was explained by BMI

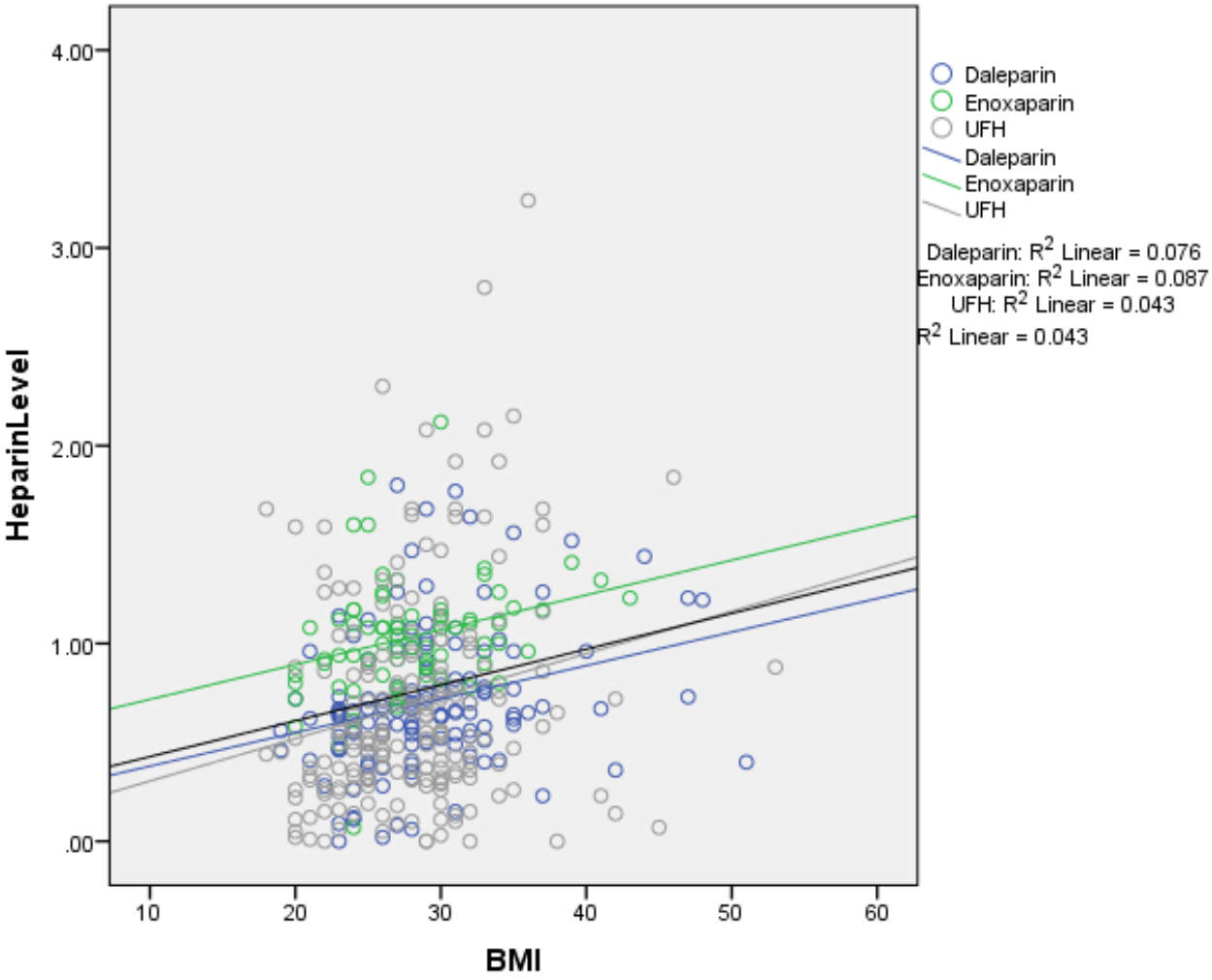
alone. Therefore, the relationship between heparin levels and BMI appears to be stronger than the relationship between heparin levels and weight (although neither is a strong relationship). These findings suggest that the increase in heparin levels with body weight is primarily mediated by greater adiposity (fatness) in heavier patients.

Summary of the diagnostic testing

The linear assumptions and collinearity were not violated.

9.3.5. Influence of BMI on heparin levels

Did heparin levels differ by BMI in patients treated with heparins?



Heparin level = 0.25 + **0.02 BMI (kg/m^2)**

N=400; R^2=0.04; F (1, 398)=18; p<0.001

Figure 7: Heparin level against BMI

Interpretation and answer

In an analysis of all patients, when type of heparin was not controlled for (Figure 7), heparin levels increased with BMI. The increase in heparin levels was 0.02 IU/ml (95% CI 0.01-0.03) for each unit increment in BMI. BMI explained 4% of variability in heparin levels (Table 2)

Summary of the diagnostic testing

The linear assumptions were not violated.

Did the relationship between BMI and heparin levels differ between patients treated with UFH, dalteparin and enoxaparin?

Heparin level =	0.44	**+ 0.02 BMI**	+ 0.20 Dalteparin	+ 0.52 Enoxaparin	**– 0 .008 BMI * Dalteparin**	**– 0.006 BMI * Enoxaparin**
Partial p		**<0.001**	0.45	0.14	**0.39**	**0.62**

N=400; R^2=0.14; F (6, 393)=10.9; p<0.001

Interpretation and answer

The relationship between BMI and heparin levels (i.e., slope of the linear regression line) did not differ among the three heparin groups (UFH, enoxaparin,

dalteparin). Because the slope did not differ, this analysis does not support the need for a separate analysis of the relationship between weight and heparin levels for each of the UFH, dalteparin, and enoxaparin subgroups.

Summary of the diagnostic testing

The linear assumptions were not violated. The effect modifiers (BMI and type of heparin) showed significant multicollinearity with type of heparin.

9.3.6. Influence of sex on heparin levels

Did heparin levels differ by sex?

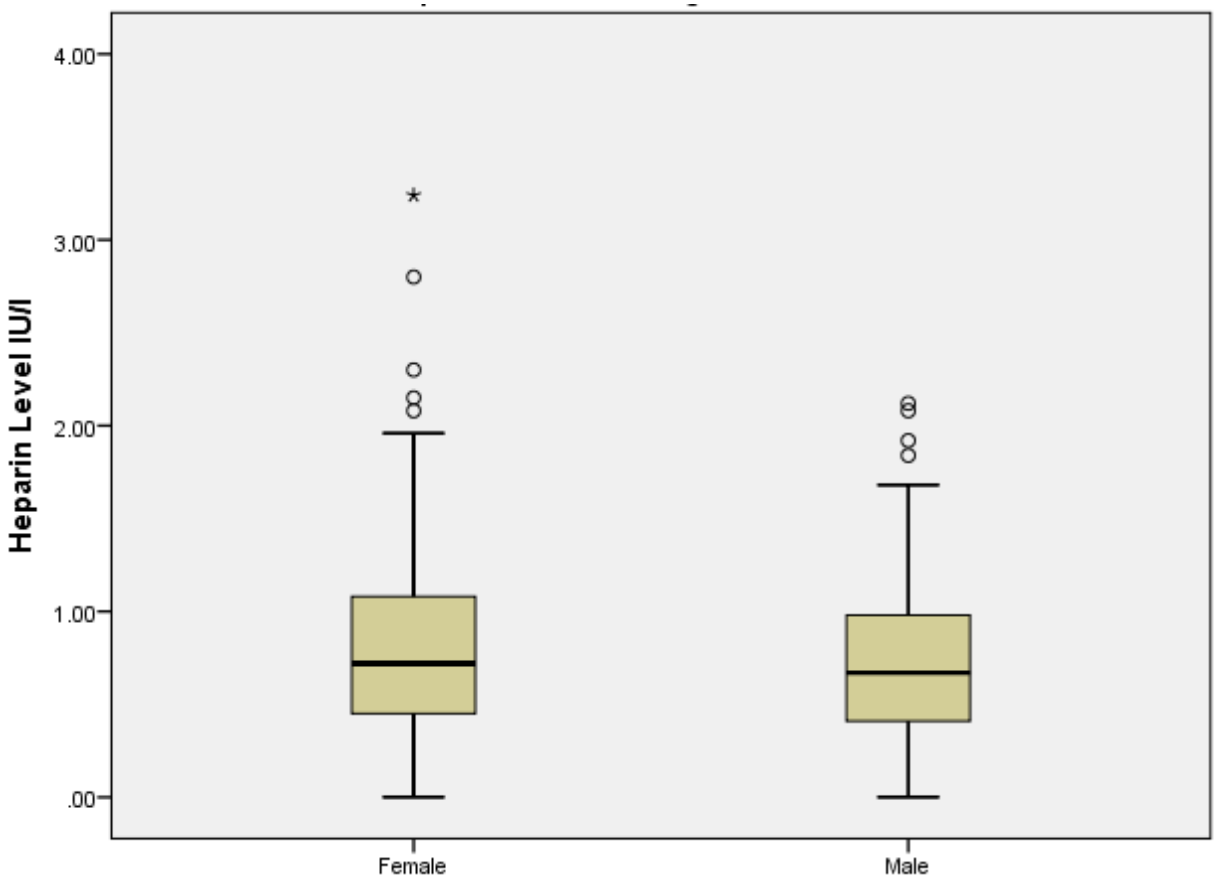


Figure 15: Heparin level in female and male patients

Heparin level = 0.72 **+ 0.10 if Female**

N=408; R^2=0.01; F (1, 406)=4.7; p=0.03

Interpretation and answer

In an analysis of all patients, when type of heparin was not controlled for (Figure 15), heparin levels was 0.10 IU/ml (95% CI 0.01-0.19) higher in female patients compared to male patients (0.82 vs. 0.72 IU/ml). Sex explained only 1% of variability in heparin levels (Table 2).

Did the relationship between heparin levels and sex differ between patients treated with UFH, dalteparin and enoxaparin?

