Fiix-prothrombin time versus standard prothrombin time for monitoring of warfarin anticoagulation: a single centre, double-blind, randomised, non-inferiority trial

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Summary

Background Rapid fluctuations in factor VII during warfarin anticoagulation change the international normalised ratio (INR) but contribute little to the antithrombotic effect. We aimed to assess non-inferiority of anticoagulation stabilisation with a warfarin monitoring method affected only by factors II and X (Fiix-prothrombin time [Fiix-PT]) compared with standard PT-INR monitoring that includes factor VII measurement as well.

Methods The Fiix trial was a single centre, double-blind, prospective, non-inferiority, randomised controlled clinical trial. Ambulatory adults on warfarin with an INR target of 2–3 managed by an anticoagulation dosing service using software-assisted dosing at the National University Hospital of Iceland, Reykjavik, Iceland, were eligible for inclusion in this study. We excluded patients undergoing electroconversion and nursing home residents. Patients were randomly assigned (1:1) to either the Fiix-PT monitoring group or the PT monitoring group by block randomisation. A blinded research INR (R-INR) based on results of the respective test was reported to the dosing staff. Participants were contacted by a study nurse at 4-week intervals to elicit information about thromboembolism or bleeding otherwise unknown to the anticoagulation management centre. The primary efficacy outcome was a composite of objectively diagnosed non-fatal and fatal arterial or venous thromboembolism, including myocardial infarction and transient ischaemic attacks, assessed in all eligible patients who were randomised (intention-to-monitor population). The safety endpoint was major bleeding or other clinically relevant bleeding, assessed in the per-protocol population. We assumed a 3% annual thromboembolism incidence and a non-inferiority margin of 2·5%. This trial is registered with ClinicalTrials.gov, number NCT01565239.

Findings Between March 1, 2012, and Feb 28, 2014, we enrolled 1156 patients. 573 patients were assigned to Fiix-PT and 575 to PT-INR monitoring after exclusion of four patients from each group for various reasons. Median follow-up was 1·7 years (IQR 1·1–1·9). During days 1–720, ten (1·2% per patient year) thromboembolic events occurred in the Fiix-PT group versus 19 (2·3% per patient year) in the PT group (relative risk [RR] 0·52, 95% CI 0·25–1·13; $p_{non\text{-}inferiority}<0·0001$). Major bleeding occurred in 17 of 571 patients in the Fiix group (2·2% per patient year) versus 20 of 573 patients in the PT group (2·5% per patient year; RR 0·85, 0·45–1·61; $p_{non\text{-}inferiority}=0·0034$). Anticoagulation stability was improved with Fiix-PT monitoring as manifested by fewer tests, fewer dose adjustments, increased time in range and less INR variability than reported with standard PT monitoring.

Interpretation Monitoring of warfarin with Fiix-PT improved anticoagulation and dosing stability and was clinically non-inferior to PT monitoring. Results from this trial suggest that during vitamin K antagonist treatment INR monitoring could be replaced by Fiix-PT and that this would lead to at least a non-inferior clinical outcome compared with monitoring with PT-INR.

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Introduction

Vitamin K antagonists have a narrow therapeutic window and must be monitored at variable intervals to ensure efficacy and minimise bleeding complications.[1] This monitoring is done by measurement of the prothrombin time (PT),[2,3] a test that is equally sensitive to warfarin induced reductions in each of the coagulation factors II, VII, and X. Reductions in factor IX also occur during vitamin K antagonist treatment but are not detected by measurement of the PT. To manage vitamin K antagonist therapy, calibrators are used to standardise the PT by calculation of an international normalised ratio (INR), which adjusts for the different sensitivities of thromboplastins used in the test.[4] Additionally, software-assisted dosing[5,6] and centralised anticoagulation dose-management centres[1,7] have led to major improvements in vitamin K antagonist treatment.

Inability to obtain stable anticoagulation with vitamin K antagonists leads to a variable need for dose adjustments and an increased risk of thrombotic and bleeding complications. Fluctuations in INR are often ascribed to food and drug interactions or patient non-compliance.[1] However, experimental data[8] suggest that INR fluctuations might also be caused by rapidly changing factor VII

