Direct Thrombin Inhibitors in Treatment and Prevention of Venous Thromboembolism: Dose – Concentration – Response Relationships

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Dissertation presented at Uppsala University to be publicly examined in Lecture hall B22, Biomedical Centre (BMC), Husargatan 3, Uppsala, Wednesday, May 24, 2006 at 10:00 for the degree of Doctor of Philosophy (Faculty of Pharmacy). The examination will be conducted in English.

Abstract

For prevention and treatment of thrombotic diseases with an anticoagulant drug it is important that an adequate dose is given to avoid occurrence or recurrence of thrombosis, without increasing the risk of bleeding and other adverse events to unacceptable levels. The aim of this thesis was to develop mathematical models that describe the dose-concentration (pharmacokinetic) and concentration-response (pharmacodynamic) relationships of direct thrombin inhibitors, in order to estimate optimal dosages for treatment and long-term secondary prevention of venous thromboembolism (VTE).

Population pharmacokinetic-pharmacodynamic models were developed, based on data from clinical investigations in healthy volunteers and patients receiving intravenous inogatran, subcutaneous melagatran and/or its oral prodrug ximelagatran. The benefit-risk profiles of different ximelagatran dosages were estimated using clinical utility functions. These functions were based on the probabilities and fatal consequences of thrombosis, bleeding and elevation of the hepatic enzyme alanine aminotransferase (ALAT).

The studies demonstrate that the pharmacokinetics of melagatran and ximelagatran were predictable and well correlated to renal function. The coagulation marker, activated partial thromboplastin time (APTT), increased non-linearly with increasing thrombin inhibitor plasma concentration. Overall, the systemic melagatran exposure (AUC) and APTT were similarly predictive of thrombosis and bleedings. The identified relationship between the risk of ALAT-elevation and melagatran AUC suggests that the incidence approaches a maximum at high exposures. The estimated clinical utility was favourable compared to placebo in the overall study population and in special subgroups of patients following fixed dosing of ximelagatran for long-term secondary prevention of VTE. Individualized dosing was predicted to add limited clinical benefit in this indication.

The models developed can be used to support the studied dosage and for selection of alternative dosing strategies that may improve the clinical outcome of ximelagatran treatment. In addition, the models may be extrapolated to aid the dose selection in clinical trials with other direct thrombin inhibitors.

Keywords: Ximelagatran, pharmacokinetic, pharmacodynamic, activated partial thromboplastin time, utility function, dosing strategy, venous thromboembolism, NONMEM

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I am not young enough to know everything

Oscar Wilde
The picture on the front cover is a computerized graph, based on X-ray crystallography, displaying part of human α-thrombin (295 amino acids, approx. 34,000 Daltons), with melagatran (430 Daltons) in the active site. Provided by Arne Svensson, AstraZeneca R&D, Möln达尔, Sweden.
This thesis is based on the following papers, which will be referred to in the text by the Roman numerals assigned below.


II. Cullberg M, Wählby U, Karlsson MO, Eriksson UG. Utility of ecarin clotting time, an ex vivo coagulation test, for pharmacokinetic analysis of the direct thrombin inhibitor melagatran. *Submitted*.


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Abbreviations

ACT     Activated clotting time
ALAT    Alanine aminotransferase
APC     Activated protein C
APTT    Activated partial thromboplastin time
AUC     Area under plasma concentration versus time curve
BW      Body weight
C       Concentration of drug in plasma
CAD     Coronary Artery Disease
CI      Confidence interval
CL      Total plasma clearance
CrCL    Creatinine clearance
CT      Computer tomography
Da      Dalton
DVT     Deep vein thrombosis
ECT     Ecarin clotting time
F       Bioavailability
FDP     Fibrin degradation product
FO      First-order
FOCE    First-order conditional estimation
GAM     Generalized additive modelling
HR      Hazard ratio
INR     International normalized ratio
i.v.    Intravenous
kₐ      Absorption rate constant
LLN     Lower limit of the normal reference range
LMWH    Low-molecular-weight heparins
Mw      Molecular weight
NONMEM  Nonlinear mixed-effects-model
PE      Pulmonary embolism
PT      Prothrombin time
OR      Odds ratio
OS      Orthopedic surgery
PD      Pharmacodynamic(s)
PiCT    Prothrombinase-induced clotting time
PK      Pharmacokinetic(s)
p.o.    Per oral
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>RefMid</td>
<td>Midpoint of the APTT normal reference range</td>
</tr>
<tr>
<td>RefWidth</td>
<td>Width of the APTT normal reference range</td>
</tr>
<tr>
<td>RSE</td>
<td>Relative standard error</td>
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<tr>
<td>s.c.</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
<tr>
<td>TAS</td>
<td>Thrombolytic Assessment System</td>
</tr>
<tr>
<td>TF</td>
<td>Tissue factor</td>
</tr>
<tr>
<td>TFPI</td>
<td>Tissue factor pathway inhibitor</td>
</tr>
<tr>
<td>t-Pa</td>
<td>Tissue plasminogen activator</td>
</tr>
<tr>
<td>TT</td>
<td>Thrombin time</td>
</tr>
<tr>
<td>UFH</td>
<td>Unfractionated heparin</td>
</tr>
<tr>
<td>ULN</td>
<td>Upper limit of the normal reference range</td>
</tr>
<tr>
<td>UWT</td>
<td>Utility weight</td>
</tr>
<tr>
<td>V</td>
<td>Apparent volume of distribution</td>
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<tr>
<td>VTE</td>
<td>Venous thromboembolism</td>
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</table>
1 Introduction

During prophylaxis and treatment with a thrombin inhibitor, as with any anticoagulant drug, it is essential that an adequate dose is given in order to avoid the occurrence or recurrence of thrombosis, without increasing the risk of bleeding and other adverse events to unacceptable levels.

This thesis deals with description and prediction of dose-concentration relationships (pharmacokinetics) and concentration-response relationships (pharmacodynamics) through mathematical models for direct thrombin inhibitors with emphasis on treatment and prevention of venous thromboembolism. Response in this context refers to the effect on coagulation biomarkers, and on clinical efficacy and safety endpoints.

1.1 Clinical drug development, dose selection and mathematical models

Clinical drug development is usually classified into different phases. Phase I studies, generally conducted in normal healthy subjects, focus on identifying tolerable doses, and on learning about the pharmacokinetic and pharmacodynamic properties of the drug. In phase IIA, the main objective is to confirm that the drug has promising efficacy in a small group of patients (‘proof of principle’). The goal of phase IIB is to learn how to use the drug in a larger group of representative patients. This is usually accomplished by dose ranging studies, with or without concomitant measurements of systemic exposure. In phase III studies the efficacy and safety of the novel drug should be confirmed against established treatment. Sheiner has viewed clinical development as two major learn-confirm cycles, the phase I-IIA, and the phase IIB-III cycles [1]. However, even if the main objective of a clinical study is confirming, there are several opportunities to learn about variation in pharmacokinetics and pharmacodynamics in patient groups to increase the likelihood of identifying dosing strategies that will result in safe and effective treatment in the individual patient.

The introduction of the population modelling approach has made this possible through the application of mathematical non-linear mixed-effects models to data obtained from relatively few samples in many individuals [2]. More specifically, population models allow characterization of (i) mean...
pharmacokinetic/pharmacodynamic parameters, (ii) extent of variability in these parameters and the sources thereof (e.g. gender, age, disease, co-medication), and (iii) relationships between pharmacokinetic (e.g. exposure) or pharmacodynamic (e.g. a biomarker) variables and clinical efficacy and safety endpoints. These models can then be used to simulate the outcome of various trial designs under different assumptions.

The usefulness of modelling and simulation in drug development and regulatory decision-making has been increasingly recognized [3-10]. Exposure-response models may, for example, be used to support (i) the use of a drug in new target populations through bridging, (ii) dose adjustment (or no need for dose adjustment) in subpopulations, (iii) new dose regimens, dosage forms and formulations, routes of administration, and minor product changes [11].

A biological marker (biomarker) has been defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [12]. The most reliable way to assess the benefit and risk of a drug therapy is through its effect on well-defined clinical endpoints. However, this approach is sometimes impractical for the evaluation of long-term disease therapies and trials that require a large number of patients. A biomarker may then be substituted for clinical response, provided that it is reasonably likely to predict clinical benefit [13]. However, the single most important use of biomarkers is selection of the dose range and doses for further investigation in the pivotal trials [14].

To further facilitate the identification of optimal dosing regimens, the use of clinical utility functions has been proposed [15-18]. Such functions serve to evaluate important desired and undesired effects of a drug on the same scale, under different assumptions of the relative severity of each outcome. In this way the observed or predicted clinical outcome of different drug therapies, or different dosing regimens of the same drug, may be compared.

1.2 Haemostasis and Thrombosis

Coagulation is an essential part of haemostasis, the process by which the body stops the flow of blood after injury. Haemostasis involves three basic steps:

(i) vascular contraction, which reduces the blood flow in the injured vessel
(ii) platelet adhesion, and subsequent platelet activation and aggregation, in response to exposure to collagen, thrombin and other factors which results in the formation of a loose platelet plug at the site of the injury (primary haemostasis), and
(iii) formation of fibrin, which interweave between the platelets, forming the mature thrombus (secondary haemostasis).

Fibrin formation results from a cascade of enzymatic reactions, known as the coagulation cascade (Figure 1). These reactions involve a series of inactive proenzymes (coagulation factors), which are converted to active forms, and cofactors such as calcium and vitamin K. Initiation of coagulation is triggered by tissue factor (TF), which is being exposed to blood after a vascular injury. TF binds to and forms a complex with circulating factor VIIa. This complex activates factor X to factor Xa, which converts prothrombin to thrombin, which in turn cleaves fibrinogen to fibrin.

The small amount of thrombin that has been generated during the initiation phase of coagulation (the extrinsic pathway) will then initiate feedback activation (the intrinsic pathway). In this amplification phase, a number of new platelets are recruited, via thrombin activation, to the primary platelet plug. At the same time, thrombin generation is sharply amplified when the thrombin that was produced during the initiation phase activates new coagulation factors (e.g. factors V, VIII). Factor IXa and factor VIIla forms the tenase complex, a potent activator of factor X, and factor Xa together with factor Va forms the prothrombinase complex, which is a potent activator of prothrombin.

The coagulation system is regulated by the action of two endogenous coagulation inhibitors:

- Tissue factor pathway inhibitor (TFPI), which inhibits the action of factor Xa via formation of a TFPI-Xa complex. This complex can also inhibit the TF-VIIa complex.
- Antithrombin, which is an inhibitor of thrombin and factor Xa.

Coagulation and thrombin generation are also regulated by thrombin itself. Thrombomodulin is an endothelial cell protein that binds to thrombin, which leads to a conformational change so that thrombin no longer cleaves fibrinogen into fibrin, but instead activates protein C to activated protein C (APC). APC finally degrades factors Va and VIIIa into inactive forms.

Fibrinolysis is the process by which fibrin is dissolved, resulting in the eventual removal of the clot. The proenzyme plasminogen is converted to plasmin in the presence of the enzyme tissue plasminogen activator (t-Pa). Plasmin breaks fibrin into small soluble fractions, fibrin degradation products (FDPs), e.g. D-dimers.

The amount of clot depends on the balance between the activity of coagulation and fibrinolysis. Excessive fibrinolysis can lead to haemorrhage, and thrombi can be formed at sites where they are not required to prevent blood loss. This may lead to obstruction of the affected blood vessel and to disruption of the circulation. Inappropriate thrombosis may occur in veins resulting in e.g. deep vein thrombosis (DVT) in the legs, or in arteries, e.g. coronary artery thrombosis, which may result in acute myocardial infarction. Intracar-
diac thrombi commonly develop due to atrial fibrillation, and may embolize to the cerebral circulation and cause a stroke.

**Figure 1.** The blood coagulation cascade. Modified from Pharmacology [19].

1.3 Venous Thromboembolism (VTE)

1.3.1 Clinical signs and symptoms

Patients with DVT may experience pain, swelling and stiffness in the affected area, but many patients are asymptomatic and remain undiagnosed unless fragments of the thrombus become detached (‘embolize’) and settle in the pulmonary vasculature. Small emboli may cause pleuritic chest pain and breathlessness, whereas massive pulmonary embolism (PE) can cause sudden death. Prevention of fatal PE is the primary reason for the prophylaxis and treatment of DVT. DVT and PE are described together as venous thromboembolism (VTE).

