Influence of Fiix-prothrombin time (Fiix-INR) on stability of warfarin anticoagulation in comparison to the Quick prothrombin time (INR)

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Lokaverkefni til BS gráðu i Læknisfræði
Háskóli Íslands
Heilbrigðisvísindasvið
Læknadeild
Áhrif Fiix-próþrombíntíma (Fiix-INR) á stöðugleika warfarínmeðferðar samanborið við próþrombíntíma (INR)

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Læknadeild
Heilbrigðisvísindasvið Háskóla Íslands
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Abstract

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\textbf{Introduction:} Prothrombin time (PT) is used to monitor warfarin therapy, by measuring the influence of coagulation factors II, VII and X on the clotting time. Experiments suggest that measuring the influence of factors II and X only may more accurately reflect the anticoagulation. Therefore a new monitoring test has been developed; Fiix-PT, which is only sensitive to the activity of factors II and X. The objective of this study was to compare the stability of anticoagulation when patients were dosed according to Fiix-PT measurements on the one hand, and according to PT on the other hand.

\textbf{Methods:} The Fiix-study was a prospective, double-blinded and randomized controlled trial with duration of two years. The participants, dosing staff and event assessors were blinded to the monitoring method. The participants were outpatients over the age of 18 with a therapeutic range of INR 2-3. In the Fiix group (n=564) warfarin was dosed according to Fiix-PT (Fiix-INR) and the control group (n=561) was dosed according to PT (INR). Patients were defined as warfarin-naïve for six months after initiating warfarin therapy, and all others were defined as warfarin-experienced. The quality of warfarin-therapy was evaluated with regards to (a) time in therapeutic range (TTR), (b) number of dose adjustments, (c) frequency of INR monitoring and (d) number of dose adjustments per monitoring test.

\textbf{Results:} Median follow-up time was 1.6 years in both groups. The total number of monitoring tests was 12,017 in the Fiix-group and 12,540 in the PT-group (a 4.2% difference). The proportions of INR measurements within the therapeutic range were 65.1\% and 62.6\% (p<0.0001), respectively. Individuals in the Fiix study arm had significantly higher TTR throughout the follow-up period. The TTR (median) increased more in the Fiix study arm, from 67.1\% at study initiation to 86.7\% at the end of study versus 70.2\% to 78.2\%, respectively, in the PT study arm (p<0.01). There were also fewer dose changes (51\% of individuals in the Fiix study arm had four or fewer dose changes in one year, versus 42\%, p=0.0039), and fewer dose adjustments for each INR measurement (0.20 versus 0.28 in the last follow-up interval, p<0.001, a 29\% dose adjustment reduction). Over the course of the study, the time between INR measurements increased from 15 days to 21 days in the Fiix arm and from 14 to 17 in the control arm. Experienced warfarin users displayed higher TTR than those defined as naïve.

\textbf{Conclusions:} Fiix-PT seems to measure anticoagulation more accurately than PT, which results in higher TTR, fewer dose adjustments and fewer monitoring tests.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACCP</td>
<td>American College of Chest Physician</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
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<td>AF</td>
<td>Atrial fibrillation</td>
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<tr>
<td>AMC</td>
<td>Anticoagulation management center/clinic</td>
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<tr>
<td>aPTT</td>
<td>Activated partial thromboplastin time</td>
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<tr>
<td>AT</td>
<td>Antithrombin</td>
</tr>
<tr>
<td>CYP450</td>
<td>Cytochrome P450</td>
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<tr>
<td>DOACS</td>
<td>Direct oral anticoagulant agents</td>
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<tr>
<td>FII</td>
<td>Factor II, prothrombin</td>
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<tr>
<td>FIIa</td>
<td>Activated factor II, thrombin</td>
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<tr>
<td>Fiix-PT</td>
<td>Fiix-prothrombin time</td>
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<tr>
<td>GI</td>
<td>Gastro-intestinal</td>
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<tr>
<td>INR</td>
<td>International normalized prothrombin time ratio</td>
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<tr>
<td>ISI</td>
<td>International sensitivity index</td>
</tr>
<tr>
<td>NSAID</td>
<td>Non-steroidal anti-inflammatory drug</td>
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<tr>
<td>OA</td>
<td>Oral anticoagulation</td>
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<tr>
<td>P&amp;P</td>
<td>Prothrombin &amp; Proconvertin, PP, Owren PT</td>
</tr>
<tr>
<td>PPP</td>
<td>Platelet poor plasma</td>
</tr>
<tr>
<td>PT</td>
<td>Prothrombin time, Quick PT</td>
</tr>
<tr>
<td>ROTEM</td>
<td>Rotational thromboelastography</td>
</tr>
<tr>
<td>TE</td>
<td>Thromboembolism</td>
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<tr>
<td>TF</td>
<td>Tissue factor</td>
</tr>
<tr>
<td>TFPI</td>
<td>Tissue factor pathway inhibitor</td>
</tr>
<tr>
<td>TIA</td>
<td>Transient ischemic attack</td>
</tr>
<tr>
<td>TMP-SMX</td>
<td>Trimethoprim-Sulfamethoxazole</td>
</tr>
<tr>
<td>TP</td>
<td>Thromboplastin</td>
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<tr>
<td>TTR</td>
<td>Time in therapeutic range</td>
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<tr>
<td>VKA</td>
<td>Vitamin K antagonist</td>
</tr>
<tr>
<td>VKD</td>
<td>Vitamin K-dependent</td>
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<td>VKOR</td>
<td>Vitamin K oxide reductase</td>
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<tr>
<td>VKORC1</td>
<td>VKOR complex 1</td>
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<tr>
<td>VTE</td>
<td>Venous thromboembolism</td>
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<tr>
<td>vWF</td>
<td>von Willebrand Factor</td>
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<td>WHO</td>
<td>World Health Organization</td>
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1 Introduction

Anticoagulation is widely used for the prevention of thrombosis and treatment of thromboembolic disorders, including patients with prosthetic heart valves, atrial fibrillation (AF) and venous thromboembolism (VTE); deep vein thrombosis (DVT) and pulmonary embolism (PE). About 50% of deaths in western societies are caused by thromboembolic disorders, and they may leave major disabilities in survivors. The majority of these events can be prevented by using anticoagulant drugs, but at the cost of increased risk of bleeding.

Patients with AF are at high risk of cerebrovascular events, especially embolic events, and the recurrence rate is high in those with a prior history of stroke or transient ischemic attack (TIA). Warfarin, the most commonly used vitamin K antagonist (VKA), has been shown to lower the risk of thromboembolic stroke in patients with AF by 64%-79% compared to placebo [1, 2]. On treatment with warfarin, these patients benefit from more risk reduction than obtainable with aspirin or no treatment [3]. However, the risk of bleeding increases as the intensity of anticoagulation increases, and the risks and benefits of anticoagulant therapy must be weighed for each patient [4].

About 1-2% of all western populations are currently on long-term anticoagulant therapy, the most common being VKAs but lately there has been an increased use of new direct oral anticoagulant agents (DOACs) such as the direct thrombin inhibitor dabigatran, and the factor Xa (FXa) inhibitor rivaroxaban [5]. The unmonitored use of dabigatran and rivaroxaban has been tested against monitored warfarin, e.g. in the RE-LY and EINSTEIN studies, respectively. These drugs have been shown to be non-inferior to warfarin and to have some benefits over warfarin therapy including a possible reduction in the incidence of intracranial hemorrhage, but with some limitations such as higher cost and lack of a reversal agent. However, when warfarin therapy is well controlled, dabigatran has not been shown convincingly to be superior to warfarin [6]. Furthermore, both dabigatran and rivaroxaban increased the incidence of gastrointestinal (GI) bleeding during clinical trials [7-9] and the frequency of myocardial infarction was increased in patients treated with dabigatran [6].