Research in context

Evidence before this study

We searched Medline between Jan 1, 1950, and Jan 1, 2012, for all studies in English using the search terms "anticoagulation", "monitoring", "coumarin", "warfarin", "vitamin K antagonist", "prothrombin time", and "international normalised ratio". No studies describing the research idea of the current study by other scientists were identified. Warfarin and other vitamin K antagonists have been dosed for more than 60 years on the basis of the prothrombin time (PT). The prothrombin time is equally sensitive to a reduction in each of coagulation factors II, VII, and X, but not to reductions in factor IX that occur during vitamin K antagonist treatment. As factor VII has a much shorter half-life than factor II and factor X, changes in factor VII cause a fast corresponding fluctuation of the PT-based international normalised ratio (INR). However, experimental data suggests that the in-vitro anti-clot forming and in-vivo antithrombotic effect of vitamin K antagonist drugs is mainly caused by lowering factor II and factor X activity and not by factor VII. Such studies include thrombin generation studies, thromboelastometric studies, small clinical studies monitoring warfarin with the native prothrombin antigen, and a rabbit study showing that disseminated intravascular coagulation induced by infusion of tissue factor was prevented by reduction of factor II and possibly factor X, but not factor VII. On the basis of these studies, we postulated that the combined effect of factor II and factor X should only be monitored during vitamin K antagonist anticoagulation and that elimination of the effect of factor VII in the test sample would increase stability of anticoagulation with at least a non-inferior clinical outcome. We invented a modified PT, the Fiix-PT, that measures only the effect of factor II and factor X in test samples. Our extensive database review of the scientific literature did not identify a similar research idea. Subsequently, we did this single centre double-blinded, randomised controlled trial to test the hypothesis, with centralised modern software-assisted dosing of warfarin.

Added value of this study

An improvement in the regulation of anticoagulation was reported and a clinically non-inferior reduction in thromboembolism in patients that were dosed based on the Fiix-PT (Fiix-INR) compared with high-quality dosing based on PT (INR) in controls. Bleeding was not increased. A post-hoc analysis suggests a possible improved long-term outcome of patients on warfarin monitored with the Fiix-PT. The new data suggest that anticoagulation variability during warfarin management is not only caused by food and drug interactions or patient non-compliance but partly by a confounding clinically irrelevant fluctuation of factor VII. Replacement of the PT with the Fiix-PT (Fiix-INR) eliminated this confounding effect of factor VII on the INR.

Implications of all the available evidence

Although a multicentre study ideally should be done to confirm the results of the Fiix-PT trial in different management settings, our results suggest that INR variability during vitamin K antagonist treatment could be reduced in practice by replacement of the PT with Fiix-PT and that this would lead to at least a non-inferior clinical outcome. Finally, the high quality of warfarin treatment in the PT control group might suggest that vitamin K antagonists should be centrally managed by dedicated staff using software-assisted dosing.

activity. Although severe reductions in factor VII can lead to a risk of bleeding, the experiments suggest that the main antithrombotic effect of vitamin K antagonists is due to stable reductions in factor II and possibly in factor X, which have much longer half-lives than factor VII, whereas the contributions of reduced factor VII activity and that of the undetected factor IX are minor.[8–10]

We have developed a modified PT assay, Fiix-PT, that is sensitive to the combined reductions of factor II and factor X and is unaffected by the activity of factor VII in the test sample.[8] This assay was based on our rotational thromboelastometric experiments,[8] which showed that in a dilute thromboplastin model, the initiation, propagation, and stabilisation phases of clotting were equally affected by reduced concentrations of factor II or factor X, but much less by factor VII or factor IX. Furthermore, thrombin generation in samples from patients receiving warfarin was detected equally well with Fiix-PT or PT-INR, suggesting that measurement of factor VIIc is not needed (Onundarson PT, unpublished). We therefore postulated that monitoring of warfarin with Fiix-PT would lead to more stable anticoagulation, with fewer dose adjustments and at least equivalent clinical outcomes.

Methods

Study design and patients

We undertook an investigator-initiated, double-blind, non-inferiority, randomised controlled trial in our anticoagulation management centre at The National University Hospital of Iceland, Reykjavik, Iceland. We invited all community-dwelling participants, aged 18 years or older, who were taking or starting short-term or long-term warfarin during the study period with an INR target of 2–3 and being managed at our anticoagulation management centre to participate in this study, irrespective of indication for anticoagulation. We excluded nursing home residents and participants being monitored at fixed weekly intervals before electroconversion of atrial fibrillation. We recruited patients consecutively as they came for their monitoring test appointments in the outpatient phlebotomy unit or in hospital wards during inpatient warfarin initiation. This study was done in accordance with the principles of the Declaration of

Helsinki at The National University Hospital of Iceland, Reykjavik, Iceland. All patients provided written informed consent. The National Bioethics Committee of Iceland (VSNb2011040019/03.15) and the Data Protection Agency of Iceland (2011040560AMK/-) approved the protocol.

