From the DEPARTMENT OF MEDICINE, SOLNA
Karolinska Institutet, Stockholm, Sweden

MONITORING OF THE ORAL ANTICOAGULANTS APIXABAN AND RIVAROXABAN TO OPTIMIZE INDIVIDUAL SAFETY AND EFFICACY

Fadiea Al-Aieshy

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Monitoring of the oral anticoagulants apixaban and rivaroxaban to optimize individual safety and efficacy

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By

Fadiea Al-Aieshy

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Principal Supervisor:
Rickard E. Malmström
Karolinska Institutet
Department of Medicine, Solna
Division of Clinical Epidemiology

Co-supervisors:
Mika Skeppholm
Karolinska Institutet
Department of Clinical Sciences, Danderyd Hospital
Division of Cardiovascular Medicine

Anton Pohanka
Karolinska Institutet
Department of Laboratory Medicine
Division of Clinical Pharmacology

Olof Beck
Karolinska Institutet
Department of Clinical Neuroscience
Centre for Psychiatry Research

Opponent:
Margareta Holmström
Linköping University
Department of Health, Medicine and Caring Sciences
Division of Diagnostics and Specialist Medicine

Examination Board:
Jaran Eriksen
Karolinska Institutet
Department of Global Public Health
Department of Clinical Science and Education, Södersjukhuset
Division of Infectious Diseases/Venhälsan

Jonas Schwieler
Karolinska Institutet
Department of Medicine, Solna
Division of Cardiology

Pär Hallberg
Uppsala University
Department of Medical Sciences
Division of Clinical Pharmacogenomics and Osteoporosis

To my family

ABSTRACT

The oral anticoagulants apixaban and rivaroxaban are administered at fixed doses without requiring routine monitoring. However, there is a dose and exposure dependency in efficacy and safety, and monitoring the anticoagulants may improve treatment outcomes. The aim of this thesis was to evaluate different laboratory methods for monitoring apixaban and rivaroxaban and to show the typical exposure levels in patients. Additionally, the study aimed to study the exposure in certain specific populations (i.e. those with obesity and renal impairment), to elucidate whether monitoring would be especially valuable for these patients.

In **studies I** and **II**, we evaluated various coagulation assays for the monitoring of apixaban and rivaroxaban. While PT-INR (venous) and aPTT were not sensitive enough to be used for this purpose, the anti-Factor Xa assay showed a strong correlation with LC/MS-MS. However, for precise estimations of apixaban/rivaroxaban concentrations in the lower range, LC-MS/MS is the preferred method to use. A large interindividual variability in trough concentrations was observed, 12-fold for apixaban (6-fold within the same dosage group) and 17-fold for rivaroxaban (17-fold for the standard dose and 3-fold for the reduced dose).

In **study III**, we explored the influence of obesity on apixaban trough and peak concentrations. A specific dose recommendation for obese patients may not be necessary given that the concentrations were largely similar to those in matched normal weight patients. However, obese patients exhibited an extensive 18-fold interindividual variability in trough concentrations. Consequently, monitoring apixaban concentrations could be valuable for specific obese patients to ensure optimal treatment outcomes.

In **study IV**, we examined the impact of renal function on apixaban exposure in AF patients. Patients with moderate renal impairment had twice as high apixaban trough concentrations compared to patients with normal renal function. This indicates that the recommended 5 mg twice daily dose might be excessive for some patients with moderate renal impairment, and that monitoring ought to be recommended.

LIST OF SCIENTIFIC PAPERS

The following four studies are included in this thesis. The articles/manuscripts can be found in full texts at the end of the thesis.

I. Skeppholm M, Al-Aieshy F, Berndtsson M, Al-Khalili F, Rönquist-Nii Y, Söderblom L, Östlund AY, Pohanka A, Antovic J, Malmström RE. **Clinical evaluation of laboratory methods to monitor apixaban treatment in patients with atrial fibrillation.** Thrombosis Research. 2015;136(1):148-53.

II. Al-Aieshy F, Malmström RE, Antovic J, Pohanka A, Rönquist-Nii Y, Berndtsson M, Al-Khalili F, Skeppholm M. **Clinical evaluation of laboratory methods to monitor exposure of rivaroxaban at trough and peak in patients with atrial fibrillation.** European Journal of Clinical Pharmacology. 2016;72(6):671-9.

III. Al-Aieshy F, Skeppholm M, Fyrestam J, Johansson F, Pohanka A, Malmström RE. **Apixaban plasma concentrations in obese and normal weight patients.**
Manuscript

IV. Al-Aieshy F, Skeppholm M, Fyrestam J, Johansson F, Pohanka A, Malmström RE. **Apixaban trough concentrations in atrial fibrillation patients with reduced renal function.**
Manuscript

CONTENTS

LIST OF ABBREVIATIONS

AF	Atrial fibrillation
AFL	Atrial flutter
aPTT	Activated partial thromboplastin time
ASC	Acute coronary syndrome
BID	Twice a day
BMI	Body mass index
DOAC	Direct oral anticoagulant
DVT	Deep vein thrombosis
EMA	European Medicines Agency
ESC	European Society of Cardiology
FDA	Food and drug administration
FXa	Factor Xa
GFR	Glomerular Filtration Rate
GFRCG	Glomerular Filtration Rate - CockCroft Gault
GFRCr	Glomerular Filtration Rate -Creatinine
GFRCyst	Glomerular Filtration Rate -Cystatin C
INR	International normalized ratio
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
NOAC	Non-vitamin K antagonists
OAC	Oral Anticoagulant
OD	Once daily
POC	Point-of-care devices
PCTL	Percentile
PE	Pulmonary embolism
PT	Prothrombin time
RCT	Randomized controlled trial
TDM	Therapeutic drug monitoring

TIA	Transient ischemic attack
SPC	Summary of Product Characteristics
VKA	Vitamin K antagonist
VTE	Venous thromboembolism
WHO	World health organization

1 INTRODUCTION

Since ancient times, humans have tried to find ways to treat diseases and conditions through natural remedies. The development in this field has expanded tremendously [1]. Today, drug therapy is the cornerstone of modern healthcare, used in the "prevention, diagnosis, alleviation, treatment or cure of disease" [2]. The drug approval process is carefully regulated, and pharmaceutical companies must present comprehensive data during the preauthorization phase of a drug. However, once available on the market, the drug is administered to a broader patient population, including patients who were excluded in the pivotal trials. [3]. Consequently, post-approval research becomes crucial. Given that drug therapy implies not only benefits but also risks, this balance is continuously tested. The anticipated benefits must outweigh the risks for a safe and rational use [4]. To maximize the positive outcomes (the benefits), each patient should be treated with the "right drug" in the "right dose" at the "right occasion" [5].

The general theme of this thesis revolved around choosing "the right dose" for the "right patient" for two drugs named apixaban and rivaroxaban. These two drugs have been introduced to the market in recent years and belong to a class of oral anticoagulants (OACs) abbreviated as NOACs or DOACs. Initially, they were named new or novel oral anticoagulants, but the terminology changed over time to non-vitamin K antagonist oral anticoagulants and direct oral anticoagulants [6, 7]. Before the introduction of these drugs, warfarin and other vitamin K antagonists (VKAs) were the only available OACs on the market. Unlike warfarin, which necessitates regular monitoring, DOACs were approved without routine monitoring [8-11]. There are generally two approaches to anticipate drug exposure levels and their corresponding therapeutic effects in an individual patient. One approach is to predict the drug levels based on the pharmacokinetics of the drug and the specific patient characteristics that may influence the levels, as is typically the case for DOACs. The second approach involves therapeutic drug monitoring (TDM) with precise drug concentration measurements or the use of an effect response marker. Most drugs do not require TDM as they have a wide therapeutic range and their effect can be clinically monitored. For example, blood pressure and blood sugar levels can be measured for patients treated with antihypertensive drugs and antidiabetic drugs, respectively. TDM is usually preferred for potent drugs and drugs with

narrow therapeutic window [12, 13]. While DOACs, compared to warfarin, have predictable pharmacokinetics and are approved for prescription without the need for routine monitoring, it is crucial to recognize that they are potent drugs with potentially serious consequences if not used at an optimal dose [8-11]. Monitoring the anticoagulant effect or measuring the concentrations of DOACs i.e. individualizing the drug therapy could improve treatment outcomes with enhanced efficacy and reduced side effects [13, 14].

