# Anticoagulation variability in relation to occurrence of thromboembolism and clinically relevant bleeding in patients on warfarin monitored with either Fiix-prothrombin time or Quick-prothrombin time

The Fiix-trial

Alma Rut Óskarsdóttir

**Lokaverkefni til B.S. gráðu í læknisfræði**
**Læknadeild**
**Heilbrigðisvísindasvið**
**Háskóli Íslands**

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# Samband breytileika warfarínmeðferðar við blóðsegamyndun og blæðingar hjá sjúklingum sem stýrt er með Fiix-próþrombíntíma eða Quick-próþrombíntíma

Fiix-rannsóknin

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# Abstract


## Anticoagulation variability in relation to occurrence of thromboembolism and clinically relevant bleeding in patients on warfarin monitored with either Fiix-prothrombin time or Quick-prothrombin time

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**Introduction:** Warfarin is challenging in use and its effect must be carefully monitored to maintain the desired anticoagulation level. The Quick prothrombin time (PT) is most commonly used to monitor the warfarin effect by measuring the effect of changes in coagulation factors (F) II, VII and X on the clotting time. Experiments suggest that rapid fluctuations in factor VII activity, mainly due to its short half-life, contribute to variation in the measured clotting time but less to the antithrombotic effect itself. The newly developed Fiix prothrombin time (Fiix-PT) is only sensitive to factors II and X and has been shown to improve anticoagulation stability. The objective of the current study is to estimate anticoagulation variability in relation to the occurrence of thromboembolic events and bleeding in patients monitored with Fiix-PT or PT.

**Methods and materials:** This study is a secondary subgroup analysis of the Fiix-trial, a single-center, double blinded, prospective, randomized controlled clinical trial. Participants were patients on warfarin, 18 years and older, with target INR range of 2.0 - 3.0. The research arm was monitored with Fiix-PT and the control arm with PT, with blinded INRs reported to the dosing staff. The two arms were further divided, based on whether participants had any clinically relevant vascular events (CRE) during the study or not. The CRE were major bleedings, other non-major clinically relevant bleedings and thromboembolism. The anticoagulation variability was evaluated with regard to test number and intervals, dosing, time within therapeutic range (TTR), anticoagulation fluctuation, and anticoagulation at the time of an event.

**Results:** There were 22,525 monitoring tests. In the Fiix arm, 115 patients suffered from CRE and 457 had no events. In the PT arm, 132 patents suffered from CRE and 439 had no events. Patients who suffered CRE had more frequent monitoring tests than patients who did not, and they also had significantly fewer tests within target therapeutic range ($p < 0.0001$ within both arms). The event groups had significantly greater dose fluctuation than the no event groups ($p < 0.0001$ within both arms) as well as lower median TTR (79% vs. 82%, $p = 0.0441$ in the Fiix arm and 75% vs. 80%, $p = 0.0004$ in the PT arm). The Fiix event group had significantly higher TTR than the PT event group. The event groups had, on top of that, significantly greater fluctuation of anticoagulation than the no event groups. The median anticoagulation (INR) at the time of major events corresponded with the risk of bleeding and thromboembolism in the Fiix arm, and with risk of thromboembolism in the PT arm.

**Conclusions:** Monitoring warfarin with the Fiix-PT and paying particular attention to patients demonstrating anticoagulation instability could improve the clinical outcome of patients on warfarin.

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# Table of contents

# List of figures



# List of tables

# List of abbreviations

| | |
|---|---|
| a | Activated |
| AF | Atrial fibrillation |
| AMC | Anticoagulation management center |
| CRB | Clinically relevant bleeding |
| CRE | Clinically relevant event |
| CYP450 | Cytochrome P450 |
| DOAC | Direct oral anticoagulant |
| F | Factor |
| INR | International normalized ratio |
| ISI | International sensitivity index |
| ITM | Intention to monitor |
| MB | Major bleeding |
| MI | Myocardial infarct |
| Owren-PT | Prothrombin-proconvertin time, PP |
| PT | Prothrombin time, Quick-PT |
| R-INR | Research INR |
| SNP | Single nucleotide polymorphism |
| TE | Thromboembolism |
| TF | Tissue factor |
| TIA | Transient ischemic attack |
| TP | Thromboplastin |
| TTR | Time within therapeutic range |
| VGR | Variance growth rate |
| VKA | Vitamin K antagonist |
| VKD | Vitamin K dependent |
| VKORC1 | Vitamin K epoxide reductase complex 1 |
| vWF | vonWillebrand factor |

# 1. Introduction

Under normal circumstances the blood flows freely through our vessels and an interruption in its flow can be dangerous. Whether it's a hemorrhage or the formation of a thrombus following an injury, it can be life threatening. Pro-hemostatic treatment and antithrombotic therapy is therefore very important, respectively. Antithrombotic drugs can be divided into two categories, antiplatelet drugs and anticoagulation drugs. During antithrombotic therapy a balance must be held, to prevent thrombus formation and not induce bleeding. Oral anticoagulation drugs of the vitamin K antagonist (VKA) type have been used for almost 70 years and although it's challenging in use, warfarin has been the most commonly used drug worldwide. Warfarin's effectiveness has been established by clinical trials for the prevention of thromboembolism, for example in atrial fibrillation (AF) patients.[1]

## 1.1. Hemostasis

Hemostasis is a homeostatic mechanism that ensures normal blood flow and blood coagulation is an essential component of it. Hemostasis involves four key components, the endothelium, platelets, the coagulation pathway and fibrinolysis. Each component and their interplay must be tightly regulated to prevent the occurrence of hemorrhagic or thrombotic events.[2]

The endothelium makes a physical barrier between the blood and subendothelial elements and its main function is to maintain the vessel wall's permeability. The endothelial cells contribute to the regulation of blood flow and blood pressure. One way to promote normal blood flow is by providing an antithrombotic surface that inhibits platelet adhesion and clotting. The expression of heparan sulfate, tissue factor pathway inhibitor and thrombomodulin inhibits the generation of thrombin, a crucial step in clot formation, and contains its activity, respectively. The endothelium also produces nitric oxide, prostacyclin and an inhibitor of adenosine diphosphate and thus prevents the adhesion and activation of platelets. The endothelium has prothrombotic properties as well. It expresses binding sites for various coagulation proteins, such as thrombin and fibrin. It also expresses tissue factor (TF) when ruptured and thus facilitates the initiation of the coagulation pathway.[3,4]

The coagulation process is a pathway of serine protease enzymes and their co-factors that interact to form a stable fibrin clot.[5] Hepatic parenchymal cells play an essential role in the formation of the coagulation factors and their cofactors.[6] The coagulation factors circulate as inactive zymogens until they are activated by proteolytic mechanisms.[5] Blood coagulation is commonly described as having two separate pathways, the intrinsic pathway and the extrinsic pathway, that both end in the common pathway. This description is called the waterfall theory of blood coagulation and was descriped by Ratnoff and Davie. It proposes that the clotting factors interact in pairs, where one factor acts as an enzyme and the other as a substrate.[7] The various clotting factors are activated in succession through this interaction and eventually lead to clot formation, as can be seen in Figure 1.

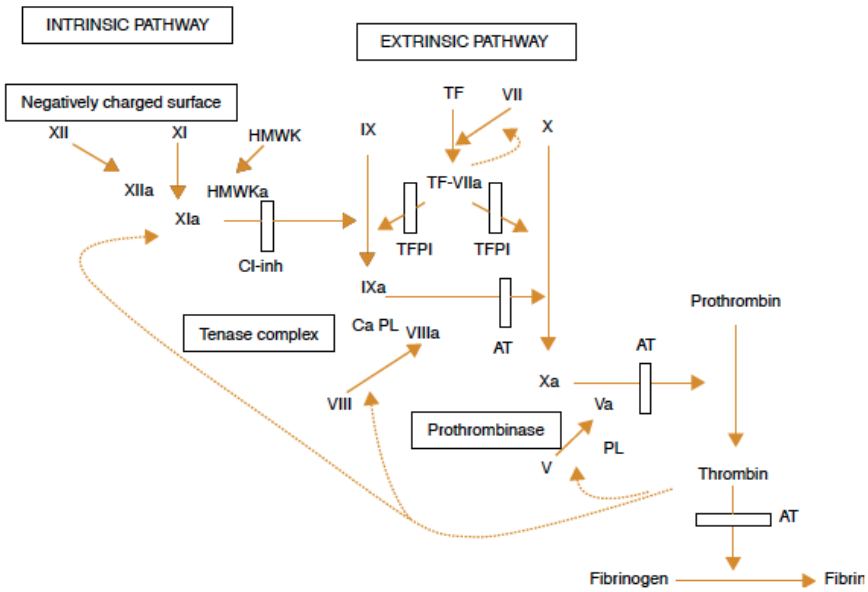


**Figure 1: The waterfall theory of blood coagulation**
The interactions of the coagulation factors lead to the formation of a fibrin clot.[5]

The intrinsic pathway can be activated when the blood comes into contact with negatively charged surfaces and the extrinsic pathway is activated when TF comes into contact with plasma.[7] TF is, as previously described, expressed by endothelial cells, but also by fibroblasts and other damaged or stimulated cells.[8] A more recent way to look at blood coagulation is the cell-based model of Roberts, Monroe and Hoffman, which focuses on the formation of protein complexes on cells, rather than the intrinsic and extrinsic pathways separated. The main cells in localizing and controlling the coagulation process are TF bearing cells and platelets.[9]

The fibrinolytic system and natural anticoagulant proteins, such as antithrombin III, activated protein C and its cofactor protein S are important to limit the clot to the wound. This regulation takes place at different stages of coagulation. Plasmin, the main fibrinolytic protease, breaks down fibrin, and if excessive, the fibrinolysis can result in hemorrhage. The natural anticoagulant proteins inactivate various coagulation factors, such as activated (a) factor (F) X, FVa and FVIIIa, and thus inhibit further formation of thrombin.[6,10]

### 1.1.1. The coagulation process

When an injury or disruption in a blood vessel wall occurs the first response of the endothelium is constriction to inhibit blood loss.[4] The endothelial cells undergo biochemical changes and become more prothrombotic. The blood becomes exposed to subendothelial elements, such as collagen, von Willebrand factor (vWF), fibronectin and TF. Circulating platelets adhere to the endothelium via glycoprotein receptors and vWF. Adherent platelets undergo degranulation and become activated. Circulating platelets interact with the adherent ones and aggregation leads to the formation of a platelet plug. This plug, the result of primary hemostasis, must be stabilized with fibrin, a product of the coagulation process.[3]