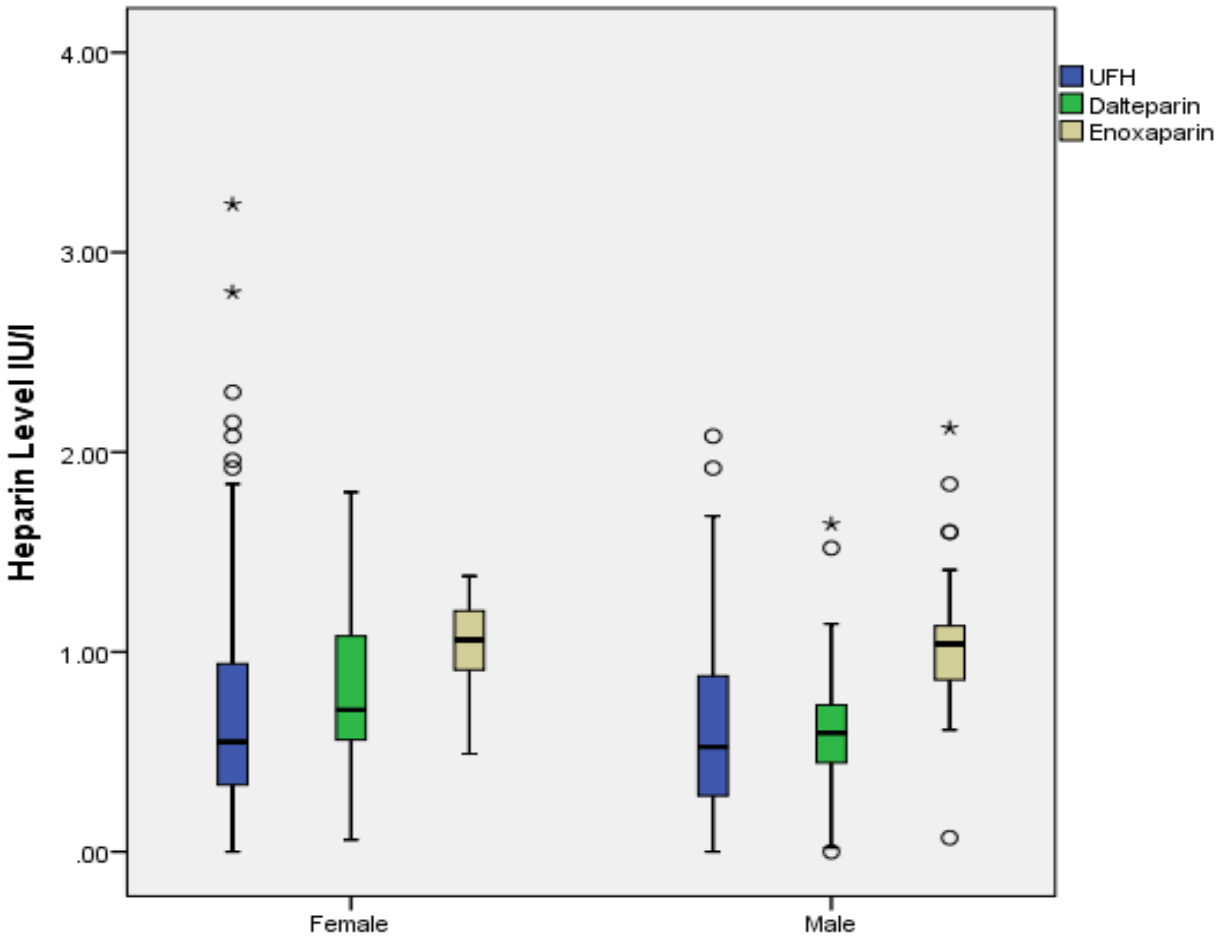


Figure 16: Heparin level in female and male patients according to type of heparin

Heparin Level = 1.00	**+ 0.12**	– 0.03	+ 0.40	**+ 0.08**	**– 0.12**
	If Female	Dalteparin	Enoxaparin	**Female * Dalteparin**	**Female * Enoxaparin**
Partial P	**0.06**	0.62	<0.001	**0.34**	**0.36**

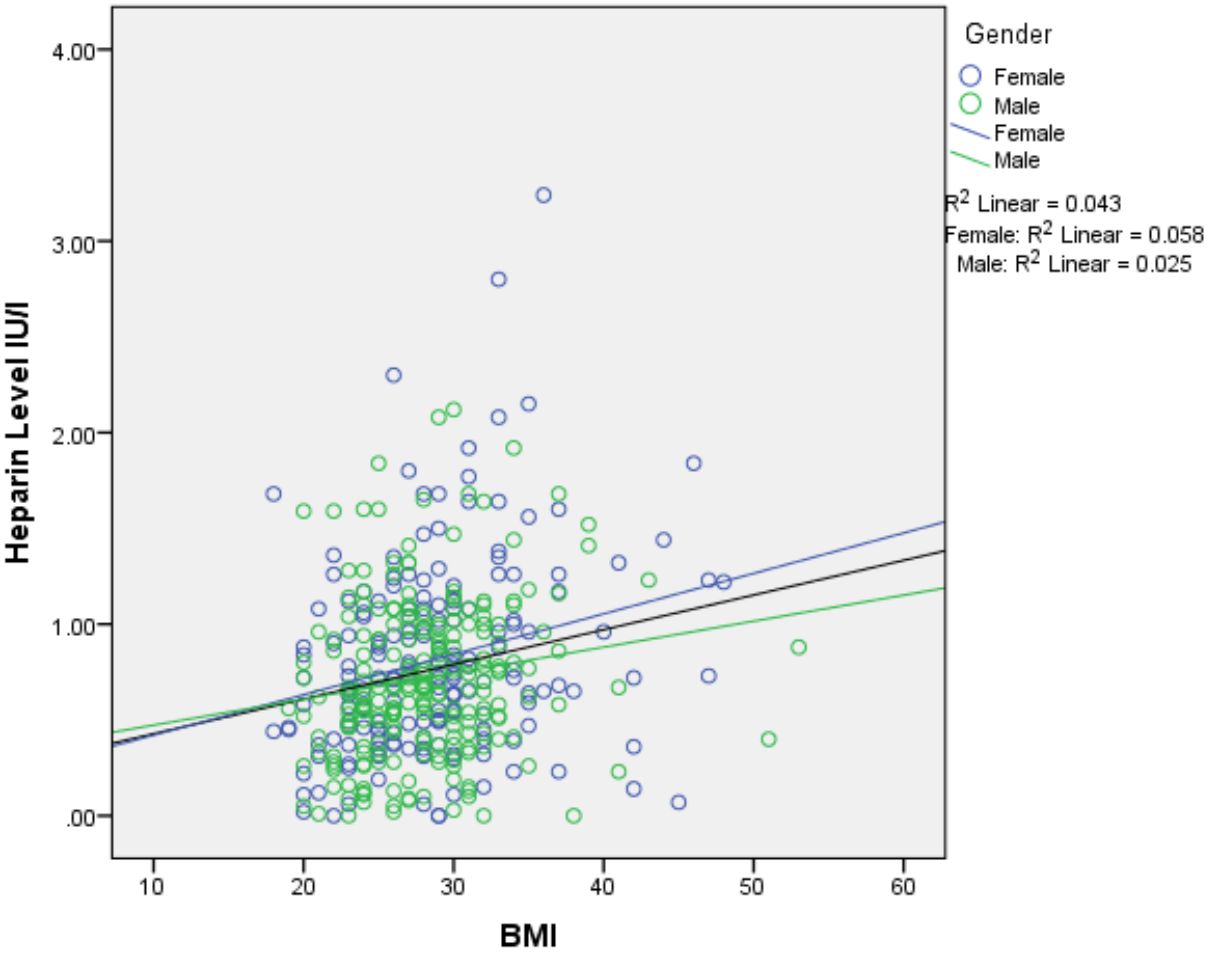
N=408; R^2=0.11; F (6, 401)= 8.7; p<0.001

Interpretation and answer

The relationship between sex and heparin levels (i.e., slope of the linear regression line) did not differ among the three heparin groups (UFH, enoxaparin, dalteparin). Because the slope did not differ, the analysis does not support the need for a separate analysis of the relationship between sex and heparin levels for each of the UFH, dalteparin, and enoxaparin subgroups.

Sex and type of heparin in combination explained 10% of variability in heparin levels (Table 2). Therefore, the proportion of the variability of heparin levels that was explained by sex (i.e., 1%) and type of heparin (i.e., 8%) appears to be additive.

Did sex influence heparin levels independently of BMI?



