The impact of body weight on laboratory parameters in patients taking rivaroxaban

Barsam, Sarah

Awarding institution:
King's College London

THE IMPACT OF BODY WEIGHT ON LABORATORY PARAMETERS IN PATIENTS TAKING RIVAROXABAN

SARAH BARSAM MBBS, MA (CANTAB), MRCP (LON)

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DECLARATION

I declare that the work undertaken in this thesis is entirely my own with the following exceptions. The standard coagulation and antiXa assays (as described in chapter 2) were undertaken by Senior Biomedical Scientists in the Blood Sciences Laboratory at King's College Hospital NHS Foundation Trust, London.

The PIVKA II analysis was undertaken by Specialist Biochemistry department at St Thomas' Hospital Foundation.

Where information from other sources has been cited, I confirm that I have referenced this accordingly.

ABSTRACT

Venous thromboembolism, VTE, is a major cardiovascular disease with significant mortality and morbidity. The current management of VTE is changing as the direct oral anticoagulants have become available with more evidence emerging on their safety and efficacy profiles. This study prospectively investigated patients taking rivaroxaban, a direct oral anticoagulant, either for venous thromboembolism treatment or for prevention post-orthopaedic surgery. The pharmacodynamic behaviour of rivaroxaban in our study population was evaluated using several standard, specialised and global coagulation assays, such as calibrated automated thrombography, rotational thromboelastography and thrombin anti-thrombin complexes. The pharmacokinetics of rivaroxaban were evaluated and a population pharmacokinetic model was developed using non-linear mixed effects modelling. Finally, patient's attitudes and beliefs, regarding rivaroxaban, were explored in the cohort of patents recruited.

In total, 101 patients were recruited. The indications for taking rivaroxaban were: 58% as treatment for an initial DVT, 26% as treatment for recurrent DVT, 12% were previously receiving long-term conventional anticoagulation (vitamin K inhibitor or low molecular weight heparin, LMWH) and switched to rivaroxaban, and 4% as primary prophylaxis following an elective total hip or knee replacement surgery. Almost three quarters of the participants were Caucasian, there was an equal male:female ratio, body weight was normally distributed. 40% of participants were obese and only 1% was under weight.

Rivaroxaban concentrations were measured by anti-Xa levels, which is an accepted surrogate measurement for rivaroxaban concentration. The concentration-time graph produced from this study is representative of those graphs published in the literature, with a peak Cmax at 2-3h post rivaroxaban ingestion and an exponential decrease with time, to a trough at approximately 24h. There was a wide distribution of rivaroxaban concentrations between time zero and 3h post ingestion, reflecting the variability in absorption. There was no association between rivaroxaban concentration and body weight, lean body weight or BMI, at any time point after rivaroxaban ingestion.

Prothrombin time, PT, was measured at each blood sampling time point. The PT did not differ significantly between groups of patients, based on various demographic characteristics or body weight categories. Spearman rank coefficient of variability between PT and anti-Xa levels, was 0.9, $p=0.00$, and a multiple regression analysis showed that this correlation was not affected by body weight.

Activated partial thromboplastin time (APTT) was normally distributed, mean 33.4 sec and correlated with rivaroxaban concentration, r=0.306, p=0.00. D-dimer, median 350 ng/ml, displayed no significant differences between any sub-group of weight, ethnicity, indication for anticoagulation or dose of anticoagulation. Clauss fibrinogen (mean 3.47 g/L) weakly correlated with rivaroxaban concentration, r=0.155, p=0.032.

Thrombin anti-thrombin complex (TAT) measurements had no association with rivaroxaban concentration. There were differences in TAT only between the orthopaedic patients and other groups, but the significance was unreliable due to small numbers of samples. The global coagulation tests: calibrated automated thrombogram (CAT), with and without the addition of contact factor inhibitor (CTI); and rotational thromboelastography (ROTEM) were performed and analysed for each sample. CAT parameters correlated significantly with rivaroxaban concentration, in particular, lag time, r= 0.60, p= 0.00, time to peak, r= 0.67, p= 0.00, peak thrombin generation, r= -0.63, p= 0.00, velocity index r= -0.66, p=0.00. The ROTEM parameters which correlated significantly with rivaroxaban concentration, were Extem CT, A10 and MCFt and Intem CT.

Only time to peak of thrombin generation correlated with lean body weight, r= 0.14, p=0.00, but no other body weight indices showed a relationship with any CAT or ROTEM parameters.

Using NOMNEM software, a population pharmacokinetic analysis was performed on the rivaroxaban concentration data collected. A one compartment model with between subject variability on rivaroxaban clearance and volume of distribution, with a combined (additive and proportional) error model, best fitted the data. Following a full co-variate analysis, creatinine clearance on rivaroxaban clearance was found to be the only significant co-variate impacting on the pharmacokinetic profile of rivaroxaban in the dataset. Body weight (BMI, actual body weight and lean body weight) was not found to have a significant effect on rivaroxaban concentration.

CONTENTS

ABBREVIATIONS

ΔOBJ change in objective function value
ANOVA analysis of variance
ACCP American College of Chest Physicians
APTT activated partial thromboplastin time
AT antithrombin
AUC area under the concentration-time curve
BD *bis die* (twice a day)
BCSH British Committee for Standards in Haematology
BMI body mass index
BSV between subject variability
CAT calibrated automated thrombinography
CI confidence interval
CL clearance
Cmax maximum concentration
CrCl creatinine clearance
CRE creatinine
CTI corn trypsin inhibitor
CV coefficient of variation
CWRES conditional weighted residuals
DRVV Dilute Russell's Viper Venom
DVT deep vein thrombosis
EDTA ethylenediamin tetraacetic acid
ETP endogenous thrombin potential
ELISA enzyme linked immunosorbent assay
F Factor
Fa activated factor
FACWTCL exponent on the weight covariate on estimates of clearance
FACLBWV exponent on the lean body weight covariate on volume of distribution estimates
GFR glomerular filtration rate
GP General Practitioner
IBW ideal body weight
INR international normalised ratio
IOV inter occasion variability
IQR interquartile range
ISTH International Society for Haemostasis and Thrombosis
IU/mL international units per millilitre (unit of measure for LMWH)
kg kilogram
Ka absorption rate constant
LBW lean body weight
Log logarithm
LMWH low molecular weight heparin
L litres
LT lag-time
mg milligram
mL millilitre
NCA non-compartmental analysis

NLME non-linear mixed effects
NONMEM NONlinear Mixed Effects Modelling (version 7.1.0) software
n number
NHCP Normal Human Control Plasma
OD *omni die* (once a day)
OBJ objective function value
OR odds ratio
PCC prothrombin concentrate
PD pharmacodynamics
PE pulmonary embolism
PK pharmacokinetics
PKPD pharmacokinetic / pharmacodynamic
PPP platelet poor plasma
Pop Population
popPK population pharmacokinetics
PRP platelet rich plasma
PT prothrombin time
PTS post thrombotic syndrome
pM picoMolar
Q inter-compartmental clearance
QOL quality of life
REC Research Ethics Committee
ROTEM rotational thromboelastometry
RUV residual unexplained variability
RVT residual vein thrombosis
SD standard deviation
SE standard error
SS steady state
ST start tail
t time
t1/2 half-life
TAT thrombin antithrombin complex
T0 first study sample (taken just before rivaroxaban dose, 0h)
T1 second study sample (taken approx. 1h post rivaroxaban dose)
T2 third study sample (taken approx. 2h post rivaroxaban dose)
TF tissue factor
TFPI tissue factor pathway inhibitor
TG thrombin generation
Tmax time to maximum concentration
TM thrombomodulin
TTP time to peak
UK United Kingdom
Vd volume of distribution
VPC visual predictive check
vs. versus
VTE venous thromboembolism

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CHAPTER 1. INTRODUCTION

1.1 VENOUS THROMBOEMBOLISM BACKGROUND

1.1.1 EPIDEMIOLOGY

Venous thromboembolism (VTE), manifested as either deep vein thrombosis (DVT) or pulmonary embolism (PE), is a common medical condition with a significant world-wide disease burden. The estimated annual incidence of VTE is 0.1% (Agnelli & Becattini, 2010) and 1.8 per 1000 person-years at 65-69 years old, increasing to one per 100 at 85-89 years old. (Kyrle & Eichinger, 2005) VTE affects 2-5% of the population during their lifetime and up to 5% of hospitalised patients with additional risk factors for VTE who have not been prescribed prophylactic anticoagulation. (Agnelli & Becattini, 2010) About 20% of patients with PE will die before or on the first day of diagnosis and 10% of diagnosed PE are immediately fatal. (Wells & Anderson, 2013) There is a 10% case fatality rate associated with late recurrent VTE following an initial PE and 5% following an initial DVT. (Wells & Anderson, 2013)

1.1.2 PATHOGENESIS OF VENOUS THROMBOSIS

1.1.2.1 VIRCHOW'S TRIAD

The framework commonly used to understand and group the pathogenesis of venous thrombosis (VTE) is an extension of the triad of Virchow, which states that thrombosis is caused by: damage to the vessel wall; decreased blood flow; and increased coagulopathic composition of blood. (Reitsma et al., 2012) Damage to a vessel wall results in exposed subendothelial ligands, including Von Willebrand factor (VWF) and tissue factor (TF). VWF contacts the receptors on the surfaces of platelets flowing through the vasculature, namely glycoprotein (GP) 1b-IX-V, causing platelet adherence, intracellular signalling and activation. Activated platelets subsequently release pro-coagulant granules, which activate thrombin, causing fibrin clot formation. The tissue factor is exposed to and combines with plasma factor VII, forming the vitamin K-dependent extrinsic tenase complex, which is crucial in initiating the coagulation cascade. (Heemskerk et al., 2002) However, vessel wall injury of the deep vein is not a common feature in DVT and the thrombosis is composed predominantly of fibrin and trapped erythrocytes, not platelets. (Heemskerk et al., 2002)

Prolonged stasis in a vein increases the chance of a deep vein thrombosis due to a lowered oxygen tension which leads to the upregulation of multiple stress-response genes including hypoxia inducible factor 1-alpha, P-selectin (CD62), and certain adhesion receptors. (Kyrle & Eichinger, 2005) The resulting endothelial environment

supports the local recruitment of monocytes, granulocytes and platelets, which express tissue factor on their cell surface and release coagulation cascade activating factors, thus, initiating coagulation. (Cushman et al., 2004; Reitsma et al., 2012)

1.1.2.2 Cell-based model of coagulation

The coagulation cascade was traditionally understood as consisting of the intrinsic, extrinsic and common pathways. However, a cell-based model has now superseded this and can be explained with three interacting phases of coagulation: initiation, propagation and amplification, involving the interaction of tissue factor bearing cells and platelets (figure 1). (Hoffman, 2003)

A small amount of thrombin is generated at the initiation phase, caused by exposed cellular tissue factor activating factor VII, which, in turn, activates factor X and IX. (Brummel et al., 2002) The propagation phase occurs when small amounts of thrombin generated at the initiation phase amplify further thrombin generation by platelet, factor V and factor VIII activation. The burst of pro-thrombinase complex (activated factor Xa-Va) is formed by the activated intrinsic tenase complex (activated factor IXa-VIIa), which forms on activated platelet phospholipid surfaces. Thrombin is the predominant catalyst in the amplification phase, which involves splitting of fibrin from fibrinogen and activation of factor XIIIa, which causes polymerisation and cross linking of the polymers to form an insoluble fibrin clot (figure 2). (Mann, 2003)

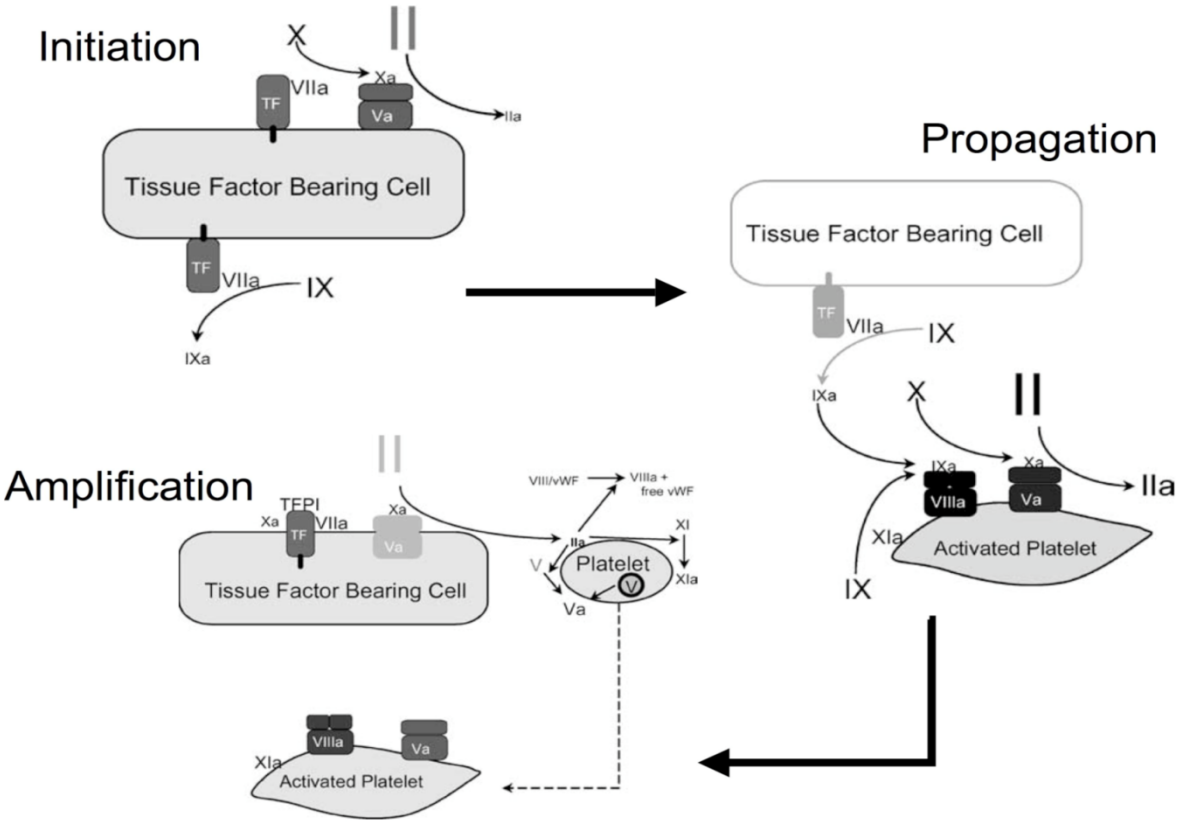

FIGURE 1 INITIATION, PROPAGATION AND AMPLIFICATION OF THE COAGULATION CASCADE

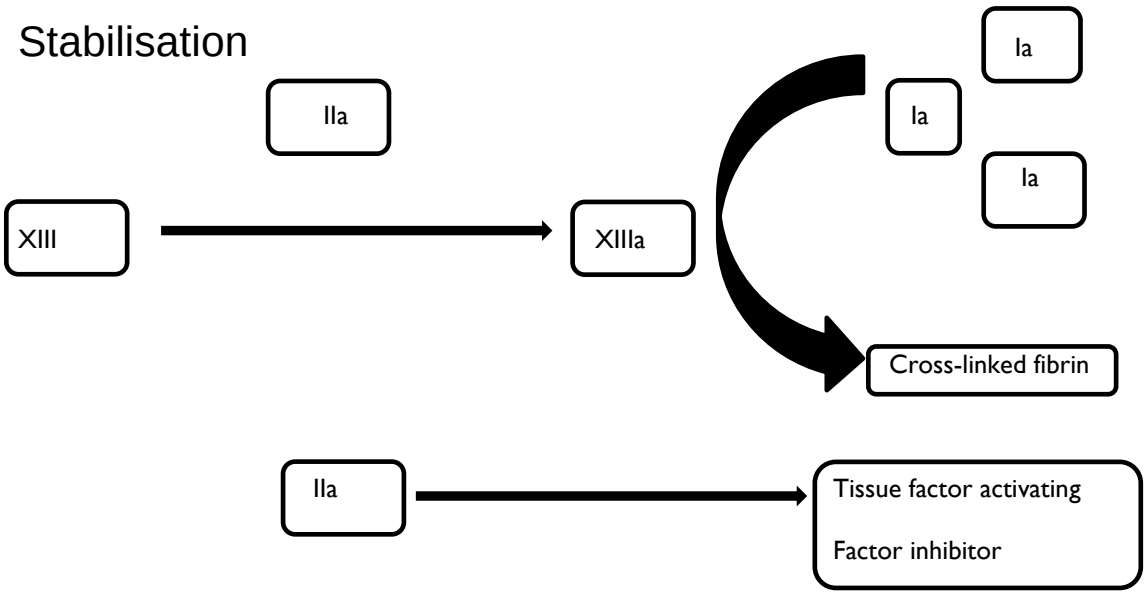

FIGURE 2 CLOT STABILISATION

1.1.2.3 Natural regulation and inhibition of coagulation

Under physiological conditions, coagulation only occurs when necessary, in a balanced and controlled fashion, at a localised injury site in the vasculature, until the damage is sealed. Thrombin generation is attenuated by various self-regulating enzymes and proteins. Tissue factor pathway inhibitor and heparan sulphate are produced by endothelial cells. Tissue factor pathway inhibitor rapidly inactivates the initiation phase of coagulation by inhibiting tissue factor-factor VIIa/Xa complex. Heparan sulphates activate antithrombin (AT), which inhibits free thrombin and factor Xa. Thrombin regulates the ongoing generation of thrombin by binding to thrombomodulin, thus activating protein C, which binds to protein S and inactivates factor Va and factor VIIa, decreasing the formation of factor Xa-factor Va and factor IXa-factor VIIIa complexes. (Mann, 2003) Additionally, there are naturally occurring fibrinolytic proteins, including tissue plasminogen activator, which convert plasminogen to plasmin, an enzyme which degrades soluble and insoluble fibrin clots. (Rijken & Lijnen, 2009) Activated protein C inactivates plasminogen activator inhibitor-1, thus enhancing fibrinolysis.

1.1.2.4 The interplay between genetic predisposition and environmental triggers

A complex interplay between genetic and environmental factors contribute to VTE predisposition. The pathophysiology and mechanisms initiating VTE are unclear, despite the breadth of knowledge regarding the risk factors. (López et al., 2004) The factors determining VTE development can be grouped into: underlying predisposition and superimposed injury or disease precipitating the acute thrombosis, which occurs once the individual's thrombosis potential is raised above a theoretical threshold. (Rosendaal, 1999) Underlying predisposing factors include inherited thrombophilic defects and genetic mutations, and acquired risk factors include acquired thrombophilic defects, immobility, surgery and hormonal effects. (Wells & Anderson, 2013) Obesity carries an increased pro-thrombotic risk due to physical immobility, increased intra-abdominal pressure, chronic inflammatory state, pro-thrombotic and pro-angiogenic hormone release. (Allman-Farinelli, 2011) Cancer is a recognised cause of VTE and VTE may be an early sign of malignancy in an otherwise asymptomatic patient. (Barsam et al., 2013) VTE is also associated with chronic inflammation and the inflammatory and malignant environment consist of pro-coagulant cytokines, chemokines and activated cells. (López et al. 2004) Release of tissue factor bearing microvesicles, which activate factor VII, from monocytes is involved in chronic inflammation. Secretion of tumour-necrosis-factor alpha (TNF alpha) and mucin, from malignant cells, activate factor X. (Heit et al., 2002; López et al., 2004) Rosendaal and colleagues have developed a model which weights the various pro-thrombotic risk factors into strong, medium and weak (table 1). (Rosendaal, FR., 1999)

TABLE 1 RISK FACTORS FOR VTE

Strength of association	Acquired risk factors	Genetic risk factors	Mixed risk factors
Strong	Major surgery	Antithrombin deficiency	High FVIII levels
	Malignancy & chemotherapy	Protein C deficiency	
	Antiphospholipid syndrome	Protein S deficiency	
	Myeloproliferative disease	Factor V Leiden homozygous	
	Paroxysmal nocturnal haemoglobinuria	Combined-thrombophilias	
	Inflammatory bowel disease		
	Pregnancy/ puerperium/ combined oral contraception		
Medium	Dehydration	Sickle cell disease	Hyperhomocystinaemia
	Long flight		
Weak	Obesity	Factor V Leiden (FVL) heterozygosity	High FIX and XI levels
	HIV infection		High fibrinogen
	Hormone replacement-therapy	Prothrombin gene-mutation (PT)	

1.1.3 Natural History of VTE

VTE is considered a chronic, as well as an acute disease because of the high risk of recurrence: 20% three years after the first event, 30% five years after and 40% 10 years after. (Prandoni et al., 2007) At least 25% VTE events occur as a second event, therefore a history of VTE is the most significant risk factor for VTE. If the first VTE was provoked by a major reversible risk factor, such as surgery, the risk of recurrence is low (3% in the first year and 10% over five years). (Prandoni et al., 2007) In contrast, if the VTE was idiopathic or if the risk factor is persistent, the risk of recurrence is 10% in the first year and 30% over five years. (López et al., 2004). Inherited hypercoagulable states are identified in a minority of patients with a VTE, namely, FVL is discovered in 20%, PT mutations in 5-10%.

DVT usually develops in the calf veins and may extend into the proximal veins, which then carries a high risk of embolization causing a PE. Fifty percent of patients with a symptomatic proximal DVT have silent co-existent PE detected on abnormal ventilation-perfusion lung scans. (Moser et al., 2014)

Venous recanalization is a slow process and residual vein thrombosis (RVT) is therefore common. Long term complications of VTE include: post-thrombotic syndrome (PTS), a chronic condition resulting from poor venous return affecting the lower limb, occurring in 50% of DVT cases, manifesting within two years of the initial event. There is an association between RVT and both PTS and recurrent VTE. Chronic thromboembolic pulmonary hypertension (CTEPH) is a complication of PE. (Fuster et al., 1984; Prandoni et al., 2007; Prandoni et al., 2015)

1.1.4 Prevention and Treatment

The goal of VTE treatment is to prevent the extension of an acute thrombosis and recurrence of VTE, in order to prevent complications such as PTS and CTEPH and to relieve the symptoms of VTE in the short term. In recent years, the prevention of VTE has become recognised as having a major positive impact on patient mortality and morbidity. Hospital acquired VTE can be prevented, if vigilant and thorough VTE prevention and risk assessments are carried out with appropriate anticoagulation prescription on admission, during hospital stay and on discharge. For acute VTE treatment, early initiation of therapeutic anticoagulation is recommended and should not be delayed pending imaging. (Kearon, 2008; Wang, Song & Jin, 2016)

Anticoagulation prevention and treatment is going through a paradigm shift. Previously, the standard of care of treatment for VTE was subcutaneous injections of low molecular weight heparins (LMWH) alone or in conjunction with oral vitamin K antagonists (warfarin). (Keeling et al., 2011) LMWH can be stopped once the INR has reached 2-3 and LMWH has been continued for a minimum of five days. (Kearon, 2008)

There are significant side effects and mortality, associated with bleeding, while on LMWH and warfarin. (Keeling et al., 2011) Warfarin is associated with >10 fold inter-individual variations in dose to achieve therapeutic anticoagulation. Its pharmacokinetics and pharmacodynamics are influenced by genetic polymorphisms, dietary vitamin K intake and absorption, concomitant medications, alcohol use, patient age, body weight and various disease states. (Ansell et al., 2008; Pérez-Andreu et al., 2009) Warfarin, thus, needs to be regularly monitored and dose adjusted to ensure it remains within the therapeutic range, which is time consuming, intrusive and expensive. (Pérez-Andreu et al., 2009)

Heparins, especially unfractionated heparin (UFH) have a risk of causing heparin induced thrombocytopenia. UFH is administered intravenously, have a narrow therapeutic window, therefore, needs regular monitoring and dose adjustment, and exhibit variability in patient response. LMWHs are administered subcutaneously and long term use has an associated risk of osteoporosis. (Kanzaki et al. 2008)

The duration of anticoagulation treatment depends on the relative risks of VTE recurrence and bleeding, both of which carry a relatively high mortality rate. A first proximal DVT or PE which occurs in the context of a transient risk factor, which has been reversed, requires three months of anticoagulation therapy. (Wang et al. 2016) If there is a persistent risk factor then long term anticoagulation is recommended and if there is an underlying malignancy then LMWH is generally used for an extended period of time. (Keeling et al., 2011; Wang et al., 2016) In patients with a first VTE which is an unprovoked, proximal DVT or a PE, extended anticoagulation may be appropriate, provided that the bleeding risk is low. (Wang et al. 2016) Following a second VTE, the risk of recurrence once anticoagulation therapy has stopped, is high, thus anticoagulation treatment is continued for at least six months, if the VTE was unprovoked. (Wang et al. 2016) A risk / benefit evaluation with a bleeding risk assessment, patient preference discussion and regular review is recommended. (Keeling et al. 2011; Kearon 2008)

1.2 Direct Oral Anticoagulant Therapy (DOAC)

1.2.1 Clinical evidence for rivaroxaban for VTE prevention and treatment

There have been four phase III trials and two phase IV trials, all demonstrating the effectiveness and safety of rivaroxaban in the prophylaxis of VTE post elective major orthopaedic surgery, in large cohorts of patients. The phase III, international trials are the Regulation of Coagulation in Orthopaedic Surgery to Prevent Deep Vein Thrombosis and Pulmonary Embolism (RECORD) trials I, II, III and IV. RECORD I and II investigated patients who were post-total hip replacement and RECORD III and IV were post-total knee replacement operation. They were randomised, double-blind, active comparator-controlled designed studies. In RECORD 1, II and III patients either received 10 mg once daily, administered 6-8h post-operation, or enoxaparin 40mg once daily, initiated 12 h pre-operation and re-started 6-8 h post-wound closure. RECORD IV compared rivaroxaban 10 mg once daily, administered 6-8 h post-operation, with enoxaparin 30 mg twice daily. The follow up period was 30-35 days.

There were 12729 patients enrolled in all RECORD trials, across 41 countries, between February 2006 and January 2008. The demographics of the subjects enrolled were 60% female, average age 64 years and 79% Caucasian.

The primary endpoint in the RECORD trials was total number of VTE events, including, symptomatic recurrent VTE, venography proven VTE, a composite of non-fatal PE or any DVT or death from any cause. (Mueck, Schwers & Stampfuss, 2013) The secondary efficacy outcomes were major VTE (proximal DVT, non-fatal PE, death from VTE. Each RECORD study showed statistically significant superiority of rivaroxaban for the prevention of total VTE and significantly reduced the all-cause mortality rate (table 2).

The principal safety outcome of the RECORD trials was clinically relevant major bleeding, from the first dose until two days post the last dose. Bleeding was classified as major if it was clinically overt and associated with a decrease in haemoglobin of ≥2 g/dl; or if it resulted in blood transfusion ≥2 units red cells; or intracranial; or retroperitoneal; or contributing to death. The rates of nonmajor clinically relevant bleeding events in each of the RECORD studies were not significantly different between the rivaroxaban and enoxaparin arms (table 2). A pooled analysis of the four studies showed that rivaroxaban was associated with an increase in major plus nonmajor clinically relevant bleeding events, up to day 12 post-operation.

Approval of rivaroxaban for post-elective total hip and total knee replacement VTE prophylaxis was granted in Europe, 2008, and USA, 2011, on the basis of the results from these RECORD trials.

TABLE 2 SUMMARY OF THE RECORD TRIALS

Variable	RECORD TRIAL			
	I	**II**	**III**	**IV**
Rivaroxaban dose	10 mg od	10 mg od	10 mg od	10 mg od
Enoxaparin dose	40 mg od	40 mg od	40 mg od	30 mg bd
Duration, days	35 +/- 5	35 +/- 5	12 +/- 2	12 +/- 2
Primary efficacy outcome, % (95% CI)	1.1 v 3.7 RR 70% (1.5-3.7, p<0.001)	2.0 v 9.3 RR 79% (5.2-9.4, p<0.001)	1.1 v 3.7 (1.5-3.7)	6.9 v 10.1 RR 31% (0.71 v 5.67 p=0.012)
Secondary efficacy outcome, % (95% CI)	0.2 v 2 AR 1.7 (1-2.5, p<0.01)	2.0 v 9.3 (p<00001)	9.6 v 18.9 RR 49% (p<0.001)	1.2 v 2 (1-2.5, p=0.124)
Principal safety outcome, % (95% CI)	0.3 v 0.1 RR 1.2% (0.92-1.6, p=0.18)	6.6 v 5.5 RR 1.22% (0.85-1.74, p=0.25)	0.6 v 0.5 RR 1.22% (0.83-1.8, p=0.77)	0.7 v 0.3 RR 1.42% (0.98-2.05, p=0.11)

RR relative risk of the specified VTE or bleeding outcome in individuals taking rivaroxaban compared to enoxaparin

1.2.2 CLINICAL EVIDENCE FOR RIVAROXABAN FOR VTE TREATMENT

The EINSTEIN DVT and PE phase III trials were open-label with blinded end-point adjudication, comparing rivaroxaban 15 mg bd for 21 days followed by 20 mg od with standard anticoagulation of enoxaparin followed by warfarin (INR 2-3), to treat proximal DVT or PE with or without DVT (Bauersachs et al., 2010; Buller, 2012). The duration of treatment was three, six or 12 months, depending on the indication. The primary efficacy outcomes from the EINSTEIN DVT and PE trials were a composite of symptomatic or venography proven recurrent DVT and non-fatal or fatal PE. The secondary efficacy outcomes were major VTE (proximal DVT, non-fatal PE, death from VTE. The principle safety outcomes for the trials were major and non-major clinically relevant bleeding. (Buller, 2012)

The studies demonstrated non-inferiority of rivaroxaban to standard therapy, and there was no difference in these outcomes for 'limited', 'intermediate' or 'extensive' clot burden. (Agnelli, 2010; Prins et al., 2013)

Rivaroxaban was associated with similar bleeding rates in the DVT trial and with 50% lower rates in the PE trial (1.1% versus 2.2% HR). (Bauersachs et al., 2010; Buller, 2012)

Table 3 summarises the main trials using rivaroxaban for VTE treatment. EINSTEIN-DVT results were 2.1 % v 3.0 % rivaroxaban v enoxaparin, in recurrent VTE events (HR 0.68, 98 % CI 0.44-1.04, p<0.001). (Buller et al. 2008) EINSTEIN-PE results were 2.1% in the rivaroxaban group and 3.0% in the standard therapy group, resulting in a hazard ratio of 0.68 (95% confidence interval 0.44-1.04), for non-inferiority with a one-sided test.

Principal safety outcomes for EINSTEIN-DVT were 0.8 % v 1.2 % major bleeding (HR 0.65, 0.33-1.3 95 % CI, p=0.21) and clinically relevant non-major bleeding was 7.3% v 7.0% for those patients given rivaroxaban v standard therapy, respectively. In the EINSTEIN-DVT extend trial, first major or clinically relevant non-major bleeding occurred in 6% on rivaroxaban and 1.2% on placebo, hazard ratio 5.19%, p<0.001. Clinically relevant non-major bleeding in DVT-extend trial was significantly more in the rivaroxaban group compared to placebo, 5.4% v 1.2%. EINSTEIN-PE trial revealed a favourable outcome for major bleeding in the rivaroxaban group, 1.1% v 2.2%, HR 0.49, 95% CI 0.31-0.79, p=0.003. However, the overall safety was similar for rivaroxaban and enoxaparin, 10.3% v 11.4% bleeding, HR 0.9, 95% CI 0.76-1.07, p=0.23. (table 3). (Bauersachs et al., 2010)

TABLE 3 SUMMARY OF THE EINSTEIN TRIALS

Variable	**EINSTEIN TRIAL**		
	DVT	**PE**	**DVT extend**
Rivaroxaban dose	15 mg bd for 3 weeks then 20 mg od	20 mg od	20mg
Comparator	Enoxaparin 40 mg & INR titrated warfarin	Enoxaparin 40 mg & INR titrated warfarin	Placebo
Duration, days	3 m, 6 m or 1 year	3 m, 6 m or 1 year	6 m or 1 year
Primary efficacy outcome, %	2.1 v 3.0 (p<0.001)	2.1 v 1.8 (p=0.003)	1.3 v 7.1 (p<0.001)
Principal safety outcome, %	0.8 v 1.2 (p=0.21)	1.1 v 2.2 (p=0.003)	0.7 v 0.0 (p=0.11)
Other Safety outcome, %	7.3 v 7.0	9.5 v 9.4	5.4 v 1.2
Total deaths through end of intended period, %	2.2 v 2.9 (p=0.06)	<0.1 v 0.1	0.2 v 0.3

A meta-analysis of nine studies of DOACs, four involving rivaroxaban, showed no significant differences in event rates between any of the DOACs and conventional anticoagulants. The combined results from the rivaroxaban studies revealed a relative risk (RR) of VTE events of 0.85 (95% CI 0.55-1.31). There was a reduction in the risk of major bleeding in rivaroxaban treated patients compared to conventional anticoagulants, RR 0.57 (95% CI 0.39-0.84), which was not the case with other DOACs. (Fox et al., 2012)

A systematic meta-analysis on the efficacy and safety of rivaroxaban for VTE treatment and secondary prevention was performed by Kakkos and colleagues. (Kakkos et al., 2014) This meta-analysis concluded that rivaroxaban was safer than warfarin for treating VTE with a reduced risk of recurrent DVT, but fatal and non-fatal PE occurred equally frequently. (Kakkos et al., 2014)

A meta-analysis of 16 trials has demonstrated a favourable prevention post total hip or knee replacement for rivaroxaban v enoxaparin compared to either dabigatran or apixaban v enoxaparin (relative risk reduction (RRR) of 0.48 v 0.71 and 0.82, respectively). (Gómez-Outes et al., 2012) However, the same meta-analysis showed a higher rate of clinically relevant bleeding in the rivaroxaban treated patients (RR 1.25 v 1.12 and 0.82, respectively). (Gómez-Outes et al. 2012) A pooled analysis from phase III trials of the prophylactic use of rivaroxaban 10 mg od, vs enoxaparin either 30 mg bd or 40 mg od, in post-operative orthopaedic patients, has shown a significant reduction in the primary composite end point of non-fatal VTE or death from cause (0.81 v 1.63%, $p<0.001$). (Turpie et al., 2012) However, this analysis also revealed a higher incidence of bleeding in the rivaroxaban group (0.44 v 0.27%). (Turpie et al., 2012)

There are no head-to-head trials of rivaroxaban compared to other DOACs. Additionally, the results from the specific trials for each DOAC and conventional anticoagulant medication as a comparator, cannot be directly compared because the exclusion criteria and duration of treatment differed. Beyer-Westendorf and colleagues investigated this by dividing the patients enrolled into EINSTEIN-DVT/PE trials into two cohorts, which could then be more fairly compared to AMPLIFY. (Beyer-Westendorf et al., 2017) The main outcome was that those patients treated with rivaroxaban who were similar to those in the AMPLIFY study, treated with apixaban, had significantly reduced recurrent VTE (RR 0.64, 95% CI 0.4-0.9) and major bleeding (RR 0.5, 95% CI 0.3-0.8). Patients treated with rivaroxaban in cohort two (not similar to those included in AMPLIFY) had similar recurrent VTE and major bleeding rates to those treated with conventional anticoagulants. (Beyer-Westendorf et al., 2017)

1.2.3 CURRENT RECOMMENDED CLINICAL USE

Rivaroxaban is licensed, National Institute for Health and Care Excellence (NICE) recommended and approved world-wide for the treatment (Ageno et al., 2012) and prevention (Antoniou, 2015) of DVT and PE in adults, at 15 mg twice a day (bd) for three weeks then 20mg once a day (od). (Buller, 2012) Rivaroxaban is also NICE recommended for thromboprophylaxis following: total hip and total knee replacement surgery, at 10 mg once a day for 35 and 12 days, respectively (Eriksson et al. 2006) (NICE 2011); and stroke/systemic embolism in non-valvular atrial fibrillation (AF) at 20 mg once a day. (Dobesh & Fanikos, 2015)

Wang and colleagues have developed clinical guidelines recommending the use of DOACs (either dabigatran, rivaroxaban, apixaban or edoxaban) for treatment of lower limb DVT or PE in patients without cancer. (Wang et al., 2016) Continued long-term rivaroxaban treatment after six to twelve months of therapy has been investigated in the EINSTEIN-extend trial. A benefit-risk analysis of the results from this trial show that for an additional year of rivaroxaban treatment, 665 fewer recurrent VTE events in 10,000 patients would have occurred compared to placebo. However, 68 more major bleeding events would have occurred. (Beyer-Westendorf et al., 2017)

Rivaroxaban is not recommended for pregnant or breast feeding women, and the evidence for its use in patients <50 kg, morbidly obese, with renal dysfunction (CrCl <15 ml/min), severe hepatic failure, recurrent VTE while on warfarin or with concomitant active cancer is minimal, therefore, it is generally not prescribed for these patients. (Mueck et al., 2013)

Clinical evidence and experience regarding long term adverse events and interactions of DOACs still remain limited, since they are relatively new drugs. (Pollack et al., 2015; Das & Liu, 2015; Asirvatham et al., 2016)

1.2.4 CHARACTERISTICS OF RIVAROXABAN COMPARED TO TRADITIONAL ANTICOAGULANTS

DOACs are categorised into direct factor Xa inhibitors and direct thrombin inhibitors. They target a single protein in the coagulation cascade and have more predictable pharmacokinetics, with reported similar bleeding rates. (Kakkos et al., 2014)

In recent years, direct oral anticoagulants (DOAC) have emerged as an effective, safe and tolerable alternative to vitamin K antagonists and LMWH, for the treatment and prevention of VTE. They overcome some of the disadvantages of the traditional anticoagulants. Table 4 summarises the advantages of rivaroxaban for an adult being treated for VTE or as VTE prophylaxis post- surgery or stroke prophylaxis for patients with AF.

Principally, it is easy-to-administer orally and at fixed dosing, does not require routine monitoring, has few drug and diet interactions and is no worse than warfarin in its bleeding risk profile. (Mekaj et al., 2015) The safety profile of rivaroxaban is better than traditional anticoagulants. A systematic review and meta-analysis of 12 randomised controlled trials which included over 100,000 patients with either non-valvular AF or VTE, revealed that rivaroxaban was associated with less major bleeding, fatal bleeding, intracranial bleeding and total bleeding as compared to warfarin. (Beyer-Westendorf et al., 2014)

The potential disadvantages are: there are no readily available measurement assays; pharmacokinetics are affected by renal and hepatic function, requiring dose adjustment; the specific factor Xa inhibitor antidote has been licensed for a short time, therefore there is little real-world data on its safety and tolerability (Siegal et al., 2015); adherence is crucial since it has a short terminal half-life; and the evidence on its long-term outcomes is yet to be established. (Burnett et al., 2016)

TABLE 4 CHARACTERISTICS OF RIVAROXABAN, LOW MOLECULAR WEIGHT HEPARIN AND WARFARIN FOR VTE TREATMENT

Quality	Rivaroxaban	LMWH	Warfarin
Route of administration	Oral	Subcutaneous	Oral
Frequency of administration	Twice a day for 21 days then once a day	Twice or once a day	Once a day
Necessity for monitoring	No but levels help to assess: haemorrhage; compliance; VTE recurrence; pre-operative bleeding risk; potential sub- or supra-therapeutic levels	In certain circumstances eg: renal impairment; pregnancy; obesity	Yes: routinely and with every medication or dietary change; before and after all procedures
Elimination half life	3-6h	1-3h	24-48h
Speed of action & onset	Rapid	Rapid	Slow
Food interaction	None known	None known	Vitamin K containing foods
PD drug interactions	Antiplatelet medication	Antiplatelet medication	Antiplatelet medication
PK drug interactions	Inhibitors and inducers of CYP3A4 and CYP2J2	None known	Inhibitors and inducers of CYP2C9
Short term complications	Bleeding, dizziness, HMB	HIT, bleeding	Bleeding, alopecia Teratogenic
Long term complications	None known	Osteoporosis	None known

HIT heparin induced thrombocytopenia; CYP cytochrome P450 isoenzyme, HMB heavy menstrual bleeding, PK pharmacokinetic; PD pharmacodynamics

1.2.5 PHARMACOKINETIC PROFILE OF RIVAROXABAN

Rivaroxaban is the compound 5-chloro-N-([5S)-2oxo-3-[4-(3-oxomorph-olin-4-yl)penyl]-1,3-oxazolidin-5-yl]methyl) thiophene-2-carboxaminde). Its molecular weight is 435.9 g/mol, 95% plasma protein binding, 7 mg/L water solubility (low-solubility) and 9.48 x 10^{-6} cm/s permeability in the large intestine (high permeability). (Mueck et al., 2014; Bayer Pharma, Leverkusen, 2013.) Rivaroxaban has a high bioavailability despite the low water solubility because it uses transporter proteins to allow its entrance into the blood stream across the stomach endothelium, and has high affinity to albumin, which transports it through the blood into tissues and organs and it has moderate lipophilicity.