The VKAs (coumarin drugs) have been the main drugs for oral anticoagulation (OA) for the past sixty years. Dicoumarol was the main drug used in
anticoagulant therapy in Iceland from its introduction in 1956 until 1992. Since then all new patients have been prescribed warfarin, although dicoumarol is still being used as a substitute drug for occasional patients with rare adverse reactions to warfarin [10].

The VKAs have a narrow therapeutic range, which can make their use in clinical practice a challenge. As their metabolism and dose response is affected by diet, genetic variables and medication, patients’ reactions to these drugs can be unpredictable. Therefore, it is important to monitor patients receiving VKAs both closely and regularly.

1.1 Blood coagulation

Hemostasis involves complex interactions between blood vessels, platelets, and the coagulation proteins and each of these components interact with the fibrinolytic system. If any of these elements are missing or dysfunctional, abnormal bleeding or thrombosis may occur.

The initial response of small blood vessels to injury is arteriolar vasoconstriction, which reduces local blood flow. The reduced flow acts to reduce blood loss, and to promote formation of the platelet-fibrin plug. Blood platelets become activated, which leads to their adhesion to the vessel wall at the site of injury, and to their aggregation. An occlusive platelet mass builds up and forms the primary hemostatic plug. The platelet plug has to be stabilized by fibrin, or otherwise it may be washed away when vasoconstriction reverses [11].

The endothelium has the ability to prevent coagulation under normal physiologic conditions. Unneeded coagulation is prevented by promoting the functions of circulating anticoagulants, by expression of thrombomodulin and heparan sulfate on healthy endothelium. By synthesizing enzymes that break down adenosine diphosphate (ADP), the endothelium can inhibit platelet activation, and it can also inhibit platelet aggregation by secreting prostacyclin and nitric oxide [12].

When the endothelium is disrupted, components of the blood make contact with substances that favor clot (hemostatic plug) formation. When exposed, collagen, von Willebrand Factor (vWF) and fibronectin in the extracellular matrix promote platelet adhesion, and tissue factor (TF) activates the coagulation pathway [12].
Under pathological conditions, the endothelium may also lose its antithrombotic properties without losing integrity of the basement membrane. Stimulation by thrombin, stress, oxidants, endotoxins or cytokines can induce the endothelial cells to express TF, which initiates the coagulation pathway, impairs fibrinolysis by secretion of plasminogen activator inhibitor (PAI-1), and reduces the expression of thrombomodulin [12].

1.1.1 Initiation of coagulation

In normal hemostasis, thrombin generation is located on the surface of activated platelets that form the primary hemostatic plug. The generation of thrombin is highly regulated to locally achieve rapid hemostasis after injury, without causing uncontrolled systemic thrombosis [12].

TF is expressed on the surface of fibroblasts in subendothelial tissue and on damaged or stimulated cells such as monocytes, macrophages and endothelial cells. A disrupted endothelium exposes TF-bearing stromal cells to circulating blood. Circulating activated (a) FVII (FVIIa) binds to exposed TF to form the TF-FVIIa complex or the “extrinsic tenase”, which activates FX to FXa and small amounts of FIX to FIXa. FXa and its co-factor FVa activate prothrombin (FII) to generate a burst of thrombin (FIIa), inducing local platelet activation and aggregation and activation of FV and FVIII on the platelet surface [12]. The pathway (Figure 1) described above

![Figure 1 - The extrinsic pathway of coagulation](image)

(A) The extrinsic pathway is started by the TF-VIIa complex. (B) The extrinsic pathway takes place on TF-bearing cells [13].
has traditionally been called the “extrinsic pathway,” the function of which is monitored by the PT assay.

Figure 2 - The intrinsic and extrinsic tenase.

The intrinsic tenase (FIXa-FVIIIa) and the extrinsic tenase (TF-FVIIa) both activate FX to FXa, which forms the prothrombinase with FVa [14].

Tissue factor pathway inhibitor (TFPI) inactivates FXa rapidly, and subsequently also inhibits the TF-FVIIa complex. TFPI and antithrombin (AT), which neutralizes FXa and thrombin, regulate the TF-triggered response and concentrate the coagulation to the site of injury. For the coagulation response to continue, TF must be exposed at a level which overcomes inhibition by TFPI and AT [15].

1.1.2 Amplification and propagation

Amplification of thrombin is acquired by the “intrinsic” tenase, which is comprised of FIXa and FVIIIa, and the aforementioned “extrinsic” tenase or TF-FVIIa complex, which activate FX to FXa (Figure 2) [14].
When sufficient Xa is formed, it forms the prothrombinase complex with FVa, which converts prothrombin (FII) to thrombin (FIIa). Thrombin subsequently activates FXI on the platelet surface, which generates additional FIXa, allowing more FIXa-FVIIIa complexes to form, and to activate additional FX to FXa (Figure 3). Activation of FVa by FXa on the platelet surface induces the continued formation of the prothrombinase complex, generating a burst of thrombin. This process, occurring on activated platelets, forms the “intrinsic pathway,” which is measured by the “activated partial thromboplastin time” (aPTT) [13].

Thrombin converts fibrinogen to fibrin monomers and activates FXIII, which cross-links the fibrin. In a positive-feedback loop, thrombin also activates FXI, FV and FVIII, influencing its own formation [11].

1.2 Vitamin-K antagonists
VKAs interfere with the vitamin K-dependent (VKD) hepatic production of four coagulation factors: FII, FVII, FIX and FX. The VKAs prevent normal gamma-carboxylation of the VKD coagulation factors by interfering with the cyclic interconversion of vitamin K and vitamin K epoxide (Figure 4).

The VKD coagulation factors depend on the gamma-carboxylation for normal procoagulant activity. The proteins require carboxylation for calcium-dependent conformational change that allows binding to cofactors on phospholipid surfaces, and treatment with VKAs such as warfarin results in hepatic production of partially carboxylated and decarboxylated proteins with impaired activity. The VKAs also
inhibit the synthesis of Proteins C, S and Z, which have a role in regulating the coagulation cascade [16].

![Diagram of the vitamin K cycle and Warfarin interactions](image)

**Figure 4 – Warfarin and vitamin K**

*Warfarin inhibits the reduction of oxidized vitamin-K, making it unusable as a co-factor for carboxylation of VKD coagulation factors [5].*

### 1.1.1 Warfarin

Warfarin is water-soluble, absorbs quickly from the GI tract and its blood concentration reaches a maximum around 90 minutes after oral administration. It is a racemic mixture of two isomers with different half-lives; R-warfarin has a half-life of 45 hours while S-warfarin has a half-life of 29 hours and is about 2.7 to 3.8 times more potent than R-warfarin [5]. The drug binds to plasma proteins in the circulation, mainly albumin, and accumulates in the liver, where the two isomers are metabolized by different pathways [17].