2 BACKGROUND

2.1 THERAPEUTIC INDICATIONS OF ORAL ANTICOAGULANTS

2.1.1 Overview

OACs are used for the prevention and treatment of thrombosis and embolism, which are the leading causes of death in the world [15]. Thrombosis occurs when a blood clot a "thrombus" is formed in a blood vessel and blocks the blood flow at the site of formation. In contrast, embolism occurs when a thrombus, travels with the blood flow and obstructs a blood vessel. The damage this causes is depending on where it occurs [16, 17].

The DOACs are approved by the European Medicines Agency (EMA) for adult patients with the following therapeutic indications: prevention of stroke and systemic embolism in atrial fibrillation (AF), prevention/treatment of venous thromboembolism (VTE) and for prophylaxis of VTE after knee/hip surgery [8–11]. In addition, rivaroxaban is also approved for acute coronary syndrome (ACS) [11] . Warfarin has a wider therapeutic use than the DOACs, as it is also used e.g. in patients with cardiac valve replacement [18].

2.1.2 Atrial fibrillation

AF is the most common cardiac arrhythmia, with a prevalence of at least 2% in the European population [19, 20]. The prevalence of AF is higher in men and increases with age, ranging from 0.1% for those aged <55 years to 9% for those aged >80 years [21, 22]. The population is getting older, and estimates are suggesting that in Europe the number of AF patients ≥55 years will double in 2060 compared with 50 years prior [23]. Several factors, besides high age, increase the risk of AF: hypertension, diabetes, high BMI/obesity, chronic kidney disease and heart failure [24, 25]. Patients with AF have an increased risk of stroke, with approximately 30% of all strokes occurring in patients with AF [26, 27]. Therefore, treatment with OACs is recommended for many of these patients. The European Society of Cardiology (ESC) recommends using the CHA_2DS_2Vasc score (Table 1) to assess stroke risk and determine whether OACs

should be initiated [24, 28]. The stroke risk is estimated to be 1.3% per year for patients with a CHA_2DS_2Vasc score of 1 and increases to 15.2% per year for those with a score of 9 [29]. OACs are recommended for female and male patients with scores of ≥3 and ≥2, respectively. However, treatment with OACs should be considered for female patients with a score of ≥2 and male patients with a score ≥1 [24].

Table 1. CHA_2DS_2Vasc score

Risk factor	***Points***
Heart failure	1
Hypertension	1
Age ≥75	2
Diabetes Mellitus	1
Stroke/transient ischaemic attack (TIA)/thromboembolism	2
Vascular disease	1
Age 65–74	1
Female sex	1

The main concern with OAC treatment is the bleeding risk. The HAS-BLED is a score used for the estimation of the bleeding risk, with one point for each of the following characteristics: Hypertension, Abnormal renal/liver function (1 point each), Stroke, Bleeding history or predisposition, Labile international normalized ratio (INR), Elderly (>65 years) and Drugs/alcohol concomitantly (1 point each) [30]. The stroke risk reduction should be weighed against the risk of bleeding before initiating treatment. However, a high bleeding score does not automatically exclude OAC treatment, as the risks of stroke and bleeding often coexist. Instead, risk factors should be identified and, if possible, treated [24].

2.1.3 Venous thromboembolism

VTE is a serious condition that represents a big global health problem, it is the third most common cause of mortality among cardiovascular diseases [31]. The incidence of VTE has increased over the last decade but varies between different populations. Among the European population, the incidence is around 104 to 183 per 100 000 person-years, and for 30% of the patients, a recurrent VTE occurs within 10 years. Factors that increase the risk for VTE are surgery, cancer, the use of oral contraceptives and obesity [32].

The term VTE includes two conditions: deep vein thrombosis (DVT) and pulmonary embolism (PE). DVT occurs typically in the extremities when a blood clot, a thrombus, is formed in a deep vein, occludes the vessel and creates distal stasis of blood. Less common, PE, an occlusion of the pulmonary artery, may occur e.g. after a DVT when a part of the deep vein thrombus embolizes and travels to the pulmonary circulation where it causes the occlusion [33].

2.2 DISPENSATION OF ORAL ANTICOAGULANTS

For over 50 years warfarin and other VKAs were the only available OACs on the market. With the introduction of the DOACs, more alternatives were now available offering more options (Table 2). Consequently, more patients are treated with OACs. In Sweden, the dispensation of OACs increased from 52% to 74% between 2012 and 2017 [34]. Other countries report similar trends [35].

Table 2. The OACs available in Sweden and the year of their introduction [8-11, 36]

Year of introduction	*OAC*
1950s	Warfarin
2011	Dabigatran
2012	Rivaroxaban
2013	Apixaban
2016	Edoxaban

The ESC recommends using DOACs as a first-line therapy [37, 38]. In 2022, apixaban and rivaroxaban were among the best-selling drugs in the world. Apixaban ranked at number six and rivaroxaban at number 16, with a total turnover of 18.3 and 7.5 billion us dollars, respectively [39]. An increasing number of patients are treated with DOACs instead of warfarin, and in Sweden, apixaban is the most used OAC as measured by the number of patients that retrieved prescriptions (Fig. 1) [40].

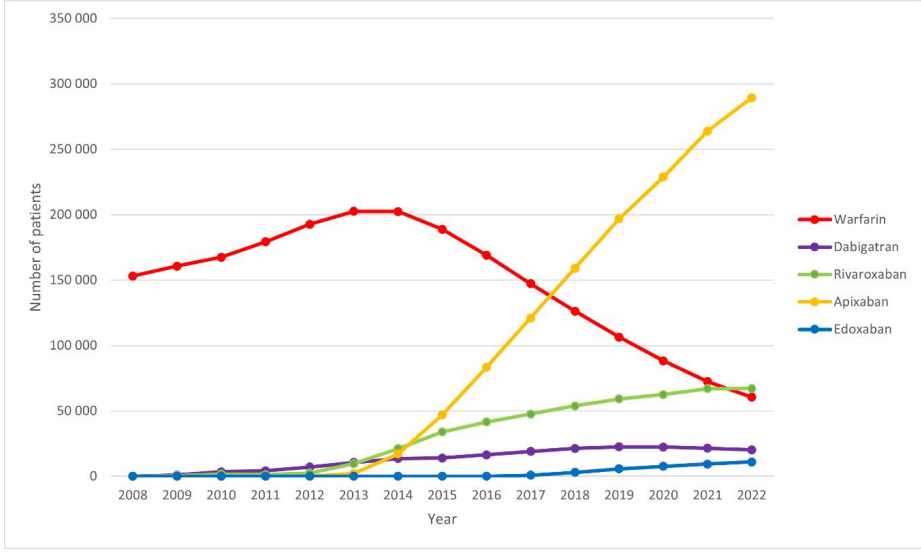

Figure 1. Number of patients with dispensed OACs in Sweden between the years 2008-2022 [40].

2.3 WARFARIN

Warfarin is a VKA that inhibits the enzyme vitamin K epoxide reductase (VKORC1), which is responsible for activating vitamin K. As a result, the vitamin K-dependent clotting factors II, VII, IX, and X are inhibited, leading to a reduction in thrombin activation and thus fibrin formation (Fig. 2) The maximum effect is observed five to seven days after initiating treatment and lasts for two to five days after discontinuing the treatment [18, 41].

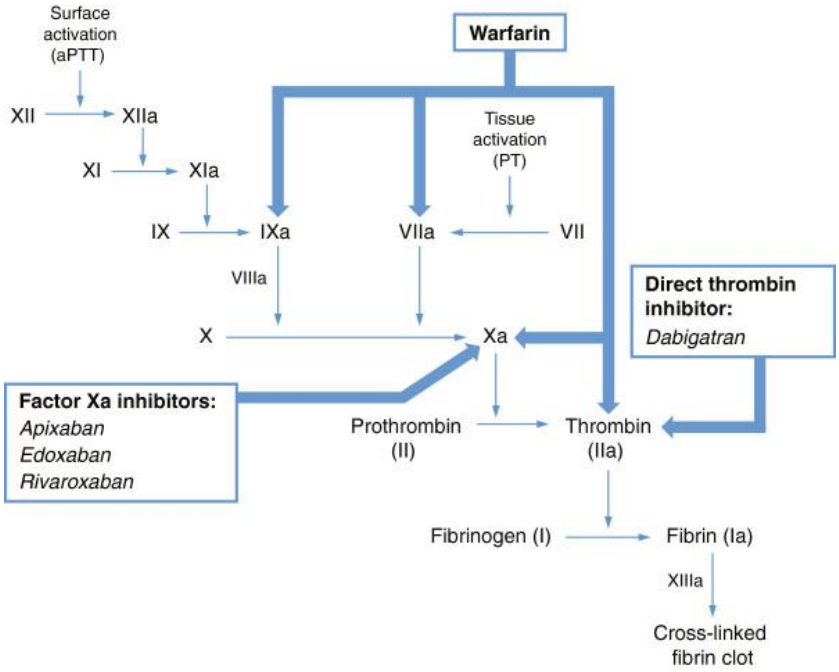

Figure 2. The coagulations cascade and sites of action for the OACs warfarin, dabigatran, rivaroxaban, apixaban and edoxaban [42]. Open access article reproduced from BioMed Central Ltd, © 2013 Stambler.