Randomisation and masking

The study nurse recruited patients and obtained informed consent and then randomly assigned patients (in a 1:1 ratio) to either the experimental monitoring Fiix-PT group or the PT monitoring control group. Block randomisation without a stratification procedure (with 48 participants in each block) was applied by each patient drawing a colour-coded card from a closed box; each box contained 24 orange cards and 24 green cards that were also numbered with an anonymous code (a case report form number) that was recorded. Each colour directed the patient to either the active Fiix-PT group or the standard PT control group. The patient then presented the colour card during phlebotomies and corresponding coloured stickers were placed on the blood sample tubes by phlebotomists. Once received in the coagulation laboratory, laboratory staff directed the sample tube to the appropriate test on the basis of the sticker colour. Results of Fiix-PT or PT were reported in the laboratory information system in a masked manner as research INR (R-INR). Only the electronically reported R-INR was available to the dosing staff, who were not involved in phlebotomies and, therefore, had no way of knowing which test had generated the R-INR and had no knowledge of each patient's colour card. Patients, dosing staff, and event outcome assessors were masked to monitoring method assignment.

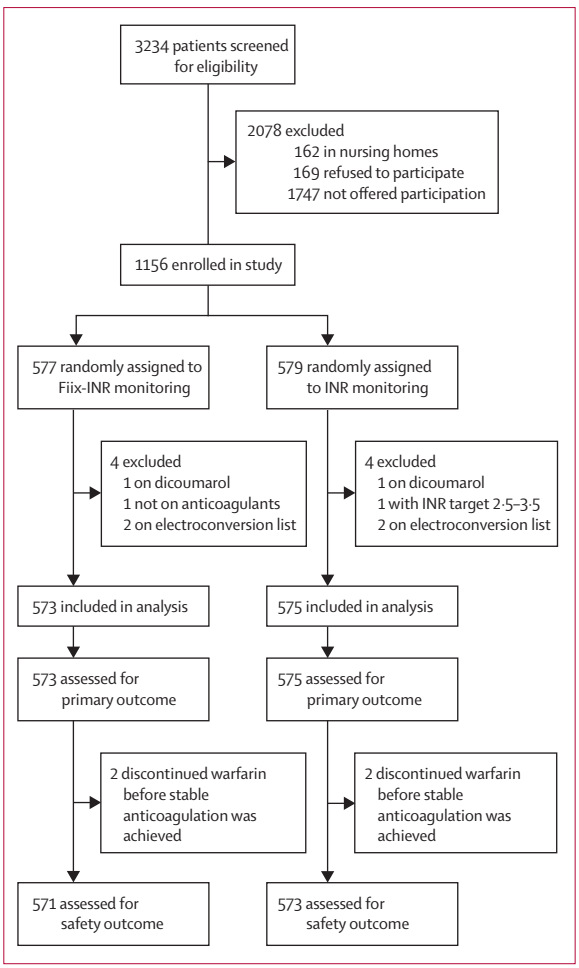

Figure 1: **Trial profile**

For the **protocol** see http://www.landspitali.is/sjuklingar-adstandendur/klinisk-svid-og-deildir/rannsoknarsvid/segavarnir/fiix-rannsoknaraaetlun/]

Procedures

We classified patients according to indications for anticoagulation. More than one indication was present in some individuals. We also recorded associated conditions and selected medication use. We calculated a CHA_2DS_2-VASC risk score for thromboembolism for patients with atrial fibrilliation.[11] Patients were registered as short term when referred as such and if treatment lasted less than 6 months and were regarded as naive to warfarin if enrolled during the first 60 days of warfarin intake.[12]

An executive committee did the trial, managed the data, and analysed the data. An independent adjudication committee consisting of three experienced physicians (a haematologist, a cardiologist, and a gastroenterologist) adjudicated all efficacy and safety endpoints. An independent safety monitoring committee reviewed study outcomes at 3-month intervals from the entry of the first patient.

Both Fiix-PT and PT tests were done at the centralised coagulation laboratory on citrated venous blood samples. The automated STA-R Evolution coagulation analyser (Diagnostica Stago, Asnieres, France) was used for both tests. PT-INR was calculated on the basis of Quick PT[2] and Fiix-INR was calculated on the basis of the new Fiix-PT, a modified PT that is only sensitive to factor II and factor X due to mixing factor II and factor X double-deficient plasma into the test sample.[8] Both tests used in-house standardisation of the thromboplastin sensitivity index (ISI) with ISI calibrators and control plasma (Danish Institute for External Quality Assurance in Health Care, Glostrup, Denmark). The calibrator is designed for PT standardisation but not for Fiix-PT standardisation.