S-warfarin is mainly metabolized by the hepatic enzyme CYP2C9 of the cytochrome P450 system, while R-warfarin is metabolized by CYP1A2 and CYP3A4. The dose-response to warfarin is highly dependent on mutations in the gene coding for CYP2C9, which affect the pharmacokinetics of warfarin. The most common
mutations are associated with an impaired ability to metabolize S-warfarin. These mutations (2C9*2 and 2C9*3) increase S-warfarin elimination half-life and patients with one or more of the mutations require lower warfarin doses [5, 18].

As mentioned earlier, warfarin exerts its action by interfering with the cyclic interconversion of vitamin K and vitamin K epoxide by inhibiting the vitamin K oxide reductase (VKOR) enzyme. A number of isoforms of the enzyme exist that conjointly are called the VKOR complex 1 (VKORC1). The most common mutations in the gene coding for the complex affect the sensitivity of the enzymes to inhibition by warfarin, leading to warfarin resistance and requiring higher doses in patients with these mutations [19, 20].

Environmental factors affect the pharmacokinetics of warfarin, such as diet, medication, and disease. Many drugs, which potentiate the effect of warfarin, are known inhibitors of CYP2C9, for example amiodarone, fluconazole, fluvastatin and isoniazid. Quinolones inhibit CYP1A2 and macrolides inhibit CYP3A4 [21]. Other drugs may interfere with warfarin’s anticoagulant effect by inhibiting absorption or increasing clearance and some may potentiate the action of warfarin by competitively binding to plasma albumin or by inhibiting its clearance [22].

Rifampin and carbamazepine potentiate clearance of warfarin by the liver causing warfarin resistance, while metronidazole and trimethoprim-sulfamethoxazole (TMP-SMX) inhibits the clearance of S-warfarin, increasing the antithrombotic effect. Second- and third-generation cephalosporins inhibit cyclic interconversion of vitamin K and thus have an anticoagulant effect like warfarin. Other broad-spectrum antibiotics may promote vitamin-K deficiency in patients by terminating the intestinal vitamin-K-producing flora and in that way increase the effect of warfarin. Drugs like aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs) which inhibit platelet function, increase the risk of warfarin-associated bleeding, not only by inhibition on platelets but also by increasing the risk of gastric erosion [5, 16].

Ginseng reduces the effect of warfarin as well as green tea, green vegetables and other food which contains high amounts of vitamin K. Similarly, inhibited production of VKD coagulation factors as happens in liver failure, or increased catabolism of these factors in hyperthyroidism, potentiates the anticoagulant response to warfarin [5, 16].
1.2.1 Time in therapeutic range and dosing

The dose of VKAs needs to be adjusted to maintain the international normalized prothrombin time ratio (INR) within a therapeutic range, most often with INR target 2.5 and range 2.0-3.0. Time spent above the recommended therapeutic range increases risk of bleeding, while time spent below the range increases risk of thromboembolic complications the drug is supposed to prevent [5].

The time spent within the therapeutic range (TTR) has been shown to be an important indicator of the quality of VKA therapy, which is reflected in the incidence of bleeding or thromboembolic events in those patients with low TTR. The TTR is in turn highly dependent on the quality of dosing. For example, compared to antiplatelet therapy with clopidogrel plus aspirin, when TTR is beneath 65% the advantage of VKA therapy is lost [8]. Poor dose management results in a higher fraction of each patient’s time spent outside the therapeutic range, which is reflected in a lower TTR and a higher risk of bleeding or thromboembolism [5].

During initiation of OA with VKAs, the concentration of each physiologically active VKD coagulation factor is affected at a different rate and to a variable degree, because of the different half-lives of the factors. The half-life of FVII is about 4-8 hours, 24-48 hours for FX, and 48-72 hours for FII. The peak effect of warfarin, which depends greatly on the plasma half-life of FII, occurs around 36 to 72 hours after administration [23].

Evidence suggests that the antithrombotic effect of warfarin mainly depends on its effect on FII and FX. Warfarin begins to have its antithrombotic effect in 6 days, although the anticoagulant effect in vitro takes 2 days [5].

In other words, the effect of warfarin becomes apparent on the INR (which tests blood coagulation), before the antithrombotic effect becomes clinically relevant. Clot-bound thrombin is an important mediator of clot growth, and interference with the activity of FII leads to less thrombin being formed to bind with fibrin, which retards clot formation [16]. Activation of FII at the start of anticoagulation therapy is highly dependent on the levels of FII and protein C. Activation of FII is not inhibited until very low levels of FII are reached. This may explain why it is recommended that patients converting from heparin therapy to VKA therapy receive heparin alongside VKA therapy for at least 48 hours after reaching the target INR [24].
The anticoagulation management center (AMC, Segavarnir) at Landspitali, University Hospital of Iceland follows a protocol based on the “American College of Chest Physicians (ACCP)” practice guidelines [5] when initiating a patient’s warfarin therapy. The protocol aims at reaching the therapeutic range in 7-10 days, no longer than 10-15 days. INR monitoring is initiated after three daily-doses of warfarin (i.e. an INR is obtained on the fourth day, that subsequently is used for the first dose adjustments). INR is monitored three times the first week, two times the second week, and weekly thereafter until the INR has stabilized within the therapeutic range, at which time the interval can be prolonged to up to 8 weeks.

The recommended starting dose for individuals under 65-70 years of age is 6 mg daily for three days.

For individuals over 70 years old, those who are malnourished, diagnosed with congestive heart failure, liver diseases, those who have recently had major surgery, and those who take medicine that potentiate the effect of warfarin, the recommended starting dose is 4 mg for three days.

In both groups, a low or no response in the INR will lead to an increase of the dose by 50% (6 mg to 9 mg and 4 mg to 6 mg), and the INR is measured again in 2-3 days. Conversely, if the INR value is raised substantially by the first dose, the dose will be decreased by 50% and another INR measurement will be performed in 2-3 days (Appendix 1).

AMCs have been shown to be more effective than manual dose therapy of individual physicians in managing patients on warfarin. These clinics have been used extensively in Scandinavia and exclusively at our medical center since 1999, when anticoagulant dosing management was moved from the hands of cardiologists and hematologists to nurses, biomedical scientists and consulting hematologists with support from the computer program DAWN AC [5, 25, 26].

A comparison between anticoagulation management at Landspitali University Hospital in the year 1992 and 2006 showed a significantly improved anticoagulation therapy. Anticoagulant dosing mainly managed by nurses and biomedical scientists resulted in higher TTR (46% vs 81% in AF patients, 62% vs 84% in VTE patients), higher proportion of monitoring tests within the therapeutic range (43% to 65%), and less variance in INR values than when dosing was managed by doctors [25].

An analysis of the RE-LY study reported a very high TTR of warfarin treatment in Scandinavia or around 77% in Sweden, 74% in Finland and 72% in
Denmark, compared to 66% in the USA and 44% in Taiwan [6]. AMCs in the USA are also associated with higher TTR compared to standard community care, although these clinics are not as prevalent as in Scandinavia.

1.3 Monitoring

Worldwide, the prothrombin time test (PT or Quick-PT) is the most common test used to monitor warfarin therapy. The Quick PT is sensitive to reduced activity of FII, FVII and FX, but is insensitive to reduction in activity of FIX. The PT is also sensitive to the activity of FV and fibrinogen (FI), which are not affected by the VKAs [14].