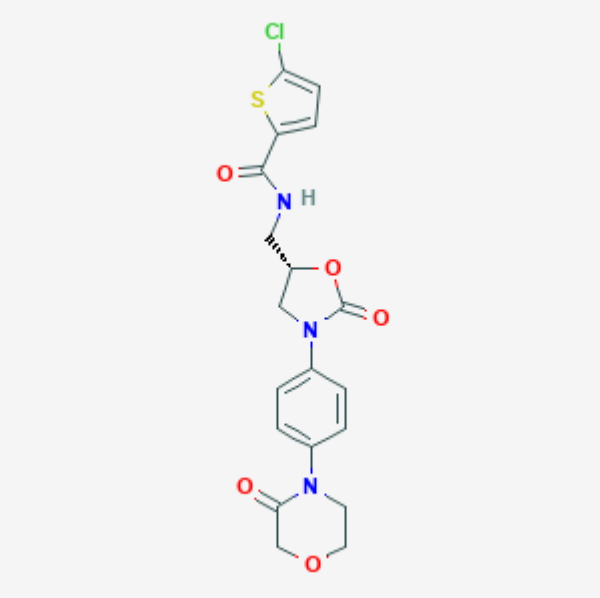

FIGURE 3 BIOCHEMICAL STRUCTURE OF RIVAROXABAN

Rivaroxaban is the active drug, therefore it does not require activation. Studies in healthy humans have shown that rivaroxaban is rapidly absorbed after oral administration, with peak plasma concentrations being reached two to four hours after tablet intake. (Weinz et al., 2009) The bioavailablity is the fraction of unchanged drug reaching the systemic circulation following administration by any route. The bioavailability of rivaroxaban is dose dependent, estimated to be 80-100% for the 10 mg dose, which is unaffected by food, 66% for the 20 mg dose if taken in the fasting state and 80-100% for the 20 mg taken in the presence of food. (Stampfuss et al., 2013; Mueck et al., 2014) The absorption of rivaroxaban is dose dependent until it reaches a steady state because it is mostly absorbed by passive diffusion across the lipid cell membrane and then through the aqueous layer adjacent to the cell. Once the drug has been distributed homogeneously across the plasma and extravascular tissues, and the elimination pathways have become saturated, steady state is reached.

Phase I studies have demonstrated no accumulation of rivaroxaban after multiple dosing (table 5). (Kubitza et al., 2005; Trujillo & Dobesh, 2014)

In vitro kinetic studies in healthy adults showed that rivaroxaban competitively inhibits human factor Xa (FXa) with an inhibition constant (Ki) 0.4 +/- 0.02 nM with 10,000 fold greater selectivity than for other serine proteases. Ki is the concentration of rivaroxaban required to decrease the maximal rate of FXa inhibition by half. FXa is a serine protease which activates prothrombin to become thrombin, in the final common pathway of the coagulation cascade. (Hoffman, 2003) Rivaroxaban has a high affinity of association for the active binding site of FXa (Kd $3x10^{-10}$ mol/l) and is a potent inhibitor of both clot-associated FXa (inhibitory concentration 50% 75 nM) and prothrombinase (factor Xa and activated factor V) activity (inhibitory concentration 50% 2.1 nM). Rivaroxaban has no direct effect on thrombin or antithrombin. (Kreutz, 2012) Its effects are targeted and its adverse effects are narrow, only known to be pertaining to increased bleeding.

The clearance of a drug is the factor which predicts the rate of elimination in relation to the drug concentration. The rate of elimination of the drug is directly proportional to the concentration in the plasma. Approximately two thirds of rivaroxaban under goes metabolic oxidation via CYP3A4 and CYP2J2 and hydrolysis, of which half is eliminated renally, and half via the hepatobiliary route. (Lang et al, 2009) The metabolites are inactive. The other third is excreted directly via the kidneys as unchanged active drug, via transporter proteins P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP). (Gnoth et al., 2011) The half-life of a drug is the time required to change the amount of drug in the body by a half, during elimination. This is the time required to attain 50% of steady state or to decay to 50% from steady state conditions. Therefore, dosing intervals must be longer than 4 half-lives to prevent drug accumulation. Rivaroxaban has a terminal elimination half-life of 5.7-9.2 h in healthy young individuals and 11-13 h in elderly individuals and the dosing interval is 12 h for the initial loading period, then 24 h to maintain steady state (table 5). (Kubitza et al., 2005; Mueck et al., 2014)

Azole antifungals and protease inhibitors are strong inhibitors of CYP3A4 and P-gp, thus, cause an increase in rivaroxaban plasma concentration for a given rivaroxaban dose. Garcia and colleagues observed a 2.6 and 2.5 fold increase in exposure, resulting in 1.7 and 1.6 fold increase in Cmax in patients taking ketoconazole 400 mg daily or ritonavir 600 mg twice daily, respectively. (Garcia et al., 2013) Strong inducers of CYP3A4 such as phenytoin, carbamazepine, phenobarbitone and St John's Wort should be avoided, or used with caution, because they will cause a decrease in rivaroxaban exposure. A recent literature search has noted several haemorrhagic and thrombotic events in patients taking rivaroxaban and clarithromycin, erythromycin, fluconazole or

ketoconazole. (Lippi & Favaloro, 2014) Patients with liver disease, Child Pugh stage B or worse, should not take rivaroxaban, due to the route of metabolism, resulting in 2.3 fold increase in mean rivaroxaban exposure. Dose adjustment to 15 mg od is recommended for patients with CrCl <30 ml/ml and if CrCl <15 ml/min, rivaroxaban should be avoided. (Trujillo & Dobesh, 2014)

Rivaroxaban has a high and reversible plasma protein binding of 92-95%, (Weinz et al., 2009) serum albumin as the main plasma blinding component. The volume of distribution (Vd) relates the amount of drug in the body to the concentration of drug in the plasma. At steady-state Vd rivaroxaban is 0.62 L/kg, indicating its low-to-moderate affinity to peripheral tissues (table 5).

Table 5 Pharmacokinetic parameters of rivaroxaban 20mg od, in healthy adults

Pharmacokinetic Variable	**Value**
Absolute bioavailability (%)	80-100
Volume of distribution (L/kg)	0.62
Maximum Xa inhibitory effect (Emax)	1
Time to Emax (h)	2.5-4
Half-life (h)	5-9
Plasma protein binding (%)	92-95
Apparent clearance (L/h)	10
Dialysable	No
Metabolism	Oxidation via CYP 3A4 & CYP2J2 and hydrolysis
Interactions	Combined P-gp inhibitors & CYP 3A4 inhibitors
Renal excretion of unchanged drug (%)	33

1.2.6 MEASURING THE CONCENTRATION OF RIVAROXABAN

1.2.6.1 THE ASSAY USED TO QUANTIFY RIVAROXABAN CONCENTRATIONS

Rivaroxaban is measured quantitatively using a calibrated anti-factor Xa chromogenic assay, which accurately gives a concentration in the range 20-660 microgram/l. (Samama et al., 2010) The chromogenic anti-Xa assay utilises FXa substrate (heparin) tagged with a chromophore which is cleaved by FXa, resulting in a colour change. The colour change is directly proportional to the concentration of FXa present in the assay, which is compared to a standard assay with a known quantity of FXa inhibitor, thus, allowing calculation of FXa inhibitor in the sample. This chromogenic assay is calibrated for rivaroxaban and has high accuracy, precision and inter-laboratory reproducibility. (Samama et al., 2010) This assay has been validated by high-performance liquid

chromatography, coupled with tandem mass spectrometry, thus, the antiXa levels determined by this chromogenic assay can be used as a surrogate marker for rivaroxaban concentration. The timing of blood sampling in relation to rivaroxaban ingestion is crucial in interpreting the results. (Samama et al., 2013) Although the anti-FXa chromogenic assays accurately measure the concentration of rivaroxaban, they do not indicate the intensity of anticoagulation, thus, do not reflect the risk of bleeding or thrombotic events. (Samama et al., 2014)

1.2.6.2 VARIABLES KNOWN TO AFFECT RIVAROXABAN CONCENTRATION

Pharmacokinetic modelling and population pharmacokinetic modelling have identified covariates which consistently affect certain pharmacokinetic parameters of rivaroxaban. (Grillo et al., 2012; Mueck et al., 2013) These include age, renal function, haematocrit, albumin and P-gp and CYP3A4 inhibitors.

1.2.6.2.1 CREATININE CLEARANCE

Kubtiza and colleagues has shown a direct correlation between decreasing creatinine clearance and decreasing rivaroxaban clearance (Pearson correlation coefficient $r = 0.83$, $p<0.001$). Consequently, the amount of unchanged rivaroxaban in the urine of patients with normal renal function, mild, moderate and severe renal impairment were 29%, 20%, 13% and 10%, respectively. (Kubitza et al., 2010) As a result, total rivaroxaban concentration, represented by the area under the concentration-time curve for individuals with renal impairment compared to those with no renal impairment, increased by 64% in severe renal impairment, 52% in moderate and 44% in mild renal impairment, which was statistically significant ($p<0.03$). The maximum concentration of rivaroxaban was increased by 35% in severe renal impairment and 27% in moderate, and the time to maximum concentration and the half-life of elimination were only slightly prolonged in patients with renal impairment. (Kubitza et al., 2010)

1.2.6.2.2 HEPATIC IMPAIRMENT

There have been few studies directly investigating the effects of hepatic function on the plasma concentration of rivaroxaban. A small (n=32), single centre, non-randomised, non-blinded study investigated the effects of Child-Pugh classified hepatic impairment on the pharmacokinetics and pharmacodynamics of rivaroxaban, after a single 10 mg dose of rivaroxaban. (Kubitza et al., 2013) Plasma concentrations of rivaroxaban in patients with mild hepatic impairment were similar to those with no hepatic impairment, but those with moderate hepatic impairment had a higher Cmax and AUC of rivaroxaban concentration and a prolonged elimination phase as compared to the healthy control group. Additionally, those with mild or moderate hepatic impairment had

prolonged half-lives. There was a weak correlation between factor inhibition of factor Xa activity and serum albumin. (Kubitza et al., 2013) however, this relationship has not been replicated, in the literature. (Gheorghiade et al., 2011; Kvasnicka et al., 2017)

1.2.6.2.3 Proteins Induced by Vitamin K Absence for Factor II (PIVKA-II)

The impact of dietary vitamin K on the anticoagulation effects and response to warfarin is well known. Vitamin K deficiency is known to enhance the effects of warfarin and increase the INR. (Pedersen et al., 1991) The activation of factor X and thrombin are linked to the vitamin K cycle of carboxylation of the coagulation factors in the cascade. Thus, in theory, vitamin K availability, which is dependent on dietary vitamin K, could affect the pharmacology of DOACs. Preliminary evidence suggests that there is an effect of vitamin K deficiency on the action of ximelagatran in rats, which is similar to that seen on the action of warfarin in humans. (Kamali & Wood, 2009) A further study by Kamali and colleagues investigated the effect of vitamin K deficiency on the pharmacological activity of rivaroxaban ex-vivo. Thirty-one patients with low vitamin K intake were compared to 28 healthy volunteers with adequate dietary vitamin K. Plasma samples were incubated ex-vivo with rivaroxaban at varying concentrations. They found that rivaroxaban produced a greater prolongation of PT ($p=0.01$) and a greater inhibition of factor Xa ($p=0.005$) in patients with poor dietary vitamin K compared to healthy volunteers. (Kamali et al., 2013)

Proteins Induced by Vitamin K Absence (PIVKA-II) assay is an enzyme-linked immunosorbent assay, which monitors uncarboxylated prothrombin and is more sensitive to minor vitamin K deficits than the prothrombin test, as reflected by an increase in PIVKA-II. (Dauti et al., 2015) It is important to ascertain the PIVKA-II level in each plasma sample because if there was an element of vitamin K deficiency then the baseline clotting tests may be prolonged independent to the action of rivaroxaban. (Funk 2012)

1.2.7 Reversal of the anticoagulation effects of rivaroxaban

The treatment of bleeding while on rivaroxaban depends on the severity of the bleed. If it is deemed clinically relevant or major / life threatening then anticoagulation reversal agents are recommended, including inactivated PCC, activated PCC and recombinant FVIIa. However, evidence for their use effectiveness is minimal. Andexanet alpha is a modified recombinant human factor Xa which has a serine-alanine mutation rendering the protease catalytically inactive. It binds to factor Xa inhibitor with high affinity, therefore, decreasing the concentration of unbound rivaroxaban, preventing them from inhibiting endogenous factor Xa.

Results from the ANNEXA-4 show that a bolus, followed by and infusion of andexanet for patients with a major bleed on rivaroxaban or apixaban, rapidly caused a decrease in factor Xa activity. The ANNEXA-4 trial interim analysis revealed a relative reduction in anti-Xa activity of 89% (95% CI 58-94) at the end of the infusion. (Connolly et al., 2016) The addition of 400mg iv bolus of Andexanet alpha to rats with a tail transection resulted in complete cessation of bleeding within two minutes. (Siegal et al., 2015; Levy et al., 2016).

Andexanet alpha also binds to tissue factor pathway inhibitor (TFPI) but only increases thrombin generation when rivaroxaban is present. However, there was a rebound increase in anti-Xa activity at four hour post infusion and 18% of patients experienced a thromboembolic event during the 30 day follow up. (Connolly et al., 2016) The study as not revealed any development of neutralising antibodies, during the year follow up after receiving Andexanet alpha. (Siegal et al., 2015)

1.3 OBESITY

1.3.1 THE EPIDEMIOLOGY OF OBESITY

Obesity, defined by the National Institutes of Health, as body mass index (BMI) ≥30 kg/m^2, has a high associated morbidity and mortality and is rapidly increasing in incidence. (Flegal, Carroll & Ogden, 2010) Obesity is a well-recognised global healthcare problem and two-thirds of all men and half of all women in England are either overweight or obese. (Jain, 2004) In England, obesity accounts for approximately 18 million days of sickness absence and 30,000 premature deaths each year. The estimated annual cost of treating obesity in the UK is £500 million and the wider costs to the economy, in terms of lower productivity and lost output, are £2 billion, each year. (de Ferranti et al., 2010; Baker, 2017)

Obesity has been shown to be associated with DVT, with a calculated hazard ratio of 2.33, in a large meta-analysis (Ageno et al., 2008) and an odds ratio of 2.39, in a large case-controlled study. (Samama, 2000) Several other studies have revealed a linear association between body weight and the risk of recurrent VTE, for example, Heit and colleagues demonstrated that a 10 point increase in BMI resulted in a 24 % increase in recurrent VTE risk (Heit et al., 2002). Eichinger and colleagues subsequently undertook a prospective study involving 1107 patients with a first unprovoked VTE. The average follow up was 46 months and 168 patients had a recurrent VTE. The mean BMI was significantly higher in patients with recurrence than those without (28.5 vs 26.9, p=0.01) and the adjusted hazards ratio for recurrence was 1.044 (95 % CI 1.024-1.079) for each point increase in BMI. Overall, this study found a 30 % increase in risk of recurrence in patients overweight and a 60 % increase in obese patients, compared to normally weighted individuals. (Eichinger et al., 2008) The Longitudinal Investigation of Thromboembolism Etiology study (LITE) of 20,374 middle-aged and elderly patients reported that, within patients with the metabolic syndrome, the predominant risk factor for VTE was abdominal obesity. (Cushman et al., 2004; Steffen et al., 2009) A study by Mazzaccoli and colleagues demonstrated a correlation between VTE incidence and epicardial fat thickness. (Mazzoccoli, Copetti & Dagostino, 2012)

However, one study revealed no increased risk of PE with body weight above the normal range, revealing a 1.08 relative risk of PE with obesity. (Kabrhel et al., 2009)

The exact impact of body weight on the pharmacokinetics and pharmacodynamics of rivaroxaban are unknown, thus, the dosing guidelines for obese patients are not fully established. The relationship between weight and global coagulation tests has not been fully determined.

1.3.2 THE EFFECT OF OBESITY ON VTE PATHOGENESIS

The physical aspects of increased body weight contribute to a higher risk of VTE due to decreased venous return and immobility. Increased BMI and waist-to-hip ratio (WHR) are associated with increased fibrinogen, which promotes fibrin formation, platelet aggregation and plasma viscosity, and increased plasminogen activating factor inhibitor-1 (PAI-1), which decreases fibrin degradation (Allman-Farinelli, 2011) Both fibrinogen and PAI-1 have been demonstrated to be higher in patients with primary and recurrent VTE, although statistical differences did not exist after controlling for BMI and other confounders. (Vayá et al., 2002)

Tissue factor (TF) is a 47 kDa transmembrane glycoprotein which initiates the coagulation cascade. It has two isoforms, an insoluble cytokine receptor, which functions as an integrin in intracellular signalling and cell migration, and a soluble isoform, which regulates angiogenesis and monocyte recruitment. Inflammatory mediators are able to induce and upregulate the soluble TF, thus increase their levels in the vasculature. (Samad et al., 2001; Samad & Ruf, 2015) Plasma TF levels have been used as a biomarker for the severity of microvascular disease in patients with type two diabetes. Additionally, mice experiments have demonstrated an association between obesity and both increased TF activity, TF mRNA levels in adipocytes and adipose-infiltrating macrophages, and elevated plasma TAT levels. (Nagai et al., 2008)

Visceral adipose tissue is metabolically active, producing pro-angiogenic and pro-inflammatory cytokines and hormone-like substances, which stimulate endothelial vasculature development within the adipose tissue, acting as a phospholipid surface for the initiation and propagation of the coagulation cascade. (Fontana et al., 2007) These cytokines and hormone-like substances also act as activators for the coagulation cascade, and attract and activate platelets. (Darvall et al., 2007)

Obesity is associated with platelet activation and increased mean platelet volume, platelet microparticles, thromboxane B2 metabolites, soluble P-selectin, and platelet derived CD40L. (Lindmark & Tenno, 2000)

Obesity is considered to be a low-level inflammatory state, which amplifies thrombosis both directly, via cytokines and indirectly, via oxidative stress. (Darvall et al., 2007; Fontana et al., 2007) Interleukein-6 (IL-6) is a cytokine produced in adipose tissues, as well as in immune cells. Its level increases in association with increasing BMI and WHR and IL-6 decreases with weight loss. (Kershaw & Flier, 2004) IL-6 induces insulin resistance, increases platelet count and aggregation, increases endothelial adhesion molecule expression and decreases adiponectin secretion. (Kershaw & Flier, 2004; Davı et al., 2014)

Leptin is a hormone found in excessive quantities in obese individuals. It induces proangiogenic, pro-inflammatory, pro-thrombotic and defective fibrinolytic mechanisms. (Darvall et al., 2007) The proangiogenic associations include adenosine diphosphate induced platelet aggregation, monocyte, smooth muscle and endothelial cell stimulation. Leptin is involved in the inflammatory process via upregulation of VEGF expression and stimulation of IL-6 receptors. Leptin secretion from hepatocytes is stimulated by tumour necrosis factor alpha (TNF alpha). Haemostatic genes are shown to be abnormally expressed in genetically obese mice, as a result of increased cytokine production, namely TNF alpha. (Loskutoff et al., 2000) Evidence for the direct pro-thrombotic actions of leptin include: decreased tissue plasminogen activator factor; increased plasminogen activator inhibitor-1 (PAI-1); and increased tissue factor. (Sierra-Honigmann et al., 1998; Samad et al., 2001) There have also been studies showing a positive correlation between PAI-1 levels and obesity (BMI, WHR), insulin resistance and triglyceride levels. (Loskutoff et al., 2000; Kershaw & Flier, 2004; Allman-Farinelli, 2011)) Additional studies have revealed positive correlations between BMI and WHR with factor VII, factor VIIIc and von Willebrand Factor. Factor VIII levels are strongly associated with VTE risk. (Reiner et al., 2001)

The global coagulation assay, thrombin generation, has demonstrated an associated increase in thrombin generation with subcutaneous adipose tissue, illustrating the impact of obesity on the coagulation system. (Prüller et al., 2012) Another study investigated 165 women with a history of VTE and found that those who were obese had prolonged lag times and higher ETP (as measured with a calibrated thrombogram), as well as increased fibrinogen, CRP and PAI-1, compared to patients of normal body weight. These findings suggest a correlation between an underlying low-grade inflammatory environment and increased thrombin generation, perhaps as a consequence of obesity, in women with an increased thrombotic tendency. However, there was no follow-up to investigate which patients developed recurrent VTE, and there was no distinction between the women with provoked and unprovoked VTE at initiation of the study. (Sonnevi et al., 2013)

Further evidence for a role for obesity in VTE has emerged from studies demonstrating an association between weight loss and decreasing markers of coagulopathy. Kershaw demonstrated decreasing PAI-1 levels with weight loss. (Kershaw & Flier, 2004)

Bariatric surgery and weight loss decrease both mortality and cardiovascular risk in morbidly obese patients. Ay and colleagues found significant reductions in thrombin generation parameters post-bariatric surgery and weight loss in a prospective longitudinal study performed with 36 severely obese patients. (Ay et al., 2010) Parameters of thrombosis which were less post-operatively compared to pre-operatively included: tissue factor,

plasminogen activator inhibitor and prothrombin fragment 1. Post-operatively, there was a significantly prolonged thrombin generation lag time phase, decreased peak thrombin generation and decreased area under the curve (AUC), compared to pre-operatively. (Ay et al., 2010) These post-operative values were not significantly different from a healthy, age and gender matched control group, with the exception of the peak thrombin generation, which remained significantly higher in the obese patients. The study suggests that the improved life expectancy and cardiovascular health associated with weight loss and bariatric surgery may be consequential upon decreased thrombin generation and less activation of the coagulation cascade. (Ay et al., 2010)

1.3.3 THE GENERAL IMPACT OF OBESITY ON THE PHARMACOKINETICS OF DRUGS

The pharmacokinetics of a specific drug depend on the physiochemical properties of that drug and the biological environment of the patient. The independent pharmacokinetic parameters: volume of distribution (Vd) and total clearance (CL) determine the drug elimination half-life ($t_{1/2}$), which, in turn, determines the maintenance dose for that drug. (Hanley et al., 2010)

The Vd describes the extent to which a drug distributes into extravascular tissues and is dependent on the physiochemical properties of that drug. (Patel et al., 2011) Drug clearance is determined by hepatic and renal physiology, which are both influenced by body weight. Certain metabolic processes may be more active in obese patients, such as glucoronidation, sulphation, increased cytochrome P450-2E1 activity and phase II conjugation, resulting in increased drug clearance. Additionally, non-diabetic obese patients have been shown to have increased glomerular filtration as a result of structural renal changes and increased renal plasma flow, resulting in increased renal clearance. (Chagnac et al., 2000) However, one study suggests that obesity-related liver lipodystrophy may result in hepatic dysfunction, thus, decreased drug clearance. (Hanley et al., 2010)

The main factors which affect the tissue distribution of drugs are: body composition, regional blood flow and the affinity of the drug for plasma proteins and/or tissue components. (Hanley et al., 2010) Obese individuals have a lower cardiac output and decreased blood supply to the adipose tissue. There are uncertain differences in drug plasma protein binding with reports of increased binding to alpha-1 acid glycoprotein in obese patients. (Benedek et al., 1983)

Obese individuals have an increased percentage of fat per kilogram of total body weight and decreased lean tissue compared to normally-weighted individuals of the same age, height and gender. (Cheymol, 2000) Han explains that since 99% of the body's metabolic processes occur within lean tissues. It is this compartment which

should be used when quantifying changes in clearance for individuals of all body weights. Han and colleagues critically evaluated drug dosing in obesity, making three crucial observations: absolute drug clearance is greater in obese individuals; clearance increases non-linearly with weight; and clearance correlates linearly with lean body weight. (Han et al., 2007)

Lean body weight closely represents fat free mass, which has been formulated through a semi-mechanistic model from individual characteristics within a population containing extremes of body weight. This model, which includes sex, body weight and height, has been shown to have good predictive performance for fat free mass. (Janmahasatian & Sarayut, 2005)

$$\text{LBW (kg), for males} = \frac{9270 \times \text{WT (kg)}}{6680 + 216 \times \text{BMI (kg m}^{-2})}$$

$$\text{LBW (kg), for females} = \frac{9270 \times \text{WT (kg)}}{8780 + 244 \times \text{BMI (kg m}^{-2})}$$

The pharmacokinetics of a specific drug are determined by multiple factors. Studies have shown that hydrophilic drugs have predictable outcomes in obese patients because they are predominantly distributed in the lean tissue compartments, therefore they can be dosed according to the ideal body weight. Lipophilic drugs, however, have unpredictable pharmacokinetics in obese patients. (Hanley et al., 2010)

The general effects of obesity on drug pharmacokinetics are summarised in table 6, but the exact influences of obesity are specific for individual drugs, depending on their characteristics (table 6).

TABLE 6 THE GENERAL EFFECTS OF OBESITY ON DRUG PHARMACOKINETICS

Pharmacokinetic parameter	Obese compared to normal-weighted individuals	Explanation
Oral absorption	Decreased	Variable gastric motility
Volume of distribution	Increased if: small molecular size, lipophilic, low protein binding	Low cardiac output, decreased blood supply, cross lipophilic membranes
Metabolism	Variable effects	Increased fatty deposits in the liver, hepatic lipodystrophy, decreased blood flow, altered cytochrome P450 activity
Elimination	Increased creatinine clearance	Increased glomerular filtration (in non-diabetic obese)

1.3.4 THE EFFECT OF BODY WEIGHT ON THE PHARMACOKINETICS OF ANTICOAGULATION MEDICATION, IN PARTICULAR, RIVAROXABAN

Body weight affects the pharmacokinetics of low molecular weight heparins (LMWH). (Patel et al., 2011) Obese and morbidly obese individuals are under-represented in clinical trials of LMWH and direct oral anticoagulants. Rondina and colleagues have shown that weight-based dosing of enoxaparin for VTE prophylaxis, in obese and morbidly obese medically-ill patients is effective at achieving optimal antiXa levels, with no bleeding events. (Rondina et al., 2010) Interestingly, this study did not show any correlation between weight or BMI and antiXa levels. Other studies comparing non-obese to obese patients taking weight-based enoxaparin for VTE treatment showed similar mean antiXa levels for the non-obese and obese groups. (Sanderink et al., 2002; Bazinet et al., 2005) However, a weight based regimen for morbidly obese patients would result in a high dose of anticoagulation. Therefore, at King's College Hospital, London, the clinical practice for obese patients is to dose LMWH in accordance with actual body weight, but to cap the dose, with each individual patient at a certain level, to prevent potential excessive bleeding. (Patel et al., 2011)

Oral direct thrombin inhibitors and direct Xa inhibitors have a predictable dose-response curve, allowing fixed dosing regimens. However, the obese sub-population has been under-represented in clinical trials, thus there is limited experience and data for these patients. There is no consensus on how, if at all, absorption of rivaroxaban is altered by weight. Cardiac function may influence blood supply to the route of administration, subcutaneous

or oral. Vd is dependent on protein binding, which is influenced by lean body weight. (Moore & Kröll, 2017) One research group demonstrated an increase in alpha (1)-acid glycoprotein (Benedek et al., 1983) and another group showed interference of protein binding by increased plasma lipids. (Wasan & Lopez-Berestein, 1993) Rivaroxaban has a low-medium distribution volume, is highly bound to plasma proteins and has moderate tissue affinity with no irreversible binding to specific organs. (Kubitza et al., 2005) These qualities confine rivaroxaban predominantly to the vascular compartment. Body weight increases with increasing adipose or muscle tissue, however, the vascular volume of the compartment remains relatively steady, irrespective of the increased body weight. (Kreutz, 2014) The active metabolic compartment determines the elimination and clearance of the drug, and this is dependent on the lean body weight rather than the actual body weight. (Mueck et al., 2014)

Analysis from the Randomised Evaluation of Long Term Anticoagulant Therapy (RE-LY) trial data of dabigatran for non-valvular atrial fibrillation (AF) revealed a significant effect of body weight on the volume of distribution of dabigatran. However, it has been reported that body weight had minor influence on the dabigatran concentration-time profile and no impact on the overall exposure of dabigatran. (Liesenfeld et al., 2011) Given dabigatran's clearance through renal mechanisms, one anticipates a clinically relevant impact of body weight on dabigatran's concentrations. A small, open-label, single-dose, group-matched study of normal weighted vs obese patients taking ximelagatran, showed no influence of obesity on melagatran plasma concentration curve, excreted product or APTT. (Sarich et al., 2003)

A recent, open-label, parallel group study of 54 patients directly compared antiXa levels, apixaban concentration and urine samples for healthy volunteers taking 10 mg apixaban. The group of lower body weight individuals had higher Cmax and AUC and the higher body weight group had lower Cmax and AUC, compared to those of normal body weight. These apixaban pharmacokinetic parameters correlated linearly with antiXa activity irrespective of body weight. Interestingly, at this dose, there was no difference in renal clearance for different body weights. (Upreti et al., 2013)

Currently, the recommendation is not to dose adjust rivaroxaban depending on weight, and to give a fixed dose to all patients. (Martin et al., 2016) In their summary of product characteristics, the manufacturers of rivaroxaban state that in patients who are at the extremes of weight (<50 kg or >120 kg), only a small influence of weight on patients' rivaroxaban plasma concentrations (<25 %) is observed and no dose adjustment is necessary. Kubitza and colleagues performed a randomised, single-blind, placebo-controlled, parallel-group study in healthy male and female volunteers to assess the PK and PD effects of 10 mg rivaroxaban in patients of extremes of body

weight compared to normal body weight. There were 48 subjects in the over-weight categories. The outcomes were no significant difference in PK parameters or FXa activity between over-weight and normal weight subjects, and 24 % increase in Cmax in subjects weighing ≤ 50 kg. (Kubitza et al., 2007) Mueck and colleagues analysed the pharmacologic properties of rivaroxaban from phase III trials of orthopaedic patients, and concluded that body weight did affect Vd of rivaroxaban but this was within the accepted interindividual variability. (Mueck et al., 2007)

Martin et al performed a thorough literature search of all DOAC publications and found limited clinical data for patients at the extreme of weight. (Martin et al., 2016) The available PK/PD evidence of decreased drug exposures, reduced peak concentrations and shorter half-lives with increasing weight, suggests the fixed dosing strategy of the direct Xa-inhibitors may not be sufficient for those in the obese category class II or III and may be too much for patients <50 kg, for their various indications. (Di Minno et al., 2015)

The ISTH has published guidelines which recommend appropriate standard dosing of the DOACs in patients with a BMI less than or equal to 40 kg/m^2 and weight less than or equal to 120 kg for VTE treatment, VTE prevention, and prevention of ischemic stroke and systemic arterial embolism in nonvalvular AF. This group also recommended that DOACs should not be used in patients with a BMI of > 40 kg/m^2 or a weight of > 120 kg. If DOACs are used in a patient with a BMI of > 40 kg/m^2 or a weight of > 120 kg, ISTH recommended checking an antiFXa peak and trough level, and if this is within the expected range, DOAC should be continued. However, if the antiXa falls outside the expected range, it should be discontinued and switched to VKA. (Martin et al., 2016)

The effect of deceased rivaroxaban concentration in obese patients and increased in under-weight patients, has been from small numbers of subjects from trials, which have not been significant and not had sufficient follow up to show any long term difference in safety or efficacy. (Di Minno et al., 2015) The decrease in rivaroxaban exposure, as stated by the manufacturers, may not be clinically significant for an overweight patient, but could be for an obese class II or class III patient, thus, they may be at risk of recurrent thrombosis. Additionally, patients of very low body weight could be exposed to too much rivaroxaban, putting them at risk of bleeding.

1.3.5 Evidence for the efficacy and safety of rivaroxaban for VTE treatment and prophylaxis of recurrent VTE for patients at the extremes of body weight

Subgroup analysis of the EINSTEIN DVT and PE trials has consistently shown no increased VTE recurrence rate for over-weight patients, and no increased bleeding for under-weight patients, taking the fixed dose of rivaroxaban, 15 mg twice a day followed by 20 mg once a day, for the treatment and prevention of recurrent VTE, with normal renal function. (Di Nisio et al., 2016)

A retrospective meta-analysis of the literature of phase III clinical trials of patients post-elective arthroplasty showed similar safety and efficacy of 10 mg daily rivaroxaban and LMWH across patients of all body weights. (Pathak et al., 2015)

Piran and colleagues investigated patients > 120 kg taking rivaroxaban 20 mg od and showed that 28% (6/21) of patients on rivaroxaban had below usual on treatment range peak concentration levels, although none had a peak plasma concentration below the median trough level for previously published PK studies. (Piran et al., 2018)

The annual Chest meeting, October 2018, had a presentation on 'efficacy and safety of DOAC in morbidly obese patients'. The preliminary results from this retrospective study revealed that, of 90 patients taking DOAC, (33 of whom were on rivaroxaban) compared to 90 patients on warfarin, all with either BMI > 40 kg/m^2 or body weight > 120 kg, there was no difference in the frequency of thromboembolic or major bleeding events. (Kalani et al., 2018) Although this is a study with few numbers, it does add to the current evidence supporting the safety of rivaroxaban in morbidly obese patients.

1.4 Population Pharmacokinetic (PPK) modelling

1.4.1 Population modelling

Individual studies are limited by small sample numbers and lack of representation of the general population. Population modelling overcomes these limitations through the application of a mathematical model which describes pharmacokinetic data obtained from a group of individuals. The method does not require each individual subject to provide sufficient data to characterise their own PK profile, since PK information is shared between individuals to develop the population PK profile. (Duffull et al., 2011) the population model integrates a covariate model, describing the relationships between PK parameters and patient characteristics and a statistical model, describing the variance in PK parameters between and within the individuals of that population and the residual variance due to biological variability. (Aarons, 1991) The information provided from each individual is pooled together and can be applied to the cohort of this study, allowing gaps of information from that individual to be filled in with information borrowed from others to develop the 'population' model. Population modelling also determines and quantifies the influence of a chosen characteristic from the pharmacokinetic profile of an individual, and, in turn, can estimate the remaining, unexplained variabilities in the pharmacokinetics of that drug between individuals and within an individual over time. Population pharmacokinetic (PPK) modelling is useful to estimate an individual's pharmacokinetic parameter for a particular drug, despite sparse sampling, in a cohort of patients.

A population model describes an observation in terms of the structural model, estimating the fixed (CL and Vd) and random effects components. It also simultaneously describes the statistical model, describing the degree of random effects, such as inter- and intra- individual and unexplained, residual variability, whilst preserving the individual's data within the population. Data from several sources can be analysed together, even if the precision of these studies differs.

Population PK plays a crucial role in developing dosing strategies for drug development and providing detailed information for a target patient group on the many variabilities in that drug pharmacokinetics. (Ette EI, 2004) The dosing strategy predicted for an individual will differ from the optimal regimen, due to inter-individual and residual variation. Additionally, the optimal regime is often determined by pharmacokinetic studies involving healthy volunteers who are rigidly standardised, performed under artificial conditions and not representative of the individual in question.

Traditional PK analysis cannot accommodate unbalanced, sparse data nor data from different sources, which population PK analysis is able to do. Population PK analysis can, additionally, offer an explanation of inter-subject variability and further, if the model is robust, it can simulate clinical scenarios. The disadvantages of population PK modelling are that different models could be developed from the same data and it is time consuming and complicated.

1.4.2 Non-Linear Mixed Effects Modelling (NONMEM)

NONMEM is a population modelling analytical software used to determine the pharmacokinetic behaviour of a drug. The mathematical model obtains final parameter estimates after the maximum likelihood of model to fit the data is achieved. The maximum likelihood of model to fit the data is calculated by non-linear equations, which are expressed linearly as approximations of the likelihood. (Sheiner, 1992)

Covariates explain some of the variability seen during the model building process and the system into which the drug is being administered. Covariates can be categorised into mechanistic and empirical. The mechanistic covariates explain variability in a population due to physiological differences, such as renal function on drug clearance. Empirical covariates describe the population characteristics which have no known physiological influence, thus, these are not relevant to the PPK for rivaroxaban in this study.

The principal steps in the PK modelling process are:

1. Analysis of the problem
2. Design and execution of the experiment
3. Collect and formal data
4. Formulate and fit model
5. Check then validate model
6. Interpret and communicate results

1.4.3 Application of PK modelling for rivaroxaban

The pharmacokinetics of rivaroxaban describes the time course of its concentration in vivo. Studies using high performance liquid chromatography-tandem mass spectrometry to measure rivaroxaban concentration, have been used to calculate the pharmacokinetics of rivaroxaban. (Kubitza et al., 2008; Kubitza et al., 2013) PPK plays a crucial role in developing dosing strategies for drug development and providing detailed information for a target

patient group. The specific covariate impacting on the drug in question can then be identified. The advantage of PPK method is that sparse data collection can be used to calculate PK parameters for the drug in question.

The pharmacodynamics of rivaroxaban, which illustrates the relationship between rivaroxaban dose and effect, is less comprehensively understood, due to both the complexity of the coagulation system in vivo and the limitations of the current laboratory tools for measuring this. Post-marketing research is essential to enable a full understanding of the effectiveness of rivaroxaban, its potential for adverse effects and how quickly and long-lasting the beneficial and adverse effects may be.

Inferences on how the pharmacokinetics (PK) of a drug determine its pharmacodynamics (PD), as measured in individuals, can be made using PK-PD population modelling. Pharmacokinetic and pharmacodynamic (PK-PD) modelling for rivaroxaban characterise the PK parameters of rivaroxaban and links them to its PD activity in a conceptual framework model. PK-PD population modelling is the application of this PK-PD model to a general population for describing data, derived from more than one individual, by borrowing information between individuals to fill in gaps in the PK-PD profiles of each individual.

Crucially, rivaroxaban is prescribed as a fixed dose for all patients, with a few exceptions. It is accepted that every individual has a specific response to any drug, which is characterised by predictable and unpredictable PK outcomes. In order to maximise an individual's response to a drug, the PK profiling must be described as comprehensively as possible, identifying as many covariates and unpredictable inter-individual variables as possible, termed co-variants. A set of statistical techniques is applied, enabling understanding of the typical response in a population and the variability in that response which arises from various factors. PK population modelling is an important tool to integrate data and knowledge, enabling quantification and understanding of the inter-patient variability of rivaroxaban exposure and response. The individual data contributes to the identification of trends and the model links the PK parameters to the drug outcome. (Mould & Upton, 2012)

1.4.2 Application of population pharmacokinetic (PPK) modelling for obesity and anticoagulation medication

There is a common risk of under dosing obese patients and over dosing under-weight patients when prescribing weight-adjusted LMWH. (Patel et al., 2011; Green et al, 2003) Green and colleagues developed a dosing strategy for enoxaparin dependent on weight, using a population pharmacokinetic (PPK) modelling approach using non-linear mixed effect model (NONMEN). (Green, 2003) Ninety-six patients taking enoxaparin were selected to

ensure that equal numbers were sub-divided evenly into three BMI weight categories: BMI < 24.9 kg/m^2; BMI 25 – 29.9 kg/m^2; and BMI > 30 kg/m^2. Green and colleagues performed PPK modelling and demonstrated that a two-compartment model with first order absorption best fitted the data. They also showed that clearance most closely depended on lean body weight and that the volume of distribution was dependent on weight. (Green, 2003). The outcomes indicated that patients over 90 kg (and over 50 years old) or over 120 kg (and under 50 years old) needed a different dosing strategy to normally weighted patients, in order to minimise bruising and ensure a consistent concentration-time profile. (Green, 2003)

Although population models for rivaroxaban already exist (Mueck et al., 2007; Mueck et al., 2011; Mueck et al., 2013; Mueck et al., 2014), none have specifically investigated body weight as a covariate on rivaroxaban clearance to a satisfactory level. It is an important analysis to make, since many patients with acute VTE are obese, yet the large clinical studies upon which our knowledge of rivaroxaban is based, exclude obese patients, this data is sparse. Additionally, the studies to date have shown a trend for patients at the extremes of under-weight to have higher rivaroxaban concentrations at a given time after a given fixed dose.

In the rivaroxaban PK modelling study by Mueck and colleagues, 2007, the body weight, expressed as body surface area, affected rivaroxaban volume of distribution (Vd), causing a Vd decrease of 6.4% per 0.1 m^2 below 1.84 m^2 body surface area. At a dose of 10 mg od or 5 mg bd, rivaroxaban concentration was affected by extremes of body weight, but the average of these effects fell within the variability of the population, thus, were not reported as being significant since they would not cause a difference in rivaroxaban exposure. (Mueck et al., 2007) Another study found that body surface area (and consequently, weight) affected the volume of distribution, thus, the pharmacodynamic effects of rivaroxaban. (Kubitza et al., 2007) A similar study has been carried out with rivaroxaban dose 20 mg od for patients with AF and LBW was found to correlate with rivaroxaban Vd. (Girgis IG., et al., 2014) The population PK method could, potentially, be used to understand if obese patients need dose adjustment, by using data from a real-world population.

1.5 COAGULATION ASSAYS TO ASSESS RIVAROXABAN PHARMACODYNAMICS

1.5.1 BACKGROUND

The clotting cascade occurs on multiple phospholipid surfaces with many enzymatic reactions occurring sequentially and simultaneously and each one producing a product which activates a further proteolytic reaction, ultimately resulting in fibrin clot formation. Rivaroxaban inhibits factor X in its free, clot bound and pro-thrombinase bound forms. Rivaroxaban, thus inhibits the clotting cascade at the initiation and propagation stages.