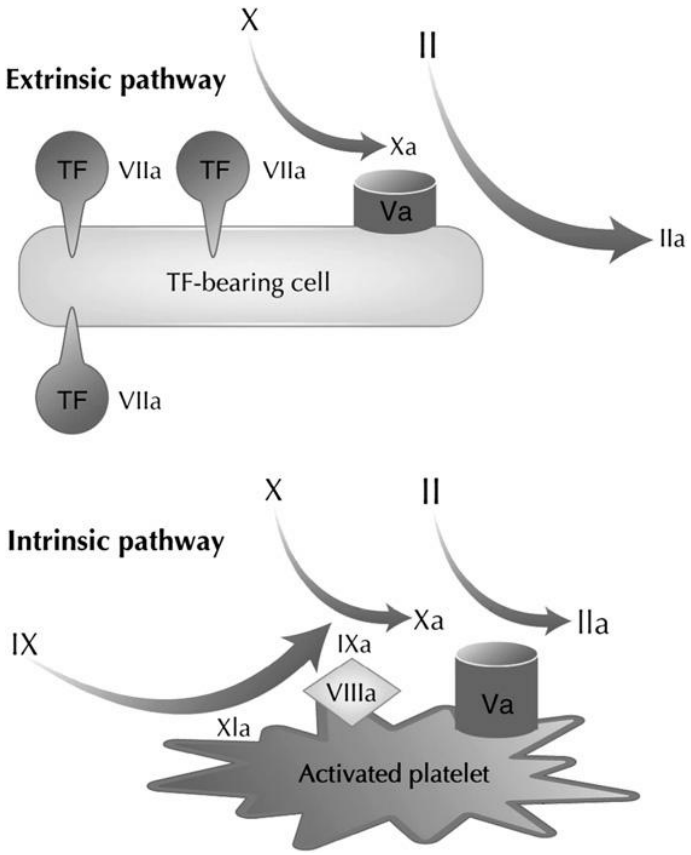


**Figure 2: The intrinsic and extrinsic pathways in the cell-based model of coagulation**
Tissue factor (TF) bearing cells and platelets play essential roles in blood coagulation. The roles of the cell-based extrinsic and intrinsic pathways are initiation of coagulation and extensive thrombin generation, respectively.[11]

The initiation step of coagulation is localized on TF bearing cells,[9] as shown in Figure 2. TF activates the extrinsic pathway of coagulation by making a complex with FVIIa. About 1% of circulating FVII is on an active form.[12] This complex is crucial to the initiation of the coagulation pathway.[5]

The TF-FVIIa complex is a tenase complex and thus activates FX to FXa. The complex also activates FIX to FIXa, but at lower levels. FIX is mainly activated via the intrinsic pathway by FXIIa and FXIa. FXa forms the prothrombinase complex with FVa, calcium and phospholipids. This prothrombinase complex converts prothrombin (FII) to thrombin (FIIa). FIXa forms another tenase complex, with FVIIIa, calcium and phospholipids, and also activates FX to FXa.[5,13]

FXa and FIXa, both activated by the TF-FVIIa complex, have different roles in initiating coagulation. The main role of FXa is to form small amount of thrombin and thus activate platelets.[14] Thrombin, generated on TF bearing cells activates platelets to a higher level of procoagulant activity. Keeping these cells separated until injury is, thus, important to inhibit unwanted initiation of coagulation.[15] The role of FIXa, however, is to provide FXa on the platelets' surface and by that enhance thrombin generation.[14] Thrombin, formed by the prothrombinase complex, also activates FV, FVIII and FIX, forming a positive feedback loop and influencing its own formation.[5]

After this amplification phase, extensive thrombin generation and clot stabilization takes place on the activated platelets' surface.[15] Thrombin converts soluble fibrinogen to fibrin monomers and they

form insoluble fibrin polymer, which seals the site of injury. The fibrin plug is then stabilized further by the thrombin activated FXIIIa.[5]

## 1.2. Vitamin K antagonists

Coumarins or VKAs are anticoagulants that affect the hepatic formation of the vitamin K dependent (VKD) coagulation factors, FII, FVII, FIX and FX, and therefore reduce their coagulation activity. VKAs also inhibit VKD the formation of the anticoagulant proteins C, S and Z, and therefore have the potential to be procoagulant. Under most circumstances, the anticoagulant effect is dominant. VKA's were the only oral anticoagulant agents for decades and their effectiveness has been well established. However, their use has to be well monitored to maintain the desired anticoagulation level.[1,16]

Vitamin K is required for the hepatic posttranslational modification of various proteins, including FII, FVII, FIX and FX. The formation of gamma-carboxy glutamic acid from glutamic acid requires the reduced form of vitamin K (vitamin $KH_2$) as a cofactor. Vitamin K reductase reduces vitamin K to vitamin $KH_2$, and the carboxylation can occur. Vitamin K 2,3-epoxide (oxidized vitamin K) is a product of the carboxylation reaction and the reduction of the vitamin K epoxide, by vitamin K epoxide reductase (vitamin K oxide reductase), forms vitamin K again. This vitamin K can be reused. VKA's strongly inhibit the vitamin K epoxide reductase and cause an accumulation of oxidized vitamin K in the liver, as well as inhibiting the vitamin K reductase. Because of the strong inhibition of the vitamin K epoxide reductase the effect of VKA's can be overcome by low doses of vitamin K.[17] This is described in Figure 3.

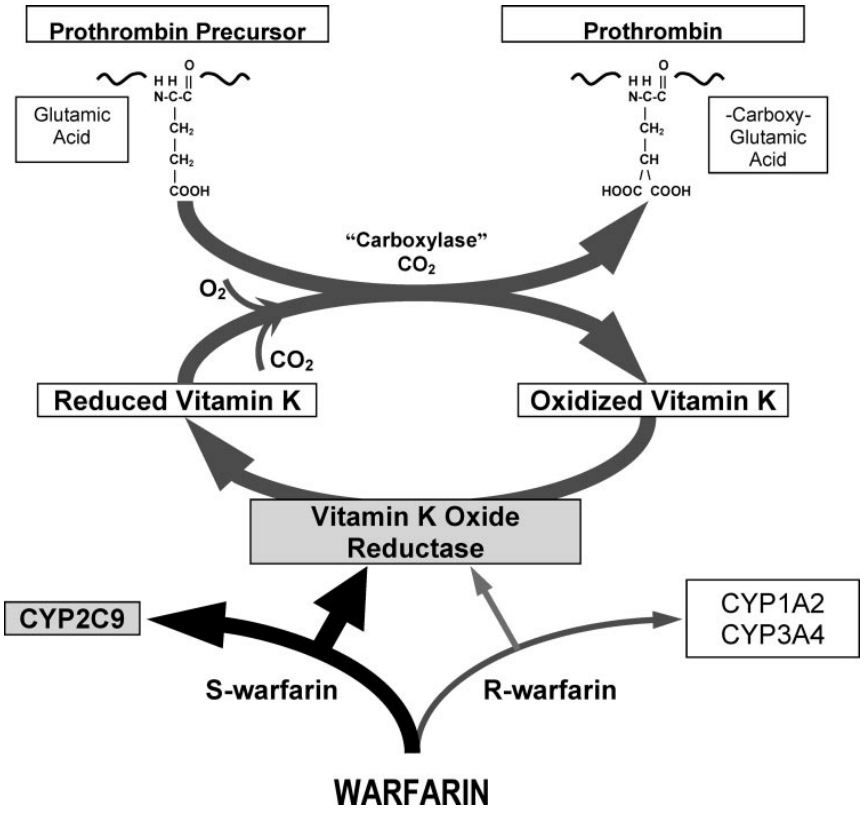


**Figure 3: The effect of warfarin on vitamin K metabolism**
Warfarin inhibits the vitamin K oxide reductase and therefore inhibits the vitamin K dependent carboxylation of glutamic acid.[1]

### 1.2.1. Warfarin

Warfarin is the most widely used oral anticoagulant. It was synthesized by Karl Link and his colleagues in 1948, after years of working to isolate an anticoagulant agent, known as dicoumarol, in sweet clover. It was initially approved as a rodenticide in the USA in 1952 and then for human use in 1954.[18] Warfarin is water soluble and easily absorbed by the gastrointestinal tract. It is metabolized in the liver, reaches maximal blood concentration about 90 minutes after oral administration and has a half-life of 36 to 42 hours. Warfarin is a racemic mixture of two isomers, the R and S enantiomers. Different enzymes of the cytochrome P450 (CYP450) system metabolize the two enantiomers. The CYP2C9 enzyme primarily metabolizes the S enantiomer, which is the more potent enantiomer, and the CYP1A2 and CYP3A4 enzymes primarily metabolize the R enantiomer. Racemic warfarin has a high degree of binding to plasma albumin, over 99% at therapeutic concentrations, and that can explain the prolonged half-life. Warfarin is usually administered at 24 hours intervals, a time shorter than its half-life.[1,19]

There is a variety of genes that affect warfarin metabolism. The primary genes are the CYP2C9 gene and the vitamin K epoxide reductase complex 1 gene (VKORC1). As previously described, CYP2C9 metabolizes the S enantiomer of warfarin and VKORC1 is the target protein for warfarin. The majority of variants in CYP2C9 are single nucleotide polymorphisms (SNP) that lead to reduced enzymatic activity. The CYP2C9*2 and CYP2C9*3 alleles are the primary non-functional alleles in Europeans, but are less frequent in Africans and Asians.[20] The reduced enzymatic activity can lead to lower dose requirements and likely increased risk of bleeding.[21] A SNP in the VKORC1 regulatory region also predicts dosing requirements. Different haplotypes of this gene predict low, median and high warfarin dose phenotypes, and are unequally distributed between ethnic races.[22]

As mentioned above the effect of warfarin can be overcome by low doses of vitamin K. Therefore food interactions must be considered during warfarin therapy as well as drug interactions. Interactions can either lead to potentiation or inhibition of the effect of warfarin. Patients on a long-term warfarin therapy are sensitive to fluctuating levels of dietary vitamin K, which is derived predominantly from plant material. A variety of drugs can affect the effect of warfarin by affecting its pharmacokinetics. Drug interactions between warfarin and commonly used drugs and drug families, such as nonsteroidal anti-inflammatory drugs, anti-infective agents and omeprazole, have been reported. Multiple diseases can also inhibit or potentiate warfarin's effect, such as hepatic failure and hypermetabolic states.[1,23]

### 1.2.2. Monitoring

The aim of warfarin therapy is keeping patients within a target therapeutic range on an international normalized prothrombin time ratio (INR) scale. That is, to keep patients within a narrow therapeutic window where maximum efficacy and safety is attained. To ensure the best therapy possible, the effect of warfarin must be carefully monitored.[1]

#### 1.2.2.1. Time within therapeutic range and variance growth rate

The time each patient spends within therapeutic range (TTR) is an important tool to assess the control and intensity of anticoagulation therapy. High TTR has been shown to correspond with reduction in

hemorrhagic and thrombotic events, especially where there is a good organization of anticoagulation treatment.[24] The TTR highly depends on the quality of the anticoagulation therapy. The benefit of oral anticoagulants over antiplatelet therapy has been studied and it seems that when the TTR is under 65%, there is no marked benefit.[25] Another important way to evaluate the quality of anticoagulation therapy is by assessing the variation in the INR in each patient over a period of time, using the variance growth rate (VGR) method. It has been shown in a retrospective study that the VGR has a strong association with clinical events up to 6 months before an event and that the predictive ability of VGR is as effective as TTR, especially for INR monitoring in the short term. The VGR can be calculated as the fluctuation around mid-target INR of 2.5 (formula A) or the fluctuation between monitoring tests (formula B1).[26] The different formulas are shown in Appendix 2.