The Prothrombin & Proconvertin test (P&P or Owren PT) is a variant that is only sensitive to FII, FVII and FX, and has for the past 60 years been the main monitoring test used in Iceland, as well as in the other Nordic countries, the Netherlands, and Japan [25].

Both tests are performed in a similar fashion. Clotting is initiated by adding calcium ions to citrated platelet poor plasma (PPP) from the patient in presence of thromboplastin (TP). The time for formation of a fibrin clot is measured in seconds. The results are standardized by conversion to INR, which takes into account the sensitivity or strength of the TP used by different reagents. This is necessary in order to standardize dosing of warfarin from one institution to the next [14].

The INR is calculated by dividing the patient’s PT by a control plasma pool PT, and the quotient is raised to a power called the “International Sensitivity Index” (ISI). The ISI denotes the ISI of the TP used at the laboratory performing the measurement, and indicates the sensitivity of a given TP to the reduction of the VKD coagulation factors, compared with World Health Organization (WHO) international reference compound. The value of the ISI is lower (closer to 1.0) for reagents that are more sensitive [16].

One study comparing the PT and P&P methods, found an “unacceptable” difference between INR values within the therapeutic range and at higher INR values. The authors speculated that the discrepancy might be due to the different dilution volumes between the testing methods. In the PT method the sample volume is 33% of the reaction mixture, but 5% in the P&P method, making it more sensitive. The authors concluded that the P&P method was superior to PT [27].
Other studies have indicated that FVII may be the predominant factor monitored by the traditional PT tests, and that native (i.e. fully carboxylated) FII levels may be a better predictor of complications during warfarin therapy [28, 29].

My mentors in this study, Páll T. Önundarson (PTÖ) and Brynja R. Guðmundsdóttir (BRG) previously evaluated the function of each VKD coagulation factor on clotting in vitro, using PT assays and rotational thromboelastography (ROTEM) with clotting initiated with trace amount of TP. The ROTEM is a method that may reflect the in vivo clotting process more accurately than clotting induced by undiluted TP. It is a more detailed study of clot formation than the routine clotting time, since it in addition measures the rate of the bulk clot formation and the final clot strength. ROTEM is usually performed by using whole blood, and the results are thus a function of platelet function, proteases, inhibitors and fibrinolysis, as well as of coagulation factors. PTÖ and BRG’s results showed a poor correlation of ROTEM parameters with the PT-INR in patient plasma, which may reflect a divergence between activity of FVII and that of the more stable FII and FX [30].

This suggests that swift changes in FVII activity during initiation and following dose changes of VKA may amplify the change in PT-INR, and does not necessarily reflect a change in antithrombotic effect or risk of bleeding. These findings are contrary to the general consensus that the PT measures the antithrombotic activity of VKAs accurately.

PTÖ and BRG concluded that FVII may be a confounding variable during monitoring warfarin therapy, and that the antithrombotic effect of FVII is of trivial importance compared to the activity of FX and FII. However, as FVII has the shortest half-life of around 4-8 hours, changes in the PT may simply reflect fluctuations in FVII activity rather than accurate changes in antithrombotic activity, even in patients on relatively stable VKA therapy. This has been shown to be the case, especially during the first few days and weeks of warfarin therapy, when a large portion of the time is spent outside the therapeutic range [28].

PTÖ and BRG therefore invented a new coagulation test, the Fiix-prothrombin time (Fiix-PT), based on the Quick PT. This new test is intended for monitoring VKA anticoagulation therapy and increasing the efficacy and safety of VKAs, by measuring the combined activity of FII and FX only. Unlike the PT and P&P tests, the Fiix-PT does not measure the activity of FVII and, therefore, less fluctuation in INR is expected.
1.4 Study objectives

The objective of the randomized controlled Fiix-trial is to (i) evaluate the efficacy and safety of Fiix-PT as a monitoring test compared to the Quick PT assay, and (ii) investigate the stability of anticoagulation in patients monitored using Fiix-PT compared to Quick PT.

Our hypothesis is that for patients on VKA anticoagulation therapy, the measurement of FVII in the PT is a source of fluctuation of the INR that confounds true assessment of the anticoagulation. Measuring the combined influence of FII and FX on clotting and eliminating all other coagulation factors might on the other hand reduce INR variation. Consequently, the efficacy and safety during VKA therapy could be improved. The new Fiix-PT test compared to PT could as a monitoring test for warfarin therapy lead to more efficient dosing, fewer blood tests and higher percent of TTR.
2 Methods

2.1 Study population

This study is a part of the recently completed Fiix-study, a clinical trial with a study population of 1155 over a two-year study period. Eligible participants were ambulatory patients over 18 years of age receiving warfarin at the AMC at Landspitali, with INR target range of 2.0-3.0, irrespective of indication for warfarin (Table 1, page 24).

After signing a document indicating an informed consent and a willingness to participate in the study, participants were randomized to either Fiix PT (test method A, Fiix INR) or PT (control method B, PT-INR). The protocol manager maintained a list over study participants and was responsible for randomization and informed consents. Each patient was labeled with a code after randomization. Each patient’s blood sample was directed to test A (Fiix-INR) or B (PT-INR) based on this code throughout the study period.

The blood samples were measured in the central coagulation laboratory, which reported all test results as “R-INR” (Research-INR) to the dosing staff (nurses, biomedical scientists and physicians), who were blinded to the type of test used to report the INR value in each patient. Dosing was according to usual practice; using DAWN AC anticoagulation management software and protocols that were based on monitoring with PT-INR (Quick-PT).

Patients with 60 days of exposure or less to VKAs before entry into the clinical trial were defined as warfarin-naïve, and this definition was kept for six months of follow-up time. Patients with more than 60 days of exposure to VKAs before entry into the trial were defined as warfarin-experienced.

This definition of warfarin-naivety has been used in a previous study by Ezekowitz et al. (2010) to compare naïve and experienced warfarin users. Other studies have used similar definitions, with the definition of naïve ranging from <30 days to <62 days of previous exposure [7, 31-33].

Because of a low number of participants in both naïve groups (Table 1), it was not possible to evaluate how those patients develop anticoagulation over longer periods than six months. However, the time that those warfarin-naïve individuals may have spent as participants of the study after that period was not excluded. Instead, we decided to allow those individuals to continue to contribute to the study, by
categorizing them as warfarin-experienced after having spent 6 months as warfarin-naïve.

2.2 The INR assays
All monitoring tests were performed by Landspitalinn coagulation laboratory biomedical scientists using STA-R Evolution® coagulation analyzing instruments from Diagnostica Stago Inc, Asnieres, France. Two STA-R Evolution® instruments were used, one for each type of INR assay.

2.2.1 PT-INR
The PT-INR was calculated based on Quick PT measurements, where 100 µL of STA-Néoplastine CI Plus reagent (thromboplastin and calcium) is added to undiluted patient plasma (50 µL) to initiate coagulation.

2.2.2 Fiix-INR
In the Fiix-INR assay, 80 µL of patient plasma is diluted with seven times the volume of STA-Owren Koller diluent. Then 80 µL of STA-Néoplastine CI Plus is added along with 25 µL of Fiix (FII and FX) depleted plasma (Haemotologic Technologies Inc.), to initiate coagulation.