Heparin Level = 0.53 + 0.02 BMI **+ 0.08 If Female**

Partial P 0.001 **0.07**

N=400; R^2=0.06; F (3, 396)=8.4; p<0.001

Figure 17: Heparin level against BMI

Interpretation and answer

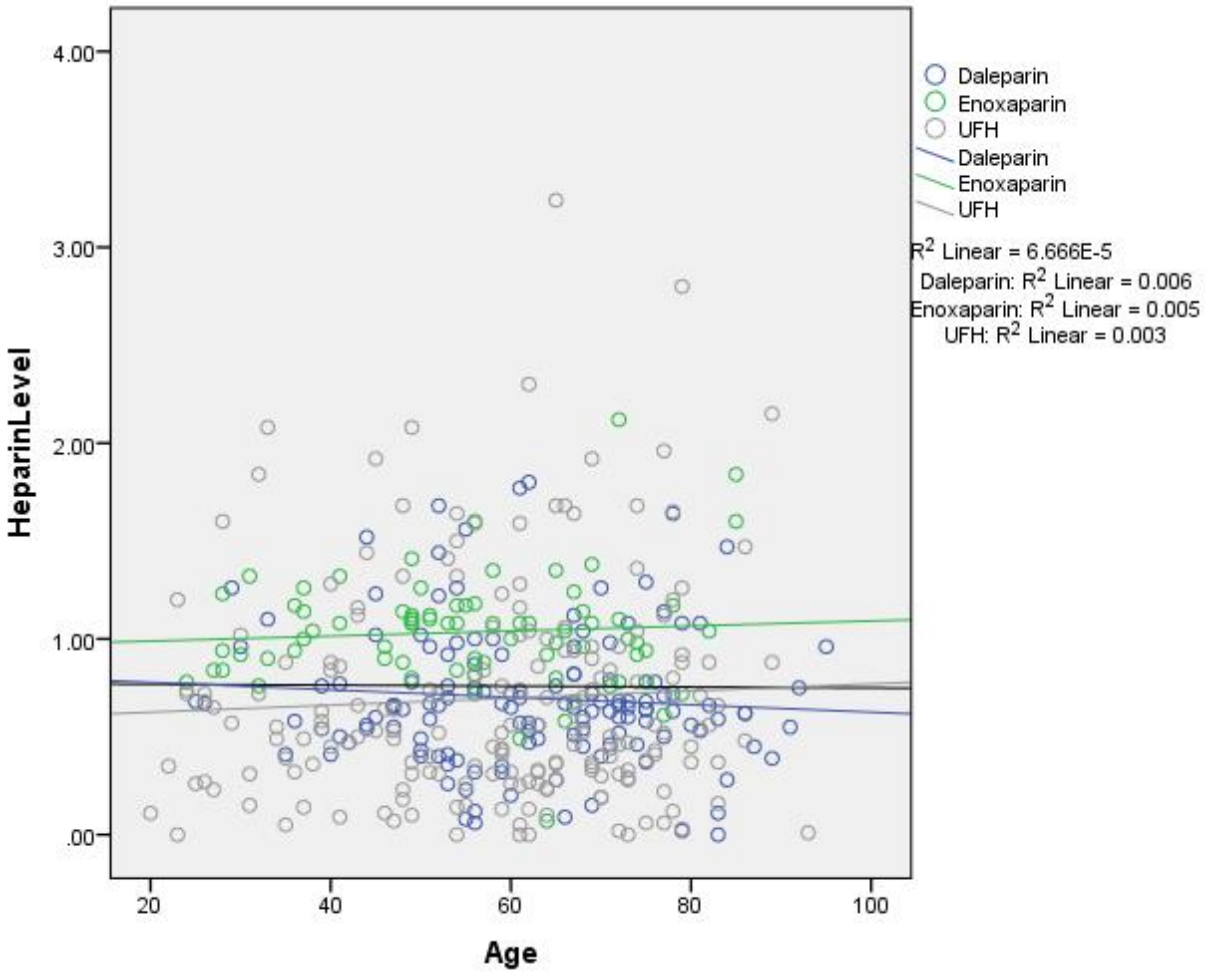
In an analysis of all patients, when type of heparin was not controlled for (Figure 17), heparin levels was not significantly different between females and males after adjusting for BMI. However, as the BMI-unadjusted increase in heparin levels and the associated P-values in females compared to males (0.10 IU/ml; p=0.03) was almost identical to the BMI-adjusted increase in heparin levels (0.08 IU/ml; p=0.07), differences in BMI between females and males does not appear to account for the higher heparin levels in females. Sex and BMI in combination explained 5% of variability in heparin levels (Table 2). Therefore, the proportion of the variability of heparin levels that was explained by sex (i.e., 1%) and BMI (i.e., 4%) appears to be additive.

Summary of the diagnostic testing

The linear assumptions were not violated.

9.3.7. Influence of age on heparin levels

Did heparin levels differ by age?



Heparin level = 0.77 **– 0.0002 Age (years)**

N= 408; R^2 = 0.0001; F (1, 406)=0.03; p=0.87

Figure 18: Heparin level against age

Interpretation and answer

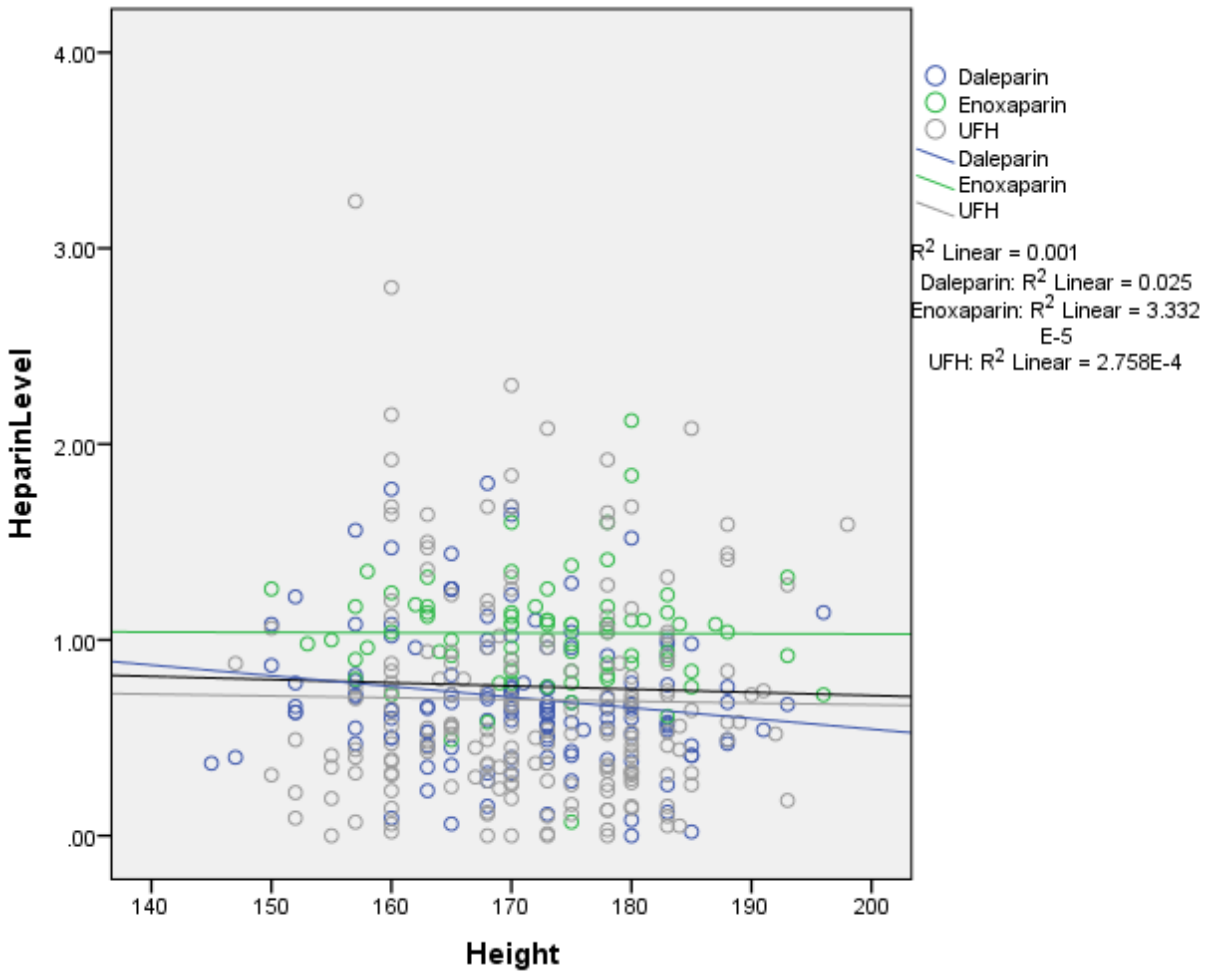
In an analysis of all patients, when type of heparin was not controlled for (Figure 18), age did not influence the heparin levels (p=0.87) (Table 2). When type of heparin was controlled for, age also did not influence heparin levels (analysis not shown; p=0.59)

Summary of the diagnostic testing

The linear assumptions were not violated.

9.3.8. Influence of height on heparin levels

Did heparin levels differ by height?



Heparin level = 1.042 **– 0.002 Height (cm)**

N=400; R^2=0.001; F (1, 398)=0.5; p=0.5

Figure 19: Heparin level against height

Interpretation and answer

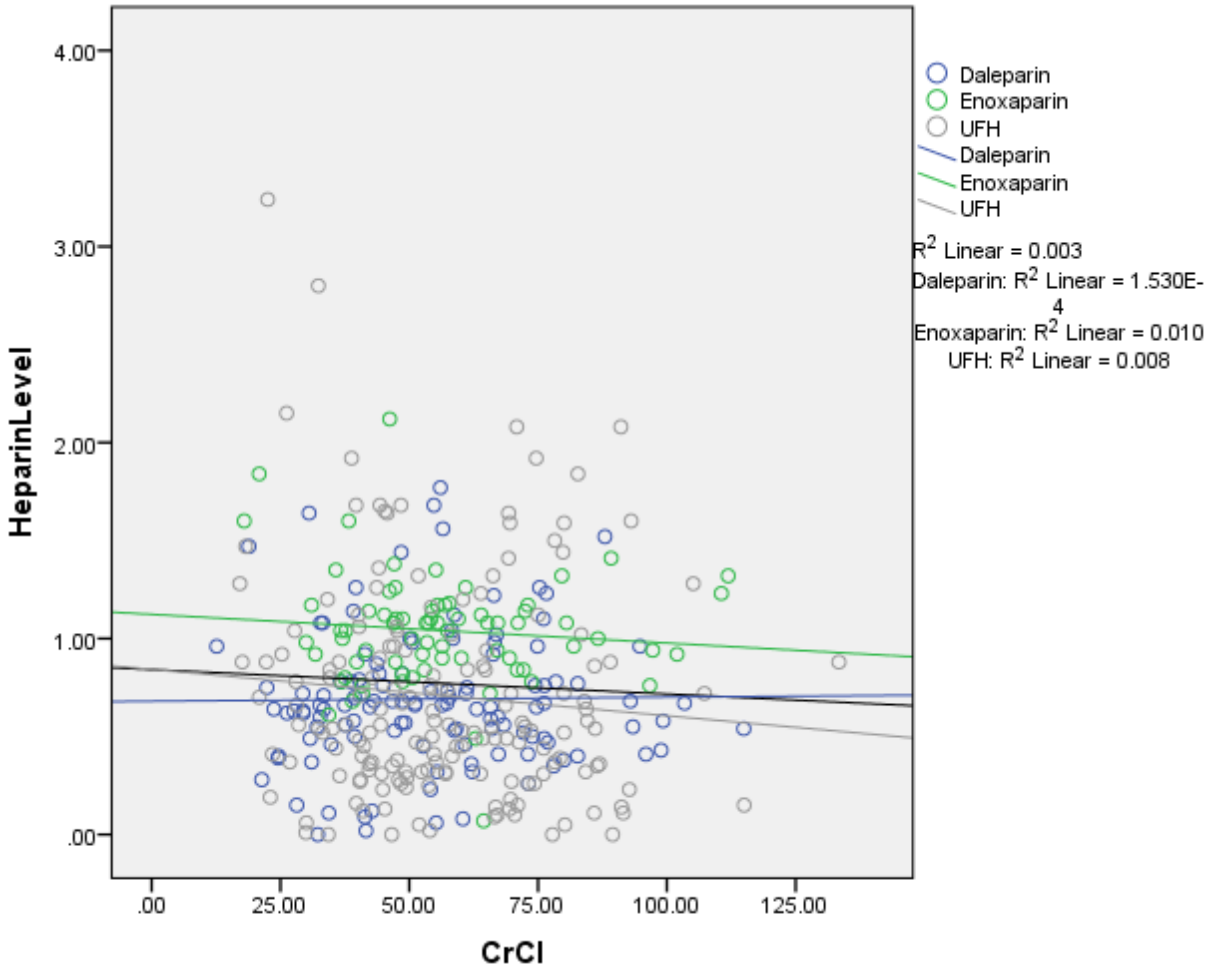
In an analysis of all patients, when type of heparin was not controlled for (Figure 17), height did not influence the heparin levels (p=0.5). In addition, height did not influence mean heparin levels after adjusting for type of heparin (analysis not shown; p=0.33).