Many patients who have experienced a DVT develop signs of chronic venous insufficiency, or post-thrombotic syndrome.
1.3.2 Diagnostic methods

The ‘gold standard’ for the diagnosis of DVT is venography (phlebography) [20]. It involves injection of radiographic contrast medium into a vein in the foot, and shows the presence as well as the extent of thrombosis. Simpler, non-invasive, but less accurate [21], methods are Doppler ultrasound techniques, which detect changes in blood flow.

After intravenous (i.v.) injection of a radioactive isotope, obstruction to blood flow in an area of the lung can be detected by scintigraphy. To exclude other causes of abnormal lung perfusion ventilation scanning, in which the patient inhales gas labelled with a radioactive isotope, may be carried out. Usually, in a patient with PE, no abnormalities in the ventilation scan are seen, and a ventilation-perfusion scan mismatch thus forms the basis of a PE diagnosis. Pulmonary angiography may also be undertaken, whereby contrast medium is injected directly into the pulmonary artery. Spiral computer tomography (CT) is being used more frequently for the diagnosis of PE.

1.3.3 Incidence, risk factors, morbidity and mortality

Deep vein thrombosis is a common complication of surgery although it can also occur as a result of immobility or in the presence of other predisposing factors, e.g. genetic disorders, cancer, pregnancy and intake of oral contraceptives.

The incidence of VTE has been estimated to 1 per 1000 person-years [22], although the true incidence is difficult to estimate as the symptoms may be subclinical.

In an epidemiological study the risk of sudden death due to PE within 30 days was estimated to 20% in patients with VTE [22]. According to a review of 25 prospective studies 8.8% (95% CI 5.0-14.1%) of recurrent VTEs were fatal during 3 months of anticoagulant therapy [23]. A similar case-fatality rate was observed in clinical studies with ximelagatran in VTE patients [24, 25]. Overall 9 out of the 133 patients (6.8%; 95% CI 3-12%) who experienced a recurrent VTE in these studies died from fatal PE.

1.4 Treatment and prevention of venous thromboembolism

There are three main classes of anticoagulants used for treatment and/or prevention of venous thromboembolism; (i) heparins, which include unfractionated heparin (UFH), low-molecular-weight heparins (LMWH), and the synthetic pentasaccharides (factor Xa inhibitors), (ii) vitamin K antagonists, such as warfarin, and (iii) direct thrombin inhibitors. Antiplatelet drugs (e.g. aspirin, clopidogrel) are generally considered only for arterial and intracar-
diac thrombosis, as these thrombi are rich in platelets, while thrombi/emboli in the venous system have relatively low platelet content. Thrombolytic drugs (e.g. t-PA) are primarily used for the treatment of acute myocardial infarction and ischemic stroke. It has been suggested that only patients with unstable massive PE should be treated with thrombolytics due to its frequent bleedings [26].

The recommended duration for the acute treatment and secondary prevention of VTE is dependent on the patient’s risk factors, but is generally between 3 and 12 months, sometimes indefinitely [27].

1.4.1 Heparins

Heparins bind to and potentiate the effect of the endogenous inhibitor antithrombin, which then inhibits thrombin and factor Xa [28].

Standard UFH (Mw 3 000-30 000 Da) is administered by continuous i.v. infusion or subcutaneous (s.c.) injection. The binding to cells and plasma proteins makes the anticoagulant effect of heparin difficult to predict. Coagulation monitoring with APTT (see section 1.5.1) is therefore required.

UFH has largely been replaced with LMWH (Mw 3 000-6 000 Da) for prevention and treatment of thrombosis due to the need for regular monitoring, the risk of bleeding, and the risk of heparin-induced thrombocytopenia, a severe immune reaction, with UFH. LMWHs are administered subcutaneously once or twice daily at fixed or weight-adjusted doses.

Fondaparinux sodium is a synthetic pentasaccharide, which is an analogue of the pentasaccharide sequence of heparin. [29]. Fondaparinux sodium is primarily used for prevention of VTE after major orthopedic surgery. It is administered s.c. once daily as a fixed dose without coagulation monitoring [30].

1.4.2 Vitamin K antagonists

Coumarin-derivatives, such as dicoumarol and the most widely used compound warfarin, act as anticoagulants by inhibiting the activation of vitamin K-dependent clotting factors (prothrombin, factors VII, IX and X) [31]. Warfarin is a highly effective drug, but the clinical use is limited by its narrow therapeutic window, and interactions with food and various medications, resulting in a need for coagulation monitoring with PT/INR (see section 1.5.2) and frequent dose adjustments [32].

1.4.3 Direct thrombin inhibitors

Direct thrombin inhibitors bind reversibly to the active site of thrombin, thereby directly inhibiting thrombin-mediated catalysis of fibrinogen into fibrin.
1.4.3.1 Hirudin
Hirudin is a peptide, originally isolated from the saliva of the medicinal leech, but is now available through recombinant DNA technology (desirudin, lepirudin). They are used for the prevention of postoperative venous thromboembolism, or in the management of thromboembolic disorders in patients with heparin-induced thrombocytopenia [33, 34]. They are administered parenterally and monitored using APTT.

1.4.3.2 Inogatran
Inogatran is a synthetic low-molecular-weight (MW 430 Da), reversible, competitive and selective thrombin inhibitor [35]. It has been clinically studied in healthy volunteers and in patients with coronary artery disease [36]. None of the four inogatran dosages tested in coronary artery disease patients was better than heparin in preventing ischemic events, and there was no relationship between event rate and inogatran dosage. Higher APTT levels during inogatran treatment were related to increased risk of death, myocardial infarction or refractory angina [37]. The clinical development of inogatran was discontinued.

Inogatran has been used to characterize the variability in APTT due to different studies, study centres and individuals in paper IV [38].

1.4.3.3 Melagatran and ximelagatran
Melagatran is a synthetic, low-molecular weight (MW=430 Da), direct, reversible, thrombin inhibitor [39]. It inhibits free and clot-bound thrombin as well as thrombin generation [40-43]. It also inhibits thrombin induced platelet aggregation [44]. Animal models of thrombosis have suggested that melagatran has a wider therapeutic window than warfarin [45].

The s.c. bioavailability of melagatran is complete [44]. Due to poor p.o. bioavailability melagatran is given orally as a prodrug, ximelagatran (MW=474 Da), resulting in a bioavailability of melagatran of about 20% in young healthy subjects [46]. Ximelagatran is metabolized in vivo, through reduction and hydrolysis to melagatran via two intermediary metabolites (Figure 2), and melagatran is not further metabolized [47]. The thrombin-inhibiting activity of ximelagatran and OH-melagatran is about 1% of that of melagatran, whereas ethyl-melagatran has about the same inhibitory potency as melagatran [44]. The prodrug and the two intermediary metabolites are rapidly cleared from plasma with half-lives of 0.3-1.5 hours and the predominant compound in plasma is melagatran, see Figure 3 [47]. The major route of elimination for melagatran is renal excretion; about 80% of an i.v. dose is excreted unchanged in the urine. The elimination half-life of melagatran is about 2 hours after i.v. or s.c. administration and 3-4 hours when given as p.o. ximelagatan to young healthy volunteers.
Figure 2. The metabolic pathways of ximelagatran for the formation of melagatran via two intermediate metabolites, ethyl-melagatran (H 338/57) and OH-melagatran (H 415/04), by reduction of the OH-group and hydrolysis of the ethyl ester.

Figure 3. Mean (SD) plasma concentrations of ximelagatran, melagatran, and the intermediate metabolites (H 338/57 and H 415/04) versus time (h) since oral dosing of ximelagatran 36 mg (n=26).
The effects of melagatran on varying coagulation assays have been described by Carlsson et al [48]. This review included TT, ECT, PiCT, APTT and PT (for explanation, see section 1.5), which responded to melagatran in decreasing sensitivity order (Figure 4).

Ximelagatran/melagatran has been used clinically in European countries for thromboprophylaxis in orthopedic surgery. In addition, ximelagatran has been given to approximately 7 000 patients in long-term studies for the treatment [25] and secondary prevention [24] of VTE, prevention of stroke in atrial fibrillation [49] and prevention of recurrent cardiovascular events after acute myocardial infarction [50]. Ximelagran was found to be effective and safe with regards to bleedings, compared to standard therapy in these studies. The phase II and III studies conducted with ximelagatran/melagatran have been reviewed by McBride [31], and the studies in treatment and secondary prevention of VTE are summarized below.

Results from the dose-guiding study (Thrombin Inhibitor in Venous Thromboembolism [THRIVE] I study) in patients with acute DVT suggested that oral ximelagatran, given in doses of 24, 36, 48, or 60 mg b.i.d. for two weeks, was well tolerated and as effective as standard treatment with LMWH and warfarin, with a similar incidence of bleeding [51]. All doses of ximelagatran appeared to be similarly effective with regard to regression of the thrombus, reduction of clinical symptoms of deep vein thrombosis (pain and oedema in the affected leg), and bleeding-related events. The pharmacokinetic and exposure-response evaluations are reported in paper III [52].
a randomized, double-blind, non-inferiority trial (THRIVE Treatment study) in patients with acute DVT, of whom approximately one third had concomitant PE, VTE recurred in 26 of the 1240 patients assigned to receive ximelagatran 36 mg b.i.d. (estimated cumulative risk, 2.1%) and in 24 of the 1249 patients assigned to receive enoxaparin/warfarin (2.0%) [25]. The absolute difference between ximelagatran and enoxaparin/warfarin was 0.2% (95% CI -1.0–1.3%). Corresponding values for major bleeding were 1.3% and 2.2% (difference, -1.0%; 95% CI -2.1–0.1%). In another double-blind trial (THRIVE III study; paper IV), 1233 patients with venous thromboembolism, who had undergone six months of standard anticoagulant therapy, were randomized to receive extended secondary prevention with ximelagatran (24 mg b.i.d.) or placebo for 18 months [24]. Symptomatic recurrent venous thromboembolism was confirmed in 12 patients assigned to ximelagatran and 71 patients assigned to placebo (hazard ratio, 0.16; 95% CI 0.09–0.30). Bleeding occurred in 134 patients and 111 patients, respectively (hazard ratio, 1.19; 95% CI 0.93–1.53). The incidence of major haemorrhage was low (six events in the ximelagatran group and five in the placebo group), and none of these haemorrhages were fatal.

Increased levels of liver enzymes in ximelagatran-treated patients were, however, observed and a potential risk for liver injury associated with ximelagatran use could not be excluded. 546 (7.9%) patients participating in the long-term studies with ximelagatran experienced elevations of alanine aminotransferase (ALAT) to more than three times the upper limit of normal reference range (>3-ULN), and there were concerns that three cases of deaths were related to ximelagatran-induced liver injury [53, 54]. The case-fatality rate in patients with ALAT-elevations to >3-ULN, following ximelagatran use, may thus be estimated to 0.55% (95% CI 0.11-1.60%).

1.4.3.4 Other
Argatroban is a synthetic direct thrombin inhibitor, which is used for the treatment and prophylaxis of thromboembolism in patients with heparin-induced thrombocytopenia. It is given i.v. and monitored with APTT [34]. Dabigatran is an oral direct thrombin inhibitor, currently undergoing clinical investigations [55-57].

1.4.4 Bleeding from antithrombotic treatment
As a consequence of their mechanism of action, all antithrombotic drugs have a potential for introducing haemorrhage.

The risk of major bleeding associated with i.v. UFH in patients with acute VTE was <3% (3 months), and LMWH was associated with less risk [58]. Overall, the rate of major bleeding events for patients on warfarin is reported to be between 1% and 4% per year [59-61]. In addition to the intensity of the anticoagulant effect, major determinants of bleeding risk include high age,
female gender, and co-morbidity [58, 62-64]. In a meta-analysis of 33 prospective studies involving 4374 patient-years of oral anticoagulant therapy (target INR 2.0–3.0), 37 of 276 major bleedings were fatal, resulting in an overall case-fatality rate of 13.4% (95% CI 9.4–17.4%) [65].

The risk of major bleedings, following treatment (36 mg b.i.d.) and secondary prevention (24 mg b.i.d.) of VTE with ximelagatran was 1.3% (6 months) and 1.0% (18 months), respectively [24, 25]. Overall 3 out of the 51 patients (5.9%; 95% CI 1.2-16.2%) who experienced a major bleeding in these studies (including the comparator groups) were judged to have died because of bleeding.

