



Age and gender differences during long-term warfarin anticoagulation monitored with Fiix-prothrombin time or prothrombin time in patients with atrial fibrillation

The Fiix-trial

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**Munurinn á langtíma warfarin blóðþynningarmeðferð
stýrðri með Fiix-próþrombín tíma eða próþrombín tíma
skipt eftir aldri og kyni sjúklinga með gáttatif**

Fiix-rannsóknin

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Ritgerð þessi er til B.S. gráðu í læknisfræði og er óheimilt að afrita ritgerðina á nokkurn hátt nema með leyfi rétthafa.

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Abstract

Age and gender differences during long-term warfarin anticoagulation monitored with Fiix-prothrombin time or prothrombin time in patients with atrial fibrillation.

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Introduction: The prothrombin time (PT, PT-INR) during monitoring of warfarin measures its influence on the activity of coagulation factors (F) II, VII and X. Rapid fluctuations occur in the PT-INR due to FVII's short half-life but these fluctuations have little effect on the antithrombotic activity of warfarin which is mainly induced by FII and possibly FX. The new Fiix-PT measures only the activity of the longer half-life FII and FX. The Fiix-trial showed that monitoring with Fiix-PT lead to less variability of anticoagulation than monitoring with PT. The objective of this study was to compare if stability of anticoagulation monitored by Quick-PT and Fiix-PT was affected differently by age and gender.

Methods and materials: This study is a part of the prospective, randomized controlled and double-blinded Fiix-trial. Patients were randomized to dosing based on Fiix-PT (Fiix-INR) monitoring (the active Fiix arm) and PT (PT-INR) monitoring (control PT arm). The participants were outpatients over 18 years old with INR 2-3 as a therapeutic range. The current subgroup study analyzed anticoagulation indicators in 815 atrial fibrillation (AF) patients on long-term warfarin therapy. The main outcome parameters were time in therapeutic range (TTR), INR variance growth rate (VGR), frequency of INR monitoring, fraction of tests in defined target INR range and number of dose adjustments.

Results: Patients in the Fiix-PT arm had 65.5% of tests in target range compared to 62.9% in the PT arm ($p = 0.0019$) and they had proportionally fewer tests with $\text{INR} < 2$ (18.9% vs 20.9%, respectively, $p = 0.0061$). There were fewer dose changes per monitoring test in the Fiix-PT arm than in the PT arm (0.26 vs 0.28, $p = 0.0428$). Males in the Fiix-PT group and PT group had 0.26 vs 0.27 median dose changes per monitoring test ($p = 0.2707$). Females in the Fiix-PT group and PT group had 0.27 and 0.32 dose changes per monitoring test ($p = 0.0292$). Females in the Fiix-PT group had 64% of tests in target range and females in the PT group had 59% ($p = 0.0001$). Females in the Fiix-PT group had 20% of tests with $\text{INR} < 2$ and females in the PT group had 24% ($p = 0.0002$). The median TTR in females in the Fiix-PT group was 80.3% compared to 75.3% in females in the PT group ($p = 0.0401$) but the median TTR in males was 80.7% vs 80.3% (n.s.). Females in the PT group had higher median daily warfarin dose than females in the Fiix-PT group, 4.2 mg vs 3.4 mg ($p = 0.0029$). Both the Fiix-PT and the PT-INR variability (VGR) were significantly higher in females than in males. There was a significant difference between the TTR (median) in the Fiix-PT and PT groups in 66-80 years old patients and in 66-74 years old patients in dose changes per test and VGR. The daily dose decreased with increasing age in both groups.

Conclusion: The Fiix-PT test improved the stability of warfarin in females. Females on warfarin monitored with the Fiix-INR had significantly higher TTR and their dose-adjustment need was reduced. Anticoagulation variability (VGR) was higher in females than in males in both groups.

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Abbreviations

ACCP	American College of Chest Physicians
ADR	Adverse drug reaction
AF	Atrial fibrillation
AMC	Anticoagulation monitor center
ATE	Arterial thromboembolism
CRE	Clinically relevant event
DOAC	Direct oral anticoagulants
F	Factor
GI	Gastrointestinal
INR	International normalized ratio
IQR	Interquartile range
ISI	International sensitivity index
ITM	Intention to monitor
NSAID	Nonsteroidal anti-inflammatory drug
PPC	Per protocol
PT	Prothrombin time
TE	Thromboembolic
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TTR	Time in therapeutic range
VGR	Variance growth rate
VKA	Vitamin K antagonist
VKD	Vitamin K-dependent
VKORCI	Vitamin K oxide reductase complex I
VTE	Venous thromboembolism
vWF	von Willebrand factor

1. Introduction

Oral anticoagulation therapy is applied to reduce the risk of cardiovascular and venous thrombosis.¹ The most common indication is non-valvular atrial fibrillation (AF).² The prevalence of AF increases with age and this is associated with a five-fold increase in the risk of cerebral infarction.³ The most common therapy to prevent embolic events in patients with AF is the oral anticoagulation drug warfarin that is a vitamin K antagonist (VKA) although new direct oral anticoagulants (DOAC) are now increasingly used.^{4,5} Studies have shown the risk reduction of cerebral infarction to be 68% in patients with AF who are treated with warfarin.⁶ This reduction in stroke frequency, however, comes at the cost of increased risk of severe bleeding, partly due to a narrow therapeutic window of warfarin.⁷ There is also a variation in response to the same dosages between individuals, due to environmental and genetic factors.⁸ Good surveillance of the dosages is therefore essential to minimize the risk of events.

1.1 The coagulation system

The coagulation system is important to repair and minimize the potential consequences of many pathological conditions, especially those that involve bleeding. The complex interaction between coagulation factors, natural anticoagulants, the fibrinolytic system, platelets and the vessel wall forms the haemostatic balance, i.e. the homeostatic mechanism responsible for maintaining the vessel wall intact. During non-pathological conditions, thrombus generation in the body is normally inhibited by antithrombogenic factors like heparin, neutral phospholipids, platelet inhibitors, coagulation inhibitors and fibrinolysis activators, which are present in the blood and endothelium. Collagen, Von Willebrand factor (vWF) and proteins, involved in platelet adhesion, are thrombogenic factors found in the subendothelial layer.⁹

The activation of the system in the body is often associated with rupture in the endothelium of the vessel wall. vWf is then released and it connects the platelets to the subendothelium which causes platelet adhesion to the vessel wall.¹⁰ This adhesion of platelets is called primary haemostasis.⁹ Thereafter activation in the platelet occurs and they secrete granules which initiates secondary haemostasis.¹¹ That includes activation of certain coagulation factors which initiates a sequence of chemical reactions between coagulation proteins. Previous models of the coagulation system assumed two separate pathways in the coagulation cascade, extrinsic and intrinsic pathways, which initiate a common pathway that leads to the cleavage of fibrinogen to form fibrin.¹² Present-day understanding, however, assume that these pathways are not separate but that the extrinsic pathway is the primary initiator of thrombin generation (the final result of this cascade) that activates coagulation factors and platelets to form enzyme complexes on activated platelet surfaces that lead to the formation of fibrin (the cell base coagulation theory).¹³

1.1.1 The coagulation factors and their interaction

Hepatic parenchyma cells produce most of the anticoagulants and precursors of the coagulation factors, but most of the procoagulants are released into the blood as inactive zymogens, which are precursors of proteolytic enzymes.^{14,15}

Various triggers can activate the coagulation factors, one example is tissue factor (TF), a proteolytic glycoprotein in the cell membranes of smooth muscle cells and fibroblasts in the subendothelium of the vessel wall.¹⁶ Damage to the endothelium releases TF to the blood which then initiates the extrinsic pathway of the coagulation cascade.¹⁷ TF forms a complex with factor (F) VIIa in the blood which binds to calcium and initially results in activation of FX which using FVa as a cofactor, cleaves prothrombin (FII) to active thrombin (FIIa).^{18,19} This thrombin in turn by a positive feedback mechanism activates FXI and the cofactors FVIII and FV. Direct activation of FX by TF-FVIIa is shut off by tissue factor pathway inhibitor (TFPI) but subsequently the TF-FVIIa complex activates FIX which using FVIIIa as a cofactor activates FX. Because there is also increased amount of FV, as a result of the positive feedback mechanism caused by thrombin, FX can activate more of FII to thrombin. The final result is therefore the amplification of the activation of FX and FII.²⁰⁻²⁵ The positive feedback loops are important for adequate thrombin formation for fibrinogen cleavage to form fibrin (Figure 1).²³ The fibrin subunits form a structure, wherein thrombin activated FXIII cross links the fibrin fragments, which stabilizes the fibrin.⁹ The fibrin fibers attach to platelets and membrane fragments in the clot because of the absence of blood flow and that causes increased amount of coagulation factors which can stick to the platelets to activate each other.²⁶ That results in thrombin generation.

Disruption in the blood flow may also increase the activity of these chemical reactions between the coagulation factors. When the blood flow is disrupted it may cause increased fibrin deposition.²⁷ No thrombin formation occurs when FIIa and FXa are removed by blood flow at a side of injury, the same does not apply to the other coagulation factors, which indicates the importance of FIIa and FXa in thrombin generation. FVII seems to be important in the regulation of thrombin generation by blood flow since when there is already a significant amount of FVII activated, variation in the wall shear rate does not have any affect. These circumstances are, however, only present in the absence of flow, since then there is enough of VIIa-TF complex and FVIIa in normal plasma to activate sufficient amount of FX and initiate coagulation. When the shear rate of the blood flow is low, like in atrial fibrillation, the coagulation system is still as active because of the interaction between FIIa and FXa despite of the removal of FX by blood flow.^{24,28} Under these circumstances there is not enough of FX for the amount of FVIIa-TF present to initiate coagulation but the FXa which is present causes increased activation of FVII by the positive feedback loop, which results in activation of the system. By this the coagulation system ignores the blood flow and initiates coagulation. When the shear rate increases, however, it eventually removes all of the FXa and no coagulation occurs. The same reason lies behind that the flow rate has increased effect on coagulation when there is increased amount of tissue factor pathway inhibitor (TFPI), since TFPI decreases the amount of FVIIa-TF.²⁴

1.1.2 Natural anticoagulants

Natural anticoagulants regulate coagulation in the body. Antithrombin and heparin interact to prevent thrombin generation.²⁹ The TF-FVIIa complex and FXa are inhibited by TFPI which thus inhibits the extrinsic pathway.²⁵ FVa and FVIIIa are inactivated by protein C and its vitamin K-dependent (VKD) cofactor, protein S. Protein C and protein S form a complex when thrombin activates protein C.^{30,31}

Thrombomodulin binds to thrombin when the endothelium is undamaged, to prevent the formation of a clot.⁹

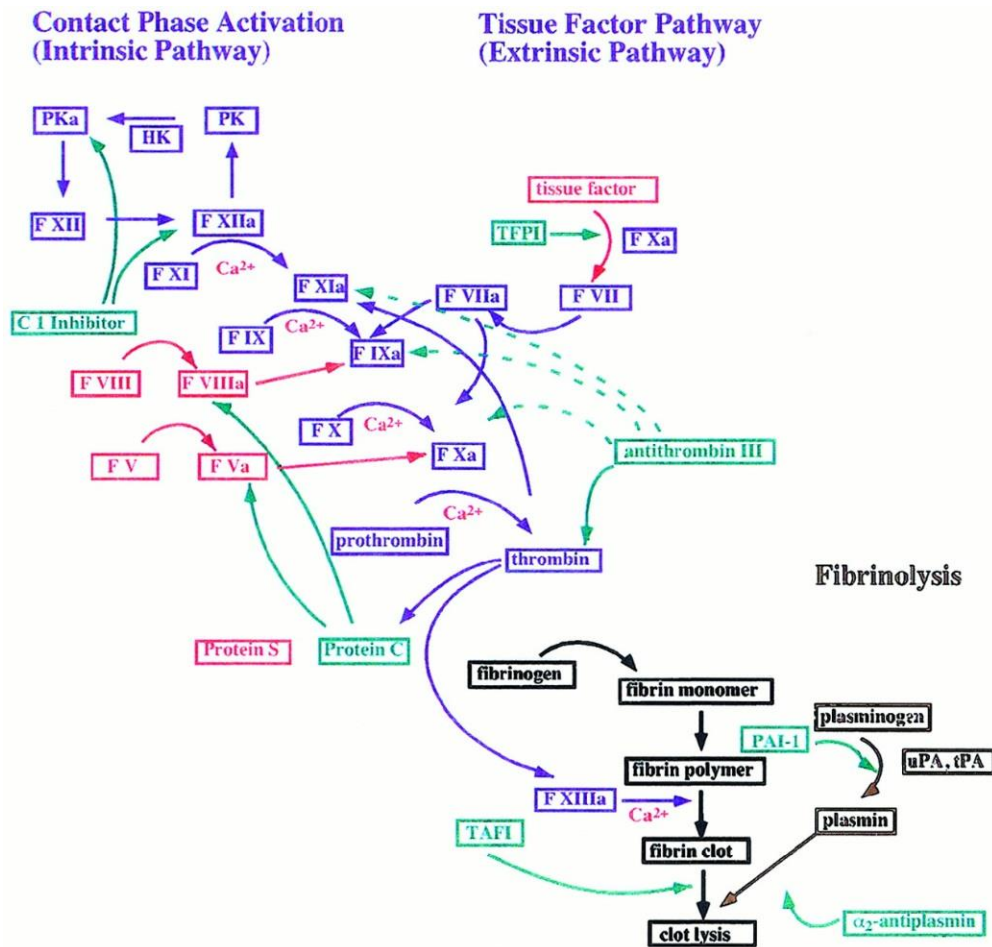


Figure 1. The coagulation system

Chemical reactions between the coagulation factors and positive feedback loops result in coagulation which is removed by the fibrinolytic system.³²

1.2 Vitamin K antagonists

Cardiovascular events may be the cause or effect of an interruption in the balance between thrombogenic and antithrombogenic factors.⁹ Increasing plasma concentration of fibrinogen with age may be related to the increasing risk of cardiovascular events with age, but other components in the coagulation system which may contribute to thrombus generation, like FV, FVII and FIX, are also increased in the plasma in the elderly.^{33,34} Other reasons for an increased risk of these events may be disorders of the vessel wall or of blood flow. Anticoagulation is used to treat or prevent such occurrences.