Summary of the diagnostic testing

The linear assumptions were not violated.

9.3.9. Influence of CrCl on heparin levels

Did heparin levels differ by CrCl?



Heparin level = 0.83 **– 0.001 CrCl (ml/min)**

N= 361; R^2 = 0.003; F (1, 359)=1.1; p=0.3

Figure 20: Heparin level against CrCl

Interpretation and answer

In an analysis of all patients, when type of heparin was not controlled for (Figure 20), CrCl did not influence heparin levels.

Summary of the diagnostic testing

The linear assumptions were not violated. Effect modifiers (products of CrCl and type of heparin) showed significant multicollinearity.

Did the relationship between CrCl and heparin levels differ between patients treated with UFH, dalteparin and enoxaparin?

Heparin level = 1.23	**– 0.002**	– 0.13	+ 0.28	**+ 0.002**	**+ 0.001**
	CrCl	Dalteparin	Enoxaparin	**CrCl * Dalteparin**	**CrCl * Enoxaparin**
Partial p	**0.13**	0.38	0.14	**0.38**	**0.71**

N=361; R^2=0.10; F (6, 354)=5.68; p<0.001

Interpretation and answer

CrCl did not influence the heparin levels, after adjusting for type of heparin. The relationship between CrCl and heparin levels (i.e., slope of the linear regression line) did not differ among the three heparin groups (UFH, enoxaparin, dalteparin). Because the slope did not differ, the analysis does not support the need for a separate

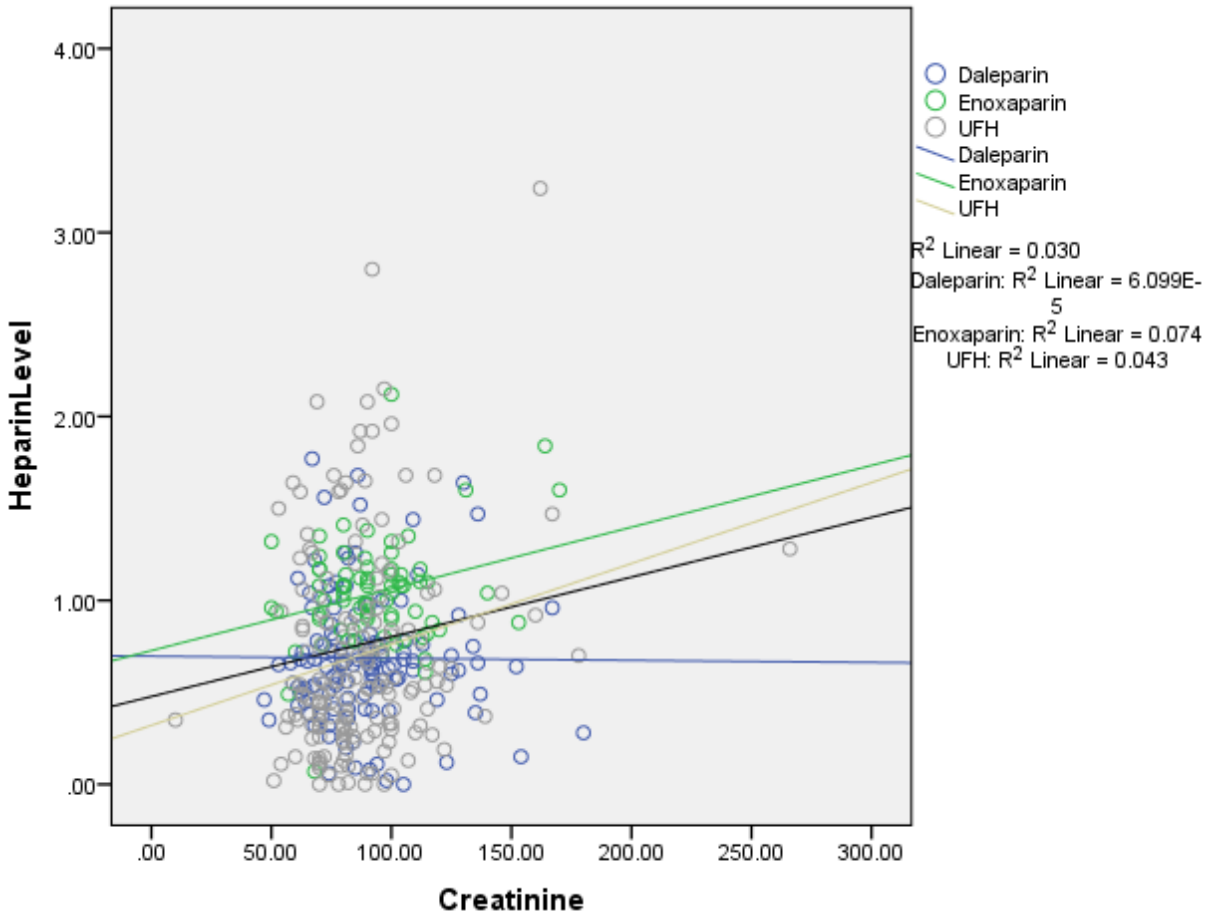
analysis of the relationship between CrCl and heparin levels for each of the UFH, dalteparin, and enoxaparin subgroups.

Summary of the diagnostic testing

The linear assumptions were not violated. The effect modifiers (CrCl and type of heparin) showed significant multicollinearity with type of heparin.

9.3.10. Influence of creatinine on heparin levels

Did heparin levels differ by creatinine in patients treated with heparins?



Heparin level = 0.48 + **0.003 creatinine (μmol/l)**

N=369; R^2=0.03; F (1, 367)=11.2; p=0.001

Figure 21: Heparin level against creatinine

Interpretation and answer

In an analysis of all patients, when type of heparin was not controlled for (Figure 21), heparin levels increased with patients' creatinine. Heparin levels increased by 0.03 IU/ml (95% CI 0.01-0.05) for every 10 μmol/l increment in creatinine. Creatinine explained 3% of variability in heparin levels (Table 2).

Summary of the diagnostic testing

The linear assumptions of linearity, are not violated.

Did heparin levels differ by creatinine between patients treated with UFH, dalteparin, and enoxaparin?

Heparin level =	0.71	**+ 0.004**	+ 0.40	+ 0.42	**– 0.005**	**– 0.001**
		Creatinine	Dalteparin	Enoxaparin	**Creatinine * Dalteparin**	**Creatinine * Enoxaparin**
Partial p		**0.001**	0.03	0.09	**0.03**	**0.68**

N=369; R^2=0.13; F (6, 362)=8.9; p<0.001

Interpretation and answer

Heparin levels increased by 0.04 IU/ml (95% CI 0.02-0.07) for every 10 μmol/l increment in creatinine. However, the relationship between creatinine and

heparin levels (i.e., slope of the linear regression line) differed according to type of heparin (UFH, dalteparin, and enoxaparin); there was a similar increase in heparin levels with increased in creatinine in patients on UFH and enoxaparin, and a significantly smaller increase (p=0.03) in heparin levels with increasing creatinine in patients on dalteparin. Because the slop differed according to type of heparin, a separate analysis was carried out for each type of heparin (see below). In the multivariable analysis, creatinine and type of heparin in combination explained 12% of variability in heparin levels (Table 2). Therefore, the proportion of the variability of heparin levels that was explained by creatinine (3%), and type of heparin (8%) appears to be additive.

For patients treated with UFH:

$$\text{Heparin level} = 0.32 + \mathbf{0.004\ Creatinine\ (\mu mol/l)}$$

$$N=172;\ R^2=0.04;\ p=0.007$$

Heparin levels for patients increased by 0.04 IU/ml (95% CI 0.01-0.08) for each 10 μmol/l increment in patient's creatinine.

For patients treated with Dalteparin:

Heparin level = 0.73 **– 0.0003 Creatinine (μmol/l)**

N=122; R^2 <0.0001; p=0.93

Patient's creatinine did not influence mean heparin levels.

For patients treated with Enoxaparin:

Heparin level = 0.71 **+ 0.003 Creatinine (μmol/l)**

N=75; R^2=0.07; p=0.02

Heparin levels for patients increased by 0.03 IU/ml (95% CI 0.01-0.06) for each 10 μmol/l increment in patient's creatinine.

Summary of the diagnostic testing

The linear assumptions were not violated. The effect modifiers (product of creatinine and type of heparin) and type of heparin showed significant multicollinearity.

Were heparin levels influenced more by creatinine or by CrCl in patients treated with heparin?

Heparin level =	0.72	+ **0.004**	+ **0.001**
		Creatinine	**CrCl**
Partial p		**0.001**	**0.80**

N=361; R^2=0.03, F (1, 367)=11.2, p=0.007

Interpretation and answer

The influence of creatinine remained after adjusting for type of heparin and CrCl, while CrCl did not. Heparin levels increased with creatinine, after adjusting for CrCl. Heparin levels increased by 0.04 IU/ml (95% CI 0.01-0.06, p=0.001) for each 10 μmol/l increment in creatinine. However, in distinction, CrCl had no effect on mean heparin levels after adjusting for creatinine (p=0.4).

Summary of the diagnostic testing

The linear assumptions and collinearity were not violated.

9.3.11. Influence of cancer on heparin levels

Were heparin levels influenced by presence of cancer in patients treated with heparin?