#### 1.2.2.2. Prothrombin time and Fiix-prothrombin time

The prothrombin time (PT or Quick-PT) test is most commonly used to monitor warfarin therapy. The PT is the time it takes to form a fibrin clot, in seconds. It is sensitive to deficiencies of the VKD FII, FVII and FX as well as FV and fibrinogen. The PT usually ranges between 10 and 14 seconds, but is prolonged with deficiencies in aforementioned factors, as well as by antibodies directed against them. Another test, less commonly used, is the prothrombin-proconvertin time (PP or Owren-PT) test. Owren-PT is similar to PT, but it is not sensitive to reduced concentration of FV and fibrinogen. It is mainly used in the Nordic countries, Holland and Japan. Both tests are performed in similar fashion, and the INR based on either Owren-PT or PT will lead to practically identical results. Both assays use citrated platelet poor plasma and coagulation is initiated using undiluted thromboplastin (TP) and recalcification. Owren-PT is done on diluted test plasma, and is considered to be more sensitive method than PT. The dilution makes the test less sensitive to factors that might interfere with the true effect of the coagulation factors.[13,27-29]

The PT results are standardized by calculation and reported as INR. This is necessary because PT results can differ when using different reagents. TPs vary in TF source and that leads to differences in sensitivities to factor deficiencies. The INR corrects for differences in TP potency, and can thus be used to report results between institutions and countries. The INR is the ratio between the patient's PT and a control PT raised to the power of the international sensitivity index (ISI). ISI is established by The World Health Organization and describes the responsiveness of the TP reagent used in the test, to reductions in the VKD coagulation factors, compared to a standard. The standard has ISI 1.0 and less sensitive TPs have higher ISI.[13]

The VKD clotting factors are reduced by warfarin at a rate proportional to their half-lives. FVII has the shortest half-life of these factors, about 3.5 hours, while FII has the half-life of 72 hours. Therefore, during the first days of warfarin therapy the PT reflects mainly the reduction of FVII, rather than a change in antithrombotic effect.[27,30] The true antithrombotic effect of warfarin requires 6 days of treatment, but the anticoagulant effect develops in 2 days. Warfarin affects the formation of the VKD factors, so its effect does not become clinically relevant until the factors already formed are used up. The antithrombotic effect of warfarin requires the reduction of FII which has relatively long half-life compared to the other VKD factors.[31] Thus, the effect of warfarin can be visible in blood coagulation tests before the antithrombotic effect becomes clinically relevant. It has been shown that the

antithrombotic effect of VKAs consist mainly of the antithrombotic effect of reducing FII and FX, and less of reducing FVII. Also, lowering FVII does not induce severe bleeding until levels are well below 5%.[16,32]

In 2009 Páll Torfi Önundarson and Brynja R. Guðmundsdóttir evaluated the roles of each VKD coagulation factor *in vitro*. Their results showed that FII and FX have predominant roles in VKA factor activity and that rapid changes in FVII activity, due to its short half-life, could exaggerate the fluctuations in the measured clotting time and, thus, the INR. These excessive fluctuations may not influence the true antithrombotic effect or risk of bleeding, but lead to redundant dose changes and too frequent monitoring. They concluded that monitoring patients with a test not sensitive to FVII could overcome this effect. Therefore, the Fiix-PT test was developed, a test only sensitive to reductions in FII and FX. Because the Fiix-PT test is not sensitive to reductions in FVII, unlike the PT and Owren-PT tests, it was believed to lead to less fluctuation in the INR and thus more efficacy and safety during VKA therapy.[27]

### 1.2.3. Dosing

The optimal therapeutic INR range can vary between individuals and is not the same for all indications. A low INR can increase the risk of thrombotic events and an INR to high can increase the risk of hemorrhage. The INR range of 2.0 - 3.0, moderate intensity INR, is effective for most indications.[1] To keep patients in the right range, blood tests are performed on regular basis and doses managed appropriately.

In Iceland, Segavarnir, anticoagulation management center (AMC), at Landspítali – The National University Hospital of Iceland manage the dose changes for patients on warfarin. The doses are managed according to the American College of Chest Physicians evidence-based clinical practice guidelines. When initiating therapy, the aim is to get the patient in the target therapeutic range in 7 to 10 days; it should not take longer than 10 to 15 days. The initial dose varies with age and underlying conditions. Another anticoagulant drug, heparin, is often administrated with warfarin during initiation until the INR is within the therapeutic range. The usual initiation dose for patients younger than 65-70 years old is 6 mg daily for three days. The following doses depend on the INR after these three days. An INR higher than the therapeutic target results in lowering of doses and an INR too low results in increased dose. Corresponding dose for patients older than 70 years and those who take drugs or have any diseases that could affect warfarin metabolism is 4 mg.[1] (Appendix 1)

All dosing at Landspítali is now computer software-assisted, using the DAWN AC ® anticoagulant therapy managing system. The software calculates a new dose and suggests the length of the interval to the next calculation. The dose is calculated based on INR target values, the current INR and previous INR history. Specialized staff reviews the doses and notifies the patients. In a study performed to compare the quality of anticoagulation using computer-assisted dosing to manual dosing by specialized staff, the TTR increased as well as risk INR values were reduced when computer-assisted dosing was used.[33]

### 1.2.4. Indications, efficacy and safety

Warfarin therapy has established its efficacy in the prevention of thromboembolic events in patients with AF, venous thromboembolic diseases, prosthetic heart valves and coronary artery disease.[34-37] Hemorrhage is the most common adverse event of warfarin. As previously described, warfarin has a narrow therapeutic range, so the quality of the anticoagulation therapy and patients characteristics have a predictive value for adverse events. The benefit of the treatment and risk of adverse events must be weighted in each case when initiating warfarin therapy.[1] Warfarin increases the frequency of hemorrhage about five times, compared to no warfarin therapy, and the frequency increases with more intense therapy. The average annual frequencies of fatal, minor and major or minor hemorrhage during warfarin therapy has been found to be 0.6%, 3.0% and 9.6%, respectively.[38]

AF is one of the most common indications for warfarin. Its effect on reducing the frequency of ischemic stroke and the risk of death of stroke has been established, especially when the treatment results in INR of 2.0 or greater.[39] One study showed the rate of hemorrhage to be 3.8% per person year in older AF patients, and the rate was highest during the first 30 days of treatment.[40]

## 1.3. Direct oral anticoagulants

Since 2009 different types of oral anticoagulants have emerged in clinical use, direct oral anticoagulants (DOACs; dabigatran, rivaroxaban, apixaban and edoxaban). DOACs inhibit FXa or thrombin (FIIa) directly and do not need to be monitored like warfarin.[41]

Many trials have been conducted to compare the new drugs to warfarin. Four phase three randomized clinical trials have been conducted to compare the efficacy and safety of the new drugs to warfarin, with focus on stroke prevention in patients with AF. The four trials showed the DOACs clinically non-inferior, in most cases, in preventing ischemic stroke, intracranial hemorrhage and all-cause mortality. They were, however, inferior to warfarin in preventing gastrointestinal bleeding. The benefit of the DOACs could be exaggerated due to the fact that they were observed primarily at AMCs that did not maintain a high TTR in the warfarin controls.[41-46]

# 2. Study objectives

After the development of the Fiix-test, a randomized clinical trial, the Fiix-trial, was conducted in order to test the hypothesis that monitoring warfarin anticoagulation with Fiix-PT would lead to improved anticoagulation stability and at least equivalent clinical outcome compared to standard INR monitoring using PT. The primary efficacy and safety endpoints were any thromboembolism, major bleedings and other non-major clinically relevant bleedings.

The aim of the current study is to estimate anticoagulation intensity and variability in relation to occurrence of thromboembolic events and clinically relevant bleeding in patients monitored with Fiix-PT or PT. The goal is to see if there is a difference in anticoagulation stability between patients who experience events and those who don't, and how this difference relates to the monitoring method.

# 3. Methods

## 3.1. Study population and conduct

This study is a secondary subgroup analysis of the Fiix-trial, a randomized controlled clinical trial conducted from March 2012 to February 2014 at Landspítali. Eligible participants were ambulatory patients 18 years and older, receiving or starting short-term or long-term warfarin therapy with INR target value of 2.0 - 3.0, irrespective of indication for warfarin. Patients being monitored weekly prior to electroconversion of AF were excluded, as well as nursing home residents. Eight patients were excluded after enrollment, resulting in 1148 participants. Every patient signed an informed consent for the study and was randomly assigned to either the research group or the control group. The research group was monitored with Fiix-PT (Fiix arm) and the control group with PT (PT arm). The study was double blinded and each patient was assigned a study-code to maintain the masking. Blood samples were measured in the coagulation laboratory at Landspítali and all results were reported as research-INR (R-INR) to the dosing staff. Both Fiix-PT and PT were measured for every patient, but only one type of result was reported as R-INR, according to which group the patient belonged to. Dosing was performed according to usual standards. DAWN AC ® anticoagulation management software was used as well as protocols based on monitoring with PT.[47]

Bearing in mind the aim of the current study, the study groups from the Fiix-trial were divided based on whether participants suffered from any clinically relevant vascular events (CRE) during the study or not. The division is described in Figure 4. Each arm, the Fiix arm and the PT arm, was divided into two main groups. The first main group included those who had CRE during the study. That group was further divided, based on the characteristics of the events. The events were clinically relevant bleedings (CRB), either major bleedings (MB) or non-major CRB and thromboembolism (TE). 13 participants, 6 in the Fiix group and 7 in the PT group, had non-major CRB as well as a MB, they were placed in the MB group. The second main group was a control group, including those who did not suffer from CRE during the study.