Until recently, VKA were the only available oral anticoagulants. VKA have a proven record of preventing stroke in patients with AF that are at increased risk of systematic arterial embolism.⁶ They have also been shown to be very effective in the primary and secondary prevention of venous thromboembolism (VTE).³⁵

1.2.1 Function of the vitamin K antagonists

Four of the coagulation factors, FII, FVII, FIX and FX, are VKD factors, and their carboxylation, which is necessary for their activity, is inhibited by warfarin, which is a VKA. It does so by inhibiting vitamin K reductase so that oxidized K-vitamin (vitamin K epoxide) is not reduced to vitamin K quinone. Vitamin K reductase also reduces vitamin K quinone, but vitamin K epoxide reductase is more sensitive to VKA (mainly the S enantiomer of warfarin) than vitamin K reductase). The reason for that the effect of VKA can be overcome by giving vitamin K is explained by this. Oxidized vitamin K cannot act as a co-enzyme for carboxylase which catalyzes the reaction of glutamic acid to gamma-carboxyglutamic acid, which is necessary for the procoagulant activity of VKD factors (Figure 1).^{8,36} For the activation of the VKD factors on the surface of activated platelets, this carboxyl group needs to be present to connect to calcium on phospholipid surfaces which is necessary for the attachment of the VKD factors to their cofactors.³⁷ The coagulation activity of those factors is therefore reduced.

1.3 Warfarin's activity and effect

Warfarin is an oral drug which is water soluble. It takes 60-90 minutes for its plasma concentration to peak after it is administered and absorbed in the upper gastrointestinal tract and the stomach.³⁸ Dosing of warfarin is difficult for various reasons but it is important that the coagulation ability in these patients is decreased but not so that it causes clinically relevant or major bleeding. Different individuals require different doses, and it is necessary to monitor the anticoagulation effect. The reasons for this are partly explained by warfarin's diverse pharmacology and the effect of environmental factors on warfarin's final result on the coagulation ability.

1.3.1 Pharmacology

Warfarin is a mixture of two enantiomers, S and R, which are metabolized by the enzymes CYP2C9, 1A2 and 3A4. CYP2C9, which is a member of the cytochrome p450 system, metabolizes S-warfarin which is 5 times more potent than R-warfarin (Figure 1). R-warfarin is metabolized by 1A2 and 3A4. The pharmacokinetic of warfarin is altered by mutations in these hepatic enzymes. Impaired ability to metabolize S-warfarin is a result of 2C9*2 or 2C9*3, the most common mutated alleles. The half-life of S-warfarin is therefore increased in individuals with these mutations, and they require lower doses. Mutations in the vitamin K oxide reductase complex I (VKORC1), a gene that encodes for certain isoforms of the enzyme, can result in warfarin resistance or can have no effect. The enzymes are then either resistant or sensitive to warfarin's inhibition. Changes in metabolism, synthesis and activity of the VKD factors can change warfarin's effect. If a patient being treated using warfarin has a mutation in FIX he has increased sensitivity to reduction of VKA, and therefore his risk of bleeding is increased beyond the usual bleeding risk.⁸

1.3.2 Environmental effects

The effect of warfarin may be affected by drugs and food. Cholestyramine reduces the absorption of warfarin which reduces its effect. Drugs that inhibit warfarin's clearance increase its effect and drugs that increase its clearance inhibit its effect. Omeprazole affects warfarin and therefore the Quick prothrombin time (PT), but only moderately, since it inhibits the clearance of R-warfarin, the less

potent isomer. Metabolic clearance of S- and R-warfarin is strongly inhibited by amiodarone and results in an increased effect of warfarin. Many antibiotics affect the pharmacokinetic and pharmacodynamics of warfarin. The effect of warfarin may be increased by salicylates of > 1.5 g/d. Nonsteroidal anti-inflammatory drugs (NSAID) inhibit platelet function and may cause gastrointestinal (GI) bleeding by increasing the risk of stomach ulcers, and therefore increase the risk of bleeding associated with warfarin. The effect of warfarin may be reduced by food products that contain high levels of vitamin K, like green tea, green vegetables and supplements with vitamin K. Other factors that may affect warfarin's activity or measured activity are imprecise testing methods, vitamin K absorption changes, warfarin absorption changes and therapeutic non-compliance.⁸

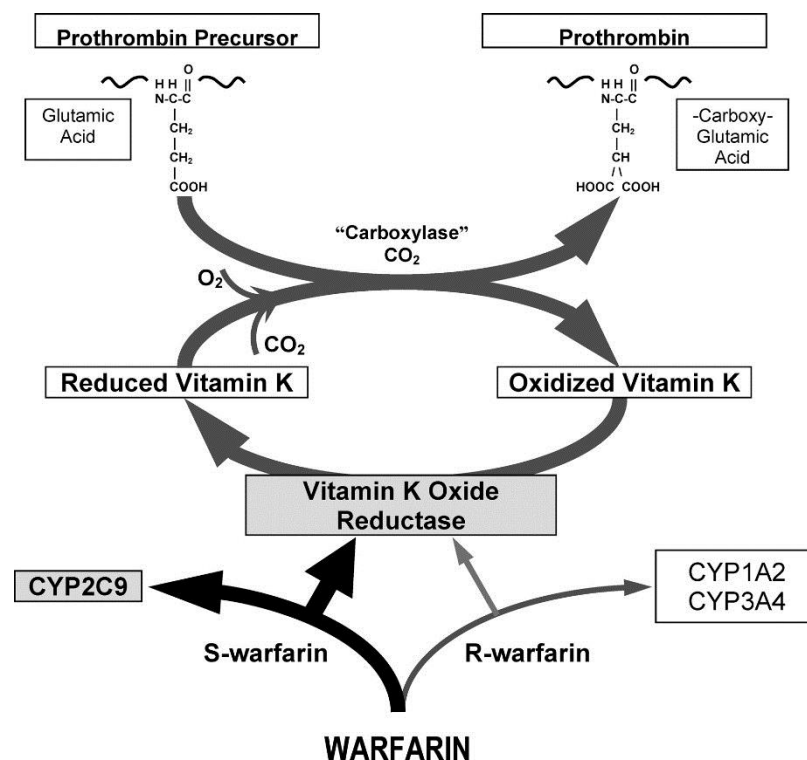


Figure 2. Warfarin and vitamin K inhibition

Warfarin inhibits vitamin K oxide reductase which results in increased amount of inactive vitamin K and coagulation factors.⁸

1.4 Monitoring and dosing of vitamin K antagonists

1.4.1 Quick-PT

The coagulation ability in individuals on VKA therapy can be monitored by measuring Quick-PT, which measures the extrinsic pathway and the common pathway. Quick-PT is defined as the time for a citrated plasma (from the patient) to clot after thromboplastin (tissue factor) and calcium are added. Since TF activates the extrinsic pathway.⁸ This test reflects the coagulation in vitro and the previous understanding of the coagulation system, but not necessarily the coagulation ability in vivo.^{39,40}

1.4.2 International normalized ratio

The influence of a reduction in VKD factors is not the same on different thromboplastins, which may be used in different laboratories measuring Quick-PT on a patient's plasma. This problem is solved by using International normalized ratio (INR) which uses international sensitivity index (ISI) which measures the activity of a given thromboplastin,⁸

The INR value is a guideline for the amount of warfarin that an individual requires but "American College of Chest Physicians (ACCP)" practice guidelines, which the anticoagulation monitor center (AMC, Segavarnir) at Landspítali, University Hospital of Iceland follows, recommends to give 4 or 6 mg, depending on health and age, at the beginning of the therapy. After the first two weeks the INR is usually monitored weekly until a stable INR value is reached. Then it is monitored at a 4-6 weeks interval and the dose adjusted as needed (appendix 2). The INR is normally around 1 in healthy individuals but the target intensity of INR in patients on VKA therapy for most indications is between 2 and 3. That range has been shown to decrease the risk of clot formation and cause the least risk of bleeding possible. Patient characteristics and indications for the use of warfarin can have an effect on INR's optimal target range. It may weigh more to have the range lower if the patient is at high risk of bleeding or after myocardial infarction, or to have the range higher if the patient has mechanical heart valve.⁸ To achieve and maintain this targeted INR it is necessary to monitor and adjust the dosages as needed. This is important to prevent events (bleeding and thromboembolism (TE)) but the reduction in activity of different VKD factors does not have the same effect on antithrombotic effect and anticoagulant effect.

1.4.3 Owren-PT

Owren-PT is a derivative of Quick-PT which is mainly used in the Nordic countries, Benelux and Japan.⁴¹ The difference lies in that in Owren-PT diluted patient plasma is mixed with plasma that is deficient of all VKD factors but has FV and fibrinogen (absorbed bovine plasma). Thus the final sample has an excessive amount of FV and fibrinogen compared to FII, FVII and FX, so the test is only sensitive to those latter factors.³⁹ The lack of sensitivity to fibrinogen and FII in the Owren-PT test may make it more specific than the Quick-PT.⁴¹ Owren-PT reflects the rate of reduction in those factors proportional to their half-lives in individuals taking warfarin.

1.4.4 Anticoagulation and antithrombotic effects

The half-lives of the coagulation factors differs, the difference between FVII, FIX, FX might be explained by the various length and glycosylation (or total absence in FVII) of their activation peptide, which must be removed for their activation which in turn causes their degradation.⁴² Since warfarin only has an effect on the carboxylation of the VKD factors, clearance of the functional factors controls the time it takes for the drug to have an effect on the coagulation.^{37,43} The first anticoagulant effect by warfarin is caused by reduction in the VKD factors that have a half-life of 24 h or less. That includes all the VKD factors except FII, which has a half-life of 60-72 h.⁸ The magnitude of FVII's effect is reflected in the Quick-PT which measures the anticoagulation effect.³⁹ FVII has the shortest half-life, 6 h, so reduction in it due to warfarin is reflected in the Quick-PT at the beginning of the therapy.⁸ Based on Wessler and Gitel's studies in rabbits it takes two days for anticoagulant effect to develop, and the reason for that may be the short half-life of FVII.⁴⁴ That is reflected in the fact that after warfarin intake

it takes 24-36 hours for the first changes in the INR to occur. Since FVII has such a short half-life, there are fluctuations in its activity. When FVII activity is measured as part of Quick-PT in vitro these fluctuations have an effect on the Quick-PT, since not all elements of the coagulation process, like cell-based surfaces, are present or have an effect.^{9,39}

When it comes to antithrombotic effect, however, reduction in FX and FII may be more important than reduction in FVII and FIX.^{40,45} TF-induced coagulation is not inhibited when FVII is selectively depressed and when FIX is selectively depressed to the same amount as during warfarin therapy in rabbits with immunodepression.⁴⁵ In the body, the amount of FVII does not start to avert thrombin generation until it is below 5%, so these fluctuations do not have much effect on the coagulation ability.^{39,45} Hence, only small amount of FIX and FVII is required for thrombin generation.⁴⁰ Clot-bound thrombin is an important mediator of increased clot formation and reduction in plasma FII is especially important for the antithrombotic effect.^{8,45} TF-induced coagulation is inhibited when FX is selectively depressed and when FII is selectively depressed to the same amount as during warfarin therapy in rabbits with immunodepression.⁴⁵ Hence a decrease in activity of FII and FX represents better the activity of VKA on coagulation in the body.³⁹ Since FII has a half-life of 60-72 h it takes six days for the antithrombotic effect of warfarin to develop in rabbits, so the clearance of FII controls the velocity of antithrombotic effect.^{8,37,40,44,45} The long half-life of FII inhibits the possibility of large loading doses.⁴³