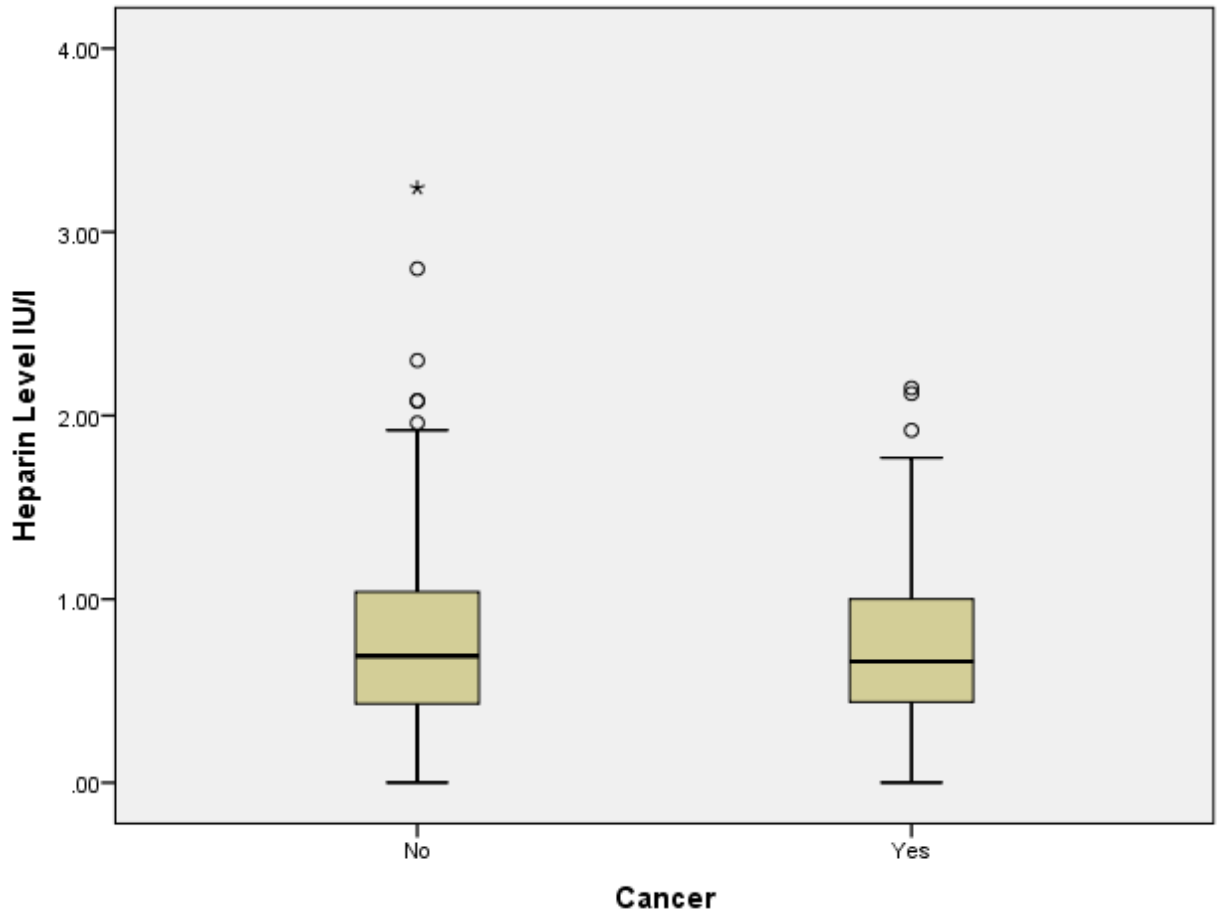


Figure 22: Heparin level in cancer and non-cancer patients

Heparin level = 0.76 **+ 0.02 if patient has cancer**

N=408; R^2=0.001; F (1, 406)=0.07; p=0.80

Interpretation and answer

In an analysis of all patients (Figure 22), when type of heparin was not controlled for, the presence of cancer did not influence heparin levels. In addition, cancer status did not influence mean heparin levels after adjusting for type of heparin (analysis not shown; p=0.27).

9.3.12. Influence of hospitalization status on heparin levels

Were heparin levels influenced by hospitalization status

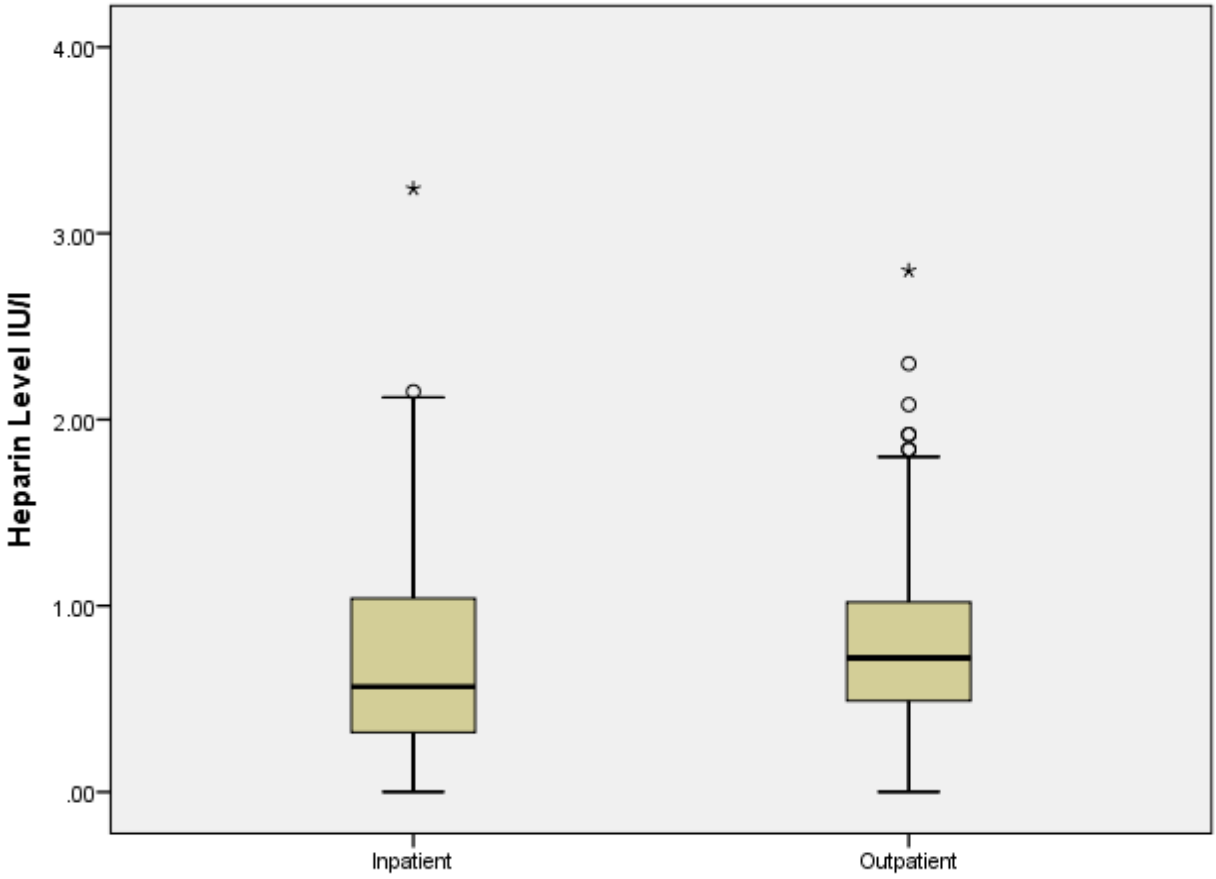


Figure 23: Heparin level against hospitalization status

Heparin level = 0.83 – **0.06 if inpatient**

N=408; R^2=0.004; F (1, 406)=1.6; p=0.21

Interpretation and answer

In an analysis of all patients, when type of heparin was not controlled for (Figure 23), heparin levels was not influenced by hospitalization status. hospitalization status only explained 0.4 % of variability in heparin levels (Table 2).

In addition, hospitalization status did not influence mean heparin levels after adjusting for type of heparin and timing of blood sample withdrawal (analysis not shown; p=0.08).

9.3.13. Influence of type of VTE on heparin levels

Were heparin levels influenced by type of VTE (DVT or PE) in patients treated with heparin?