1.5 Biomarkers for anticoagulants

There are a variety of coagulation tests available for anticoagulants, which formally meet the definition of a biomarker. The value of APTT and ECT as such have, however, been questioned as they only demonstrate that \textit{ex vivo} clot formation has been altered, not that an \textit{in vivo} process has been affected [66]. Nevertheless, they may be useful as predictors of clinical outcome, reflecting a pharmacologic response, even though they may have little or no value as indicator of normal or biological pathogenic processes.

1.5.1 Activated partial thromboplastin time (APTT)

APTT is an assay that measures the activity of all coagulation factors in the intrinsic pathway (V, VIII, XI, and XII). After incubation of citrated plasma with a reagent containing phospholipids and a contact activator, calcium is added and the clotting time is registered. APTT has long been used to monitor treatment with UFH [67], but it is not affected by LMWH at therapeutic doses [68]. The recommended APTT-prolongation in treatment of VTE with UFH is 1.5–2.5 times [27]. APTT is prolonged by direct thrombin inhibitors [48, 69].

Association between an increased risk of VTE and a short APTT has been found in several studies [70-73]. The risk of recurrent VTE among patients with an APTT-ratio (to reference APTT) $\leq 0.95$ was estimated to 0.56 (95% CI 0.38-0.84), compared to patients with an APTT-ratio $<0.95$ [74].

1.5.2 Prothrombin time and INR

Prothrombin time (PT) is an assay that measures the activity of vitamin K-dependent factors (prothrombin, factors VII, IX and X), i.e. the extrinsic pathway. Vitamin K antagonists result in synthesis of less functional vitamin K-dependent clotting factors and thus prolong PT. PT is slightly prolonged by direct thrombin inhibitors [48].
The International Normalized Ratio (INR) is a standardization of the PT test. It is calculated according to \( \text{INR} = \left( \frac{\text{PT}_{\text{patient}}}{\text{PT}_{\text{normal}}} \right)^{\text{ISI}} \), where ISI is a reagent- and instrument-specific correction factor [75]. A target INR range of 2.0–3.0 is recommended for long-term treatment of VTE [27].

1.5.3 Ecarin Clotting Time

Ecarin clotting time (ECT) is a coagulation test, which was first introduced for monitoring of hirudin therapy [76]. The assay uses a protease extracted from the snake \textit{Echis carinatus} venom, which cleaves prothrombin to generate meizothrombin, which in turn autocatalyses to \( \alpha \)-thrombin [77, 78]. The ECT is sensitive to a series of direct thrombin inhibitors \textit{in vitro}, but not to heparin [79, 80]. It has been shown to be well correlated to the concentration of melagatran in plasma from healthy volunteers \textit{in vitro} and \textit{ex vivo} studies [48].

1.5.4 Other

The activated clotting time (ACT) uses a similar test principle as APTT, except that it is carried out in whole blood. It is insensitive for thrombin inhibitors at high plasma levels [48, 81].

Prothrombinase-induced clotting time (PiCT) is a newly developed clotting assay that allows activation of factor V and formation of the prothrombinase complex (intrinsic pathway). It yields comparable results with direct thrombin inhibitors and UFH, and has been suggested as suitable for monitoring of both drug groups [82].

Thrombin time (TT) is a screening test for the fibrinogen–fibrin polymerization step. TT is linearly related to plasma melagatran concentrations in the lower therapeutic range, but samples with high therapeutic concentrations need to be diluted before analysis by the traditional method [48].
2 Aims

The overall aim of this thesis was to develop mathematical models that describe the pharmacokinetics and pharmacodynamics of direct thrombin inhibitors, and their relationship to clinical efficacy and safety endpoints, in order to estimate optimal dosages for treatment and secondary prevention of venous thromboembolism (VTE). Specific aims were to

- Describe the pharmacokinetics of melagatran given as p.o. ximelagatran or s.c. melagatran in patients undergoing orthopaedic surgery, and in patients who have experienced a deep vein thrombosis and/or a pulmonary embolism
- Evaluate the performance of ecarin clotting time (ECT) when used as a surrogate for plasma melagatran concentration measurements in pharmacokinetic evaluation
- Characterize the relationship between plasma concentration and activated partial thromboplastin time (APTT) for inogatran and melagatran in healthy subjects and/or in patients with thrombosis
- Develop models that describe the relationship between melagatran exposure (AUC) and
  - the change in thrombus size and the probability of bleeding in acute treatment of VTE
  - the probability of VTE recurrence, bleeding, and ALAT-elevation in extended secondary prevention of VTE
- Develop models that describe the relationship between APTT and the probability of VTE recurrence and bleeding in extended secondary prevention of VTE
- Predict the clinical efficacy and safety outcome following varying dosing regimens of ximelagatran in secondary prevention of VTE
- Illustrate how utility functions based on the probabilities and consequences of clinical events can be used to select dosing strategies.
3 Materials and Methods

3.1 Clinical studies

3.1.1 Inogatran (paper I)

APTT and plasma inogatran concentration data from five phase I studies and two clinical multicentre studies in patients with unstable angina pectoris or non-Q-wave myocardial infarction, were evaluated. A total of 3296 pairs of concentration-APTT samples were obtained before, during and after i.v. infusion of inogatran at varying doses. Major study design features are given in Table 1, and subject characteristics in Table 2.

Table 1. Design features of the clinical studies with inogatran (paper I)

<table>
<thead>
<tr>
<th>Study</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical phase</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>IIA</td>
<td>IIB</td>
</tr>
<tr>
<td>Number of centres</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>61</td>
</tr>
<tr>
<td>Administration</td>
<td>i.v. inf (10 min)</td>
<td>i.v. inf (10 min)</td>
<td>i.v. inf (4 hrs)</td>
<td>i.v. inf (10 min)</td>
<td>i.v. inf (10 min)</td>
<td>i.v. inf (4 hrs)</td>
<td>i.v. inf (72 hrs)</td>
</tr>
<tr>
<td>Conc range</td>
<td>0.10–7.06</td>
<td>0.02–2.41</td>
<td>0.02–2.38</td>
<td>0.02–0.92</td>
<td>0.02–2.42</td>
<td>0.03–1.91</td>
<td>0.04–9.10</td>
</tr>
<tr>
<td>Population</td>
<td>Healthy subjects</td>
<td>Healthy subjects</td>
<td>Healthy subjects</td>
<td>Healthy subjects</td>
<td>Healthy subjects</td>
<td>CAD</td>
<td>CAD</td>
</tr>
<tr>
<td>Number of individuals</td>
<td>22</td>
<td>16</td>
<td>16</td>
<td>12</td>
<td>12</td>
<td>49</td>
<td>899</td>
</tr>
<tr>
<td>Number of samples</td>
<td>183</td>
<td>155</td>
<td>477</td>
<td>249</td>
<td>32</td>
<td>286</td>
<td>1913</td>
</tr>
</tbody>
</table>

CAD= Coronary artery disease patients

a Plasma inogatran concentration (µmol/L)
Table 2. Subject characteristics of healthy volunteers and patients in studies with inogatran (paper I)

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects/patients</td>
<td>78</td>
<td>948</td>
</tr>
<tr>
<td>Gender (♂/♀)</td>
<td>78/0</td>
<td>652/296</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>28 (20–39)</td>
<td>66 (32–81)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>76 (66–86)</td>
<td>79 (50–121)</td>
</tr>
<tr>
<td>Baseline APTT (s)</td>
<td>34 (28–49)</td>
<td>29 (15–60)</td>
</tr>
</tbody>
</table>

For categorical variables the number of individuals is given and for continuous variables the median (range) is given.

3.1.2 Melagatran/Ximelagatran (papers II-IV)

Two phase II dose-guiding studies (one including s.c. melagatran) and one phase III study with ximelagatran were included in the analyses. One of the phase II studies was conducted in patients undergoing elective hip or knee replacement surgery (‘METHRO I study’, paper II) and one in patients with acute deep vein thrombosis (‘THRIVE I study’, paper III). In the phase III study patients received ximelagatran or placebo as secondary prevention of VTE following standard treatment for 6 months (‘THRIVE III study’, paper IV). Summary characteristics of the studies are given in Table 3. The major differences between the study populations are that the orthopedic surgery population was older and included more women, as compared to the VTE patient populations.
Table 3. Characteristics of the clinical studies with melagatran/ximelagatran including demographics of the pharmacokinetic study population

<table>
<thead>
<tr>
<th></th>
<th>METHRO I study Paper II</th>
<th>THRIVE I study Paper III</th>
<th>THRIVE III study Paper IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical phase</td>
<td>II</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>Melagatran doses (mg s.c. b.i.d.)</td>
<td>1, 2, 4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ximelagatran doses (mg p.o. b.i.d.)</td>
<td>6, 12, 24</td>
<td>24, 36, 48, 60</td>
<td>24</td>
</tr>
<tr>
<td>Treatment time</td>
<td>8–11 days</td>
<td>12–16 days</td>
<td>18 months</td>
</tr>
<tr>
<td>Study population</td>
<td>Orthopedic surgery</td>
<td>Acute VTE</td>
<td>VTE</td>
</tr>
<tr>
<td>Number of patientsa</td>
<td>98 (102)</td>
<td>264 (277)</td>
<td>596 (617)</td>
</tr>
<tr>
<td>Gender (Ƃ/ƃ)</td>
<td>37/61</td>
<td>151/113</td>
<td>322/274</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>72 (53–80)</td>
<td>62 (20–85)</td>
<td>58 (18–87)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>78 (53–108)</td>
<td>82 (50–118)</td>
<td>82 (46–147)</td>
</tr>
<tr>
<td>CrCLb (ml/min)</td>
<td>65 (45–127)</td>
<td>86 (35–183)</td>
<td>104 (35–271)</td>
</tr>
<tr>
<td>Number of samplesc</td>
<td>931 C + 1772 ECT</td>
<td>1836 C</td>
<td>3595 C</td>
</tr>
<tr>
<td>Evaluationd</td>
<td>PK, PD</td>
<td>PK, ER</td>
<td>PK, PD, ER, AR</td>
</tr>
</tbody>
</table>

For categorical variables the number of patients is given and for continuous variables the median (range) is given.

a Number included in pharmacokinetic evaluation (number randomised to ximelagatran)
b Creatinine clearance [83]
c Number of samples included in the pharmacokinetic evaluation (C=plasma melagatran concentration, ECT=Ecarin clotting time)
d Evaluations included in this thesis (PK=pharmacokinetic, PD=pharmacodynamic, ER=exposure – clinical response, AR= APPT – clinical response)

3.1.3 Ethical conduct

All studies were performed in accordance with the Declaration of Helsinki and Good Clinical Practice and were approved by the relevant ethics committees. Written informed consent was obtained from all healthy volunteers and patients included.
3.2 Measurements and variables

3.2.1 Drug concentration in plasma (papers I-IV)

3.2.1.1 Blood sampling schedules

In the inogatran studies (paper I) frequent venous blood samples were drawn in healthy volunteer studies A–E, during and after the infusion of inogatran, while sparse sampling schedules were applied in patient studies F–G. Details are given in paper I [38].

In orthopedic surgery patients (paper II) venous blood samples for determination of plasma melagatran concentration (from about half of the patients) and ECT (from all patients) were to be drawn in 5 ml citrated tubes at the following time points:

- prior to surgery (baseline ECT)
- on the day of surgery at 3, 6 and 10 hours after the first s.c. injection
- on the post-operative day at 0.5, 1, 2, 4 and 8 hours after the third s.c. injection
- on the first and final day of p.o. administration at 2, 4, 6, 8 and 10 hours after the morning dose.

In the acute VTE study (paper III) one blood sample was to be collected per time point at 0, 2, 4, 6, and 8 hours post dose on day 1 or day 2 (5 samples), and at a single time point between 2 and 8 hours post dose on days 3 to 5, days 6 to 9, and days 12 to 16 (3 samples).

In the phase III VTE secondary prevention study (paper IV) one blood sample was to be collected before the first dose, and one between 1 and 10 hours post dose at 0.5, 3, 6, 9, 12, 15, and 18 months of ximelagatran treatment in all patients. In addition two samples were collected, separated by at least 30 min, at 1–4 h or 4–8 h post dose at 0.5 and 18 months in patients from four preselected countries.

3.2.1.2 Bioanalytical methods

3.2.1.2.1 Inogatran (paper I)

Plasma was recovered after centrifugation of venous blood samples, drawn in 5 ml heparinized tubes. The concentration of inogatran was determined using reversed-phase liquid chromatography (LC) and mass spectrometry. The lower limit of quantification was 0.10 μmol/L in study A, and 0.02 μmol/L in studies B-G.