The benefit of rivaroxaban is that it has relatively predictable pharmacokinetics which are closely correlated with the pharmacodynamic effects, thus does not require routine laboratory monitoring. However, the availability of a reliable and readily accessible standardised laboratory assay for measurement and monitoring of the effect/response to FXa inhibitors is important in certain clinical situations such as, overdose, acute bleeding, need for urgent surgery, or patient non-adherence. Ideally, the results from this assay should be available within 30-60mins of venepuncture. Furthermore, for those at the extremes of weight, these assays would provide a useful measure of whether patients are being under or over-anticoagulated, and therefore have the potential to be suitable laboratory markers for the direct Xa inhibitors when questions regarding dosing for those at the extremes of weight arise. (Garcia et al., 2013; Samama et al., 2014)

Standard coagulation laboratory tests provide rapid results targeted at specific areas of haemostasis using non-physiological triggers in a plasma system. However, research has shown that the same coagulation factor deficiency or thrombophilic defect results in very different phenotypic bleeding or thrombotic presentations. The impact of genetics and the environment on the coagulation system is profound and, to date, there is still no one valid test to accurately reflect the phenotypic state of the overall haemostatic thrombotic system. The current standard coagulation tests are of limited use because they do not indicate the haemostatic potential in an individual and many have been developed for a specific purpose.

1.5.2 PROTHROMBIN TIME (PT)

Prothrombin time (PT) is a commonly used assay in the clinical setting. It is the time for citrated (thus calcium free) plasma to coagulate after the addition of calcium and thromboplastin. PT is the only established, standardised assay, which has been shown to directly, linearly correlate with rivaroxaban concentration, and it is sensitive at high concentrations, therefore is useful in situations of haemorrhage or over dose. (Tripodi, 2013)

A recent study developed a computer model for blood coagulation, incorporating the intrinsic and extrinsic coagulation pathways and blood flow to evaluate the efficacy and safety of rivaroxaban using known kinetic constants and various concentrations of tissue factor in PT and APTT assays. Rivaroxaban effects on PT were more pronounced at lower TF concentrations. (Burghaus et al., 2011)

However, there is no standardisation between laboratories or across different reagents for PT, and there is a large variability in the sensitivities of thromboplastin reagents, resulting in large inter-laboratory variability of PT in monitoring rivaroxaban concentration. Additionally, at low concentrations, rivaroxaban has a relatively weak effect on PT, thus its effect may not be detected in patients on the prophylactic orthopaedic dose. (van Veen et al., 2013)

The general limitations of PT as a measure for rivaroxaban activity are, firstly, that PT is triggered when only 5% of all physiologically relevant thrombin is formed, and 50-90% decrease in the concentration of functional prothrombin is necessary to prolong PT, therefore, it is not a reflection of the activity of the whole coagulation system. (Molenaar et al., 2012) Secondly, PT does not predict clinical bleeding or clotting phenotype. (Lindhoff-Last et al., 2010) finally, the relationship between PT and rivaroxaban concentration is dependent on the reagent used, thus, the results are not comparable across laboratories.

1.5.3 Activated Partial Thromboplastin Time

Activated partial thromboplastin time (APTT) is reflective of the activity and quantity of coagulation factors II, V, VIII, XII and fibrinogen. APTT is the measured time from activation by silica or ellagic acid, to an initial clot formation. However, the prolongation of APTT is first evident when an individual coagulation factor is reduced by 70-80% (Lindhoff-Last et al., 2010) and when very little of the overall thrombin has been formed. (Rand et al., 1996) Additionally, APTT does not reflect or predict the overall bleeding or clotting potential of an individual.

Heparin affects APTT in a linear, concentration dependent manner. Rivaroxaban has been shown to prolong APTT, even at low concentrations, in a curvilinear concentration-response relationship but its effect varies depending on the reagent used and this correlation has not been consistently replicated across studies. (Samama et al., 2010; Hillarp et al., 2011) Rivaroxaban has less of an effect on APTT than on PT. (Lindhoff-Last et al., 2010)

1.5.4 D-Dimer and clauss fibrinogen

D-dimers are fragments released from factor XIII cross linking fibres within a fibrin clot, following plasmin lysis. High D-dimer reflects a high rate of fibrin degradation, thus, a high rate of thrombin formation. A normal D-

dimer reflects a low risk of VTE, and, together with a low clinical probability score, can reliably exclude the possible diagnosis of DVT. (Wells & Anderson, 2013) However, due to low specificity, a high D-dimer cannot identify people at high risk of thrombosis. (Nice, 2012)

Clauss fibrinogen measures the free fibrinogen concentration in plasma. At a given time, this is not dynamic and remains constant despite fluctuations in clot formation potential. (Khor & Van Cott, 2010)

1.5.5 THROMBIN GENERATION AND THE GLOBAL COAGULATION ASSAYS

1.5.5.1 BACKGROUND

The overall thrombin generation capacity and its enzymatic activity determine the coagulability of blood. Measurements of thrombin generation and thrombin enzymatic work potential provide a method for quantifying the composite effect of the multiple factors that determine coagulation capacity and influence of the environment on these factors. Thrombin generation has been shown to be a sensitive surrogate variable for bleeding or thrombotic tendency. (Baglin, 2005; Mann, 2003) This is measured by global coagulation assays, such as Calibrated Automated Thrombogram (CAT) and Rotational Thromboelastography (ROTEM). (Funk, 2012)

1.5.5.2 CALIBRATED AUTOMATED THROMBOGRAM (CAT)

1.5.5.2.1 THE MECHANISM OF THE CAT

Hemker and colleagues have developed a method to measure thrombin generation which was reproducible and accurate. (Hemker et al., 2003) The thrombin generation assay measures the ability of plasma to generate thrombin following in-vitro activation of coagulation with tissue factor. A thrombin generation curve is produced which reflects both the initiation and propagation phase of coagulation, as well as the anti-coagulant and fibrinolytic phases. (Hemker et al., 2000) CAT measures thrombin generation in whole or platelet poor plasma. (Bloemen et al., 2013)

The CAT test is performed in triplicate on the sample and on the calibrator, using a microtitre plate. In the measurement wells, tissue factor and phospholipid vesicles are added to initiate coagulation, then a combination of calcium chloride and fluorogenic substrate is added to the sample. A known amount of substrate converting activity is added to the plasma in the wells and a low-affinity fluorogenic substrate for thrombin is added. Thrombin causes splitting of the substrate, thus, the thrombin activity in the clotting plasma is continuously monitored via the colour change. The developing fluorescence is recorded by a fluorometer and the fluorescence

output values are converted into thrombin generation curves by an integrated software, comparing the fluorogenic activity to a constant known thrombin activity in a simultaneously run non-clotting sample.

The calibration wells contain the thrombin calibrator, which consists of thrombin bound to alpha2-macroglobulin, which cannot be split by plasma protease inhibitors, thus, these wells do not clot. The calibration wells have a standard known amount of substrate converting activity, thus, the fluorogenic substrate is converted at a constant rate by the added thrombin calibrator. (Hemker et al., 2000; Hemker et al., 2003; Hemker, Raed, 2004)

The parameters of the thrombin generation curve are described in terms of lag time, peak height, area under the curve, time to peak and start tail (figure 3).

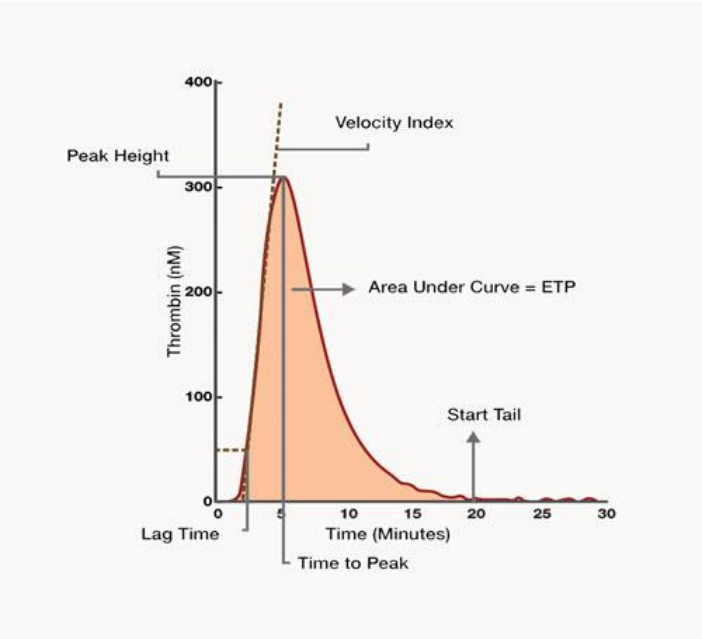

FIGURE 4 AUTOMATED CALIBRATED THROMBOGRAPHY CURVE

The lag time is the time needed for the thrombin concentration to reach one sixth of the peak thrombin concentration and the end of the lag period represents the thrombin burst. The area under the curve represents the endogenous thrombin potential (ETP) and this, together with the peak thrombin concentration signify the thrombotic potential of that individual.

1.5.5.2.2 VARIABILITIES WITHIN THE CAT ASSAY

Sample preparation is important with CAT and two types of samples can be used: platelet poor or platelet rich plamsa (PPP and PPR, respectively). This study used platelet poor plasma for analysis with CAT because the plasma can be frozen and run in batches, whereas the platelet rich plasma must be analysed as a fresh sample,

within one hour of collection. However, PPP is dependent on the phospholipid concentration used. (Hemker et al., 2003) Low or high tissue factor concentration can be used, the former is associated with greater intra- and inter-assay variability and more influenced by contact activation. (Luddington & Baglin, 2004) If the pre-analytical conditions of CAT are not standardised they have large, significant influence on the data, resulting in unacceptable inter-laboratory and inter-individual variations. (Loeffen et al., 2012; Bloemen et al., 2013) The pre-analytical variables include: concentration of tissue factor used; (Gerotziafas et al., 2005; Dargaud & Yesim, 2012a); temperature during storage and operation and the length of time during which the samples are pre-heated (De Smedt & Hemker, 2011); collection syringe and vessels; and the time between blood sampling and CAT analysis. Most studies have shown a consistent, significant difference in the results of blood collected using plastic versus siliconized glass and using a syringe versus a butterfly needle. (Loeffen et al., 2012)

Contact activation is difficult to control for, and is the predominant variable causing imprecision, particularly when there is a long lag time. Contact activation can be minimised, but not eliminated, by free flowing techniques and more so, by the addition of contact factor inhibitor (CTI). (Luddington & Baglin, 2004) A study using CTI at various concentrations has shown that CTI reduces the effect of thrombin generation due to contact factor activation and, at a final concentration of 18.3 microgram/mL in whole blood, this effect is completely inhibited. (Luddington & Baglin, 2004) However, this study was in normal healthy individuals and in DVT patients who had completed their anticoagulation therapy. Other studies have been inconclusive and there is no consensus regarding the routine use of CTI.

The variation of assay conditions and the inconsistency of reagents used between laboratories results in intra- and inter-laboratory variations which are too large to be able to create a reliable reference range or valid inter-laboratory comparisons, thus, CAT is not used in clinical situations.

1.5.5.2.3 CAT RESULTS AND THROMBOSIS

Thrombin generation, as measured with CAT, has been evaluated as a potential tool to risk-stratify patients with a history of VTE, in order to help determine the likelihood of recurrent VTE. The CAT parameter, endogenous thrombin potential (ETP), has been shown to be 93% sensitive to the pro-thrombotic state associated with VTE and women using the oral contraceptive pill. (Dargaud et al., 2006) Besser and colleagues, suggested that patients with increased peak thrombin and endogenous thrombin potential have increased risk of recurrence. (Besser et al., 2008) Besser also showed a correlation between ETP and unprovoked recurrent DVT (Besser et al., 2008)

Hron and colleagues further demonstrated that peak thrombin generation can be used as a cut off marker to determine those at low risk of recurrence. (Hron et al., 2013) Further studies are needed to investigate the relationship between clinical phenotype, disease and CAT results. (Haas, Schutgens & Kluft, 2011)

1.5.5.3 ROTATIONAL THROMBOELASTOMETRY

Rotational Thromboelastometry (ROTEM) is another global coagulation assay which rapidly assesses the dynamics of coagulation and quantitatively measures clot formation. It has a point-of-care application, delivering a representation of the overall platelet function and activity of the coagulation proteases and inhibitors, within 30min. ROTEM consists of four stationary chambers with disposable cuvettes in which whole blood is placed and different reagents added. The software gives a graphic representation of clot formation and lysis, via detection of the increasing impedance of a pin rotating in whole blood as a clot forms. (Luddington, 2005) ROTEM detects changes in all the phases of coagulation and fibrinolysis and measures the firmness of the clot. ROTEM is highly dependent on platelet function, fibrinogen content and integrity, fibrin polymerisation, cross linking and fibrinolysis, as well as thrombin generation and activity. (Franz, 2009)

Clotting time (CT) is the time from the start, when blood is mixed with the activator reagent until the waveform (representing the clot formation) reaches 2 mm above baseline. Clot formation time (CFT) is the time from 2 mm above baseline to 20 mm above baseline (clot of firmness of 20 mm). Maximum clot firmness (MCF) is the maximum stabilisation of the clot by polymerised fibrin, thrombocytes and FXIII, as is represented by the maximum amplitude of the waveform. Maximum lysis at specific time points after MCF is the reduction of clot firmness. The alpha angle is the tangent at 2 mm amplitude. (figure 4)

Recent reports have discovered inconsistencies in ROTEM results dependent on sample stability, patient age (Sankarankutty et al., 2012) and gender (Gorton et al., 2000). Additionally, assays have not been standardised between centres.

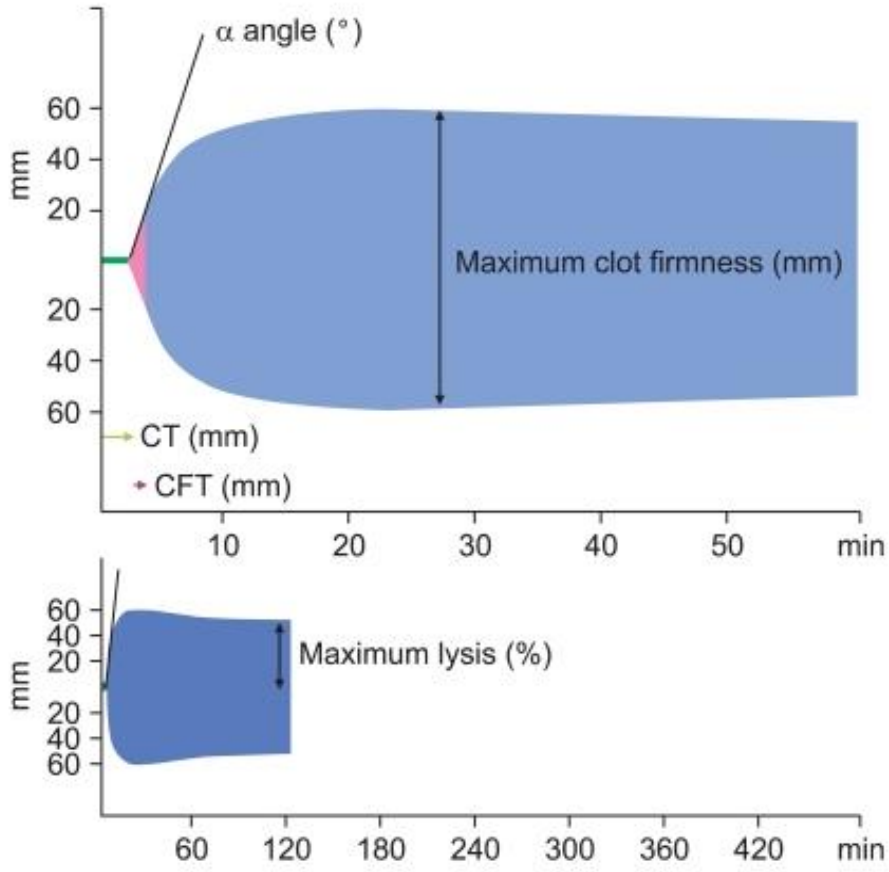

FIGURE 5 ROTEM TRACE AND PARAMETERS, FOR A HEALTHY VOLUNTEER SAMPLE, RUNNING FOR 2 HOURS

1.5.6 THROMBIN ANTI-THROMBIN COMPLEX (TAT)

The enzyme-linked immunosorbent assay, ELISA, measures thrombin-anti-thrombin (TAT) complex concentrations in plasma. TAT complexes are formed following the neutralisation of thrombin by anti-thrombin and they are a surrogate marker for thrombin generation. (Mann, 2003; Brummel-Ziedins, Pouliot, & Mann, 2004)

A large research study showed that there is minimal value in TAT as a diagnostic tool for VTE. (Speiser, W. 1990) However, it has been shown to be useful as a marker for a hypercoagulable state in patients with certain conditions, such as: AF (de Boer, Karin 1989; Wu et al, 2015); DIC (Teitel, Bauer & Lau, 1982); pregnancy and hypertension (de Boer & Karin, 1989); DVT (Bouman et al., 2014); and post thrombotic syndrome (Bouman et al., 2014). There has been a computer generated model of whole blood thrombin potential, using TAT, which is reliable and replicable for individuals, but requires complex software and cannot compare results between individuals. (Brummel-Ziedins et al., 2004)

Additionally, TAT is more sensitive to the effects of 10 mg daily rivaroxaban than 40 mg daily enoxaparin, for prophylaxis post-orthopaedic surgery (Oswald et al., 2014) and, in this context, has been shown to not be affected by warfarin 1mg daily or aspirin 325 mg daily. (Bern et al., 2015)

Green et al 2010, found a statistically significant increase in TAT levels in patients from before to during orthopaedic surgery. There was a significant decrease in TAT levels from peri-operative to post-operative levels only for those patients who took rivaroxaban 10 mg, 6-8h post-operation, and not for those patients who had dalteparin 2500 U or 5000 U, (depending on body the weight) 6-8h post-operation. (Green et al., 2010)

1.6 PATIENT ADHERENCE TO RIVAROXABAN

Patient non-adherence to prescribed medication is a significant contributing factor to medication failure. (Andersson Sundell & Jönsson, 2016) Patients' beliefs about medicines are complex and diverse and are often grouped into two categories: perceptions of necessity and concerns about negative effects. Adequate adherence to rivaroxaban is especially important since it has a short half-life and patients will have less frequent healthcare follow-up and monitoring. A subgroup analysis of the Einstein trial data used an anticoagulant-specific questionnaire (ACTS) and a generic questionnaire (TSQM II) to assess patient satisfaction, post VTE, while taking rivaroxaban compared to enoxaparin/warfarin. (Prins et al., 2015) Overall, the rivaroxaban treated patients reported greater satisfaction in the 'burdens' sections of ACTS and in all subscales of TSQM II, especially the 'convenience' and 'global satisfaction' subscales. (Prins et al., 2015) Patients in the >40 to <65 year-old and 65-75 year-old sub-groups showed better satisfaction in the ACTS 'burdens' subset while taking rivaroxaban compared to the traditional anticoagulants. (Prins et al., 2015)

Research has shown that medication non-adherence is a result of rational decisions by the patient, thus, understanding these cognitive processes will enable more effective prescribing. Clinical experience with rivaroxaban is limited, therefore patients cannot access a plethora of published literature and support information to aid them with their views and beliefs regarding its benefit. As part of the wider research study, the patients' attitudes and beliefs about medication in general and specifically rivaroxaban has been investigated using a belief about medication questionnaire (BMQ). The questionnaire is based on the BMQ developed by Horne et al, (Horne & Weinman, 1999) and has been used in the past to investigate enoxaparin. (Patel et al., 2012) BMQ consists of two sections. Section one contains four questions relating to the nature of medicines and their general harm and four questions relating to the views about how medicines are used by doctors and

their general overuse. The second section contains five questions pertaining to the necessity of taking rivaroxaban and five questions pertaining to concerns regarding rivaroxaban. In this way, the BMQ instrument provides an opportunity to explore this important issue.

1.7 HYPOTHESIS

The hypothesis of this research project is that patients of extreme low body weight require lower doses of rivaroxaban and obese patients require higher doses of rivaroxaban than patients within the normal range of body weight (all determined by either actual body weight, lean body weight or BMI) to achieve the same rivaroxaban concentration when following the standard 10 mg once a day, or 20 mg once or twice a day dosing regimen. The differences in the pharmacokinetics of rivaroxaban between patients of differing weights is investigated in terms of the rivaroxaban clearance and volume of distribution.

1.8 AIMS

This study was established to determine if rivaroxaban should be used as it is currently recommended, as a fixed dose without the need for routine coagulation monitoring, if body weight affects the pharmacodynamics of rivaroxaban to an extent which renders underweight patients over anticoagulated and overweight patients under anticoagulated. This study addresses the above question in two sections. Firstly, following the assessment of several coagulation tests to determine which correlate with rivaroxaban concentration, dosing and timing, the influence of body weight, measured as actual body weight, lean body weight and BMI, on the pharmacodynamics of rivaroxaban, in conjunction with other covariates such as age, gender, ethnicity and indication for anticoagulation. Secondly, pharmacokinetic (PK) modelling for rivaroxaban was performed to evaluate the influence of body weight on rivaroxaban concentration and dosing for patients being treated for VTE or having prophylaxis for VTE post elective total hip replacement (THR) or total knee replacement (TKR) surgery. As an adjunct, this study also evaluated the level of patient adherence to rivaroxaban using a standardised questionnaire.

CHAPTER 2. MATERIALS AND METHODS

The purpose of chapter 2 is to describe the general methodology and specific laboratory methods used in this study, including the rational and limitations of each investigation.

2.1 STUDY DESIGN AND PARTICIPANTS

The steps involved in this study were:

1. Literature review of previous work
2. Optimal design work to inform study design and power
3. Ethics and trust research and development approval to conduct the study at King's College Hospital London. This study was approved by the Brent Research Ethics Committee (REC reference 12/LO/1951) and local Research and Development department at King's College Hospital NHS Foundation Trust. (Appendix)
4. Recruit eligible patients into the study, collect samples then perform laboratory investigations on the fresh samples required and freeze certain other samples
5. Thaw and perform thrombin generation testing (without and with the addition of CTI) and thrombin-anti-thrombin investigations
6. Send off light-protected samples to collaborating laboratory for PIVKA sampling
7. Evaluate data, perform statistical analysis and prepare data for modelling
8. Model data for PK analysis

2.1.1 STUDY SETTING

This study was carried out at the thrombosis centre at King's College Hospital, London. The patients who consented to be in the study were assessed and bled either in a private room in the DVT clinic, in the general haematology out-patient clinic room or on the orthopaedic ward.

2.1.2 RECRUITMENT OF PARTICIPANTS

Study participants for the VTE group were identified from patients referred to the outpatient DVT service or to the anticoagulation service upon hospital discharge. The identified patients were selected and only those meeting the inclusion criteria were contacted. Adult patients of 18 years and older were eligible to participate in the study if they met the following inclusion criteria: willing and likely to comply with the study; normal baseline coagulation; creatinine clearance of at least 30 ml/min, using the Cockcroft-Gault method of calculation; no

significant liver dysfunction; no documented allergy to rivaroxaban; and not taking certain other medications, namely clarithromycin, telithromycin, HIV protease inhibitors, ketaconazole, itraconazole, voriconazole and posaconazole. The eligible patients were taking rivaroxaban either for DVT treatment and secondary prevention, or for VTE primary prophylaxis post elective hip or knee replacement surgery.

Eligible patients were phoned and asked to participate, explaining the aims, methods and background to the study. Those who were willing to participate were explained the research study and provided with the patient information leaflet (PIL) to read. (Appendix 1)

Study participants for the orthopaedic group were identified soon after their operation on the ward and were given the patient information leaflet on the study on day 2-3 following their operation. At least 12h following this, usually on the subsequent hospital visit, or the following day for orthopaedic patients, the PIL was fully explained and if the patient agreed to participate in the study, they were recruited and if possible, a date was arranged for them to return for blood tests. If it was not possible to arrange a date at that time, the patient was telephoned at home a few days later to arrange a visit.

On the day of the patient's visit, following recruitment, full informed written consent was obtained.

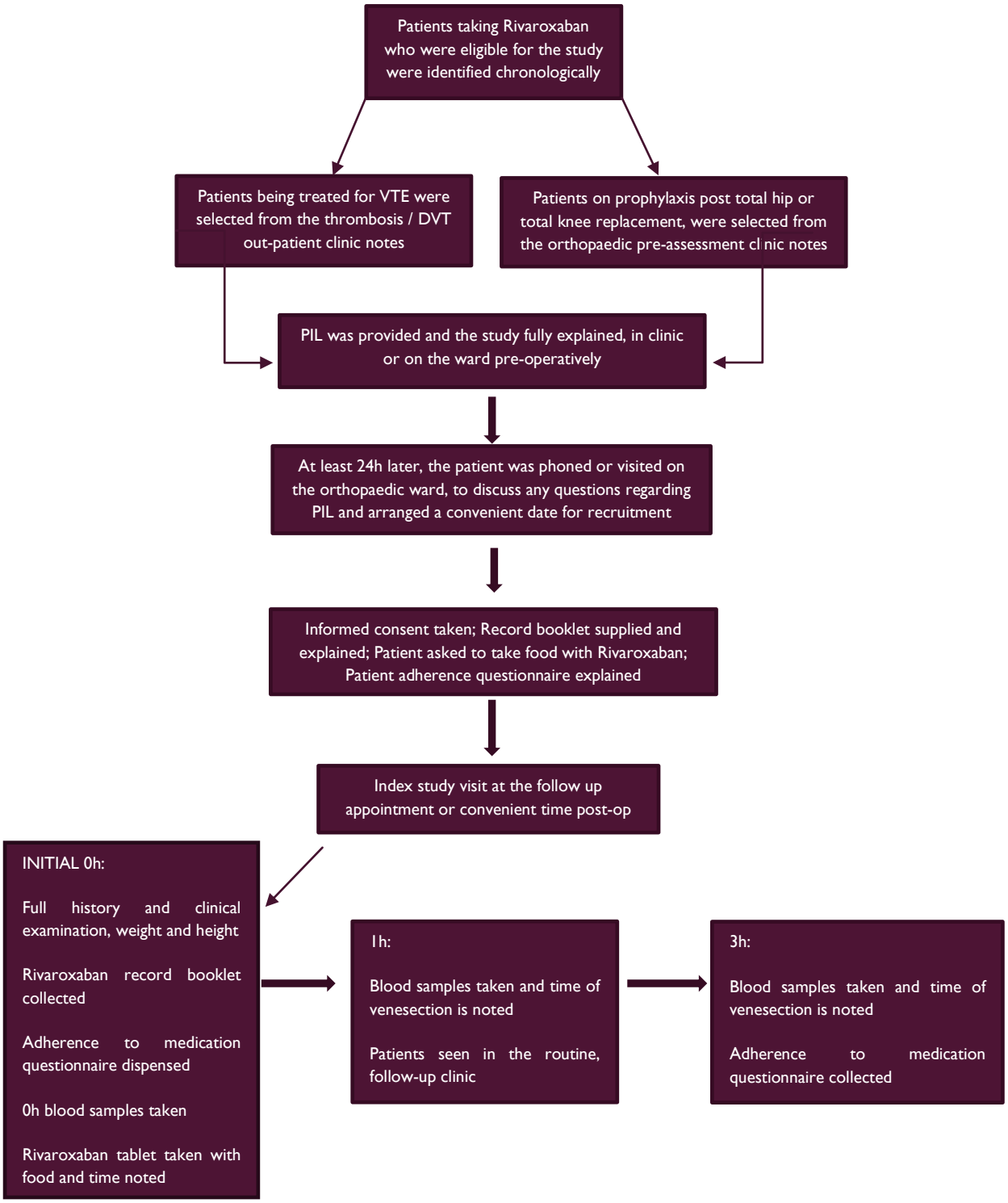

FIGURE 6 FLOW DIAGRAM OVERVIEW OF THE METHODOLOGY OF THIS STUDY

2.2 PATIENT ASSESSMENT

2.2.1 CLINICAL HISTORY AND EXAMINATION

Participants were seen at a convenient time for them, either during their regular thrombosis clinic review or on the same day as their out-patient physiotherapy session post orthopaedic operation. A full history and examination were done, including, a thrombotic/bleeding history assessment. All participants were questioned regarding provoking factors for VTE and a smoking, alcohol and past medical history were taken. The participants' height and weight were measured, in order to calculate the body mass index (BMI) and lean body weight (LBW). A note was made of the nature and quantity of the most recent food intake, in relation to the administration of rivaroxaban.

2.3 DRUG ADHERENCE QUESTIONNAIRE

The participants were asked to complete a Rivaroxaban adherence questionnaire and return it that same day or post it back in their own time. This consisted of two sections, the first pertaining to patient views on the general harm and overuse of medicines by prescribers and the second pertaining to beliefs about the specific necessity or specific concerns regarding Rivaroxaban. The questionnaire is scored on a five point likert scale. (Appendix 5)

2.4 BLOOD SAMPLES

2.4.1 SAMPLE COLLECTION

The patient attending VTE clinic was consulted separately for the study. Following an assessment, a venous sample was taken, the time was noted as 0 h. Immediately following this, rivaroxaban was taken with food. Venous blood samples were taken again at 1h and 3 h after the rivaroxaban dose, noting the exact time of each sample. Each sample was taken from the antecubital fossa vein, using a 23 gauge butterfly needle with 19 cm tube length (Terumo Europe NV, Leuven, Belgium), using a light tournequet. The first 10ml of blood from each venepuncture was used for full blood count, PIVKA and biochemistry evaluation, not for coagulation tests. The 2nd blood draw was performed at uniform speed and 18ml was collected into a 20 ml syringe. This was divided into six equal aliquots of 3 ml, five of which were transferred into separate CTAD vacutainer tubes with 0.109 M trisodium citrate (BD Diagnostics, Plymouth, UK) and one into a CTAD vacutainer with 0.109 M trisodium citrate (BD Diagnostics, Plymouth, UK) plus 1mg of 1ml/ml corn trypsin inhibitor (CTI) 1.45 μM (final

concentration in whole blood) in preparation for the calibrated automated thrombogram, as per the current recommendation of the official communication of the subcommittee on the control of anticoagulation of the ISTH. (Luddington & Baglin 2004)

2.4.2 Protein Induced by Vitamin K Absence II (PIVKA 11)

Two-three millilitres of blood were collected into a Z Serum Sep Clot Activator tube and placed in a Hettich 46R Rotina Centrifuge, (Tuttlingen, Germany) at 3040 g for seven minutes at room temperature. The top three-quarter supernatant was decanted into a plastic tube wrapped in foil to prevent light exposure and frozen at -40 °C until the time of analysis. All samples were clearly labelled and couriered in an enclosed, dark freezer box to St Thomas' Hospital to be analysed for PIVKA II activity within 4 h of receipt of the box.

2.4.3 Standard laboratory coagulation tests

2.4.3.1 Prothrombin Time

The sample was centrifuged in a Hettich 46R Rotina Centrifuge, (Tuttlingen, Germany) at 3040 g for seven minutes at room temperature and the top three-quarter supernatant was decanted and stored for a maximum of 8 h. The samples were analysed by the Pathology Department at King's College Hospital. The two reagents used were Neoplastine® Plus, lyophilised thromboplastin from rabbit cerebral tissues, and a calcium containing solvent. 50 uL of plasma was incubated at 37°C for 240 seconds. 100ul of Neoplastine-® was added and the time to clot formation was measured.

2.4.3.2 Activated partial Thromboplastin Time

The sample was centrifuged at 3040 g for seven minutes at room temperature and the top three-quarter supernatant was decanted and stored for a maximum of 4 h. The samples were analysed by the pathology department at King's College Hospital. The two reagents used were Cephascreen-®, which contains cephalin prepared from rabbit cerebral tissues and a polyphenolic activator in a buffered medium, and calcium chloride 0.025 M. 50 ul of Cephascreen-® was added to 50 ul of plasma and incubated at 37°C for 240 seconds. 50 ul calcium chloride 0.025 M was added and the time to clot formation was measured.

2.4.3.3 D-Dimer assay

The immune-turbidometric method is based on a change in turbidity, which results in a change in absorbance, of a micro particle suspension measured by photometry. The sample was centrifuged at 3040 g for seven minutes at room temperature and the top three-quarter supernatant was decanted and stored for a maximum of 8 h.

The samples were analysed by the pathology department at King's College Hospital. The two reagents used were a Tris buffer and STA- ® Owren-Koller and STA-® Liatest-® Control. These reagents were mixed gently at room temperature to create a suspension of microlatex particles coated by covalent bonding with two different mouse monoclonal anti-human D-dimer antibodies. 25ul plasma was diluted with 25ul STA-® Owren-Koller reagent and 100 ul D-Di buffer and they were incubated together at 37°C for 240 seconds. 150 ul STA-® Liatest reagent was added and the reaction was monitored with an automated conversion of absorbance to D-dimer. The D-dimer level of the plasma was displayed on the analyser screen.

2.4.3.4 Clauss Fibrinogen assay

The sample was centrifuged at 3040 g for seven minutes at room temperature and the top three-quarter supernatant was decanted and stored for a maximum of 8 h. The samples were analysed by the pathology department at King's College Hospital. The STA-® Fibrinogen reagent, 80 NIH units/ml of lyophilised titrated human calcium thrombin with a heparin inhibitor, was reconstituted with 5 ml of distilled water and left for 30 min at room temperature. The patient plasma was loaded onto the instrument where the dilutions were automatically prepared in STA-® Owren-Koller buffer. The time to clot was measured and converted to a fibrinogen level, which was displayed on the analyser screen.

2.4.4 Rivaroxaban concentration, Factor Xa activity

The sample was centrifuged at 3040 g for a double spin at seven min each spin, at room temperature and the top three-quarter supernatant was decanted frozen in a plastic tube at -40°C, within one hour of venepuncture. The samples were assayed in batches by the pathology department at King's College Hospital, at least every fortnight. One reagent contained 4.5 µMol of a chromogenic substrate, CBS 02.44, in 4 ml vial of MAPA-Gly-Arg-pNA, HCl. The second reagent contained 1.0 iU bovine Factor Xa per 4 ml vial. Calibration was performed with STA-Rivaroxaban Calibrator and STA-Owren-Koller buffer. The patient plasma was tested undiluted and loaded into the instrument, resulting in a reading on the analyser screen in ng/ml.

2.4.5 Global coagulation tests

2.4.5.1 Calibrated Automated Thrombography

The Thrombinoscope ™ assay (Thrombinoscope BV, maastrichts, Netherlands) was used to measure thrombin generation, as described by Hemker et al, 2003. Fresh platelet poor plasma was produced by double centrifugation at 4750 g for 10 min on both occasions and decanting the top three-quarter supernatant after

each centrifugation, initially into a polypropylene tube then finally into a plastic tube suitable for freezing at -40˚C. The PPP was frozen at -40˚C within 1h of venepuncture. PPP was thawed in 37˚C waterbath immediately prior to analysis, within one month of collection.

The reagents used were PPP-Reagent Low and FluCa-kit, together which contain 1 pM tissue factor and 4 µM phospholipids. FluCa-kit consists of Fluo-Buffer, containing calcium chloride, and Fluo-Substrate, containing a fluorogenic substrate.

20 ul of the PPP-Reagent Low was added to test wells 1-3 of the immulon 96-well micro titre plate (Thermo Labsystems, Franklin, USA). The calibration wells were run in triplicate in which 20 ul of thrombin calibrator was placed into wells 4-6 of the same row. 80 ul of thawed patient PPP was added to each well of that patient nominated row. The PPP with CTI were treated as separate patient samples and run in separate rows to the PPP without CTI. The plate was placed inside the CAT and incubated for 10 minutes. The fluo-buffer was warmed and 40 ul fluo-substrate was added and placed on the vortex. 20 ul of the mixed fluo-reagent was dispensed automatically by the fluorometer, into each well, which automatically triggered coagulation.

Fluoroscan Ascent measured the fluorescence over time. The filters in place were 390 nm excitation and 460 nm emission. The Thrombinoscope BV software (version 3.0.0.30) converted the signal into nM thrombin generated over time and adjusted both for the filter and the thrombin bound to alpha 2 macroglobuin.

Controls using reconstituted PPP were run with each plate. Non-human control plasma, NHCP (Technoclone, Vienna, Austria), was used for both intra- and inter-assay variability assessments. NHCP was reconstituted as per manufacturer's instructions, pooled and frozen to -40C before use. Intra-assay variability was assessed using NHCP in one plate of 16 assays and each assay was run in triplicate. Inter-assay variability was assessed using pooled, frozen, human control plasma and NHCP, run in eight triplicate assays each.

2.4.5.2 Rotational Thromboelastometry

Rotational Thromboelastometry (ROTEM®) was performed by the principle researcher within 4 h of venepuncture. The citrated vacutainer containing whole blood together with the ROTEM reagents were placed onto the ROTEM plate and warmed for five minutes to 37˚C. The extrinsic coagulation system was measured using r-ex-TEM reagents, and the intrinsic coagulation system, using r-in-TEM reagents. R-ex-TEM contains an optimised concentration of tissue factor as an activator, phospholipids and heparin inhibitor. In-TEM contains partial thromboplastin phospholipid made of rabbit brain and ellagic acid as activators. Star-TEM is added to re-

calcify the reagents and they are mixed with the sample whole blood to commence the coagulation process. A pin is suspended within the cup, which is connected to an optical detector system. The pin is oscillated relative to the cup, which is stationary, and the transmitted impedance of the rotation is detected and a computerised trace is generated.

This study used the four chambers for two Extem and two Intem reagents, thus, two samples were run on each tray. Extem assess Factors VII, X, V, II, I, platelets and fibrinolysis. Intem measures Factors XII, XI, IX, VII, X, V, II, I, platelets and fibrinolysis.

The ROTEM® analyser continuously and automatically monitors the coagulation process, providing a graphical trace (figure 4) and individual values. Controls using ROTROL N, QCexN and QCinN were preformed once a week to calibrate the assay.

2.4.5.3 Thrombin-Antithrombin Assay

The sample was centrifuged at 3040 g for double spin at seven minutes each spin, at room temperature and frozen at -40˚C within one hour of venepuncture. The Thrombin-Antithrombin Complex (TAT) Human ELISA Kit (Abcam) was used and the materials were supplied by the manufacturer. The reagents and working standards were prepared according to the manufacturer's instructions, with various dilutions of the TAT standard prepared immediately before use. The TAT plasma samples were thawed in a water bath at 37˚C. Each sample was assayed in duplicate and each 96 well plate strip had 36 individual samples. The plate strip was annotated and a diagrammatic representation was labelled. 50 µl of TAT standard or plasma sample was added to each well and the bubbles carefully removed. The timer was started following the addition of the last sample, sealing tape was placed on the top of the wells and the plate was left to incubate at room temperature for 2 h. The wells were washed six times using a plate washer (ELX500 BioTek) and the excess water decanted. 50 µl of 1X Biotinylated TAT Antibody was added to each well and incubated at room temperature for 1h. The wells were washed and 50 µl of 1X SP conjugate was added to each well and incubated for 30 min. 50 µl of Chromogen substrate was added to each well and incubated for 20 minutes or until optimal blue density. 50 µl of Stop solution was added and the absorbance was read from a microplate reader at 450 nm immediately. The ASCENT software calculated a standard curve and intra- and inter-assay coefficients of variability for every assay.

2.5 DATA ANALYSIS AND STATISTICAL METHODS

2.5.1 DATA COLLATION AND ANALYSIS

The data was collected from the laboratory analysers and entered directly onto a master Excel spread sheet. The derived parameters, creatinine clearance, BMI and lean body weight were calculated from the raw data using the equations below.

Creatinine clearance was estimated using the Cockroft-Gault equations: (Cockcroft & Donald, 1976)

For males: $\frac{(140\text{-age}) \times \text{weight, kg} \times 1.23}{\text{creatinine, µmol/L}}$

For females: $\frac{(140\text{-age}) \times \text{weight, kg} \times 1.04}{\text{creatinine, µmol/L}}$

The Cockroft-Gault equations take into account weight and age and have been shown to have the least discordance rates as compared to the gold standard of a timed 24 h urine collection method for calculating the actual creatinine clearance (CrCL). (Fernandez et al, 2016) Glomeular filtration ate (GFR) is the flow rate of filtered fluid through the kidney, and CrCL is the volume of plasma cleared of creatinine pe unit time. CrCL is the standard used for drug dosing and it is a useful measurement for approximating GFR. It is not feasible to collect urine over 24 h for every patient, in order to calculate the creatinine clearance. The estimated GFR from other methods, such as the Modification of diet in renal disease study (MDRD), does not take into account weight, is only validated for chronic kidney disease and it underestimates GFR in healthy subjects.

The value for weight which was used in this situation was lean body weight (LBW).

The lean body weight was calculated for each individual, using the formulae: (Han et al. 2007)

For males: $\frac{9270 \times \text{actual body weight}}{6680 + (216 \times \text{BMI})}$

For females: $\frac{9720 \times \text{actual body weight}}{8780 + (244 \times \text{BMI})}$

A master spread sheet for every relevant data point was created on excel. The demographic data was originally noted down on the patient record sheets at the time of the study investigation, then transferred to excel, creating numerical values for certain categorical data such as ethnicity and sex. The numerical continuous data were inputted directly into excel and for some of these data sets, categorical groups were created either according to known data groups, such as WHO BMI categories, or determined by the graphical representation of the histograms, such as time after dose.

Data was pasted from excel into PRISM with no alterations, for initial statistical analysis and graphical representation. The data from excel was organised into cases in IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA), firstly by defining the characteristics of the variables then entering the data from excel into the data view for each case, according to the strict regulations of IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA).

2.5.2 STATISTICAL ANALYSIS

The IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA) software was used to perform all statistical analyses.

The continuous variables, such as age, weight, height, creatinine, antiXa levels, were evaluated to determine their distribution, by measuring the skew and kurtosis values. Those continuous variables which were normally distributed were described using mean and standard deviations (SD), and those which were not normally distributed were described using median and inter-quartile range (IQR). Categorical data, such as ethnicity, sex, groups for indications for anticoagulation, dose of rivaroxaban, were described with percentages and frequency charts.