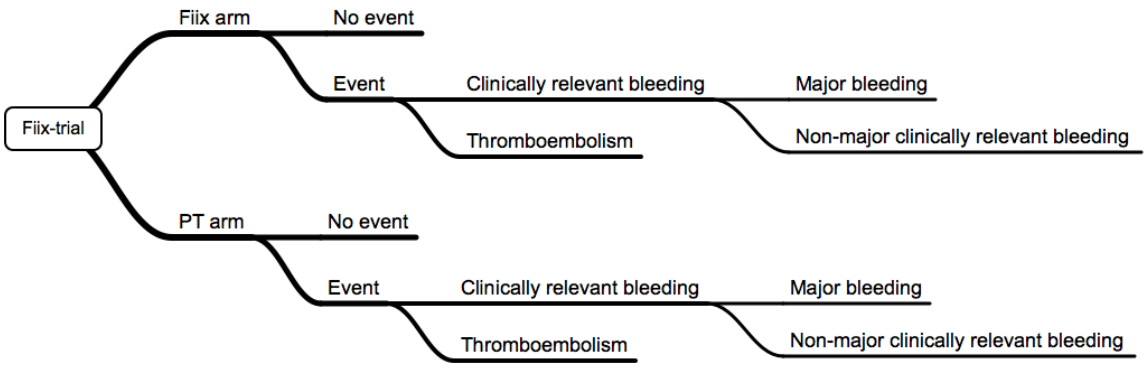


**Figure 4: Classification of study groups**
The study groups were divided based on the characteristics of the events the patients experienced.

A special adjudication committee assessed every event and classified them as previously described. MB was defined according to the ISTH criteria. MB is defined as a fatal bleeding, symptomatic bleeding in a critical area or organ, or a bleeding leading to a transfusion of two or more units of whole blood or red cells.[48] Other non-major CRB was defined as bleeding not meeting the criteria for MB. That includes bleeding associated with medical intervention, unscheduled physician contact, temporary cessation of treatment, or discomfort, such as pain, or other impairment of activities of daily life. TE events were diagnosed non-fatal and fatal arterial or venous TE, including myocardial infarct (MI) and transient ischemic attacks (TIA). TIAs were included if they had been diagnosed by a treating physician, but imaging studies were not mandatory.[47]

During a 3.5-month period 16 months into the Fiix-trial a laboratory calibration problem occurred, causing the Fiix-INR to be reported 0.2 decimal points too high. This may have led to unnecessary and aberrant dose reductions in the Fiix arm.[47] This period was excluded in the current study and patients who were only in the Fiix-trial during this period are not considered a part of this study population**.** One patient was excluded in the Fiix arm and four in the PT arm, resulting in 1143 participants, 572 in the Fiix arm and 571 in the PT arm.

## 3.2. Coagulation assays

Coagulation laboratory biomedical scientists performed all monitoring tests at Landspítali. Two STA-R Evolution ® coagulation analyzing instruments from Diagnostica Stago Inc, Asnieres, France, were used to perform the tests, one instrument for each type of assay. The STA-R instruments measure the time it takes for the blood to clot, by measuring the variation in the amplitude of an oscillating magnetic ball. Increase in viscosity of the plasma being tested results in reduction in the amplitude.

The PT-INR was calculated using the Quick-PT method. To initiate coagulation, 100 µl of STA-Néoplastine CI Plus reagent (TP and calcium) was added to 50 µl of undiluted patient plasma.

The Fiix-INR was calculated using 80 µl of patient plasma, diluted with seven times the volume of STA-Owren Koller dilutent. To initiate coagulation 80 µl of STA-Néoplastine CI Plus reagent (TP and calcium) and 25 µl of Fiix (FII and FX) depleted plasma (Haematologic Technologies Inc.) was added to the diluted plasma.

The only difference between the two methods is the reagents used and the dilution of the plasma. For both assays, the INR was calculated, as previously described, by the formula:
$INR = (\text{patient PT}/\text{mean normal PT})^{ISI}$.

## 3.3. Calculations and statistical analysis

For this analysis, following factors were assessed by calculation; test number and intervals, dosing, TTR, fluctuation of anticoagulation and anticoagulation at the time of major events.

Number of monitoring tests was counted for each patient, as well as number of observation days. Days between monitoring tests were counted, and the average number of days for each patient used, test frequency rate was calculated by dividing the number of monitoring tests per each patient by the number of months they spent in the study.

Number of dose changes was counted for each patient and subsequently the number of dose changes in each patient per year. Calculated annual dose changes per monitoring test in each patient

were calculated by dividing the dose changes per monitoring test in each patient by the number of years they spent in the study. Variability between doses was calculated with the VGR formula B1. The VGR reflects the fluctuation in dose sizes over a period of time.[26]

To calculate percent TTR, the Rosendaal formula was used to calculate a daily INR.[49] The total days in range were counted and divided by the days each patient spent in the study.

INR fluctuation was calculated using two different VGR formulas, A and B1.[26] Formula A was used to calculate the fluctuation around a mid-target of 2.5, and formula B1 to calculate the INR fluctuation between adjacent tests.

Anticoagulation at the time of event was assessed for major events, MB and TE. To have a comparison, the average anticoagulation of each patient who did not experience any events was calculated. If there was a lack of an INR value at the time of event, the value from the last monitoring test before the event was used, that was the case for two patients in the Fiix MB group and one patient in the PT MB group.

All analysis is shown based on intention to monitor (ITM) analysis. All events were counted from the day of enrollment until 5 days after final discontinuation of warfarin or study completion, regardless of short-term or long-term discontinuation of therapy. INR values from the whole period were used. Only patients with three or more monitoring tests were included in the analysis, except when counting number of monitoring tests and observation time, as well as anticoagulation at the time of events. After this exclusion, 28 patients fell out, 12 in the Fiix arm (3 Fiix event, 9 Fiix no event) and 16 in the PT arm (3 PT event, 13 PT no event).

The Mann-Whitney non-parametric test, a variation of the t-test, was used to compare individual-based, continuous data between two groups and the Kruskal-Wallis test for more than two groups. The Fisher exact test or the Chi-square tests were used to compare categorical data for two groups or more, respectively. The Farrington-Manning test was used for non-inferiority calculations, using non-inferiority margin of 0.025. All p-values less than 0.05 are considered statistically significant. All statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA) and R (R Foundation for Statistical Computing, Vienna, Austria).

## 3.4. Permissions

This study is in accordance with the Helsinki declaration at Landspitali Iceland. The protocol for the Fiix-trial was approved by the National Bioethics Committee of Iceland (VSNb2011040019/03.15) and the Data Protection Agency of Iceland (2011040560AMK/-) and can be downloaded from: http://www.landspitali.is/sjuklingar-adstandendur/klinisk-svid-og-deildir/rannsoknarsvid/segavarnir/fiix-rannsoknaraaetlun/. The Fiix-trial was registered at www.clinicaltrials.gov as NCT01565239.

# 4. Results

## 4.1. Study population and distribution of events

The following results are based on analysis of 22,525 monitoring tests, 11,026 from patients monitored with Fiix-PT and 11,499 from patients monitored with PT. In the Fiix arm, 115 patients had suffered from CRE (112 with three or more monitoring tests) and 457 had no CRE (448 with three or more monitoring tests). In the PT arm, 132 patients had CRE (129 with three or more monitoring tests) and 439 had no CRE (426 with three or more monitoring tests).

Patient characteristics on enrollment are shown in Table 1 and the treatment description in Table 2. These groups are defined post-hoc based on event occurrence and are therefore not randomized, but the whole monitoring arms after randomization irrespective of occurrence of CRE are shown in Supplementary table 1, which shows that no baseline differences were present. (Appendix 3) The total ITM observation time was 133 patient years in the Fiix event group, 551 in the Fiix no event group, 155 in the PT event group and 536 in the PT no event group.

**Table 1: Patient characteristics**

Patients with and without clinically relevant events[a] monitored with either Fiix-prothrombin time (Fiix arm) or prothrombin time (PT arm).