Warfarin also reduces protein C and S. Protein C has a short half-life, therefore at the beginning of warfarin therapy there is reduced amount of FVII, which has little effect on thrombosis, and there is reduced amount of protein C, which causes increased coagulation. That may result in hypercoagulation at the beginning of warfarin therapy. Heparin is always given along with warfarin at the beginning of therapy for these reasons.⁴⁵

1.4.5 Fiix-PT

Fluctuations in FVII activity can result in fluctuations in the INR that may cause dose changes in VKA that are unnecessary and it makes it more difficult to get a stable INR. The Fiix-PT test was developed because of these properties. It only measures the effect of FII and FX by mixing platelet poor plasma immunodepleted of FII and FX with plasma from the patient on anticoagulation.³⁹

1.5 Time in therapeutic range and variance growth rate

The time in therapeutic range (TTR) reflects the VKA effect, control and intensity, and the quality of dose management.⁴⁶ The TTR value is a good predictor of events, like bleeding or TE, but some studies suggest TTR and INR are not sufficient or dependable predictors.^{8,46} The variation that occurs in the INR (and is not measured by TTR) may also have an important influence on events (bleeding or TE), since increased variation increases risk 3-6 months before an adverse TE or bleeding event. A method of measuring this variability is the variance growth rate (VGR). The VGR is as good as TTR to predict events during short term INR monitoring.⁴⁶ The quality of dose management has an effect on the TTR and poor dose management may result in lower TTR and therefore increased risk of events.⁴⁷ Increased risk of mortality, ischaemic stroke and other TE events is associated with 10%

increase in time out of range.⁴⁸ The risk of events is still high in well controlled population and the achievement and maintenance of good control is challenging.⁴⁸ Improvement in the anticoagulant tests could improve the TTR and VGR and decrease the risk of events.

1.6 New oral anticoagulants

Recent studies have shown that to prevent ischaemic stroke in patients with AF the new direct oral anticoagulants (DOACs), dabigatran (RE-LY), rivaroxaban (ROCKET AF), apixaban (ARISTOTLE) and edoxaban (ENGAGE), appear to be non-inferior to warfarin in studies that all used warfarin control groups that were suboptimally managed.⁴⁹⁻⁵² Although the risk of gastrointestinal hemorrhage may be increased, they appear to decrease the risk of intracranial haemorrhage, when compared to warfarin.⁵ The quality of warfarin dose management matters, since if the dose management is high-quality the risk of events is less than when the therapy is not as well-controlled.⁴⁷ Some studies have shown that the risk of TE and major bleeding in patients treated with apixaban remained the same as in warfarin even when the mean TTR exceeded 68%.⁵³ Another concern is that antidotes for patients on these new drugs do not exist and they are very expensive.⁵⁴

1.7 Study objectives

The basis for the Fiix-PT test is the hypothesis that fluctuations in the INR measured with Quick-PT or Owren's PT that are due to FVII's short half-life do not reflect the true anticoagulation in patients on VKA anticoagulation therapy. By measuring only the combined activity of FII and FX on coagulation with the new Fiix-PT, true anticoagulation would be observed and anticoagulation variability would be reduced. The aim of the Fiix-trial conducted in 2012-2014 was to test the hypothesis that monitoring warfarin with the Fiix-PT would increase the stability of anticoagulation therapy compared to standard monitoring with Quick-PT (INR).⁵⁵ The trial confirmed the hypothesis and demonstrated that the Fiix-PT was clinically at least non-inferior to standard PT-INR monitoring and reduced long-term TE by over 50%. Fiix-PT monitoring also lead to more stable dosing, fewer blood tests, higher percent of TTR and lower VGR, hence increased stability.

The main objective of the current analysis of data from the Fiix-trial was to compare if age and gender influence anticoagulation differently depending on the monitoring method.

2. Methods

2.1 Study population

In the randomized blinded Fiix non-inferiority trial, 1148 patients were prospectively observed for occurrence of TE and bleeding and for anticoagulation differences.⁵⁵ Patients on warfarin therapy at the anticoagulation dosing management center (AMC) at Landspítali and who were 18 years and older were qualified to participate. The patients were eligible independent of indication for warfarin but had to have INR target of 2.0-3.0. The study period was from March 1st 2012 to February 28th 2014. The initial randomized population in the Fiix-study was 1155 but 8 participants were excluded before analysis of the data was carried out. One patient who was randomized to the Fiix arm that was not on warfarin therapy, one that was on dicumarol and two that were on electroconversion list were excluded. The 4 patients who were randomized to the PT arm and were excluded included one who was on dicumarol, two on electroconversion list and one with INR target 2.5 – 3.5.

Participants were randomized to Fiix-PT or PT monitoring arms, after they accepted and signed informed consent. Thereafter, each participant was assigned a specific code and based on this code, the blood samples from the patients were directed to test A (Fiix-INR) or B (PT-INR) throughout the study period. All the tests were done in the central coagulation laboratory and the results were reported to the dosing staff (nurses, biomedical scientists and physicians) as a blinded R-INR and the patients were dosed according to that. Protocols that were based on the monitoring with PT-INR (Quick-PT) and the DAWN AC anticoagulation management software were used for the dosing.

In the current study, all 815 individuals on long-term anticoagulation with AF in the Fiix-trial were studied in order to compare the influence of gender and age on the anticoagulation outcome. Naïve patients (had been on warfarin therapy < 180 days) were excluded as well as patients with other indications for warfarin to get a more stable population.

2.2 The INR assays

STA-R evolution® coagulation analyzing instruments from Diagnostica Stago Inc, Asnieres, France, were used to perform both monitoring tests. Landspítalinn coagulation laboratory biomedical scientists performed the tests.

2.2.1 PT-INR

Quick-PT was used to calculate the PT-INR. That is conducted by adding 100 µL of STA-Néoplastine CI Plus reagent (thromboplastin and calcium) to undiluted patient plasma (50 µL) to initiate coagulation.

2.2.2 Fiix-INR

25 µL of Fiix (FII and FX) depleted plasma (Haemotologic Technologies INC.) is added to the test sample in the Fiix-INR test. 80 µL of the patient plasma was diluted (before adding the coagulation factors) with seven times the volume of STA-Owren Koller diluent. To initiate coagulation, 80 µL of STA-Néoplastine CI Plus are added.

The INR is calculated with the formula: $INR = (\text{patient PT} / \text{mean normal PT})^{ISI}$, in both tests.

2.2.3 Instrument

The STA-R coagulation analyzing instruments measures the variation in the amplitude of an oscillating magnetic ball, which depends on how much the blood is coagulated where an increase in viscosity of the plasma causes a reduction in the amplitude. In that way the STA-R instrument can measure the time for blood to coagulate.

2.5 Statistical analysis

During analysis the study group was categorized by age in days and by gender. The age range was split into quartiles so that the same number of patients was in each quartile. If two participants were exactly the same age they were put into the same quartile. This was done with the Fiix-PT and PT groups.

The following outcome parameters were studied: TTR, VGR, frequency of monitoring tests, days between monitoring tests in each patient, daily warfarin dose, frequency of dose changes and dose changes per monitoring test in each individual. Only values from patients with more than two monitoring tests were included in these calculations. Other outcome parameters were number of monitoring tests at certain INR intervals and number of observation days per patient which included values from all of the patients.

TTR was calculated by dividing the time a person spends within the target range, INR between two and three (two and three included), with the time that the individual spent in the study. The Rosendaal linear interpolation method assumes that consecutive INR measurements are linear over time and that was used to calculate a daily INR (since there was not an INR value each day for each participant).⁵⁶ The TTR for each group is the median of TTR for each patient in that group.

VGR was calculated with the Fihn (method A) method and Cannegieter (method B1) method in the statistical program R. Method A evaluates the difference between the patient's INR value and a target INR value of 2.5 per time in weeks between the present and previous INR values. Method B1 evaluates the difference in two adjacent INRs in a patient per time in weeks between the two INR measurements.⁴⁶

In order to estimate the contribution of FVII to the R-INR the Fiix-PT INR measured in all samples was subtracted from the Quick-PT INR which was also measured in all samples. A negative difference would mean that the Quick-PT INR were lower which would mean more activity of the coagulation factors and therefore more activity of FVII than if the value were positive, or closer to zero. If the difference were zero it would mean that the activity of FVII is not having any affect on the PT, it does not increase or decrease the coagulation. If all the differences are transformed to positive values it could reflect how much affect FVII is having, either decreasing or increasing the coagulation that the PT measures.

The whole period that a patient spent in the study was considered in the analysis independent of whether they were temporarily discontinued from warfarin or not dosed according to Landspítali's AMC protocol, defined as intention to monitor (ITM).

12 AF patients in the Fiix-PT group who had been on warfarin for less time than 180 days were excluded. 7 AF patients in the Fiix-PT group who had less than 3 INR tests were excluded for part of the analysis. 6 patients in the Fiix-PT group were excluded because they had been on warfarin

for less than 180 days and they had less than 3 tests. 10 AF patients in the PT group who had been on warfarin for less time than 180 days were excluded. 15 AF patients in the PT group who had less than 3 INR tests were excluded for part of the analysis. 4 patients in the PT group were excluded because they had been on warfarin for less than 180 days and they had less than 3 tests.

The Mann-Whitney U test was used to compare individual-based data between two groups. The Mann-Whitney test compares ranks without assuming a Gaussian distribution, but it is a variation of the t-test. When comparing more than three groups, the Kruskal-Wallis non-parametric one-sided ANOVA test was used, which is an extension of the Mann-Whitney test. Fischer's exact (Chi-squared with Yates' correction) test was used to compare count data. All p-values ≤ 0.05 were considered significant. Microsoft Excel, the statistical program R and GraphPad Prism 6.0 were used to perform all statistical analysis.

2.6 Approvals

The Fiix-trial was conducted by academic investigators in accordance with the Helsinki declaration at Landspítali/The National University Hospital of Iceland AMC in Reykjavik, Iceland. The protocol 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-rannsoknaraetlun/>. The study was registered at www.clinicaltrials.gov as NCT01565239.

3. Results

3.1 Patients, study conduct and clinical description

The subgroup of long-term AF patients enrolled in the Fiix-trial was studied in the current project. Their characteristics on enrollment are seen in Table 1. The number of patients in the PT arm was slightly higher than in the Fiix-PT arm. The median (IQR) observation time in the Fiix-PT arm and PT arm was 1.4 (1.1-1.6) years per patients and 1.4 (1.0-1.6), respectively. There were no statistically significant differences in any of the patient characteristics on enrollment between the Fiix-PT and PT arms (Table 1). The median age was 73.7 (66.2-79.6) years in the Fiix-PT arm and 74.6 (67.1-80.5) years in the PT arms. The median CHA₂DS₂-VASC scores were identical in AF patients in both groups, 3 (2-4) in the Fiix-PT arm and 3 (2-4) in the control arm. In the Fiix-trial warfarin experienced patients with AF comprised 69% (n = 396) of the Fiix-PT arm and 73% (n = 419) of the PT arm. The Fiix-PT group comprised of 265 males and 131 females and the PT group of 292 males and 127 females.

The age quartiles in the Fiix-PT group were all the same size (n = 99) and in the PT group the 1st included 106, the 2nd 105 and the 3rd and 4th 104. The median age in the Fiix-PT groups was 62.3 in the 1st (age range 32-66), 69.8 in the 2nd (age range 66-73), 76.8 in the 3rd (age range 73-79) and 83.2 in the 4th (age range 79-95). The median age in the PT groups was 62.5 in the 1st (age range 31-67), 70.3 in the 2nd (age range 67-74), 77.4 in the 3rd (age range 74-80) and 84.2 in the 4th (age range 80-95). There were no significant differences in any of the non-endpoint discontinuations from the study and no patient was lost to follow-up (Table 2). Patient characteristics and treatment description according to age are shown in supplementary tables 1 and 2.

Table 1. Patient characteristics on enrollment

Patients on long-term warfarin anticoagulation for atrial fibrillation monitored with either Fiix-PT or Quick-PT.