3.2.1.2.2 Melagatran (papers II–IV)

The melagatran concentration was determined in plasma recovered from citrated blood samples, after centrifugation and storage at -20°C. The melagatran concentration was determined by liquid chromatography-mass spectrometry after solid phase extraction of melagatran from plasma [84]. The lower limit of quantification was 0.01 μmol/L.
3.2.2 Ecarin Clotting Time (paper II)

Blood samples were drawn as described in section 3.2.1.1. ECT was determined using dry-chemistry test cards impregnated with a low concentration of ecarin and the Thrombolytic Assessment System (TAS) [85, 86].

3.2.3 Activated Partial Thromboplastin Time (papers I and IV)

The blood sampling schedules used in paper I and paper IV are described in section 3.2.1.1. In the studies reported in paper I, plasma from citrated blood samples was separated and APTT was measured according to the standard method of the local laboratory. All samples from healthy volunteers were analysed by the same laboratory using the reagent PTT-Automate 10™ (Diagnostica Stago). The normal reference range of this laboratory was 30-42 s. In study F three additional laboratories were used, with normal reference ranges of 23-34 s, 24-35 s, and 30-42 s, respectively. In study G APTT was determined locally at 61 different centres. The lower limit of normal (LLN) values varied between 18 and 30 s (median 25 s), and upper limit of normal (ULN) values between 30 and 46 s. The width of the reference intervals (RefWidth) varied between 6 and 20 s and the midpoint (RefMid) between 26 and 38 s for the different laboratories.

In the phase III study (paper IV) a central laboratory analysed all APTT samples, which were drawn in citrated tubes. A standard method was applied with Dade Actin FSL Activated PTT Reagent. The reference range was 22-34 s.

3.2.4 Thrombosis evaluation (papers III-IV)

3.2.4.1 Change in thrombus size (paper III)

Venograms, performed before randomization and at the end of the 2-week study treatment period, were evaluated by 2 independent radiologists who were blinded to the treatment allocation. The change in thrombus size was classified as regression, no change, or progression, where a difference between the start and end of the treatment of more than ±2 cm was regarded as a change. Further information is given in Eriksson et al [51].

3.2.4.2 Recurrence of thrombosis (paper IV)

Standardized bilateral compression ultrasonography of the legs and ventilation-perfusion lung scanning were used for confirmation of clinically suspected VTE recurrences. All suspected recurrent VTE events were adjudicated by a central, independent, blinded endpoint committee.
3.2.5 Bleeding evaluation (papers III–IV)

In paper III severe and minor bleeding events were recorded throughout the study [51]. Severe bleeding was defined as intracerebral, intraocular, intraspinal, retriperitoneal, or excessive bleeding as judged by the local investigator.

In paper IV [24] major and minor bleedings were recorded throughout the study. Major bleeding was defined as a fatal haemorrhage, a clinically overt haemorrhage associated with a decrease in the haemoglobin level of at least 20 g per litre or a need for transfusion of at least 2 units of blood, a retroperitoneal or intracranial haemorrhage, or any bleeding warranting permanent cessation of the study treatment. All other bleeding events were regarded as minor. All bleeding events that were reported to be major were adjudicated by a central, independent, blinded endpoint committee.

3.2.6 ALAT (paper IV)

Blood samples for ALAT analysis (and other clinical chemical analysis) were collected approximately monthly and were analyzed at a central laboratory, applying the normal reference range 0-48 U/L. A cut-off value of ALAT elevation to >3-ULN any time during the study was selected as a potential signal for hepatic injury.

3.3 Data analysis

3.3.1 Population modelling approach

The population modelling approach was used for the pharmacokinetic and pharmacodynamic analyses. This approach applies nonlinear mixed-effects models to repeated measurements from a group of individuals [87]. Such models include fixed effect parameters (θ:s), which describe the typical value (population mean) of a parameter (e.g. drug clearance (CL)) and its relation to patient covariates (e.g renal function), and random effect parameters at two or more levels.
In *Figure 5* an example of a mixed effects model is represented. If $\text{CL}_i$ denotes drug clearance for individual $i$, and clearance is linearly related to renal function (creatinine clearance; CrCL), then the typical value of clearance for individual $i$ ($\text{TV}(\text{CL}_i)$) may be written as follows (lower left panel):

$$\text{TV}(\text{CL}_i) = \theta_1 + \theta_2 \cdot \text{CrCL}_i$$

Due to random interindividual variability, the clearance of individual $i$ (filled circle) will differ from its typical value (unfilled circle) by a factor $\eta_i$:

$$\text{CL}_i = \text{TV}(\text{CL}_i) + \eta_i$$

The $\eta_i$s are assumed to be symmetrically distributed with a mean value of 0 and a standard deviation of $\omega$ (upper left panel).

If, for example, the pharmacokinetics is characterized by a one-compartment model, the predicted plasma drug concentration ($C_{ipred}$) after an i.v. bolus injection is described as follows:
\[ C_{\text{ipred}} = \frac{\text{Dose}}{V_i} \cdot e^{-t_{ij} \cdot CL/V_i} \]

where \( V_i \) is the volume of distribution for individual \( i \). If \( t_{ij} \) represents the time point \( j \) for individual \( i \), then the expected concentration would be as represented by the unfilled circle in the lower right panel if \( \eta_i \) were 0, and by the filled circle if \( \eta_i \) were different from 0.

Due to other variability sources than differences between individuals (e.g. sampling time error, measurement error and model misspecification), the observed concentration (squared symbol; \( C_{\text{obs}} \)) will differ from the individual model-predicted concentration (\( C_{\text{ipred}} \)) by a residual error factor, denoted by \( \varepsilon \):

\[ C_{\text{obs}} = C_{\text{ipred}} + \varepsilon_{ij} \]

The \( \varepsilon \):s are assumed to be symmetrically distributed with a mean value of 0 and a standard deviation of \( \sigma \) (upper right panel).

The population approach was exemplified above by a linear covariate model, and by additive error models, for simplicity. Other models may also be used (e.g. multiplicative and exponential models), as shown in the applications below.

Empirical Bayes’ estimates of individual pharmacokinetic and pharmacodynamic parameters were calculated based on the population model, the individual covariates, and the individual measurements of plasma concentration, APTT or ECT. The AUC during a dosing interval at steady state was calculated as F·dose/CL (for definitions, see section 3.3.2.1).

### 3.3.2 Pharmacokinetic models (papers II–IV)

#### 3.3.2.1 Structural models

The pharmacokinetics of melagatran, following s.c. administration of melagatran, or p.o. administration of ximelagatran, was described by a one-compartment model with first-order absorption:

\[ C(t) = \frac{F \cdot D \cdot k_a (e^{-CL/V} - e^{-k_a t})}{V(k_a - CL/V)} \]

where \( D \) is the administered melagatran (or ximelagatran) dose, \( F \) is the bioavailability fraction, \( k_a \) is the absorption rate constant, \( CL \) is the clearance and \( V \) is the apparent volume of distribution. Following ximelagatran ad-
ministration, F should be interpreted as the fraction of the ximelagatran dose systemically available as melagatran (Fm). When both administration routes were applied (paper II) the observed melagatran concentration in plasma was modelled as the sum of the concentrations in the s.c. and the p.o. central compartments. The one-compartment model was compared to a two-compartment model for s.c data (paper II) and p.o. data (paper III).

3.3.2.2 Inter-individual error models
Exponential error models were used for the interindividual error:

\[ P_i = TV(P_i) \cdot e^{\varepsilon_i} \]

where \( P_i \) denotes the pharmacokinetic parameter (e.g. CL) for patient \( i \), and \( TV(P_i) \) is the typical value for that patient.

3.3.2.3 Residual error models
In paper II a proportional and a combined additive+proportional inter-individual error models were compared:

Proportional error model:  \( C_{\text{obs}} = C_{\text{pred}} + \varepsilon \cdot C_{\text{pred}} \)

Combined model:  \( C_{\text{obs}} = C_{\text{pred}} + \varepsilon_1 + \varepsilon_2 \cdot C_{\text{pred}} \)

In papers III–IV a proportional error model was used. In paper IV the size of \( \varepsilon \) was allowed to differ between the patients as follows:

\( C_{\text{obs}} = C_{\text{pred}} + \varepsilon \cdot e^{\varepsilon_i} \cdot C_{\text{pred}} \)

3.3.2.4 Covariate analyses
In paper II the influence of gender, age, body weight, creatinine clearance and type of surgery on the pharmacokinetic parameters CL, \( V_{sc} \), \( V_{po} \), F were estimated.

In papers III and IV the influence of gender, age, body weight, creatinine clearance, smoking habits, and concomitant medication (provided that at least 20 patients were treated with the drug combination during the whole study period) on the pharmacokinetic parameters CL/F and V/F were quantified. In paper III only \( \beta_1 \)-blockers fulfilled this criterion, while in paper IV 13 classes of co-medications were evaluated. In addition, the influence of dose was evaluated in paper III, as was the influence on F of covariates affecting both CL/F and V/F after reparameterization of the model.

Relationships between covariates and individual pharmacokinetic parameters were first explored by the generalized additive modelling (GAM) procedure [89], and by visual inspection of plots of individual parameters versus covariates. Formal testing was then performed by inclusion of covariates,
one by one, in the NONMEM model. A significance level of $p \leq 0.05$ was used in the forward inclusion steps to generate the full model. This model was then reduced by eliminating covariate-parameter relationships, one by one, and only those covariate effects that were significant at the $p \leq 0.001$ level were retained in the final model. Lastly, the influence of single patients on the covariate effect was investigated by bootstrap of the GAM, by plots of Cooks’ distances, and by plots of Studentized residuals of the GAM fit [90]. If these exploratory methods indicated that a single patient was driving a covariate–parameter relationship, formal testing of the covariate effect was repeated in NONMEM after omission of the suspected influential patient. If the covariate effect was not statistically significant without that single patient, the covariate was to be excluded from the model.

In paper II a slightly modified approach was used (see section 3.3.9.1). Due to the application of more accurate $\Delta$OFV values for assessment of statistical significance, significance levels of $p \leq 0.10$ and $p \leq 0.05$ were used in the forward inclusion and backwards elimination steps, respectively. In addition, refinement of models was carried out by assessment of presumed clinical relevance of found relationships, and checking for any influential patients by looking at the individual contributions to the OFV [91].

3.3.3 Pharmacodynamic models

3.3.3.1 APTT (papers I and IV)

In paper I different pharmacodynamic models were first fitted to APTT and inogatran plasma concentration data, pooled from the studies in healthy volunteers (studies A–E). The following structural models were compared ($C =$ inogatran concentration):

- Model 1: $\text{APTT} = \theta_1 + \theta_2 \cdot \log(C+1)$
- Model 2: $\text{APTT} = \theta_1 + \theta_2 \cdot C^{\theta_3}$
- Model 3: $\text{APTT} = \theta_1 + \theta_2 \cdot C/(\theta_3 + C)$
- Model 4: $\text{APTT} = \theta_1 + \theta_2 \cdot C^{\theta_3}/(\theta_4 + C^{\theta_3})$
- Model 5: $\text{APTT} = \theta_1 + \theta_2 \cdot C + \theta_3 \cdot C/(\theta_4 + C)$.

Additive and proportional inter-individual error models were compared, as were proportional and additive + proportional residual error models. Interoccasion variability was modelled by the method proposed by Karlsson & Sheiner [92]. The best model was selected for subsequent analysis of pooled patient data (studies F–G). The influence of the following covariates was then evaluated on APTTbaseline and Emax: diagnosis, gender, age, body weight, smoking habits, hypertension, diabetes, cardiac failure, clinical outcome day 30, and centre-specific normal reference values for the APTT method (LLN,
ULN, RefMid, RefWidth). In addition, the effect of aspirin was evaluated in study D.

In paper IV a linear model, a power model (model 2), an $E_{\text{max}}$ model (model 3) and a combined linear + $E_{\text{max}}$ model (model 5) were compared. Exponential interindividual and proportional residual error models were used. The influence of gender, age, and body weight on APTT$_{\text{baseline}}$ and APTT$_{\text{slope}}$ were investigated.

The covariate analyses were conducted using stepwise forward inclusion–backward elimination procedures, as described in section 3.3.2.4.

### 3.3.3.2 ECT (paper II)

A linear model model was used to describe the relationship between plasma melagatran concentration and ECT. A proportional interindividual error model was used and, in addition, the covariance between the individual $\eta$s for ECT$_{\text{baseline}}$ and ECT$_{\text{slope}}$ parameters was estimated. Additive and proportional residual error models were compared.