As warfarin has been on the market for many years, there is vast knowledge and experience. The treatment efficacy, adverse effects and how to handle bleeding are all aspects that are well documented and understood. However, warfarin has rather complex pharmacokinetics and pharmacodynamics, and needs to be monitored extensively. Treatment with warfarin requires regular monitoring of PT-INR with target values of 2.0-3.0 (Fig. 3), which is the interval with the most

optimal balance between the efficacy of stroke prevention and safety regarding the bleeding risk [18, 43, 44].

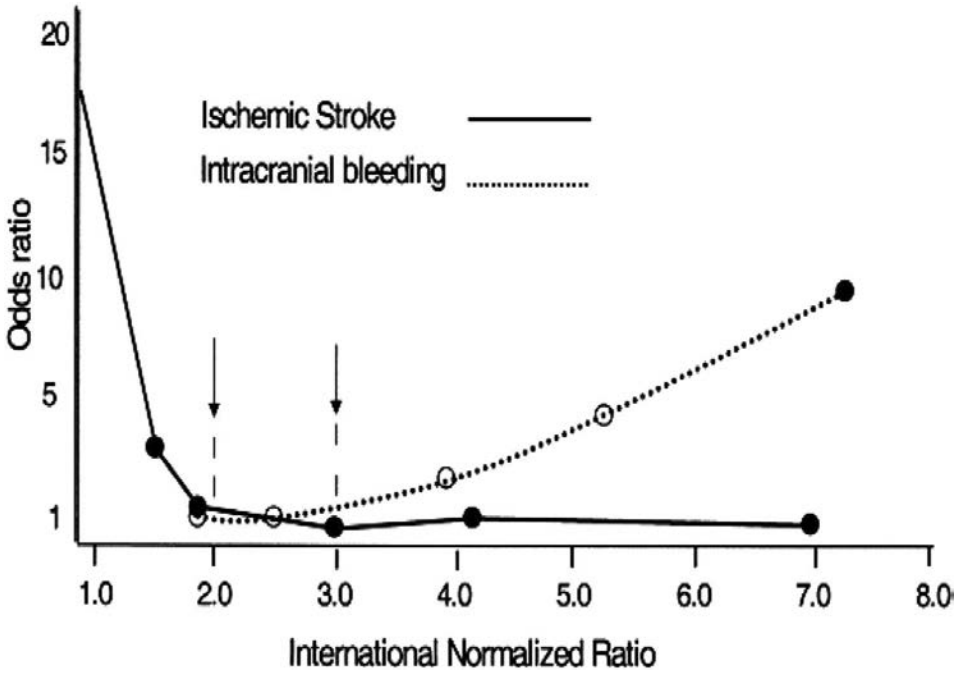

Figure 3. Odds ratio for ischemic stroke and intracranial bleeding in relation to INR [45]. Reproduced with permission from Wolters Kluwer Health, INC. © 2011 by the American College of Cardiology Foundation, the American Heart Association, Inc., and the European Society of Cardiology.

2.4 DIRECT ORAL ANTICOAGULANTS

There are four approved DOACs on the market today: the direct thrombin inhibitor dabigatran, and the direct factor Xa (FXa) inhibitors apixaban, rivaroxaban and edoxaban (Fig. 2) [8-11]. In the pivotal trials, RELY for dabigatran, ROCKET for rivaroxaban, ARISTOTLE for apixaban and ENGAGE for edoxaban, the DOACs were at least non-inferior compared to warfarin in AF patients [46-50].

Similar results were also seen in other indications and versus other standard-of-care comparators [50]. The DOACs differ in pharmacodynamics and pharmacokinetics, including bioavailability, metabolism pathways, renal excretion, half-life and dose regimen etc. Dabigatran is the DOAC most dependent on renal function for its elimination, and apixaban is the least dependent. Dabigatran and apixaban are administered twice daily (bid), while a once-daily (OD) dosing regimen is used for rivaroxaban and edoxaban [39-42]. The main benefits of DOACs compared to warfarin are that the effects occur faster since they are direct-acting and that there is no need for routine monitoring. The risk of food-drug interactions and drug-drug interactions are also supposedly less with DOACs than with warfarin [51].

Although monitoring of the anticoagulant effect or concentration measurements of DOACs is currently not recommended as routine, there are several clinical situations where monitoring would be required or helpful. In short, any time when it is reasonable to determine whether the patient is optimally treated, meaning not under- or overtreated, either chronically or in connection to an emergency situation. For example, when adverse effects or treatment failure occurs, in connection with a surgical procedure linked to bleeding risk, for assessment of patient compliance, to navigate drug interactions, overdose or if an antidote should be administered. Monitoring the plasma concentrations or the effect of DOACs may increase the drug benefits and decrease the risks when used in non-emergency situations as well. Particularly in patients with characteristics that differ from those included in the pivotal trials and that potentially could affect the drug levels e.g. extremes in body weight, renal impairment and concurrent use of drugs that potentially could interact [52-55].

2.4.1 Monitoring of direct oral anticoagulants

TDM is defined as "the regular measurement of serum levels of drugs requiring close "titration" of doses in order to ensure that there are sufficient levels in the blood to be therapeutically effective, while avoiding potentially toxic excess; drug concentration in vivo is a function of multiple factors" [56]. With the use of TDM, drug therapy can be more individualized. This is of value mostly for drugs

with narrow therapeutic intervals and where there may be clinically significant differences in the pharmacokinetics of the drug between patients [57]. DOACs are potent drugs and the ability to monitor the treatment and to find a therapeutic interval with the best efficacy for preventing a thromboembolic event with an acceptable risk for bleeding would provide improved benefits for the patient. There is limited information published on the dose-concentration-effect relationships for DOACs. For dabigatran, there is published data describing a clear correlation between dabigatran trough concentrations and its efficacy and safety (Fig. 4), and thus monitoring and adjusting the dose accordingly should improve safety and efficacy [58, 59]. A similar correlation was published also for edoxaban [60]. According to data filed to the U.S. Food and Drug Administration (FDA), there was a direct relationship between apixaban concentrations and the risk for major bleeding [61], which also was observed for rivaroxaban [62]. These analyses have not been published in their entirety, but based on what has been published on dabigatran and edoxaban and what has been analyzed and stated by the FDA it is hence clear that concentration-dependent safety and efficacy exist for DOACs.

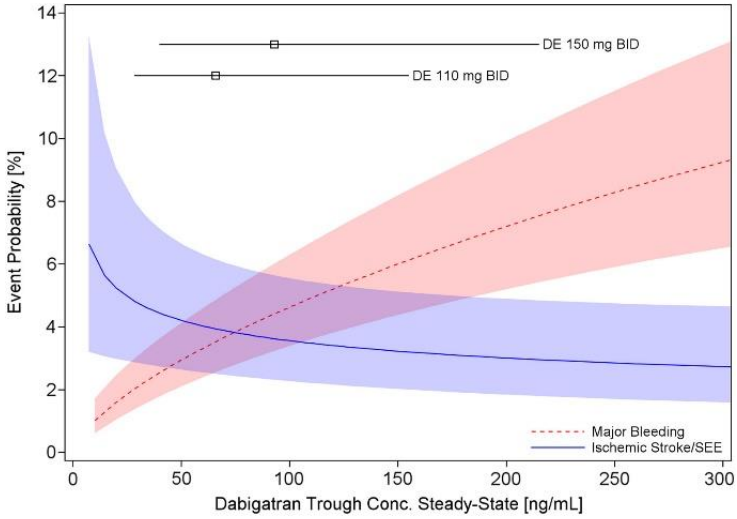

Figure 4. Probability of major bleeding event and ischemic stroke/systemic embolic event in relation to dabigatran trough concentration [58]. The optimum balance between efficacy and safety is seen in the plasma dabigatran concentration interval 50-100 ng/mL. Reproduced with permission from Elsevier. © 2014 by the American College of Cardiology Foundation.