	<i>Fiix-PT arm^a</i>	<i>PT-arm^a</i>	<i>P-value^b</i>
Number	396	419	
AF without prior arterial thromboembolic event	296 (74.7)	308 (73.5)	0.7462
AF with prior cerebral thromboembolic event or TIA	95 (24.0)	105 (25.1)	0.7846
AF with prior peripheral arterial embolism	5 (1.3)	6 (1.4)	0.9249
	73.7 (66.2-79.6)	74.6 (67.1-80.5)	0.3211
Age in years (median, IQR^c)			
Years of warfarin treatment prior to enrollment - median (IQR^c)	4.3 (1.6-8.5)	3.7 (1.2-8.1)	0.1004
CHA₂DS₂-VASC risk score in AF patients – median (IQR^c)	3 (2-4)	3 (2-4)	0.8083
Percent with score 0 (low TE risk)	17 (4.3)	16 (3.8)	0.8685
Percent with score 1 (moderate TE risk)	33 (8.3)	44 (10.5)	0.3484
Percent with score ≥2 (high TE risk)	346 (87.4)	359 (85.7)	0.5454
Percent with score ≥3	252 (63.6)	271 (64.7)	0.8128
Additional indications for warfarin – no. (%)			
Ischaemic heart disease total	5 (1.3)	9 (2.1)	0.4823
Acute MI	5 (100.0)	9 (100.0)	-
Other IHD	0	0	-
Congestive heart failure	1 (0.3)	0	0.9775
Atrial septal defect	0	0	-
Artificial heart valves	2 (0.5)	2 (0.5)	0.6564
Rheumatic mitral valve disease (mitral stenosis)	1 (0.3)	0	0.9775
Venous thromboembolism (VTE)	21 (5.3)	20 (4.8)	0.8528
Deep vein thrombosis	9 (42.9)	5 (25.0)	-
Pulmonary embolism	12 (57.1)	15 (75.0)	-
Pulmonary hypertension	0	0	-
Associated conditions– no. (%) :			

Smoker	39 (9.8)	37 (8.8)	0.7047
High blood pressure	252 (63.6)	275 (65.6)	0.6013
Ischaemic heart disease	114 (28.8)	124 (29.6)	0.8603
Peripheral vascular disease	21 (5.3)	23 (5.5)	0.9701
History of congestive heart failure	59 (14.9)	64 (15.3)	0.9587
Diabetes mellitus	61 (15.4)	51 (12.2)	0.2158
Cancer	63 (15.9)	72 (17.2)	0.6929
- active cancer chemotherapy	9 (14.3)	11 (15.3)	-

Select drug use – no. (%):

Acetylsalicylic acid	93 (23.5)	95 (22.7)	0.8479
Clopidogrel	5 (1.3)	6 (1.4)	0.9249
Non-steroidal antiinflammatory drugs	41 (10.4)	43 (10.3)	0.9422
Amiodarone	48 (12.1)	49 (11.7)	0.9364
H ₂ blockers and proton pump inhibitors	87 (22.0)	79 (18.9)	0.3093
Any other drugs	380 (96.0)	400 (95.5)	0.8611

^a Fiix-PT denotes Fiix-prothrombin time and PT denotes Quick prothrombin time. ^b Mann-Whitney non-parametric test was used for numerical rows and Fisher's exact test for categorical values. ^c IQR denotes interquartile (25-75%) range.

Percentages do not total 100 due to presence of more than one indication in some patients or rounding of numbers.

Table 2. Treatment description according to monitoring method

	<i>Fiix –PT arm^a</i>	<i>PT arm^a</i>	<i>P-value^b</i>
	<i>n = 396</i>	<i>n = 419</i>	
	<i>n (%)</i>	<i>n (%)</i>	
Observation time			
Total intention-to-monitor observation years	521	534	
Intention-to-monitor observation years per patient – median (IQR ^c)	1.4 (1.1-1.6)	1.4 (1.0-1.6)	0.1012
Number of monitoring tests – no			
	8384	8802	
Discontinuation from study – n (%)			
Total	59 (14.9)	62 (14.8)	0.9540
Anticoagulation discontinued	19 (4.8)	19 (3.6)	0.9904
Voluntary discontinuation	5 (1.3)	3 (0.7)	0.6631
Switched to direct oral anticoagulant	14 (3.5)	13 (3.1)	0.8814
INR target changed	3 (1.0)	8 (1.9)	0.2625
Lost to follow-up	0	0	-
Other reason	18 (4.5)	19 (4.5)	0.8722

^a Fiix-PT denotes Fiix-prothrombin time and PT denotes Quick prothrombin time. ^b Mann-Whitney non-parametric test was used for numerical rows and Fisher's exact test for categorical values. ^c IQR denotes interquartile (25-75%) range.

Percentages do not total 100 due to rounding of numbers.

Clinically relevant arterial thromboembolic or bleeding events (CRE) occurred in 92 in the Fiix-PT arm and 93 in the PT arm ($p = 0.7876$) and are shown in Table 3. Arterial thromboembolic events (ATE) occurred in 10 in the Fiix-PT arm vs 16 in controls ($p = 0.3949$). Clinically relevant bleeding occurred in 82 in the Fiix-PT arm and 77 in the PT arm ($p = 0.4529$). Thereoff, major bleeding occurred in 15 in the Fiix-PT arm and 16 in the PT arm ($p = 0.8727$).

CRE occurred in 59 males and 33 females in the Fiix-PT group and 64 males and 29 females in the PT group ($p = 0.6014$ and $p = 0.9968$ within the Fiix-PT and PT groups, respectively). ATE occurred in 4 males and 6 females in the Fiix-PT group vs 11 males and 5 females in controls ($p = 0.1357$ and $p = 0.8462$ within the Fiix-PT and PT groups, respectively). Clinically relevant bleeding occurred in 55 males and 27 females in the Fiix-PT arm and 53 males and 24 females in the PT group ($p = 0.9215$ and $p = 0.9647$ within the Fiix-PT and PT groups, respectively). Major bleeding occurred in 11 males and 4 females in the Fiix-PT arm and 10 and 6 females in the PT group ($p = 0.7960$ and $P=0.7183$ within the Fiix-PT and PT groups, respectively).

Table 3. Thromboembolic and clinically relevant bleeding events in the study population.

	<i>Fiix-PT arm^a</i>			<i>PT arm^a</i>		
	<i>Total</i>	<i>Males</i>	<i>Females</i>	<i>Total</i>	<i>Males</i>	<i>Females</i>
	n = 396	n = 265	n = 131	n = 419	n = 292	n = 127
Total events – no (%)	92 (23.2)	59 (22.3)	33 (25.2)	93 (22.2)	64 (21.9)	29 (22.8)
Arterial thromboembolism – no (%)	10 (2.5)	4 (1.5)	6 (4.6)	16 (3.8)	11 (3.8)	5 (3.9)
All clinically relevant bleeding – no (%)	82 (20.7)	55 (20.8)	27 (20.6)	77 (18.4)	53 (18.2)	24 (18.9)
Major bleeding	15 (3.8)	11 (4.2)	4 (3.1)	16 (3.8)	10 (3.4)	6 (4.7)
Other clinically relevant bleeding	67 (16.9)	44 (16.6)	23 (17.6)	61 (14.6)	43 (14.7)	18 (14.2)

^a Fiix-PT denotes Fiix-prothrombin time and PT denotes Quick prothrombin time. There were no statistically significant differences between any of the groups in any of the listed variables by Chi-square.

3.2 Outcome parameters in relation to gender

3.2.1 Test frequency

The median test frequency per patient per month in the Fiix-PT and PT arms was 1.3 in both arms ($p = 0.6732$). Females in the Fiix-PT group and the PT group had more frequent monitoring tests, median 1.4 and 1.5, respectively, than males (1.2 vs 1.3, $p = 0.0110$ in Fiix-PT group and $p = 0.0003$ in PT group). Thus, males had 13% and 14% less frequent testing than did females, respectively.

The interval between monitoring tests was longer in males, Fiix-PT 24.4 and PT 24.2, than in females, Fiix-PT 21.3 and PT 20.4 ($p = 0.0110$ within Fiix-PT, $p = 0.0003$ within PT). More detailed results are shown in Table 4.

3.2.2 Number of tests and fractions of tests within defined INR ranges

With Fiix-PT, the number of monitoring tests was reduced by 5.0% (Table 2). As shown in Table 4 about four percent more monitoring tests fell within target range in the Fiix-PT arm (65.5% vs 62.9% in the PT arm, $p = 0.0019$).

Males in both study arms had a higher fraction of tests in range (Fiix-PT group with 66%, PT group with 65%) than females (Fiix-PT group with 64%, PT group with 59%) ($p = 0.0160$ in the Fiix-PT group and $p < 0.0001$ in the PT group). Females (64%) in the Fiix-PT arm had significantly higher number of tests within target range (8% more) than females (59%) in the PT group ($p = 0.0001$). There were fewer tests in the Fiix-PT arm with low INR <2 (18.9% in the Fiix-PT group vs 20.9% in the PT group, $P=0.0010$) but this difference was caused by a significant difference in females only (20% vs 24% in the Fiix-PT and PT groups, respectively, $p = 0.0002$). Males in the PT group had fewer tests with INR < 2 than females in that group; 20% vs 24% ($p = 0.0001$). There was not a significant difference between males and females in the Fiix-PT arm (19% vs 20%, $p = 0.3542$). There was a significant difference between the genders in both arms in fraction of tests with INR higher than 3 but not between the arms, neither in the total comparison nor for the genders.

3.2.3 Time in therapeutic range

The median TTR was possibly higher in the Fiix-PT arm than in the PT arm, 80.7% vs 78.6% ($p = 0.0857$, n.s., Table 4, Figure 3. a). Males had higher TTR than females (80.3% vs 75.3%, respectively) in the PT arm ($p = 0.0015$), but not in the Fiix-PT arm (males 80.7% vs females 80.3%, $p = 0.2164$, Figure 3. b). Females in the Fiix-PT group had significantly higher TTR than females in the PT group (80.3% vs 75.3%, respectively, $p = 0.0401$, Figure 3. b).

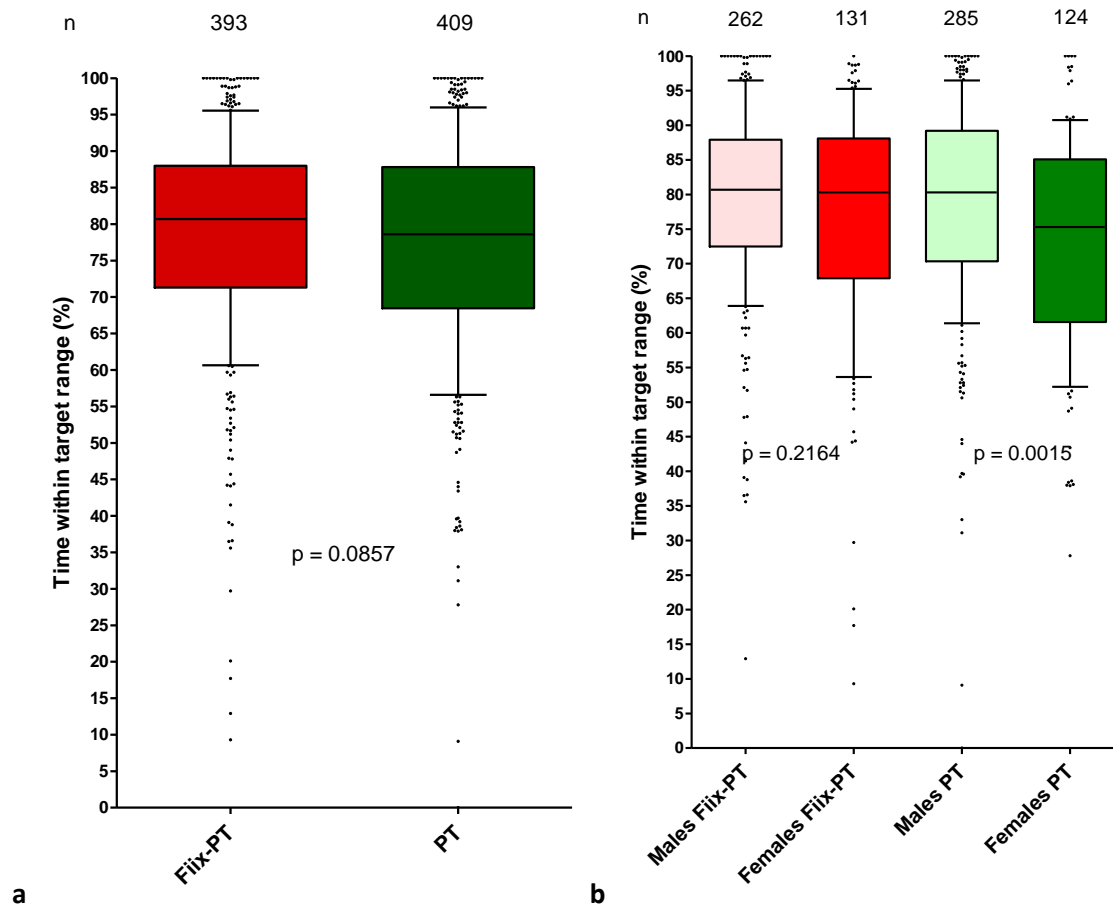


Figure 3. Time in therapeutic range (TTR)

The left panel (a) shows TTR for the total Fiix-PT arm and PT arm and the right panel (b) shows TTR for in males and females subgrouped according to study arms. Fiix-PT denotes Fiix-prothrombin time and PT denotes Quick prothrombin time. Median, interquartile range and 10-90th percentiles for each group based on TTR for each patient. Mann-Whitney non-parametric test was used.