Both assays are performed in the same fashion, the only difference being the reagent being used and the dilution of the plasma. The STA-R coagulation analyzing instruments measure the time needed for blood coagulation, by measuring the variation in the amplitude of an oscillating magnetic ball. The reduction in amplitude corresponds to an increase in viscosity of the plasma being tested. An electromagnetic field created alternatively on each side of the ball maintains a swinging motion when the viscosity is constant, i.e. when coagulation has not occurred.

In both assays, the INR is calculated by the following formula: \( \text{INR} = \frac{\text{Patient PT}}{\text{mean normal PT}} \times \text{ISI} \).

2.3 Statistical analysis
For this analysis, we determined (i) the TTR, (ii) frequency of dose changes, (iii) frequency of monitoring tests, and (iv) dose changes per monitoring test in each individual during the study, at consecutive six-month intervals. Within each period, only those patients with three or more monitoring tests were included.
TTR is reported as the percent of time each participant spent with INR between (and including) 2 and 3 during the study period. A daily INR was calculated using the linear interpolation method of Rosendaal et al [34], which assumes that changes between consecutive INR measurements are linear over time. This method allows us to allocate a specific INR value to each day that a participant did not have an actual INR measurement. The TTR was calculated for each patient by counting the total days in range for each time period and dividing by the total number of therapeutic days.

The number of dose changes and the number of monitoring tests was counted for each individual within each time period, and the frequency calculated by dividing the number of observation days. By reporting the frequency, any variation in the number of observation days between individuals has been corrected for, making comparison between groups easier.

Every extent of time where each patient was either (i) not on warfarin or (ii) not dosed according to Landspitali’s AMC protocol was excluded from the analysis. This includes any time that each participant may have spent as an inpatient during cardioversion or perioperatively relating to other surgery. The period when each patient started warfarin therapy again after a pause and had not reached a stable INR value was also excluded. A stable INR was defined as at least two consecutive INR measures within the target range (INR 2-3).

Table 1 shows that 30 individuals were excluded from the analysis in total, 13 individuals in the Fiix group and 17 individuals in the PT group. Sixteen were excluded because they spent the whole follow-up time as inpatients (Fiix: 5, PT: 11) and another fourteen because they had fewer than three monitoring tests (Fiix: 8, PT: 6).

When comparing individual-based data between two groups, such as TTR, number of monitoring tests per month, and number of dose adjustments, the Mann-Whitney non-parametric test was used. The Mann-Whitney test is a variation of the t-test, which compares ranks without assuming a Gaussian distribution. When comparing data for the two groups as a whole, the Chi-Square test was used. All statistical analysis was performed using Microsoft Excel and GraphPad Prism 6.0.
3 Results

The following analysis is based on 24,559 INR values collected from 1,125 patients enrolled in the Fiix trial over the two-year study period. There were 12,017 INR values in 564 individuals in the Fiix-group and 12,540 values for 561 individuals in the PT-group. There were 4.2% fewer monitoring tests (INRs) in the Fiix study arm (p=0.0159).

The median follow-up time was 1.6 years in both groups. The main indications for warfarin were AF (Fiix: 61.4% and PT: 68.2%) and VTE (23.7% and 22.3%), as shown in Table 1. The mean age (±1 SD) of the study population was 69.7 (11.2) and 69.9 (11.8). The mean age of experienced warfarin users was higher than that of patients defined as warfarin-naïve (Fiix: 70.2 vs 66.2, PT: 70.8 vs 63.1). The mean age of experienced users was similar between the study arms (70.2 vs 70.8).
Table 1 - Participant characteristics

<table>
<thead>
<tr>
<th></th>
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<td>Patients enrolled</td>
<td>577</td>
<td>578</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Patients excluded</td>
<td>13</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients assessed</td>
<td>564</td>
<td>492</td>
<td>72</td>
<td>561</td>
<td>503</td>
<td>58</td>
</tr>
<tr>
<td>Age - mean (SD)</td>
<td>69.7 (11.2)</td>
<td>70.2 (11.1)</td>
<td>66.2 (11.1)</td>
<td>69.9 (11.1)</td>
<td>70.8 (11.8)</td>
<td>63.1 (15.0)</td>
</tr>
<tr>
<td>Male sex – n (%)</td>
<td>348 (62)</td>
<td>314 (64)</td>
<td>34 (47)</td>
<td>370 (66)</td>
<td>337 (67)</td>
<td>33 (57)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>381 (68.0)</td>
<td>345 (70.1)</td>
<td>36 (50.1)</td>
<td>385 (68.6)</td>
<td>360 (71.6)</td>
<td>25 (43.1)</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>345 (61.4)</td>
<td>315 (64.0)</td>
<td>30 (41.7)</td>
<td>366 (65.2)</td>
<td>343 (68.2)</td>
<td>23 (39.7)</td>
</tr>
<tr>
<td>Atrial flutter</td>
<td>2 (0.4)</td>
<td>2 (0.4)</td>
<td>0</td>
<td>3 (0.5)</td>
<td>2 (0.4)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>19 (3.4)</td>
<td>15 (3.1)</td>
<td>4 (5.6)</td>
<td>9 (1.6)</td>
<td>9 (1.8)</td>
<td>0</td>
</tr>
<tr>
<td>Heart failure</td>
<td>2 (0.4)</td>
<td>2 (0.4)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Atrial septal defect</td>
<td>3 (0.5)</td>
<td>2 (0.4)</td>
<td>1 (1.4)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Biological aortic valve</td>
<td>1 (0.2)</td>
<td>1 (0.2)</td>
<td>0</td>
<td>2 (0.4)</td>
<td>1 (0.2)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Mechanical mitral valve</td>
<td>2 (0.4)</td>
<td>2 (0.4)</td>
<td>0</td>
<td>1 (0.2)</td>
<td>1 (0.2)</td>
<td>0</td>
</tr>
<tr>
<td>Mechanical aortic valve</td>
<td>6 (1.1)</td>
<td>6 (1.2)</td>
<td>0</td>
<td>4 (0.7)</td>
<td>4 (0.8)</td>
<td>0</td>
</tr>
<tr>
<td>Mitral valve disease</td>
<td>1 (0.2)</td>
<td>0</td>
<td>1 (1.4)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cerebrovascular disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>43 (7.5)</td>
<td>41 (8.5)</td>
<td>2 (2.8)</td>
<td>49 (8.7)</td>
<td>40 (8.0)</td>
<td>9 (15.5)</td>
</tr>
<tr>
<td>Cerebral embolism</td>
<td>12 (2.1)</td>
<td>11 (2.2)</td>
<td>1 (1.4)</td>
<td>16 (2.9)</td>
<td>12 (2.4)</td>
<td>4 (6.9)</td>
</tr>
<tr>
<td>Cerebral thrombosis</td>
<td>3 (0.5)</td>
<td>3 (0.6)</td>
<td>0</td>
<td>7 (1.2)</td>
<td>7 (1.4)</td>
<td>0</td>
</tr>
<tr>
<td>TIA</td>
<td>12 (2.1)</td>
<td>11 (2.2)</td>
<td>1 (1.4)</td>
<td>13 (2.3)</td>
<td>12 (2.4)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Ischemic stroke not further defined</td>
<td>16 (2.8)</td>
<td>16 (3.3)</td>
<td>0</td>
<td>13 (2.3)</td>
<td>9 (1.8)</td>
<td>4 (6.9)</td>
</tr>
<tr>
<td>Peripheral arterial embolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial embolism</td>
<td>6 (1.1)</td>
<td>5 (1.0)</td>
<td>1 (1.4)</td>
<td>2 (0.4)</td>
<td>2 (0.4)</td>
<td>0</td>
</tr>
<tr>
<td>Venous thromboembolism (VTE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>133 (23.7)</td>
<td>100 (20.3)</td>
<td>33 (45.9)</td>
<td>123 (22.3)</td>
<td>99 (20.0)</td>
<td>24 (41.3)</td>
</tr>
<tr>
<td>Deep vein thrombosis</td>
<td>70 (12.4)</td>
<td>57 (11.6)</td>
<td>13 (18.1)</td>
<td>59 (10.5)</td>
<td>49 (9.7)</td>
<td>10 (17.2)</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>61 (10.9)</td>
<td>42 (8.5)</td>
<td>19 (26.4)</td>
<td>64 (11.4)</td>
<td>50 (9.9)</td>
<td>14 (24.1)</td>
</tr>
<tr>
<td>Pulmonary hypertension</td>
<td>2 (0.4)</td>
<td>1 (0.2)</td>
<td>1 (1.4)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other indications</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dissection of SMA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (0.2)</td>
<td>1 (0.2)</td>
<td>0</td>
</tr>
</tbody>
</table>