Comparisons of the means of two sets of values from one continuous variable, such as the difference between antiXa levels in men and women, was performed with the independent-samples T-test. The paired T-test was used to compare data sets from the same individual, thus, was employed to determine if there was a difference between adding CTI to PPP and not adding CTI to the same PPP CAT sample.

Two sets of normally distributed continuous data were compared using one-way ANOVA with post-hoc Tukey tests for comparisons between multiple data sets. ANOVA was also used to compare categorical contingencies with continuous outcomes, such as the differences between antiXa levels between the groups of patients taking rivaroxaban at various doses. One way ANOVA was deemed appropriate for these analyses because there was

a known effect of rivaroxaban on the coagulation system, which only causes decreased, not increased coagulation.

Multiple sets of non-parametric continuous data were compared using Krusal-Wallis test.

The relationships between two categorical data sets, both with only two categorical outcomes, such as gender (male/female) were analysed using Pearson's chi-square test. Those frequencies which were less than five, such as the results from the orthopaedic group of patients, were analysed with Fisher's Exact test rather than chi square. For analysis of several categorical variables, such as BMI category and indications for taking rivaroxaban, the results of the chi-square test were compared between the various categories.

Pearson's correlation test was used to ascertain the relationships between normally distributed continuous variables, such as antiXa results and creatinine clearance and Spearman's rho correlation coefficient was used for non-parametric variables, such as D-dimer results. The r value was used to understand the relationship between the two continuous variables, since it determines the direction and strength of correlation. R squared would not be as appropriate since the analyses are not determining the proportion of explained variance.

The Cronbach alpha constant was used to evaluate the reliability of the questionnaire items within a particular section or subsection, representing a common concept. Therefore, it indicates how accurately the questionnaire measures the variable of interest. IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA) was used to calculate the Cronbach's alpha using the case processing summary of SPSS and a score > 0.5 was deemed acceptable.

2.5.3 Non-Linear Mixed Effects Population Modelling, NONMEM

The aim of population pharmacokinetic and pharmacodynamic studies is to obtain accurate estimates of the desired parameters in the model being developed. Therefore, if the study design is suboptimal in terms of when the samples were drawn, then the final model developed may be inaccurate or imprecise. It was decided that the optimal anti-Xa sampling times for this study were just prior to the next dose of rivaroxaban (trough), one hour after and three hours after the dose of rivaroxaban.

The statistical methods used in the NONMEM analysis are described in detail in chapter 5.

CHAPTER 3. INFORMATION ON THE STUDY POPULATION

Chapter 3 describes this study population in terms of its demographic and baseline haematological and biochemical characteristics. Comparisons between this study population and previous populations of participants in the published rivaroxaban trials and literature reports, are described.

3.1 BACKGROUND AND LITERATURE REVIEW

3.1.1 THE DEMOGRAPHICS OF THE INDIVIDUALS ENROLLED INTO THE PUBLISHED RIVAROXABAN TRIALS

The principal clinical trials which led to the licensing of rivaroxaban were RECORD I, II and III and ENSTEIN-DVT, EINSTEIN-PE and EINSTEIN-ext. (refer to chapter 1.2.1). The trials were conducted in 39 countries at 336 sites. Exclusion criteria were: creatinine clearance <30 ml/min; clinically significant liver disease; active bleeding or high risk of bleeding; systolic blood pressure >180 mmHg; diastolic blood pressure >110 mmHg; pregnancy or breast feeding; use of P450 3A4 inhibitor or inducer; life expectancy < three months. The pooled demographic information from the EINSTEIN trials are shown in table 7. (Di Nisio et al., 2016)

TABLE 7 DEMOGRAPHIC CHARACTERISTICS OF THE POOLED EINSTEIN TRIALS, INVESTIGATING RIVAROXABAN VERSUS LOW MOLECULAR WEIGHT HEPARIN FOR VTE

Characteristic	**Subjects taking rivaroxaban 20 mg once a day, n=4150**
Age, median (range), y	58 (18-97)
Female, %	44.5
Weight, mean (SD), kg	82.5 (18.9)
BMI, mean (SD), kg/m^2	28.1 (5.7)
Creatinine clearance, % >80 ml/min 50-79 ml/min 30-49 ml/min <30 ml/min	 66.8 25 7.8 0.2
Body weight, % <50 kg 50-100kg >100 kg	 2 81 17
BMI, % <25 kg/m^2 25-30 kg/m^2 30-35 kg/m^2 >35 kg/m^2	 30 40 20 10

It is important to note that 2 % of subjects in these trials weighed less than 50 kg, of whom only 10 % were male. 10 % had a BMI greater than 35 kg/m^2. 40 % of those patients with BMI >35 kg/m^2 were male and 50 % of those with BMI <25 kg/m^2were male. The age distribution was evenly spread between the BMI categories. (Di Nisio et al., 2016)

Several phase I studies indicate that Cmax, volume of distribution and clearance of rivaroxaban remain consistent despite differences in ages.

3.2 Demographics of the study population, results

3.2.1 Characteristics of the study population

Recruitment into this study commenced June 1st 2013 and the last participant was recruited on May 16th 2014. During this time, 101 participants were recruited and 193 samples were obtained.

The baseline characteristics of the study participants are shown in table 8. The majority, 59, participants were receiving rivaroxaban as treatment for an initial DVT, 26 were being treated for a recurrent DVT, 12 were being treated with long term anticoagulation and had switched from warfarin or heparin to rivaroxaban and 4 were prescribed rivaroxaban for prophylaxis following an orthopaedic surgery. The distribution of dosing amongst the study population was weighted heavily towards treatment dose, with 58 taking 20 mg once a day and 38 taking 15 mg twice a day. Four patients were taking 10 mg once a day for elective post-orthopaedic prophylaxis and one participant was prescribed 15 mg once a day because of decreased renal function (table 8).

The proportion of orthopaedic patients compared to VTE treatment and prophylaxis was four compared to 97 because it was much more difficult to recruit from the orthopaedic population than the VTE population. The VTE patients were recruited from DVT clinic and had scheduled appointments at DVT clinic in the future, during which it was convenient for them to volunteer and participate in the study. The orthopaedic patients, however, were recruited at pre-admissions clinic or on the ward before their operation, during which there is little time to fully consent and recruit them. The orthopaedic patients did not routinely return to hospital following discharge, therefore it was inconvenient for them to attend for the sole purposes of the study, exacerbated by the fact that most of the patients were not fully mobile for weeks following their operation. The patients who agreed to participate often had routine hospital appointments to attend following discharge, during which time they could partake in the study. There were proportionally less orthopaedic patients than VTE treatment patients who managed to remain in the study for the full five hours, perhaps because they had arranged transport especially for the purpose of the study and had no other reason to remain in hospital. The VTE patients, however, partook in the study around their clinic appointments and various other ongoing investigations in hospital at the time of the study, thus, were able to remain in hospital for the full five hours.

The proportion of patients who supplied three antiXa samples, at the trough, one hour and three hours was 24 %, there were 44 % who supplied two anti-Xa samples at the trough and one hour, and there were 32 % who only managed to supply one sample at the trough time point (table 8).

Almost three quarters of the participants were Caucasian. The 'other' category of ethnicity consisted of three Greek/Cypriots and two Asians. 58 % of the participants were female (table 8).

Body weight, lean body weight, and BMI were normally distributed. The most prevalent BMI category was over weight (BMI 25-29.9 kg/m^2), only one participant was underweight (BMI 16-18.5 kg/m^2) and 27 were of normal body mass index (BMI 18.5-24.9 kg/m^2). Forty-one participants were classified as obese (BMI ≥30 kg/m^2), of whom six were morbidly obese (BMI ≥40 kg/m^2). The mean body weight of the orthopaedic patients was 75.1 kg (+/- 5.3), those with acute first episode VTE 84.8 kg (+/- 22.0) and patients with recurrent DVT was 97.9 kg (+/- 26.3). The mean body weights of the Caucasian and Black ethnic groups were similar, 89.8 kg (+/- 24.9) and 86.7 kg (+/- 16.0), respectively, but that of the 'other' group was significantly less, 68.1 kg (+/- 11.5) (table 8).

Eight patients had coronary artery disease (CAD), of which three also had diabetes mellitus (DM); three patients had inflammatory bowel disease (IBD); two had myeloproliferative disease (MPD); one had sickle cell anaemia (SSA); three had hypothyroidism; seven had cancer in the past or cancer in remission; and ten had some form of detected inherited thrombophilia (table 8).

TABLE 8 DEMOGRAPHIC CHARACTERISTICS OF THIS STUDY SAMPLE

Patient demographics		**N=101**
Age, years, mean (range)		52 (20-86)
Male/ female, %		42 / 58
Body weight, kg, mean (+/- SD)		88.0 (23.4)
Lean body weight, kg, mean (+/- SD)		57.0 (11.3)
BMI category, %:	16-18.49 kg/m^2	1
	18.5-24.9 kg/m^2	27
	25-29.9 kg/m^2	32
	30-34.9 kg/m^2	21
	35-39.9 kg/m^2	14
	≥40 kg/m^2	6
Indication for rivaroxaban, %:	Acute first event	58
	Acute recurrent event	26
	Switched from traditional anticoagulant	12
	Elective orthopaedic VTE prophylaxis	4
Dose of rivaroxaban, %:	20mg once a day	38
	15mg twice a day	57
	10mg once a day	4
	15mg once a day	1
Samples at each time point, %:	trough only	32
	1h & trough	44
	3h & 1h & trough	24
Ethnicity, %:	White	74
	Afro-Caribbean	21
	Other	5
Medical history (%):	CAD	8
	DM	3
	IBD	3
	Hypothyroidism	3
	Past history of cancer	7
	Inherited thrombophilia	10
Smoker / non-smoker, %		27 / 73

3.2.2 PATIENTS AT THE EXTREME OF BODY WEIGHTS

The study population had a wide range of body weights, with a normal distribution. There were two individuals who weighed less than 60 kg and 21 individuals above 100 kg (figure 6).

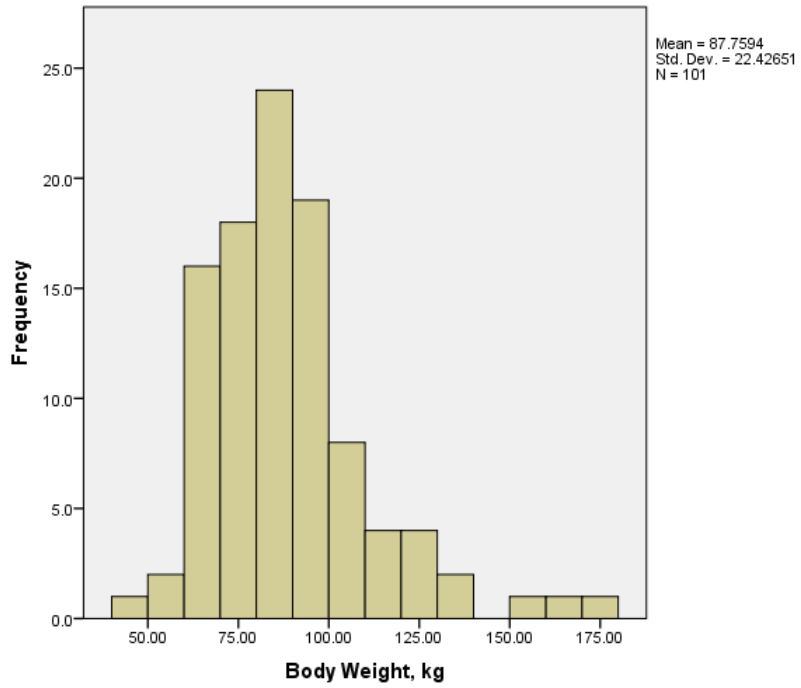

FIGURE 7 THE DISTRIBUTION AND FREQUENCY OF THE BODY WEIGHTS OF THE SAMPLE POPULATION

The two individuals weighing less than 60 kg were: a 44 kg, 84 year-old, Caucasian lady with a creatinine clearance of 30.4 ml/min. The 25 h trough antiXa was 59.9 ng/ml which rose to 237.6 ng/ml at 1h post 20 mg rivaroxaban. The second individual was a 51.1 kg, 78 year-old, Caucasian female with a creatinine clearance of 74.3 ml/min, who had a trough rivaroxaban concentration of <20 ng/ml at 27 h and a level of 134.2 ng/ml at 1.3 h post 20 mg.

There were nine individuals weighing more than 120 kg amongst our study population. Five were female, four were under 50 years old and five were 50-60 years old. Seven of these were Caucasian and two were Afro-Carribean. All nine individuals started rivaroxaban in the DVT clinic, having presented to Accident and Emergency with new symptoms of DVT and not taking anticoagulant medication at the time. Five patients were taking 15 mg bd rivaroxaban and four were taking 20 mg od at the time of sampling. Five of these patients had had a VTE in the past and four were presenting with their first VTE. Of the five females, three reported

menorrhagia, but there were no other side effects reported. Three patients had Factor V Leiden, one was heterozygous for PTG20120A mutation and one had raised Factor VIII.

3.3 Laboratory test results and their correlations with rivaroxaban concentration

3.3.1 General coagulation test results: Activated Partial Thromboplastin Time (APTT), D-dimer and Clauss fibrinogen

APTT was measured once during the investigation, simultaneously with the trough antiXa sample. APTT was normally distributed, mean 33.4 sec (+/- 5.75 sec). The Pearson correlation coefficient (r) for APTT with rivaroxaban concentration was 0.306, $p<0.0001$, (table 9), showing a significant, but not strong positive correlation between them.

D-dimer values were mostly obtained from the first sample, therefore at trough rivaroxaban levels. There was no consistency as to when the D-dimer value was measured in respect to timings of the VTE event or anticoagulation treatment schedule, however, every patient had been treated with rivaroxaban (and often with LMWH or warfarin previously) for at least one week. The distribution of D-dimer was slightly positively skewed, thus the non-parametric Kruskal-Wallis was used to evaluate the differences in the distribution of these parameters across the categories. The median D-dimer was 350 ng/ml (range 270-7970 ng/ml), (table 9).

Eight D-dimer levels were greater than 3000 ng/ml. Of these eight, three were orthopaedic patients (out of five orthopaedic patients) and four had recurrent DVT. Using the non-parametric Kruskal Wallis test to compare the mean D-dimers for the subgroups: indication for anticoagulation; BMI; and ethnic categories, revealed no significant difference within each subgroup ($p=0.378$, 0.177, 0.271, respectively). There was no significant correlation between D-dimer and rivaroxaban concentration, since the Spearman rank correlation coefficient p value was > 0.05 (table 9).

Clauss fibrinogen levels were taken at a similar time as the trough antiXa levels. Ninety-nine results were obtained because two samples were insufficient to run. Clauss fibrinogen was normally distributed and significantly correlated with rivaroxaban concentration, although with a low Pearson's correlation coefficient, thus a weak correlation, $r=0.155$, $p=0.032$, (table 9). There was no significant difference between the Clauss

fibrinogen results for patients in the various categories of BMI or ethnicity, but there were significant differences between the indication for anticoagulation groups (p=0.008).

TABLE 9 LABORATORY COAGULATION PARAMETERS MEASURED AND THEIR CORRELATIONS TO RIVAROXABAN CONCENTRATIONS

Laboratory parameter, n=100	**Mean (+/-SD) #** **Median (range) ***	**Pearson # or Spearman *** **correlation coefficient, r (p)**
APTT, sec	# 33.4 (5.8)	# 0.306 (<0.001)
D-dimer, ng/ml	* 350 (270-7970)	* 0.031 (0.7)
Clauss fibrinogen, g/L	# 3.47 (0.8)	# 0.155 (<0.001)

APTT, activated partial thromboplastin time

SD standard deviation

3.3.2 Proteins Induced by Vitamin K Absence (PIVKA)

The results for every sample sent for PIVKA, n=101 samples, was < 0.2 AU/ml (normal range is <0.2 AU/ml), thus, there was a negligible amount of PIVKA present in every sample, thus no patient had vitamin K deficiency, as estimated by the laboratory assay performed. This suggests an absence of effect on the rivaroxaban concentration.

3.3.3 Biochemistry and general haematology laboratory test results

One patient did not have biochemistry laboratory results available because the sample was insufficient for analysis because that patient was difficult to venesect. The estimated creatinine clearance (CrCL) was calculated from the serum creatinine concentration, age and weight, obtained on the day of investigation, correlated significantly with rivaroxaban concentration, r= -0.176, p=0.015 (table 10). Haemoglobin values were normally distributed for our study population and were taken at time 0 h of study sampling. Pearson correlation demonstrates a negative association, but non-significant correlation, between haemoglobin and rivaroxaban concentration, r=-0.169, p=0.09 (table 10).

TABLE 10 LABORATORY PARAMETERS MEASURED AND THEIR CORRELATIONS TO RIVAROXABAN CONCENTRATIONS

Laboratory parameter	Number of samples	Mean (+/-sd)	Pearson correlation coefficient, r (p)
CrCl,ml/min	100	76.7 (27.9)	-0.176 (<0.001)
Creatinine, µmol/L	100	82.5 (24.0)	0.098 (0.3)
Haemoglobin, g/L	101	136.2 (17.1)	-0.169 (0.1)
Platelet count, 10^9/L	101	266.4 (86.7)	0.047 (0.6)
White cell count, 10^9/L	101	6.9 (2.1)	0.074 (0.4)
Albumin, g/l	100	42.2 (3.3)	-0.93 (0.4)

3.4 DISCUSSION

3.4.1 COMPARISON OF THIS STUDY COHORT TO THOSE OF CLINICAL TRIALS COHORTS

The majority of participants in this study were being treated for DVT, either acute or long term, with only four patients prescribed rivaroxaban for primary prophylaxis post-orthopaedic operation. The population of patients enrolled in this study differed from those in the EINSTEIN trials in their weight distribution. This study had 21 % of patients weighing more than 100 kg compared to the EINSTEIN trials, where 17 % weighed >100 kg. 20 % of this study population had BMI >35 kg/m^2 compared to 10 % of the EINSTEIN trial population. However, many other characteristics were comparable, including the proportion of under-weight patients: 2 % in our population, and 2 % in the EINSTEIN trials weighed <50 kg; 28 % of our population and 30 % of EINSTEIN trial had BMI <25 kg/m^2. (Di Nisio et al., 2016)

The mean age of our study population was 52 years-old (range 20-86), which was similar to that of the EINSTEIN trials and the pooled RECORD 1-4 studies populations. (Kwong & Turpie, 2015) Our study population, EINSTEIN and RECORD trials had a similar proportion of female: 42 %, 45 % and 60 %, respectively. (Di Nisio et al., 2016; Kwong & Turpie, 2015) Pooled results from the phase III RECORD 1-4 studies, showed mean body weight 80 kg, mean BMI 29 and 79 % were Caucasian. (Kwong & Turpie, 2015) These demographics are similar to our patient population.

Our study consisted of 74 % Caucasian, which does not reflect the demographic spread within King's College Hospital. Perhaps the bias towards Caucasians was a result of attitudes and beliefs in medicines, which is further investigated in section seven of this thesis. There were 21 % African participants and five percent other (Asian and Cypriot) in this study population.

3.4.2 The influence of age, renal function, hepatic function and ethnicity on the pharmacokinetic properties of rivaroxaban, as reported in the literature

Data from phase II dose ranging studies investigating rivaroxaban 10 mg once daily or 5 mg twice daily for thromboprophylaxis after total hip replacement (Mueck et al., 2008) or 20 mg daily for acute DVT (Mueck et al., 2011) were collated into population pharmacokinetic analyses using non-linear mixed effects modelling (NONMEM), in order to develop a population pharmacokinetics model. They both showed an oral one-compartment, first-order absorption rate model to accurately describe the pharmacokinetics of rivaroxaban. Using this population pharmacokinetic (PPK) model to predict the behaviour of 10 mg once a day of rivaroxaban in healthy subjects of extreme body weight, age and renal impairment, the plasma concentration-time profiles fell within the 90 % confidence intervals for those parameters of the studied patient population, thus, were deemed to be consistent. (Mueck et al., 2007)

For the DVT population, there was a dose-proportional profile, affected to a moderate degree, by age and renal function and the influence of body weight was small. However, the average of these effects remained within the overall variability of the population. (Mueck et al., 2011)

The orthopaedic population has shown a moderate influence of age and renal function on clearance, and of body weight, albumin and haematocrit on volume of distribution. These alterations, on average, fell within the variability of the predicted population PK, suggesting that variations of these factors should not cause significant alterations in rivaroxaban exposure. (Mueck et al., 2008)

However, all studies have shown an influence, which on an individual case-by-case basis may be significant.

3.4.2.1 Age

Phase I studies in individuals aged >75 years showed an increase in rivaroxaban concentration, with 41 % higher area under the curve (AUC) values. However, phase III studies have subsequently revealed that these effects can

be attributed to reduced clearance of rivaroxaban in elderly individuals, thus no dose adjustment is recommended based solely on age. (Kubitza et al., 2013)

3.4.2.2 Renal and hepatic function

Pharmacokinetic studies with rivaroxaban have demonstrated a direct impact of renal function on the Cmax and elimination half-life of rivaroxaban, in both normally weighted and obese patients. In phase I studies, individuals with mild renal impairment (creatinine clearance 50-80 mL/min) had increased rivaroxaban concentrations by 1.4 fold, moderate renal impairment (CrCL 30-49 mL/min) had increased rivaroxaban concentration 1.5 fold, and severe renal impairment (CrCL 15-29 mL/min) had 1.6 fold increased rivaroxaban concentration, which correlated with a decrease in clearance from 2.4 L/h in healthy individuals to 0.5 L/h. (Kubitza et al., 2010b)

In phase III studies, patients with mild hepatic impairment had an average of 1.2 fold increase in total rivaroxaban concentration compared to healthy subjects. (Kubitza et al., 2013) Patients with moderate hepatic impairment had a 2.3 fold increase in rivaroxaban concentration, 1.3 fold increase in peak Cmax rivaroxaban concentration and prolonged elimination half-life by approximately 2 h. (Kubitza et al., 2013) Additionally, there was a significant correlation between unbound plasma rivaroxaban and serum albumin. Hepatic disease can lead to coagulopathy, which is a contraindication to anticoagulant medication, therefore cirrhotic patients classified as Child-Pugh B and C should not take rivaroxaban. (Kubitza et al., 2013)

3.4.2.3 Ethnicity

There have been no reported significant differences in rivaroxaban concentration observed between various ethnic groups, including Chinese, African, Caucasian, Hispanic.

3.4.2.4 Summary of reported variables which influence rivaroxaban effects

In conclusion, creatinine clearance is known to affect rivaroxaban exposure, clearance, AUC and Cmax. (Kreutz, 2014; Kubitza et al., 2010) There is emerging evidence supporting variations of rivaroxaban effects in patients at the extremes of body weight, and at the upper extreme of age and hepatic function. However, all studies to date have reported that these effects are moderate and not sufficiently significant, and often not statistically significant, thus, no dosing alteration is required. (Kubitza et al., 2013a; Kubitza et al., 2013b) The impact on the clinical use of rivaroxaban depends on its safety profile, which has been shown to be consistently safe across a wide range of patients of varying ages, weights and indications. (Mueck et al., 2011; Siegal et al., 2014; Wang et al., 2016)

CHAPTER 4 THE PHARMACODYNAMICS OF RIVAROXABAN

Chapter 4 outlines the parameters available to measure the pharmacodynamic effects of rivaroxaban, including their relative benefits and limitations in vivo. The chapter evaluates the results of this study and compares them to the available literature with regards to the relevant clinical outcomes and effects of rivaroxaban.

4.1 BACKGROUND

The pharmacodynamics (PD) of a drug are difficult to accurately measure because no in vitro test can replicate exactly the effects in vivo. The clotting cascade is a complex inter-play between multiple enzymatic activations, deactivations, feedback interactions and eventually clot formation and lysis, which all occur in succession and in parallel. The acceptable measurements of rivaroxaban pharmacodynamics must linearly, directly and closely correlate with plasma rivaroxaban concentration. These include prothrombin time (PT) and antiXa activity.

AntiXa activity has the greatest correlation and is accepted as a surrogate marker for plasma rivaroxaban concentration.

PT is still not consistent between laboratories, is dependent on the reagents used, and does not reflect the overall thrombotic or bleeding potential of an individual or the state of the coagulation system. Thus, PT cannot be consistently or reliably used as a PD marker.

The global coagulation tests, CAT and ROTEM are representative of the pro-thrombotic potential of an individual. CAT reflects the thrombin generating potential, which determines the potential to form clots in vivo, and ROTEM represents the overall clot formation and lysis in vivo, indicating the platelet function and contribution to clot formation, and plasmin activity in clot lysis. Both CAT and ROTEM parameters demonstrate increased coagulability in patients with VTE. (Baglin, 2005; Spiezia, et al, 2008; Casutt, et al., 2012)

4.2 RIVAROXABAN CONCENTRATION, ANTIXA RESULTS

The mean rivaroxaban concentration was 148.8 ng/ml (SD +/-128.4 ng/ml). The actual range is not known since the analyser reports all antiXa levels less than 20 ng/ml as <20 ng/ml and all levels more than 500 ng/ml as >500 ng/ml. The plasma rivaroxaban concentrations measured were taken a specific time points after rivaroxaban ingestion, and the results are recorded at this time post dose (figures 8A and 8B). Figure 8 illustrates the distribution of rivaroxaban plasma concentration, as it peaks between one and three hours post-ingestion,d exponentially decreases to trough levels and after approximately 24 h, as it is eliminated from the body. There does not appear to be a clear differentiation in rivaroxaban concentrations between the dosing categories.

Figure 8 reveals three outlier samples with higher than expected rivaroxaban concentrations for a given time post dose, at greater than 12 h. There are also three samples which have lower than expected rivaroxaban concentrations at four hours post dose.

The two outlier results correspond to a Caucasian 72 year-old female of 71 kg and lean body weight 43 kg, with a creatinine clearance of 43.6 ml/min. This individual was taking 15mg rivaroxaban twice a day for an acute DVT and had a trough level of 386.75 ng/ml at 14.25 h post dose. The second outlier had a trough antiXa of 343.62 ng/ml at 26.4 h post 20mg dose of rivaroxaban. This participant was a 58 year-old, Caucasian, 93.3 kg male with lean body weight 66.7 kg and creatinine clearance 60.6 ml/min. The third outlier was a Caucasian male of 96 kg, lean body weight 65.7 kg and creatinine clearance 41.4 ml/min. This participant had taken 15mg rivaroxaban 13.7 h prior to the 20 mg dose and had a rivaroxaban level of 272.2 ng/ml at 24 h (figure 8A).

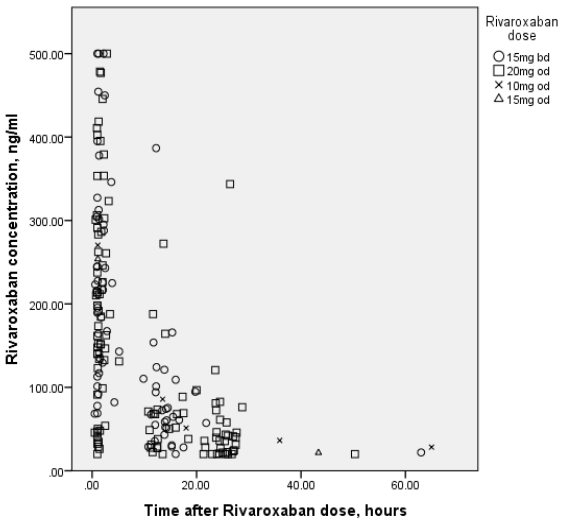

FIGURE 8A RIVAROXABAN CONCENTRATION VS TIME AFTER RIVAOXABAN DOSE, FOR THE VARIOUS DOSES TAKEN, 10MG OD, 15MG OD, 15MG BD, 20MG OD

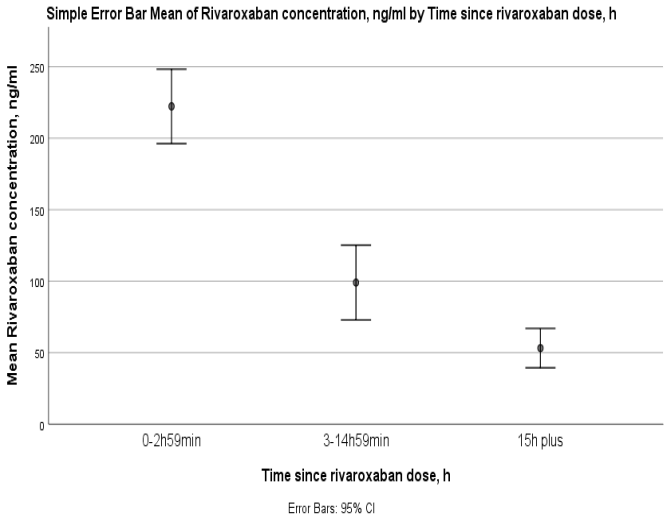

FIGURE 8B ERROR BAR CHART FOR RIVAROXABAN CONCENTRATION VS TIME AFTER RIVAROXABAN DOSE

Od once a day, bd twice a day

4.2.1 THE EFFECT OF BODY WEIGHT ON RIVAROXABAN CONCENTRATION

When the results were grouped according to BMI and time after dose, within each time group, there was a trend for those patients in the lowest BMI group to have the lowest mean rivaroxaban concentrations, but this was not statistically significant (figure 9). BMI was normally distributed and there was one individual in the lowest BMI group, 27 in the second group (BMI 18.5-24.9 kg/m^2), 32 in the third (BMI 25-29.9 kg/m^2), 21 in the fourth (BMI 30-34.9 kg/m^2), 14 in the fifth (BMI 35-39.9 kg/m^2) and 6 individuals had BMI >40 kg/m^2 (figure 9).

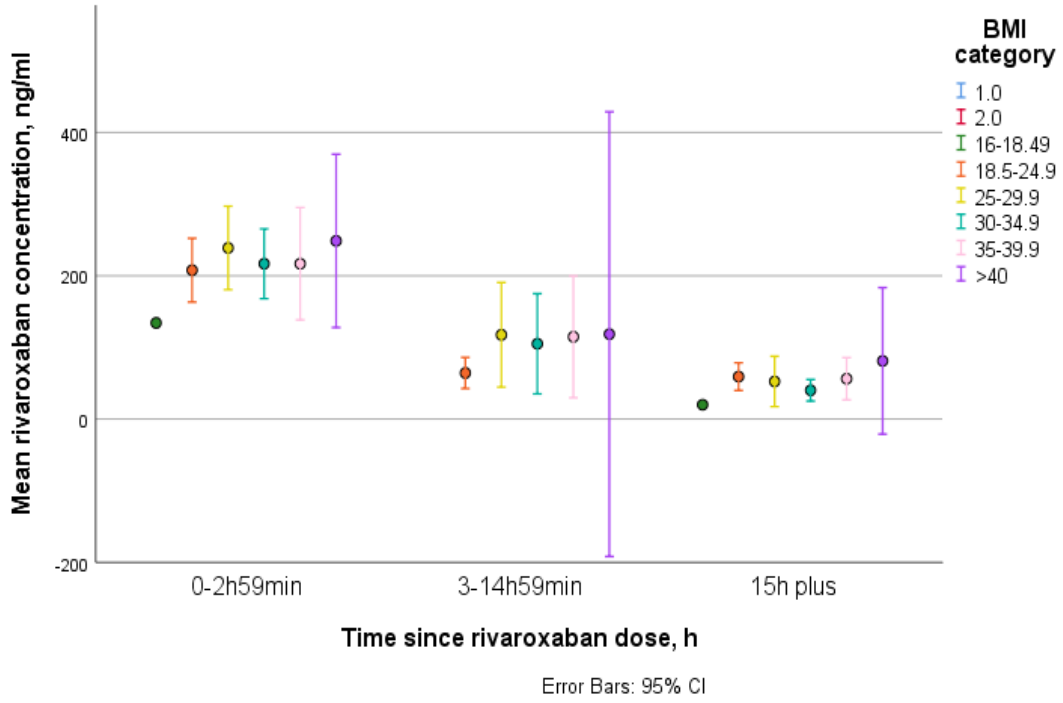

FIGURE 9 MEAN RIVAROXABAN CONCENTRATIONS AT CERTAIN TIME POINTS POST-DOSE, GROUPED INTO BMI CATEGORIES

4.2.2 Use of antiXa levels during rivaroxaban treatment

The manufacturers of rivaroxaban state that the drug plasma levels do not need to be monitored, since there a large therapeutic index. Current guidance recognises this, but states that, in situations when it is important to know if rivaroxaban is present in the plasma in clinically relevant levels, a normal antiXa measurement can exclude this. (Ikeda & Tachibana, 2015) Thus, if an urgent or invasive procedure is required, or in the situation of a haemorrhage or major trauma, antiXa measurement is suggested. (Oswald et al., 2014) AntiXa levels are also recommended if a rivaroxaban concentration quantitation is required, for example, if a patient has had a bleed or recurrent VTE while taking rivaroxaban. (Asmis et al., 2012; Tripodi, 2013)

4.3 Prothrombin Time (PT) in assessing rivaroxaban concentration

4.3.1 Background and literature review of Prothrombin Time (PT) in assessing rivaroxaban effects

Phase I multiple ascending dose studies of rivaroxaban on healthy subjects, using non-linear mixed effect modelling (NONMEM) demonstrated a direct linear correlation between PT and plasma rivaroxaban concentration with low inter-individual variability. (Mueck et al., 2007) This was further replicated in phase II studies on orthopaedic patients, post-total hip replacement surgery, taking either 10 mg once a day or twice a day, demonstrating a simple linear intercept model between PT and rivaroxaban concentration with a strong correlation. (Mueck et al., 2008) If studies can demonstrate consistency between the PT values of patients on rivaroxaban, within a patient and between laboratories and if there is an agreed calibrator for rivaroxaban on the PT assay, then PT could potentially be used to monitor the effects of rivaroxaban, in cases of bleeding, overdose, emergency surgery and non-adherence or recurrent VTE while on rivaroxaban. (Samama et al., 2013)

4.3.2 Results of Prothrombin Time (PT) and its correlations with rivaroxaban concentration

Prothrombin time (PT) was measured in association with every antiXa level. PT frequency distribution was positively skewed, since most samples were taken one to two hours post rivaroxaban administration, therefore at the peak of drug concentration. Median PT was 16.95 sec, (range 12.1-36.9 sec). There were no statistical differences between the mean PT values of Caucasian, African or other ethnic groups, p=0.561; acute, recurrent, long term or orthopaedic VTE groups, p=0.054; or between the 6 BMI categories, p=0.859, using the non-

parametric Kruskal-Wallis test. The two-tailed Spearman rank coefficient for PT with rivaroxaban concentration, was 0.9, p=0.00, n=194, (figure 10).

Multiple regression analysis of rivaroxaban concentration demonstrated no effect of actual or lean body weight on the correlation between PT and rivaroxaban concentration.

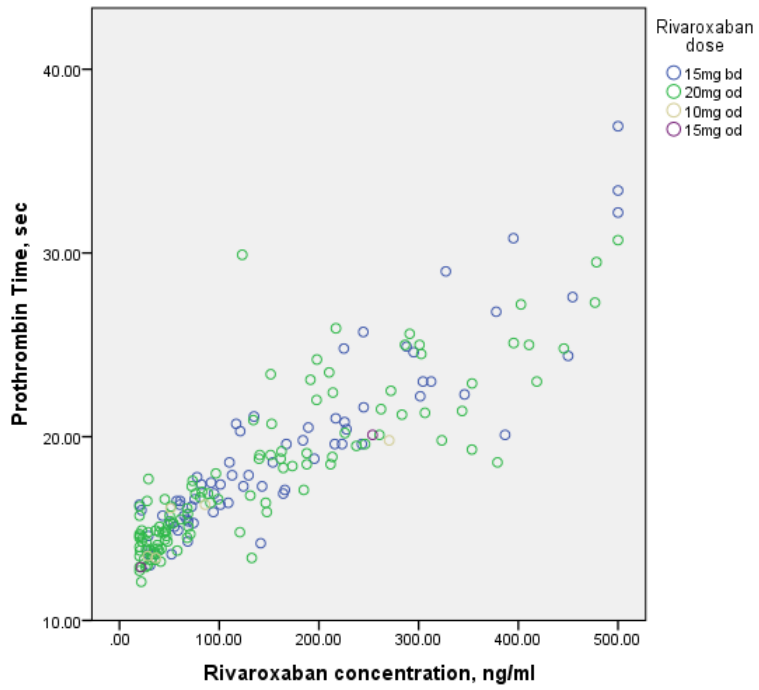

FIGURE 10 PROTHROMBIN TIME VERSUS RIVAROXABAN CONCENTRATION, FOR THE VARIOUS DOSES OF RIVAROXABAN TAKEN

4.3.3 THE USE OF PROTHROMBIN TIME (PT) MONITORING DURING RIVAROXABAN TREATMENT

Current guidance recognises that rivaroxaban does not need to be routinely monitored, as compared to warfarin. (Burnett et al., 2016) However, there are situations in which PT is a suggested tool for assessing the activity of rivaroxaban and informing the long-term treatment decisions. These situations include haemorrhage, diminished renal or hepatic function, overdose, drug interactions or advanced age. (Garcia et al., 2013) In these cases, a normal PT can exclude the possibility of excessive rivaroxaban plasma concentration. (Molenaar et al., 2012) The translation of plasma rivaroxaban concentration into the efficacy of its anticoagulant effect is still not clear, although, at present, the PT can be used as a surrogate marker of the anticoagulant effect, as long as certain reagents are employed in the assay. (Rodgers et al., 2013)

4.4 THROMBIN ANTI-THROMBIN COMPLEXES (TAT)

4.4.1 BACKGROUND

The inhibition of thrombin by antithrombin results in the formation of stable thrombin-antithrombin complexes (TAT). An enzyme-linked immunosorbent assay (ELISA) has been developed to detect TAT in platelet poor plasma. (Elgue et al., 1990) The reference range which has been determined for healthy subjects is 0.85-3.2 µg/l and for patients with a recent VTE 3-25 µg/l. (Pelzer et al., 1988)

There was a high range of coefficients of variability (CV) for the thrombin-anti-thrombin (TAT) complex results. Following a thorough literature review, it was decided to include only those results with CV less than 25% into the statistical analyses. The techniques, nature of storage and thawing were consistent for all samples and the individual characteristics of those samples with low CVs were similar to those with high CVs.

4.4.2 RESULTS

The TAT analysis was performed in two separate wells on the same plate, for each TAT sample, thus, the mean TAT value for each sample could be calculated. A standard deviation for each TAT result and the CV for the combined two TAT results from each sample were calculated by the Abcam TAT software.

In total, 380 TAT tests were carried out. Therefore, there were 190 samples and 190 mean TAT results. 272 TAT results had CV less than 25 %. The TAT data were not normally distributed. The median (and range) for the total 190 mean TAT results was 4.48 ng/ml (1.00 - 42.93 ng/ml) and for those with CV less than 25 % (n = 139), the median was 4.73 ng/ml (range 1.00-36.07 ng/ml).

Spearman rho analysis revealed no significant correlations, between TAT and any parameter including: age ($p=0.71$), body weight ($p=0.65$); lean body weight ($p=0.30$); rivaroxaban concentration ($p=0.15$); D-dimer ($p=0.96$); or fibrinogen ($p=0.52$). Kruskal-Wallis test for differences between the means of multiple categories, revealed significant differences between the groups of indication for rivaroxaban and between the doses of rivaroxaban taken. Further analysis demonstrated that these significant differences in the mean TAT values were between those patients on prophylactic rivaroxaban post orthopaedic surgery and acute VTE, $p=0.007$ and between those patients taking 15 mg bd and 10 mg od, $p=0.007$, and between 20 mg od and 10 mg od, $p=0.004$. However, the numbers were small in the orthopaedic (10 mg dose) group therefore the statistical analyses were unreliable. There were no significant differences between mean TAT levels for lean body weight categories ($p=0.553$), BMI categories ($p=0.160$) or ethnic groups ($p=0.757$).

4.5 GLOBAL COAGULATION TESTS

4.5.1 BACKGROUND

Thrombin generation is due to a complex enzymatic mechanism occurring within the plasma, triggered by injury, inter-playing with the inactivation of thrombin and lysis of the clot. Hemker described the importance of the endogenous thrombin potential (ETP), determined by the area under the curve, in reflecting the overall thrombotic potential of an individual, and detecting both hypo- and hyper-coagulability. (Hemkerl & Béguin, 1995) Calibrated Automated Thrombography (CAT) measures endogenous thrombin potential in vitro. (RE Rotational thromboelastography (ROTEM) performed on whole blood reflects the overall haemostatic interplay between thrombin generation, vessel wall interaction and platelet activation.

This study investigated the effects of various doses of rivaroxaban taken for different indications, on CAT and ROTEM parameters, analysed in vivo, at certain recorded time points following rivaroxaban. The effects on the global coagulation test parameters were analysed in the context of the individual and compared to the effects on PT and APTT.

4.5.3 CALIBRATED AUTOMATED THROMBOGRAM (CAT)

4.5.3.1 BACKGROUND: THE USE OF CAT IN PATIENTS WITH VTE AND IN PATIENTS TAKING RIVAROXABAN

The thrombin generating capacity of plasma is one of the main determinants of haemostasis and thrombosis. The thrombotic response is proportional to the concentration of thrombin and time during which it can activate the substrates (assuming that the substrates are not exhausted). This is represented by the area under the concentration-time curve.