| | Fiix arm | Fiix arm | PT arm | PT arm | P-value[b] |
|---|---|---|---|---|---|
| | Event | No event | Event | No event | |
| **N (% of all in each arm)** | 115 (20) | 457 (80) | 132 (23) | 439 (77) | - |
| **Age in years - median (IQR[c])** | 75 (66-79) | 70 (63-78) | 74(67-80) | 71 (63-78) | 0.0154 |
| **Male sex – n (%)** | 71 (62) | 285 (62) | 80 (61) | 298 (68) | 0.2303 |
| Years of warfarin treatment prior to enrollment – median (IQR) | 5.1(2.5-8.9) | 3.2 (0.7-7.8) | 3.4 (0.7-8.7) | 3.4 (0.8-7.8) | 0.0080 |
| **Indication for warfarin - n (%)** | | | | | |
| **Heart disease** | | | | | |
| **Atrial fibrillation total** | 88 (76.5) | 320 (70.0) | 101 (76.5) | 328 (74.7) | 0.2352 |
| AF without prior arterial thromboembolic event | 62 (70.5) | 245 (76.6) | 71 (70.3) | 246 (75.0) | 0.8958 |
| AF with prior cerebral thromboembolic event | 26 (29.5) | 70 (21.9) | 28 (27.7) | 78 (23.8) | 0.1864 |
| AF with prior peripheral arterial embolism | 0 (0) | 5 (1.6) | 2 (2.0) | 4 (1.2) | 0.6513 |
| $CHA_2DS_2$-VASC[d] risk score in AF patients – median (IQR) | 3 (2-4) | 3 (2-4) | 3 (3-5) | 3 (2-4) | 0.0240 |
| Percent with score 0 (low TE risk) | 1.1 | 5.3 | 1.0 | 4.9 | 0.1105 |
| Percent with score 1 (moderate TE risk) | 6.8 | 9.1 | 5.9 | 12.2 | 0.1752 |
| Percent with score ≥2 (high TE risk) | 92.0 | 85.6 | 93.1 | 82.9 | 0.0073 |
| Percent with score ≥3 (high TE risk) | 73.9 | 60.3 | 75.2 | 70.0 | 0.0061 |
| **Ischemic heart disease total** | 5 (4.3) | 19 (4.2) | 6 (4.5) | 10 (2.3) | 0.3620 |
| Acute MI | 5 (100.0) | 18 (94.7) | 5 (83.3) | 10 (100.0) | - |
| Other ischemic heart disease | 0 (0.0) | 1 (5.3) | 1 (16.7) | 0 (0.0) | - |
| **Congestive heart failure as only indication** | 0 (0.0) | 1 (0.2) | 0 (0.0) | 0 (0.0) | - |
| **Atrial septal defect** | 1 (0.9) | 7 (1.5) | 0 (0.0) | 3 (0.7) | 0.3610 |
| **Artificial heart valves** | 4 (3.5) | 6 (1.3) | 4 (3.0) | 6 (1.4) | 0.2468 |
| **Rheumatic mitral valve disease** | 0 (0.0) | 1 (0.2) | 0 (0.0) | 1 (0.2) | - |
| **Arterial thromboembolism without known AF total** | 4 (3.5) | 32 (7.0) | 7 (5.3) | 26 (5.9) | 0.5293 |
| Cerebral thromboembolism or TIA | 4 (100.0) | 26 (81.3) | 7 (100) | 26 (100.0) | - |
| Peripheral arterial thromboembolism | 0 (0.0) | 6 (18.8) | 0 (0.0) | 0 (0.0) | - |
| **Venous thromboembolism total** | 28 (24.3) | 109 (23.9) | 25 (18.9) | 99 (22.6) | 0.6653 |
| Deep vein thrombosis alone | 5 (17.9 ) | 55 (50.5) | 8 (32.0) | 43 (43.4) | - |
| Pulmonary embolism | 23 (82.1) | 54 (49.5) | 17 (68.0) | 56 (56.6) | - |
| **Pulmonary hypertension** | 0 (0.0) | 2 (0.4) | 1 (0.8) | 0 (0.0) | - |
| **Associated conditions – n (%)** | | | | | |
| Smoker | 9 (7.8) | 58 (12.7) | 13 (9.8) | 48 (10.9) | 0.4553 |
| High blood pressure | 72 (62.6) | 265 (58.0) | 94 (71.2) | 250 (56.9) | 0.0223 |
| Ischemic heart disease | 36 (31.3) | 117 (25.6) | 33 (25.0) | 123 (28.0) | 0.5660 |
| Peripheral vascular disease | 10 (8.7) | 21 (4.6) | 13 (9.8) | 23 (5.2) | 0.0684 |
| History of congestive heart failure | 21 (18.3) | 51 (11.2) | 17 (12.9) | 57 (13.0) | 0.2412 |
| Diabetes | 12 (10.4) | 66 (14.4) | 16 (12.1) | 50 (11.4) | 0.4736 |
| Cancer | 20 (17.4) | 67 (14.7) | 22 (16.7) | 75 (17.1) | 0.7556 |
| -active cancer chemotherapy | 3 (2.6) | 8 (1.8) | 5 (3.8) | 9 (2.1) | 0.5474 |
| **Select drug use – n(%)** | | | | | |
| Acetylsalicylic acid | 28 (24.3) | 93 (20.4) | 30 (22.7) | 88 (20.0) | 0.7125 |
| Clopidrogel | 2 (1.7) | 10 (2.2) | 1 (0.8) | 7 (1.6) | 0.7224 |
| Non-steroidal anti-inflammatory drugs | 17 (14.8) | 43 (9.4) | 19 (14.4) | 47 (10.7) | 0.2173 |
| Amiodarone | 9 (7.8) | 44 (9.6) | 12 (9.1) | 40 (9.1) | 0.9471 |
| $H_2$ blockers and proton pump inhibitors | 21 (18.3) | 103 (22.5) | 39 (29.5) | 84 (19.1) | 0.0578 |
| Any other drugs | 113 (98.3) | 412 (90.2) | 119 (90.2) | 406 (92.5) | 0.0317 |

Percentages do not total 100 due to rounding of numbers or presence of more than one indication in some patients. [a]Major bleeding, other non-major clinically relevant bleeding and thromboembolism. [b]Kruskal-Wallis test for continuous data and Chi-square test for categorical data. P-values <0.05 are considered significant. [c]IQR denotes interquartile (25-75%) range. [d]The $CHA_2DS_2$-VASC risk score indicates the risk of thromboembolic events in AF patients, based on underlying diseases and condition.

**Table 2: Treatment description**

Patients with and without clinically relevant events[a] monitored with either Fiix-prothrombin time (Fiix arm) or prothrombin time (PT arm).

| | Fiix arm<br><br>Event | Fiix arm<br><br>No event | PT arm<br><br>Event | PT arm<br><br>No event |
|---|---|---|---|---|
| **n** | 115 | 457 | 132 | 439 |
| **Total intention-to-monitor observation years** | 133 | 551 | 155 | 536 |
| **Intention–to-monitor observation years per patient – median (IQR[b])** | 1.3 (0.7-1.6) | 1.4 (0.9-1.6) | 1.4 (0.8-1.6) | 1.4 (0.9-1.6) |
| **Non-major event related discontinuation from study – n (%)** | | | | |
| **Total** | 17 | 102 | 14 | 88 |
| Anticoagulation discontinued | 5 | 67 | 5 | 48 |
| Voluntary discontinuation | 0 | 5 | 1 | 4 |
| Switched to direct oral anticoagulant | 4 | 12 | 4 | 11 |
| INR target changed | 3 | 3 | 1 | 7 |
| Lost to follow-up | 0 | 0 | 0 | 0 |
| Other reason | 5 | 15 | 3 | 18 |

[a]Major bleeding, other non-major clinically relevant bleeding and thromboembolism. [b]IQR denotes interquartile (25-75%) range.

## 4.2. Clinically relevant events in the two study arms

In the Fiix arm 105 patients experienced CRB, 19 MB and 86 other non-major CRB. Ten patients experienced TE in the Fiix arm. In the PT arm, 113 patients experienced CRB, 21 MB and 92 other non-major CRB. Nineteen patients suffered from TE in the PT arm.

The rate of CRE were numerically reduced in the Fiix arm (p = 0.0066 for non-inferiority), especially TE events (p = 0.0002 for non-inferiority), as can be seen in Table 3.

**Table 3: Clinically relevant events in the two study arms**
Clinically relevant events in patients monitored with either Fiix-prothrombin time (Fiix arm) or prothrombin time (PT arm).

| | **Fiix arm** n (fatal) | **PT arm** n (fatal) | **P-value for non-inferiority**[a] |
|---|---|---|---|
| No clinically relevant event | 457 | 439 | - |
| All clinically relevant events | 115 | 132 | 0.0066 |
| All clinically relevant bleeding | 105 | 113 | 0.033 |
| Major bleeding | 19 (1) | 21 (3) | 0.0142 |
| Gastrointestinal | 12 | 10 | 0.0264 |
| Intracranial | 2 | 5 | 0.0002 |
| Other major bleeding | 5 | 6 | 0.0015 |
| Other non-major clinically relevant bleeding | 86 | 92 | 0.0411 |
| All thromboembolism | 10 (1) | 19 (3) | 0.0002 |
| ATE[b] | 10 (1) | 18 (3) | 0.0003 |
| VTE[c] | 0 | 1 | 0.0002 |

[a]Farrington-Manning test with non-inferiority margin 0.025. P-values <0.05 are considered significant. [b]Arterial thromboembolism. [c]Venous thromboembolism.

## 4.3. Surrogate anticoagulation indicators

Surrogate anticoagulation indicators in patients monitored with either Fiix-PT or PT in relation to presence or absence of clinically relevant events are shown in Table 4.

### 4.3.1. Test number and intervals

Patients experiencing events had significantly more frequent monitoring tests than patients with no events. In the Fiix arm the median number of monitoring tests per patient per month was 1.5 with CRE vs. 1.3 with no event ($p = 0.0180$) and 1.6 vs. 1.3 ($p < 0.0001$) in the PT arm. Consequently, patients with CRE had significantly fewer days between monitoring tests ($p = 0.0166$ in the Fiix arm and $p < 0.0001$ in the PT arm). The event groups had significantly fewer tests within therapeutic range (INR 2.0 – 3.0) than the no event groups (61.2% vs. 66.8% within the Fiix arm and 59.1% vs. 63.8% within the PT arm, $p < 0.0001$ within both arms). The event groups also had significantly more tests with INR > 3 than the no event groups, and the Fiix event group had significantly more tests with INR < 2 than the Fiix no event group. There was no significant difference between the event groups of the two study arms in any of the variables above. However, the patients in the Fiix arm who did not suffer from CRE had significantly more tests within TTR and significantly fewer tests <2 than the patients in the PT arm who did not suffer from CRE ($p < 0.0001$). The distribution of tests within each event subgroup is shown in Supplementary table 2. (Appendix 3)

### 4.3.2. Dosing

There was no significant difference in warfarin dose size between the groups within each arm; they ranged from 4.5 to 4.8 mg. The PT event group had significantly more dose changes and calculated annual dose changes, than the PT no event group ($p = 0.0002$ and $p = 0.0017$, respectively). There was a significant difference between the two groups within each arm in dose fluctuation (calculated with VGR formula B1), 0.18 vs. 0.03 within Fiix arm and 0.12 vs. 0.03 within PT arm ($p < 0.0001$ within both arms). There was no significant difference between the two study arms.

### 4.3.3. Time within target range

As shown in Table 4, the total CRE groups had significantly lower TTR than the no event groups within each arm (79% vs 82%, $p = 0.0441$ in Fiix arm and 75% vs 80%, $p = 0.0004$ in PT arm). In the Fix arm, patients with CRB had significantly lower TTR than that observed in patients without events. In the PT arm all clinical event subgroups had lower TTR than the no event patients.

Overall, the Fiix arm spent more time within target range than the PT arm. When the two arms were compared, the TTR was a numerically higher in all the Fiix subgroups than in the PT arm. However, the difference was only significant between the total event groups of the two study arms ($p = 0.0405$).