The influence of the following covariates on ECT$_{\text{baseline}}$ and ECT$_{\text{slope}}$ were estimated using stepwise forward inclusion–backward elimination procedures, as described in section 3.3.2.4: Gender, age, body weight, creatinine clearance and type of surgery.

### 3.3.4 Performance of ECT for estimation of pharmacokinetic parameters (paper II)

A combined pharmacokinetic-pharmacodynamic model was fitted to all ECT-concentration-time data. All concentration points were then deleted and individual Bayes’ parameters were re-estimated using ECT observations only. As a measure of bias, potentially introduced by omitting the concentration data, the geometric mean values of the individual ratios between ECT- and concentration- derived pharmacokinetic parameters were calculated, and correlation plots between the parameters were used as indicators of precision.

### 3.3.5 Exposure-clinical response models (papers III–IV)

The relationship between AUC of melagatran and the probability of the clinical events, listed below, were evaluated using logistic regression methods [93].

---

34
Paper III
- Change in thrombus size, modelled as a thricotomous categorical variable (regression, no change, or progression)
- Bleeding-related event.

Paper IV
- Recurrent VTE
- Major bleeding
- Any bleeding, defined as major and/or minor bleeding
- ALAT-elevation $>3$ ULN.

In paper III linear, logarithmic and $E_{\text{max}}$ logit models were compared. In paper IV the models tested were generally linear. However, when judged more appropriate, non-linear models (power model, $E_{\text{max}}$ model, and sigmoid $E_{\text{max}}$ model) were also fitted to the data. In order to identify potential confounding factors, the influence of the following patient covariates were first assessed in the placebo group: Gender, age, body weight, creatinine clearance, and whether the index event was the first VTE event or not (as a predictor for VTE only). Thereafter, the analyses were repeated in the whole study population in a step-wise forward inclusion – backwards elimination manner, using an approach similar to that applied in the pharmacokinetic and pharmacodynamic covariate analyses. For patients lacking pharmacokinetic data, pharmacokinetic parameters were imputed from the final population models and patient covariates.

3.3.6 APTT-clinical response models (paper IV)
Linear logit regression models were used to describe the relationship between the probability of experiencing a clinical event (recurrent VTE, major bleeding, any bleeding) and the variables average APTT, average APTT-change from baseline, and average APTT-ratio ($\text{APTT}/\text{APTT}_{\text{baseline}}$) over a steady state dose interval. Patient covariates, found to be statistically significant predictors of clinical events in the placebo group, were tested for significant effects in the APTT-clinical response models.

3.3.7 Estimation of clinical utility (papers III–IV)
In paper III, the clinical utility function comprised the sum of the probabilities of thrombus regression and a bleeding-related event.
In paper IV, clinical utility functions were derived based on the sum of the probability of a recurrent VTE and the probability of a major bleeding event, assigning different relative weights to these events, as functions of AUC and other relevant patient covariates. The average value of the utility function was calculated for the whole study population, as well as for special
patient subgroups based on the population pharmacokinetic model and the exposure-response models for varying ximelagatran doses and placebo. Furthermore, in order to predict what theoretically might be gained by applying an individualized dosing strategy, a target AUC was identified which minimizes the total risk, under the assumption that the exposure-response models are true, and the outcome of a completely individualized dosing strategy was estimated. This hypothetic dosing strategy was based upon the best known predictor of melagatran exposure, i.e. creatinine clearance. Optimal doses were also estimated using utility functions which, in addition to VTEs and major bleedings, included the risk of any bleeding and ALAT-elevations to >3-ULN. Assumptions behind one of these functions, in which VTE, major bleedings and ALAT-elevations to >3-ULN were given the relative weights 1:1:0.1, were based on the estimated case-fatality rates for VTE (6.8%), major bleedings (5.9%) and ALAT-elevations (0.55%).

3.3.8 Software
The software program NONMEM version V, or VI beta) was used for all pharmacokinetic and pharmacodynamic analyses [88]. The first-order (FO) method was applied in papers I–II, and the first-order conditional estimation (FOCE) algorithm in paper III and IV (pharmacodynamic evaluation). For the pharmacokinetic analyses in paper IV, the first-order conditional estimation algorithm (FOCE INTER), assuming interaction between the residual error and the interindividual error, was used.

The post-processor XPOSE (version 2.0 in papers I–III, version 3.0 in paper IV) [90] was used for model diagnostic purposes and for exploration of possible covariate relationships by the GAM procedure [89].

For the logistic regression analyses (exposure-clinical response, APTT–clinical response), the likelihood algorithm in NONMEM version V was used (papers III–IV).

3.3.9 Statistics
3.3.9.1 Model comparison
In NONMEM parameters are estimated through a maximum likelihood approach, whereby an objective function is evaluated which expresses the likelihood of the data given the model parameters. The objective function is an extended least squares function, which is approximately proportional to minus two times the logarithm of the likelihood of the data [88].

For comparison of models, likelihood ratio tests were applied. In NONMEM the difference in objective function value ($\Delta$OFV) between two hierarchical models are approximately $\chi^2$-distributed with the number of degrees of freedom equal to the difference in number of parameters between the two
models. Under this assumption, a ΔOFV of 3.84 corresponds to a \( p \)-value of 0.05 at one degree of freedom. Corresponding ΔOFV values are 6.64 and 10.83 for \( p=0.01 \) and \( p=0.001 \), respectively.

The ΔOFV-values described above were used in all papers, with the exception of paper II. In that paper a covariate randomization procedure was first undertaken in order to define more accurate ΔOFV values, required for attainment of desired significance levels [94]. These ΔOFV values were then applied as cut-offs for inclusion of parameter-covariate relationships in the model.

### 3.3.9.2 Calculation of confidence intervals

Calculations of confidence intervals (CI) were generally based on standard errors (SE) of the parameters, and the assumption of normal distribution.

For evaluation of the influence of concomitant medication, and treatment time in paper IV, 90\% CIs were constructed for each factor by fixing it to different values and mapping of the likelihood profile [95]. Calculation of CIs for the utility functions was based on SEs of the logistic regression parameters, with the assumption that thrombosis and bleedings were independent events.
4 Results

4.1 Pharmacokinetic results (papers II–V)

Following s.c. administration of melagatran or oral administration of ximelagatran the pharmacokinetics of melagatran was described by a one-compartment disposition model, with first-order absorption. Peak melagatran concentrations were reached within 1 hour following s.c. administration of melagatran and at about 2 hrs after p.o. ximelagatran. Observed and model predicted plasma concentrations from the three clinical studies are given in Figure 6, Figure 7, and Figure 8.

The model parameter estimates for the pharmacokinetic parameters were in good agreement across the different studies (Table 4). The mean bioavailability (F) of melagatran, following p.o. administration of ximelagatran was 21% on the first oral treatment day after surgery, and increased slightly (to about 24%) during the course of about 6 days. Mean volume of distribution (V) was 23 L following s.c. administration and 34 L following p.o. administration in orthopedic surgery patients and 176-191 L (V/F) in VTE patients. Total plasma clearance (CL) of melagatran was 5 L/h in the surgery population and CL/F was approximately 30 L/h in the VTE populations. Based on these clearance and volume parameters, the population mean half-life was 3.1 (s.c.) and 4.6 (p.o.) hours in the surgery population. Corresponding half-lives following p.o. dosing to VTE populations were 4.5 hours (paper III) and 4.2 hours (paper IV).

The AUC-distribution in all studies at the different doses is given in Figure 9. The total variability in dose-adjusted melagatran AUCs, following oral ximelagatran was 36%, 45%, and 44% in papers II, III and IV, respectively. The single most important factor determining melagatran oral clearance, and thus exposure, was the patient’s renal function, as estimated from creatinine clearance (Table 5). The relationships between clearance and creatinine clearance were similar in the different studies (Figure 10).

The other patient factors that significantly influenced the pharmacokinetic parameters were similar across studies. Women had 20% lower CL/F and 27% higher V/F than men in the THRIVE III study (paper IV). Women had 19% higher oral bioavailability and 19 % lower volume of distribution after s.c. administration than men in the METHRO I study, but these covariate effects were not included in the final model as they were less than 20%, and therefore not considered clinically relevant (paper II). Patients taking β1-
blocking agents (predominantly women) had 28% higher oral bioavailability in the THRIVE I study (paper III), but not in the THRIVE III study (paper IV). Gender and body weight was included in the final models as a covariate on the apparent volume of distribution in the THRIVE III and the THRIVE I studies, respectively.

Apart from the effect of $\beta_1$-blocking agents in the THRIVE I study, there was no significant influence of any of the following concomitant medications (paper IV): ACE inhibitors, selective $\beta_1$-receptor blockers, diuretics, dihydropyridine derivatives, organic nitrates, HMG-CoA reductase inhibitors, anilides, thyroid hormones, benzodiazepine derivatives, glucocorticoids, sulfonamides, urea derivatives, platelet inhibitors excluding heparin, and selective $\beta_2$-stimulating agents. The estimated effects on AUC were less than ±15% and the 90% CI were within ±30% for all of the investigated co-medications.

Smoking did not influence the pharmacokinetics of melagatran, and no dose dependencies were found.

Figure 6. Observed and model-predicted plasma melagatran concentration versus time since first oral dose in orthopedic surgery patients. METHRO I study (paper II).
Figure 7. Observed and model-predicted plasma melagatran concentration versus time since dose day 1 in VTE patients. THRIVE I study (paper III).

Figure 8. Observed and model-predicted plasma melagatran concentration versus time since latest ximelagatran dose (24 mg b.i.d.) in VTE patients. THRIVE III study (paper IV).
Table 4. Pharmacokinetic parameters of melagatran following s.c administration of melagatran or p.o. administration of ximelagatran