The parameters measured by the Calibrated Automated Thrombogram (CAT) include: lag time, representing the time to initial thrombin generation; peak thrombin generation, representing the maximum amount of thrombin formed; time to peak (ttpeak), representing the mean time taken to reach the maximum thrombin formation; endogenous thrombin potential (ETP), which is measured by the area under the thrombin generation curve, representing the product of the overall thrombin activity and the total time for which the thrombin remains active; and the velocity index (Velin) is the rate index of the propagation phase of thrombin generation, calculated by dividing the peak thrombin by the difference of the time to peak and lag time.

In platelet poor plasma, the thrombin generation curve reflects all clotting factor deficiencies except for factor XIII and is sensitive to the action of anticoagulant drugs.

There has only been one international multicentre study investigating the use of a standardised protocol for thrombin generation measurement. The CAT equipment, method and reagents used were the same as in this present study, (CAT, Stago) with tissue factor concentration of 1 pM and the addition of CTI at 1.45 μM final concentration. (Dargaud & Yesim, 2012a) The intra- and inter-individual CV % and the inter-centre CV % were determined for samples at room temperature and those pre-heated to 37°C. The CV %s were more for those corresponding samples which had been pre-heated. The CVs were all <15 % at room temperature for every CAT parameter and there were detectable differences between CAT parameters for normal, hyper- and hypo-coagulable plasmas. However, the sample size was small and only six healthy male volunteers donated plasma for the normal pooled plasma, one for the hypocoagulable plasma and five for the hypercoagulable plasma. (Dargaud & Yesim, 2012a)

The baseline capacity to form thrombin varies enormously between individuals and is quoted as 15% coefficient of variation. Hemker and colleagues, using the same techniques and reagents as this study, determined normal values and variabilities for ETP, peak thrombin generation and lag time, and determined the inter- and intra-variability for the CAT (table 11). (Hemker et al., 2003)

TABLE 11 REFERENCE VALUES FOR CAT USING PLATELET POOR PLASMA

Parameter	Inter-individual CV%	Intra-individual CV%	Mean (+/-SD)
ETP, nM/min	15	4.5	1,879 (284)
Peak Thrombin, nM	13	5.5	458 (60)
Lag Time, min	47	8.1	3.1 (1.4)

ETP, endogenous thrombin potential, CAT, calibrated automated thrombography

Luddington and colleagues suggest that the addition of CTI decreases the variability of the results by eliminating false activation of the clotting cascade due to contact factor activation in vitro. (Luddington & Baglin, 2004) Dargaud and colleagues suggest a final concentration of CTI of 1.45 μM. (Dargaud & Yesim, 2012b)

Bloemen and colleagues investigated the inter-individual variabilities in the CAT parameters of platelet poor plasma (PPP) for differing recombinant tissue factor and phospholipid reagent concentrations, using direct and

indirect antithrombin-mediated inhibitors of thrombin and factor Xa, in heathy volunteers. The coefficients of variation before inhibition were 16 % for peak thrombin generation and 18 % for ETP, and after addition of the anti-coagulant drugs, increased to between 24-43 % for peak and 20-24 % for ETP. Concentrations of the anticoagulant drugs needed to cause 50 % inhibition of peak or ETP varied enormously. (Bloemen et al., 2013) Previously, Gerotziafas had demonstrated a lower baseline intra- and inter-variability of 9 %. (Gerotziafas et al., 2005)

Douxfils and colleagues spiked pooled normal human platelet poor plasma (PPP) with various concentrations of rivaroxaban. The CAT parameter most sensitive to rivaroxaban was peak thrombin generation. (Douxfils et al., 2012) Gerotziafas and colleagues had previously performed similar experiments, revealing a strong correlation between rivaroxaban concentration and increasing lag time, time to peak and decreasing peak thrombin and ETP. (Gerotziafas et al., 2007)

CAT is sensitive to the effects of pH, ion concentrations, temperature, calcium concentration, albumin concentration, haemoglobin values, fibrinogen concentration (Loeffen et al., 2012) and pre-heating conditions (De Smedt & Hemker, 2011)

CAT has been used in research studies investigating the thrombin potential in patients developing thrombosis and in patients with underlying heritable pro-thrombotic conditions. Hron and colleagues demonstrated an increase in peak thrombin generation and ETP in patients with recurrent VTE. (Hron et al., 2013) A previous study prospectively investigated patients with a first VTE and measured CAT at two to three months following completion of anticoagulation therapy. Patients with higher ETP went on to develop significantly higher rates of unprovoked recurrent VTE than those with lower ETP. Additionally, ETP was the only parameter associated with unprovoked recurrent thrombosis in a multivariate model and remained a significant predictor of recurrence after adjustment for various confounding factors. (Besser et al., 2008)

In whole blood samples, lag time was prolonged by rivaroxaban, but ETP and peak height were less responsive to rivaroxaban than in PPP. However, the addition of prothrombin complex concentrate (PCC) to whole blood and PPP samples caused a more rapid and complete normalisation of ETP and peak height in the whole blood than in PPP. (Dinkelaar et al., 2013)

Herrmann and colleagues used CAT to investigate the effects of rivaroxaban in vivo, in patients taking 10 mg for primary VTE prophylaxis post orthopaedic surgery. (Herrmann et al., 2014) Lag time, ETP and peak height were

reduced by rivaroxaban, and normalised by PCC, factor 8 inhibitor bypassing activity (FEIBA) and recombinant factor VIIa.

4.5.3.2 CAT RESULTS

4.5.3.2.1 INTRA- ASSAY AND INTER-INDIVIDUAL ASSAY VARIABILITY

The CAT does not have an integrated system to measure the intra-assay and inter-individual assay variability of the thrombin generation recorded, therefore this was performed manually, to ensure reproducibility of the method described in the previous section. The intra-assay variability is the variability within the test and the inter-individual variability is the variability of the test between individuals.

A healthy control (female aged 36 years) was bled every four weeks from the start of this research project. Four plasma samples were obtained on each occasion and immediately frozen. 21 plasma samples from the control were thawed together, within eight weeks of the blood being drawn, and they were analysed in triplicate, along with triplicate of reconstituted human control plasma (NHCP, Technoclone, Vienna, Austria), and triplicates of calibrators, in a 48-well CAT plate.

Inter-individual assay variability was assessed using human control plasma (NHCP, Technoclone, Vienna, Austria), which was reconstituted as per the manufacturer's instructions, pooled and frozen prior to every use with every row and plate performed. One NHCP sample was thawed within eight weeks of freezing, and run, in conjunction with the patient samples, in 8 rows of 3 samples and 3 calibrators. The variance of 10 consecutive NHCP results were compared to give the inter-individual assay CV %.

Table 12 summarises the results for the four principal CAT parameters lag time, ETP, peak thrombin generation and time to peak thrombin formation. Intra-assay coefficient variations (CV) were between 6.4 and 15.9 % without CTI and between 7.2 and 21.6 % with CTI. Inter-individual CVs were 4.5-14.6 % without CTI and 14.1 – 77.5 % with CTI. The inter-individual CV of the test, using normal human control plasma (NHCP) was between 13.1 and 27.7 %.

TABLE 12 INTRA-ASSAY AND INTER-INDIVIDUAL ASSAY VARIABILITY FOR CAT

	Lag time (min)	ETP (nM/min)	Peak thrombin (nM)	ttPeak (min)
Intra-assay without CTI				
Mean control plasma	6.3	1302.6	243.3	9.6
Mean SD	0.4	88.4	38.7	0.7
Coefficient variation (%)	6.4	6.8	15.9	7.0
Intra-assay with CTI				
Mean control plasma	5.4	2250.4	332.9	8.9
Mean SD	0.6	113.6	15.4	1.2
Coefficient variation (%)	8.4	19.8	21.6	7.2
Inter-individual assay				
Mean NHCP	5.7	1505.7	206.6	10.8
Coefficient of variation (%)	27.7	25.8	13.1	17.6
Inter-individual trough no CTI				
Mean of subjects	6.8	1222.9	146.9	10.5
Mean SD	0.8	83.8	32.6	0.9
Coefficient of variation (%)	8.7	14.6	4.5	11.2
Inter-individual at trough + CTI				
Mean of subjects	14.3	656.8	24.2	32.8
Mean SD	0.3	14.2	0.3	2.3
Coefficient of variation (%)	46.0	46.2	77.5	14.1

NHCP normal human control plasma, CTI corn trypsin inhibitor, ETP endogenous thrombin potential, ttPeak time to peak thrombin

4.5.3.2.2 CAT PARAMETERS OVER TIME

The thrombographs produced from the CAT software showed the skewed parabola shaped curve, with peak thrombin generation being achieved rapidly after the initiation of thrombin generation, following the initial lag time. The time to trough, representing the fibrinolysis of the clot, was longer than the time to peak, representing the initiation, amplification and propagation phases of clot formation. The thrombograms for the samples corresponding trough rivaroxaban concentration compared to those samples taken at peak rivaroxaban concentrations have shorter lag times, shorter time to peak, higher peaks, greater area under the curve and shorter start tail.

The trend over time of thrombin generation, following the last dose of rivaroxaban administration, is in keeping with the anticoagulant effect of rivaroxaban, as the concentration peaks at 2-4 h post-dose, then falls until baseline at 24-36 h. This is illustrated by each CAT parameter measured, including lag time (figure 11) and mean peak thrombin formation (figure 12).

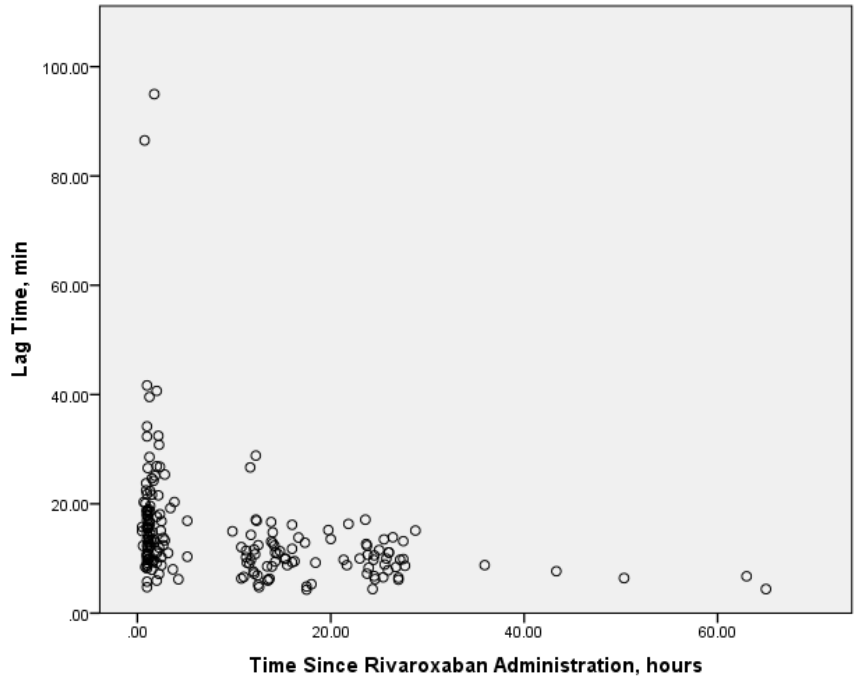

FIGURE 11 LAG TIME VERSUS TIME SINCE RIVAROXABAN ADMINISTRATION

4.5.3.2.4 CAT PARAMETERS MEASURED WITH AND WITHOUT THE ADDITION OF CORN TRYPSIN INHIBITOR

Table 13 summarise the principal findings of the CAT parameter of the patients recruited into the study, with CTI and without CTI added to the plasma prior to investigating the thrombin generation. The effect of rivaroxaban on every CAT parameter was exaggerated by the addition of CTI, thus lag time was prolonged, ETP was decreased and peak thrombin generation was less following the addition of CTI. The absolute differences between the means for the CAT parameters measured with and without the addition of CTI are all significantly different when assessed with the two-tailed, paired sample T-test (table 13). The mean CAT parameter values with and without CTI are compared, using the two-tailed, paired t-test. The inhibition of rivaroxaban on the thrombin generation parameters measured in samples with CTI is so marked that the relative influence of background variability becomes significant, and small differences cannot be attributed to the anticoagulant effect of rivaroxaban (figure 12).

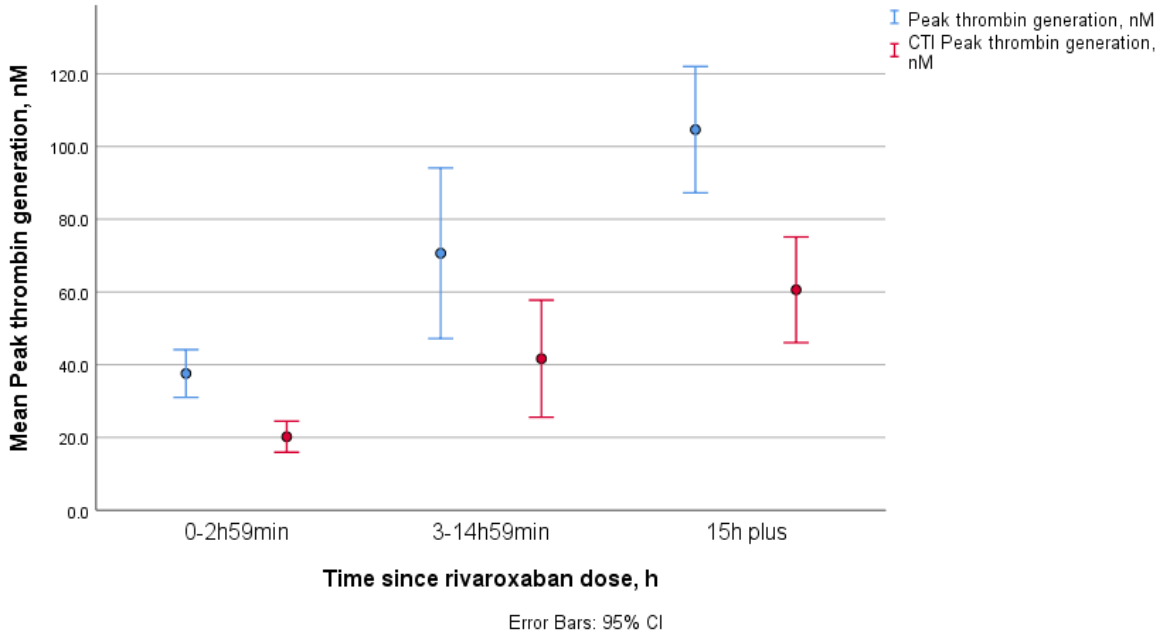

FIGURE 12 ERROR BAR OF MEAN PEAK THROMBIN FORMATION VERSUS TIME SINCE RIVAROXABAN DOSE, WITHOUT & WITH CTI

TABLE 13 MEAN CAT PARAMETERS WITH AND WITHOUT CTI ADDED AT SPECIFIC TIME POINTS FOLLOWING RIVAROXABAN DOSE.

MEAN (+/- SD)	TIME AFTER DOSE 0-3H		TIME AFTER DOSE 3-15H		TIME AFTER DOSE >15H		2-TAILED T-TEST WITH VS WITHOUT CTI (95% CI)
	with CTI n=87	without CTI n=99	with CTI, n=34	without CTI, n=41	with CTI, n=44	without CTI, n=52	
Lag time, min	24.5 (13.9)	17.9 (13.0)	16.1 (6.5)	11.8 (5.3)	13.1 (4.4)	9.8 (3.2)	19.5 (12) v 15.6 (10) P <0.001
ETP, nM/min	299.1 (390.3)	605.3 (463.5)	575.9 (506.2)	966.6 (507.6)	775.7 (429.5)	1159 (410.0)	484 (472) v 776 (510) P < 0.001
Peak, nM	20.2 (20.0)	45.2 (42.1)	41.7 (46.1)	87.6 (78.1)	59.5 (47.3)	128.6 (93.2)	35 (39) v 62 (55) P < 0.001
Velindex, nM/min	2.1 (3.6)	8.3 (14.1)	5.8 (11.5)	21.9 (29.7)	10.6 (12.3)	38.3 (49.1)	5.2 (9) v 12.1 (16) P < 0.001
ttPeak, min	38.4 (18.0)	27.5 (15.0)	27.4 (9.2)	19.5 (8.8)	21.5 (15.9)	15.1 (5.8)	31.7 (16) V 24.1 (13) P < 0.001

ETP, endogenous thrombin potential; Velindex, velocity index; ttPeak, time to peak thrombin formation.

4.5.3.2.5 CAT PARAMETERS CORRELATION WITH RIVAROXABAN CONCENTRATION

There are significant correlations between the CAT parameters measured and rivaroxaban concentrations, (table 14). The strongest of these correlations were in ttPeak (positive correlation) and Velindex (negative correlation), indicating that the greater the concentration of rivaroxaban in the plasma, the longer the propagation phase of the clotting cascade, thus, the longer it takes to reach maximum thrombin formation. This relationship, which also exists for lag time, does not differ between patients taking various doses of rivaroxaban or between ethnic, sex, age or BMI/body weight groups of patients.

The addition of CTI to the sample resulted in a stronger correlation between the CAT parameter and rivaroxaban concentration, although the two-sample paired T-test revealed no statistically significant difference

between any of the CAT-rivaroxaban concentration correlations. The addition of CTI did make the CAT parameters significantly more sensitive to the anticoagulant effects of rivaroxaban on thrombin generation, but the statistical significance remained at p=0.000 for every parameter with and without CTI (table 14).

TABLE 14 CORRELATION COEFFICIENTS BETWEEN CAT PARAMETERS AND RIVAROXABAN CONCENTRATIONS WITHOUT AND WITH CTI ADDED

CAT parameter	Pearson correlation coefficient, r, without CTI (p)	Pearson correlation coefficient, r, with CTI (p)
Lag time, min	0.602 (< 0.001)	0.616 (< 0.001)
ETP, nM/min	-0.546 (< 0.001)	-0.645 (< 0.001)
Peak, nM	-0.626 (< 0.001)	-0.655 (< 0.001)
ttPeak, min	0.665 (< 0.001)	0.748 (< 0.001)
Velindex, nM/min	-0.658 (< 0.001)	-0.697 (< 0.001)

ETP, endogenous thrombin potential; Velindex, velocity index; ttPeak, time to peak thrombin formation.

There were strong, statistically significant correlations between rivaroxaban concentration and CAT lag time, ETP, peak, ttpeak and velocity index, as illustrated by lag time vs rivaroxaban concentration (figure 13).

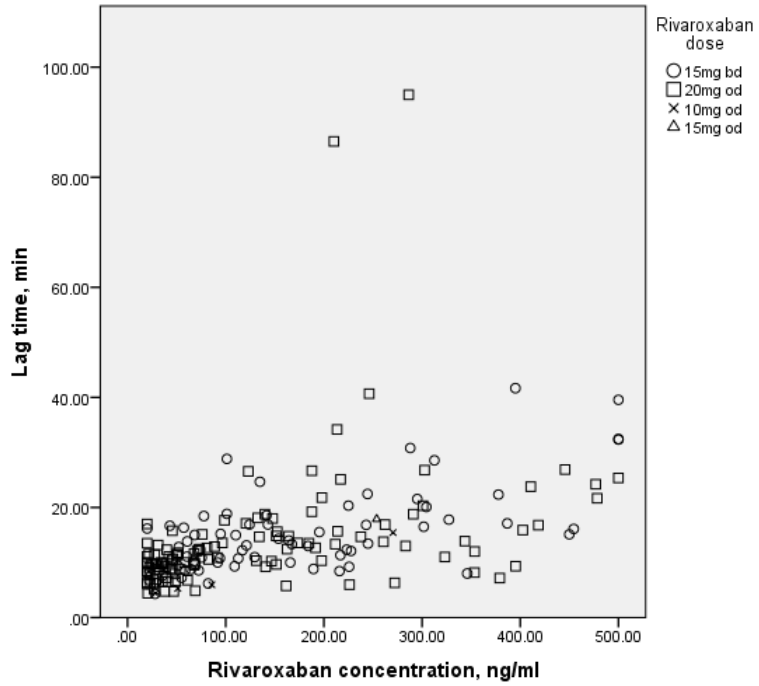

FIGURE 13 CAT LAG TIME VERSUS RIVAROXABAN CONCENTRATION FOR PATIENTS TAKING DIFFERING DOSES OF RIVAROXABAN

4.5.3.2.3 THE EFFECT OF BODY WEIGHT ON THE CAT PARAMETERS

The CAT parameters were normally distributed. The independent T-test comparing mean CAT parameters for patients in differing groups according to BMI, revealed statistically significant differences only between: ETP for BMI categories five versus six (p=0.02), four versus seven (p=0.03) and seven versus eight (p=0.02). All other comparisons for all other CAT parameters and BMI combinations were not statistically different.

Pearson's two tailed correlation for all CAT parameters with weight and lean body weight showed no correlation for weight but there were correlations with lean body weight (LBW). LBW and time to peak thrombin generation correlated, r= -0.18, p=0.01; LBW with velocity of thrombin formation, r=0.14, p=0.004.

There was no observed trend for any CAT parameters analysed for the BMI categories, as illustrated by the graph of lag time versus time after dose for the BMI categories (figure 14).

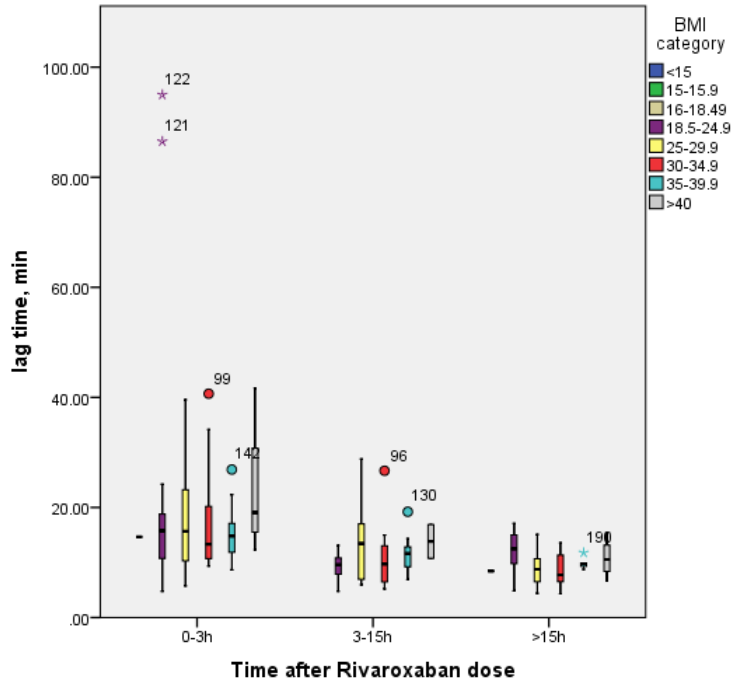

FIGURE 14 ERROR BAR CHART FOR MEAN LAG TIME VERSUS TIME AFTER DOSE FOR THE BMI CATEGORIES

4.5.4 ROTEM

4.5.4.1 BACKGROUND

Thromboelastometry graphically represents the viscoelastic changes that occur during coagulation, quantifying clot initiation, formation, stability and lysis. It was designed as a rapid point of care monitor at the bedside and peri-operatively. It measures the tension caused on a rotating pin in activated whole blood as a clot forms then lyses.

There is still no consensus regarding standardisation between laboratories, validity and translation to represent a hyper- or hypo-coagulable state since differences in the results have been reported relating to blood sampling, whole or platelet-poor blood, reagent used, patient age and gender. (Luddington, 2005) ROTEM is used to guide blood product and drug administration during cardiac and hepatic surgery including monitoring antifibrinolytic therapy, activated prothrombin complex concentrates and recombinant activated factor VII treatment. (Luddington, 2005)

Dias and colleagues investigated the effects of rivaroxaban on human pooled plasma in vitro, assessed by thromboelastography (TEG). (Dias et al., 2015) TEG is a viscoelastic measurement of whole blood clotting, which is similar to ROTEM, and both tests have been shown to be sensitive to the anti-coagulant effects of heparin and

anti-platelet agents. Dias and colleagues demonstrated that rivaroxaban causes a prolonged initiation phase of coagulation, as illustrated by a prolonged TEG activated clotting time and a prolonged R time in the kaolin assay. Additionally, the prolonged R time caused by rivaroxaban rapidly shortens back to the control level following the addition of ecarin. (Dias et al., 2015)

Oswald and colleagues performed a prospective observational study on 188 patients taking rivaroxaban 10 mg or enoxaparin 40 mg post-major orthopaedic surgery. ROTEM parameters measured revealed a significant difference in the prolongation of CT Extem in patients taking rivaroxaban, outside of the normal reference range, compared to enoxaparin (change in Extem CT 15 v 5 ($p<0.0001$). (Oswald et al., 2014)

Fox and colleagues studied 121 patients taking rivaroxaban for VTE treatment and prevention and measured the rivaroxaban peak levels together with ROTEM analysis. There was no reliable correlation between CT ROTEM parameters and rivaroxaban concentration, thus, there is currently no clinical use for ROTEM in the assessment of rivaroxaban induced anticoagulation. (Fox et al., 2016)

4.5.4.2 RESULTS

The results from the ROTEM parameters in our study population reflect the expected prolonged time to initiation of clot formation and time to maximum clot formation, decreased clot amplitude and maximum clot formation, under the influence of rivaroxaban.

The Extem parameters were more affected by rivaroxaban than the Intem parameters. With the exception of CT, the Extem parameters are normally distributed, thus, reported as mean +/- SD, whereas Intem CT, CFT and MCFt are positively skewed, thus reported as median and inter-quartile range (table 15).

TABLE 15 ROTEM PARAMETERS AT VARIOUS TIME POINTS AFTER RIVAROXABAN ADMINISTRATION, MEAN (+/-SD); # MEDIAN (RANGE)

Parameter	0-3 h		3-15 h		>15 h	
	Extem, n=96	Intem, n=96	Extem, n=40	Intem, n=40	Extem, n=53	Intem, n=53
CT, sec	#119 (350)	#230 (497)	#84.5 (105)	#196 (166)	#77.0 (219)	#173 (219)
A10, mm	62 (6.2)	58 (6.0)	64 (6.0)	59.6 (5.9)	61.7 (6.2)	58.9 (5.8)
A20, mm	67 (5.3)	63 (5.6)	69 (5.1)	64.5 (5.2)	67.1 (5.4)	64.2 (5.3)
CFT, sec	68 (16.3)	#67 (172)	61 (15.0)	#63.0 (60.0)	67.0 (17.6)	#63.0 (167)
MCF, mm	68 (5.4)	64 (5.7)	69 (4.9)	64.7 (5.2)	67.7 (5.2)	64.7 (5.2)
MCFt, sec	1401 (222)	#1284 (1265)	1393 (221)	#1240 (845)	1439 (189)	#1305 (23)
AUC	6736 (530)	6365 (549)	6860 (481)	6437 (511)	6715 (508)	6442 (533)

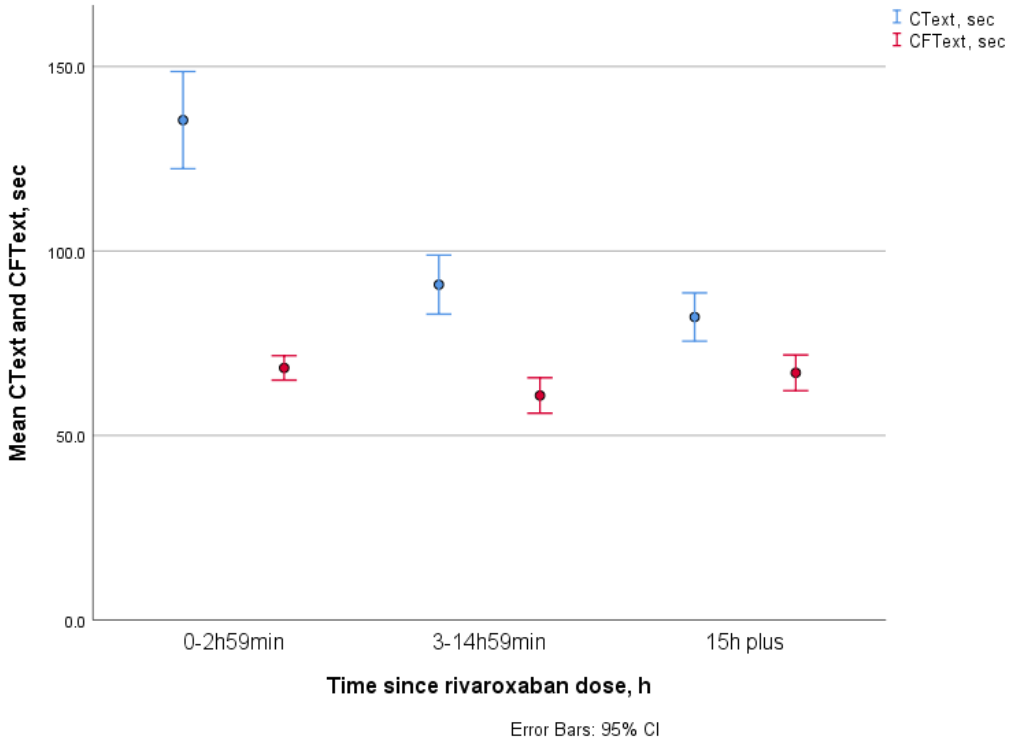

FIGURE 15 ERROR BAR CHART OF MEAN ROTEM PARAMETERS, CT, A10 AND CFT AT CLUSTERED TIME POINTS AFTER RIVAROXABAN DOSE

CT clotting time, A10 time to 10mm amplitude clot, CFT clot formation time

The mean Extem ROTEM parameter mean Extem-CT is greater at time 0-3 h post dose and decreases as rivaroxaban exposure decreases with increasing time post-dose (figure 15, table 16).

TABLE 16 CORRELATIONS BETWEEN EXTEM AND INTEM ROTEM PARAMETERS AND RIVAROXABAN CONCENTRATION

ROTEM parameter	Correlation coefficient, r (p value)	Correlation coefficient (p value)
	Extem	**Intem**
CT, sec	*0.736 (< 0.001)	*0.672 (< 0.001)
A10, mm	#0.148 (0.043)	*0.093 (0.205)
MCFt, sec	#-0.161 (0.028)	*0.031 (0.676)
MCF, mm	#0.087 (0.239)	*0.011 (0.128)
CFT, sec	#0.014 (0.854)	*0.025 (0.732)

Pearson correlation coefficient, for normally distributed values, * Spearman correlation coefficient for non-parametric values

The Spearman rho correlation coefficients for Extem-CT and Intem-CT parameters and rivaroxaban concentration, demonstrate a significant linear correlation (figure 16, table 16). The Pearson correlation coefficients were significant only for Extem-MCFt and Extem-A10 (table 16).

Non-parametric independent Kruskal-Wallis tests showed a significant difference in the distribution of CFTint, $p = 0.005$; CText, $p = 0.004$ and CFText, $p = 0.015$, across the categories of indication for anticoagulation. CFTint and CFText were significantly differently distributed across various ethnic groups, $p < 0.001$ and $p = 0.004$, respectively. There were no differences for sex, age categories or BMI categories (data not shown).

There was a significant correlation between Extem-CT and PT (Spearman's rho correlation coefficient 0.721, $p < 0.001$), and between Intem-CT and APTT (Spearman's rho correlation coefficient 0.213, $p = 0.003$) (table 16).

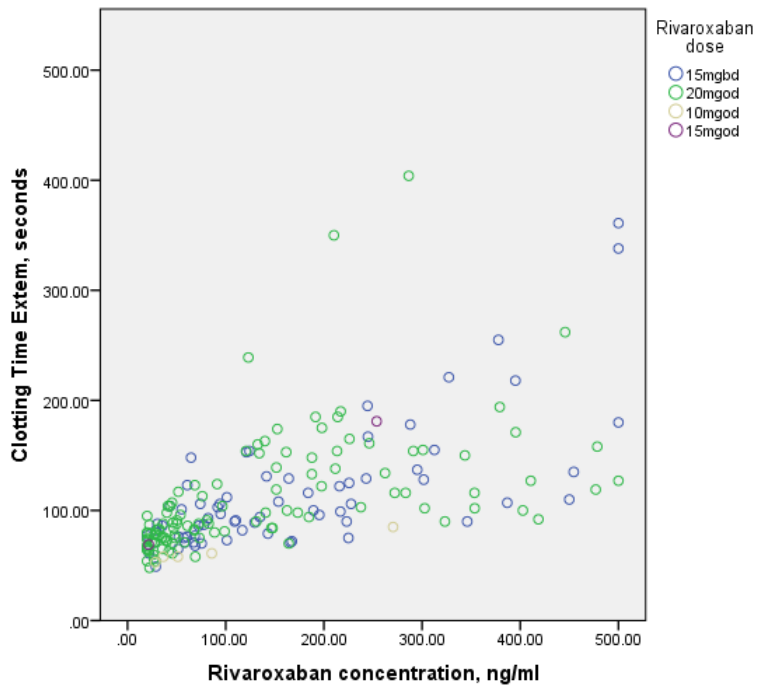

FIGURE 16 CT EXTEM VS RIVAROXABAN CONCENTRATION FOR VARIOUS DOSES OF RIVAROXABAN

4.5.4.2.1 THE EFFECT OF BODY WEIGHT ON THE ROTEM PARAMETERS

The Kruskal-Wallis test demonstrated a difference in distribution of CFTint, MCFint and CFText across BMI categories, p = 0.02, 0.19, 0.04, respectively.

Spearman-rho two tailed correlation for all ROTEM parameters showed no correlation for absolute body weight but there were correlations with lean body weight (LBW). ROTEM intem parameters which correlated with LBW included: A10, r = -0.18, p = 0.01; CFT r = 0.18, p = 0.01; MCF, r = -0.16, p = 0.03. The ROTEM extem parameters which correlated with LBW included: A10, r = -0.17, p = 0.017; MCF, r = -0.15, p = 0.03.

4.5.4.3 DISCUSSION

The relationship between body weight and ROTEM global parameters for patients taking rivaroxaban in the study is small but, with more data points, may be significant. The ROTEM analysis in this study confirms the known relationship between rivaroxaban concentration and prolongation of CTextem, in particular, but also CTintem and MCFt extem. (Oswald, et al., 2014; Casutt, Konrad & Schuepfer, 2012) This reflects the global effect that rivaroxaban is known to have on the clotting cascade, namely, to increase the initiation and propagation phases.

The potential effects of weight on the coagulation assays may therefore be masked by the significant effects of rivaroxaban. Additionally, the potential effects of weight on the pharmacokinetics of rivaroxaban may be masked by the coagulation assay sensitivities to rivaroxaban. It may be helpful to dose normalise rivaroxaban prior to analysis with CAT and ROTEM.

4.6 Discussion

4.6.1 How do the results from this study compare to the literature for antiXa measurements and rivaroxaban

Phase I studies in healthy volunteers showed maximum Factor Xa inhibition at three hours with residual detectable inhibition lasting at least 12 h post dose. The half-life in adults aged 18-65 years was five to seven hours and in over 65 year olds was 11-13h. (Kubitza et al., 2005)

Population modelling has determined rivaroxaban trough and peak levels in patients treated for VTE, taking 15 mg twice a day (bd) or 20 mg once a day (od). The trough was determined to be 26 µg/L (6-87 µg/L) and peak was 270 µg/L (189-491 µg/L), respectively. (Mueck et al., 2014) A similar analysis was performed on patients undergoing total hip or knee replacement surgery, taking prophylactic doses of rivaroxaban, either 5 mg bd or 10 mg od. The median peak was 125 µg/L and median trough was 9 µg/L, which did not differ significantly between 5 mg bd or 10 mg od dosing. (Mueck et al., 2008)

4.6.2 Thrombin-Antithrombin (TAT) as a surrogate marker of thrombin formation in assessing the effects of rivaroxaban

TAT has been used to study thrombin formation in whole blood of healthy volunteers, during the process of coagulation. (Rand et al., 1996; Brummel et al., 2002)

TAT has not been demonstrated to be consistently useful in monitoring coagulation activation due to its large inter- and intra-variability, partly because of its very short half-life. A study by Leroy-Matheron investigated the effect of the type of anticoagulant used in the collection tube and suggested that prothrombin fragments one and two were consistently affected, D-dimers were not affected and TAT enzyme-linked immune-assay (ELISA) had no pattern. (Leroy-Matheron & Gouault-Heilmann, 1994)

One study on 13 healthy volunteers demonstrated that TAT was not a useful marker of thrombin generation, as assessed by whole blood stimulated by tissue factor, in contrast to thrombin generation, which revealed a

direct correlation between the maximum active levels and the computer generated predicted levels. (Brummel-Ziedins et al., 2004) However, this study did reveal a low intra-individual variation in TAT, of 11.6% and a large inter-individual variation of 25.2%.

A further study revealed an increase in TAT levels in patients with chronic heart failure, which was shown to decrease following rivaroxaban, although this was not shown to be statistically significant. (Rand et al., 1996)

Green et al, 2010, used TAT to investigate the anticoagulant efficiency of rivaroxaban in orthopaedic patients. Evidence from this study suggested an increase in TAT during surgery. Green and colleagues measured TAT in patients pre-operatively, peri-operatively and 24 h post-operatively, who had been given rivaroxaban 10 mg 6-8 h post-surgery. They demonstrated a significant increase in TAT levels peri-operatively and a subsequent, significant decrease in TAT levels, post-operatively, after rivaroxaban administration. Additionally, rivaroxaban reduced TAT levels more than dalteparin. (Green et al., 2010)

A recent single-centre phase one clinical trial investigated the effects of bariatric surgery on TAT levels, and on the pharmacokinetics of rivaroxaban, in obese patients taking prophylactic rivaroxaban 10 mg 24 h pre-surgery and three days post-surgery. The pharmacokinetics (AUC, Cmax, half-life) were not significantly affected by bariatric surgery. TAT levels decreased one to three hours after taking rivaroxaban and increased 24 h after rivaroxaban dose, but the effect of the bariatric surgery on the pharmacodynamics of rivaroxaban, as determined by TAT levels, cannot be ascertained from this study. (Kröll et al., 2017)

Many TAT samples produced two very different test results, despite them being run on the same plate simultaneously. There is an inbuilt inconsistency in the TAT investigation method since the pipetting cannot occur simultaneously and there is a time lag from the first pipetted sample on the plate until the last. Secondly, TAT is very user and observer dependent. The reaction is sensitive to minor condition variables such as temperature, air bubbles and movement. Many of the TAT results in this study had high CV, therefore were not included in the analysis. The outcome of the limited analysis in this study, was that there was no correlation between any factor and TAT levels, including rivaroxaban concentration. This is not reflected in the literature and may be a result of inadequate reagents or poor technique.

4.6.3 How do the CAT results from this study compare to the literature?

Thrombin generation tests measure the ex-vivo potential of plasma to generate thrombin over time and provide information on the initiation, amplification and propagation stages of thrombin generation, as well as the fibrinolytic phase.

CAT is responsive to the effects of rivaroxaban and has been used to assess the use of prothrombin complex (PCC) antidote for reversing the effects of rivaroxaban. (Dinkelaar et al., 2013) Dinkelaar and colleagues spiked normal pooled plasma and whole blood samples from healthy volunteers with rivaroxaban at various concentrations and PCC was then added. PT and CAT (Schneider et al., 2015) were measured before and after PCC. In the platelet poor plasma (PPP) only the endogenous thrombin potential (ETP), as represented by area under the curve (AUC), was decreased by rivaroxaban and normalised by PCC, but PT was unaffected. The extent of ETP normalisation was dependent on both the rivaroxaban concentration and tissue factor (TF) concentration. PT, Cmax, peak height and lag time were lengthened by rivaroxaban and inversely proportional to TF concentration used. These parameters were not normalised by PCC at any PCC or TF concentration. As a control, PT was lengthened by coumarin and subsequently normalised rapidly by PCC. (Dinkelaar et al. 2013)

Green et al., 2010, demonstrated a significantly increased thrombin generation peri-operatively compared to pre-operatively, and a subsequent decrease with rivaroxaban treatment post-operatively, for elective total hip or knee replacement surgery. (Green et al., 2010)

This current study is consistent with the literature, showing a prolonged lag time and time to peak thrombin generation, and a decreased ETP and peak thrombin generation for patients with peak rivaroxaban concentration compared to those with trough rivaroxaban levels.

Thrombin generation studies focusing on the effects of obesity have consistently demonstrated an increased thrombin generation in patients of greater body weight. One study demonstrated a decreased thrombin generation potential in adults post-bariatric surgery. (Ay et al., 2010) A second study has revealed a greater thrombin potential in women over-weight compared to those of normal weight. (Sonnevi et al., 2013) The relationship between obesity and thrombosis is multifaceted and a study has shown that trunk obesity is related to increased inflammation, thrombin generation and thrombosis. (Prüller et al., W 2012)

This current study has evaluated all CAT parameters for patients of varying actual body weight, lean body weight (LBW) and BMI. The ETP was different between certain BMI categories, but not all. LBW correlated negatively

with time to peak and positively with velocity of clot formation, suggesting that patients with a larger LBW have increased propagation of clot formation, that those with lesser LBW.