3.2.4 Variance growth rate

The median INR fluctuation (variability) between tests measured as VGR (method B1) was 0.20 in the Fiix-PT arm and 0.24 in the control PT arm (Figure 4. b). Thus, the VGR was 20% higher in the PT arm, $p = 0.0810$, n.s. Females (0.25 in the Fiix-PT group, 0.30 in the PT group) had higher INR VGR (B1) than males (0.18 in the Fiix-PT arm, 0.21 in the PT arm) in both arms ($p = 0.0371$ in Fiix-PT arm and $p = 0.0056$ in the PT arm, Figure 4. b). Using VGR method A (fluctuation around mid INR target) a similar trend was observed although the difference between the genders was only significant within the PT arm (Figure 4. a). More detailed results are shown in Table 4.

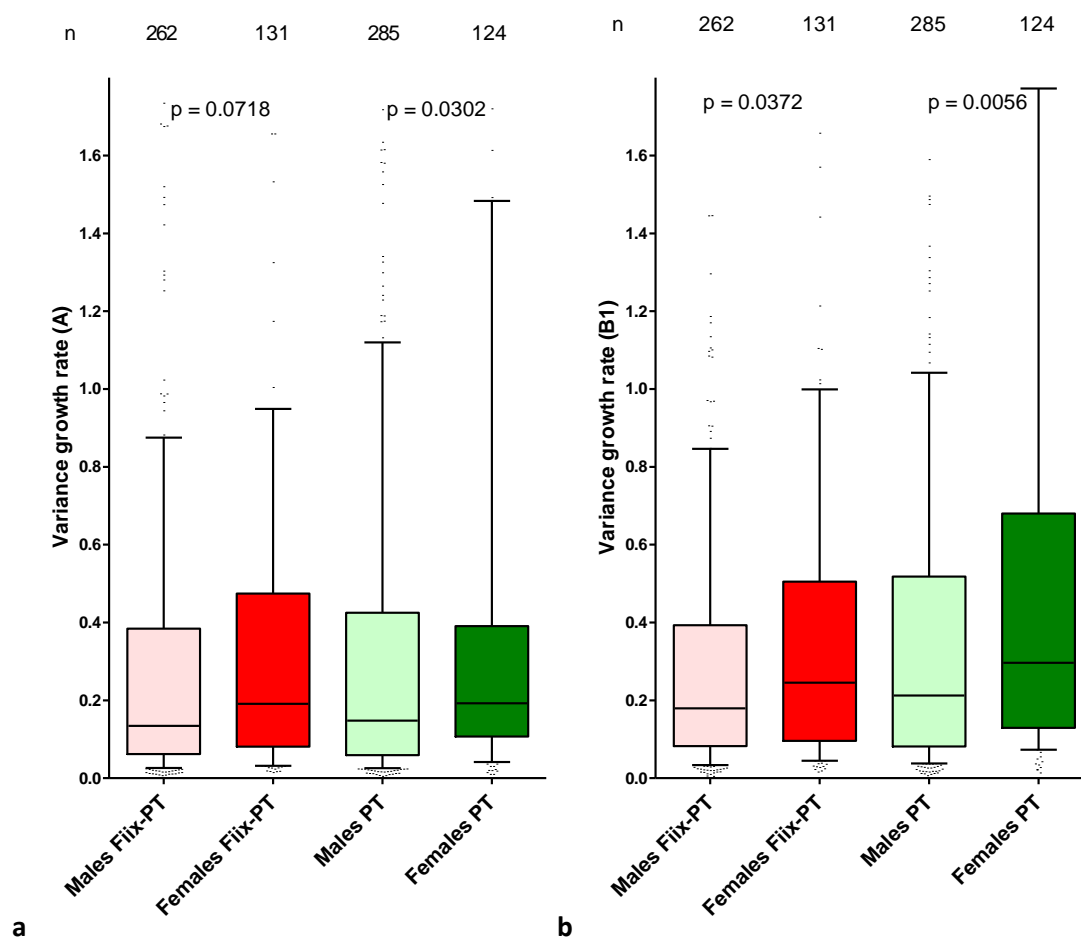


Figure 4. Variance growth rate (VGR) in females and males

The left panel (a) shows INR VGR based on method A (fluctuation around mid target) and the right panel (b) shows VGR based on method B1 (fluctuation between two adjacent INRs) FVII in males and females subgrouped according to study arms. Fiix-PT denotes Fiix-prothrombin time and PT denotes Quick prothrombin time. Median, interquartile range and 10-90th percentiles for each group based on INR VGR for each patient. Mann-Whitney non-parametric test was used.

3.2.5 Daily dose and dose changes

The median daily warfarin dose was almost identical in both arms, 4.4 mg in the Fiix-PT arm and 4.5 mg in the PT arm ($p = 0.3874$). Females in the PT group had a median daily warfarin dose of 4.7 mg which was significantly higher than females in the Fiix-PT group (3.4 mg, $p = 0.0029$) but there was not a significant difference between the males (Figure 5). Males had a higher daily dose than females in both arms (the Fiix-PT arm with $p < 0.0001$ and the PT arm with $p = 0.0323$).

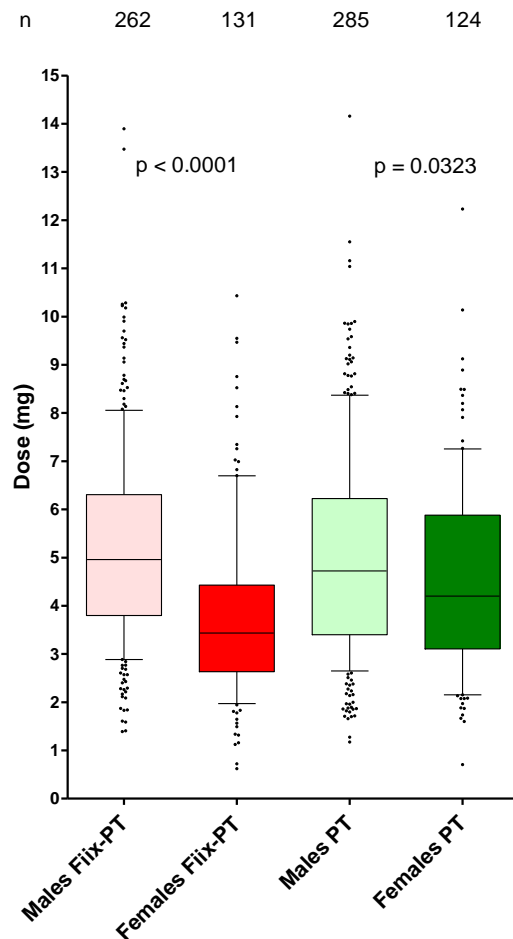


Figure 5. Daily warfarin dose in males and females

The figure shows daily dose in mg FVII in males and females subgrouped according to study arms. Fiix-PT denotes Fiix-prothrombin time and PT denotes Quick prothrombin time. Median, interquartile range and 10-90th percentiles for each group based on daily warfarin dose in mg for each patient. Mann-Whitney non-parametric test was used.

The median number of dose changes during the observation period was 5.6 in the Fiix-PT arm and 6.2 in the PT arm ($p = 0.0822$). Females in the PT group had more dose changes than males in the PT group (7.4 vs 5.8, $p = 0.0006$). There were more dose changes in females in the PT group compared to females in the Fiix PT group (7.4 vs 6.0, $p = 0.0342$, Figure 6. a).

Dose changes per monitoring test reflect the dose change rate better than simply counting the number of dose changes in each. The median dose changes per monitoring test was reduced 7% in the Fiix-PT arm compared to the PT arm, 0.26 and 0.28, respectively ($p = 0.0428$). However, this was explained only by a difference in females in the subgroup analysis (males: Fiix-PT 0.26 vs PT 0.27, females: Fiix-PT 0.27 vs PT 0.32, 16% less dose changes per test in females in the Fiix-PT group, $p = 0.2707$ in males and $p = 0.0292$ in females, Figure 6. b). Males in the PT group had 0.27 dose changes per monitoring test and females had 0.32, (16% less, $p = 0.0094$, Figure 6. b). There was not a significant difference between dose changes per test in males and females in the Fiix-PT arm. More detailed results are shown in Table 4.

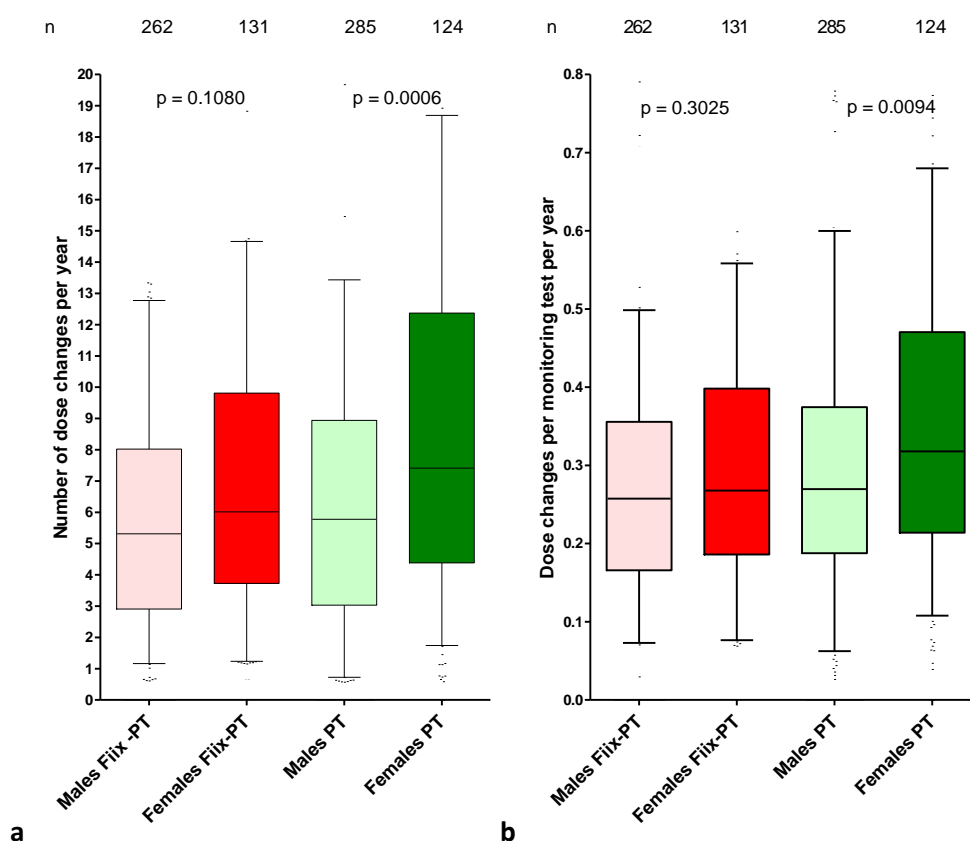


Figure 6. Dose change frequency in males and females

The left panel (a) shows number of dose changes per year and the right panel (b) shows dose changes per monitoring test per year in males and females subgrouped according to study arms. Fiix-PT denotes Fiix-prothrombin time and PT denotes Quick prothrombin time. Median, interquartile range and 10-90th percentiles for each group based on number of dose changes for each patient. Mann-Whitney non-parametric test was used.