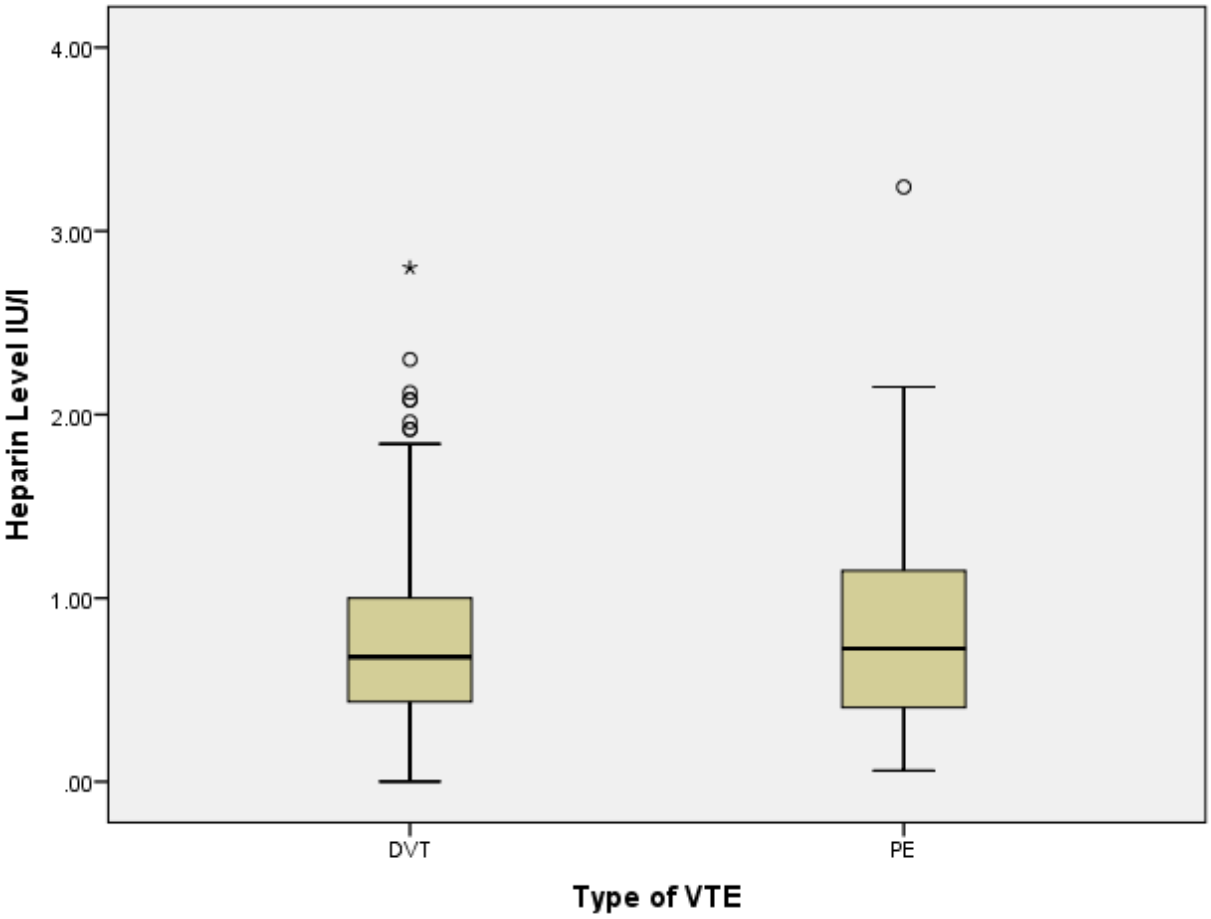


Figure 24: Heparin level against type of VTE in patients treated with heparins

Heparin level = 0.83 – **0.09 (if DVT)**

N=408; R^2=0.006; F (1, 406)=2.3; p=0.13

Interpretation and answer

In an analysis of all patients, when type of heparin was not controlled for (Figure 24), heparin levels was not influenced by type of VTE (Table 2). In addition, type of VTE did not influence mean heparin levels after adjusting for type of heparin (analysis not shown; p=0.15).

9.3.14. Influence of platelet count on heparin levels

Were heparin levels influenced by platelet count?

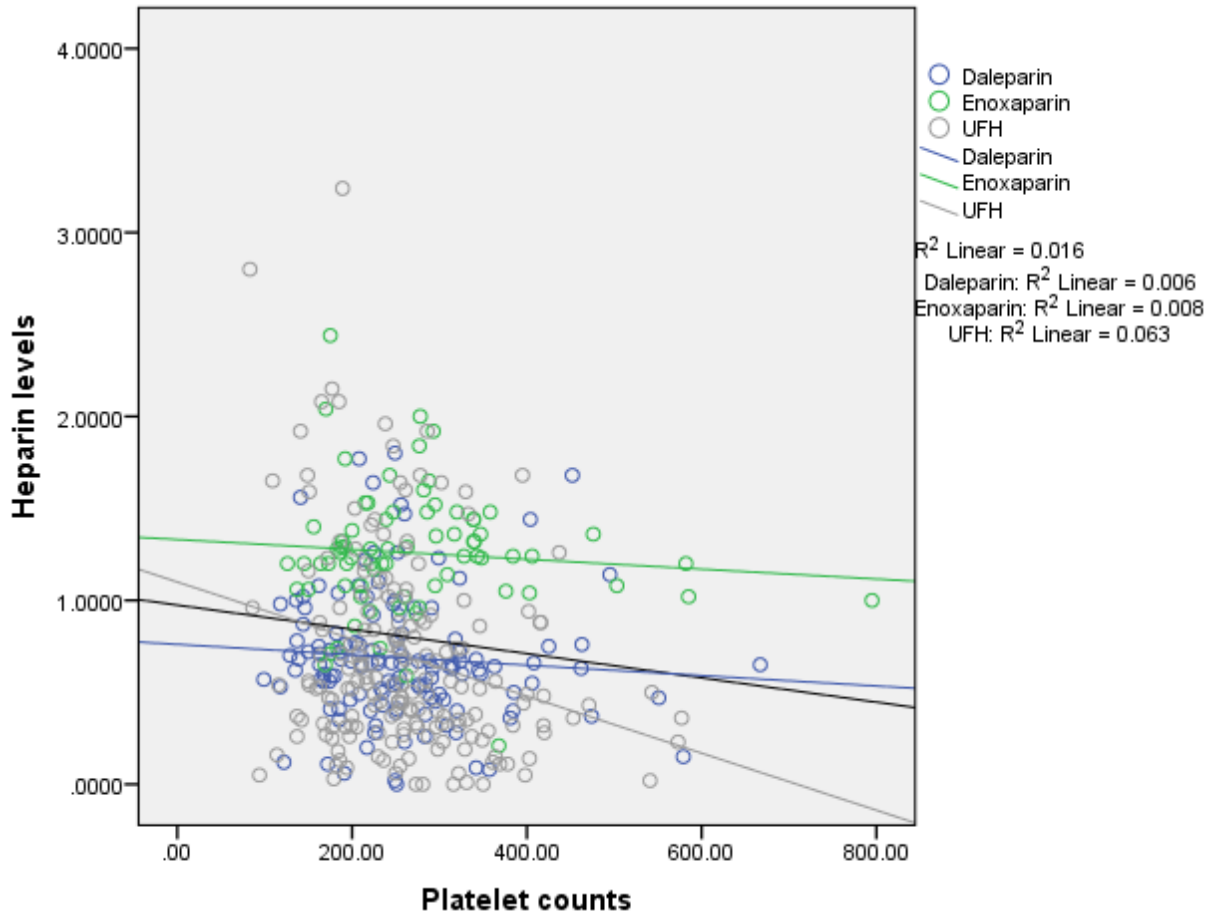


Figure 25: Heparin level against platelet count in patients treated with heparins

Heparin level = 0.95 – **0.001 Platelet count**

N=388; R^2=0.02; F (1, 386)= 8.5; p=0.04

Interpretation and answer

In an analysis of all patients, when type of heparin was not controlled for (Figure 25), mean heparin levels decreased with platelet count. The decrease in heparin levels was 0.1 IU/ml (95% CI – 0.12 to – 0.02) for each 100 X 10^9/L increase in platelet count, with platelet count explaining 2% of the variability in heparin levels (Table 2).

Summary of the diagnostic testing

The linear assumptions were not violated.

Did the relationship between platelets and heparin levels differ between patients treated with UFH, dalteparin and enoxaparin?

Heparin level =	1.10	**– 0.002**	– 0.34	+ 0.01	**+ 0.001**	**+ 0.001**
		Platelet count	Dalteparin	Enoxaparin	**Platelet count* Dalteparin**	**Platelet count* Enoxaparin**
Partial p		**0.001**	0.03	0.92	**0.02**	**0.03**

N = 388; R^2 = 0.14; F (6,381) =11.37 p <0.001

Interpretation and answer

Heparin levels decreased with patients' platelets count, after adjusting for type of heparin. Heparin levels decrease by 0.2 IU/ml (95% CI – 0.23 to – 0.08) for every

100 X 10^9/L increase in platelet count. In addition, the relationship between platelet count and heparin levels (i.e., slope of the linear regression line) differed according to type of heparin (UFH, dalteparin, and enoxaparin); Because the slope for patients treated with UFH differed significantly from the slopes for patients treated with Dalteparin (p=0.02) as well as those treated with enoxaparin (p=0.03), a separate analysis was carried out for each type of heparin (see below). The proportion of the variability of heparin levels that was explained by platelet count (i.e., 2%), type of heparin (i.e., 8%), and by the platelet x type of heparin interaction (2%) appears to be additive.

For patients treated with UFH:

Heparin level = 1.10 **– 0.002 platelet count**

N= 183; R^2 = 0.06; p=0.001

For patients treated with Dalteparin:

Heparin level = 0.76 **– 0.0002 platelet count**

N= 130; R^2 = 0.006; p=0.41

For patients treated with Enoxaparin:

$$\text{Heparin level} = 1.12 - \mathbf{0.0002\ platelet\ count}$$

$$N= 75;\ R^2 = 0.01;\ p=0.33$$

Interpretation and answer

Heparin levels decreased as platelet count increased in patients on UFH by 0.2 IU/ml (95% CI – 0.06 to – 0.24) for every 100 X 10^9/L, but were not influenced by platelet count in patients on dalteparin or enoxaparin.

Summary of the diagnostic testing

The linear assumptions were not violated. The effect modifiers (product of platelet counts and type of heparin) and type of heparin showed significant multicollinearity.

9.4. Influence of heparin levels on VTE and bleeding outcome

9.4.1. Relationship between proportion of patients in the therapeutic categories (*subtherapeutic, therapeutic and supratherapeutic heparin levels)* and type of heparin

What proportion of patients who were treated with UFH or LMWH had subtherapeutic, therapeutic and supratherapeutic heparin levels?

The proportion of patients who were treated with UFH or LMWH and had subtherapeutic, therapeutic and supratherapeutic heparin levels is shown in Table 3. The proportion in each of the three therapeutic categories did not differ between patients who were treated with UFH or LWMH (dalteparin or enoxaparin as a combined group (χ^2=0.8, p=0.66) (Table 3).

What proportion of patients who were treated with dalteparin or enoxaparin had subtherapeutic, therapeutic and supratherapeutic heparin levels?

Among the LMWH group, there was a significant association between whether patients were treated with dalteparin or enoxaparin and the therapeutic category of their heparin levels (χ^2=16.36 p=0.0003). The proportion of patients in the subtherapeutic range was significantly higher in the dalteparin group compared to the enoxaparin group (40% vs. 4%, χ^2=40.56, p<0.00001), whereas the proportion

of patients in the supratherapeutic range was significantly higher in the enoxaparin group compared to the dalteparin group (57% vs. 18%, χ^2=32.45, p<0.0001). The proportion of patients in the therapeutic range did not difference between the dalteparin and enoxaparin groups (42% vs. 39%, χ^2=0.19, p=0.67) (Table 3).

Table 3: Classification of patients based on anti-Xa levels

Heparin level	Number (%)				
	All patients	**UFH**	**LMWH**	**Dalteparin**	**Enoxaparin**
Subtherapeutic	103 (25)	45 (24)	58 (27)	55 (40)	3 (4)
Therapeutic	163 (40)	74 (38)	89 (41)	59 (42)	30 (39)
Supratherapeutic	142 (35)	74 (38)	68 (32)	25 (18)	43 (57)
Total	**408 (100)**	**193 (100)**	**215 (100)**	**139 (100)**	**76 (100)**

9.4.2. Influence of therapeutic categories on VTE and bleeding outcomes

Was there a difference in the proportion of patients with subtherapeutic versus non-subtherapeutic heparin levels who had recurrent VTE during 90-days follow-up?