The important, relevant outcome for clinicians is the relationship between VTE recurrence and thrombin generation potential. One study has not shown any association between the peak thrombin generation or the ETP and recurrent DVT, in patients with the pro-thrombotic factor V Leiden mutation. The AUREC study identified a subset of patients (approximately a third of the overall first, unprovoked VTE population studied) with a low risk of recurrence four years after anticoagulation cessation. In these patients, the peak thrombin concentration was < 400 nmol/l. Additionally, in patients with ETP >100%, the risk of recurrence was 1.6-fold. (Kyrle & Eischer, 2013) Besser et al., demonstrated a predictive value of thrombin generation measurement and recurrent unprovoked VTE following an initial unprovoked VTE. (Besser et al., 2008)

Conversely, Bloemen et al., 2013, have shown that at ETP less than or equal to 350 nM/min there is an increased risk of bleeding. (Bloemen et al., 2013) This could be a surrogate marker for bleeding risk for patients taking rivaroxaban, since rivaroxaban concentration level has not been shown to predict bleeding risk.

Schneider et al., investigated the effects of age on CAT parameters in 132 healthy adults, using 1 þmol/L and 5 þmol/L final concentration of tissue factor. They demonstrated a non-linear significant increase in thrombin generation parameters with age. (Schneider et al., 2015)

Thrombin generation has also been shown to be more predictive of recurrent VTE in patients with a lack of the anti-coagulants, protein C and S, than PT or APTT. (Tripodi et al. 2009)

This study performed CAT with and without the addition of CTI because it is not clear from the literature the importance of CTI, however, the evidence suggests that it may improve the sensitivity of the CAT. Our results showed that the effects of rivaroxaban on thrombin generation were exaggerated by the addition of CTI and the correlations of rivaroxaban concentration and CAT parameters with and without CTI were significantly different. However, the p value for the correlations were all less than 0.00 despite no addition of CTI, suggesting no significant benefit to adding CTI.

The clinical utility of a coagulation test is determined by its ability to improve health outcome and depends on the performance of the test for the specific clinical condition applied. The test must be precise, therefore reproducible, and accurate, therefore detect the disease-attributable variance. CAT has a high degree of intra-individual variability, and physiological thrombin generation has a normal high degree of inter-individual variability.

Standardisation of CAT and its reagents is required to make conclusions about the value of its measurements in a particular population. (Brummel-Ziedins et al. 2004) In conclusion, CAT is more sensitive to the changes in the physiological coagulation system caused by rivaroxaban, than PT or APTT. However, in order for it to be a useful clinical test it should have a measurable predictive value for clinical outcome, which, as yet, has not been demonstrated.

4.6.4 Comparison of the ROTEM results from this study to those of the current literature

Schneider and colleagues investigated which demographic variables may affect ROTEM parameters in healthy adults. There was a positive correlation between age and maximum clot firmness and alpha angle, and a negative correlation between age and clotting time. (Schneider et al., 2015)

Casutt, Konrad and Schuepfer investigated the changes in ROTEM parameters 2.5 h after 10 mg rivaroxaban in healthy male adults. The Extem and Intem clotting time (CT), Extem clot formation time (CFT) and Extem alpha angle changed significantly ($p<0.05$) after rivaroxaban. The numbers were small, but there was a visual correlation between Extem-CT and PT and between Intem-CT and APTT. (Casutt, Konrad & Schuepfer, 2012)

Adelman et al. investigated healthy volunteers using modified ROTEM to evaluate the effects of rivaroxaban. They showed a linear correlation between rivaroxaban concentration and CT and time to maximum velocity (tmaxVel), for low tissue factor (lowTF) and for pro-thrombinase induced clotting time modified ROTEM analyses. Low TF-ROTEM CT and tMaxVel had high sensitivity and specificity for detecting rivaroxaban to a concentration level cut off point at 0.2 µg/ml. (Adelmann et al., 2013) A study by Korber and colleagues showed that rivaroxaban increased Extem-CT in healthy volunteers, in a concentration-dependent fashion, but did not correlate with quantitative rivaroxaban levels. The addition of recombinant FVIIa (but not PCC) decreased Extem-CT, although this reached statistical significance only in the therapeutic rivaroxaban concentration samples. (Körber et al., 2013)

One study on ten men with an ischaemic stroke from Texas, USA, taking rivaroxaban investigated the effects of rivaroxaban 20 mg once a day on thromboelastography. It demonstrated a decrease in all parameters measuring clot strength and increase in time to clot formation, within 2-4 h. All TEG™ parameters normalised at 18 h post rivaroxaban, except the time to clot firmness at 2 mm, suggesting some element of ongoing inhibition of factor Xa. (Bowry et al., 2014)

Oswald and colleagues investigated the comparative effects of 10 mg rivaroxaban and 40 mg enoxaparin on ROTEM parameters in patients who were post-orthopaedic surgery. (Oswald et al., 2014) The samples were taken 16 h post rivaroxaban dose, thus, at the trough and at 4h post enoxaparin dose, thus, at the peak. The Extem-CT in the rivaroxaban group was prolonged as compared to the normal reference values, to the enoxaparin group and to the baseline value. In contrast, Intem-CT, although it was increased in both groups, was not above the normal reference and was not significantly different between rivaroxaban and enoxaparin groups. The baseline values of CFT, MCF and alpha angle changed significantly after taking rivaroxaban or enoxaparin. (Oswald et al., 2014) Oswald suggests that Extem-CT can differentiate direct Xa inhibitors from other anticoagulants.

Herrmann and colleagues used ROTEM and CAT to investigate the effects of rivaroxaban in vivo, in patients taking 10 mg rivaroxaban for primary VTE prophylaxis post orthopaedic surgery. (Herrmann et al., 2014). In contrast to Oswald et al., none of the ROTEM parameters, in this study, were significantly altered by rivaroxaban. (Herrmann et al., 2014) Casut, Konrad and Schuepfer showed a slight correlation between CT-extem and PT and between CT-intem and aPTT, but there were a significant number of patients taking rivaroxaban 10 mg once a day, who had normal ROTEM parameters. (Casutt, Konrad & Schuepfer, 2012)

To date, there has only been two published studies investigating the applicability of ROTEM to monitor the effects of rivaroxaban on VTE patients. The first study involved 13 patients taking rivaroxaban 20 mg once a day. At around the peak of rivaroxaban concentration (time 2.5 h post dose), Extem-CT was prolonged, together with PT and at the trough concentrations (24 h post-dose) there was residual anti-Xa activity but no prolongation of Extem-CT. (Chojnowski et al., 2015) The second study involved 121 consecutive VTE patients taking rivaroxaban once daily. Levels were measured together with ROTEM analysis at 3-5h post-dose. There were significant positive correlations between rivaroxaban concentration and both PT (r=0.796), APTT (r=0.425) and CT (r=0.328). However, this correlation was not sufficiently large to be a useful measure of the anticoagulant effect of rivaroxaban. (Fox et al., 2016)

Rotational Thromboelastography (rTEG^{TM}) is a similar investigation to ROTEM and has been used to detect rivaroxaban. A study in healthy volunteers revealed a lack of sensitivity of rTEG to the various direct oral anticoagulant medications, until ecarin was added, which resulted in a dramatic shortening of the R time for those volunteers taking antiXa (rivaroxaban) compared to dose-proportional shortening for those taking anti-thrombin (dabigatran). (Dias et al., 2015)

The results from this study are consistent with previous published data from VTE patients taking rivaroxaban, which differs from healthy volunteers, probably due to the pro-thrombotic nature of the patient's blood compared to healthy adults. This study is consistent with published data on patients taking rivaroxaban, demonstrating that Extem-CT is the ROTEM parameter which most closely correlates with rivaroxaban concentration. The Extem-CT levels at the trough time points in this study were still elevated above the Ki (0.4 nM) range (Oswald et al., 2014) thus, this parameter is sensitive to the residual effects of rivaroxaban 15h post-dose. However, the baseline ROTEM parameters for patients in this study, prior to the commencement of rivaroxaban, were not collected, thus, no conclusions regarding the effect of rivaroxaban on the ROTEM parameters for an individual, can be made.

The predictive value of ROTEM, or its use as a surrogate marker for the anticoagulation effect of rivaroxaban, in terms of their risk of recurrent VTE or risk of bleeding, or determining the level of anticoagulation achieved, is still undetermined, however, it is a useful test to demonstrate how profoundly anticoagulated (over- or under) a patient who is taking rivaroxaban is.

4.6.5 SUMMARY OF THE PHARMACODYNAMIC EFFECTS OF RIVAROXABAN

The most reliable, accessible and available pharmacodynamic test to detect the anticoagulant activity of rivaroxaban, currently, is PT. However, the global, in vivo anticoagulant activity of rivaroxaban is not reflected by PT. This study, along with others in the literature, has shown that CAT ttPeak and velocity index parameters and ROTEM clotting time parameters, correlate strongly with rivaroxaban concentration. Besser and colleagues, 2008, demonstrated that CAT ETP has a predictive value in determining the chances of recurrent, unprovoked VTE. Our study did not investigate clinical outcomes but this research question should be addressed in the future.

CHAPTER 5. POPULATION PHARMACOKINETIC MODELLING OF RIVAROXABAN

Chapter 5 focuses on the use of non-linear effects modelling to create a population pharmacokinetic model for rivaroxaban, which is then used to determine which co-variates affect certain pharmacokinetic parameters. The specific question of this study 'does weight influence the pharmacokinetic parameters of rivaroxaban?' is addressed in this chapter. The results will be discussed in the context of previously published work.

5.1 INTRODUCTION

Population analysis is a set of statistical techniques that can be used to learn about the typical response in a population to a particular drug, as well as the variability in response that arises from different factors. Population analysis aims to describe data, which may be sparse, arising from a group of individuals. Data is borrowed from the individuals in the group, and the influence of certain characteristics and of unexplained variability, on the PK parameters, can be quantified. Population modelling is a tool used to identify and describe relationships between a subject's physiological characteristics and observed drug exposure or response. The population PK model describes the relationship between rivaroxaban concentration and time. It requires accurate information on dosing, measurements and covariates. The model consists of three components: the structural model, which describes the time course of a measured response, the stochastic model, which describes the variability or random effects in the observed data, and the covariate model, which describes the influence of factors such as demographics or disease on the individual time course of the response.

5.1.1 PHARMACOKINETIC MODELS FOR RIVAROXABAN PUBLISHED TO DATE

To date, several pharmacokinetic (PK) studies have been conducted by the manufacturers of rivaroxaban, as summarised in table 17. Mueck and colleagues defined the population PKPD model for healthy male volunteers using data from a phase one trial of rivaroxaban, utilising non-linear mixed effects modelling, NONMEM. (Mueck et al., 2007) The conclusions were that rivaroxaban has a predictable, dose-proportional PK and PD, with a linear correlation between PT and rivaroxaban concentration. (Mueck et al., 2007)

Muek and colleagues subsequently collated data from patients enrolled in two phase 2b studies investigating rivaroxaban for the thromboprophylaxis following total hip replacement. (Mueck et al., 2008) The results were pooled and NONMEM was used to analyse the pharmacokinetic profile. A one-compartment model with was

found to represent rivaroxaban pharmacokinetics (table 17). Volume of distribution, Vd, of rivaroxaban was affected by body weight and clearance, CL, was affected by the study day, age, renal function, serum albumin and haematocrit, (table 17). (Mueck et al., 2008) Rivaroxaban clearance decreased by: 1 % each year after age 66; 0.3 % per 1 ml/min less than the median creatinine clearance; 2.2 % per 0.1 g/dl less than the median albumin; and 1.2 % per 1 % less than the median haematocrit. The effects of these mechanistic, biochemical parameters on the pharmacokinetics of rivaroxaban are important and would be expected to affect the pharmacodynamics of rivaroxaban at the extremes of these parameters.

NONMEM was employed to simulate rivaroxaban concentration-time profiles after a 10 mg dose for patients of extreme age, weight and creatinine clearance. The pharmacokinetics of these patients fell within the predicted 90 % confidence intervals, thus, the effects of these factors on rivaroxaban plasma concentration was not shown to differ significantly from a patient of average age, body weight or creatinine clearance. A decrease of 6.4% Vd per 0.1m^2 below median body surface area, a decrease of 1% CL per year older than 66, a decrease of 0.3% CL per 1ml/min less than the median CrCL, a decrease of 2.2% CL per 0.1g/dl less than median albumin and a decrease of 1.2% per 1% haematocrit less than the median haematocrit (table 17). (Mueck et al., 2008a) There were no significant differences between the once a day or twice a day dosing regimens in terms of Cmax or Ctrough, and both Cmax and Ctrough linearly correlated with PT. (Mueck et al., 2008a)

Mueck and colleagues later analysed the data from two phase 2 studies, OD1Xa-DVT and EINSTEIN DVT, assessing the efficacy and safety of rivaroxaban for the treatment of acute DVT, in a population PK model (table 17). They concluded that the pharmacokinetics of rivaroxaban were predictable and consistent across all doses evaluated. Only creatinine clearance below 50 ml/min affected the area under the concentration-time curve and maximum concentration for AF patients taking 15 mg or 20 mg once a day. Renal function and age affected the clearance and body weight, age and gender affected the volume of distribution, but these were within the limits of observed inter-individual variations in this population. (Mueck et al., 2011; Kubitza et al., 2013)

The impact of impaired renal function, together with certain drugs affecting the hepatic metabolism pathway of rivaroxaban, was investigated by Grillo et al, 2012. There was a demonstrable increase in rivaroxaban exposure in patients taking erythromycin, a P-gp and CYP3A4 inhibitor and with impaired renal function. (Grillo et al. 2012)

There have been many studies investigating the effects of rivaroxaban on patients with atrial fibrillation, AF. A large, randomised, multicentre double-blind phase III study with patients with non-valvular AF at moderate risk of a stroke has shown PK rivaroxaban to be represented by an oral one compartment, first order absorption rate constant. The PK outcomes were CL 6.1 L/h and Ka 1.24 /h. Age caused a 1.1% decrease in CL per year increase from the median, Cr caused a 1.9% decrease in CL per 0.1 mg/dL increase from the median and LBW caused a 1.2% increase in Vd per 1kg increase from the median. (Girgis et al., 2014)

Subsequent studies, since these population PKPD and dose-finding studies, have shown minimal variation in plasma concentration of rivaroxaban for patients of differing weight, age, renal function and dosing regimen. (Bayer Shering Pharma http://www.ema.europa.eu/docs/en_GB/document_library; Arcelus et al., 2014)

The aim of this study was to develop a population pharmacokinetic model of rivaroxaban to assess whether body weight has an impact on rivaroxaban drug exposure. This study describes the population-PK model development and evaluation for rivaroxaban and the results reveal the outcomes in terms of which mechanistic and descriptive factors influence the PK of rivaroxaban. NONMEN software allows simultaneous evaluation of data from all individuals in a population. Nonlinear refers to the fact that the dependent variable is nonlinearly related to the model parameters and independent variables. Mixed effects refers to the mixture of fixed and random effects parameterisation. NONMEN brings data and models together, discovering the population pharmacokinetic parameters for the structural, statistical and covariate models which describe the data, using an estimation model and finding the sources of variability in the population.

TABLE 17 SUMMARY OF THE ORTHOPEADIC PRIMARY PROPHYLAXIS (*) AND THE VTE TREATMENT (#) STUDIES FOR RIVAROXABAN PHARMACOKINETICS

CrCl creatinine clearance, AUC area under the curve, PT prothrombin time, Cmax maximum effect at 50% concentration, PK pharmacokinetic, PD pharmacodynamic, Vd volume of distribution, T1/2 half life

Study	Dose of rivaroxaban and comparator	Methods	Kinetic Findings	Compartment	Conclusions
***Kubitza et al. (2006)**	10 mg rivaroxaban vs placebo	Single-blinded, randomised	Age impacts CrCL (AUC α 1/CrCL). antiXa α PT. >75 yr increase AUC but no change C_{max}. All parameters normalise to within 10% of baseline, within 24h post dose.	N/A	Increasing age decreased CrCL. Rivaroxaban anticoagulant effects were short lived.
***Kubitza et al. (2007)**	10 mg rivaroxaban vs placebo	Single-blind, randomised, placebo-controlled	Body weight <50 kg result in increased C_{max} by 24 % & increased PT by 15%, AUC unchanged. Body weight >120kg C_{max} & AUC unaffected.	N/A	Effects were more pronounced in low body weight.
***Mueck et al. (2007)**	5 mg od vs bd vs tds vs 10 mg bd vs 20 mg bd vs 30 mg bd	Phase I multiple ascending dose study, using NONMEM	Linear correlation antiXa & PT.	Oral two compartment model with first-order absorption & elimination.	Predictable, dose-proportional PK & PD.
***Mueck et al. (2008a)**	10 mg od vs 10 mg bd	Phase II double-blind, randomised studies, using NONMEM	Cmax 125 ng/ml C trough 9 ng/ml AUC 1170 ng.h/ml Vd 58 l CL 5-8 l/h Linear correlation antiXa & PT. 90% of intervals of 10 mg od antiXa levels overlapped with 10 mg bd intervals.	Oral one compartment model.	Age, renal function, albumin, haematocrit & body surface area influenced rivaroxaban CL. PK & PD were predictable with od & bd doses.
#Kubitza et al. (2010)	10 mg rivaroxaban od	Two-centre non-blinded cohort study, matched by age & gender	AUC for concentration-time & for PT-time increased as renal function decreased.	N/A	Rivaroxaban CL decreases with decreasing renal function.
#Mueck et al. (2011)	15 mg od versus 15 mg bd	Population PK study of rivaroxaban in patients in 2 phase II studies	Cmax 270 ng/ml Ctrough 25 ng/ml AUC 2870 ng.h/ml Vd 68.7 l CL 7.16 l/h Linear correlation antiXa & PT AUC increased dose dependently.	Oral one compartment model.	Predictable PK. Age & renal function influenced CL. Vd was influenced by weight, age & sex.
***Kubitza et al (2013)**	10 mg rivaroxaban od vs placebo	Single-blind, randomised study, effects of age & gender on the PK of rivaroxaban	CrCL decreases with age AUC were 41% higher in the elderly than young ($p<0.001$). AUC was slightly lower in females. No change in C_{max}, or T1/2.	N/A	Increasing age decreased CrCL. No effect of gender.

5.2 METHODS

5.2.1 RIVAROXABAN RECORD BOOKLET

All participants in this study were asked to complete a rivaroxaban record booklet indicating the times and dates of any missed doses and the times, dates and amounts of rivaroxaban taken during the weeks prior to blood sampling. This information was crucial for inputting data into NONMEM (version 7.1.0) and coding the software to analyse the results according to time after dose, thus developing a pharmacokinetic model for rivaroxaban.

5.2.2 DATA ENTRY AND FORMATTING

In total, 101 study participants taking rivaroxaban provided 193 raw data points from this study. The dose, timings, demographic and laboratory information were entered into a microsoft excel spreadsheet. This spreadsheet was converted into a comma delimited ('.csv') file and specifically formatted to be compatible with the code used to instruct NONMEM (version 7.1.0) software to analyse the data. (Beal et al., 1998)

The majority of antiXa levels determined from the samples, were at time points less than three hours post rivaroxaban dose and the majority of orthopaedic patients provided one or two samples, as compared to VTE patients, who provided two or three samples.

Anti-Xa levels which were below or above the limit of quantification (BLQ or ALQ) were determined to be <10% of the total data. BLQ and ALQ data are a valuable source of information and when used in the PK analysis, aid in defining the model and increase the precision with which the parameters can be estimated.

The options within the pharmacokinetic discipline, for handling this type of data were to: remove them from the data set; fix them all to the value limit; set them to half the BLQ value; use the M3 error method, as described by Beal (Beal, 2001); or use the absolute values recorded on the analyser, which may include negative values. Keizer and colleagues have demonstrated that when BLQ is incorporated as a continuous dataset and represents less than 10% of the total data, all BLQ methods showed a similar performance. (Keizer 2010) A more recent study by Keizer and colleagues evaluated the methods above and for the linear one compartment model, parameters CL and Vd, no systematic bias was observed by any of the methods where BLQ was less than 10% or even up to 20%. The M3 method showed the highest variation in estimates. The 'all data' incorporation method gave the lowest RMSE values and gave the most stable model estimation. However, the results for 'all data', set to half the BLQ and the M3 method, were comparable if the BLQ were less than 10%, as in our data set. (Keizer et al., 2015)

The decision was made, based on the fact that there were <10 % of values that fell BLQ, to report values <20 ng/ml as 10 ng/ml, unless the absolute data value was known, in which case it was entered as the absolute value. If the analyser reported the values as <20 ng/ml and the absolute values were not available, then the result was recorded as half the BLQ (10 ng/ml). In the cases when the ALQ was reported as > 500 ng/ml and no absolute value was available, the recorded value was 500 ng/ml.

5.2.3 DEVELOPMENT OF RIVAROXABAN STRUCTURAL BASE MODEL

The methods used to approximate the population PK/PD model have been developed including the first-order with conditional estimation (FOCE) method and the FOCE method with interaction (FOCEI). The FOCE methods allow for hypothesis testing during model building and generally produce less biased model parameter estimates. Several structural base models were explored, with regards to the best fit for the data, obtained from this study. The first order conditional method with interaction in NONMEM was used to estimate the base model. Statistical improvement in the fit of the model to the data was performed with the objective function. Finally, the precision of the parameter estimates and residual variability, were assessed. Several one compartment base models were explored with random effects (ETA's) placed on clearance (CL), volume of distribution (Vd), and absorption rate constant (Ka). A mix of additive and / or proportional error models were explored as part of the base model development. A non-parametric bootstrap was run on this base model as an additional investigation to further confirm the statistical properties of the base model.

5.2.4 EVALUATION OF RIVAROXABAN BASE MODEL

Once a model has been fit to the pharmacometric data, it is crucial to evaluate the goodness of that fit. Initially, the performance of the model was visually assessed using the standard goodness of fit plots. These include population /individual observed versus population / individual predicted concentrations of rivaroxaban. Goodness of fit plots were used to visually assess the statistical improvement in the fit of the model to the data. The goodness of fit plots assess the residual difference between the observed function (minus twice the log-likelihood of the data) and the predicted values, as determined by the precision of the parameter estimates of the model and the residual variability. A residual is the difference between observed and model predicted values. (Bonate 2005) Positive residuals indicate the model under-predicting the actual observations, whereas negative residuals indicate that the model is over-predicting observations. Residuals are assumed to be normally distributed, with a mean of zero. An unbiased model would have a mean value near zero and residuals less than two would be considered good. Graphical examination of systematic trends of residuals provides a valuable insight into how

well a particular model is performing. A model which is describing the data well, would have population / individual observed versus population / individual predicted concentrations, when plotted, being a mirror image of one another, with no systemic trends and the line of unity running through the centre of the points.

The NONMEM user guide suggests the use of the weighted residuals (WRES), the weighted difference between the model prediction and the data, as one model diagnostic to evaluate model misspecification. However, the shift in using the first order with conditional estimation (FOCE) in NONMEM has led to a new diagnostic residual tool, the conditional weighted residuals (CWRES). The CWRES are calculated in a similar manner to the WRES but have the advantage of being directly related to a term in the objective function used in the FOCE method of model fitting, thus giving more detailed information about the fit of a model to data.

As part of a residual analysis, histogram analysis of residuals, in this case CWRES, exhibiting a normal distribution, provide further evidence that a robust base model has been developed. Finally, as well as evaluating the model estimates for the pharmacokinetic parameters in question and whether the estimates are reasonable (subjective), a non-parametric bootstrap was applied in order to learn about the statistical properties of the distribution of the data and to quantify uncertainty in the parameter estimates, i.e. median and standard errors. (Parke et al., 1999)

The base model was chosen and taken forward for full covariate analysis.

5.2.5 PARAMETERISATION OF THE BASE MODEL WITH COVARIATES

Modelling of the pharmacokinetics of a drug is dependent on the relative influences of covariates. As many potential covariates as possible were identified from previous research studies and these were investigated to ascertain how much they could be impacting upon the inter-subject variability in the pharmacokinetics of rivaroxaban. The dosing schedule of rivaroxaban may need to be adjusted for a certain covariate for patients with an extreme value, depending on the degree of influence which that particular covariate has on the pharmacokinetic parameter.

The final model includes those covariates which have been identified as having a relevant influence on the pharmacokinetic parameter, by statistical tests, assessment of the biological, mechanistic and clinical relevance and prior knowledge of the modelled system. The model which was developed for rivaroxaban pharmacokinetic behaviour in this study incorporated the covariates which underlies the biological behaviour of rivaroxaban, for example creatinine clearance (CrCL) was identified as a relevant covariate for rivaroxaban clearance because

rivaroxaban is known to be partially renally cleared, thus, CrCL is a mechanistic co-variate. A graphical analysis initially identified trends of influence by certain covariates. A full covariate analysis was done by adding each covariate as a univariate, stepwise addition, to the base-model of the PK parameter.

5.2.6 Evaluation of the final model

The first order conditional method with interaction in NONMEM was used to estimate the final models. The final model developed was further assessed using non-parametric bootstrapping to evaluate the statistical properties of the model. Following the inclusion of the covariates into the final model, the difference in variability in the parameter was evaluated and checked visually with a visual predictive check (VPC).

5.2.7 Visual Predictive Check (VPC)

The visual predictive check (VPC) graphically assessed the predictive capability of the final model. The observed data was plotted against an independent variable, such as time, to determine if the central trend and variability were able to be reproduced by this model. (Bergstrand et al. 2011)

5.3 Results

5.3.1 Base model

Population models provide a means of characterising the extent of between-subject (the differences in exposure form one patient to another) and between-occasion (the differences between dose exposures within the same patient) variability. Unexplained variability includes the variability between the parameter values of a subject and the population value of that parameter (BSV), and the unexplained residual variability (RUV). Random effects are represented by ETA, reflecting the difference between the subject's parameter value and the population parameter value.

Covariates which explain variability are identified and the influence on BSV is quantified. Several one compartment base models were explored with ETAs (random effects) placed on the covariates clearance, ETA1, volume of distribution, ETA2, and concentration constant, ETA3, with a mix of additive and / or proportional error models. The base model was developed from a one compartment model. The base model which was selected to be the best was: rivbasemodel3blq, which had ETA on CL and Vd, and omega block for CL and Vd, with a combined error model.

5.3.1.1 GOODNESS OF FIT EVALUATION OF THE BASE MODEL

Standard goodness of fit plots were produced to graphically evaluate and further confirm the appropriateness of this one compartment base model (figures 17 and 18). The straight lines represent the lines of identity and the curved lines represent the linear regression line.

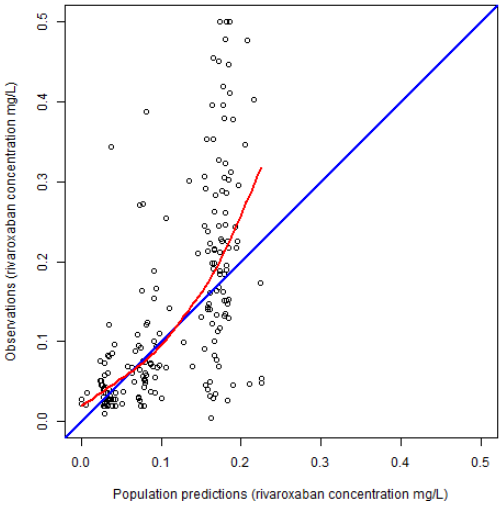

FIGURE 17 OBSERVED VERSUS POPULATION PREDICTED RIVAROXABAN CONCENTRATIONS, USING THE BASE MODEL DEVELOPED

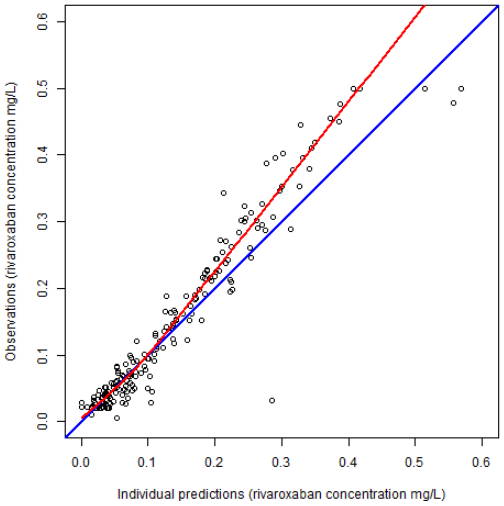

FIGURE 18 OBSERVED VERSUS INDIVIDUAL PREDICTED RIVAROXABAN CONCENTRATIONS

Figure 17 demonstrates that the base model predicts the actual observed rivaroxaban concentrations at low and average values, but it tends to under predict at high concentrations. The observed and individual predicted graph lines generally follow the same linear correlation, with a slight deviation at higher concentrations, illustrating that this model is under-predicting rivaroxaban concentrations only at higher actual concentrations, otherwise it accurately predicts rivaroxaban concentrations (figure 18).

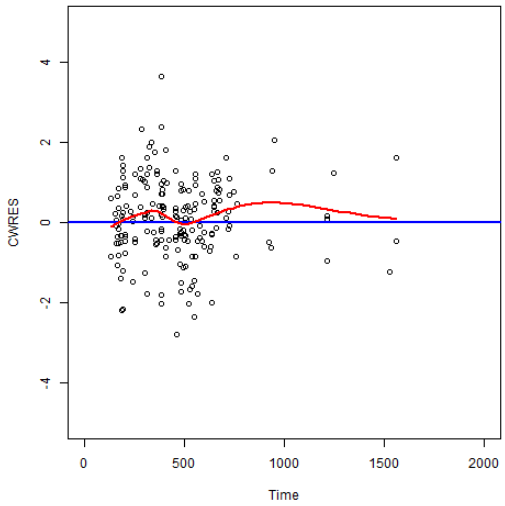

FIGURE 19 CONDITIONAL WEIGHTED RESIDUALS (CWRES) VERSUS POPULATION PREDICTIONS RIVAROXABAN CONCENTRATIONS

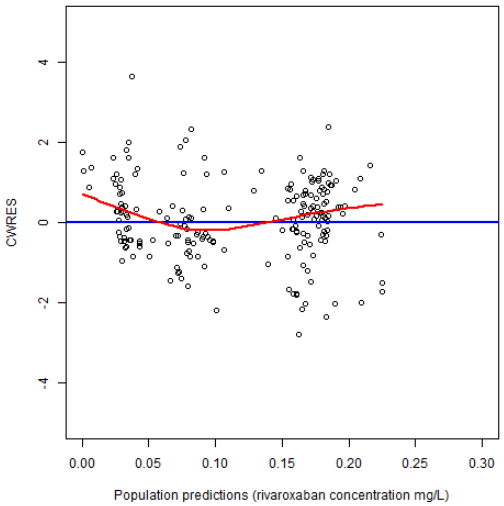

FIGURE 20 CONDITIONAL WEIGHTED RESIDUALS (CWRES) VERSUS TIME AFTER RIVAROXABAN DOSE

At the extremes of the population prediction rivaroxaban concentrations, the conditional weighted residuals slightly deviate from zero but otherwise they follow the zero CWRES line of identity (figures 19 and 20). This suggests that this one compartment model accurately describes the data.

Figure 20 illustrates the goodness of fit of the base model to the observed data for time after dose, thus, confirming that this base model was a stable base model to take forward in the population PK evaluation.

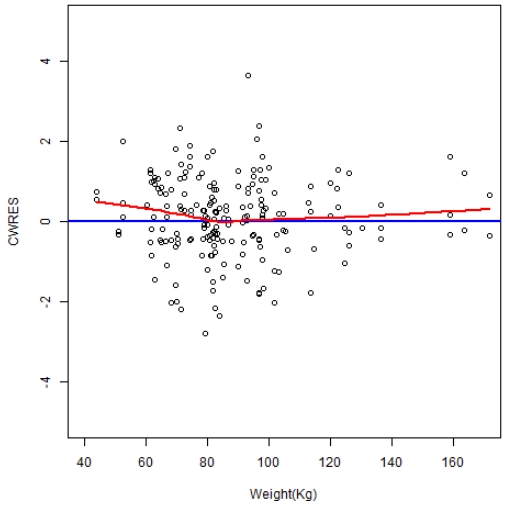

FIGURE 21 CONDITIONAL WEIGHTED RESIDUALS (CWRES) VERSUS BODY WEIGHT (BW)

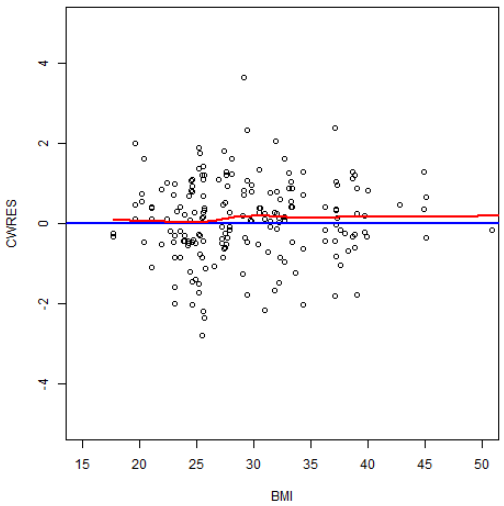

FIGURE 22 CONDITIONAL WEIGHTED RESIDUALS (CWRES) VERSUS BODY MASS INDEX (BMI)

The red lines follow the blue lines, thus the parameter values follow the CWRES for both body weight and BMI graphs (figures 21 & 22). In both the body weight and LBW graphs CWRES deviate slightly at the lower extremes of weight (figures 21 and 23).

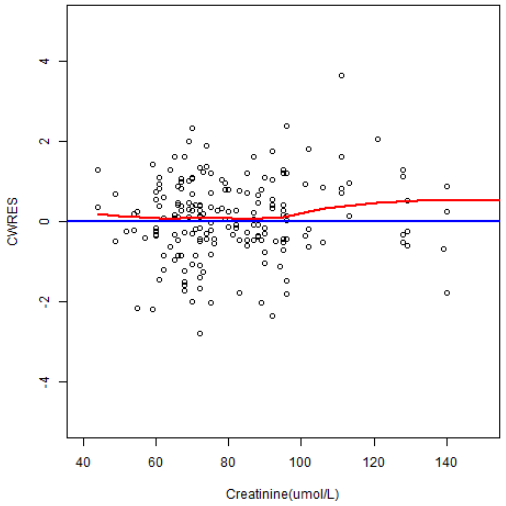

FIGURE 23 CONDITIONAL WEIGHTED RESIDUALS (CWRES) VERSUS SERUM CREATININE

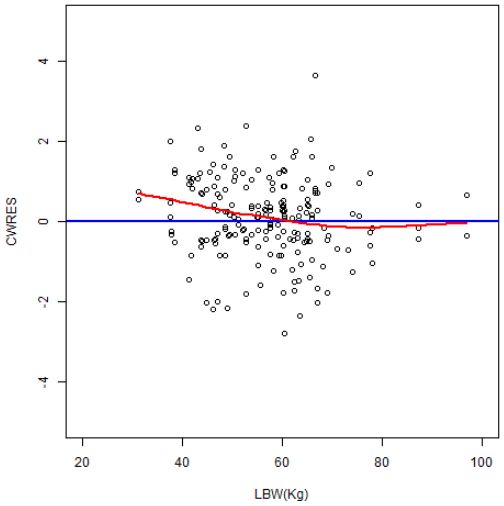

FIGURE 24 CONDITIONAL WEIGHTED RESIDUALS (CWRES) VERSUS LEAN BODY WEIGHT (LBW)

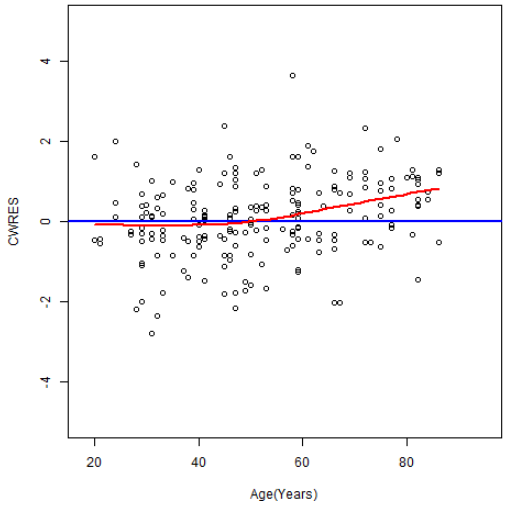

FIGURE 25 CONDITIONAL WEIGHTED RESIDUALS (CWRES) VERSUS AGE

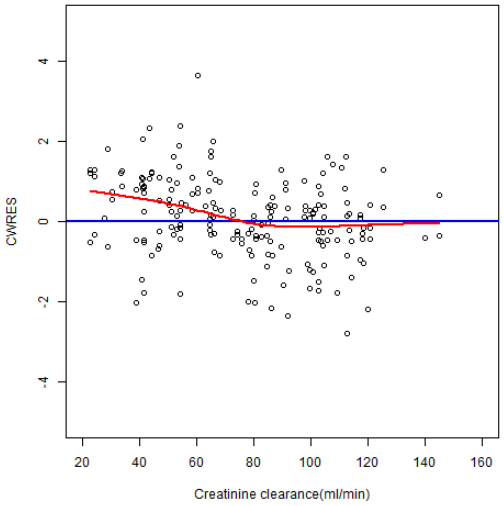

FIGURE 26 CONDITIONAL WEIGHTED RESIDUALS (CWRES) VERSUS CREATININE CLEARANCE (CRCL)

CWRES actual values fell within the predicted for most values of creatinine and creatinine clearance, except at the upper extreme of creatinine, which corresponded to the lower extreme of creatinine clearance, where there was some deviation (figures 23 and 26).

At the upper extreme of age, the CWRES deviated from the line of identity slightly, but is still considered a good fit because this base model captured most of the observed data, thus was considered a stable model to use in the subsequent modelling process (figure 25). Since creatinine depends on age, and creatinine clearance depends on age and creatinine, these plots are expected to mirror each other slightly (figures 23, 25 and 26).

The two pharmacokinetic parameters which were chosen for the population modelling, were clearance (CL), ETA1, and volume of distribution (Vd), ETA2.

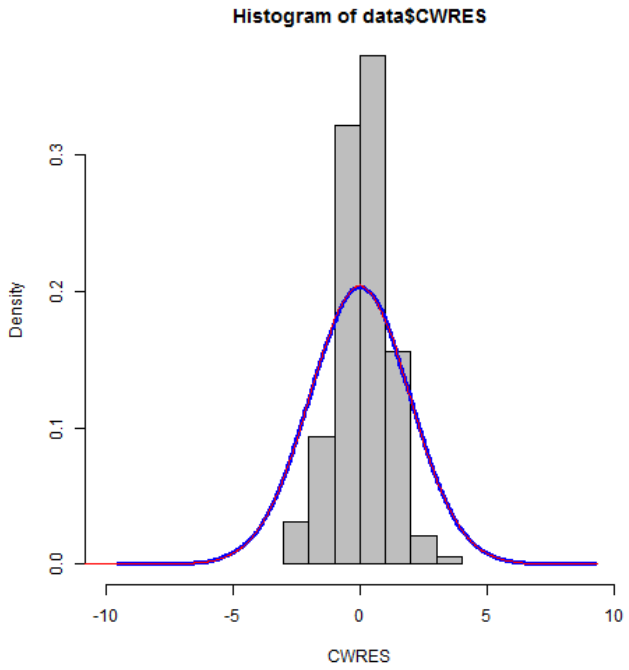

FIGURE 27 HISTOGRAM OF CONDITIONAL WEIGHTED RESIDUALS (CWRES)

The blue line in figure 27 represents the actual standard deviation of CWRES, and this is coincident with the red line, which represents the SDs of CWRES, as determined from the base model, thus, all of the observed data are incorporated into this one compartment base model. This confirms that this base model is stable and good to use subsequently for the covariate analysis.

5.2.2 COVARIATE ANALYSIS

A full covariate analysis was performed, by adding the mechanistic covariate being considered in a univariate fashion to the PK parameter. The mechanistic covariates which were tested were age, ethnicity, body weight, lean body weight, BMI, creatinine and creatinine clearance. Following the univariate analysis of these co-variates, only CrCL on CL (BLQ7) was shown to be significant, thus, was taken forward. The change in the base model objective function with the addition of creatinine clearance covariate caused a -14.65 change in CL, age caused -6.91 and LBW caused -1.45. Volume of distribution was not significantly changed by any of the covariates tested.

A full covariate graphical analysis was performed to confirm these findings. The graphical analysis of the random effects of clearance (ETA1) versus the mechanistic covariates selected, are illustrated in figures 28A and 28B. These show a slight trend with creatinine clearance to increase to a plateau then decrease as these parameters increase. No trend was observed with age, thus, this does not appear to affect the clearance of rivaroxaban (figures 29A and 29B).