2.4.2 Dosing and monitoring of apixaban in subgroups

There are two approved dosing regimens for apixaban, the standard dose of 5 mg bid and the reduced dose of 2.5 mg bid. The reduced dose is recommended in AF patients with two of the following characteristics: age ≥80 years, body weight ≤60 kg or serum creatinine ≥133 µmol/L (Table 3). In Europe, a dose reduction is also recommended in AF patients with glomerular filtration rate (GFR) estimated with the Cockcroft Gault Formula (CG) between 15-29 mL/min [8]. Interestingly, dose reduction based only on renal function is not recommended in the FDA-approved information [63].

Table 3. Dose recommendations of apixaban according to the European SPCs [8].

Indication		***GFRCG***	
		15-29 ml/min	***<15 ml/min***
AF	Lower dose (2.5 mg bid) if serum creatinine ≥133 µmol/L and age ≥80 years and/or body weight ≤60 kg	Lower dose (2.5 mg bid)	Contraindicated
VTE	-	Use with caution	Contraindicated

GFRCG = Glomerular filtration rate estimated using Cockcroft gault

2.4.2.1 Obesity

Obesity is usually measured by the Body Mass Index (BMI), which is calculated by the patient's weight (kg) divided by the square of the height (m); kg/m^2. Underweight is classified as BMI <18.50, normal range BMI 18.50-24.99, overweight ≥25.00 and obese ≥30 kg/m^2 [64]. According to the World Health

Organization (WHO), obesity and overweight are increasing globally, for both women and men across all ages [65]. In the adult population aged 18 and over, the prevalence of overweight and obesity were 39% and 13%, respectively in 2016 [66]. The numbers were higher in Sweden, with 56% of the population being overweight and 21% being obese. The prevalence of obesity has more than doubled between 1975 and 2016 [67]. As obesity increases the risk for VTE and AF, it also increases the likelihood of a treatment indication for apixaban or other OACs [68-70].

Low body weight constitutes a criterion for dose reduction when in combination with at least another of the characteristics mentioned above (section 2.4.2), as increased drug levels are expected. On the other hand, the manufacturer does not recommend adjusting the dose for patients with high body weight [8]. For certain drugs, it is important to consider the possibility that absorption, volume of distribution and clearance may differ in obese leading to altered drug exposure, effects or treatment failure compared to patients with normal weight [71, 72].

Patients with high body weight were not excluded in the randomized controlled trials (RCTs) for apixaban, however, separate analyses on high body weight were not presented in all these trials. In some trials, the efficacy and safety of apixaban in obese patients have been compared to conventional treatment with sufficient results [73-78]. However, the conventional treatment is not problem-free as it is associated with many challenges and complications. Moreover, there is very limited data regarding apixaban exposure levels in obese patients or patients with high body weight.

2.4.2.2 Renal impairment

About 27% of apixaban is eliminated in unchanged form through the kidneys and thus increased apixaban concentrations could be expected with worsening renal function [8]. The risk for AF increases with age, and so does renal impairment [22, 79]. Therefore, it is not uncommon for patients treated with apixaban to have

renal impairment. In addition, patients with impaired renal function are at increased risk of both bleeding and thromboembolism [80]. All these factors make this group particularly important to study, as treatment can be optimized and improved.

In ARISTOTLE, AVERROES and AMPLIFY patients with GFRCG <25 mL/min were excluded, whereas in ADVANCE patients were excluded if GFRCG was <30 mL/min [48, 73, 75, 77]. In the RCT comparing apixaban with warfarin, patients with severe renal impairment were underrepresented as only 1.5% (n=137) had GFRCG between 25-30 mL/min. The risk for both serious bleeding and stroke/embolism during treatment with apixaban (and warfarin) increased with worsening renal function [48].

3 RESEARCH AIMS

The overall aim of this thesis can be summarized in two parts. In the first part, we aimed to evaluate different laboratory methods to monitor apixaban and rivaroxaban and to characterize the typical exposure of these drugs in patients. For the second part, we focused on apixaban treatment as this has become the most used OAC in Sweden [40], and studied specific patient characteristics and the exposure of apixaban to elucidate whether monitoring could potentially enhance individual safety and efficacy.

The specific aims for each study were:

Study I -To evaluate different laboratory methods for measuring the effect and exposure of **apixaban**, and to show the typical concentration intervals in patients with AF.

Study II -To evaluate different laboratory methods for measuring the effect and exposure of **rivaroxaban**, and to show the typical concentration intervals in patients with AF.

Study III -To study **apixaban** exposure in obese patients compared with normal weight patients with AF, Atrial flutter (AFL) or VTE.

Study IV -To study **apixaban** exposure in AF patients with renal impairment compared with normal renal function.

4 MATERIALS AND METHODS

This section is a summary of the materials and methods used in the thesis. Detailed descriptions are available in the published papers/manuscripts.

4.1 STUDY DESIGN AND POPULATIONS

All four studies were non-interventional clinical studies, and thus no drug therapy modification was made for the included study patients. The patients were recruited from the cardiology department at Danderyd Hospital, an emergency hospital in Sweden with 500 nursing places and 4500 employees [81]. During one single patient visit, blood sampling and various measurements were performed at steady state (i.e. a minimum of three days after initiation of DOAC treatment). The included patients were treated according to the recommendations in the EMAs SPC for each DOAC [8, 11].

Patients were excluded under the following conditions:

- Non-compliance with the dosing recommendations specified in the SPCs for the studied DOAC.
- History of bariatric surgery.
- Treated with drugs that could interact pharmacodynamically or pharmacokinetically with the studied DOAC (studies I and II).
- Treated with drugs that could interact pharmacokinetically with the studied DOAC (studies III and IV).

In this thesis, a total of 306 patients treated with either apixaban (n=235) or rivaroxaban (n=71) were included. In Table 4 an overview of each study population is presented.

Table 4. Overview of the included patients in each study.

Study	*Indication -DOAC*	*Dose*	*n*	*Age, years* *mean ± SD*	*Weight, kg* *mean ± SD*	*GFR mL/min* *Median (range)*	*Sex* *% males*
I	AF -apixaban	5 mg bid	60	71 ± 6.9	85 ± 18.5	83 (45-139)[a]	64%
		2.5 mg bid	10	80 ±3.9	70 ± 19.6	43 (32-133)[a]	
II	AF -rivaroxaban	20 mg OD	61	72 ±8.1	87 ± 13.7	85 (45-182)[a]	55%
		15 mg OD	10	77 ± 4.2	67 ± 12.8	48 (40-63)[a]	
III	AF/AFL/VTE -apixaban	*5 mg bid:*					73%
		Obese	40	58 ± 11.9	124 ± 21.4	88 (51-114)[b]	
		Normal weight	40	58 ± 12.0	75 ± 8.5	86 (60-114)[b]	
IV	AF -apixaban	*5 mg bid:*					62%
		- Normal renal function	39	60 ± 8.4	85 ± 13.1	104 (78-126)[c]	
		- Moderate renal impairment	40	78 ± 5.1	80 ± 9.4	55 (35-74)[c]	
		2.5 mg bid:					
		- Severe renal impairment	6	74 ± 10.7	82 ± 11.3	27 (21-34)[c]	

[a]GFRCG, estimated glomerular filtration rate, calculated using the Cockcroft-Gault formula [82].

[B]Absolute GFRCr IBW (ideal body weight), [C]Absolute GFRCr TBW (total body weight), estimated glomerular filtration rate, calculated using LM Revised [83, 84].

In **study I,** apixaban concentrations were measured in 70 AF patients at trough (median 12.3 hours after last intake of apixaban dose). The levels were measured using Liquid Chromatography with tandem mass spectrometry (LC-MS/MS) and were compared to coagulation assays: anti-Factor Xa (anti-FXa) assay, prothrombin time-international normalized ratio (PT-INR) and activated partial thromboplastin time (aPTT).