Overall, in patients treated with UFH, dalteparin or enoxaparin, there was no significant difference in the proportion of patients with subtherapeutic and non-subtherapeutic (i.e., therapeutic or supratherapeutic) heparin levels groups who had recurrent VTE at 90 days (4.9% vs. 2.3%, χ^2=1.83, p=0.18) (Table 4). However, a text of interaction suggested that the association between subtherapeutic heparin levels and recurrent VTE differed between patients treated with UFH and LMWH (p=0.02).

When the analysis was restricted to patient in the UFH group, there was no significant difference in the proportion on of patients with subtherapeutic and non-subtherapeutic heparin levels who had recurrent VTE at 90-days (0% vs. 3.4%, χ^2=0.15, p=0.70). The five recurrent VTE episodes in patients who had non-subtherapeutic heparin levels occurred on days 20, 30, 33, 35, and 45.

When the analysis was restricted to patient in the LMWH group, the proportion of patient with recurrent VTE was significantly higher in patients with

subtherapeutic compared with non-subtherapeutic heparin levels (8.6% vs. 1.3%, χ^2=7.26, p=0.01). The five recurrent VTE episodes in the subtherapeutic group occurred on days 1, 4, 14, 27, and 33 (all in dalteparin arm) and the two recurrent VTE episodes in the non-subtherapeutic group occurred on days 22 (enoxaparin arm) and 29 (dalteparin arm).

Table 4: Therapeutic category of heparin levels and recurrent VTE at 90 days

Type of heparin	***Number of patients***	***Recurrent VTE***	
		(number)	***(percent)***
UFH+LMWH			
Subtherapeutic	103	5	4.9%
Non-subtherapeutic	305	7	2.3%
UFH			
Subtherapeutic	45	0	0%
Non-subtherapeutic	148	5	3.4%
LMWH			
Subtherapeutic	58	5	8.6%
Not-subtherapeutic	157	2	1.3%

Was there a difference in the proportion of patients with supratherapeutic versus non-supratherapeutic heparin levels who had bleeding (major or minor) during the initial 10-days follow-up?

Overall, in patients treated with UFH, dalteparin or enoxaparin, the proportion of patient with bleeding was higher in patients with supratherapeutic compared with non-supratherapeutic heparin levels (6.3% vs. 1.5%, χ^2=7.65, p=0.01) (Table 5). Although the test of homogeneity suggests that two groups are identical, a subgroup analysis is presented below. Although test of interaction suggested that the association between supratherapeutic heparin levels and bleeding did not differ between patients treated with UFH and LMWH (p=0.13), we have still chosen to present an exploratory subgroup analysis below.

When the analysis was restricted to patients in the UFH group, the proportion of patients with bleeding was significantly higher in patients with supratherapeutic compared with non-supratherapeutic heparin levels (9.4% vs. 0.8%, χ^2=8.53, p=0.003). The seven bleeding events in patients who had supratherapeutic heparin levels occurred on days 1, 3, 4, 5, 6, 7 and 7 and the single bleeding event in patients who had non-supratherapeutic heparin levels occurred on day 4. All eight bleeding events were judged to be minor.

When the analysis was restricted to patients in the LMWH group, there was no significant difference in the proportion of patients with supratherapeutic and non-supratherapeutic heparin levels who had bleeding (2.9% vs. 2.0%, χ^2=0.28, p=0.59). The two bleeding events in patients who had supratherapeutic heparin levels occurred on days 2 and 4 (all in enoxaparin arm) and the three-bleeding event in patients who had non-supratherapeutic heparin levels occurred on days 2, 3, and 7 (all in the dalteparin arm). The two bleeding events in patients who had supratherapeutic heparin levels were minor. Of the three bleeding events in patients who had non-supratherapeutic heparin levels, one was minor and two were major.

Table 5: Association between category of heparin levels and bleeding events at 10 days

Type of heparin	*Number of patients*	*Bleeding events*	
		(number)	*(proportion)*
UFH+LMWH			
Supratherapeutic	142	9	6.3%
Non-supratherapeutic	266	4	1.5%
UFH			
Supratherapeutic	74	7	9.4%
Non-supratherapeutic	119	1	0.8%
LMWH			
Supratherapeutic	68	2	2.9%
Non-supratherapeutic	147	3	2.0%

9.4.3. Effect of heparin levels on VTE and bleeding outcomes

As a continuous variable, did heparin levels influence recurrent VTE during the 90-days follow up?

In a univariable analysis that included patient who were treated with UFH or LMWH, heparin levels did not influence the odds of recurrent VTE events at 90-days follow up. The OR for recurrent VTE was 0.49 (95% CI: 0.12 to 2.02, p=0.32) for each 1 IU/ml increase in heparin levels.

When the analysis was restricted to the type of heparin (UFH and LMWH), heparin levels did not influence the risk of recurrent VTE in patients treated with UFH, however the risk of recurrent VTE decreased with increasing heparin levels in patients treated with LMWH. The OR for recurrent VTE for each 1 IU/ml increment in heparin levels was 1.46 (95% CI: 0.37 to 5.76, p=0.59) in patients treated with UFH, and 0.04 (95% CI: 0.003 to 0.55, p=0.02) in patients treated with LMWH (Table 6).

In a multivariable analysis that included patient who were treated with UFH or LMWH, after controlling for antiplatelet use and diagnosis of cancer at baseline (predefined), heparin levels did not influence the risk of recurrent VTE at 90-days

follow up. The OR for recurrent VTE was 0.46 (95% CI: 0.11 to 1.87, p=0.28) for each 1 IU/ml increase in heparin levels.

When the analysis was restricted to the type of heparin (UFH and LMWH), heparin levels did not influence the risk of recurrent VTE in patients treated with UFH, however the risk of recurrent VTE decreased with increasing heparin levels in patients treated with LMWH. The OR for recurrent VTE events for each 1 IU/ml increment in heparin levels was 1.17 (95% CI: 0.29-4.66, p=0.83) in patients treated with UFH and 0.05 (95% CI: 0.004-0.68, p=0.02) in patients treated with LMWH (Table 6).

Table 6: Association of recurrent VTE with heparin levels

Data set	*OR for each 1.0 IU/ml increment in heparin levels*	*95% CI*	*P value*
Univariable analysis			
UFH+LMWH	0.49	0.12-2.02	0.49
UFH	1.46	0.37-5.76	0.59
LMWH	0.04	0.003-0.55	0.02
Multivariable analysis			
UFH+LMWH	0.46	0.11-1.87	0.28
UFH	1.17	0.29-4.66	0.83
LMWH	0.05	0.004-0.68	0.02

Covariates in the multivariable model are antiplatelet use at baseline, and diagnosis of cancer at baseline. P value for HL test= 0.37 for UFH+LMWH; 0.54 for UFH; and 0.99 for LMWH.

As a continuous variable, did heparin levels influence bleeding events (major or minor) during the initial 10-days follow up?

In a univariable analysis that included patient who were treated with UFH or LMWH, increasing heparin levels increased the risk of bleeding events during the initial 10-days follow up. The OR for bleeding events was 3.62 (95% CI 1.49-8.77, p=0.004) for each 1 IU/ml increase in heparin levels. When the analysis was restricted to the type of heparin (UFH and LMWH), increasing heparin levels increased the risk of bleeding events in patients treated with UFH, but not in patients

treated with LMWH. The OR for bleeding for each 1 IU/ml increase in heparin levels was 3.32 (95% CI 1.30-8.46, p=0.01) in patients treated with UFH and 3.77 (95% CI 0.42-33.92, p=0.24) in patients treated with LMWH (Table 7).

In multivariable analysis that included patient who were treated with UFH or LMWH, after controlling for age, antiplatelet use at baseline, and diagnosis of cancer at baseline (predefined), increases in heparin levels still increased the risk of bleeding events during the initial 10-days follow up. The OR for bleeding events was 3.37 (95% CI 1.49-9.22, p=0.005) for each 1 IU/ml increase in heparin levels. When the analysis was restricted to the type of heparin (UFH and LMWH), increments in heparin levels increased the risk of bleeding events in patients treated with UFH, but not in patients treated with LMWH. The OR for bleeding for each 1 IU/ml increase in heparin levels was 3.39 (95% CI 1.25-9.19, p=0.02) in patients treated with UFH and 4.69 (95% CI 0.50-44.33, p=0.18) in patients treated with LMWH (Table 7).

Table 7: Association of bleeding with heparin levels

Data set	*OR for each 1.0 IU/ml increment in heparin levels*	*95% CI*	*P value*
Univariable analysis			
UFH+LMWH	3.62	1.49-8.77	0.004
UFH	3.32	1.30-8.46	0.01
LMWH	3.77	0.42-33.92	0.24
Multivariable analysis			
UFH+LMWH	3.37	1.49-9.22	0.005
UFH	3.39	1.25-9.19	0.02
LMWH	4.69	0.50-44.33	0.18

Covariates in the multivariable model are antiplatelet use and diagnosis of cancer at baseline. P value for HL test= 0.30 for UFH+LMWH; 0.33 for UFH; and 0.28 for LMWH.

10. Discussion

These analyses have enabled us to answer several important questions about the use of SC UFH and SC LMWH for acute treatment of thrombosis, and particularly acute treatment of VTE.

First, our findings support treating patients with a dose of SC UFH or SC LMWH that is directly proportional to patient's weight rather than according to other more complex equations.[1,18-20] This is because, although there was a statistically significant increase in heparin levels with increasing weight in patients treated with UFH and LMWH, the magnitude of this increase was not clinically important. We might have found that heavy patients had much higher heparin levels than lighter patient because heavier patients received a much higher absolute dose of heparin. If that had been the case, it would have suggested that heparin dose either should not be varied with patient weight (unlikely), or that the increase in dose should be proportionally less than the increase in body weight (more likely). An example of the latter might be treating patients ≤80 kg with 100 units/kg twice-daily (e.g., 7,000 units if 70 kg) and treating patient >80Kg with a dose of 16,000 units plus 50 units for each kg above 80 kg (e.g., 16,000 units plus 3,000 units if 140 kg). However, the small systematic increase in heparin levels that we saw with increases in patient weight (weight only accounted for 4% of the variability in heparin levels) would not

justify a much more complex calculation for heparin dose. The stronger association between (1) BMI and heparin levels, compared to (2) weight and heparin levels, suggests that the heavier patients were more likely to have slightly higher heparin levels because they have a higher percentage of body fat; consequently, after adjusting for BMI in my analyses, weight was no longer significantly associated with heparin levels. Furthermore, the small systematic increase in heparin levels with weight (or BMI) did not appear to differ according to whether patients were treated with UFH or LMWH (or dalteparin compared to enoxaparin).