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**Table 4: Surrogate anticoagulation indicators**
Warfarin patients monitored with Fiix-prothrombin time (Fiix arm) or prothrombin time (PT arm) in relation to presence or absence of clinically relevant events[a].

| | **Fiix arm Event** | **Fiix arm No event** | **P-value[b]** Within Fiix arm | **PT arm Event** | **PT arm No event** | **P-value** Within PT arm | **P-value** Fiix event vs. PT event | **P-value** Fiix no event vs. PT no event |
|---|---|---|---|---|---|---|---|---|
| **Test number and intervals** | | | | | | | | |
| Number of monitoring tests –n | 2382 | 8644 | - | 2925 | 8574 | - | - | - |
| Number of monitoring tests per patient – median (IQR[c]) | 20 (12-27) | 18 (13-25) | 0.1793 | 23 (14-29) | 18 (12-25) | 0.0036 | 0.1966 | 0.6321 |
| Number of tests within defined Fiix-INR or INR ranges | | | | | | | | |
| 2-3 – n (%) | 1457 (61.2) | 5770 (66.8) | <0.0001 | 1728 (59.1) | 5468 (63.8) | <0.0001 | 0.1291 | <0.0001 |
| <2 – n (%) | 515 (21.6) | 1581 (18.3) | 0.0003 | 648 (22.2) | 1804 (21.0) | 0.2137 | 0.6644 | <0.0001 |
| >3 – n (%) | 410 (17.2) | 1293 (15.0) | 0.0077 | 549 (18.8) | 1302 (15.2) | <0.0001 | 0.1527 | 0.6927 |
| Number of observation days per patient – median (IQR) | 487 (270-569) | 508 (325-584) | 0.3894 | 508 (287-574) | 508 (323-579) | 0.4562 | 0.8137 | 0.9507 |
| Test frequency rate (tests per patient per month) – median (IQR) | 1.5 (1.1-2.0) | 1.3 (0.9-1.8) | 0.0180 | 1.6 (1.2-2.1) | 1.3 (0.9-1.7) | <0.0001 | 0.1707 | 0.5328 |
| Days between monitoring tests in each patient – median (IQR) | 20.9 (15.4-27.9) | 23.3 (17.2-32.0) | 0.0166 | 19.2 (14.6-25.4) | 24.2 (17.9-32.4) | <0.0001 | 0.1586 | 0.5347 |
| **Dosing** | | | | | | | | |
| Daily warfarin dose in mg – median (IQR) | 4.5 (3.2-6.1) | 4.8 (3.4-6.5) | 0.2521 | 4.5 (3.3-6.3) | 4.7 (3.4-6.4) | 0.4942 | 0.6931 | 0.9025 |
| Number of dose changes in each patient per year – median (IQR) | 6.7 (3.5-10.9) | 5.3 (2.9-9.2) | 0.1116 | 7.9 (5.1-12.6) | 5.8 (3.1-9.9) | 0.0002 | 0.0540 | 0.3671 |
| Calculated annual dose changes per monitoring test in each patient – median (IQR) | 0.32 (0.19-0.46) | 0.26 (0.17-0.43) | 0.0987 | 0.33 (0.24-0.52) | 0.28 (0.19-0.43) | 0.0017 | 0.1989 | 0.3561 |
| Between dose variability (VGR-B1[d]) | 0.18 (0.01-2.24) | 0.03 (0.01-0.40) | <0.0001 | 0.12 (0.02-1.46) | 0.03 (0.01-0.20) | <0.0001 | 0.7423 | 0.8351 |
| **Percent time within target range** | | | | | | | | |
| Any clinically relevant major vascular event – median (IQR) | 79 (67-86) | 82 (71-91) | 0.0441 | 75 (62-84) | 80 (69-89) | 0.0004 | 0.0405 | 0.0805 |
| - TTR total clinically relevant bleeding | 79 (67-85) | - | 0.0405 | 76 (64-84) | - | 0.0079 | 0.1697 | - |
| - TTR major bleeding only | 76 (55-85) | - | 0.1528 | 73 (39-84) | - | 0.0295 | 0.4857 | - |
| - TTR other non-major clinically relevant bleeding | 79 (67-86) | - | 0.0923 | 76 (65-85) | - | 0.0402 | 0.2373 | - |
| - TTR thromboembolism only | 80 (57-94) | - | 0.8017 | 62 (56-80) | - | 0.0007 | 0.1427 | - |

[a]Major bleeding, other non-major clinically relevant bleeding and thromboembolism. [b]Mann-Whitney test for continuous data and Fisher's exact or Chi-square test for categorical data. P-values <0.05 are considered significant. [c]IQR denotes interquartile (25-75%) range. [d]Variance growth rate formula B1.

## 4.4. Fluctuation of anticoagulation

When calculated with formula A (fluctuation around mid-target INR), the VGR was always significantly higher in the event groups than in the no event group, within the PT arm. There was one exception in the Fiix arm, where there was not a significant difference between the TE group and the no event group. When formula B1 (fluctuation between adjacent tests) was used to calculate the VGR, it was significantly higher in the total event groups than in the no event groups, within each arm. All event subgroups, except Fiix TE and, surprisingly, PT MB also had significantly higher fluctuation than the no event groups. There was a significant difference in INR fluctuation between the TE groups of the two arms (0.20 vs. 0.50, p = 0.0051) as well as between the no event groups (p = 0.0146). The results are shown in Table 5.

**Table 5: Fluctuation of anticoagulation**

Fluctuation measured as INR variance growth rate (INR-VGR) in patients with and without clinically relevant bleeding events or thromboembolism, monitored with either Fiix-prothrombin time (Fiix arm) or prothrombin time (PT arm)

| | **Fiix arm Event** | **Fiix arm No event** | **P-value[a] Within Fiix arm** | **PT arm Event** | **PT arm No event** | **P-value Within PT arm** | **P-value** Fiix event vs. PT event | **P-value** Fiix no event vs. PT no event |
|---|---|---|---|---|---|---|---|---|
| **INR fluctuation around mid-target INR[b] (VGR-A[c]) – median (IQR[d])** | 0.23 (0.10-0.64) | 0.12 (0.06-0.34) | 0.0001 | 0.27 (0.12-0.71) | 0.15 (0.06-0.36) | <0.0001 | 0.2628 | 0.2913 |
| - All clinically relevant bleeding | 0.23 (0.10-0.63) | - | 0.0001 | 0.25 (0.12-0.68) | - | 0.0003 | 0.7006 | - |
| - Major bleeding only | 0.46 (0.14-0.89) | - | 0.0030 | 0.29 (0.12-0.87) | - | 0.0359 | 0.7513 | - |
| - Other clinically relevant bleeding | 0.23 (0.09-0.62) | - | 0.0022 | 0.24 (0.10-0.57) | - | 0.0014 | 0.5614 | - |
| - Thromboembolism | 0.13 (0.08-0.84) | - | 0.4621 | 0.54 (0.26-0.86) | - | 0.0001 | 0.0849 | - |
| **INR fluctuation between tests (VGR-B1[e]) – median (IQR)** | 0.23 (0.12-0.54) | 0.17 (0.08-0.38) | 0.0041 | 0.35 (0.14-0.77) | 0.21 (0.09-0.52) | 0.0011 | 0.0643 | 0.0146 |
| - All clinically relevant bleeding | 0.24 (0.13-0.55) | - | 0.0018 | 0.31 (0.12-0.77) | - | 0.0115 | 0.3180 | - |
| - Major bleeding only | 0.31 (0.15-0.97) | - | 0.0154 | 0.59 (0.07-1.36) | - | 0.1547 | 0.9747 | - |
| - Other clinically relevant bleeding | 0.23 (0.12-0.53) | - | 0.0134 | 0.30 (0.13-0.71) | - | 0.0269 | 0.2821 | - |
| - Thromboembolism | 0.20 (0.07-0.26) | - | 0.6843 | 0.50 (0.27-0.90) | - | 0.0029 | 0.0051 | - |

[a]Mann-Whitney test for continuous data. P-values <0.05 are considered significant. [b]INR 2.5. [c]Variance growth rate formula A. [d]IQR denotes interquartile (25-75%) range. [e]Variance growth rate formula B1.

## 4.4. Anticoagulation at the time of major events

Anticoagulation intensity (INR) at the time of major events (MB and TE) is shown in Figure 6. The median INR value in the Fiix MB group was 3.2 vs. 2.5 in the PT MB group (p = 0.5601).

There was a less difference between the two study arms regarding TE. The median INR at the time of TE event was slightly under the target therapeutic range in both groups, the Fiix TE group had a median INR value of 1.8 and the PT TE group 1.9 (p = 0.6128).

The event groups varied from the no event groups, within both arms. The median INR value of the average anticoagulation in the Fiix no event group was 2.5. There was a significant difference between the Fiix MB group and the Fiix no event group (p = 0.0467) as well as between the Fiix TE group and the Fiix no event group (p = 0.0024). The median INR value of the average anticoagulation in the PT no event group was also 2.5. There was no significant difference between the PT MB group and the PT no event group (p = 0.5123), but there was a significant difference between the PT TE group and the PT no event group (p = 0.0059).

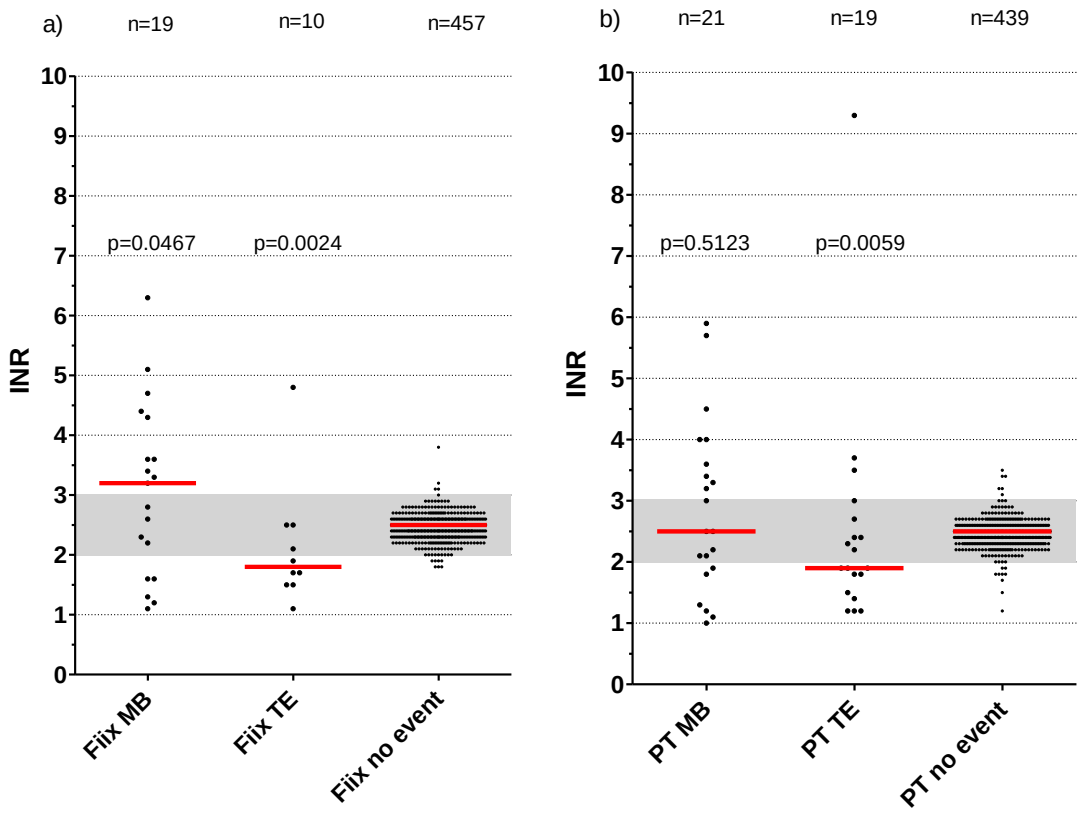


**Figure 5: INR at the time of major events**
The INR values at the time of major bleedings and thromboembolism compared with the average anticoagulation of each patient in the no event groups. MB: major bleeding, TE: thromboembolic event. The dotplots display the distribution of the values, the red line is the median, and the gray zone is the therapeutic target range. N is the number of patients in each group. P-values are shown compared to the no event groups. P-values <0.05 are considered significant. a) Fiix arm. b) PT arm.