In **study II**, blood samples from 71 AF patients were collected at trough (n=71) and peak (n=30) after a mean of 24.9 hours and 2.5 hours after last intake of rivaroxaban dose, respectively. Rivaroxaban levels were measured using LC-MS/MS and were compared to coagulation assays: anti-FXa, PT-INR (venous and point of care [POC] assay -CoaguChek) and aPTT.

A matched-paired analysis was performed in **study III**, comparing apixaban concentrations in 40 obese patients (mean BMI of 39.4 kg/m2) and 40 normal weight patients (mean BMI 23.4 kg/m^2). The patients were treated with apixaban 5 mg bid, regardless of indication, and were matched for sex, age and relative GFR creatinine (GFRCr)**.** Relative GFRCr is body surface adjusted to the average area for an adult, and was calculated using the revised Lund-Malmö equation [83]. Apixaban concentration was measured using LC-MS/MS at trough (n=40 pairs) and peak (n=22 pairs), on average 12.5 hours and 3.1 hours after last apixaban dose intake, respectively.

Study IV was a three-arm group study, including apixaban-treated AF patients with different levels of renal function. Patients in the normal- and moderately-impaired renal function groups were excluded in the screening process if fulfilling more than one of the characteristics for dose reduction (section 2.4.2). The patients were enrolled based on their last measured relative GFRCr and apixaban dose as follows:

- Normal renal function: >80 mL/min/1.73m^2, 5 mg bid, n=39
- Moderate renal impairment: 30-59 mL/min/1.73m^2, 5 mg bid, n=40
- Severe renal impairment, 15-29 mL/min/1.73m^2, 2.5 mg bid, n=6

At the time of the patient visit, creatinine was measured and subsequently new updated values for relative GFRCr were generated. Renal function was also estimated using cystatin C-based CAPA equation (GFRCys) [85]. The primary analysis was to compare apixaban exposure in patients with normal renal function to patients with moderate renal impairment, while an exploratory analysis was performed for patients with severe renal impairment. Apixaban trough concentrations were measured using LC-MS/MS, on average 12.1 hours after the last intake of apixaban.

4.2 LABORATORY METHODS

In this thesis several laboratory analyses were used to measure apixaban and rivaroxaban exposure and/or effects. Direct measurements of drug concentrations in plasma were conducted using LC-MS/MS, while indirect measurements, i.e. an effect converted to a concentration, were performed with an anti-FXa assay, while functional measurements included aPTT and PT-INR. In the subsequent sections, a short overview of each of these methods is presented.

4.2.1 LC-MS/MS methodology

Plasma concentrations of apixaban and rivaroxaban were analyzed using LC-MS/MS at the Department of Clinical Pharmacology, Karolinska University Hospital. The method has a measurement range of 2-500 ng/mL. A typical chromatogram of all DOACs that are analyzed using this method is shown in Fig. 5. LC-MS/MS is a highly sensitive and precise method, but slightly more time-consuming than anti-FXa assay and not available everywhere [86, 87].

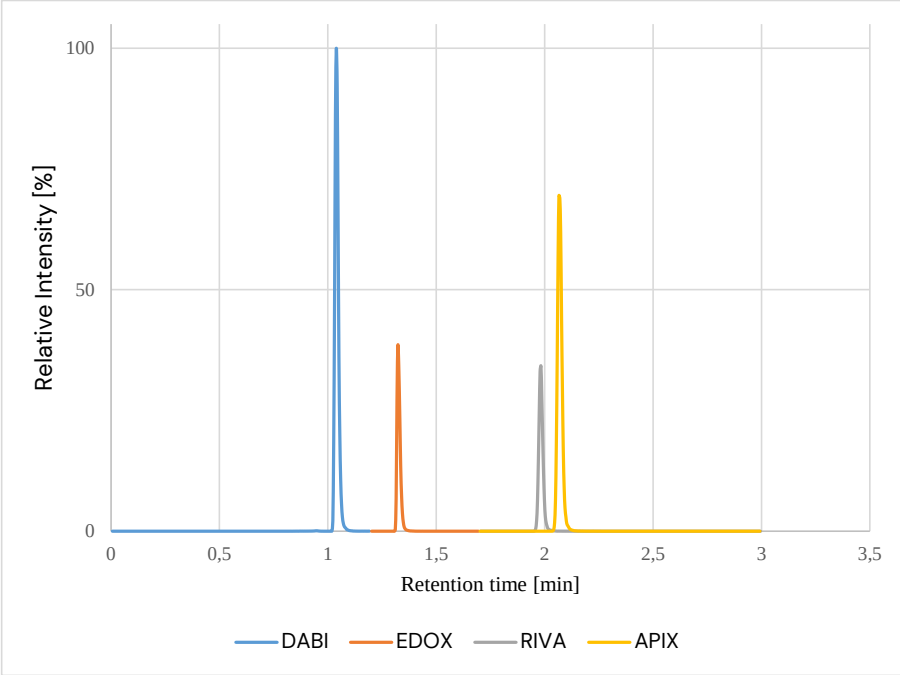

Figure 5. LC-MS/MS chromatogram of the DOACs.

4.2.2 Anti-FXa assay

Apixaban and rivaroxaban exposure were measured using the chromogenic assay STA® Liquid Anti-FXa (Diagnostica Stago, Asnieres, France) calibrated with apixaban/rivaroxaban plasma calibrators and analysed on Sysmex® CS-2100 (Sysmex, Kobe, Japan). This was performed at the Department of Clinical Chemistry, Karolinska University Hospital. The method has according to the method description a measurement range of 10-200 ng/mL.

The principle of this assay is the inhibition of exogenous factor Xa by the drug. Factor Xa is added and will be partially blocked by apixaban/rivaroxaban in the blood. The residual factor Xa is measured, using a photometric method and the results are converted to a corresponding plasma drug concentration [88].

4.2.3 PT-INR

The PT measures the time it takes to form a fibrin clot after factor VII activation in the extrinsic pathway. It measures the activity of the factors VII, V, X, prothrombin and fibrinogen [89]. Due to variability in methods used between different laboratories, the PT is presented as PT-INR, which is a ratio between the PT of the patient sample divided by a control PT [90].

PT-INR was analysed at the Department of Clinical Chemistry, Karolinska University Laboratory. For studies I and II, on Sysmex® CS2100i instrument (Sysmex, Kobe, Japan) using Owren reagent SPA + ® (Diagnostica Stago, Asnieres, France). As new instruments were introduced, Sysmex® CS-5100 instruments were used for studies III and IV with MRX Owren´s PT reagent (Medirox). The normal reference range of PT-INR is ≤1.2.

4.2.4 aPTT

The aPTT measures the time it takes to form a fibrin clot from the initiation of the intrinsic pathway through activation of factor XII. A prolonged aPTT is seen when there are reduced levels of factors XII, IX, XI, VIII, X, V, prothrombin, and fibrinogen [89].

This analysis was conducted at the Department of Clinical Chemistry, Karolinska University Laboratory. For studies I and II, the Sysmex® CS2100i instrument (Sysmex, Kobe, Japan) was used with Automate® reagent (Diagnostica Stago, Asnieres, France). For the studies performed later (studies III and IV), new instruments were introduced: Sysmex® CS-5100 (Sysmex, Kobe, Japan) using Dade Actin FS APTT reagent (Siemens). The normal reference range for aPTT is ≤40 seconds.

4.3 STATISTICAL ANALYSIS

Pearson's correlation coefficients were used to estimate simple correlations between variables.

Differences between two independent/unpaired samples were evaluated by unpaired sample t-test (normal distribution) and Wilcoxon's Rank Sum test/Mann-Whitney test (non-normal distribution). For categorical data, the chi-square test was used for the unpaired samples. Differences between dependent/paired samples were evaluated with t-test for paired samples (normal distribution) or the Wilcoxon signed rank test (non-normal distribution). For categorical data, McNemar's test was used for the paired samples. Normality distribution was assessed visually through histograms, and through testing with Shapiro-Wilk test or Kolmogorov-Smirnov test.

A p-value <0.05 was considered statistically significant. Statistical analyses were performed using The JMP package version 10.0 (SAS Institute, Cary, C, USA) and IBM SPSS statistics version 25 (IBM Corp, USA).