Table 4. Comparison of anticoagulation indicators in males and females in relation to monitoring method

	Fiix-PT arm vs PT arm ^a n = 396 vs 419	P-value ^b	Fiix-PT males vs PT males ^a n = 265 vs 292	P-value ^b	Fiix-PT females vs PT females ^a n = 131 vs 127	P-value ^b	Fiix-PT males vs females ^a n = 265 vs 131	P-value ^b	PT males vs females ^a n = 292 vs 127	P-value ^b
Number of monitoring tests per patient – median	20 vs 20	0.56409	20 vs 19	0.4762	21 vs 23	0.7861	20 vs 21	0.0233	19 vs 23	0.0053
Fraction of tests within defined R-INR ranges (%)										
INR 2-3 – %	65.5 vs 62.9	0.0019	66 vs 65	0.0808	64 vs 59	0.0001	66 vs 64	0.0160	65 vs 59	<0.0001
INR <2 – %	18.9 vs 20.9	0.0061	19 vs 20	0.1810	20 vs 24	0.0002	19 vs 20	0.3542	20 vs 24	0.0001
INR >3 – %	15.6 vs 16.2	0.2906	15 vs 15	0.4137	17 vs 17	0.4553	15 vs 17	0.0334	15 vs 17	0.0173
Number of observation days per patient – median	527 vs 514	0.1012	540 vs 517	0.1847	511 vs 503	0.3028	540 vs 511	0.4242	517 vs 503	0.3734
Test frequency per patient per month – median	1.3 vs 1.3	0.6732	1.2 vs 1.3	0.9711	1.4 vs 1.5	0.2954	1.2 vs 1.4	0.0110	1.3 vs 1.5	0.0003
Days between monitoring tests in each patient - median	23.4 vs 22.6	0.6732	24.4 vs 24.2	0.9711	21.3 vs 20.4	0.2954	24.4 vs 21.3	0.0110	24.2 vs 20.4	0.0003
Daily warfarin dose in mg – median	4.4 vs 4.5	0.3874	5.0 vs 4.7	0.2186	3.4 vs 4.2	0.0029	5.0 vs 3.4	<0.0001	4.7 vs 4.2	0.0323
Number of dose changes in each patient per year - median	5.6 vs 6.2	0.0822	5.3 vs 5.8	0.4001	6.0 vs 7.4	0.0342	5.3 vs 6.0	0.1080	5.8 vs 7.4	0.0006
Dose changes per monitoring test per year in each patient - median	0.26 vs 0.28	0.0428	0.26 vs 0.27	0.2707	0.27 vs 0.32	0.0292	0.26 vs 0.27	0.3025	0.27 vs 0.32	0.0094
Percent TTR of each patient – median	80.7 vs 78.6	0.0857	80.7 vs 80.3	0.4430	80.3 vs 75.3	0.0401	80.7 vs 80.3	0.2164	80.3 vs 75.3	0.0015
INR fluctuation between tests (variance growth rate) – B1 – median	0.20 vs 0.24	0.0810	0.18 vs 0.21	0.2420	0.25 vs 0.30	0.1207	0.18 vs 0.25	0.0372	0.21 vs 0.30	0.0056
INR fluctuation around mid target (variance growth rate) – A – median	0.15 vs 0.16	0.5168	0.13 vs 0.15	0.6765	0.19 vs 0.19	0.5493	0.13 vs 0.19	0.0718	0.15 vs 0.19	0.0302

^a Fiix-PT denotes Fiix-prothrombin time and PT denotes Quick prothrombin time. ^b Mann-Whitney non-parametric test was used for numerical rows and Fisher's exact test for categorical values. Percentages do not total 100 due to rounding of numbers.

3.2.6 Activity of FVII

Although patients were monitored with either Fiix-PT or PT both tests were actually measured in all patients. In order to assess the contribution of FVII to the INR we subtracted the Fiix-INR from the PT-INR and plotted up the difference.

The median (IQR) for the positive differences between the Fiix-PT INR and PT INR to estimate the activity of FVII was higher in females than males ($p = 0.0010$, figure 7. a). The median positive difference was 0.17 (0.08-0.30) for females in the Fiix-PT group and 0.21 (0.09-0.38) for females in the PT group (figure 7. b). FVII thus had 24% more effect in females in the PT group than females in the Fiix-PT group. The median was 0.16 (0.07-0.28) for males in the Fiix-PT group and 0.20 (0.09-0.35) for males in the PT group, 25% more in the PT group (Figure 7. a). The median was significantly higher in the PT arm for both males and females ($p < 0.0001$ for both genders). The median was higher for females than males in the PT arm ($p = 0.0011$). The difference was not significant between genders in the Fiix-PT arm.

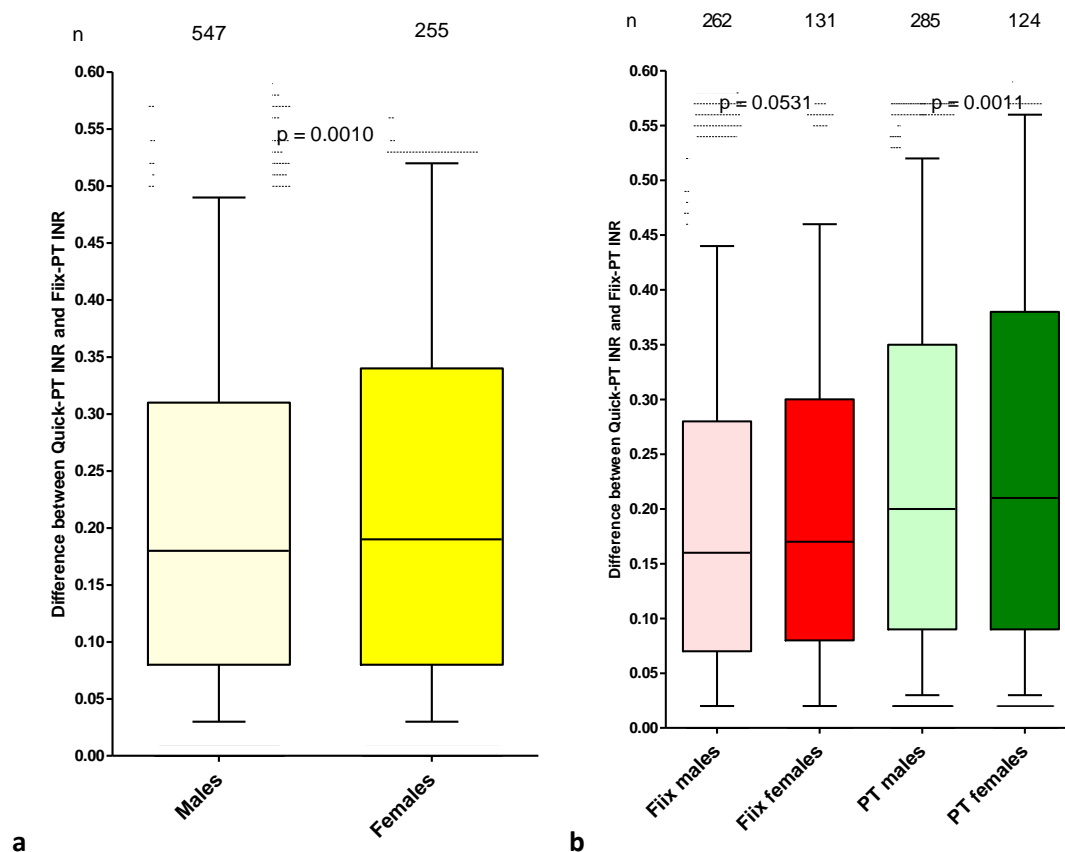


Figure 7. The effect of FVII

The left panel (a) shows the effect of FVII in males and females and the right panel (b) shows the effect of FVII in males and females subgrouped according to study arms. Fiix-PT denotes Fiix-prothrombin time and PT denotes Quick prothrombin time. Median, interquartile range and 10-90th percentiles for each group based on the difference between Quick-PT INR and Fiix-PT INR for each sample which might reflect the effect of FVII. Mann-Whitney non-parametric test was used.

3.3 Outcome parameters in relation to age

3.3.1 Time in therapeutic range and variance growth rate

The TTR was 77.4, 81.9, 83.5 and 77.1% in the 1st, 2nd, 3rd and 4th age quartiles, respectively, in the Fiix-PT arm ($p = 0.0025$ by ANOVA, figure 8. b). The TTR was 80.2, 76.7, 80.2 and 77.8% in the 1st, 2nd, 3rd and 4th age quartiles, respectively in the PT arm ($p = 0.3900$ by ANOVA). There was a significant difference in the TTR between the Fiix-PT and PT arms in the 2nd and 3rd quartiles ($p = 0.0166$ vs $p = 0.0345$, respectively) but not the other quartiles. There was not a significant difference between any of the groups when the PT arm had a higher numeric TTR value. Figure 8. A, which shows TTR for the total Fiix-PT arm and PT arm, is shown for comparison to Figure 8. b. There was a significant difference in INR VGR between the 2nd quartiles in the Fiix-PT and PT arms (0.18 vs 0.29, $p = 0.0068$) but not the other quartiles.

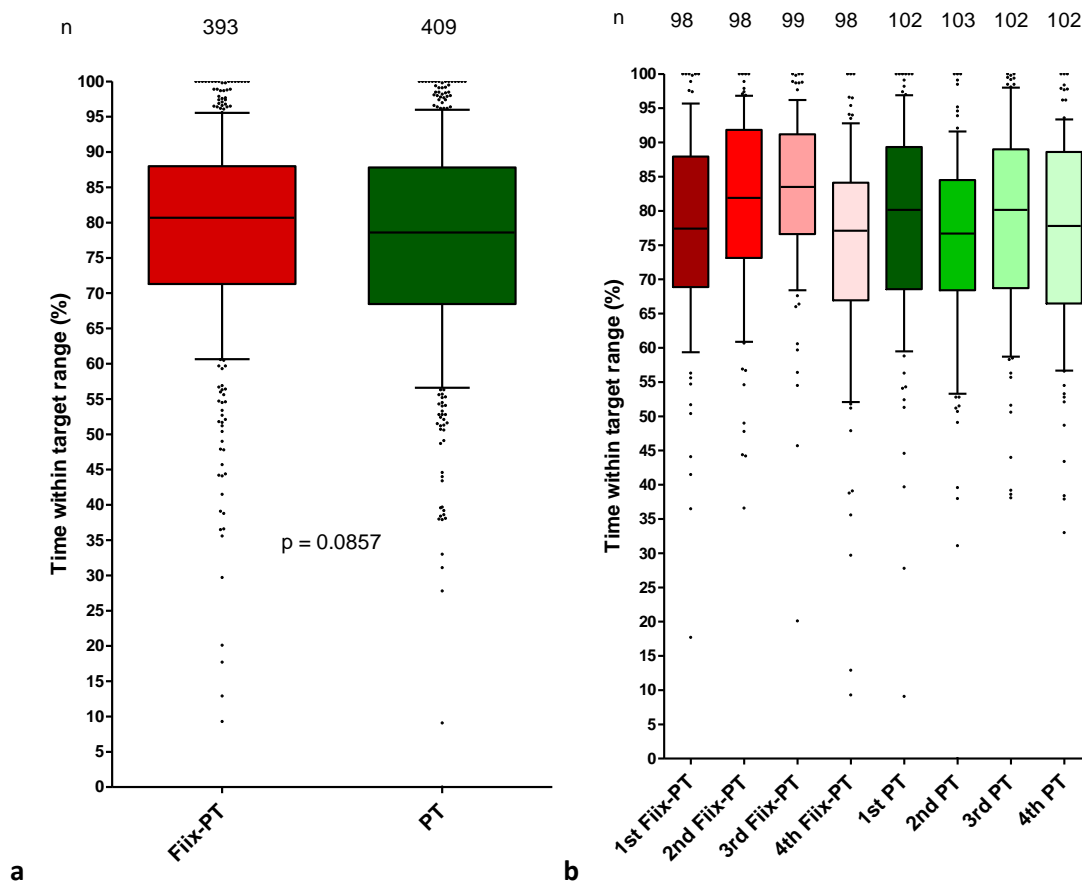


Figure 8. Time in therapeutic range (TTR)

The left panel (a) shows TTR for the total Fiix-PT arm and PT arm and the right panel (b) shows TTR by age divided into quartiles with 1st denoting the youngest quartile, 2nd the second youngest quartile, 3rd the second oldest and 4th the oldest quartile. Fiix-PT denotes Fiix-prothrombin time and PT denotes Quick prothrombin time. Median, interquartile range and 10-90th percentiles for each group based on TTR for each patient. Mann-Whitney non-parametric test was used in the left panel (a).

3.3.2 Daily dose and dose changes

The daily dose decreased with age in both arms ($p < 0.0001$ by ANOVA in both arms, Figure 9). The number of dose changes per monitoring test were 0.27, 0.25, 0.25 and 0.27 in the 1st, 2nd, 3rd and 4th age quartiles, respectively in the Fiix-PT arm ($p = 0.1775$ by ANOVA, Figure 10). The dose changes per monitoring test was 0.27, 0.30, 0.27, 0.28 in the 1st, 2nd, 3rd and 4th age quartiles, respectively in the PT arm ($p = 0.3490$ by ANOVA). There was a significant difference in dose changes per test between the Fiix-PT and PT arms only between the 2nd quartiles ($p = 0.0182$).