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Paper II METHRO I study (n=98)</th>
<th>Paper III THRIVE I study (n=264)</th>
<th>Paper IV THRIVE III study (n=596)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structural model parameters (θ)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL (θ₁)</td>
<td>5.17 (4.2%) ³</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CL/F (θ₂)</td>
<td>-</td>
<td>27.3 (2%) ³</td>
<td>31.6 (2%) ³</td>
</tr>
<tr>
<td>CrCL on CL/F (θ₃)</td>
<td>0.0450 (40%) ³</td>
<td>0.0107 (6%) ³</td>
<td>0.00894 (5%) ³</td>
</tr>
<tr>
<td>Female gender on CL/F (θ₄)</td>
<td>-</td>
<td>-</td>
<td>-0.203 (11%)</td>
</tr>
<tr>
<td>Vₑₑₑₑ (L)</td>
<td>23.3 (5.8%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vₑₑₑₑ (L) (θ₅)</td>
<td>34.0 (7.2%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>V/F (L) (θ₆)</td>
<td>-</td>
<td>176 (3%)</td>
<td>191 (4%)</td>
</tr>
<tr>
<td>BW on V/F (θ₇)</td>
<td>-</td>
<td>0.0072 (20%) ³</td>
<td>-</td>
</tr>
<tr>
<td>Female gender on V/F (θ₈)</td>
<td>-</td>
<td>-</td>
<td>-0.270 (12%) ³</td>
</tr>
<tr>
<td>Fₛₛₛₛ (L)</td>
<td>1 ³</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fₛₛₛₛ (θ₉)</td>
<td>0.211 (6.8%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tim on Fₛₛₛₛ (θ₁₀)</td>
<td>0.000223 (37%) ³</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>β₁-blockers on Fₛₛₛₛ (θ₁₁)</td>
<td>-</td>
<td>1.28 (22%) ³</td>
<td>-</td>
</tr>
<tr>
<td>kaₑₑₑₑ (h⁻¹)</td>
<td>4.6 ³</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>kaₛₛₛₛ (h⁻¹)</td>
<td>1.24 (16%)</td>
<td>1.18 (~12%)</td>
<td>0.883 (~8%)</td>
</tr>
<tr>
<td><strong>Inter-patient variability (σε)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL (%)</td>
<td>19 (23%)</td>
<td>19 (22%)</td>
<td>27 (9%)</td>
</tr>
<tr>
<td>Vₑₑₑₑ (%)</td>
<td>17 (59%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vₑₑₑₑ (%)</td>
<td>34 (29%)</td>
<td>-</td>
<td>12 (36%)</td>
</tr>
<tr>
<td>Fₛₛₛₛ (%)</td>
<td>26 (21%)</td>
<td>21 (17%)</td>
<td>-</td>
</tr>
<tr>
<td>kaₑₑₑₑ (%)</td>
<td>100 (37%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>kaₛₛₛₛ (%)</td>
<td>85 (43%)</td>
<td>~90 (~36%)</td>
<td>~110 (~16%)</td>
</tr>
<tr>
<td><strong>Residual variability (σφ)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 (5.4%)</td>
<td>29 (3%)</td>
<td>28 (3%)</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as parameter estimates (Relative SE); s.c., subcutaneous route of administration; p.o., oral route of administration; CL, total plasma clearance of melagatran; V, volume of distribution; F, bioavailability; ka, absorption rate constant; ³ CL = θ₁ + θ₂ (Clₑₑₑₑ×68), where CL is the typical value for melagatran plasma clearance (L/h), and CrCL is the creatinine clearance (mL/min). ³ CL/F = θ₃(1 + θ₄·(CrCL–86)), where CL/F is the typical value of melagatran oral clearance (L/h) and CrCL is creatinine clearance (mL/min). If CrCL > 120 mL/min then CL = θ₁·(1 + θ₂·34). ³ CL/F = θ₅(1 + θ₆·(CrCL–104))(1+θ₇·SEX), where CL/F is the typical value of melagatran oral clearance (L/h), CrCL is creatinine clearance (mL/min), and SEX is 0 for males and 1 for females. If CrCL > 135 mL/min then CL/F = θ₅(1 + θ₆·31)(1 + θ₇·SEX). ³ Vₛₛₛₛ = θ₈(1 + θ₉·(BW–82)), where Vₛₛₛₛ is the typical value of oral volume of distribution (V/F; L/h) and BW is body weight (kg). ³ Vₛₛₛₛ = θ₈(1+θ₉·SEX), where Vₛₛₛₛ is the typical value of oral volume of distribution (V/F; L/h) and SEX is 0 for males and 1 for females. ³ Fixed. ³ Fₛₛₛₛ = θ₉ + θ₃(time-40), where Fₛₛₛₛ is the typical value for the oral bioavailability at the time (hrs) elapsed since the first drug administration. ³ Fₛₛₛₛ = θ₃ in patients without concomitant β₁-blockers and θ₃θ₉ in patients with β₁-blockers. ³ Proportional error.
Figure 9. Distribution of melagatran AUC after b.i.d. administration of s.c. melagatran or p.o. ximelagatran. Orthopedic surgery (METHRO I study, paper II); acute VTE treatment (THRIVE I study, paper III); secondary VTE prevention (THRIVE III study, paper IV). The boxes represent 25th and 75th percentiles, the whiskers 10th and 90th percentiles, and the lines within the boxes medium (solid) and mean (dotted) values.
Table 5. Contribution of patient demographics to total variability in CL_{po} a) (paper IV)

<table>
<thead>
<tr>
<th>Patient covariate(s)</th>
<th>p-value</th>
<th>Explained fraction of inter-patient variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>No covariates</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CrCL</td>
<td>&lt;0.001</td>
<td>46%</td>
</tr>
<tr>
<td>Gender</td>
<td>&lt;0.001</td>
<td>16%</td>
</tr>
<tr>
<td>Weight</td>
<td>&lt;0.001</td>
<td>15%</td>
</tr>
<tr>
<td>Age</td>
<td>&lt;0.001</td>
<td>13%</td>
</tr>
<tr>
<td>CrCL &amp; gender</td>
<td>Gender &lt;0.001</td>
<td>55%</td>
</tr>
<tr>
<td>CrCL &amp; weight</td>
<td>Weight &gt;0.05</td>
<td>46%</td>
</tr>
<tr>
<td>CrCL &amp; age</td>
<td>Age &gt;0.05</td>
<td>46%</td>
</tr>
</tbody>
</table>

a) No covariate effects on any other PK parameter were included in these models.

Figure 10. Models for the relationship between oral melagatran clearance (CL/F) and creatinine clearance, estimated using data from varying clinical studies. For comparison F=0.24 (F at the end of the treatment period) was used for calculation of CL/F in the orthopedic surgery patients (METHRO I study).
4.2 Pharmacodynamic results (papers I–II and IV)

4.2.1 APTT (papers I and IV)

The overall population mean (SE) of the observed baseline APTT in the pooled analysis of data from seven clinical studies was 29.2 (0.01) s, but large differences were observed across individuals. In young healthy volunteers the mean baseline value was 35.2 (95% CI 34.3-36.1), while it was 31.1 (95% CI 30.4-31.7) in patients from matching study centres. The major source of the overall variability was attributed to interindividual differences, while the variance between studies (within centre) contributed to less than one tenth of the total variance in APTT when no drug is present (Table 6). Intercentre variability was also significant, but less than interindividual variability.

APTT was non-linearly related to the plasma concentration of the thrombin inhibitors inogatran and melagatran (Figure 11). A combined linear and E\text{max} model best described the relationship for inogatran (paper I; Table 7), and a power model best described the relationship for melagatran in the THRIVE III study (paper IV; Table 8). The lower limit of the normal reference range (LLN) was included in model to adjust for the method-differences when pooling data from several studies and centres (paper I).

The shape of the models are similar in the concentration region studied, see Figure 11. This graph also illustrates that young healthy subjects tend to have higher baseline APTT (patient’s age; \(p=0.004\)) and a higher degree of response to inogatran, at the same plasma concentration, compared to CAD patients. Baseline APTT and the response to melagatran were also higher in young (up to 58 yrs) than in elderly patients in the THRIVE III study (\(p<10^{-7}\) for intercept and slope parameters; paper IV).

Table 6. Variability estimates for baseline APTT.

<table>
<thead>
<tr>
<th>Variability component</th>
<th>Standard deviation ((\sigma))</th>
<th>Standard error (\text{SE}(\sigma))</th>
<th>Significance level ((p))^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study centre</td>
<td>1.8 s</td>
<td>0.27 s</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>Study</td>
<td>1.3 s</td>
<td>0.34 s</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>Individual</td>
<td>3.6 s</td>
<td>0.16 s</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>Occasion + residual</td>
<td>1.4 s</td>
<td>0.01 s</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

a) Based on increase in OFV when the variability component was fixed to zero.
Table 7. Population parameter estimates (RSE) of the pharmacodynamic model in patients: \( \text{APTT} = \text{APTT}_{\text{baseline}} + \text{APTT}_{\text{slope}}C + E_{\text{max}}C/(\text{EC}_{50} + C) \), where \( C \) is the plasma inogatran concentration in \( \mu \text{mol/L} \) and \( \text{APTT} \) is in s (paper I).

<table>
<thead>
<tr>
<th>Pharmacodynamic parameter</th>
<th>Covariate expression</th>
<th>Parameter estimate (RSE)</th>
<th>Random interpatient variability [%CV (RSE)]</th>
<th>Residual variability [%CV (RSE)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTT_{baseline}</td>
<td>( \text{APTT}_{\text{baseline}} = \theta_1 + \theta_2(\text{LLN-25}) )</td>
<td>( \theta_1 = 29.2 ) (0.3%) ( \theta_3 = 0.36 ) (17%)</td>
<td>11 (18%)</td>
<td></td>
</tr>
<tr>
<td>APTT_{slope}</td>
<td>( \text{APTT}_{\text{slope}} = \theta_2 )</td>
<td>( \theta_2 = 5.8 ) (24%)</td>
<td>NE</td>
<td></td>
</tr>
<tr>
<td>( E_{\text{max}} )</td>
<td>( E_{\text{max}} = \theta_3 + \theta_7(\text{LLN-25}) )</td>
<td>( \theta_3 = 31 ) (16%) ( \theta_7 = 0.67 ) (33%)</td>
<td>28 (8)</td>
<td></td>
</tr>
<tr>
<td>( \text{EC}_{50} )</td>
<td>( \text{EC}_{50} = \theta_4 )</td>
<td>( \theta_4 = 0.72 ) (0.17%)</td>
<td>NE</td>
<td></td>
</tr>
<tr>
<td>( r^2 )</td>
<td></td>
<td>0.37 (49%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RSE, relative standard error
*\( r \) is the correlation coefficient between the \( \eta \)s of \( \text{APTT}_{\text{baseline}}, \text{APTT}_{\text{slope}} \), and the RSE is the relative standard error of the covariance of these \( \eta \)s.

Table 8. Population parameter estimates (RSE) of the pharmacodynamic model: \( \text{APTT} = \text{APTT}_{\text{baseline}} + \text{APTT}_{\text{slope}}C \), where \( C \) is the plasma inogatran concentration in \( \mu \text{mol/L} \) and \( \text{APTT} \) is in s (paper IV).

<table>
<thead>
<tr>
<th>Pharmacodynamic parameter</th>
<th>Covariate expression</th>
<th>Parameter estimate (RSE)</th>
<th>Random interpatient variability [%CV (RSE)]</th>
<th>Residual variability [%CV (RSE)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTT_{baseline}</td>
<td>( \text{APTT}<em>{\text{baseline}} = \theta_1(1+\theta_3(\text{age-58})) ) \ if age &lt;58, otherwise ( \text{APTT}</em>{\text{baseline}} = \theta_1 )</td>
<td>( \theta_1 = 27.0 ) (2%) ( \theta_3 = -0.00607 ) (63%)</td>
<td>14 (26%)</td>
<td></td>
</tr>
<tr>
<td>APTT_{slope}</td>
<td>( \text{APTT}<em>{\text{slope}} = \theta_2(1+\theta_4(\text{age-58})) ) \ if age &lt;58, otherwise ( \text{APTT}</em>{\text{slope}} = \theta_2 )</td>
<td>( \theta_2 = 23.7 ) (4%) ( \theta_4 = -0.0248 ) (25%)</td>
<td>12 (36%)</td>
<td></td>
</tr>
<tr>
<td>( r^2 )</td>
<td></td>
<td>0.51 (42%)</td>
<td></td>
<td>19 (11%)</td>
</tr>
</tbody>
</table>

RSE, relative standard error
*\( r \) is the correlation coefficient between the \( \eta \)s of \( \text{APTT}_{\text{baseline}}, \text{APTT}_{\text{slope}} \), and the RSE is the relative standard error of the covariance of these \( \eta \)s.
4.2.2 ECT (paper II)

ECT was linearly related to the plasma melagatran concentration (Table 9, Figure 12). Individual ECT\textsubscript{baseline} and ECT\textsubscript{slope} parameters were correlated, but no patient factors were identified, which significantly affected these parameters.
Table 9. Parameter estimates (RSE) for the pharmacodynamic model:
ECT = ECT_{baseline} + ECT_{slope}·C, where C is the plasma melagatran concentration in µmol/L and ECT is in s.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (RSE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structural model parameters (θ)</strong></td>
<td></td>
</tr>
<tr>
<td>ECT_{baseline} (s)</td>
<td>41.4 (2.2%)</td>
</tr>
<tr>
<td>ECT_{slope} (s · L/µmol)</td>
<td>586 (3.2%)</td>
</tr>
<tr>
<td><strong>Inter-patient variability (σθ)</strong></td>
<td></td>
</tr>
<tr>
<td>ECT_{baseline} (%)</td>
<td>14 (44%)</td>
</tr>
<tr>
<td>ECT_{slope} (%)</td>
<td>16 (28%)</td>
</tr>
<tr>
<td>τ (ECT_{baseline}·ECT_{slope})</td>
<td>0.92 (37%)</td>
</tr>
<tr>
<td><strong>Residual variability (σε)</strong></td>
<td>18 (5.8%)</td>
</tr>
</tbody>
</table>

RSE, relative standard error
d Proportional error (expressed as %)
^τ is the correlation coefficient between the η:s of ECT_{baseline}, ECT_{slope}, and the RSE is the relative standard error of the covariance of these η:s.

Figure 12. Relationship between ECT and plasma melagatran concentration

4.3 Performance of ECT (paper II)
Individual clearance and bioavailability parameters, estimated from ECT values correlated well with those estimated from plasma concentration (Figure 13) and the potential bias was small (<10%) for all parameters, suggesting that ECT is a useful surrogate for melagatran plasma concentration in pharmacokinetic evaluations.
Figure 13. Bayesian pharmacokinetic parameters based on plasma melagatran concentration and ECT. The linear regression lines (dotted) and lines of identity (unbroken) are also included.
4.4 Relationship between melagatran exposure and clinical response (papers III–IV)

4.4.1 Acute treatment of VTE (paper III)

The distribution of melagatran exposure was similar, irrespective of clinical response. The mean AUC (SE) was 3.69 (0.16), 4.08 (0.42), and 3.52 (0.41) h·µmol/L in patients experiencing regression (n=152), no change (n=51), and progression (n=18), respectively. Corresponding values were 3.96 (± 0.31) and 3.76 (± 0.15) h·µmol/L in patients with (n = 32) and without (n = 232) bleeding-related episodes, respectively. No significant relationships were found between AUC of melagatran and thrombus extension \( p=0.59 \), or the probability of a bleeding-related event \( p=0.77 \), and the estimated exposure-response models were all relatively flat.