# 5. Discussion

## 5.1. Main findings

The current study not only confirmed that patients on warfarin with clinically relevant hemorrhagic or thromboembolic vascular events (CRE) had reduced anticoagulation stability and increased need for dose changes than those without such events, but also found that the overall anticoagulation stability was improved when PT monitoring was replaced with Fiix-PT monitoring. Moreover, the lowest anticoagulation intensity and highest INR fluctuation was observed in patients monitored with the PT-INR that had suffered from TE. This is in agreement with the previous finding that the long-term thromboembolism incidence was significantly reduced by over 50% in the more stable Fiix-PT arm.[47] Taken together, the findings confirm the hypothesis that the Fiix-PT monitoring improves both the stability and clinical outcome of warfarin treatment.

### 5.1.1. Anticoagulation intensity and variability

The current study results show that patients who experience CRE have more frequent monitoring tests and shorter intervals between monitoring tests than patients who don't experience events. They also have fewer tests within target therapeutic range of INR 2.0 - 3.0 and spend less time in that range than patients with no events. Furthermore, patients experiencing events also have more frequent dose changes and larger dose changes. All these findings indicate less stability of anticoagulation in the CRE groups and more difficulty adjusting the warfarin dose.

It is of course not surprising that patients with CRE had significantly fewer tests within therapeutic range than those without events. Too low INR (<2) leads to insufficient antithrombotic effect and therefore increases the risk of thromboembolism. Likewise, an INR too high (>4) indicates excessive anticoagulation and therefore increases the risk of bleeding.[1,50] However, in the Fiix-PT arm there were fewer tests with low INR than in the control arm and this coincided with the apparent 50% reduced long-term incidence of TE.[47] This supports the hypothesis that the difference in the two monitoring tests influences the difference in TE rates.

The test frequency rate and days between monitoring tests correspond to each other. The fact that patients in the CRE groups had more frequent monitoring tests, shows that their anticoagulation was less stable than in those without CRE. Patients with unstable anticoagulation need to have more monitoring tests in attempt to get them into the therapeutic range.

Because there was only a significant difference in number of dose changes and calculated annual dose changes within the PT arm and not the Fiix arm, it indicates that the PT event group had more unstable anticoagulation than the no event group, and that the difference in stability was greater than within the Fiix arm. The Fiix event group did not have significantly more dose changes than the no event group, but it had significantly larger dose changes. The fact that there was no difference in dose size between groups suggests that the median drug dose does not have a predictive value for events by itself, but rather the dose fluctuation does. The fluctuation in doses being significantly higher in patients suffering from events suggests that patients with high dose fluctuation should be identified and subsequently better monitored.

The overall bleeding rates in the Fiix-trial were low, compared to bleeding rates in most DOACs studies,[42-45] but similar in both the trial arms, reflecting the high TTR in both study arms.[47] As previously described the TTR is an important tool to assess the control and intensity of anticoagulation therapy. Most studies of anticoagulation control in AF patients report TTR or percentage of INRs in range, and those outcomes can be used to predict adverse events. A 7% improvement in TTR has been found to reduce the rate of major hemorrhage by 1 event per 100 patient years and 12% improvement to reduce the rate of TE to the same extent.[50] In a study conducted in Iceland in 2007 the TTR of INR 2.0 - 3.0 was 81% for patients with AF and 84% for patients with venous thromboembolism in a per protocol analysis.[33] That is higher than that observed in most other countries.[25] In the current study the median TTR of the Fiix no event group was 82% and 80% in the PT no event group in an ITM analysis (i.e. including periods of warfarin discontinuation). That is consistent with the best results of reported TTR from dose management centers in Northwestern Europe.[51] As a low TTR can be a predictor of clinical events, it is no surprise that the TTR in the event groups was lower than in the no event groups and that the Fiix-PT improved the TTR, compared with PT. Interestingly, the PT thromboembolism group had TTR of 62%, similar to that observed in control patients in studies on new direct oral anticoagulants.[41,46] This is associated with a doubling of TE incidence in the PT group compared to the Fiix group.

### 5.1.2. Fluctuation of anticoagulation

The variability or fluctuation of VKA anticoagulation can be measured with the VGR method. As described earlier in a retrospective study, the VGR is strongly associated with clinical events.[26] Therefore, a greater fluctuation in the event groups than in the no event groups is consistent with previous findings and it is believed to be shown for the first time in a prospective manner in the current study. As mentioned above the Fiix-PT reduced the long-term TE rate greatly in the Fiix-trial, but the bleeding incidence was similar in the two arms.[47] The significantly greater fluctuation in the PT TE group than in the Fiix TE group is in an agreement with those findings. It is also interesting that the Fiix TE group, which had only a 1.1% annual TE incidence, was the only group in the Fiix arm that did not have significantly greater fluctuation than the no event group, but the PT TE group had greater fluctuation than the PT no event group. That could imply that a certain number of TE will always occur, no matter how well the anticoagulation is monitored.

### 5.1.3. Anticoagulation at the time of events

The INR at the time of a major vascular event can be informative. When an anticoagulated patient has a major event, a blood test is supposed to be done to find out whether the anticoagulation could be the reason for the event. Based on previous knowledge clinically relevant bleeding is more likely to occur in patients with INR above the therapeutic range, and TE in patients with INR below the therapeutic range but CRE of course also occur at therapeutic levels.[1]

In this study, an elevated Fiix-INR appeared to indicate hemorrhagic risk better than the PT-INR did whereas low Fiix-INR and low PT-INR (<2) appeared to indicate TE risk equally, although the small number of events must be kept in mind. Both of the TE groups had median INR values lower than the therapeutic target range. Studies suggest that there is only a brief warning period during

which a slightly elevated INR can predict a bleeding event[52] and that excess mortality is associated with high INR values.[53] Only the Fiix MB group had median value higher than the therapeutic range. It may suggests that the Fiix-INR better predicts hemorrhagic events than the PT, possibly as it is not confounded by the influence of low FVII that raises the INR but has little influence on the hemorrhagic risk.

## 5.2. Study strength and limitations

This study is a subgroup analysis of a single center prospective randomized controlled clinical trial. The participants were blinded to which monitoring arm of warfarin they belonged. Each participant was assigned a study-code and all blood samples were color-coded. The protocol manager managed all information about the patients as well as listing the results of the monitoring tests before reporting it to the dosing staff. The dosing staff was also blinded to which group each patient belonged, thus eliminating experimental bias. Also, the assessment of clinical events was by a blinded adjudication committee.[47] The analysis in this study was based on ITM analysis, as previously described. Events occurring when warfarin was temporarily halted are counted in.

As the subgroups compared in this study are defined post-hoc some differences in patient characteristics are observed and they may point to clinical patient risk factors that influence clinical outcome. The limited size of the study does not allow further subgroup analysis of clinical outcome but it is evident that the patients in the event groups were older and had higher blood pressure than the no event groups. They also, understandably, had higher $CHA_2DS_2$-VASC risk score, a score that indicates the risk of thromboembolic events in AF patients, based on underlying diseases and condition. Moreover, the Fiix event group had more other drug use than the other groups. A study, conducted to evaluate the risk factors for hemorrhage and thromboembolism during long-term anticoagulation therapy found, in contrast to our findings, that malignant disease was associated with hemorrhage and thromboembolism, but age, sex, hypertension, AF and stroke were not significantly associated with major hemorrhage.[54] Interestingly, the patients in the Fiix event group had been longer than the other groups on warfarin treatment prior to enrollment, but previous studies have found the rate of events to be highest during the initiation of therapy.[55]

As mentioned above, a calibration problem occurred during a 3.5-month period during the Fiix-trial led to the Fiix-INR being reported out erroneously high, which may have led to unnecessary dose reductions in the Fiix arm.[47] Excluding this period in the current analysis takes away not only many monitoring tests, but also five patients. However, keeping this period in the current analysis of surrogate outcome parameters in relation to CRE would have given a wrong image of the difference between the two arms.[47]

It is important to note that monitoring and managing warfarin with Fiix-PT was based on a protocol and a dosing software algorithm that is designed for the highly variable PT-INR. This includes a limitation to the testing interval length. Despite that, PT-INR monitoring was consistently less stable by multiple parameters.

The population of the Fiix-trial was small considering multicentric DOACs studies, and the subgroups in this study were therefore even smaller. To get a better view of the true effect of the Fiix-PT over PT and greater statistical power, a larger multicentric study should ideally be conducted.

However, the single center design also has benefits, such as totally identical management of both arms, except for the active arm being monitored using a test that is not affected by factor VII in the test samples.

## 5.3. Conclusions

Patients on warfarin, who suffered vascular events, had increased anticoagulation variability. Also, PT monitoring of warfarin was associated with higher variability of anticoagulation than monitoring with Fiix-PT. Monitoring warfarin with the Fiix-PT and paying particular attention to patients demonstrating anticoagulation instability could, therefore, improve the clinical outcome of patients on warfarin.

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# Appendix 1

## Segavarnir – stuttar leiðbeiningar (12/10/2010)

**Stefna skal að því, að að ná meðferðarmarkmiðum á 7-10 dögum og ekki lengri tíma en 10-15 dögum.**

Hjá sjúklingum, sem eru að hefja meðferð með warfaríni er ráðlagt að hefja mælingar á INR eftir 3 daglega skammta. Mæla skal INR x3 fyrstu vikuna, x2 í annarri viku og síðan vikulega þar til INR er orðið stabilt. Ráðlagður upphafsskammtur warfaríns hjá sjúklingum yngri en 65-70 ára eru 6 mg daglega í 3 daga. Næsta dag skal mæla INR og áframhaldandi skammtar ráðast af því hvernig INR bregst við. Sé lítil eða engin svörun skal auka skammt í 9 mg daglega (eða um 50%) og mæla að nýju eftir 2-3 daga.