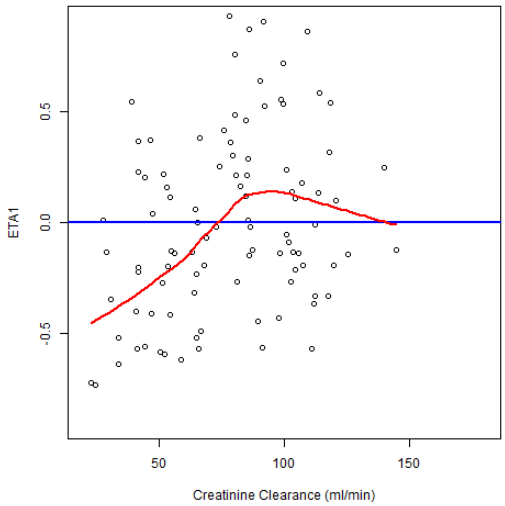

FIGURE 28A ETA1 (CL) VERSUS CREATININE CLEARANCE BEFORE

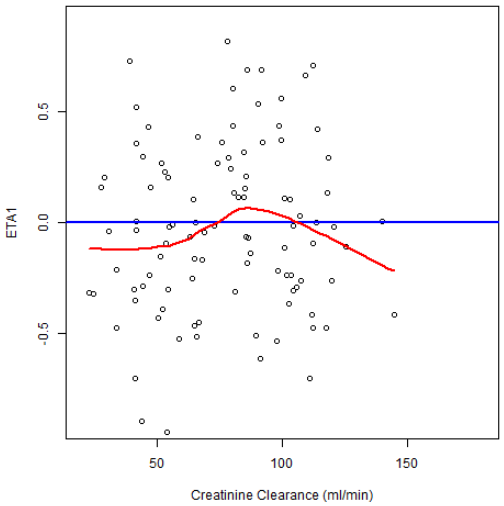

FIGURE 28B ETA1 (CL) VERSUS CREATININE CLEARANCE AFTER

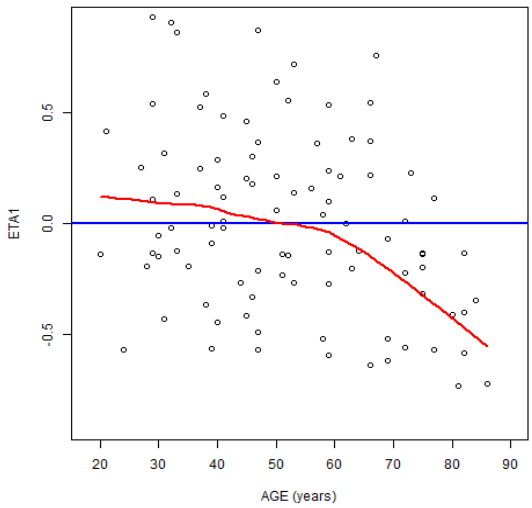

FIGURE 29A ETA1 (CL) VERSUS AGE BEFORE

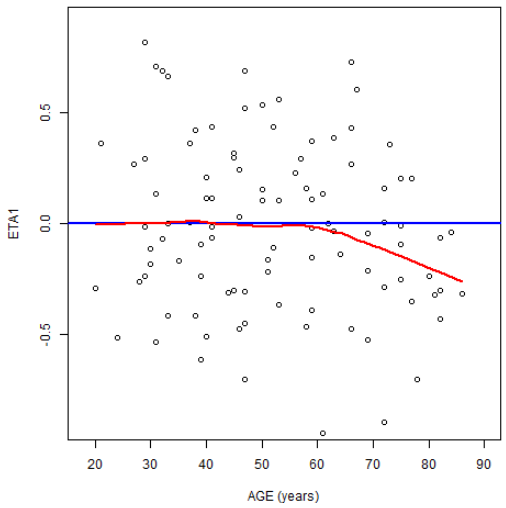

FIGURE 29B ETA1 (CL) VERSUS AGE AFTER

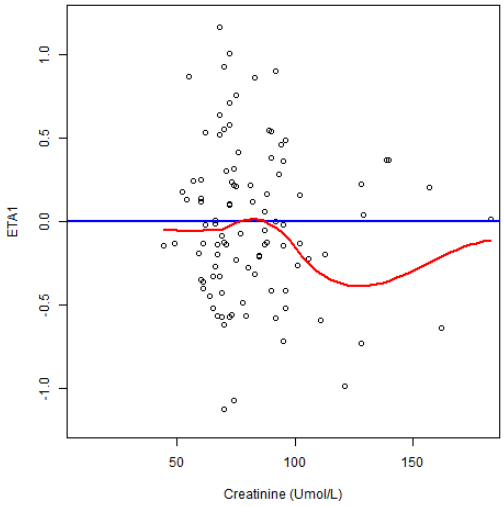

FIGURE 30A ETA1 (CL) VERSUS CREATININE BEFORE

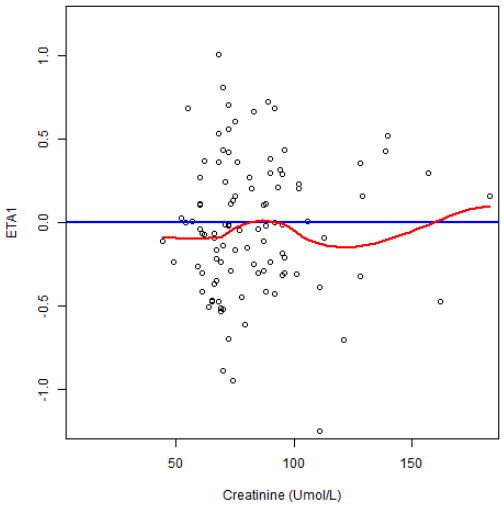

FIGURE 30B ETA1 (CL) VERSUS CREATININE AFTER

Graphical analysis from figures 31-32 reflect the trends of the selected covariates on the random effects of volume of distribution (ETA2). Creatinine clearance shows a trend for increasing volume of distribution to a peak then decreasing as creatinine clearance increases (figures 31A and 31B). Age does not seem to affect the volume of distribution of rivaroxaban (figures 32A and 32B).

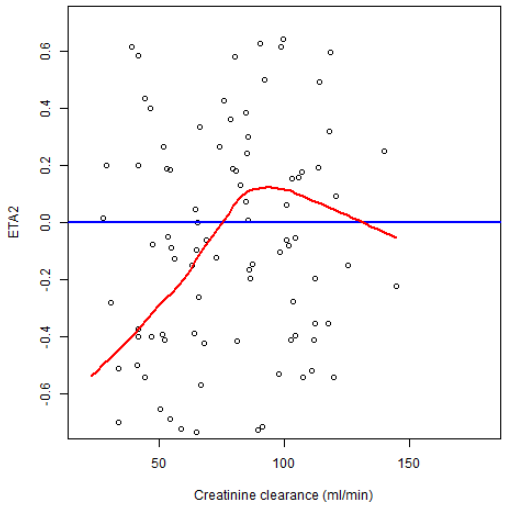

FIGURE 31A ETA2 (VD) VERSUS CREATININE CLEARANCE BEFORE

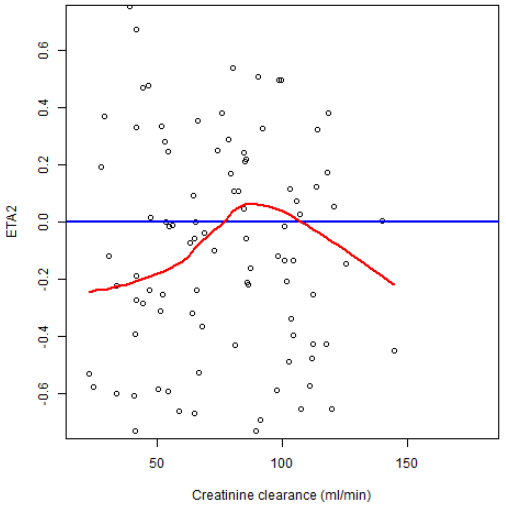

FIGURE 31B ETA2 (VD) VERSUS CREATININE CLEARANCE AFTER

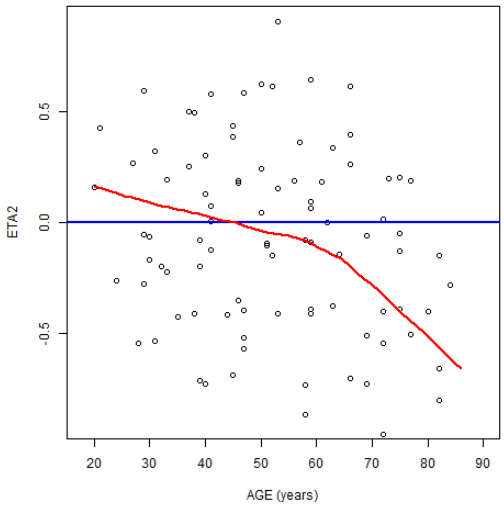

FIGURE 32A ETA2 (VD) VERSUS AGE BEFORE

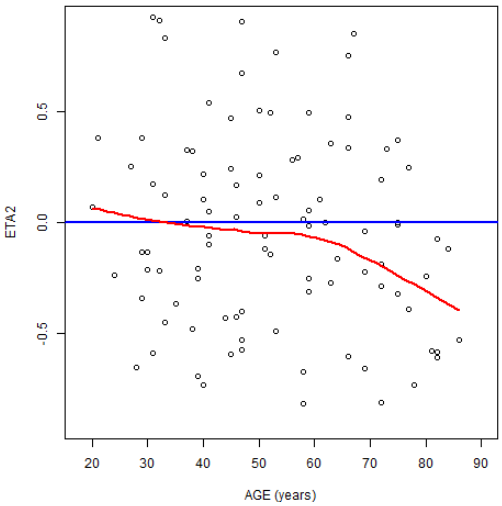

FIGURE 32B ETA2 (VD) VERSUS AGE AFTER

The final model was created, based on the stability and confirmatory tests of the one compartment base model, using the covariate estimates, as illustrated in table 18.

TABLE 18 COVARIATE ESTIMATES FOR A ONE COMPARTMENT MODEL FOR RIVAROXABAN PHARMACOKINETICS

Parameter	One compartment base model (SE %)
CL (l/h)	8.59 (7)
Vd (l)	104 (13)
Ka (/h)	1.32 (24)
ω_{CL} (%CV)	56 (25)
ω_{Vd} (%CV)	66 (52)
Proportional error (%)	28.6 (84)
Additive error (ng/mL)	0.018 (38)
Objective function	-853.7

The final model, taken forward, was evaluated with a nonparametric bootstrap. The bootstrap analysis was done on the CrCL and CL model and the parameter estimates produced are tabulated in table 19. The bootstrap analysis shows that this one compartment with CrCL on CL model was the best model.

TABLE 19 FINAL PK MODEL USING BOOTSTRAPPING TO EVALUATE THE STATISTICAL SIGNIFICANCE OF THE COVARIATE MODEL

Parameter	Estimate from final co-variate model (SE %)	1000 Bootstrap median (2.5th-97.5th) from the co-variate model
CL (l/h)	8.86 (7)	8.57 (7.58-9.79)
Vd (l)	101 (12)	95 (75.3-125)
Ka (/h)	1.21 (34)	1.21 (0.72-2.13)
ω_{CL} (%CV)	48 (99)	64.5 (53.8-78.2)
ω_{Vd} (%CV)	60 (247)	82.1 (38.7-96.1)
Proportional error (%)	31 (215)	52.7 (5.62-67.2)
Additive error (ng/mL)	0.016 (112)	0.016 (0.006-0.025)
Objective function	-868.4	/

5.2.3 NONMEM RESULTS OF BODY WEIGHT AND RIVAROXABAN PHARMACOKINETICS

Neither body weight nor BMI correlate with rivaroxaban concentration. Although the majority of the data points are in the lesser spectrum of the rivaroxaban concentration range, these plots are considered to be of sufficient data to enable an observed a trend, if it existed. The systematic trends in random effects error before inclusion of lean body weight or body weight as a covariate on clearance or on volume of distribution are not removed following inclusion of these covariates into the final model.

5.2.4 VISUAL PREDICTIVE CHECK OF THE FINAL PK MODEL FOR RIVAROXABAN

A final visual predictive check (VPC) was conducted to ensure the final model developed for rivaroxaban PK included all of the observed data. The concentration time graph developed from the observed data is shown as the red trace on figure 30. Simulated data is represented by the blue line. The 5th, 50th and 95th prediction intervals from the simulated concentrations were plotted against time after dose, in blue, with the observed data superimposed in red. The visual graphical representation of this final model does describe the observed data, thus, this model can be predictive for other data sets.

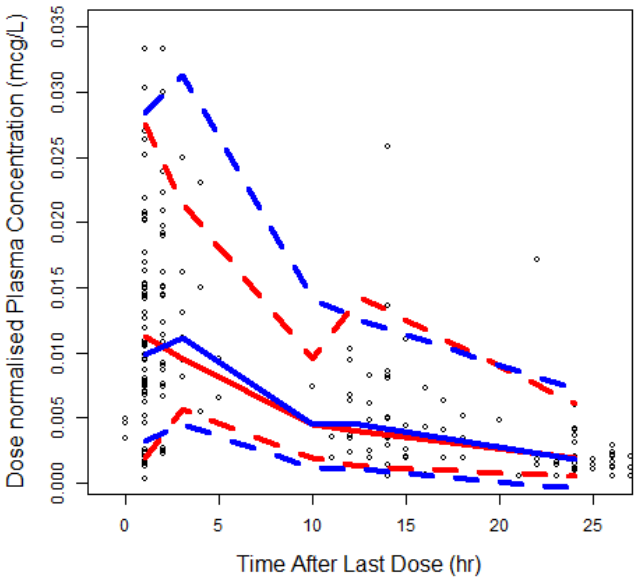

FIGURE 33 VISUAL PREDICTIVE CHECK OF DOSE NORMALISED PLASMA CONCENTRATIONS VERSUS TIME AFTER LAST DOSE

The final model parameter estimates were used to simulate the data sets. The red lines in figure 33 represent the 5th, 50th and 95th percentiles from the observed data and the blue lines represent the 5th, 50th and 95th percentile data form the simulated data set. The final model is describing the observed data, since the lines mostly overlap. The large variation in the dose normalised plasma concentration of rivaroxaban between ingesting the tablet and 2 hours post dose represents the large inter-patient variability in the absorption of rivaroxaban. From approximately two hours post dose until the trough, the inter-patient variability is less and the curves for predicted and observed follow each other closely (figure 33).

5.3 DISCUSSION

5.3.1 POPULATION PHARMACOKINETIC (PPK) STUDIES OF RIVAROXABAN, TO DATE

Mueck et al., 2008, determined that a one compartment model with parameters described in terms of first-order absorption rate constant, Vd and CL, best fit the PK of rivaroxaban for patients taking either 5 mg bd, 10 mg od or bd and 20 mg od or bd, of rivaroxaban post-orthopaedic operation. The maximum and minimum concentrations of rivaroxaban were not significantly affected by the dosing regime. The steady state CL was similar to this current study, although the Vd was less (table 20). (Mueck et al. 2008)

A population PK-PD study in patients being treated with rivaroxaban for DVT and an exposure simulation for patients with atrial fibrillation (AF), using samples from ODIXa-DVT and EINSTEIN-DVT studies, supported the evidence from the previous orthopaedic study. Rivaroxaban CL was influenced by age and renal function. Rivaroxaban Vd was influenced by age, body weight and gender. (Mueck et al. 2011)

Kubitza et al, 2013, specifically investigated the effect of age and gender on the pharmacokinetics and pharmacodynamics of rivaroxaban in a randomised, double-blind, placebo-controlled, parallel-group study, using 10 mg od rivaroxaban. AUC was 41% (p=0.001, 90% CI 1.2-1.6) higher in the group aged >75, which was attributable to the decreased renal elimination. Cmax rivaroxaban was not significantly different between the age groups and all pharmacodynamic parameters returned to baseline at 24 h. There was no difference in PK or PD parameters for gender. (Kubitza et al., 2013)

Girgis et al, 2014, used data from the ROCKET-AF population, taking either 15 mg od or 20 mg od of rivaroxaban, and compared them to the sparse PK and PD data from the phase II EINSTEIN studies on patients with DVT treated with rivaroxaban 20mg od, to create a population PK/PD model. NONMEM with first-order absorption constant was used. The concentration-PD relationships were consistent and correlated linearly with PT and antiXa levels. Age had a moderate effect on the baseline antiXa level and there was a near-linear correlation between PT and antiXa activity. This study supported previous studies showing that CrCL reduces AUC and Cmax, therefore the dose should be reduced in moderate renal impairment to 15 mg od. (Girgis et al., 2014)

TABLE 20 COMPARATIVE POPULATION PHARMACOKINETIC STUDIES OF RIVAROXABAN

Study & dose rivaroxaban	CL, l/h	Parameter effect on CL	Vd, l	Parameter effect on Vd
One compartment, first-order, 10 mg od, 15 mg or or bd, 20 mg od (current study)	8.57 (7.58-9.79~)	Renal function: CrCL caused -6.91 change in base model objective function CL	95 (75.3-125 95% CI)	No significant covariate
Two-compartment, first order Healthy males, 5 mg od, bd or tds, or 10 mg, 20 mg or 30 mg bd (Mueck et al., 2007)	9.2 (inter-individual variability 17%)	None were assessed	70 (residual variability 25%)	None were assessed
One compartment first order model Post-orthopaedic, 10 mg od or 10 mg bd (Mueck et al. 2008a)	7.51 (4.1*) Inter-individual variability 32.4 (23% CV)	Study day: 5.46 day1 Age: 1% / y > median Renal function: 0.3% / ml/min < median Serum albumin: 2.2% / 0.1g/dl < median Haematocrit: 1.2% decrease per 1% < median	58.2 (4.9*)	Body weight: 6.4% decrease / 0.1m^2 below median BSA
One compartment, first-order model, Non-valvular AF, 20 mg od & 15 mg od in moderate renal impairment (Girgis et al., 2014)	6.10 (3.9% #)	Age: -0.011 (26% #) Cr: -0.194 (34% #)	79.7 (6.1% #)	LBW: 0.0118 (32% #) Age: -0.0013 (187% #)
One compartment model DVT treatment, 15 mg bd or 20 mg od (Mueck et al. 2011)	7.16 (3.7% #) Inter-individual variability 39.9 (7.6% CV)	Age: -0.007 (15% #) 1.05% / y > median Cr: -0.269 (18% #) 1.94% / 0.1 mg/dL < median	68.7 (3.8% SE)	LBW: 0.0082 (17% #) 1.18% increase in Vd per 1 kg increase from median Age: -0.0049 (21% #) 0.13% decrease in Vd per 1 year increase from median

Cr , creatinine, CL clearance, Vd volume of distribution at steady state, LBW lean body weight, BSA body surface area, AF atrial fibrillation, DVT deep vein thrombosis, od once a day, bd twice a day, tds three times a day, **#** standard error,* residual standard error

A comparison of this study population modelling pharmacokinetic results and previous studies of patients taking rivaroxaban for DVT prevention, DVT treatment or AF, is summarised in table 20. The clearance, CL, of rivaroxaban is similar between studies, however, the volume of distribution, Vd, of rivaroxaban, as determined from this current study is larger than those determined from the previous studies. Perhaps this is a reflection that our patient population had a proportionally larger overall body weight compared to the previous studies.

Additionally, age was found to be an independent co-variate for both clearance and volume of distribution in the previous studies by Meuck et al., 2008a, Mueck et al., 2008b; Mueck at al., 2011 and Girgis et al., 2014) but not in this current study (table 20). Mueck and colleagues, 2011, determined lean body weight, LBW, which includes age in the equation, to be a significant co-variant. Perhaps, it is the age which is driving this effect, rather than the body weight. (Mueck et al., 2011)

In this study, weight did not emerge as a significant co-variate on either Vd or CL. The randomised single-blind, placebo-controlled, parallel-group study in healthy volunteers taking 10 mg rivaroxaban, revealed a 24% increase in Cmax rivaroxaban in subjects weighing < 50 kg, resulting in a 15% increase in PT, compared to subjects weighing 70-80 kg. The AUC was no different in subjects weighing > 120 kg or < 50 kg. (Kubitza et al., 2007) Subsequent phase 2 and 3 studies, investigating rivaroxaban in the clinical setting, have confirmed that weight does not have a clinically significant impact on rivaroxaban pharmacokinetics or pharmacodynamics.

Creatinine clearance was a significant co-variate on clearance, CL, in this study. Several studies have consistently shown a linear increase in rivaroxaban exposure with decreasing renal function. (Kubitza et al., 2010b) Creatinine clearance has weight in its equation. It may, therefore be, the weight component of the creatinine clearance which is determining the effect on the clearance. Rivaroxaban is actively secreted into the glomerulus, as well as being passively, renally excreted. Perhaps, if there were more data points, especially at the extremes of body weight, then a pattern and relationship between weight and CL or Vd would emerge.

The impact of body composition on drug exposure profiling is crucial to understanding the most appropriate dosing regimens for a particular drug in patients of extremes of body weights. Body composition is defined as the differentiation of lean tissue, where 99% of the metabolic processes occur, from body fat, which is metabolically inactive. Clearance (renal and hepatic) is the pharmacokinetic parameter which has the most significant influence on drug exposure, thus, understanding the relationship between body composition and drug clearance can help to form quantitative predictions for that drug concentration-time profile. There are few

studies utilising body composition, thus LBW, as a covariate in the analysis of drug clearance and dosing. Han and colleagues re-analysed 80 studies, re-investigating the effect of body composition on inter-individual variability in drug clearance. Sixty-five percent of these studies failed to disagree with the hypothesis that body composition is sufficient to explain inter-individual variability in drug clearance. (Han et al., 2007)

5.3.2 Current evidence for increased exposure of rivaroxaban in under-weight patients, and under-exposure in over-weight patients

Patients with extremes of body weights are under-represented in clinical trials and in pre-clinical dose-determining studies, therefore the optimal dosing for both safety and efficacy in this subgroup of patients remains unknown.

To date, there have been no large randomised prospective trials investigating the efficacy and safety of rivaroxaban in the obese population, thus, the data has been extrapolated from sub-group analyses. (Buller, 2012; Prins et al., 2013) Additionally, none of the phase 3 clinical trials reported the number of patients enrolled with a BMI greater than 40 kg/m^2. However, a large sub-study performed on more than 8000 patients from the EINSTEIN DVT and EINSTEIN PE trials revealed no association between body weight or BMI and risk of recurrent VTE, major bleeding or clinically relevant bleeding. (Di Nisio et al., 2016)

The population PK modelling studies for rivaroxaban have been in healthy adult volunteers involved in phase one and two studies and small-scale cohort studies inadequately powered to detect differences in efficacy and safety outcomes between normal-weighted and over-weight patients. The effect of extreme age and gender on the pharmacology and tolerability of rivaroxaban has been investigated in healthy subjects taking 10 mg once a day compared to placebo. (Kubitza et al., 2006) Body weight has been shown to have limited influence on the safety, tolerability, pharmacokinetics, or pharmacodynamics of rivaroxaban in healthy subjects. (Kubitza et al., 2007; Kubitza et al., 2013)

The majority of pharmacokinetic evidence, which has formed the basis of our guidance, has arisen from sub-group analyses of the main trials, not from specific pharmacokinetic-pharmacodynamic population studies. (Burnett et al., 2016) A sub-group analysis of the EINSTEIN and DVT studies, focusing on patients in the group weighing >100 kg, revealed minimal effects of body weight on the safety, tolerability and risk of recurrent VTE in patients taking rivaroxaban compared to those taking VKA (Buller, 2012; Prins et al., 2013). A randomised, single-blinded placebo study with 10 mg rivaroxaban once a day, found that individuals in the weight category

>100 kg had similar rivaroxaban pharmacokinetics to those in the normal weight category. However, individuals weighting less than 50 kg had 24% increased maximum concentration of rivaroxaban and 15% prolonged PT. These parameters all returned to within 10% of baseline after stopping rivaroxaban for 24 h. (Kubitza et al., 2007) The maximum concentration and area under the curve remained similar in individuals weighing more than 120 kg compared to normally weighted individuals, receiving rivaroxaban for VTE treatment. (Graff et al., 2007)

A recent study investigated the effect of extremes of body weight on the clinical outcome with rivaroxaban 15 mg twice a day for three weeks, followed by 20 mg once a day, for the treatment of VTE in 167 patients in a tertiary thrombosis centre in UK, with similar demographics to those of our study population. (Arachchillage et al., 2016) 12% weighed <50 kg and 26% weighed >120 kg. There was no significant difference in the rivaroxaban concentrations of those patients in the normal weight (median 308 ng/ml) and over-weight (median 281 ng/ml) categories, but those in the under-weight (<50 kg) category had significantly higher rivaroxaban levels at a given time point post rivaroxaban dose (460 ng/ml). This was reflected in the PT and APTT results. No association between rivaroxaban levels or body weight and the clinical outcomes of recurrent VTE or bleeding rates were found. (Arachchillage et al., 2016)

Piran and colleagues, 2018, investigated the peak and trough rivaroxaban levels in patients weighing >120 kg, taking DOAC. There were 21 patients on rivaroxaban, and none had a level falling below the median trough, while 6 (28%) fell below the 5th percentile peak. (Piran et al. 2018)

5.3.4 Results from this study for the pharmacokinetics of rivaroxaban, determined by body weight

This study has developed a population PK model for rivaroxaban, which describes the mechanisms of actions in the extremes of body weight, to be similar to those in patients of normal body weight. A one compartment model was determined to represent the PK of rivaroxaban, and within this model, creatinine clearance was found to have a mechanistic effect on the clearance of rivaroxaban. Thus, dosing of rivaroxaban should take into account the patient's creatinine clearance, but not necessarily other demographics or laboratory parameters.

However, the clinical implications, in terms of recurrent VTE due to under-coagulation, or bleeding due to over-coagulation, are yet to be conclusively determined. More studies looking into the clinical outcomes relating to rivaroxaban concentration and PK modelling to further expand the knowledge of rivaroxaban pharmacokinetics

behaviour in patients of extremes of body weight, are required to fully understand the potential effects of rivaroxaban in these small populations of patients.

5.3.5 CURRENT GUIDANCE ON THE USE OF RIVAROXABAN IN OBESE PATIENTS

Current guidance of the ISTH on the use of DOACs in obese patients makes three recommendations (Martin et al., 2016): (i) to use appropriate standard dosing of DOACs in patients up to 40 kg/m^2 and weight 120 kg for VTE prevention and treatment and prevention of ischemic stroke and systemic arterial embolism in NVAF, (ii) not to use DOACs in patients with a BMI >40 kg/m^2 or weight > 120 kg due to limited data and available PK/PD evidence suggesting decreased drug exposure, peak concentration and shorter elimination half-lives with increasing weight, and (iii) if DOACs are used in patients with a BMI >40 kg/m^2 or weight > 120 kg, then to check drug-specific peak and trough level. If the drug level is reported back within the expected range, continuation of the DOAC seems reasonable. The guideline authors suggest, if the drug level is found to be below the expected range, then the guidance committee suggest changing to a VKA rather than adjusting the dose of the DOAC. No guidance is provided by the SSC on what to do in patients with a weight <50kg. (Martin et al., 2016) The ISTH guidance implies that all DOACs have a similar pharmacokinetic profile. Whilst it is acknowledged that the DOACs have more predictable pharmacokinetics compared to VKAs, each DOAC has its own distinct pharmacokinetic profile, worthy of individual consideration, especially when considering patients at the extremes of weight.

5.4 LIMITATIONS OF OUR PK MODELLING

The limitations of the PK modelling in this study include a lack of data points for many patients, thus the modelling is based on many subjects providing only one or two data points, rather than the ideal three. Modelling was still possible because there were nearly two hundred points in total. The second limitation is that comparisons between orthopaedic and non-orthopaedic populations were difficult because of the limited data points obtained from the orthopaedic population. This limited the ability to draw conclusions about prophylactic versus treatment doses and forms of rivaroxaban.

Evidence suggests that the administration of rivaroxaban with food affects its absorption at doses of 15 mg and above. The food intake was recorded by the patient in their booklet, for between one and three weeks prior to the date of study investigation. These recordings were not robust because they were heavily reliant on the

memory and recording of the subject. The food intake on the day of investigation was accurately recorded by the study investigator. Due to these inconsistencies, the food record was not factored into the modelling process, thus, potentially disregarding a source of bias.

5.5 SUMMARY

The population pharmcokinetics of rivaroxaban, as determined by this study, are consistent with previous studies, revealing a one compartment, first order model. Clearance was determined to be 8.5 l/h and volume of distribution 95l (75 – 125, 95% CI). No effect of body weight on rivaroxaban pharmacokinetics, was shown, however, lean body weight integrates age into the equation and age is known to be a strong predictor of creatinine clearance. Additionally, weight is known to affect thrombin generation and this study has shown a large effect of rivaroxaban on thrombin generation, thus, perhaps, obscuring any potential effect weight may have on the anticoagulant effects of rivaroxaban.

CHAPTER 6 PATIENT'S VIEWS ON THE RIVAROXABAN PRESCRIBED

6.1 BACKGROUND

6.1.1 WHAT DETERMINES ADHERENCE TO MEDICINES?

The prescription of a medicine is one of the most common interventions in healthcare. A growing interest in clinical medicine and clinical practice is the attitudes of patients to taking medicine and the consequences of non-adherence, which is stated to be up to 50% of patients with chronic conditions. (Cutler, 2010) Adherence is the extent to which the behaviour of the patient, with regards to taking medicine, is in accordance with the recommendations from the prescriber. Non-adherence can be categorised into intentional, when the patient makes a decision not to follow treatment advice, and non-intentional, when the patient may have not understood, forgotten or been unable to take the medicine. Adherence is a complex topic, which has been conceptualised in a common-sense model by Leventhal and colleagues. They suggest that patients base their decision on whether to seek medical advice and then, consequently, to adhere to the prescribed treatment, on five principal components of their condition: what is the condition; how long will the condition last; what caused the condition; how will the condition affect the patient; is there a cure. (Home, 1997)

Horne has further explained adherence in terms of a conceptual map comprising: patient perceptions and behaviour; patient-provider interactions and healthcare communication; societal policy and practice; interventions. This theory helps to explain the multiple interacting factors which determine a patient's views, attitudes and perceptions on the importance and potential harm of taking certain medicines. There are external influences including views and communications by healthcare providers, prescribers, peers, common practice and internal influences such as previous experiences and beliefs. (Horne et al., 2005)

One study has shown that medication beliefs were more powerful predictors of reported adherence than sociodemographic factors. (Horne & Weinman, 1999) A further study involving 1214 post-orthopaedic THR patients, showed that 64.7% were non-compliant with the medical thromboprophylaxis prescribed. (Gao et al., 2016) The VTE rate in the 'good compliant' group compared to the 'non-compliant' group was 4.2% vs 9.6%, p=0.013, suggesting that compliance is a risk factor for recurrent VTE. This study did not distinguish between traditional anticoagulants (LMWH) and rivaroxaban. (Gao et al., 2016)

A recent study investigated the attitudes of Swedish adults towards medicines and the relation of their beliefs to medicines and to natural remedies or over the counter medication. (Andersson, Sundell & Jönsson, 2016) Of 7099 respondents, those using only herbal remedies believed strongly that prescription and over the counter medicines are harmful and overprescribed. Perceived sensitivity to medicines was higher among patients using herbal remedies only. The beliefs in the benefits of medicines was higher in respondents using prescription and over the counter medicines compared to those using herbal remedies. (Andersson, Sundell & Jönsson, 2016)

Direct oral anticoagulant medication has advantages over traditional anticoagulation (heparins and vitamin K antagonsits) of easy-to-administer, oral, once a day, safe, tolerable, no need for monitoring and few side effects. The expected sequelae is an increased adherence rate and patient satisfaction.

6.1.2 Overview of the Belief about Medicines Questionnaire (BMQ) used in this study

The belief about medicines questionnaire consists of two sections with eight statements in each. It is a validated tool which addresses participant's perceived general harm and overuse of medication in general, in section one and the necessity and harm of a specific medication, in section two. The second section was adapted for the context of rivaroxaban to specify the beliefs regarding its necessity and the perceived concerns about taking a certain medicine. There was a section at the end of the questionnaire for the individuals to make comments. (appendix 6)

Responses to each statement were scored on a five-point Likert scale (1=strongly disagree and 5=strongly agree). The questionnaire was divided into two sections, for the purpose of results evaluation and analysis: section 1 included items relating to general overuse and general harm of medicines. Section two consisted of two subsections, the first related to specific necessity for rivaroxaban and the second related to beliefs about specific concerns regarding rivaroxaban. (appendix 6)

The necessity-concern scale in the BMQ was developed by Horne and Weinman. (Horne & Weinman, 1999) It has consistently shown a correlation between high necessity scores and higher reported adherence because those patients who score high in necessity and low in concerns are accepting of medicines. Additionally, patients whose concern scores exceeded necessity scores, were sceptical and the reported adherence rates were significantly lower, which was described by the differential framework. (Clifford et al., 2008)

6.2 MEDICATION ADHERENCE STUDY OBJECTIVES

This study assessed the pharmacokinetics of rivaroxaban in patients who were either being treated for VTE or having prophylaxis for recurrent VTE or VTE post-orthopaedic operation. The pharmacokinetic interpretation depends on the correct dose of rivaroxaban having been taken. In this study, the peak rivaroxaban concentration was measured, in conjunction with many laboratory and specialist coagulation investigations, following administration of rivaroxaban at time 0h during the study, thus non-adherence to that dose would be an unlikely scenario. The objectives of understanding the study populations' beliefs in adherence to rivaroxaban and medicines in general, would support and help any recommendations made on the optimal dosing strategy for this population. Additionally, any differences in these beliefs between ethnicity, VTE treatment and prevention sub-groups and age would be important.

6.3 METHODS FOR COMPLETING THE BELIEF IN MEDICINES QUESTIONNAIRE (BMQ)

6.3.1 ADMINISTRATION OF BMQ

Participants were informed about the BMQ at the time of blood sampling and were given the questionnaire to fill in and complete in their own time, either during the study day in between blood sampling episodes, or at their leisure at home. The participants were informed that the BMQ results were only identifiable by their study identity number and only the principal study investigator would know the results.

A return, stamp-addressed envelope was provided if the participant preferred to fill in the questionnaire at home, after the study day. If the participant was unable to read or understand the questionnaire, a relative or translator was asked to assist. The completed questionnaire was checked on the study day, and any unanswered questions were re-presented to the participant. A list of all participants who opted to take the BMQ home after the study day, was made and these participants were phoned to encourage completion and to aid with sending it back in the post.

Those patients who had elective orthopaedic surgery will have been prescribed rivaroxaban for the least amount of time before completing the BMQ (less than a week), unless they completed it at home following the surgery. The patients who had a VTE and were prescribed rivaroxaban, will have completed the BMQ after at least two weeks of rivaroxaban. Additionally, the orthopaedic patients were on the wards, therefore the medical staff

would administer the rivaroxaban to the patient together with the other prescribed medicines. In contrast, those patients who were prescribed rivaroxaban for VTE treatment were out-patients and would decide if and when to take the medicine, on the advice of the medical staff involved.

The responses were recorded and entered into an Excel spreadsheet. This spreadsheet was transferred onto an SPSS spreadsheet and analysed using the statistical program, SPSS version 21. The timing of completing the BMQ, with respect to how long the participant had taken rivaroxaban for, was recoded.

6.3.2 ANALYSIS

The eight items in each section were scored on a five-point likert scale, from one (strongly disagree) to five (strongly agree). This BMQ uses multiple item measures of a concept. In order to be able to make valid comparisons between the grouped concepts of overuse, harm, necessity and concern scores, each item within the specific group needs to have internal consistency. Cronbach's alpha score is the coefficient for measuring the internal consistency or inter-relatedness of a measurement, which reflects its reliability. (Cronbach, 1951) Cronbach alpha is a property of the scores from a test on a specific set of participants and it must be calculated for each sample investigated. For this study, there were four items in each group and a Cronbach's alpha score of >0.7 was selected to determine each internal consistency for the subscale. (Tavakol & Mohsen, 2011)

The Cronbach's alpha score for the overuse subsection of section one of the questionnaire, was 0.807, for the general harm subsection of section one, it was 0.723.

The Cronbach's alpha coefficients for section two of the questionnaire, were 0.798 for the subsection specific necessity and 0.640 for the subsection specific concern. Deleting only item 6, 'the rivaroxaban is a mystery to me', and item 10, 'rivaroxaban protects me from getting worse', caused an increase in Cronbach's alpha.

The necessity-concerns differential was calculated by the difference between the patient's perceived necessity for treatment, the average necessity subscale score, from their apparent concerns, the average concern subscale score, for each participant. A positive score indicates that necessity outweighs concerns, and this is a strong predictor of medication adherence. The same is true for the converse.

6.4 RESULTS

A total of 101 questionnaires were distributed to the study subjects and 85 were completed and returned, thus, the response rate was 84.1%. Of the returned questionnaires, 60.5% of participants were male, 57% had an acute VTE, 25.6% had a recurrent VTE, 12.8% were on long term anticoagulation and 4.7% were orthopaedic patients. The mean age of the participants was 52.5 years (+/- 17.2).

6.4.1 SUMMARY OF BMQ RESULTS

The necessity-concern differentials were positive for all categories (table 22) The group of recurrent VTE had the highest score in the 'specific concern' scale (table 22). These individuals were being treated for at least the second occasion with anticoagulant medication. Those patients who were on long-term anticoagulation but had switched from a traditional medication to rivaroxaban, scored the highest in the 'specific necessity' scale and NC differential, (table 22). Orthopaedic patients, who were taking a low dose of prophylactic rivaroxaban for a short period, had the lowest scores in both section one and section two subscales, and had the lowest NC differential (table 22). However, the number of individuals in the orthopaedic group was too small to be statistically significant.

TABLE 21 MEAN SCORES FOR THE DERIVED SCALES OF THE BMQ QUESTIONNAIRE, DIVIDED INTO INDICATIONS FOR ANTICOAGULATION CATEGORIES

BMQ subscale	**Acute VTE, mean (+/-SD), n= 49**	**Recurrent, VTE mean (+/-SD), n= 21**	**Long-term, mean (+/-SD), n= 11**	**Orthopaedic, mean (+/-SD), n= 4**
General overuse	11.14 (+/- 3.0)	10.97 (+/- 3.8)	10.27 (+/- 2.9)	8.17 (+/- 2.0)
General harm	8.83 (+/- 2.4)	8.35 (+/- 3.3)	8.67 (+/- 1.8)	7.75 (+/- 2.1)
Specific necessity	15.96 (+/- 3.1)	15.94 (+/- 3.9)	16.57 (+/- 4.8)	11.00 (+/- 3.8)
Specific concern	12.31 (+/- 3.1)	13.27 (+/- 4.4)	11.73 (+/- 2.8)	9.25 (+/- 3.0)
NC differential	3.65	2.67	4.84	1.75

6.4.2. SECTION 1: GENERAL OVERUSE AND GENERAL HARM

Each item was normally distributed, with a range of one to five. 'Medicines are poisons' scored the lowest and 'if doctors had more time with patients they would prescribe fewer medicines' scored the highest.

Statistical analysis, using a 2-tailed independent T-test, revealed a statistical difference in the subscale 'most medicines are addictive' between acute and long-term anticoagulation categories, p=0.03 (95% CI -1.26 to- 0.76). Patients with acute VTE generally scored the highest in most subscales of section one, reflecting a more negative attitude to medicine over-use and harm.

6.4.2. SPECIFIC NECESSITY AND SPECIFIC CONCERN

Analysis for this section was performed separately for the two subsections: specific necessity and specific harm because they represent different beliefs. The combined scores for the specific necessity and specific concern items were analysed and used to determine the necessity-concern differential.

The lowest scoring item was 'rivaroxaban disrupts my life' and the highest scoring was 'Rivaroxaban protects me from becoming worse'. This suggests a generally more positive than negative attitude to rivaroxaban in the study cohort, (table 23).

TABLE 22 DESCRIPTIVE STATISTICS FOR SPECIFIC NECESSITY AND SPECIFIC HARM LIKERT SCALES FROM THE BMQ QUESTIONNAIRE

Specific Necessity	**Mean Score (SD)**
My health depends on rivaroxaban	3.49 (1.10)
My life would be impossible without rivaroxaban	2.48 (0.93)
Without rivaroxaban I would be very ill	2.98 (1.04)
My health in the future depends on rivaroxaban	3.00 (0.97)
Rivaroxaban protects me from becoming worse	3.87 (0.85)

Specific Concern	**Mean Score (SD)**
Having to take rivaroxaban worries me	2.45 (1.01)
I sometimes worry about the long term effects of rivaroxaban	3.00 (1.11)
Rivaroxaban disrupts my life	1.92 (0.80)
Rivaroxaban is a mystery to me	2.62 (1.04)
I sometimes worry about becoming too dependent on rivaroxaban	2.35 (1.05)

Comparing the means of the specific necessity items using two-tailed T-test, between all of the categories for indication or rivaroxaban, revealed a statistically significant difference in the 'my health in the future depends on rivaroxaban' between the acute VTE group and the recurrent VTE group, p=0.033. The acute VTE v orthopaedic groups had significantly different means for three items: 'my health depends on rivaroxaban', p=0.048; 'without rivaroxaban I would be very ill', p=0.009; and 'rivaroxaban protects me from becoming worse', p=0.020. Analysis of the 'specific concern' items revealed significant differences between the means of 'rivaroxaban is a mystery to me', p=0.001 and 'having to take rivaroxaban worries me', p=0.041, for the acute VTE group and the orthopaedic group.

6.5 Discussion

This sub-study utilised BMQ as a method for assessing our patient population's attitudes to and beliefs in medicines and rivaroxaban. The advantages of self-reporting as a method to assess medication adherence include: convenient, inexpensive, simple and informative. However, if the questions are leading, asked by a prescriber, or perceived to result in personal judgement, then over-estimations of adherence often occur.

The study population, on a whole, did not feel that medicines were either over used or harmful and they were more positive towards the necessity for taking rivaroxaban than negative in their concerns.