4.4 ETHICAL CONSIDERATIONS

All four studies were performed in accordance with the Declaration of Helsinki and approved by the Ethical Review Board in Stockholm, Sweden. The Dnr number for each study was as follows:

- **Study I**: Dnr 2012/1232-31/4
- **Study II**: Dnr 2012/1232-31/4 and Dnr 2014/390-32
- **Study III**: Dnr: 2019-03169
- **Study IV**: Dnr 2021-03598

The most important ethical consideration of this thesis involves respecting the personal integrity of the study patients. Before study participation, oral and written informed consent was obtained. Patients were informed that their participation was voluntary and declining participation, would not impact their health care. Further, they were informed that they at any time and without giving any explanation could withdraw their consent. Patient name and Swedish personal identity number, which is a unique number for each individual, were pseudonymized and provided with a code number. All patient documents were classified and locked up. The participant could not be identified in the published data in scientific medical journals. However, for certain patients, the medical records were screened and patients who met the inclusion criteria were contacted via a telephone call. While some patients might find this as a potential threat to their personal integrity, efforts were made to minimize this by selectively reading only the most relevant parts of the medical records.

There was a minimal risk of physical harm for the participants. All patients were treated according to clinical routine. Thus, no patient started, finished, or did in any way receive modifications in their drug therapy. Venous blood sampling was performed for all study patients. Besides the risk of developing a local reaction (e.g. hematoma), the harm of such a procedure for the individual patient is very limited. The possible benefits of these studies outweigh these minimal risks of harm.

There was no immediate benefit for the patients who participated in this research. However, increased knowledge about therapeutic levels of DOACs could in the longer-term lead to safer and more efficient drug therapy.

5 RESULTS

In this section, the main findings from the four included studies in this thesis are presented. A detailed description is available in the published papers/manuscripts.

5.1 METHODS TO MONITOR APIXABAN AND RIVAROXABAN

Analytical results from studies I and II are presented in this section.

5.1.1 Anti-FXa-assay

The anti-FXa assay correlated significantly ($p < 0.001$) with apixaban trough concentrations ($r=0.98$) and with rivaroxaban trough ($r=0.96$) and peak ($r=0.95$) concentrations, measured with LC-MS/MS. For trough concentrations in the lower range of ≤50 ng/mL for apixaban ($n=11$, $r=0.88$) and ≤30 ng/mL for rivaroxaban ($n=30$, $r=0.89$), the correlation was weaker but remained statistically significant ($p < 0.001$). At the lower concentration range, the anti-Fxa assay underestimated the concentration by 17% (all values: 6%) and 36% (all values: 15%) for apixaban and rivaroxaban, respectively.

5.1.2 aPTT

The correlations between aPTT and trough concentrations of apixaban ($r=0.32$) and rivaroxaban ($r=0.24$), measured with LC-MS-MS, were significant but weak. Despite treatment with apixaban or rivaroxaban, the majority of the aPTT values were within the normal range, below 40 s. A normal aPTT value was observed in 90% of the apixaban-treated patients and in 83% of the rivaroxaban-treated patients.

5.1.3 PT-INR

5.1.3.1 Venous

The correlations between venous PT-INR and apixaban trough concentrations or rivaroxaban trough/peak concentrations, measured with LC-MS/MS, were non-significant. Most of the patients treated with apixaban had normal PT-INR values ≤1.2 (86%). Among the patients treated with rivaroxaban, 70% and 30% had normal values of venous PT-INR at trough and peak measurements, respectively.

5.1.3.2 POC assay

A significant ($p < 0.001$) correlation was observed for rivaroxaban peak PT-INR POC assay and rivaroxaban peak concentrations measured with LC-MS/MS ($n=30$, $r=0.64$). Three percent had normal values of ≤1.2 measured at peak. For the trough concentrations, the correlation was non-significant, and 67% had normal PT-INR POC values.

5.2 RIVAROXABAN CONCENTRATIONS -LC/MS/MS

Rivaroxaban trough concentration varied 17-fold overall (see Table 5 for a detailed description by rivaroxaban dose). There were non-significant differences between the two dosage groups, for both trough and peak concentrations. For patients sampled both at trough and peak ($n=30$), there was a 17-fold variation at trough and a 3-fold variation at peak. The peak and trough concentrations correlated significantly ($r=0.52$) with higher concentrations at peak (median [range]: 233 ng/mL [120-375] vs. 33 ng/mL [5-84]), $p < 0.001$.

Among patients treated with the 20 mg OD dose, rivaroxaban concentrations at trough (n=61) and peak (n=24) did not correlate with body weight, age or GFRCG. Trough concentrations were significantly ($p < 0.05$) higher in females than males, while for peak it was non-significant.

Table 5. Rivaroxaban trough concentrations measured with LC-MS/MS, divided by dosage group

Study	***20 mg OD***		***15 mg OD***	
II	*Median (range)*	*10^{th}-90^{th} PCTL*	*Median (range)*	*10^{th}-90^{th} PCTL*
	Variability	*Variability*	*Variability*	*Variability*
Trough, n=71	34 (5-84)[1]	13-62	30 (22-66)[1]	22-65
	16.8	4.8	3.0	3.0

[1]Non-significant difference between the two dosage groups

PCTL = Percentile

Variability= interindividual variability, calculated as the ratio between the highest and lowest measured concentrations within each dosage group.

5.3 APIXABAN CONCENTRATIONS -LC/MS/MS

5.3.1 Trough concentrations overview

In studies I, III and IV, apixaban concentrations were measured. For patients treated with 5 mg bid, the correlation between apixaban trough concentrations and gender or age was non-significant (study I). An overview of the measured apixaban trough concentrations is presented in Table 6.

Table 6. Overview of apixaban trough concentrations (ng/mL) measured in studies I, III and IV, using LV-MS/MS. In study I, 60 patients were treated with the standard dose and 10 patients with the reduced dose.

Study	***5 mg bid***		***2.5 mg bid***	
	Median (range)	*10^{th}-90^{th} PCTL*	*Median (range)*	*10^{th}-90^{th} PCTL*
	Variability	*Variability*	*Variability*	*Variability*
I	77 (29-186)[1]	47-121	48 (15-83)[1]	17-80
	6.4	2.6	5.5	4.7
III				
Obese, n=40	59 (11-201)[2]	39-130		
	18.3	3.3		
Normal weight n=40	52 (31-151)[2]	34-83		
	4.9	2.5		
IV *-renal function*				
Normal, n=39	60 (16-171)[3]	35-124		
	10.7	3.6		
Moderately impaired, n=40	129 (41-295)[3]	74-222		
	7.2	3.0		
Severely impaired, n=6			82 (62-109)	
			1.8	

PCTL = Percentile

Variability= interindividual variability, calculated as the ratio between the highest and lowest measured concentrations

[1] Mann-Whitney test, $p < 0.001$

[2] Wilcoxon signed rank test, $p < 0.05$

[3] Mann-Whitney test $p < 0.001$

5.3.2 Body weight -obesity

In **study I**, body weight did not correlate significantly with apixaban trough concentrations in AF patients treated with apixaban 5 mg bid. The patients in this study had a mean (min-max) body weight of 85 kg (54-143) and BMI of 28 kg/m^2 (19-46).

In **study III**, 40 obese and normal weight pairs were matched for age, sex and renal function, and thus there were no differences between the groups for these characteristics. However, 65% of the obese patients had comorbidities such as diabetes, hypertension, and/or heart failure, while for those with normal weight the proportion was lower (35%), $p < 0.05$.

The obese patients had slightly but significantly higher median trough concentrations than the patients with normal weight (Table 6). Apixaban peak concentrations were not significantly different in obese patients compared to normal weight patients: 125 ng/mL (range: 82-278) vs. 114 ng/mL (range: 76-335).

An 18-fold variability in trough concentrations was observed within the obese group, whereas the normal weight group exhibited a 5-fold variability. The variability in peak concentration was less pronounced for both groups. Apixaban trough and peak concentrations had weak and non-significant correlations with BMI and weight.

5.3.3 Renal function

In studies I and III, the primary focus was not renal function, but subgroup analyses performed in these studies indicated that renal function affects apixaban levels. In **study I**, AF patients treated with apixaban 5 mg bid with GFRCG <82.5 mL/min (n=30) had significantly higher trough concentrations than patients with GFRCG >82.5 mL/min (n=30). The median trough concentrations were 92 ng/mL (range: 46-186) compared with 75 ng/mL (range: 29-126), in patients below and above GFRCG 82.5 mL/min, respectively. Apixaban trough

concentrations did not correlate significantly with GFRCG in study I, whereas in contrast a significant correlation was observed in **study III**. In study III, renal function, estimated as either relative GFRCr or absolute GFRCr ideal body weight (IBW), correlated with apixaban trough concentrations in patients treated with apixaban 5 mg bid ($r=-0.5$, $p<0.001$), yielding higher trough concentrations upon lower GFR.