3.1 Time in therapeutic range

3.1.1 TTR before and after enrollment

The boxplot below (Figure 5) displays the median individual TTR, interquartile range and 10-90th percentile in all patients, naïve and experienced, in the Fiix study arm (red) and PT study arm (gray) three months before enrollment into the study and three months after enrollment.
Figure 5 – TTR at three months before and after enrollment.

Fiix study arm in red, PT study arm in grey. Median, interquartile range and 10-90th percentiles.

The median TTR for both groups improved significantly during the first three months of follow-up (67.1% vs 80.2% and 70.2% vs 80.1% respectively, p<0.0001). The TTR before enrollment is based on all available INR measurements for both groups in that period, i.e. the time each individual may have spent as an inpatient or off warfarin for a short period is included. Such periods are not included during the post-enrollment period, which is “per protocol”. However, results for the post-enrollment period, done as an “intention to monitor analysis” with all tests included irrespective of patients being on warfarin at the time of testing, are similar (not shown).

3.1.2 TTR for whole study period

The TTR was higher in the Fiix group than in the PT group throughout the follow-up time, except during the third six-month period, see 3.1.4. Figure 6 compares the TTR during the whole study period for individuals in the Fiix arm and the PT arm (median and interquartile range). The overall median TTR was higher in the Fiix group (82.2% vs 80.2%, p=0.016).
Based on INR measurements for all participants. Median, interquartile range.

Similarly, the Fiix-group had a higher proportion of INR values within range overall during the whole two-year study period, or 65.1% compared to 62.6% in the PT-group (p<0.0001).

3.1.3 TTR: Naïve vs experienced warfarin users

Most patients in the study were experienced warfarin users with only 72 (12.7%) and 58 (10.3%) of patients being defined as naïve in the Fiix arm and the control arm, respectively. As shown in Figure 7, the naïve warfarin users in both arms had a similar TTR during the first 6 months of the study (75.6% vs 76.0%, respectively). During the same period, experienced users in both the Fiix arm and the PT arm had significantly higher TTR than the naïve groups (75.6% vs 85.2%, p<0.001 and 76.0% vs 81.4%, p<0.01, respectively).
3.1.4 TTR: Warfarin experienced users

As shown in Figure 8, the median TTR was consistently higher in the Fiix arm than in the PT arm, except for the third six-month period when surprisingly no difference was found, as presented and discussed in the sections below. The Fiix group displays a significantly higher TTR than the PT group at 0-6 months (85.2% vs 81.4%, p<0.01), 6-12 months (84.2% vs 79.9%, p<0.001) and 18-24 months (86.7% vs 78.2%, p<0.01). At 12-18 months, the Fiix group dips down to the same TTR as the PT group (p=0.47).
Figure 8 – TTR: Experienced warfarin users.

Based on individual TTR during consecutive six-month intervals. Median individual TTR.

When the “dip” became evident during data analysis we noted that the median Fiix-INR was slightly prolonged during a three-and-a-half month period in the summer of 2013, from May 22nd 2013 to September 11th 2013. As this prolongation may have affected dosing of warfarin and, hence, TTR adversely in the Fiix-group, we re-analyzed the data after excluding this period. After exclusion, the median TTR for the Fiix group was identical during both years of the observation period, supporting a calibration error in the Fiix-PT in the second year (Figure 9).
Figure 9 - TTR: Fiix experienced patients.
First and second year based on individual observation time. Median, interquartile range and 10-90th percentiles.

With the 3.5-month period excluded (Figure 9, box at right), the median TTR was higher than the median value with the period included (box in the middle, p=0.05), and similar to the median TTR for the first year (box at left).

Table 2 - Number of days between tests and TTR based on six-month intervals

<table>
<thead>
<tr>
<th>Fiix vs PT</th>
<th>0-6 mo</th>
<th>6-12 mo</th>
<th>12-18 mo</th>
<th>12-18 mo*</th>
<th>18-24 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean days between test</td>
<td>Fiix 19</td>
<td>PT 19</td>
<td>Fiix 22</td>
<td>PT 19</td>
<td>Fiix 20</td>
</tr>
<tr>
<td>Median days between test</td>
<td>Fiix 15</td>
<td>PT 14</td>
<td>Fiix 20</td>
<td>PT 15</td>
<td>Fiix 15</td>
</tr>
<tr>
<td>Percent days in range (mean)</td>
<td>Fiix 83</td>
<td>PT 80</td>
<td>Fiix 83</td>
<td>PT 78</td>
<td>Fiix 79</td>
</tr>
<tr>
<td>Percent days in range (median)</td>
<td>Fiix 71</td>
<td>PT 68</td>
<td>Fiix 91</td>
<td>PT 71</td>
<td>Fiix 71</td>
</tr>
<tr>
<td>Mean INR</td>
<td>Fiix 2.55</td>
<td>PT 2.52</td>
<td>Fiix 2.54</td>
<td>PT 2.52</td>
<td>Fiix 2.61</td>
</tr>
<tr>
<td>Median INR</td>
<td>Fiix 2.49</td>
<td>PT 2.42</td>
<td>Fiix 2.46</td>
<td>PT 2.42</td>
<td>Fiix 2.54</td>
</tr>
</tbody>
</table>

*3.5 month period excluded

Table 2 is a sub-analysis of monitoring test intervals during the observation time (as opposed to individual patient TTR). The analysis shows the same trend as Figure 9 when the 3.5-month summer period is excluded. In this separate analysis the days in range and number of days between monitoring tests during the four six-month intervals are shown. During the third six-month period, the median days between tests in the Fiix-group rises from 15 to 18 days when the “dip” period is removed. Similarly, the mean days in range increases from 79% to 80% in the Fiix group and decreases from 80% to 79% in the PT group. The median INR also decreases.
significantly in both groups, from 2.54 to 2.47 (p=0.0008) in the Fiix group and from 2.46 to 2.41 in the PT group (p=0.04).

### 3.2 Monitoring tests per month

Figure 10 displays the mean number of monitoring tests (mean, SEM) performed for each individual per month at consecutive 6-month intervals. The difference is only significant at the 7-12 month interval.