4.4.2 Secondary prevention of VTE (paper IV)

The AUC distributions in all patients, and in groups of patients with and without clinical events, are shown in Figure 14. Results from the logistic regression analysis are given in Table 10 and Figure 15 (left panel).

**Figure 14.** AUC of melagatran, in patients with and without clinical events. The boxes represent 25th and 75th percentiles, the whiskers 10th and 90th percentiles, and the lines within the boxes medium (solid) and mean (dotted) values.
Table 10. Logistic regression models for exposure-clinical response relationships (paper IV)

<table>
<thead>
<tr>
<th>Clinical event and influential factors</th>
<th>Logit function</th>
<th>Parameter estimate (SE)</th>
<th>OR(^a) (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VTE</strong></td>
<td>( \theta_1 + \theta_2 \cdot \text{Gender}^b + \theta_3 \cdot \text{AUC} )</td>
<td>( \theta_1 = -1.69 (0.153) )</td>
<td>-</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>( \theta_2 = -0.914 (0.258) )</td>
<td>0.40 (0.24-0.66)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td>( \theta_3 = -1.05 (0.205) )</td>
<td>0.35 (0.23-0.52)</td>
</tr>
<tr>
<td><strong>Major bleeding</strong></td>
<td>( \theta_1 + \theta_2 \cdot \text{AUC} )</td>
<td>( \theta_1 = -5.20 (0.512) )</td>
<td>-</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>( \theta_2 = 0.379 (0.239) )</td>
<td>1.46 (0.91-2.33)</td>
</tr>
<tr>
<td>AUC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Any bleeding</strong></td>
<td>( \theta_1 + \theta_2 \cdot \text{Gender}^b + \theta_3 \cdot \text{AUC} )</td>
<td>( \theta_1 = -1.70 (0.117) )</td>
<td>-</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>( \theta_2 = 0.429 (0.145) )</td>
<td>1.54 (1.16-2.04)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td>( \theta_3 = 0.091 (0.058) )</td>
<td>1.10 (0.98-1.23)</td>
</tr>
<tr>
<td>AUC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ALAT&gt;3-ULN</strong></td>
<td>( \theta_1 + \theta_2 \cdot \text{AUC} )</td>
<td>( \theta_1 = -4.62 (0.411) )</td>
<td>-</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>( \theta_2 = 2.91 (1.09) )</td>
<td>-</td>
</tr>
<tr>
<td>( E_{\text{max}} ) for AUC</td>
<td>( \theta_1 + \theta_2 \cdot \text{AUC} )</td>
<td>( \theta_1 = 1.04 (1.17) )</td>
<td>-</td>
</tr>
<tr>
<td>( E_{\text{50}} ) for AUC</td>
<td>( \theta_3 + \text{AUC} )</td>
<td>( \theta_3 = 0.84 (1.01) )</td>
<td>-</td>
</tr>
</tbody>
</table>

SE, standard error
\(^a\) Odds ratio for women/men and per unit (h·µmol/L) increase in AUC, respectively
\(^b\) Men=0, women=1

4.4.2.1 VTE recurrence

Women had significantly lower risk for a recurrent VTE than men. 20 out of 298 women and 51 out of 313 men experienced a VTE in the placebo group (OR = 0.37, \( p=2.0\cdot10^{-4} \)). Corresponding numbers in the ximelagatran group were 3 out of 281 women and 9 out of 331 men. Only gender and AUC significantly influenced the risk of a recurrent VTE. The OR for women/men was estimated to 0.40 (\( p=2.0\cdot10^{-3} \)), and the OR for each unit (h·µmol/L) increase in AUC was 0.35 (\( p<10^{-3} \)).
Figure 15. Estimated probability (95% CI) of recurrent VTE (a), major bleeding (b) and any bleeding (c) as functions of AUC (left panel) and APTT-ratio (APTT/APTTbaseline) (right panel)
4.4.2.2 Bleeding

No patient covariates were identified that significantly influenced the risk of a major bleeding. The OR was 1.46 per unit increase in melagatran AUC ($p=0.077$).

There was a tendency for higher incidence of any bleeding (major and/or minor) in women in both treatment groups. 64 out of 298 women and 47 out of 313 men experienced a bleeding in the placebo group (OR = 1.55, $p=0.038$). Corresponding numbers in the ximelagatran group were 74 out of 281 women and 60 out of 331 men. Drug exposure was not demonstrated to significantly influence the risk of bleeding. The estimated OR was 1.10 for each unit increase in melagatran AUC ($p=0.124$) when the influence of gender was included in the model (OR (women/men) = 1.54, $p=0.003$). No other patient factors significantly affected the risk of bleeding.

4.4.2.3 ALAT-elevations

The incidence of ALAT-elevations to more than 3·ULN was 37/612 in the ximelagatran group and 6/611 patients in the placebo group. With the exception of melagatran AUC ($p=9.0\cdot10^{-7}$), no patient covariates were identified that significantly influenced the risk of ALAT-elevations. The logit model that best described the relationship between the probability of ALAT-elevations above 3·ULN and AUC was an $E_{\text{max}}$ model (Figure 16).

![Figure 16. Estimated (95% CI) probability of an ALAT-elevation to >3·ULN as a function of melagatran AUC.](image-url)
4.5 Relationship between APTT and clinical response (paper IV)

The probability of recurrent VTE was significantly related to all of the APTT-variables evaluated, but the risks of bleedings were not significantly related to any of them. The average APTT-ratio (APTT/APTT_{baseline}) over a dosing interval was a better predictor than was the average absolute APTT or the average APTT change from baseline for all events. The logistic regression models for the probability of clinical events against the APTT-ratio are shown in Table 11 and Figure 15 (right panel).

Overall, the probability of recurrent VTE and bleedings were statistically associated with the APTT-ratio and AUC to a similar degree. The APTT-ratio was a slightly better predictor than AUC for VTE, but a slightly poorer predictor than AUC for bleeding.

Table 11. Logistic regression models for the relationships between APTT-ratio (APTT/APTT_{baseline}) and clinical response (paper IV)

<table>
<thead>
<tr>
<th>Clinical event</th>
<th>Logit function</th>
<th>Parameter estimate (SE)</th>
<th>OR^a (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VTE</strong></td>
<td>( \theta_1 + \theta_2 \cdot \text{Gender} + \theta_3 \cdot 10 \cdot (\text{APTT-ratio}-1) )</td>
<td>( \theta_1 = -1.66 \ (0.151) )</td>
<td>-</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>( \theta_2 = -0.944 \ (0.258) )</td>
<td>0.39 (0.23-0.65)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td>( \theta_3 = -0.533 \ (0.092) )</td>
<td>0.59 (0.49-0.70)</td>
</tr>
<tr>
<td>APTT-ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Major bleeding</strong></td>
<td>( \theta_1 + \theta_2 \cdot 10 \cdot (\text{APTT-ratio}-1) )</td>
<td>( \theta_1 = -5.12 \ (0.598) )</td>
<td>-</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>( \theta_2 = -0.533 \ (0.092) )</td>
<td>0.59 (0.49-0.70)</td>
</tr>
<tr>
<td>APTT-ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Any bleeding</strong></td>
<td>( \theta_1 + \theta_2 \cdot \text{Gender} + \theta_3 \cdot 10 \cdot (\text{APTT-ratio}-1) )</td>
<td>( \theta_1 = -1.72 \ (0.125) )</td>
<td>-</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>( \theta_2 = 0.439 \ (0.144) )</td>
<td>1.55 (1.17-2.06)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td>( \theta_3 = 0.056 \ (0.035) )</td>
<td>1.06 (0.99-1.13)</td>
</tr>
<tr>
<td>APTT-ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SE, standard error

^a Odds ratio for women/men and per 0.1 units increase in APTT-ratio, respectively

^b Men=0, women=1

4.6 Estimation of clinical utility (papers III-IV)

The estimated clinical utility function for acute treatment of VTE (paper III) was positive across the entire range of AUC values observed in this dose-guiding study (Figure 17).
Figure 17. Estimated (± 95% CI) clinical utility of the ximelagatran treatment as a function of melagatran exposure (AUC). Utility function = Probability of thrombus regression – Probability of bleeding-related event (paper III).

In the secondary prevention of VTE indication (paper IV), the estimated clinical utility function versus melagatran AUC is gender-specific, because of the higher risk for VTE in men. In Figure 18 utility functions for the sum of the probabilities of a VTE and a major bleeding are shown. The minimum value is found at 3.2 h·µmol/L (2.5%), and 2.5 h·µmol/L (1.9%), for men and women, respectively.

Figure 18. Estimated (± 95% CI) clinical utility function as a function of melagatran exposure (AUC). Utility function = Probability of VTE + Probability of major bleeding (paper IV).
The average probability of a VTE and/or a major bleed is shown against varying fixed twice daily doses of ximelagatran in Figure 19. Included are also utility function curves, based on the utility weight (UWT) for a major bleeding relative to a VTE between 0.5 and 10. The twice daily ximelagatran dose that is expected to give the best clinical net benefit varies between 15 and 41 mg, depending on the weight used. For equal weights, the optimal dose is estimated to 34 mg if the same dose is given to all patients. However, the average utility function value following a fixed dose of 24 mg is expected to be favourable, compared to placebo, independent of the weight used.

The estimated total risk of a VTE and/or a major bleeding is low, compared to the risk following placebo, even in special subgroups of patients who are supposed to have an increased risk of bleedings or VTE, inherently or as a consequence of altered pharmacokinetics (Table 12). A completely individualized dosing regimen, based on creatinine clearance and targeting an AUC value of 2.9 h-μmol/L in each patient, was predicted to result in a relative risk reduction of 21%. According to the population pharmacokinetic model this target AUC would be achieved with a dose of 39 mg in a patient with a typical value of CL/F.

Figure 19. Average probability (Pr) of recurrent VTE and major bleeding, respectively, and average utility curves for different utility functions against varying fixed twice daily doses of ximelagatran. Utility function = Pr(VTE) + UWT - Pr(major bleeding). The dose that minimizes the utility function value is shown in the legend.
Table 12. Estimated probability (Pr) of clinical events and average utility function value (Pr(VTE) + Pr(major bleeding)) in special patient subgroups following placebo, a fixed dose of 24 mg ximelagatran b.i.d, and a hypothetic, completely individualized dosing regimen with an average dose of 39 mg b.i.d.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Pr(VTE)</th>
<th>24 mg fixed</th>
<th>39 mg indiv</th>
<th>Pr(major bleeding)</th>
<th>24 mg fixed</th>
<th>39 mg indiv</th>
<th>Average utility function value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>Placebo</td>
<td>24 mg fixed</td>
<td>Placebo</td>
<td>39 mg fixed</td>
<td>Placebo</td>
</tr>
<tr>
<td>All patients</td>
<td>612</td>
<td>100</td>
<td>11.59</td>
<td>2.26</td>
<td>0.74</td>
<td>0.55</td>
<td>1.24</td>
</tr>
<tr>
<td>Men</td>
<td>331</td>
<td>54</td>
<td>15.58</td>
<td>3.43</td>
<td>1.02</td>
<td>0.55</td>
<td>1.09</td>
</tr>
<tr>
<td>Women</td>
<td>281</td>
<td>46</td>
<td>6.89</td>
<td>0.88</td>
<td>0.42</td>
<td>0.55</td>
<td>1.43</td>
</tr>
<tr>
<td>Age&lt;40</td>
<td>90</td>
<td>15</td>
<td>10.94</td>
<td>2.72</td>
<td>0.70</td>
<td>0.55</td>
<td>1.01</td>
</tr>
<tr>
<td>Age&gt;75</td>
<td>47</td>
<td>7.7</td>
<td>12.06</td>
<td>1.06</td>
<td>0.79</td>
<td>0.55</td>
<td>2.00</td>
</tr>
<tr>
<td>Weight&lt;60 kg</td>
<td>34</td>
<td>6</td>
<td>7.40</td>
<td>0.86</td>
<td>0.55</td>
<td>0.55</td>
<td>1.47</td>
</tr>
<tr>
<td>Weight&gt;100 kg</td>
<td>77</td>
<td>13</td>
<td>13.21</td>
<td>3.49</td>
<td>0.80</td>
<td>0.55</td>
<td>0.98</td>
</tr>
<tr>
<td>Men with CrCL&gt;80</td>
<td>268</td>
<td>44</td>
<td>15.58</td>
<td>3.91</td>
<td>1.04</td>
<td>0.55</td>
<td>0.98</td>
</tr>
<tr>
<td>Men with CrCL&lt;50</td>
<td>7</td>
<td>1.1</td>
<td>15.58</td>
<td>0.52</td>
<td>0.79</td>
<td>0.55</td>
<td>2.37</td>
</tr>
<tr>
<td>Women with CrCL&lt;50</td>
<td>14</td>
<td>2.3</td>
<td>6.89</td>
<td>0.08</td>
<td>0.32</td>
<td>0.55</td>
<td>3.82</td>
</tr>
</tbody>
</table>
If the risk of ALAT-elevations is taken into account, by assigning it a relative weight of 0.1 in the utility function, the expected optimal fixed dose is reduced from 34 mg to 33 mg b.i.d (Figure 20). If, in addition, the risk of any bleeding is included with a relative weight of 0.05, then the optimal fixed dose is further reduced to 32 mg (VTE:Major bleeding: Any bleeding: ALAT-elevation weighted as 1:1:0.05:0.10).