Second, on balance, our findings support treating males and females with the same weight-adjusted dose of SC UFH or SC LMWH. Although, heparin levels were 0.1 IU/ml higher in females compared to males, this increase does not appear to be large enough to justify using different weight-adjusted dosing regimens for males and females. In addition, sex only accounted for 1% of the variability in heparin levels. A possible explanation for the slightly higher heparin levels in females compared to males, and in patients with higher BMI, is that, relative to body weight (and heparin dose), females and fatter individuals are expected to have a lower blood volume (i.e., per kg); as heparin is mainly distributed in the plasma compartment, a lower relative blood volume is expected to be associated with higher heparin levels.

Third, the effect of renal function on heparin levels was minor, with some unexpected findings. CrCl was not significantly associated with heparin levels in patients treated with UFH, dalteparin or enoxaparin. It is not surprising that CrCl was not associated with heparin levels in patients treated with UFH because UFH is predominantly metabolized rather than excreted by the kidneys.[15] LMWH is predominantly excreted by the kidneys and, therefore, heparin levels are expected to be higher with renal impairment in patients treated with dalteparin and enoxaparin.[11,15] It is very likely that at least part of the reason that heparin levels did not increase with reduced CrCl in the LMWH patients is that patients with marked renal impairment (i.e., creatinine >200 μmol/l) were excluded. This is expected to reduce ability to detect an association between heparin levels and CrCl in two ways. First, heparin levels might not increase until renal function is very impaired (i.e., relationship was not present in the patients in this analysis). Second, regression has difficulty detecting a relationship when the range of the independent variable is narrow (i.e., relationship was present but could not be detected in this analysis). Two findings relating to heparin levels and renal function were unexpected. First, there was a detectable relationship between heparin levels and creatinine level even though there was no detectable relationship between heparin levels and CrCl. This suggests that it is not necessary to calculate CrCl to determine if renal impairment is

likely to cause heparin retention; instead, serum creatinine level can be used. Second, there was an association between heparin levels and creatinine in patients treated with UFH; this suggests that renal impairment may also result in retention of UFH.

Fourth, there was a small decrease in heparin levels as platelet count increased in patients who were treated with UFH, but no decrease in heparin levels in patients treated with LMWH. A possible explanation for this is that UFH may bind to platelets, or to platelet factor 4 (PF4) which is released from platelets, more than LMWH.[11,15] If higher levels of PF4 accounts for the inverse association between heparin levels and platelet count in the UFH patients, this release of PF4 could have happened before (in-vivo) or after (ex-vivo) blood was drawn for measurement of heparin levels.

Of note, age, height, type of VTE, hospitalization status and diagnosis of cancer had no influence on heparin levels. It had previously been suggested that UFH may be eliminated more rapidly in patients who present with symptomatic PE rather than DVT.[21,22] Our finding that heparin levels, in both patients who were treated with UFH and LMWH, were not lower in patients with PE compared to DVT, and does not support the notion that more rapid elimination of UFH or LMWH in patients with PE.

I will now address some methodological issues that relate to the analyses in this thesis. In the FIDO study, attempts were made to standardize the day that blood was drawn for the sub-study (on Day 3), and the interval between when a SC injection was given and when blood was drawn (6 hours). The reason for this was to reduce variability in heparin levels due to differences in the timing of blood sampling; for example, heparin levels could systematically increase the more days that patients are being treated (i.e., accumulation), or could peak 4 hours after a SC injection. In the context of the questions that we wanted to address, difference in heparin levels due to differences in timing of blood sampling would be "noise" that could obscure important relationships. To reduce the potential for sample timing noise, for patients to be eligible for the current analysis, blood had to have been drawn between 3.0 and 9.9 hours after the injection of UFH or LMWH, and on the 2nd to 6th day (inclusive) after starting UFH or LMWH. As an additional precaution, we also examined if the day of sampling, or the interval between injection and blood sampling, influenced heparin levels. We found that there was no systematic difference in heparin levels according to the day of sampling, but that there was a small systematic decrease in heparin levels as the interval between injection and sampling increased (with no evidence of an increase in heparin levels to an initial peak over the time that samples were drawn in this sub-study). Because there was a small systematic decrease in

heparin levels as the interval between injection and blood sampling increased, we adjusted for this time interval in all analysis (i.e., as a co-variable that was forced into the regression model).

Because patients treated with LMWH are expected to have a more predictable pharmacokinetic response compared to patients treated with UFH, it was anticipated that a larger proportion of heparin levels in the LMWH patients would be in the therapeutic range. However, this was not the case; the proportion of patients in the therapeutic range did not differ according to whether patients were treated with UFH or LMWH. Unexpectedly, the analysis found that there was a marked difference in heparin levels in patients treated with dalteparin and enoxaparin. Heparin levels were, on average, 0.3 IU/ml or 49% higher in patients treated with enoxaparin compared to dalteparin. As we used the same heparin levels therapeutic range (0.6 to 1.0 IU/ml) for enoxaparin and dalteparin, patients treated with dalteparin were more likely to be subtherapeutic compared to patients treated with enoxaparin, and patients treated with enoxaparin were more likely to be supratherapeutic compared to patients treated with dalteparin. There were too few episodes of bleeding in the LMWH group to be able to make a meaningful comparison of the risk of bleeding with enoxaparin (2/76 patients) compared to dalteparin (3/139 patients).

While, overall (all patients combined), the analysis did not find an association between heparin levels and recurrent VTE, a subgroup analysis suggested that there was an association between subtherapeutic heparin levels and VTE in patients treated with LMWH. Similarly, while, overall (all patients combined), the analysis did find an association between heparin levels and bleeding, a subgroup analysis suggested that this association between supratherapeutic heparin levels and bleeding was only seen in patients treated with UFH. Nevertheless, our confidence in both of these subgroup associations is weak because: 1) they are subgroup findings, and subgroup findings are often misleading;[39] 2) tests of interaction did not suggest that the association between supratherapeutic heparin levels and bleeding outcome differed significantly between the UFH and LMWH subgroups; 3) the CI around the estimates are wide because the number of events in the analysis was small; and 4) these analyses were part of many analyses, increasing the chance of a statistically significant finding due to multiple testing. Arguments in favor of the subgroup findings are: 1) some previous studied have reported similar findings [25,29]; 2) the subgroup analyses were prespecified [40,41]; and 3) the findings are biologically plausible.

Current clinical practice guidelines do not advocate monitoring heparin levels in patients who are treated with LMWH. However, monitoring of heparin levels in

patients who are treated with LMWH is suggested if there is concern that usual weigh-adjusted dosing may deliver too high a dose to some patients, such as those with marked renal impairment or extreme obesity.[42] While we think this is reasonable, particularly in patients with marked renal impairment, our results are not particularly supportive of monitoring heparin levels in these subgroups, at least over the range of renal function and body weight included in our analysis; there was very little association between heparin levels and each of renal function and body weight in our analysis. Physicians should also be aware that variation in the heparin levels in patients treated with UFH or LMWH were poorly explained by the baseline characteristics that were examined in this thesis, which may suggest that variables with a greater influence on heparin levels have yet to be determined.

It was somewhat puzzling in this analysis that higher heparin levels increased the risk of bleeding in patients treated with UFH but not in those treated with LMWH. While bleeding with heparins is mainly due to their inhibitory effect on the coagulation cascade, the interaction of heparin with platelets and endothelial cells may also contribute to bleeding.[43,44] These interactions may occur more often with UFH than with LMWH, which may explain why heparin levels increased bleeding in patients treated with UFH and not LMWH.

10.1. Strength and Limitations:

Strength of this analysis are: 1) the objectives were defined prior to analysis, which reduces the risk of "data dredging" and selective reporting of results; 2) use of regression modeling that allowed adjustment for important co-variables when examining the associations between i) patient's characteristics and heparin levels, and ii) heparin levels and clinical outcomes (i.e., VTE and bleeding); 3) that patients were studies prospectively, which facilitates completeness of data recording, standardized follow-up, and standardized assessment of outcomes; 4) blind adjudication of outcomes, that reduces the risk of biased interpretation; 5) few losses to follow-up, which minimizes missing data that are usually "missing not at random" and, therefore, can bias associations; and 6) that heparin levels were measured in a central laboratory, reducing variability due to inter-laboratory differences.[45,46,47]

Limitations of this analysis are: 1) the finding in this thesis have not been validated using an external data set and, therefore, should be interpreted as an exploratory analysis until such validation occurs; 2) failure to detect associations between baseline characteristics and heparin levels, and particularly between heparin levels and clinical outcomes (recurrent VTE or bleeding) may have been due to lack of power; 3) only 57% of patients in the FIDO study had heparin levels available for analysis, with evidence of differences between included and non-included patients;

4) the analysis included many comparisons, so "multiple testing" and chance may have resulted in some statistically significant differences.

10.2. Conclusion

In patients who are treated with weight-adjusted UFH or LMWH, the type of heparin had the greatest influence on heparin levels (higher in the enoxaparin group), with higher heparin levels being associated to a lesser degree with greater BMI (true for patients treated with UFH, dalteparin, or enoxaparin), female sex (true for patients treated with UFH, dalteparin, or enoxaparin) and lower platelets counts (true for patients treated UFH). Although it was possible to identify factors that were associated with heparin levels in patients who had been treated with weight-adjusted UFH or LMWH, none of these associations were strong enough to suggest that variable other than weight should influence SC heparin dosing.

Subtherapeutic heparin levels were associated with a higher risk of recurrent VTE in patients treated with LWMH, but not UFH. Supratherapeutic heparin levels were associated with a higher risk of bleeding in patients treated with UFH, but not LMWH. Indirectly, these findings suggest that adjusting UFH or LMWH dose in response to heparin levels might improve clinical outcomes.

11. References

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