**Figure 10 - Monitoring tests per month: Experienced patients.**

Mean, SEM.
3.3 Dose changes

In Figure 11 the mean number (±1 SEM) of dose changes are shown. At 0-6 months, individuals in the Fiix group have had fewer dose changes (2.7 vs 3.1, p<0.05). The same applies to the groups at 7-12 months (2.7 vs 3.3, p<0.001), 13-18 months (2.6 vs 2.7, p=0.68), and >18 months (1.1 vs 1.8, p<0.001).

![Figure 11 - Dose changes: Experienced patients. Six-month intervals. Mean, SEM.](image)

Figure 12 compares Fiix and PT experienced warfarin users in the first year of observation, left, and second year of observation, right. Using the same data as in Figure 11, the graph shows a cumulative percentage of individuals with each number of dose changes per year. For example, according to these results, 51% of Fiix experienced warfarin users have 4 or fewer dose changes in the first year of study, and 42% in the PT group (p=0.0039). In the second year, the same number of dose changes applies to around 70% in the Fiix group and around 65% in the PT group (p=0.09).
Figure 12 – Dose changes: Cumulative percent of individuals.
Cumulative percentage of individuals with each number of dose changes, first and second year of observation.
3.4 Number of dose changes per monitoring test

Both the number of monitoring tests and the number of dose changes are highly variable between individuals. Therefore, the number of dose changes for each individual is shown in context with the number of monitoring tests in Figure 13 and Figure 14, in order to determine the mean number of dose changes per test. Results for those individuals who may have had a very low or a very high number of monitoring tests have thus been adjusted for.

3.4.1 Dose changes per monitoring test: Warfarin naïve patients

The scattergraph shown above (Figure 13) displays the number of dose changes per monitoring test for each individual defined as naïve in the Fiix group and PT group. The red lines and error bars depict the mean and SEM for each group. The Fiix group has fewer dose changes per test than the PT group (mean: 0.46 vs 0.51 p=0.12).

Figure 13 - Dose changes per test: Naïve patients.

Mean, SEM.
3.4.2 Dose changes per monitoring test: Warfarin experienced patients

Figure 14 – Dose changes per test: Experienced patients.

Mean, SEM.

Warfarin-experienced individuals in the Fiix study arm had significantly fewer dose changes per monitoring test during the first (0.31 vs 0.35, p<0.05), second (0.37 vs 0.42, p<0.01) and fourth (0.20 vs 0.28, p<0.001) six-month intervals (Figure 14).


4 Discussion

4.1 Summary

In this study we compared the new Fiix-PT test and the traditional Quick-PT, with regards to the secondary outcomes of the Fiix-trial; TTR, frequency of INR monitoring and number of dose adjustments. The development of Fiix-PT was based on former results that suggested that the activity of FVII might be confounding the results of the Quick-PT. Our hypothesis is that by measuring only FII and FX, improved stability of anticoagulation could be achieved. Our findings support this hypothesis. The Fiix-PT thus seems to be a superior measurement of the anticoagulant effect of warfarin compared to Quick-PT. The new test measures only the VKD coagulation factors II and X and does not measure FVII, unlike the Quick-PT. Furthermore, the patient plasma is diluted more in the Fiix-PT than in the Quick-PT, making the Fiix-PT more sensitive [27].

The participants randomized to INR monitoring with the Quick-PT had very high individual TTR compared to other published studies [4, 6, 8, 32, 33, 35], although the TTR in participants randomized to the Fiix-PT was even higher. Additionally, participants randomized to the Fiix-PT needed fewer monitoring tests and fewer dose adjustments than the control group.

TTR is an important indicator of the quality of anticoagulation therapy, and at Landspitali’s AMC the quality is very high [25]. According to the results of Wallentin et al (2010), which analyzed data from the RE-LY trial based on TTR between treatment centers, the TTR in Iceland is among the highest in the world [6]. However, the TTR presented here may not reflect the “true” TTR of our total clinic population, since the TTR is usually lower in clinical practice than in clinical trials, due to less selection bias [4, 35].

4.2 Main study findings

Based on our results, individuals randomized to INR monitoring with the Fiix-PT assay have higher TTR, undergo fewer monitoring tests and are subject to fewer dose changes than individuals monitored using the traditional Quick-PT. Except for one 3.5-month period during the summer of 2013 where the quality of anticoagulation in the Fiix-PT group dipped down to the level of the Quick-PT (see discussion in pages 36-37), the Fiix-PT seemed to lead to a better warfarin treatment.

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4.2.1 Time in therapeutic range

Both study groups were very similar or even identical at randomization, as can be seen from the TTR at three months before enrollment (Figure 5). However, a significant improvement could already be seen in both groups only three months following enrollment, with the median TTR being raised from about 70% to around 80% in both groups.

This measurable and significant rise in TTR in such a short time period came as a surprise, although this can, to some extent, be attributed to a form of compliance bias. As the participants are well aware that they are taking part in a clinical trial, they may be inclined to please the researchers with increased adherence to the intervention [36]. In this case, the participants may have shown increased compliance by taking their correct dose of warfarin at the right time. Another explanation could be that the TTR at three months before enrollment is not analyzed per protocol, i.e. the INR values for intervals of time that participants may have spent as inpatients etc., were not excluded. However, a similar difference was seen when TTR for the groups at three months following enrollment was analyzed as intention to monitor.

When the median individual TTR of the Fiix and PT (with naïve and experienced participants combined) for the whole study period is compared (Figure 6), there is a significant difference. The median TTR in the Fiix group is higher (82.2% vs 80.2%, p=0.016). For the same period, there is a significantly higher ratio of INR values within the therapeutic range in the Fiix group (65.1% vs 62.6%, p<0.0001).

Warfarin-naïve patients were similar between the study arms with regards to mean age and indications for warfarin therapy (Table 1). Both naïve groups had higher proportions of VTE than their respective group of experienced warfarin users. With that in mind, it should not come as a surprise that the naïve groups had lower median TTR, as VTE patients are often administered short-term anticoagulation therapy. Most individuals with VTE are administered a three-month anticoagulant therapy, with the duration of further treatment depending on the risk of recurrent VTE [37]. Thus, the difference seen between the naïve and experienced cohorts in Figure 7 may be explained by the different proportion of participants with AF and VTE.

Fiix experienced warfarin users have higher TTR than PT experienced users in all six-month intervals, except for the 13-18 month interval, as shown in Figure 8. A
three-and-a-half month period where a calibration error affected the Fiix-PT sensitivity is currently the most likely explanation. The median individual TTR was higher the second year with the period excluded (Figure 9). In addition, Table 2 showed a significant difference in both the mean and median days in range and the median INR when the three-and-a-half month period was excluded.

Another way to look at the interval of the suspected calibration error in the Fiix group is by comparing the mean number of days between monitoring tests, median INR and the mean and median days in range. As shown in Table 2, the median INR is higher for both groups when the interval is included in the analysis. The difference is significant in both cases, and the numerical difference is higher in the Fiix group. This may explain the dip in median individual TTR for the 13-18 month period, since a higher median INR represents a higher proportion of INR values above the therapeutic range, and therefore affects the mean and median days in range.

4.2.2 Frequency of monitoring tests

When comparing the frequency of monitoring tests (Figure 10), the mean value was identical in the first interval, significantly lower in the Fiix group at the second interval, and similar in the other two. This may reflect the tendency of the monitoring staff to summon patients for INR monitoring routinely, regardless of the stability of anticoagulation.