In **study IV**, we proceeded to specifically examine how renal function affects apixaban levels. The three included groups had the following renal function as measured per the day they were sampled:

- Normal renal function, median relative GFRCr 85 mL/min/1.73m^2 (range: 72-111).
- Moderate renal impairment, median relative GFRCr 51 mL/min/1.73m^2 (range: 31-67).
- Severe renal impairment, median relative GFRCr 23 mL/min/1.73m^2 (range 22-28).

In study IV, patients with moderate renal impairment had substantially higher trough concentrations than patients with normal renal function (Table 6), $p<0.001$. This difference was also observed when men and female patient groups were analysed separately. In patients treated with apixaban 5 mg bid, the concentrations correlated with renal function, $p<0.001$. This correlation was observed for renal function estimated as relative/absolute GFRCr ($r=-0.6$), relative/absolute GFRCys ($r=-0.7$) and GFRCG ($r=-0.6$).

6 DISCUSSION

Apixaban and rivaroxaban, like other DOACs, are approved for treatment at a fixed dose without the prerequisite of routine monitoring. However, being able to assess the anticoagulant plasma concentration and/or effect may still improve treatment outcomes for the individual patient, i.e. enhancing efficacy and reducing adverse effects. In addition, in certain clinical situations being able to monitor the DOACs is necessary and simplifies handling and decision making. When we started this project, little was known about measurements of DOACs, what methods to use and how they correlated in clinical samples. We validated methods for the concentration measurements of apixaban and rivaroxaban in plasma and correlated the plasma levels to measures of drug effect through different coagulation tests. Further, the clinical utility of plasma concentration tests was explored as we studied how the drug levels were affected by different patient characteristics.

Based on the findings in this thesis, the following aspects will be discussed:

1. Laboratory methods to use for monitoring apixaban and rivaroxaban.
2. The typical exposure range of apixaban and rivaroxaban
3. Apixaban concentrations in obese
4. Apixaban concentrations in patients with renal impairment.

6.1 LABORATORY MEASUREMENTS OF APIXABAN AND RIVAROXABAN

As we show here, apixaban and rivaroxaban can both be monitored using LC-MS/MS, a highly sensitive method and now considered to be the golden standard. Unfortunately, it is not available everywhere and usually not around the clock [86, 91]. Therefore, finding an easier and more readily available method for monitoring DOACs was of importance. At the initiation of studies I and II, there was limited knowledge on this subject. While some had evaluated various coagulation assays in comparison to LC-MS/MS, the value of examining these laboratory methods using real-world data with actual patient samples was

evident, as opposed to solely relying on plasma samples spiked in vitro with a known amount of DOAC. In addition, any results could differ between laboratories and the type of regents that were used [92, 93]. We demonstrated a very good correlation between anti-FXa assay and LC-MS/MS, suggesting that this method can be used for estimating the anticoagulant effect of both apixaban and rivaroxaban. The manufacturer for each drug also reports utility for the anti-FXa assay [8, 11]. According to the SPC for apixaban, the anti-FXa assay is strongly correlated with the plasma concentration levels [8]. The anti-FXa assays have been tested in several studies using spiked apixaban/rivaroxaban plasma samples, in studies involving healthy volunteers and in patient studies [94-106]. However, it is important to note that for the lower concentrations of apixaban and rivaroxaban, the anti-FXa-assay was not reliable [97-99]. This observation aligns with the results found in in our studies (studies I and II), where the anti-Xa assays underestimated concentrations by 17 and 36%, respectively in apixaban and rivaroxaban trough concentrations below 50 and 30 ng/mL. Being able to estimate the precise concentration at these lower ranges is probably most important in emergency situations. In the guidelines provided by the International Council for Standardization in Haematology (ICSH), it is recommended to consider administering an antidote for patients experiencing serious bleeding and for those who will undergo acute surgery with high bleeding risk when DOAC trough concentrations exceed 50 ng/mL and 30 ng/mL, respectively. This recommendation implies that concentrations above these levels are linked to a clinically relevant effect, posing an increased risk of bleeding [54]. However, there has been some questioning of this threshold due to a lack of data [107]. And at an individual level, these thresholds may look very different. Nevertheless, measuring the exposure levels is likely safer than not doing so for the individual patient [108, 109].

We also examined the routine coagulation assays aPTT and PT-INR (venous), comparing their results with the concentrations measured using LC-MS/MS, and found that they lacked the required sensitivity to be used for the purpose of monitoring apixaban and rivaroxaban. Other studies confirm these results, as shown in both spiked plasma samples and in patients [94, 95, 100-102, 110]. There is a dose-dependent prolongation of aPTT in patients treated with rivaroxaban according to the SPCs. However, the method is not recommended to be used for assessing the effect [11]. The manufacturer of apixaban also states

that these tests are unsuitable for assessing the effect of the drug [8]. We found in studies I and II that the majority of patients treated with apixaban/rivaroxaban had normal values of aPTT and PT-INR (venous) measured at trough. For rivaroxaban peak samples measured with PT-INR (POC), only three percent had normal values, while for PT-INR (venous) 30% had normal values. Other studies also show a better correlation for PT-INR for the high/peak concentrations than for the trough concentrations, and with more promising results for the POC assay than venous PT-INR [98, 99, 111-113]. This is likely attributed to the sample dilution, with the POC assay being more concentrated and thus more sensitive [114].

It has been suggested that elevated PT-INR and prolonged aPTT can be used as indicators of high DOAC drug concentration. However, it is important to note that a) high DOAC plasma concentrations may occur at completely normal values for PT-INR and aPTT and b) an elevated PT-INR and prolonged aPTT may occur when DOAC plasma concentrations are within the commonly seen therapeutic range [115]. This approach is associated also with other challenges as there are variations between laboratory methods and regents [113]. Currently, two methods are available at the Karolinska University Hospital, Stockholm, Sweden for the assessment of the concentrations of apixaban and rivaroxaban: the LC-MS/MS, which covers the entire concentration range, and the anti-FXa assay, which is less reliable for the lower concentration range (<30 ng/mL) and only up to 200 ng/mL. However, the anti-FXa assay offers quicker results and around-the-clock analysis [116].

6.2 TYPICAL EXPOSURE RANGE

6.2.1 Apixaban

Prior to our study (study I), there was minimal information available regarding the typical exposure interval for apixaban in real-life patients with AF. Our study was the first to describe and contribute valuable insights in this field. The manufacturer reports apixaban median (5^{th}-95^{th} percentile) concentration levels (ng/ml) for AF patients of 79 (34-162) and 103 (41-230) at trough and 123 (69-

221) and 171 (91-321) at peak, for 2.5 mg and 5 mg bid, respectively. This means AF patients treated with the higher dose of apixaban (5 mg bid) had 30% higher trough- and 39% higher peak-plasma concentrations than patients treated with the lower dose (2.5 mg bid) [8]. A higher exposure for patients treated with the higher dose was also observed in our study (study I) but with an even greater difference between the two dosage groups (60%). Others also report this difference between the two dosages [117]. The point of dose reduction can be discussed. In general, doses are reduced in order not to achieve a too high exposure that would have been the case in certain conditions given standard dosing. For apixaban, these conditions are stated in the SPCs; conditions that may both increase the risk of thrombosis and bleeding. It can certainly be debated whether these patients should have lower exposure to apixaban than other patients or not.

6.2.2 Rivaroxaban

Data on rivaroxaban levels in real-life AF patients was sparse when we conducted study II. Concentration levels (mean, 5^{th}-95^{th} percentile) from the pivotal ROCKET trial on AF patients treated with 20 mg OD, showed larger variation between patients at trough (44, 12–137 ng/mL) than peak (249, 184–343 ng/mL). In patients treated with the reduced dose of 15 mg OD, the mean (5^{th}-95^{th} percentile) plasma concentrations in AF patients were 57 (18-136) ng/mL at trough and 229 (178-313) ng/mL at peak [118]. This corroborates the results from our study (study II), showing a higher variability for the trough- than peak-concentrations. The plasma concentrations did not differ much between the two dosage groups in the pivotal trial, and this was also seen in our study. Other studies report similar levels [119, 120]. This is in apparent contrast to what was observed for apixaban as discussed above.