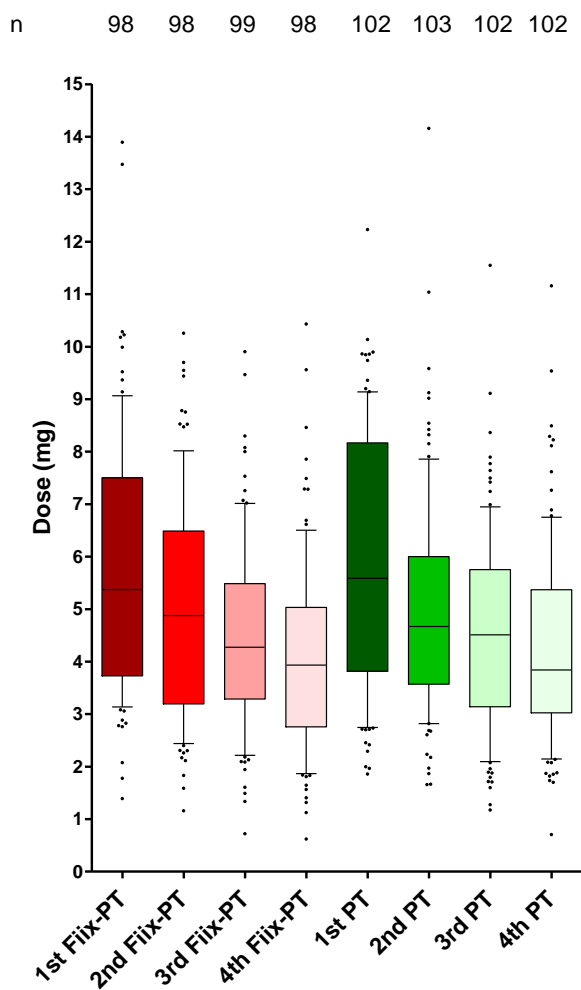


Figure 9. Daily warfarin dose by age

The figure shows daily warfarin dose in mg by age divided into quartiles with 1st denoting the youngest quartile, 2nd the second youngest quartile, 3rd the second oldest and 4th the oldest quartile. Median, interquartile range and 10-90th percentiles for each group based on daily dose in mg for each patient. Fiix-PT denotes Fiix-prothrombin time and PT denotes Quick prothrombin time.

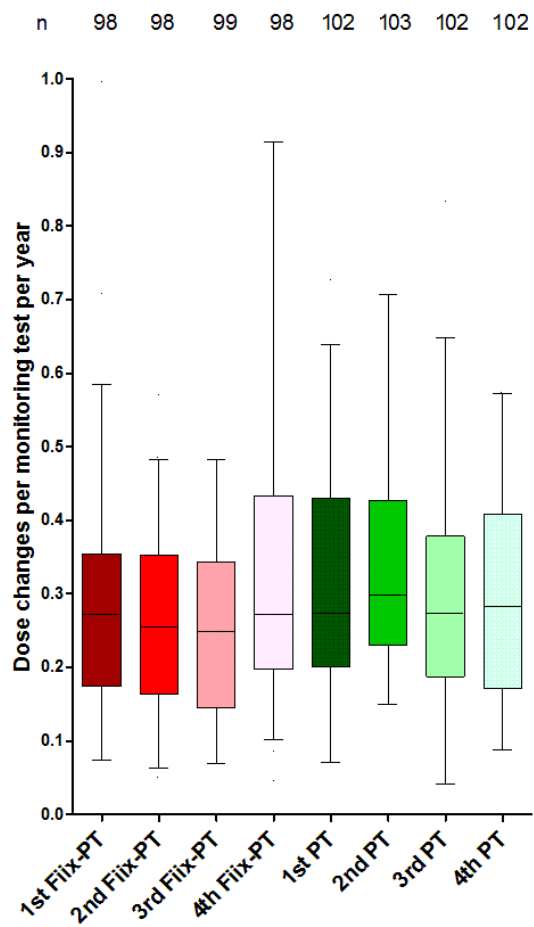


Figure 10. Dose changes per monitoring test per year.

The figure shows dose changes per monitoring test per year by age divided into quartiles with 1st denoting the youngest quartile, 2nd the second youngest quartile, 3rd the second oldest and 4th the oldest quartile. Fiix-PT denotes Fiix-prothrombin time and PT denotes Quick prothrombin time. Median, interquartile range and 10-90th percentiles for each group based on dose changes per monitoring test per year for each patient.

4. Discussion

In this subgroup analysis of the Fiix-study the difference between anticoagulation therapy monitored with Fiix-PT compared to PT-INR depending on age and gender was analyzed in warfarin experienced patients with AF. The clinical safety and efficacy could not be estimated sufficiently due to lack of statistical power but the number of events was grossly similar between the study groups. There was possibly a difference in ATE but it was not significant for this subgroup of experienced AF patients only. The analysis is therefore primarily based on surrogate outcome parameters that assess the stability and intensity of the anticoagulation.

4.1 Summary of study findings

In this study we found that the observed improvement in warfarin management during Fiix-INR monitoring in patients on long-term warfarin for AF was surprisingly attributable solely to an improvement of treatment in females. The detailed findings are discussed below.

4.2 Study findings according to gender

4.2.1 Test frequency, number of tests and fraction within defined range

Overall, with Fiix-PT monitoring anticoagulation was more stable as manifested by a higher fraction of tests within the INR target range and a lower fraction with INR <2 than during standard monitoring. However, this difference was all due to differences in anticoagulation in females whereas males were identically anticoagulated in both monitoring arms. Compared to males, females in both monitoring arms had higher test frequency per patient, shorter interval between tests. However, females in the Fiix-PT group also had fewer tests with INR < 2 than did females in the PT group. Previous studies suggest that when the INR value is out of range in female patients it is usually due to too low INR. Females may therefore be at increased risk of TE.^{57,58} In our study females in the PT monitoring arm were more often outside the target INR range than males and both sexes in the Fiix arm, mainly due to low INR. Based on similar findings for the Quick-PT it has been suggested that a higher therapeutic range is needed in females than in males to decrease the risk of ischemic stroke.⁵⁸ However, our data with Fiix-PT monitoring suggests that the therapeutic range likely would be identical in males and females although we do not have clinical data to support this.

4.2.2 Time in therapeutic range and variance growth rate

There was a trend to a higher TTR in the total Fiix-PT monitoring arm compared to the PT arm, although the difference was not significant. However, the TTR in the current subgroup analysis was lower than in the subanalysis of AF patients in the Fiix study, the most likely explanation being that the current analysis was based on intention-to-monitor (ITM) data, which also includes data from periods where patients were not monitored or dosed according to the protocol or temporarily discontinued from warfarin therapy.⁵⁵ In the Fiix trial where a significant difference was found between the monitoring arms the analysis was based on per protocol (PPC) data collected from the patients only when on treatment. A confirmatory PPC analysis of the current data should therefore be done on the

current material. It should also be noted that the current analysis was based on warfarin experienced patients only and that also could explain part of the difference.

Based on lower TTR results, again the anticoagulation appeared to be of worse quality in females (75.3%) than in males (80.3%) in the PT arm but no gender difference was observed in the Fiix-PT arm (TTR 80.7% vs 80.3%, respectively). Lower TTR in females has been reported previously during standard monitoring of warfarin.^{59,60} The reason for this gender difference during standard monitoring is not known but it is interesting that with Fiix-PT monitoring no such difference occurred suggesting that the explanation lies in part within the influence of FVII on the traditional PT.⁶¹ In support of this our result showed that females in both monitoring arms had significantly higher VGR than males (VGR 0.25-0.30 vs 0.18-0.21) and that the VGR may have been higher in the PT arm albeit not statistically significant (VGR 0.30 vs 0.25, $p = 0.12$).

4.2.3 Daily warfarin dose and dose changes

There were fewer dose changes per test per year in the Fiix-PT arm than the PT arm, largely attributable to more dosing instability in females in the PT arm. Dosing stability was similar in females in the Fiix monitoring group and in males in both groups. The fact that there are fewer dose changes per test in the Fiix-PT arm may indicate that fewer tests need to be done with the Fiix-PT test than the PT test. The daily doses of warfarin were higher in males than in females in both monitoring arms. This confirms findings from previous studies that have suggested that the maintenance dose for females is usually lower than the dose required for males to keep the INR value within the therapeutic range.^{8,54}

Surprisingly, females in the Fiix-PT monitoring group received a lower daily warfarin dose than did females in the PT group. That is interesting since the fraction of tests that had INR higher than 3 is identical in females in both arms. It seems that females that were monitored according to Quick-PT were overdosed which should have resulted in higher fraction of tests which were above 3 than in females in the Fiix-PT group. The doses did not differ according to monitoring method in males.

Previous studies have concluded that the risk of TE in females with AF not on warfarin therapy is greater than that of males.⁶² Due to low prevalence of events in this subgroup study no interpretation on a clinical event difference in the groups can be done. The fluctuations in the INR and dosing instability are probably more important than the daily dose when it comes to events.

4.2.4 Activity of FVII

The only practical difference between the Fiix-PT and the PT during monitoring of warfarin is the measurement of the effect of FVII in the test sample with the PT. This difference was investigated further by comparing medians of the difference between the Fiix-INR and PT-INR in all patient samples. Both of the sexes in the PT arm had higher medians in the positive difference between Fiix-PT INR and Quick-PT INR than both sexes in the Fiix-PT arm which may indicate that FVII is having more effect on the INR values in the PT arm than in the Fiix-PT arm. That suggests that the fluctuations of FVII are having an effect on the PT measurements and doses in the PT arm. When the PT is measured with Quick-PT on samples from patients dosed according to Fiix-PT INR, then FVII is measured but the dosing is not affected by its fluctuations so the Quick-PT also gets more stable. The fact that females in the PT group had a higher median difference than males means that FVII could be

having more effect in them or rather fluctuations in FVII might be greater in females. Possible reasons for this difference in influence of FVII on the monitoring test could be more sensitivity to vitamin K or warfarin in females or variance between the sexes in the effect of vitamin K on the coagulation factors. FVII would be most affected of all the coagulation factors and it would probably be the first to be affected due to its short half-life. This means that females could have more variation in the activity in all of the VKD coagulation factors than males. That may explain why females in the PT group had less stability than all of the other groups since FVII's reactions to changes in vitamin K or warfarin are much more than the reaction of the other VKD factors, because of its short half-life. The interquartile and 10-90% ranges were broadest in females in the PT group which might indicate that there is more variation in FVII between individual females.

4.2.5 Difference between males and females

If the intake of vitamin K in females were more variable than in males it could cause increased fluctuations in the VKD factors. This would first be reflected by changes in FVII and in the standard PT. Indeed, studies have shown that if the vitamin K intake is variable it may affect the INR and that providing a stable vitamin K intake, which can be achieved by using guidelines, increases tests that are in target INR range.^{63,64} Patients on VKA therapy may also be more sensitive than individuals not on VKA therapy to changes in vitamin K intake and multivitamins containing K (1)-vitamin may affect the INR in them.^{64,65} Studies that have examined the difference in vitamin K intake habits suggest that females ingest more amount of vitamin K daily than males, but despite that the absorption, plasma concentration and function of vitamin K does not differ between males and females.^{66,67} Females are also more likely to use more than one medication at a time.⁶⁸ Other studies have shown that mental health is associated with food intake in females but not in males. Less frequent consumption of vegetables is associated with increased stress, and vice versa, in females but not in males.⁶⁹ One study measured that the average daily frequency (evaluated with a semi-quantitative food frequency questionnaire) of vegetables in females ranged from 0.91-2.43 but 0.53-0.72 in males.⁷⁰ One study found that the variation in nutrition intake between subjects was less in females than males and the variation between individuals was less than the day-to-day variation within individual.⁷¹ These studies suggest that the habit or pattern of vitamin K intake could vary between males and females.

If females were more sensitive to changes in the amount of vitamin K in the body than males it could possibly also explain the difference in the fluctuations. Research on the effect of changes in vitamin K in rats suggest that female rats have 2-3 times more hepatic menaquinones (vitamin K) than male rats on the same diet. The resistance to vitamin K deficiency is greater in female rats than male rats and female rats have to be on vitamin K deficient diet for a longer period than males for changes to occur in levels in FVII and PIVKA (descarboxy prothrombin).^{72,73} Female rats need less vitamin K than male rats.⁷³ According to human studies, females have higher percent undercarboxylated osteocalcin, a vitamin K biochemical marker. That means that they could possibly have less vitamin K activity than males.⁷⁴

The reaction to warfarin or the habit of warfarin intake could also vary between the genders and explain the difference. The proportional daily warfarin dose is equally increased in both genders in the presence of the rs2108622 polymorphism.⁷⁵ There are conflicting results on the adherence to

warfarin of males and females with some showing more non-adherence in males and others concluding that females are less adherent to long-term therapy.^{68,76} The latter, could explain our results, since if females are less adherent it could also indicate irregular warfarin intake and increased fluctuation in FVII.