The Landspitali AMC’s protocol of warfarin therapy recommends that all patients on a constant dose should be monitored at least every four to six weeks (Appendix 1). Patients who require frequent monitoring tend to have less stable anticoagulation. The Fiix-PT seems to measure anticoagulation more accurately and to improve the quality of anticoagulation therapy. In addition to decreasing the cost of warfarin therapy, there are obvious benefits for those patients that are difficult to manage. Because of the invasive process of blood tests, a substantial discomfort accompanies frequent INR monitoring. By increasing the accuracy of monitoring, improving the stability of anticoagulation, and thus requiring fewer monitoring tests, the Fiix-PT may help in minimizing that discomfort.
4.2.3 Dose adjustments

The number of dose adjustments in each group is shown in Figure 11. Individuals in the Fiix group require fewer dose changes per six-month interval, with the difference being significant at three of four intervals. The mean value becomes considerably lower for both groups at the >18 month interval. As pointed out before, the median follow-up time for both study arms is 1.6 years. The number of dose changes for each interval does not represent the frequency, and the mean value may therefore be influenced by the shorter follow-up time for most individuals in this interval. Even so, the mean number of dose changes is significantly lower in the Fiix group.

A cumulative representation of this data was shown in Figure 12, which shows the percentage of individuals within each group with each number of dose changes for the two years of follow-up. For example, 51% of individuals in the Fiix-group require four dose changes or less for the first year in study, versus 42% in the PT-group (p=0.0039). The values for the second year of observation may be influenced by the variance in follow-up times between individuals, with those individuals contributing few days in the study also having few dose changes by default.

4.2.4 Dose changes per monitoring test

By looking at the number of dose changes per monitoring test (Figure 13, Figure 14), the influence of those individuals with very few (or a high number of) follow-up days on the number of dose changes may be adjusted for. Both naïve groups had similar number of dose changes per monitoring test, with the Fiix naïve group having a lower mean (p=ns). On the other hand, the experienced groups followed the same trend as has been reported before, with the Fiix group being significantly lower in all intervals except for the 13-18 month interval.

Interestingly, warfarin-experienced patients in the Fiix study arm seem to require fewer dose changes per monitoring test than experienced patients in the PT study arm. This may suggest an increased stability with time, which is reflected in the need for fewer dose adjustments in the Fiix arm. However, the number of dose changes per test is at its lowest point in both groups at the >18 month interval, in addition to the number of dose changes and the number of monitoring tests. This may reflect a possible selection bias, with the individuals (n=237 and n=240) that contributed more than 18 months to the study being more stable than those that
dropped out after less than 18 months of follow-up time. Nevertheless, if the stability of anticoagulation of the study groups were the same during all intervals, the mean number of dose changes per monitoring test would be expected to be similar in all intervals. Hence, we conclude that the stability of anticoagulation was improved more in the Fiix arm.

4.3 Study strengths and limitations
This study was a substudy of a large prospective and blinded randomized controlled clinical trial, the Fiix-trial. The protocol manager maintained a detailed list of study participants, which included indications for warfarin and days each participant spent as an inpatient. To minimize the chance for error, the protocol manager was also responsible for logging results from monitoring tests and dose adjustments, receiving only limited help from two other researchers.

Participants were randomized to either a test arm or a control arm, and the dosing staff were blinded to which group each participant belonged, which practically eliminates experimenter bias. The current substudy did not measure clinical outcomes, as those will be reported in a separate analysis of the Fiix-trial. Nevertheless, the increased TTR observed indicates an improvement in both efficacy and safety of warfarin therapy for those monitored with Fiix-PT. Also, the reduced need for dose adjustments in the Fiix study-arm suggests increased clinical stability of those patients.

This was a single-center study, only including outpatients receiving warfarin therapy at the AMC of Landspitali. Nursing-home patients, who represent the least healthy and oldest population, were excluded. Therefore, any clinical conclusions drawn based on the research may not reflect the excluded population. An international multi-center randomized controlled trial with different coagulation center TTRs would have more statistical power, and more external validity. Such a study would be much more expensive however, and the study protocol would be difficult to standardize between treatment centers.

The original objective of this substudy was to evaluate the stability of warfarin therapy of those patients receiving warfarin for the first time, i.e. of warfarin-naïve participants in the Fiix-trial. Unfortunately, there were relatively few warfarin-naïve patients enrolled. Those patients were eventually deemed too few to evaluate for
longer period than six months regarding TTR, dose changes and frequency of INR monitoring, mostly because of short follow-up times.

The sudden dip in TTR in the Fiix group in the summer of 2013 came as a surprise. The fact that this change only appears in the Fiix group and not in the PT group, suggests a confounding variable that affects the groups unevenly. Therefore, it is highly unlikely that a factor affecting both groups is responsible, for example a different dosing routine. With the only difference between the groups being the monitoring test used to measure the INR, this suggests a temporary variation in the process of the Fiix-INR assay. An error in the ISI calibration following the arrival of a new batch of the Fiix-reagent is a possibility, and is currently considered the most plausible explanation.

4.4 Conclusions

We conclude that the Fiix-PT is superior to the traditional Quick-PT for monitoring warfarin therapy, with regards to the secondary outcomes of the Fiix-trial. The Fiix-PT led to increased TTR, reduced number of INR tests, reduced frequency of INR monitoring, and fewer dose adjustments. An analysis of the primary outcomes will further address the possible non-inferiority of the Fiix-PT with regards to clinical endpoints, such as the effect of the monitoring method on reducing thromboembolic events and mortality, and minimizing serious bleeding.
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Appendix 1

Segavarnir – stuttar leiðbeiningar (12/10/2010)

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

Hjá sjúklingum, sem eru að hefja meðferð með warfaríni er ráðlagt að hafja 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 svörur 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, liffrarsjúklingum, þeim sem nýlega hafa gengist undir stórar skurðaðgerðir eða hjá þeim, sem taka lyf, sem vitað er að auka námsliða fyrir warfaríni (t.d. amiodarone, sýklalýf) er 3 daglegir 4 mg skammtar. Áframhaldandi skammtar ráðast af því hvernig INR bregst við (mælt á 4. degi). Sé lítil eða engin svörur 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 hættu notkun heparíns 2 – 3 dógu 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 tinna fresti (ACCP guidelines, 8, útgáfa, Chest 2008;133;160-198).

Minnkun warfarínsskammtur 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 skömmum og mæla INR oftar og halda síðan áfram meðferð með viðeigandi skömmum þegar INR er komið niður í meðferðarmörk. Ef hækkun að INR er aðeins litilsháttur (u.b.b. 0,5-1.0) upp fyrir meðferðarmörk og hækkunin tengist augljösum orsókum er ekki ráðlagt að breyta skammti. Ef INR er lækkad 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 skömmum þegar INR er komið niður í meðferðarmörk (ACCP guidelines, 8, útgáfa, Chest 2008;133;160-198).
Lækknun á Kóvarskammti þegar INR hækkað

INR <5 >4 - minnka skammt næsta dag (frá 50% háð því hversu mikil blæðingahættu sj. er) og halda síðan afbrónt 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.


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

> Hemoglóbínlækknun > 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, lifefindafræðinga og hjúkrunarfæðinga um eftirlit segavarna LSH á blóðþynnnum sjúklingum (sept. 2008)).