6.3 MONITORING APIXABAN IN SUBGROUPS

In the pivotal trial ARISTOTLE, comparing apixaban with warfarin in AF patients, apixaban had superior efficacy and lower risk for serious bleeding [48]. Similarly,

in the pivotal trial AMPLIFY, apixaban had non-inferior efficacy and a lower risk for serious bleeding compared to treatment with low molecular heparin followed by warfarin [75]. The efficacy and safety of apixaban after drug approval, show similar favorable results as those in the pivotal trials [121]. However, it is crucial to acknowledge that apixaban is compared to a treatment that is associated with many challenges. Warfarin has in various studies shown to be the main cause of emergency visits due to adverse events [122, 123], and even though apixaban is associated with fewer risks, it still ranks high among the drugs causing emergency visits [123]. In the annual report for 2022, published by the Swedish Medical Product Agency on spontaneously reported adverse reactions by healthcare personnel, apixaban was the second most frequently reported substance for serious adverse reactions [124]. This is partly related to apixaban being the most used OAC in Sweden [40]. In the annual report for 2015, warfarin was the most used OAC and the substance with the most reported adverse reactions [125]. However, these findings suggest that there is room for improvement in apixaban treatment.

One possible approach is that reduction of apixaban adverse reactions and optimization of its efficacy may be achieved by assessing apixaban exposure/effect to individualize treatment and dosage. A concentration effect/adverse event relationship is highly likely, as shown in the pivotal trials for edoxaban and dabigatran [58, 60]. This relationship has also been shown for all DOACs in smaller studies [126-128]. Even though we currently lack a precise therapeutic interval for apixaban and other DOACs, there is likely a preferable concentration range. Monitoring the drug exposure, may improve treatment outcome. The intraindividual variability of the DOACs is much smaller than the interindividual variability [129, 130]. Hence, there is no requirement for extensive monitoring as with warfarin. Warfarin has a high intra-individual variability as its effect can vary extensively over time, thus monitoring its effect is required regularly. In contrast, as the DOAC concentration appears to be stable within individual patients [130], monitoring could be useful when initiating DOAC treatment and in cases where patient characteristics that could potentially affect the drug levels are altered. [131].

The recommended fixed dose of apixaban is likely suitable for the majority of the patients [8]. However, when comparing all the patients included in this thesis, a huge 27-fold variability was observed for the trough concentrations ranging between 11-295 ng/mL (Table 6) in patients treated with 5 mg bid (n=219).

Certain subpopulations may have more advantages from TDM than others. We studied two subpopulations treated with apixaban: patients with obesity and patients with renal impairment. The following sections will discuss these two subgroups in detail.

6.3.1 Obese patients

At low body weights below 60 kg, in combination with other characteristics, a reduced dose is prescribed [8]. The reduced dose was used in the pivotal studies on AF patients to reduce the risk of bleeding for patients with two or more of the criteria: age ≥80 years, body weight ≤60 kg or serum creatinine ≥133 µmol/L [48, 132]. A dose adjustment for high body weight or obesity is not recommended by the manufacturer. However, an increase in the volume of distribution is expected with higher body weight, e.g. 22% higher for a body weight of 90 kg compared with 70 kg [132]. In addition, obesity can affect other pharmacokinetic processes such as the metabolism and elimination of drugs. Consequently, altered apixaban exposure might be expected in obese patients [72, 133].

We observed in study III, similar median apixaban levels in obese patients and normal weight patients but noted much larger interindividual variability in trough concentrations among the obese patients. Interestingly, also warfarin treatment was more complicated in obese patients compared to non-obese patients, requiring higher doses and longer treatment duration to achieve a target INR [134]. It seems to be more difficult to predict the exposure in obese patients, and thus monitoring apixaban levels could be recommended to facilitate this.

6.3.2 Patients with renal impairment

Patients with reduced renal impairment have an increased risk for thromboembolic events and bleedings and are commonly treated with apixaban and other OACs as the risk for both AF and renal impairment increases with higher age [79, 135-137]. Since approximately 27% of apixaban is eliminated in unchanged form through the kidneys, an increase in apixaban exposure is expected with worsening renal function [8]. All three studies included in this thesis that examined apixaban demonstrated the significance of renal function on apixaban exposure levels. In study IV, the emphasis was specifically on renal function. When comparing all measured apixaban trough concentrations in this thesis for patients treated with 5 mg bid, those with moderately reduced renal function (median relative GFRCr: 51 ml/min/1.73m^2, range: 31-67 ml/min/1.73m^2), exhibited the highest trough concentrations. The median trough concentration was 129 ng/mL in these patients compared with 60 ng/mL in patients with normal renal function, and 77 ng/mL in study I with a mixed cohort of patients regarding renal function (not all had normal function), and 52-59 ng/mL in normal weight and obese patients in study III (table 6).

A declining renal function with higher age often goes hand in hand [79]. An analysis of subjects from the Aristotle trial showed that patients with only one and not two of the dose adjustments criteria: age ≥80 years, body weight ≤60 kg and creatinine ≥133 µmol/L, had an optimal treatment with apixaban 5 mg bid. Studies support that age alone have no clinically relevant influence on apixaban pharmacokinetics [138, 139], which we also observed in study I. Dose adjustments should be implemented not only based on the pharmacokinetics of apixaban and the SPC criteria for dose adjustments but also by assessing apixaban levels [140]. In study IV, the patients in the normal- and moderately impaired renal function groups were all treated with apixaban 5 mg bid, while the patients in the severely impaired renal function group were treated with the reduced dose, all according to the recommendations in the SPCs approved by EMA [8]. Despite following the recommendations, the patients with moderately impaired renal function had significantly higher, roughly twice as high trough concentrations as the other groups of patients.

A dose adjustment based on renal function for AF patients is only recommended when GFRCG is below 30 mL/min [8]. To achieve similar concentrations for patients with moderately impaired renal function as those in patients with normal renal function, adjusting the dose might be considered. But this should be done for the right reasons as one study showed that the use of a reduced apixaban dose without a renal cause (defined as the absence of creatinine ≥133 μmol/L) was associated with worse effect and no benefit in safety [141]. However, another study showed that apixaban levels in patients treated with the reduced off-label apixaban dose had adequate concentration levels [140]. Nevertheless, monitoring apixaban levels is necessary for these patients, but as there is not an established therapeutic interval for apixaban, data interpretation is difficult. Adjusting the dose for patients with moderate renal impairment and apixaban values in the higher range is likely rational and beneficial, particularly for those with a history of bleeding or currently treated with drugs that could interact with apixaban.

7 CONCLUSIONS

The following conclusions can be drawn from this thesis:

- Monitoring of apixaban and rivaroxaban can be performed using LC-MS/MS and anti-Fxa assay. LC-MS/MS covers the entire concentration range, while the anti-Fxa assay lacks precision at lower concentration levels.

  Venous PT-INR and aPTT are unreliable for monitoring apixaban and rivaroxaban.

- A substantial interindividual variability in trough concentrations was observed among patients treated with apixaban and rivaroxaban.

- Obese and normal weight patients exhibited similar apixaban trough and peak concentrations. However, there was extensive variability at trough among obese patients. Monitoring apixaban in obese patients may thus be of importance at the individual level to identify subjects far from the typical exposure range.

- Apixaban trough concentrations were twice as high in patients with moderate renal impairment as in patients with normal renal function. We recommend monitoring at moderate renal impairment and considering dose reduction when plasma levels are very high, as the appropriateness of the current dosing regimen seems questionable.

8 POINTS OF PERSPECTIVE

The overarching theme of this thesis, as introduced on the initial page of this compilation chapter, revolves around choosing "the right dose" for the "right patient". We examined and validated methods (studies I and II) to monitor apixaban and rivaroxaban, and as a result, we now have methods to assess the concentrations of these anticoagulants. This advancement facilitates individualized treatment, where the individual concentrations can be compared with the typical exposure ranges. However, it would be highly beneficial to establish a true therapeutic concentration interval with the best balance between efficacy and safety. This would also increase the knowledge of when and how to adjust the dose of the DOACs.

We demonstrated in studies III and IV the importance of measuring apixaban concentrations in obese patients and in patients with renal impairment. Identifying other patient characteristics requiring monitoring is fundamental. Addressing these knowledge gaps in future research may decrease the risks associated with apixaban and rivaroxaban and enable a better and safer treatment.

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