There were differences between the subgroup patient groups, namely, the orthopaedic group scored lower on general overuse and general harm and they were less concerned about rivaroxaban than other sub-groups, although they also felt less strongly about the necessity for taking rivaroxaban. However, the number of participants in the orthopaedic group was small, thus, cautious inferences should be made from these statistical results. These differences may be understood to reflect the underlying reason why certain patients were prescribed rivaroxaban. In the VTE group, rivaroxaban was curative and potentially life-saving of the disease they were symptomatic with. In the orthopaedic group, rivaroxaban was prophylactic, thus, the patients' beliefs were influenced by the potential benefit and risk avoidances rather than treatment benefits of rivaroxaban. The orthopaedic patients were asymptomatic from VTE and many were not fully informed about the reasons and importance of taking rivaroxaban post-operatively.

Additionally, many of those being treated for VTE had previously taken LMWH subcutaneously, which is less favourable than rivaroxaban, compared to the orthopaedic sub-population of patients who had not experienced this as a contrast. Consequently, the views of the 'treatment for VTE' subgroup were more positive to taking

rivaroxaban than those of the 'prevention of VTE, orthopaedic' subgroup. Mengiardi and colleagues (2011) followed 213 patients injecting LMWH in the community and found a self-reported non-compliance rate of 17.1%, with 38.9% of patients stating that self-administration of the injections required some effort. (Mengiardi et al., 2011) Similarly, Spahn and colleagues (2002) followed 207 orthopaedic patients post-knee arthroscopy, reporting 34.8% had problems with self-injection, initially. (Spahn et al., 2002) Wilke and colleagues showed a positive preference of patients towards oral thromboprophylaxis following major orthopaedic surgery. (Wilke & Müller, 2010)

The NC differentials were positive for all subgroups of participants taking rivaroxaban for various indications, suggesting that the adherence rates should be high. The orthopaedic group had the lowest NC and those on long-term anticoagulation had the highest NC, reflecting the difference in attitude to medicine for prophylactic medicines compared to therapeutic medicine.

The literature reports higher adherence rates for medications in patients with acute compared to chronic illnesses and adherence rates generally decrease after six months of treatment. (Osterberg & Lars, 2005)

The subgroup analysis of EINSTEIN VTE demonstrated a significant difference in patient satisfaction between the rivaroxaban and enoxaparin/warfarin groups, patients in the former reporting much better satisfaction than those in the latter. (Prins et al., 2015)

Rahme and colleagues reported that fewer than one in five elderly patients discharged home after an orthopaedic operation received thromboprophylaxis. (Rahme et al., 2008) The implications of the results, from our study and others, suggest that more information should be given to orthopaedic patients regarding their risk of VTE and the importance of prophylactic anticoagulation. Ongoing thromboprophylactic education for the orthopaedic medical staff is essential so they can impart their knowledge to the patients. Leaflets and contact numbers of haematology medical professions should be available to further inform and encourage adherence to thromboprophylaxis post-operation.

Gao and colleagues analysed 1214 orthopaedic patients in China and discovered a strong association between complete non-compliance to the medical thromboprophylaxis post-operation and VTE incidence, but no correlation with partial non-compliance. (Gao et al., 2016)

The logical argument would be that medicines which are monitored are better adhered to. Therefore, adherence to rivaroxaban and other DOACs would be assumed to be less than the traditional anticoagulants, because the

levels of the DOACs are not regularly or routinely monitored and non-adherence is therefore difficult to detect, objectively. However, the lack of invasiveness, either intravenous blood sampling or subcutaneous injections, with DOACs, makes them more favourable and, therefore, easier to adhere to.

6.6 IMPLICATIONS FOR THE FUTURE

There are no reported studies in the literature discussing the long-term recurrent thrombotic complications of an acute VTE because of non-adherence. However, this is a likely cause and should be addressed.

INR monitoring of warfarin does not decrease the ongoing non-adherence to taking warfarin, and more sophisticated and effective measures to ensure adherence need to be developed. (Ten Cate 2013) Rivaroxaban adherence is good, and can be further improved with education, accessible, evidence-based-information and positive reinforcement. Many medical practitioners are still sceptical about DOACs because they cannot guarantee effective anticoagulation and drug concentration levels are not readily accessible to many clinicians. It is, therefore, not possible to be reassured of the level of anticoagulation and the clinician-patient trust in DOAC adherence is paramount.

This BMQ was administered to individuals who had consented to and were participating in this study, thus, they were a select group of patients willing to give up their free time for medical research. It is likely that these individuals were more adherent to and pertain positive beliefs about medications than the general population. There may also be biased reporting, since the individuals were aware that the BMQ was for a study on rivaroxaban.

It is important for healthcare professionals to understand patients' beliefs and experiences about rivaroxaban and to reinforce the necessity of adherence, particularly during the immediate post-operation and post-DVT period, when the risk of VTE and life-threatening VTE is high.

Future work into why patients are adherent or non-adherent and what would encourage patients to be more adherent is important. Honest, transparent, open discussions with up-to-date evidence-based information which is simple, accessible and relevant, is crucial in fostering the clinician-patient relationship, which will encourage increased adherence.

CHAPTER 7 CONCLUSIONS AND IMPLICATIONS FOR THE FUTURE OF CLINICAL PRACTICE

7.1 CONCLUSIONS

The primary aim of this study was to describe the pharmacokinetics of rivaroxaban using a pharmacokinetic-population model. The predominant question being addressed was 'does body weight affect the pharmacokinetics of rivaroxaban?'.

Venous thromboembolism (VTE) is expected to double in the next forty years, and it carries a significant morbidity and mortality burden. Obesity is an increasingly serious and often life-threatening condition. It is no longer the case that the minority of patients being treated with anticoagulation for VTE treatment or prevent are of normal weight, in fact, a large proportion are over-weight and obese. Clinical trials on rivaroxaban have excluded patients in extremes of body weight and, consequently, the pharmacokinetic (PK) models do not currently provide sufficient evidence for clinicians prescribing for patients in this subgroup. Weight is a well-recognised covariant in the pharmacokinetic profile of LMWH and warfarin. (Patel et al., 2011) In the small sub-group analyses of the rivaroxaban trials, weight has been shown to either impact on rivaroxaban concentrations, but within acceptable limits (Di Nisio et al., 2016) or to have no impact on the composite primary efficacy endpoints when analysed across subgroup weight strata. (Turpie et al., 2012) There are, however, few real-life studies on rivaroxaban, especially including patients at the extremes of body weight (Arachchillage et al., 2016)

Guidelines suggest that the clinical impact of rivaroxaban in patients at the extremes of body weight is still unknown because evidence is lacking. (Burnett et al., 2016) The pharmacokinetics and pharmacodynamics of rivaroxaban may be affected by body weight, therefore, in patients of body weight <50kg or >120kg, rivaroxaban should not be prescribed, unless traditional anticoagulants cannot be used. (Burnett et al., 2016) ISTH recommends not to use DOACs in patients with a BMI >40 kg/ m2 or weight > 120 kg, and if they are used in these patients, drug-specific peak and trough levels should be within the expected range. No guidance on dose adjustment is given, instead, a switch to warfarin in recommended if the levels are out-of-range. (Burnett et al., 2016)

The specific, distinct pharmacokinetic profile of rivaroxaban needs further investigation and clarification, in particular, with real-world data. (Kwong & Turpie, 2015) Population PK modelling is useful to determine the PK

profile of rivaroxaban for the small sub-population of patients at extremes of body weight, where data is sparse. (Duffull et al. 2011) This study has confirmed the known pharmacokinetic model of rivaroxaban and built on the existing data with real-world patient data. This study also supports the increasing body of evidence which demonstrates an insignificant effect of body weight on rivaroxaban absorption, yet a small effect on volume of distribution. (Mueck et al., 2007; Mueck et al., 2008a; Mueck et al., 2008b; Kubitza et al. 2010; Moore & Kröll, 2017)

Thrombin generation as measured with CAT was a reliable tool for assessing the effects of rivaroxaban, although there was a high inter-individual variability, these outcomes are in line with those reported in the current literature. (Bowry et al., 2014) ROTEM parameters were influenced by the presence of rivaroxaban, but generally not in a dose dependent manner, again, consistent with the current literature. (Fox et al., 2016)

A relatively well-performing one compartment PK model accurately described the data with good prediction at the low/average rivaroxaban concentrations, but less accurately predicts at the high rivaroxaban concentrations. The final model described the observed data but showed a large inter-patient variability in the absorption of rivaroxaban. This model suggests that the single best co-variant predicting rivaroxaban exposure is creatinine clearance. Creatinine clearance was the only co-variate found to have a significant mechanistic effect on the clearance of rivaroxaban, thus, on drug exposure, in this study. Interestingly, the creatinine clearance formula used in this study, includes weight as a factor, thus, weight may, to some extent have an influence on rivaroxaban clearance.

Weight did not feature as a significant covariant, and the small effect of weight observed on rivaroxaban exposure was a consequence of few data points at the extremes of body weight. Current evidence suggests that in obese patients, changes in absorption and Vd are attributed to the pharmacological properties of a specific drug. (Moore & Kröll, 2017)

A recent, retrospective study, involving 180 obese patients, 90 of whom were taking DOAC, and 33 on rivaroxaban and 90 were on warfarin, reported no difference in the incidence of thrombotic or major bleeding events between DOAC and warfarin groups. (Kalani et al., 2018) Our current study was a cross-section and did not follow the patients forward to assess their clinical outcomes while taking rivaroxaban. The yellow card scheme, to date, has reported 3-5% adverse events, in the form of arterial or venous thrombosis, of patients while on rivaroxaban.

This current study had 101 patients of a population representing a wide demographic spread, a wide spread of ages and with an equal proportion of male and female individuals. However, it is important to recognise that the current and previous studies, do not have many samples from patients at the extremes of body weight, thus statistical conclusions are difficult to ascertain. This study also only had 4 patients in the orthopaedic group, taking 10 mg rivaroxaban, which resulted in interesting, although non-statistically significant differences in many of the comparative analyses.

The BMQ results highlights the positive attitude of patients to rivaroxaban, on the background of a generally positive attitude to medicines and doctors prescribing (Prins et al., 2015). The NC differential was greater for those patients taking long term anticoagulation therapy compared with the orthopaedic population (4.84 +/- 4.3 vs 1.75 +/- 2.9). Specific necessity and specific concern were greater for those patients on long term therapy compared to the orthopaedic population (16.57 +/- 4.8 vs 11.0 +/- 3.8; 13.27 +/-4.4 vs 9.25 +/- 3.0, respectively).

7.2 Implications for the future

The key question of this study was does the dosing regime of rivaroxaban need to be adjusted in accordance with the body weight. In order to address this, this study investigated how rivaroxaban can be measure in vitro, how rivaroxaban affects the global coagulation assays, the best suited population pharmacokinetic (PK) model, including which covariants affect the selected PK parameters and what the patients' attitudes are to taking medicines and, specifically, rivaroxaban.

7.2.1 Is individualised rivaroxaban dosing necessary?

The crucial implication of this study is that individualised dosing regimens of rivaroxaban are not necessary for patients of differing body weight. This study has supported the current literature consensus that body weight does not have a significant impact on the pharmacokinetics of rivaroxaban, thus, dose adjustments for individuals of differing body weight is not required. The most recent published guidelines recommend rivaroxaban for first line treatment in VTE without evidence of cancer. (Kearon, C., et al., 2016) There are 54 recommendations, based on 20 studies classified as strong evidence, but no high quality evidence. (Kearon, C., et al., 2016)

Most previous studies, however, used healthy volunteers, were performed under artificial conditions and were not representative of the individual population of interest. It is these, which the current guidance for rivaroxaban prescribing and dosing is based on.

Obesity is becoming more prevalent and can no longer be considered a minority demographic. There is, however, still insufficient knowledge about the pharmacokinetic parameters as a function of body composition, predominantly because obese patients are excluded from clinical trials. More patients at the extremes of body weight need to be included in all pharmacokinetic and pharmacodynamic studies and body weight should be investigated as a specific covariate in population PK-PD studies. Population PK-PD studies including and specifically addressing the effects of body weight on rivaroxaban exposure will enable accurate and individualised dosing. From this study, clinicians can be reassured that the individualised dosing regimen for rivaroxaban based on body weight is not necessary. However, caution must be taken when prescribing for patients at the extremes of body weight and a low threshold for assessing rivaroxaban levels must be taken.

7.2.2 What precautions must clinicians take when prescribing rivaroxaban?

The crucial, clinical implications of patients having suffered with VTE or undergoing a high thrombotic risk operation, therefore taking rivaroxaban, namely, thrombosis or bleeding, were not investigated in this study. The risk of thrombosis or bleeding while taking rivaroxaban are increased if the dose is either too small or too large, either because of individual variation in sensitivity to the drug or due to under- or over- rivaroxaban level range. Further clinical outcome studies are required to investigate and understand the relative bleeding or thrombotic risk in the sub-populations of under- and over-weight patients.

30% of patients requiring anticoagulation therapy have CKD. (Covic, A., et al., 2018) Renal function must be closely monitored for patients on rivaroxaban and levels should be taken if there is any deterioration in renal function. (Trujillo & Dobesh, 2014) Patients with moderate CKD (eGFR 59-30 l/min / 1.73m^2) should be closely monitored with antiXa levels and patients with severe CKD should avoid rivaroxaban. (Covic, A., et al., 2018)

7.2.3 Future directions

Evidence must increase, in terms of anecdotal patient experiences on rivaroxaban and more importantly, large clinical trials, with high evidence base, need to be carried out to ascertain the safety, efficacy and tolerability of rivaroxaban in as many real-world patient sub-populations as possible. Real-world data is urgently needed to confirm and clarify both the pharmacokinetic, pharmacodynamic and clinical outcome measures of rivaroxaban, in order that clinicians can best individualise the dosing regime, applicability of prescription, monitoring and long-term use of rivaroxaban. It is currently first line recommended treatment in patients with VTE without cancer,

therefore there are many patients currently, and who will be initiating treatment with rivaroxaban, each day, thus, there is a big pool of real-world data potentially available to collect.

Additionally, global coagulation assays in the clinical setting could potentially guide clinicians to more accurately prescribe rivaroxaban on an individual basis, by providing detailed information on the pharmacodynamic effects of rivaroxaban. Currently, however, these assays are not validated across sites, are complicated and laborious to run and are dependent on the reagent used.

It remains unknown if our findings of the small effects of weight on rivaroxaban levels, ROTEM and CAT parameters and volume of distribution will be replicated in further studies. The multitude of effects which body weight has on drug pharmacokinetics is specific to each drug, and patients have different thrombotic potential and phenotype to healthy volunteers. Thus, more research is required, in the form of both pharmacokinetic studies and real-world trials, investigating patients taking rivaroxaban for VTE prevention and treatment.

Identifying patients who will respond to rivaroxaban and maintain a steady, within-therapeutic-range rivaroxaban level, while creatinine clearance of rivaroxaban remains stable, will be an important advancement of anticoagulation therapy because these patients could be safely treated without monitoring. Those patients who may metabolise rivaroxaban faster or slower than the majority of the population, thus, would have levels out-of-therapeutic range, should be identified and the dose required should be ascertained or rivaroxaban levels should be measured, for monitoring purposes. This, in turn, will aim to prevent on-rivaroxaban thrombotic events or bleeding. Ultimately, this will substantially reduce the cost burden of VTE to the healthcare system. The field of thrombosis and haemostasis is continuing to develop and consequently, patients are being offered more attractive, less dangerous and more effective medication for VTE prophylaxis and treatment.

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APPENDIX 1: PATIENT INFORMATION LEAFLETS

25th January 2013

King's College Hospital NHS
NHS Foundation Trust

KING'S COLLEGE HOSPITAL NHS FOUNDATION TRUST
King's College Hospital
Denmark Hill
London SE5 9RS

Dr Sarah Barsam
Direct fax: 020 3299 4689
Email: sarah.barsam@nhs.net

Patient Information Sheet for Orthopaedic Patients

We would like to invite you to take part in a research study. Before you decide if you want to take part you need to understand why the research is being done and what it would involve for you. Please read the following information carefully. You may ask us if there is anything that is not clear or if you would like more information.

Take time to decide whether you would like to take part or not.

Title: The impact of body weight on response to rivaroxaban

Please read Part 1. If the information given there interests you and you are considering taking part in the study, please read the additional information in Part 2 before making any decision.

After your operation, whilst you are on the ward, a member of the research team will go through the information sheet with you and answer any questions you may have.

Part 1

Purpose of the study

Following a hip or knee replacement, the body is at risk of developing blood clots in the veins. Patients are routinely given the medicine rivaroxaban, for up to 4 weeks following the surgery, to prevent blood clots from forming.

Rivaroxaban works by reducing the *stickiness* of the blood, stopping these blood clots from forming.

Currently very little information exists on what influence body weight has, on response to rivaroxaban. It is important we have a better understanding of this, so that rivaroxaban is used in the best way.

The purpose of this study is to find out how your body weight is affecting your response to the rivaroxaban therapy.

Why have I been invited?

You have been invited to take part in this study because you will be prescribed rivaroxaban following your operation.

Do I have to take part?

No. It is up to you to decide. If you do decide to take part you are free to withdraw at any time without giving a reason. Whatever you decide it will not affect the care that you receive.

What will happen to me if I take part?

If you wish to take part in the study you will be asked to sign a consent form.

After your orthopaedic surgery

Following your hip or knee replacement, when you are ready to go home, you will be asked to take rivaroxaban treatment (one tablet once a day). We would like to see you around two weeks after your surgery, whilst you are still taking rivaroxaban, in order to take blood samples from you. This visit to the hospital will be an **additional** visit, unless you are being seen by the physiotherapy department. If you decide to participate and this is the case, you will be reimbursed for your travel expenses.

If you decide to take part in this study, we will ask you to have **three** blood tests at a single visit to the hospital. These blood tests will measure the effect rivaroxaban is having on your blood. The blood tests will be taken at different times during your visit. This will help us to determine how the rivaroxaban effects are changing with time.

All blood tests will be taken from a vein in your arm by a trained member of the research team. This blood test will be similar to other blood tests you may have had at your GP or other clinics. We will ask you if you prefer to have the blood taken from a particular arm.

A small quantity of blood (six tablespoons) will be taken at the clinic visit, as follows:

- two table spoons of blood will be taken on arrival at clinic,
- two table spoons of blood will be taken 1 hour later and,
- a further two table spoons will be taken 3 hours after you arrived in clinic.

In addition, you will be weighed and will be asked about any additional medications you may be taking.

If you decide to participate in the study, it is expected that your appointment in the will last **3 hours** in total – due to the tests being done.

You will also be asked to keep a daily record of the time you take the rivaroxaban tablet each day prior to seeing the researcher - you will be provided with a record booklet.

The following flow diagram shows what a typical visit would involve, if you decide to take part in the study:

On arrival	You will see the researcher in clinic and the following information will be updated / collected: - weight - height - other medications you may be taking - time of your last rivaroxaban dose - blood tests taken

	You will then be asked to take your usual dose of rivaroxaban in clinic
	
1 hour later	A blood test will be taken one hour later by the researcher and you will be given a voucher for the hospital coffee shop You will also see the physiotherapist and the bone doctor (if this is part of your routine clinical care)
	
3 hours after arriving in clinic	A final blood test will be taken

On the day of your clinic visit, you should not take the rivaroxaban until you are told to do so, by the researcher (Dr Barsam).

As rivaroxaban should be taken after food, on the day of the clinic visit, the researcher will ask you when you last had some food, and if necessary you will be offered a small snack to eat, before taking the rivaroxaban in clinic.

In addition, as part of this study, you will also be asked to complete a short questionnaire which explores your views about having to take rivaroxaban.

This is being done, as currently very little information exists on patient's views, around having to take rivaroxaban following bone surgery. The questionnaire should take no longer than 10 minutes to complete.

Expenses and payment

Travel costs will be provided for the visit to meet the research team that are not on the same day as your usual outpatient appointments at the physiotherapy or orthopaedic clinic.

Whilst you are waiting for all the blood tests to be taken, we will give you a voucher to get refreshments from the hospital shop / coffee shop.

What are the possible disadvantages and risks of taking part in the study?

As with any blood test, there could be discomfort and/or bruising at the site from where the blood is taken.

Your appointment at the hospital could take longer than if you were not in the study.

What are the possible benefits of taking part?

If you needs any routine blood tests done you will not have to go to the blood test area of the hospital for these, you can have them done, as part of the study.

The results from this study may provide valuable information on the impact body weight has on response to rivaroxaban therapy.

Overall, we cannot promise that the study will be of direct benefit to you.

What happens when the research stops?

You will continue to see the physiotherapist and/or orthopaedic doctor as per usual.

What if there is a problem?

Any complaint about the way you are being dealt with during the study or any possible harm you might suffer will be dealt with. The detailed information on this is given in Part 2.

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be kept strictly confidential. The details are included in Part 2.

Part 2

What if relevant new information becomes available?

If any new information on this medicine becomes available, then your doctor will let you know. If this happens a member of the research team will discuss with you whether you should continue in the study. If you decide to

continue in the study, you may need to sign a new consent form. If you decide to withdraw from the study, you will continue to see your usual doctor and the care you receive will not be affected.

What will happen if I don't want to carry on with the study?

If you decide to withdraw from the study, please contact the research team. You are free to withdraw at any time, without giving a reason. Withdrawing from the study will not affect the care that you receive. If you wish, any stored blood that can be identified as yours will be destroyed. All data collected up to the time of your withdrawal from the study will be used.

After entering the study, should you lose the ability to give consent you will be withdrawn from the study at this time. We will use any blood or information collected up to the time of your withdrawal from the study.

What if there is a problem?

If you are worried about any part of the study, you should speak to a member of the research team (contact names and telephone numbers are at end of this sheet).

If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints procedure. Details can be obtained from the hospital.

In the event that something goes wrong and you are harmed due to someone's negligence, you may have grounds for legal action but you may have to pay for your legal costs.

The normal National Health Service complaints mechanisms will be available to you.

What will happen to any blood samples I give?

All blood samples collected will be securely stored in a freezer at the hospital. Only members of the research team will have access to these samples. Each research sample will be identified with a unique number. It will only be possible for members of the research team to link the samples back to you.

Samples will not be moved out of the UK; but they may be transferred to a different laboratory in the UK for testing if equipment at the hospital is unavailable e.g. breakdown of equipment.

At the end of the study all blood samples will be destroyed.

What will happen to the questionnaire I complete?

The information from the questionnaire will be entered onto a computer, so it can be analysed. The information from your completed questionnaire will be anonymised and will not be shared with your clinical team.

Will genetic tests be done?

No.

What will happen to my data?

All information collected during the study will be kept strictly **confidential**. Your data will be **anonymised** using a unique number.

The data collected will only be used for this study. The data will be stored securely on a password protected computer at the hospital. Only members of the research team will be able to access the data.

The data will be retained for 5 years and then destroyed. Use of the data in other studies will not be possible without further ethical approval and consent from you.

Involvement of the General Practitioner (GP)

We would like to inform your GP that you are taking part in this study. We will only inform your GP with your consent.

Informing your hospital doctor (orthopaedic doctor)

We would like to inform your hospital doctor that you are taking part in this study. We will only inform your hospital doctor with your consent.

What will happen to the results of the research study?

If you decide to participate in this study, we will ask you if you wish to be informed of any results arising from the research. When results are obtained we will send you a letter providing an outline of the broad scientific results of the study, with references for any work that has been published in medical journals.

It is hoped that results will be presented at scientific meetings and published in medical journals. At no time will it be possible to identify that you have taken part in this study.

This study will be described in the researcher's (Dr Sarah Barsam) MD thesis.

Who is sponsoring the research?

King's College Hospital Foundation NHS Trust is sponsoring the research.

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favorable opinion by the Harrow Research Ethics Committee.

Further information and contact details

1) General information about research

National Electronic Library for Health www.library.nhs.uk/trials

The National Research Register (UK database of research projects) www.nrr.nhs.uk

INVOLVE (Promoting public involvement in the NHS) www.invo.org.uk

2) Sponsor website

King's College Hospital www.kch.nhs.uk and www.kingshealthpartners.org

3) Specific information about this research study

Dr. Sarah Barsam 020 3299 4190

Jignesh Patel 0203 299 2828

Dr. Lara Roberts 0203 299 7633

Dr. Raj Patel 020 3299 3418

Mr. Venu Kavarthapu 020 3299 2185

Prof. Roopen Arya 020 3299 3570

25th January 2013

King's College Hospital NHS
NHS Foundation Trust

KING'S COLLEGE HOSPITAL NHS FOUNDATION TRUST

King's College Hospital

Denmark Hill

London SE5 9RS

Dr Sarah Barsam

Mobile tel: 07794510609

Direct fax: 020 3299 4689

Email: sarah.barsam@nhs.net

Patient Information Sheet For VTE Patients

We would like to invite you to take part in a research study. Before you decide if you want to take part you need to understand why the research is being done and what it would involve for you. Please read the following information carefully. You may ask us if there is anything that is not clear or if you would like more information.

Take time to decide whether you would like to take part or not.

Title: The impact of body weight on response to rivaroxaban

Please read Part 1. If the information given there interests you and you are considering taking part in the study, please read the additional information in Part 2 before making any decision.

At your next visit to see the deep vein thrombosis (DVT) nurse, or haematology doctor, a member of the research team will go through the information sheet with you and answer any questions you may have.

Part 1

Purpose of the study

Following a deep vein thrombosis, patients are prescribed medicines which reduce the *stickiness* of the blood. The purpose of these medicines are to reduce the risk of further blood clots from forming.

Rivaroxaban works by reducing the *stickiness* of the blood, stopping these blood clots from extending and reforming.

Currently very little information exists on what influence body weight has, on response to rivaroxaban. It is important we have a better understanding of this, so that rivaroxaban is used in the best way.

The purpose of this study is to find out how your body weight is affecting your response to the rivaroxaban therapy.

Why have I been invited?

You have been invited to take part in this study because you are taking rivaroxaban for your blood clot.

Do I have to take part?

No. It is up to you to decide. If you do decide to take part you are free to withdraw at any time without giving a reason. Whatever you decide it will not affect the care that you receive.

What will happen to me if I take part?

If you wish to take part in the study you will be asked to sign a consent form.

After you have been taking the rivaroxaban for at least three weeks, the researcher will phone you, to see if you might be interested in participating in the study at your next routine clinic visit.

If you decide to take part in this study, we will ask you to have **three** blood tests over the course of one of your follow-up visits.

The purpose of these blood tests will be to measure the effects of rivaroxaban on your blood at different time points. This will help us to determine how the rivaroxaban effects are changing with time.

All blood tests will be taken from a vein in your arm by a trained member of the research team. This blood test will be similar to other blood tests you may have had at your GP or other clinics. We will ask you if you prefer to have the blood taken from a particular arm.

A small quantity of blood (six tablespoons) will be taken at the clinic visit, as follows:

- two table spoons of blood will be taken on arrival at clinic,
- two table spoons of blood will be taken 1 hour later and,
- a further two table spoons will be taken 3 hours after you arrived in clinic.

In addition, you will be weighed and will be asked about any additional medications you may be taking.

If you decide to participate in the study, it is expected that your appointment in the will last **3 hours** in total – due to the additional tests being done.

You will also be asked to keep a daily record of the time you take the rivaroxaban tablet each day prior to seeing the researcher - you will be provided with a record booklet.

The following flow diagram shows what a typical visit would involve, if you decide to take part in the study:

On arrival	You will see the researcher in clinic and the following information will be updated / collected: - weight - height - other medications you may be taking - time of your last rivaroxaban dose - blood tests taken You will then be asked to take your usual dose of rivaroxaban in clinic
	
1 hour later	A blood test will be taken one hour later by the researcher and you will be given a voucher for the hospital coffee shop You will also see the doctor or DVT nurse specialist, as part of your routine clinical care

3 hours after arriving A final blood test will be taken

in clinic

On the day of your clinic visit, you should not take the rivaroxaban until you are told to do so, by the researcher (Dr Barsam).

As rivaroxaban should be taken after food, on the day of the clinic visit, the researcher will ask you when you last had some food, and if necessary you will be offered a small snack to eat, before taking the rivaroxaban in clinic.

You will not be required to have these blood tests done at subsequent visits to the thrombosis clinic.

In addition, as part of this study, you will also be asked to complete a short questionnaire which explores your views about having to take rivaroxaban.

This is being done, as currently very little information exists on patient's views, around having to take rivaroxaban following a deep vein thrombosis. The questionnaire should take no longer than 10 minutes to complete.

Expenses and payment

Travel costs will be provided for all visits to meet the research team that are not on the same day as your usual outpatient appointments at the thrombosis / haematology clinic.

Whilst you are waiting for all the blood tests to be taken, we will give you a voucher to get refreshments from the hospital shop / coffee shop.

What are the possible disadvantages and risks of taking part in the study?

As with any blood test, there could be discomfort and/or bruising at the site from where the blood is taken.

The time you spend at the hospital, could be longer than if you were not in the study.

What are the possible benefits of taking part?

The usual blood tests which you will have as part of your routine care will be taken in the haematology clinic – so you will not have to go to the blood test area of the hospital for these.

The results from this study may provide valuable information on the effect weight has on response to rivaroxaban therapy.

Overall, we cannot promise that the study will be of direct benefit to you.

What happens when the research stops?

You will continue to see the haematology doctor or DVT nurse as per usual.

What if there is a problem?

Any complaint about the way you are being dealt with during the study or any possible harm you might suffer will be dealt with. The detailed information on this is given in Part 2.

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be kept strictly confidential. The details are included in Part 2.

Part 2

What if relevant new information becomes available?

If any new information on this medicine becomes available, then your doctor will let you know. If this happens, a member of the research team will discuss with you whether you should continue in the study. If you decide to continue in the study, you may need to sign a new consent form. If you decide to withdraw from the study, you will continue to see your usual doctor and the care you receive will not be affected.

What will happen if I don't want to carry on with the study?

If you decide to withdraw from the study, please contact the research team. You are free to withdraw at any time, without giving a reason. Withdrawing from the study will not affect the care that you receive. If you wish, any stored blood that can be identified as yours will be destroyed. All data collected up to the time of your withdrawal from the study will be used.

After entering the study, should you lose the ability to give consent you will be withdrawn from the study at this time. We will use any blood or information collected up to the time of your withdrawal from the study.

What if there is a problem?

If you are worried about any part of the study, you should speak to a member of the research team (contact names and telephone numbers are at end of this sheet).

If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints procedure. Details can be obtained from the hospital.

In the event that something goes wrong and you are harmed due to someone's negligence, you may have grounds for legal action but you may have to pay for your legal costs.

The normal National Health Service complaints mechanisms will be available to you.

What will happen to any blood samples I give?

All blood samples collected will be securely stored in a freezer at the hospital. Only members of the research team will have access to these samples. Each research sample will be identified with a unique number. It will only be possible for members of the research team to link the samples back to you.

Samples will not be moved out of the UK; but they may be transferred to a different laboratory in the UK for testing if equipment at the hospital is unavailable e.g. breakdown of equipment.

At the end of the study all blood samples will be destroyed.

What will happen to the questionnaire I complete?

The information from the questionnaire will be entered onto a computer, so it can be analysed. The information from your completed questionnaire will be anonymised and will not be shared with your clinical team.

Will genetic tests be done?

No.

What will happen to my data?

All information collected during the study will be kept strictly **confidential**. Your data will be **anonymised** using a unique number.

The data collected will only be used for this study. The data will be stored securely on a password protected computer at the hospital. Only members of the research team will be able to access the data.

The data will be retained for 5 years and then destroyed. Use of the data in other studies will not be possible without further ethical approval and consent from you.

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We would like to inform your GP that you are taking part in this study. We will only inform your GP with your consent.

Informing your hospital doctor (orthopaedic doctor)

We would like to inform your hospital doctor that you are taking part in this study. We will only inform your hospital doctor with your consent.

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It is hoped that results will be presented at scientific meetings and published in medical journals. At no time will it be possible to identify that you have taken part in this study.

This study will be described in the researcher's (Dr Sarah Barsam) MD thesis.

Who is sponsoring the research?

King's College Hospital Foundation NHS Trust is sponsoring the research.

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favorable opinion by the NRES Comittee, London, Harrow Research Ethics Committee.

Further information and contact details

1) General information about research

National Electronic Library for Health www.library.nhs.uk/trials

The National Research Register (UK database of research projects) www.nrr.nhs.uk

INVOLVE (Promoting public involvement in the NHS) www.invo.org.uk

2) Sponsor website

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3) Specific information about this research study

Dr. Sarah Barsam 07794510609

Jignesh Patel 0203 299 2828

Dr. Lara Roberts 0203 299 7633

Mr. Venu Kavarthapu 020 3299 2185

Dr. Raj Patel 020 3299 3418

Prof. Roopen Arya 020 3299 3570

APPENDIX 2: CONSENT FORMS USED IN THE STUDY

King's College Hospital NHS
NHS Foundation Trust

King's College Hospital NHS Foundation Trust
King's College Hospital
Denmark Hill
London SE5 9RS

Dr Sarah Barsam

Direct fax: 020 3299 4689
Email: sarah.barsam@nhs.net

25/09/2012

CONSENT FORM, VTE patients

Title of Study: The impact of body weight on response to rivaroxaban

Name of Researcher: Dr Sarah Barsam

Please initial each box	Initials
1. I confirm that I have read and understand the information sheet dated 25th September 2012 Version 1, for the above study. I have had the opportunity to consider the information and ask questions.	☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.	☐
3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the research team and also by regulatory research and ethical authorities in the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	☐
4. I agree to my GP being informed of my participation in the study.	☐
5. I am happy to complete the questionnaire as part of the study	☐
6. I agree to take part in the above study.	☐

_____	_____	_____
Name of Patient	Signature	Date

I confirm that I have explained the study to the participant and have answered their questions honestly and fully.

_____	_____	_____
Name of Person taking consent	Signature	Date

Patient would like information on the broad results of the study: Yes / No

When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes

APPENDIX 3: ETHICS APPROVAL FOR THE STUDY

NHS
Health Research Authority

NRES Committee London - Harrow
Bristol Research Ethics Committee Centre
Level 3, Block B
Whitefriars
Lewins Mead
Bristol
BS1 2NT
Telephone: 01173 421383
Fax: 01173 420455

01 February 2013

Professor Roopen Arya
Director of the King's Thrombosis Centre and Consultant Haematologist
King's College Hospital Foundation NHS Trust
Denmark Hill
London
SE5 9RS

Dear Professor Arya

Study title:	**The impact of body weight on laboratory response to rivaroxaban therapy**
REC reference:	**12/LO/1951**
IRAS project ID:	**115654**

Thank you for your letter of 29 January 2013, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Libby Watson, nrescommittee.london-harrow@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management

APPENDIX 4: KING'S COLLEGE HOSPITAL R&D APPROVAL

15 May 2013

King's College Hospital NHS
NHS Foundation Trust

Research and Development Department
First Floor Jennie Lee House, 34 Love Walk
Kings College Hospital NHS Trust
London SE5 8AD

Tel: 020 3299 9000
Fax: 020 3299 3445
Minicom: 020 3299 9009
www.kch.nhs.uk
kch-tr.research@nhs.net

Direct tel: 020 3299 1981
Direct fax: 020 3299 5515

Professor Roopen Arya
Professor of Thrombosis & Haemostasis
4TH Floor Hambledon Wing
King's College Hospital
Denmark Hill
London
SE5 9RS

Dear Professor Arya,

STUDY TITLE: IMPACT OF BODY WEIGHT ON LABORATORY RESPONSE TO RIVAROXABAN THERAPY

In accordance with the Department of Health's Research Governance Framework for Health and Social Care, all research projects taking place within the Trust must receive a favourable opinion from an ethics committee and approval from the Department of Research and Development (R&D) prior to commencement.

- ETHICS REF: 12/LO/1951
- **Sponsor: King's College Hospital NHS FT**
- **Funder: Bayer**
- **End date (as per IRAS application): 01/02/2014**
- **Protocol: V1 dated 25/09/2012**

Site: King's College NHS Foundation Trust
R&D approval Date: 15 May 2013

R&D have reviewed the documentation submitted for this project and I am pleased to inform you that we are approving the work to proceed within King's College Hospital NHS Foundation Trust**. The study has been allocated the Trust R&D registration number** KCH13-078. **Please quote this registration number in any communications with the R&D Department regarding your project.**

CONDITIONS OF NHS PERMISSION FOR RESEARCH:

- **The Principal Investigator must notify R&D of the actual end date of the project.**

The Principal Investigator is responsible for ensuring that Data Protection procedures are observed throughout the course of the project.

- **The project must follow the agreed protocol and be conducted in accordance with all Trust Policies and Procedures especially those relating to research and data management.**
- **R&D must be notified of any changes to the protocol prior to implementation. Please submit a copy of the progress report on the anniversary of the Ethics favourable opinion** (01 February)

If appropriate it is recommended that you register with the Current Controlled Trials website; http://isrctn.org/

Please ensure that you are aware of your responsibilities in relation to The Data Protection Act 1998, NHS Confidentiality Code of Practice, NHS Caldicott Report and Caldicott Guardians, the Human Tissue Act 2004, Good Clinical Practice, the NHS Research Governance Framework for Health and Social Care, Second Edition April 2005 and any further legislation released during the time of this study.
Members of the research team must have appropriate substantive or honorary contracts with the Trust prior to the study commencing. Any additional researchers who join the study at a later stage must also hold a suitable contract.

IF THE PROJECT IS A CLINICAL TRIAL UNDER THE EUROPEAN UNION CLINICAL TRIALS DIRECTIVE THE FOLLOWING MUST ALSO BE COMPLIED WITH:

1. The EU Directive on Clinical Trials (Directive 2001/20/EC) and UK's implementation of the Directive: The Medicines for Human Use (Clinical Trials) Regulations 2004;
2. The EU Directive on Principles and Guidelines for Good Clinical Practice (EU Commission Directive 2005/28/EC); and UK's implementation of the Directive: The Medicines for Human Use (Clinical Trials) Amendment Regulations 2006;

AMENDMENTS

Please ensure that you submit a copy of any amendments made to this study to the R&D Department.

ANNUAL REPORT

It is obligatory that an annual report is submitted by the Chief Investigator to the research ethics committee, and we ask that a copy is sent to the R&D Department. The yearly period commences from the date of receiving a favourable opinion from the ethics committee.

Should you require any further information please do not hesitate to contact us.

In line with the Research Governance Framework, your project may be randomly selected for monitoring for compliance against the standards set out in the Framework. For information, the Trust's process for the monitoring of projects and the associated guidance is available from the Trust's intranet or on request from the R&D Department. You will be notified by the R&D Department if and when your project has been selected as part of the monitoring process. No action is needed until that time.

Many thanks for registering your research

project Yours sincerely,

Taffy Bakasa
Research Governance Specialist

APPENDIX 5: ADAPTED BELIEFS ABOUT MEDICINES QUESTIONNAIRE (BMQ) USED IN THIS STUDY

Participant Number:

Understanding your views on having to take rivaroxaban

You have been asked to complete this questionnaire, as you are currently having rivaroxaban tablets. Currently, very little information exists on patient's views about having to take this medicine.

As part of the rivaroxaban study, we want to explore your views about medicines use, in particularly rivaroxaban, and would like you to complete this questionnaire.

The questionnaire compromises of two main sections. The first section will investigate your views about medicine use in general. The second section will investigate your views about rivaroxaban therapy specifically.

Instructions

- In both sections, a series of statements have been made.
- A 5-point rating scale is used, going from strongly agree to strongly disagree. Please consider each statement in turn and tick the box that best describes your views to that statement.
- At the end of the questionnaire, there is an opportunity for you to write any other comments you may have

Please answer every question. It will take approximately 10 minutes to complete.

Your answers will be completely anonymous and will be kept confidential.

There are no "right" answers to the questions – we are simply interested in your views.

THANK YOU FOR TAKING THE TIME TO COMPLETE THIS QUESTIONNAIRE

Section 1: General questions relating to medicine use in general

This section explores your views and concerns (if any) about taking medicines in general.

Please answer every question by ticking the box that best describes your views to each statement.

Please remember, there are no "right" answers to the questions, we are simply interested in your views.

		Strongly agree	Agree	Uncertain	Disagree	Strongly disagree
G1	Doctors use too many medicines					
G2	People who take medicines should stop their treatment for a while every now and again					
G3	Most medicines are addictive					
G4	Natural remedies are safer than medicines					
G5	Medicines do more harm than good					
G6	All medicines are poisons					
G7	Doctors place too much trust on medicines					
G8	If doctors had more time with patients they would prescribe fewer medicines					

SECTION 2: SPECIFIC QUESTIONS RELATING TO RIVAROXABAN THERAPY

This section explores your views and concerns (if any) specifically around rivaroxaban therapy.

Please answer every question by ticking the box that best describes your views to each statement.

Please remember, there are no "right" answers to the questions, we are simply interested in your views.

		Strongly agree	Agree	Uncertain	Disagree	Strongly disagree
S1	My health depends on rivaroxaban					
S2	Having to take rivaroxaban worries me					
S3	My life would be impossible without rivaroxaban					
S4	Without rivaroxaban I would be very ill					
S5	I sometimes worry about the long term effects of rivaroxaban					
S6	The rivaroxaban is a mystery to me					
S7	My health in the future depends on rivaroxaban					
S8	The rivaroxaban disrupts my life					
S9	I sometimes worry about becoming too dependent on rivaroxaban					
S10	Rivaroxaban protects me from becoming worse					

If you have any other comments you would like to make, please make them below:

THANK YOU VERY MUCH FOR COMPLETING THIS QUESTIONNAIRE