*Figure 20.** Average probability (Pr) of recurrent VTE, major bleeding, and ALAT>3-ULN, respectively, and average value for the utility function Pr(VTE) + Pr(major bleeding) + 0.1 Pr(ALAT>3-ULN) against varying fixed twice daily doses of ximelagatran. The dose that minimizes the utility function value is estimated to 33 mg b.i.d.
5 Discussion

The population pharmacokinetic models, derived from data obtained in patients undergoing orthopedic surgery, and in patients experiencing a VTE, demonstrate that the pharmacokinetics of melagatran following p.o. administration of ximelagatran is consistent across different studies and patient populations. The total variability (C.V.) in melagatran exposure was between 36% and 45%. The most important predictor of melagatran exposure was renal function, as estimated by calculated creatinine clearance. The linear models with a breakpoint at high creatinine clearances (‘hockey-stick’ models) appear to be more credible than a linear model without a breakpoint, as the clearance predicted at zero creatinine clearance (approximately 2 L/h) is close to the non-renal clearance value estimated previously [96]. The small differences observed in CL/F between the study populations could be attributed to demographic differences, as the orthopedic surgery study included older patients and more women, who in general have lower renal function, than did the VTE studies. The influence of other patient factors on the melagatran pharmacokinetics was also similar, although manifested in different covariates. Volume of distribution was, for example, related to body weight in one study and to gender in another. The estimated effect of β₁-blockers on the bioavailability observed in the phase II study might, at least partly, be confounded by the fact that more women were taking β₁-blockers, as no influence of β₁-blockers were found in the larger phase III study and as women had lower CL/F and V/F than men in this study.

The covariate analysis in the phase III study showed no influence of concomitant medications on melagatran pharmacokinetics. A major limitation of these covariate analyses is that the co-medications were classified according to therapeutic groups (ATC-codes), which reduces the likelihood of identifying interactions caused by individual drugs. In addition, the doses and dosing times of the co-medications were not considered, although it may be presumed that the dosing regimens used are representative of those applied in clinical practice. Evaluation of drug interactions from population pharmacokinetic studies should be considered as an adjunct to other, more specific in vitro and in vivo drug interaction studies.

There was a good linear correlation between plasma melagatran concentration and ECT, and neither baseline ECT nor the ECT response to melagatran was influenced by any of the patient covariates evaluated. This makes ECT well suited as a surrogate for plasma melagatran concentration meas-
urements, as demonstrated in paper II. It may also be expected that ECT measurements would perform well if therapeutic drug monitoring had been required.

The APTT assay is less sensitive to the effect of thrombin inhibitors. APTT increased in a non-linear fashion with increasing plasma concentrations of inogatran and melagatran. The evaluation in paper I demonstrates the importance of using standardized methods, or adjusting for methodological differences, when pooling data from different studies or study centres. The information available in the normal reference range may be utilized for this purpose by including it as a covariate in the pharmacodynamic model.

The results reported in papers I and IV demonstrate that baseline APTT is lower, and the APTT response is less sensitive to inogatran and melagatran at the same plasma concentration in elderly patients than in young healthy subjects and patients. This may be explained by age-related changes in the haemostatic system such as increased clotting factors [97]. The clinical implications of these age-related differences are not known, but the findings suggest that the elderly need slightly higher plasma concentrations of thrombin inhibitors than younger patients to achieve the same degree of anticoagulation.

Although APTT is expected to reflect the degree of anticoagulation, average APTT (or APTT-change or APTT-ratio) over a dose interval was not a better predictor than melagatran AUC for the bleeding risk. The reasons for this may be that the melagatran plasma concentration versus APTT curve is relatively flat at high concentrations, and that the APTT assay variability adds imprecision to the data, despite the analyses being conducted within the same laboratory, and thereby countering the potential of APTT for predicting bleeding events. If APTT were to be used for coagulation monitoring and dose adjustments, evaluation of sampling times that are expected to be the best predictor of VTE and bleeding risks could be performed through simulations using the population pharmacokinetic and pharmacodynamic models developed.

In the phase II study, no statistically significant relationships were found between melagatran AUC and change in thrombus size or risk of bleeding-related events. There may be different explanations for this, including too few patients studied, a too short treatment period, and too indiscriminatory endpoints. In the larger phase III study, a highly statistically significant relationship was, however, found between melagatran AUC and the probability of recurrent VTE, and borderline statistically significant relationships to the risks of bleeding. In addition, the risk of ALAT-elevations to >3-ULN were significantly related to melagatran AUC. An ordinary $E_{\text{max}}$-model described this relationship equally well as a sigmoid $E_{\text{max}}$-model, and was superior to a linear model. This might support the hypothesis that only some patients are prone to experience ximelagatran-induced ALAT-elevations, although the proportion of patients affected may vary with the cut-off level applied. A
threshold exposure level might exist, below which no patients will experience drug-related ALAT-elevations to >3-ULN, but such a threshold level could not be defined possibly because of the gap of data in the lowest exposure region.

The exposure-clinical response analyses should be interpreted with caution, as they were exploratory and not based on randomized comparisons. It is therefore not possible to separate to what extent exposure independently affects the probability of clinical events from the influence of other patient factors, which co-vary with exposure. Attempts were, however, made to adjust for confounding factors by utilizing placebo data from the phase III study. Due to relatively few clinical events, the power to identify such confounding factors is, however, still limited. All models should be validated to assess their predictability. The exposure-response models described in this thesis have not been formally validated against new independent data. However, the models for major bleedings and ALAT-elevations were consistent with models derived in other patient populations (unpublished observations), which supports their validity.

The benefit-risk profile of a drug therapy is often difficult to establish in a quantitative manner, as it is highly dependent on individual judgements of the treating doctor and the patient. In paper IV, the clinical utility of varying doses was estimated under different assumptions about the relative severity of a major bleeding versus a VTE in patients receiving ximelagatran as secondary prevention of VTE. The models predict that the clinical utility of ximelagatran 24 mg b.i.d. is favourable, compared to placebo, in the overall study population for a wide range of weights of a major bleeding relative to a VTE. Based on fatality rates, observed in ximelagatran studies [24, 25], and other studies in VTE patients [23, 58], it seems reasonable to assign the same weights to a major bleeding and a VTE. Under this assumption the clinical utility was favourable also in patient subgroups who might be expected to have an increased risk of VTE or bleeding, obviating the requirement for dose adjustment in these patients. A hypothetic, completely individualized dosing regimen, based on the patient’s creatinine clearance, was predicted to reduce the total risk of a VTE and/or a major bleeding by 20%, and any more practically feasible individualization strategy is expected to have less impact on the clinical utility. It should, however, be emphasized that the potential to improve the clinical outcome by individualization depends on the composition of the population studied, as well as on the average dose given. If more renally impaired patients were included, or a higher dose given, the impact of individualization would be greater. It should be noticed, though, that the patient subpopulations that were predicted to have the lowest benefit-risk ratio using a fixed dose of 24 mg b.i.d. were men with high body weight or good renal function, i.e. those with the highest risk of lack of efficacy, and not those with the highest risks of major bleeding. The inclusion of the probability of any bleeding and ALAT-elevations in the
clinical utility function had only minor influence on the estimated optimal
dose, under the conditions explored. However, the estimated risk of a fatal
outcome for a patient with an ALAT-elevation of >3-ULN is based on a low
number of events, and is thus relatively uncertain.

The models developed may be used to simulate the clinical outcome fol-
lowing alternative ximelagatran dosing strategies in patient populations with
varying demographic properties and using different utility functions. Other,
non-fatal, clinical consequences of VTEs, bleedings and ALAT-elevations
may be considered by varying the weight assigned to the probability of each
event. However, the estimated optimal dosing strategy in one indication
must not be extrapolated to other indications due to different risk profiles.
Assuming similar relationships between the biomarkers and clinical re-
response, the models may also be used for dose selection in clinical studies
with other direct thrombin inhibitors.
6 Conclusions

- The pharmacokinetics of melagatran was predictable, well correlated to renal function, and consistent across studies and patient populations.
- ECT is a useful surrogate for melagatran plasma concentrations in pharmacokinetic evaluations.
- Characterization of APTT variability demonstrated the importance of adjusting for assay-differences when pooling data from several studies or study centres, and a modelling approach to handle this was proposed.
- Relationships between APTT and the plasma concentration of inogatran and melagatran were described by non-linear models. Elderly patients had lower baseline APTT and appeared to have less pronounced effects of the thrombin inhibitors than younger patients or healthy subjects, which might imply that the elderly need higher plasma concentrations than younger patients to achieve the same anticoagulant effect.
- Clear relationships between melagatran AUC and thrombus size or bleeding risk could not be established to support dose selection in pivotal studies for acute treatment of VTE, but the estimated clinical utility was positive in the studied exposure range.
- Overall, melagatran AUC and APTT were similarly predictive of thrombosis and bleeding in long-term secondary prevention of VTE.
- The identified relationship between the risk of ALAT-elevation and melagatran AUC suggests that the incidence approaches a maximum at high exposures.
- The models support a fixed ximelagatran dosing regimen in secondary prevention of VTE without the need for individualization. This conclusion is based on predicted favourable clinical utility, compared to placebo, in the overall study population as well as in special patient subgroups (n.b. severe renal impairment not studied).
- The models developed can be used for selection of dosing strategies that are expected to increase the likelihood of an optimal clinical outcome in future potential clinical trials with ximelagatran and other direct thrombin inhibitors.
7 Populärvetenskaplig sammanfattning

Vid förebyggande och akut behandling av blodpropp är doseringen av det blodförtunnande läkemedlet av avgörande betydelse för att undvika uppkomst och tillväxt av proppar utan att riskera blödningar. De läkemedel som används mest idag (t ex warfarin) kräver täta kontroller och justeringar av dosen eftersom effekten varierar mellan olika patienter samt påverkas av kosten och andra läkemedel.

Syftet med denna avhandling har varit att beräkna vilka doseringar av en ny sorts läkemedel, s.k. trombinhämmare (ximelagatran) som kan förebygga blodpropp i ben och lungor på ett effektivt och säkert sätt. Detta har gjorts med hjälp av matematisk-statistiska modeller som beskriver sambandet mellan given dos och koncentration av det verksamma ämnet (melagatran) i blodet, samt mellan koncentration och respons, där respons innefattar mätt på blodets koagulationsförmåga, klinisk effekt och biverkningar. Vidare har s.k. nyttofunktioner använts för att på ett objektivt sätt försöka väga ihop riskerna för, och konsekvenserna av, blodpropp, blödningar och förhöjda levervärden.

Resultat från patienter som medverkat i utprovningen av olika ximelagatran-doser visar att blodkoncentrationen av melagatran är förutsägbar, och till stor del bestäms av patientens njurfunktion. Modellerna ger stöd för att samma dos kan ges till alla patienter, utom till dem med gravt nedsatt njurfunktion, och att denna dos ger en god balans mellan effekt och biverkningar jämfört med placebo ("sockerpiller") i olika patienter.

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