Ráðlagður upphafsskammtur hjá öldruðum, veikburða, vannærðum, hjartabiluðum, lifrarsjúklingum, þeim sem nýlega hafa gengist undir stórar skurðaðgerðir eða hjá þeim, sem taka lyf, sem vitað er að auka næmi fyrir warfaríni (t.d. amiodarone, sýklalyf) er 3 daglegir 4 mg skammtar. Áframhaldandi skammtar ráðast af því hvernig INR bregst við (mælt á 4. degi). Sé lítil eða engin svörun skal auka skammt í 6 mg (50%) og mæla að nýju eftir 2-3 daga (ACCP guidelines, 8, útgáfa, Chest 2008;133;160-198).

**Ef hækkun verður mikil strax – minnka skammt um 50% og mæla aftur eftir 2 – 3 daga (ekki viðurkennd regla).**

Sé sjúklingur á heparíni eða LMWH (Klexan, Fragmin) samhliða warfaríni má hætta notkun heparíns 2 – 3 dögum eftir að INR hefur sannanlega verið > 2 (eftir að INR hefur mælst >2 tvisvar sinnum með 2-3 daga millibili).

Mælt er með því að INR mælingar hjá sjúklingum, sem taka stöðugan skammt af warfaríni séu að jafnaði ekki sjaldnar en á fjögurra til sex vikna fresti (ACCP guidelines, 8, útgáfa, Chest 2008;133;160-198).

**Minnkun warfarínsskammts hjá sjúklingum sem fara til útlanda (breytt loftslag). Dæmi um minnkun: 4 mg minnka í 3,72 mg (7,5%), 4,40 mg minnka í 4 mg (10%) (ekki viðurkennd regla).**

**Hjá sjúklingi með INR yfir sett meðferðarmörk en < 5,0 og engin merki um blæðingu** er ráðlagt að minnka skammt eða sleppa 1-2 skömmtum og mæla INR oftar og halda síðan áfram meðferð með viðeigandi skömmtum þegar INR er komið niður í meðferðarmörk. Ef hækkun á INR er aðeins lítilsháttar (u.þ.b. 0,5-1.0) upp fyrir meðferðarmörk og hækkunin tengist augljósum orsökum er ekki ráðlagt að breyta skammti. Ef INR er lækkað eða hækkað (+/- 0,5) láta standa óbreytt í eitt skipti.

**Hjá sjúklingum með INR > 5,0 en < 9,0 og engin merki um blæðingu** er ráðlagt að stoppa warfarínmeðferð í 2-3 daga, mæla INR oft, og halda síðan meðferð áfram með viðeigandi skömmtum þegar INR er komið niður í meðferðarmörk (ACCP guidelines, 8, útgáfa, Chest 2008;133;160-198).

## Lækkun á Kóvarskammti  þegar INR hækkað

INR <5 >4 -  minnka skammt næsta dag (frá 50% háð því hversu mikil blæðingahætta sj. er) og halda síðan áfram með 10 – 20% minni skammti. Mæla INR innan sjö daga.
INR > 5 - ekkert Kóvar í einn dag og minnka skammt um 15 - 20%. Mæla INR e. viku.
INR > 6 - ekkert Kóvar í tvo daga og minnka skammt um 20 - 30%. Mæla INR e. 5 – 7 daga.
INR > 10 - ekkert Kóvar í þrjá daga. Mæla INR eftir þrjá daga. Kóvarskammtur minnkaður um 30% - 50 % þegar INR hefur lækkað í meðferðargildi (unnið af starfsfólki Segavarna).

**Segavörnum ber sérstaklega að fylgjast með hemoglóbíni og MCV og láta vakthafandi lækna sannanlega yfirfara svör ekki síðar en í lok dags**:

Hemoglóbínlækkun > 25 g/L eða ef ný anemia er komin fram (Hb <108 hjá konum eða < 120 hjá körlum).

MCV lækkun > 5 fl eða MCV < 80 fl.

(Leiðbeiningar fyrir lækna, lífeindafræðinga og hjúkrunarfræðinga um eftirlit segavarna LSH á blóðþynntum sjúklingum (sept. 2008)).

# Appendix 2

## Variance growth rate formulas

Following formulas were used to calculate the VGR.[26]

Variance growth rate Fihn (method A)

$$\sigma^2 = \frac{1}{n}\sum_{i=1}^{n}\frac{(\mathrm{INR}_i - \mathrm{target}_i)^2}{\tau_i}$$

Variance growth rate Cannegieter (method B1)

$$\sigma^2 = \frac{1}{n}\sum_{i=1}^{n}\frac{(\mathrm{INR}_{i+1} - \mathrm{INR}_i)^2}{\tau_{i,i+1}}$$

# Appendix 3

## Supplementary tables

**Supplementary table 1: Patient characteristics after enrollment**

Patients monitored with either Fiix-prothrombin time (Fiix arm) or prothrombin time (PT arm). Results of randomization.

| | Fiix arm | PT arm | P-value[a] |
|---|---|---|---|
| **N (% of all in each arm)** | 572 | 571 | |
| **Age in years - median (IQR[b])** | 71 (64-78) | 72 (64-79) | 0.3403 |
| **Male sex – n (%)** | 356 (62) | 378 (66) | 0.1954 |
| Years of warfarin treatment prior to enrollment – median (IQR) | 3.7 (0.9-8.2) | 3.4 (0.8-7.8) | 0.4660 |
| **Indication for warfarin - n (%)** | | | |
| **Heart disease** | | | |
| **Atrial fibrillation total** | 408 (71.3) | 429 (75.1) | 0.1607 |
| AF without prior arterial thromboembolic event | 307 (75.2) | 317 (73.9) | 0.5526 |
| AF with prior cerebral thromboembolic event | 96 (23.5) | 106(24.7) | 0.4391 |
| AF with prior peripheral arterial embolism | 5 (1.2) | 6 (1.4) | 0.7732 |
| $CHA_2DS_2$-VASC risk score in AF patients – median (IQR) | 3 (2-4) | 3 (2-4) | 0.7894 |
| Percent with score 0 (low TE risk) | 4.4 | 4.0 | 0.8632 |
| Percent with score 1 (moderate TE risk) | 8.6 | 10.7 | 0.3495 |
| Percent with score ≥2 (high TE risk) | 87.0 | 85.3 | 0.4856 |
| Percent with score ≥3 (high TE risk) | 63.2 | 64.3 | 0.7736 |
| **Ischemic heart disease total** | 24 (4.2) | 16 (2.8) | 0.2597 |
| Acute MI | 23 (95.8) | 15 (93.8) | - |
| Other IHD | 1 (4.2) | 1 (6.3) | - |
| **Congestive heart failure as only indication** | 1 (0.2) | 0 (0.0) | 1.0000 |
| **Atrial septal defect** | 8 (1.4) | 3 (0.5) | 0.2243 |
| **Artificial heart valves** | 10 (1.7) | 10 (1.8) | 1.0000 |
| **Rheumatic mitral valve disease** | 1 (0.2) | 1 (0.2) | 1.0000 |
| **Arterial thromboembolism without known AF total** | 36 (6.3) | 33 (5.8) | 0.8040 |
| Cerebral thromboembolism or TIA | 30 (83.3) | 33 (100.0) | - |
| Peripheral arterial thromboembolism | 6 (16.7) | 0 (0.0) | - |
| **Venous thromboembolism total** | 137 (24.0) | 124 (21.7) | 0.3979 |
| Deep vein thrombosis alone | 60 (43.8) | 51 (41.1) | - |
| Pulmonary embolism | 77 (56.2) | 73 (58.9) | - |
| **Pulmonary hypertension** | 2 (0.3) | 1 (0.2) | 1.0000 |
| **Associated conditions – n (%)** | | | |
| Smoker | 67 (11.7) | 61 (10.7) | 0.6392 |
| High blood pressure | 337 (58.9) | 344 (60.2) | 0.7178 |
| Ischemic heart disease | 153 (26.7) | 156 (27.3) | 0.8941 |
| Peripheral vascular disease | 31 (5.4) | 36 (6.3) | 0.6148 |
| History of congestive heart failure | 72 (12.6) | 74 (13.0) | 0.8598 |
| Diabetes | 78 (13.6) | 66 (11.6) | 0.3269 |
| Cancer | 87 (15.2) | 97 (17.0) | 0.4220 |
| -active cancer chemotherapy | 11 (1.9) | 14 (2.5) | 0.5526 |
| **Select drug use – n(%)** | | | |
| Acetylsalicylic acid | 121 (21.2) | 118 (20.7) | 0.8844 |
| Clopidrogel | 12 (2.1) | 8 (1.4) | 0.4996 |
| Non-steroidal anti-inflammatory drugs | 60 (10.5) | 66 (11.6) | 0.5724 |
| Amiodarone | 53 (9.3) | 52 (9.1) | 1.0000 |
| $H_2$ blockers and proton pump inhibitors | 124 (21.7) | 123 (21.5) | 1.0000 |
| Any other drugs | 525 (91.8) | 525 (91.9) | 1.0000 |

Percentages do not total 100 due to rounding of numbers or presence of more than one indication in some patients. [a]Mann-Whitney test for continuous data and Fisher's exact test for categorical data. P-values <0.05 are considered significant. [b]IQR denotes interquartile (25-75%) range.

**Supplementary table 2: Number of tests within defined INR ranges**

Number of tests within defined INR ranges (%) for different types of clinical events[a]

| | Fiix arm MB | Fiix arm non-major CRB | Fiix arm TE | Fiix arm no event | PT arm MB | PT arm non-major CRB | PT arm TE | PT arm no event |
|---|---|---|---|---|---|---|---|---|
| **INR 2 - 3** | 154 (54) | 1226 (62) | 77 (61) | 5770 (66.8) | 179 (56) | 1368 (60) | 181 (55) | 5468 (63.8) |
| **INR <2** | 65 (23) | 418 (21) | 32 (25) | 1581 (18.3) | 73 (23) | 498 (22) | 77 (23) | 1804 (21.0) |
| **INR >3** | 66 (23) | 326 (17) | 18 (14) | 1293 (15.0) | 66 (21) | 409 (18) | 74 (22) | 1302 (15.2) |

[a]Thromboembolism and clinically relevant bleeding. MB: major bleeding, TE: thromboembolism, NMCRB: non-major clinically relevant bleeding