4.3 Study findings according to age

The Fiix-INR did not seem to change the median daily warfarin dose compared to the PT-INR in any of the age groups but a similar progressive decrease in daily dose was observed in the upper three quartiles. It is well known that the VKA dose decreases with increasing age and that was very clear in both arms in this analysis.^{8,54} This is usually explained by more drug interactions in the elderly although with ageing the clearance of warfarin decreases.^{77,78} Some studies, however, suggest that warfarin's pharmacokinetics does not vary between age groups. They suggest that the lower requirement in elderly may rather be explained by decreased hepatic metabolism of warfarin.⁷⁹

The two intermediate age quartiles in the Fiix-PT arm had the highest TTR, significantly higher than the youngest and the oldest age groups in the Fiix-PT arm which had a TTR similar to all of the age groups in the PT arm. This can be assumed since there was not a significant difference between these quartiles in the Fiix-PT arm and corresponding quartiles in the PT arm or any quartiles within the PT arm. The reason for why the 3rd and especially the 2nd quartiles, which both include patients around 70 years old, were the only ones that got increased stability with the Fiix-INR is unknown. However, younger patients have been shown to have lower TTR and some suggest the reason being difficulty in coordinating frequent monitoring and dose changes while working a full-time job.⁸⁰ Non-adherence to warfarin does not correlate with older age but it does correlate with number of hospitalizations and hospitalization frequency increases with increasing age.^{81,82} Non-adherence could therefore be more in younger patients as well as in the oldest group.⁷⁶

If there were a correlation between increased age and increased fluctuations in FVII it would most likely mean decreased anticoagulation intensity and stability with the Quick-PT test with age but this was not observed. As vegetable consumption decreases with age which could mean reduced vitamin K intake.⁸³ If the vitamin K intake becomes more irregular with age it would mean decreased stability with the Quick-PT in contrast to the Fiix-PT which could lead to more dose interference. However, we did not confirm this in a convincing manner.

4.4 Study strengths and limitations

The Fiix-study was prospective, double blind and randomized which most likely reduces the risk of some biases and should correct some confounders. There was not a significant difference between the patient characteristics in the two arms. The study conduct, dosing and the statistical analysis were all done independently. Since this subgroup study was only based on warfarin experienced patients who have AF these results should be applied to that group specifically. Some further analysis needs to be done to study the difference between males and females and age that are not experienced and have other indications than AF. The difference between the two study arms as a whole was studied in the Fiix-trial.⁵⁵

The study population was not large limiting the ability to assess clinical endpoints during subgroup analysis due to lack of statistical power. This was the main reason for why conclusions about clinical safety (bleeding) and efficacy (TE) could not be drawn from the subgroups study in this project. The overall results with Fiix-PT monitoring, however, suggest improved anticoagulation stability over that attained with PT which is likely to translate into improved efficacy and safety.

All randomized clinical trials come with the risk of not representing reality completely due to patient selection. Our results may therefore not apply to every warfarin experienced patient with AF although in this study few exclusion criteria were actually used. The study does not represent the oldest and the weakest patients residing in nursing homes. There were no pediatric or adolescent participants so the results should not be inferred for those populations. The youngest age group (1st quartiles) had a broad range of age but the majority was over 60 years old. This age distribution reflects the patient group on long-term warfarin therapy, but since the objective of this study was to analyze the tests in regard to age, these young patients could distort the results that would really be found in patients around 60 years old.

Finally, all of the patients were monitored according to the AMC protocol at Landspitali and may therefore not completely reflect practices at other monitoring centers. The analysis was based on ITM patients which means that some of them did not follow the protocol during the whole study period. The ITM analysis might in some way be a strength since this rather reflects the reality for some patients but also a disadvantage since periods when warfarin was not ingested or not monitored and dosed according to the protocol are included.

4.5 Conclusion

The Fiix-PT test improved the stability of anticoagulation therapy in females with AF on long-term warfarin therapy. No significant difference was observed in the stability of anticoagulation in males depending on monitoring method. Difference in fluctuations in FVII between the sexes could possibly explain this as the new test is not affected by the variable FVII. It is not clear why the stability of monitoring with these two tests differ between the genders and this needs further investigation.

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Appendix 1

Supplementary tables

Supplementary table 1. Patient characteristics on enrollment according to gender.

	<i>Fiix –PT arm^a</i>	<i>Fiix-PT arm^a</i>	<i>PT arm^a</i>	<i>PT arm^a</i>	<i>P-value^b</i>	<i>P-value^b</i>
	<i>males</i>	<i>females</i>	<i>males</i>	<i>Females</i>	<i>Within Fiix arm / Within PT arm</i>	<i>Fiix vs. PT</i>
						<i>Males / Females</i>
Number	265	131	292	127		
AF without prior arterial thromboembolic event – no (%)	202 (76.2)	94 (71.8)	222 (76.0)	86 (67.7)	0.4006 / 0.0987	0.9645 / 0.5682
AF with prior cerebral thromboembolic event or TIA – no (%)	61 (23.0)	34 (26.0)	65 (22.3)	40 (31.5)	0.6041 / 0.0598	0.9106 / 0.3974
AF with prior peripheral arterial embolism – no (%)	2 (0.8)	3 (2.3)	5 (1.7)	1 (0.8)	0.4184 / 0.7756	0.5271 / 0.6364
Age in years - median (IQR^c)	72.8 (65.2-79.4)	74.9 (69.5-80.9)	73.6 (66.3-79.7)	76.3 (69.6-82.2)	0.0062 / 0.0070	0.3290 / 0.4792
Years of warfarin treatment prior to enrollment - median (IQR^c)	4.5 (1.6-8.9)	4.1 (1.2-8.2)	4.1 (1.3-8.4)	3.3 (1.1-7.1)	0.2461 / 0.1810	0.1789 / 0.3228
CHA₂DS₂-VASC risk score in AF patients – median (IQR^c)	3 (2-4)	4 (3-5)	3 (2-4)	4 (3-5)	<0.0001 / <0.0001	0.8834 / 0.8131
Percent with score 0 (low TE risk)	17 (6.4)	0	16 (5.5)	0	0.0069 / 0.0158	0.7738 / -
Percent with score 1 (moderate TE risk)	31 (11.7)	2 (1.5)	38 (13.0)	6 (4.7)	<0.0001 / 0.0178	0.7324 / 0.2618
Percent with score ≥2 (high TE risk)- ≥2	217 (81.9)	129 (98.5)	238 (81.5)	121 (95.3)	<0.0001 / 0.0205	0.9951 / 0.2618
Percent with score ≥3	142 (53.6)	110 (84.0)	165 (56.5)	106 (83.5)	<0.0001 / <0.0001	0.5438 / 0.9531
Additional indications for warfarin – no (%)						
Ischaemic heart disease total	3 (1.1)	2 (1.5)	7 (2.4)	2 (1.6)	0.8829 / 0.8673	0.4216 / 0.6364
Acute MI	3 (100.0)	2 (100.0)	7 (100.0)	2 (100.0)	- / -	- / -
Other IHD	0	0	0	0	- / -	- / -
Congestive heart failure	1 (0.4)	0	0	0	0.7188 / -	0.9613 / -
Atrial septal defect	0	0	0	0	- / -	- / -
Artificial heart valves	1 (0.4)	1 (0.8)	2 (0.7)	0	0.8076 / 0.8699	0.9328 / 0.7065
Rheumatic mitral valve disease (mitral stenosis)	0	1 (0.8)	0	0	0.7188 / -	- / 0.9876

Venous thromboembolism (VTE)						
	12 (4.5)	9 (6.9)	13 (4.5)	7 (5.5)	0.4592 / 0.8271	0.8717 / 0.8461
Deep vein thrombosis	6 (50.0)	3 (33.3)	3 (23.1)	2 (28.6)	- / -	- / -
Pulmonary embolism	6 (50.0)	6 (66.7)	10 (76.9)	5 (71.4)	- / -	- / -
Pulmonary hypertension	0	0	0	0	- / -	- / -
Associated conditions – no. (%) :						
Smoker	29 (10.9)	10 (7.6)	26 (8.9)	11 (8.7)	0.3893 / 0.9149	0.5070 / 0.9409
High blood pressure	165 (62.3)	87 (66.4)	193 (66.1)	82 (64.6)	0.4862 / 0.8486	0.3931 / 0.8566
Ischaemic heart disease	84 (31.7)	30 (22.9)	103 (35.3)	21 (16.5)	0.0889 / 0.0002	0.4222 / 0.2597
Peripheral vascular disease	15 (5.7)	6 (4.6)	15 (5.1)	8 (6.3)	0.8313 / 0.8051	0.9320 / 0.7380
History of congestive heart failure	44 (16.6)	15 (11.5)	48 (16.4)	16 (12.6)	0.2282 / 0.3917	0.9508 / 0.9267
Diabetes mellitus	47 (17.7)	14 (10.7)	36 (12.3)	15 (11.8)	0.0929 / 0.9892	0.0948 / 0.9294
Cancer	44 (16.6)	19 (14.5)	45 (15.4)	27 (21.3)	0.6954 / 0.1876	0.7888 / 0.2096
- active cancer chemotherapy	8 (18.2)	1 (5.3)	10 (22.2)	1 (3.7)	- / -	- / -
Select drug use – no. (%):						
Acetylsalicylic acid	68 (25.7)	25 (19.1)	76 (26.0)	19 (15.0)	0.1846 / 0.0029	0.9985 / 0.4747
Clopidogrel	5 (1.9)	0	6 (2.1)	0	0.2696 / 0.2381	0.8709 / -
Non-steroidal antiinflammatory drugs	19 (7.2)	22 (16.8)	26 (8.9)	17 (13.4)	0.0054 / 0.2247	0.5522 / 0.5551
Amiodarone	32 (12.1)	16 (12.2)	37 (12.7)	12 (9.4)	0.9013 / 0.4366	0.9328 / 0.6075
H ₂ blockers and proton pump inhibitors	54 (20.4)	33 (25.2)	49 (16.8)	30 (23.6)	0.3373 / 0.1312	0.3258 / 0.8821
Any other drugs	251 (94.7)	129 (98.5)	281 (96.2)	119 (93.7)	0.1298 / 0.3737	0.3737 / 0.0963

^a Fiix-PT denotes Fiix-prothrombin time and PT denotes Quick prothrombin time. ^b Mann-Whitney non-parametric test was used for numerical rows and Fisher's exact test for categorical values. ^c IQR denotes interquartile (25-75%) range.

Percentages do not total 100 due to presence of more than one indication in some patients or rounding of numbers.

Supplementary table 2. Treatment description according to monitoring method and gender.

	<i>Fiix –PT arm^a Males</i>	<i>Fiix –PT arm^a Females</i>	<i>PT arm^a Males</i>	<i>PT arm^a Females</i>	<i>P-value^b Within Fiix arm / Within PT arm</i>	<i>P-value^b Fiix vs PT Males / Females</i>
	<i>n = 265</i>	<i>n = 131</i>	<i>n = 292</i>	<i>n = 127</i>		
	<i>n (%)</i>	<i>n (%)</i>	<i>n (%)</i>	<i>n (%)</i>		
Observation time						
Total intention-to-monitor observation years	349	172	375	159		
Intention-to-monitor observation years per patient – median (IQR ^c)	1.5 (1.1-1.6)	1.4 (1.1-1.6)	1.4 (1.0-1.6)	1.4 (1.0- 1.6)	0.4242 / 0.3734	0.1847 / 0.3028
Number of monitoring tests - no	5393	2991	5839	2963		
Discontinuation from study – no (%)						
Total	43 (16.2)	16 (12.2)	46 (15.8)	16 (12.6)	0.3654 / 0.4926	0.9710 / 0.8687
Anticoagulation discontinued	17 (6.4)	2 (1.5)	15 (4.0)	4 (2.5)	0.0585 / 0.8689	0.6419 / 0.6516
Voluntary discontinuation	4 (1.5)	1 (0.8)	1 (0.3)	2 (1.6)	0.8829 / 0.9784	0.3132 / 0.5131
Switched to direct oral anticoagulant	9 (3.4)	5 (3.8)	8 (2.7)	5 (3.9)	0.9395 / 0.7315	0.8390 / 0.7852
INR target changed	1 (0.4)	2 (1.5)	8 (2.7)	0	0.5318 / 0.1423	0.0612 / 0.4915
Lost to follow-up	0	0	0	0	- / -	- / -
Other reason	12 (4.5)	6 (4.6)	14 (4.8)	5 (3.9)	0.8041 / 0.9359	0.9582 / 0.9581

^a Fiix-PT denotes Fiix-prothrombin time and PT denotes Quick prothrombin time. ^b Mann-Whitney non-parametric test was used for numerical rows and Fisher's exact test for categorical values. ^c IQR denotes interquartile (25-75%) range.

Percentages do not total 100 due to rounding of numbers.

Appendix 2

Segavarnir – stuttar leiðbeiningar (12/10/2010)

Stefna skal að því, 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 stöðugt. 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 oft og halda síðan áfram meðferð með viðeigandi skömmtum þegar INR er komið niður í meðferðarmörk. Ef hækkun á INR er aðeins lítilsháttar (u.p.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)).