Standardised PT ratios and Fiix-PT ratios were reported electronically as R-INR to dosing staff (nurses, biomedical scientists, and physicians) who adjusted doses according to usual practice, aiming for an R-INR of 2–3, with the DAWN anticoagulation software (4-S, Milnthorpe, UK)[5] and in-house protocols designed for monitoring with traditional PT based on the American College of Chest Physicians guidelines.[6,10] The maximum recommended interval between monitoring tests was 6 weeks.

All thromboembolism and bleeding events reported to the anticoagulation management centre by patients, health-care workers, and hospital units were recorded.

	Fiix-PT group (n=573)	PT group (n=575)
Age (years)	71 (64–78)	72 (64–79)
Sex		
Male	357 (62%)	379 (66%)
Female	216 (38%)	196 (34%)
Ethnic origin		
Caucasian	>99%	>99%
Years of warfarin treatment before enrolment	3·7 (0·9–8·2)	3·4 (0·8–7·8)
Warfarin experienced (>60 days on warfarin when enrolled)	492 (86%)	506 (88%)
Short-term warfarin treatment*	53 (9%)	45 (8%)
Indications for warfarin		
Heart disease	445 (78%)	446 (78%)
Atrial fibrillation	408 (71%)	429 (75%)
Atrial fibrillation without previous arterial thromboembolic events	307 (75%)	317 (74%)
Atrial fibrillation with previous cerebral thromboembolic events or transient ischaemic attack	96 (24%)	106 (25%)
Atrial fibrillation with previous peripheral arterial embolism	5 (1%)	6 (1%)
CHA_2DS_2-VASC risk score in patients with atrial fibrillation	3 (2–4)	3 (2–4)
Score 0 (low thromboembolic risk)	7·0	5·6
Score 1 (moderate thromboembolic risk)	7·4	13·6
Score ≥2 (high thromboembolic risk)	85·6	80·8
Ischaemic heart disease	24 (4%)	16 (3%)
Acute myocardial infarction	23 (96%)	15 (94%)
Other ischaemic heart disease	1 (4%)	1 (6%)
Congestive heart failure as only indication	1 (<1%)	0
Atrial septal defect	8 (1%)	3 (<1%)
Artificial heart valves	10 (2%)	10 (2%)
Rheumatic mitral valve disease	1 (<1%)	1 (<1%)
Arterial thromboembolism without known atrial fibrillation	36 (6%)	33 (6%)
Cerebral thromboembolism or transient ischaemic attack	30 (83%)	33 (100%)
Peripheral arterial thromboembolism	6 (17%)	0
Venous thromboembolism	140 (24%)	128 (22%)
Deep vein thrombosis alone	60 (43%)	53 (41%)
Pulmonary embolism	78 (56%)	74 (58%)
Pulmonary hypertension	2 (1%)	1 (<1%)
Associated conditions		
Smoker	68 (12%)	61 (11%)
High blood pressure	339 (59%)	349 (61%)
Ischaemic heart disease	152 (27%)	158 (27%)
Peripheral vascular disease	31 (5%)	36 (6%)
History of congestive heart failure	132 (23%)	123 (21%)
Diabetes	79 (14%)	66 (11%)
Cancer	87 (15%)	100 (17%)
Active cancer chemotherapy	11 (13%)	14 (14%)
Drug use		
Acetylsalicylic acid	124 (22%)	121 (21%)
Clopidogrel	12 (2%)	8 (1%)
Non-steroidal anti-inflammatory drugs	60 (10%)	66 (11%)
Amiodarone	53 (9%)	53 (9%)
Hydrogen blockers and proton pump inhibitors	125 (22%)	125 (22%)
Any other drugs	528 (92%)	533 (93%)

Data are median (IQR) or n (%). Percentages may not total 100% owing to presence of more than one indication in some patients or rounding of numbers. *Short-term warfarin is defined as less than 6 months receiving warfarin treatment. Fiix-PT=Fiix-prothrombin time. PT=prothrombin time.

Table 1: **Baseline characteristics**

All participants were instructed to report any new health-related events as soon as possible. The study nurse contacted all participants at 4-week intervals using a checklist to elicit information about thromboembolism or bleeding otherwise unknown to the anticoagulation management centre. After a recorded event, the next follow-up call was 4 weeks later. We assessed causes of death on the basis of information obtained from hospital records, physicians, autopsy reports, and death certificates. Deaths were coded as caused by bleeding, thromboembolism, or other causes when neither could be confirmed.

Outcomes

The primary efficacy outcome was calculated in the intention-to-monitor population and was a composite of objectively diagnosed non-fatal and fatal arterial or venous thromboembolism, including myocardial infarction during the whole 720 day study period. We included transient ischaemic attacks of any kind if they had been diagnosed by a treating physician, but imaging studies were not mandatory. We did not include superficial thrombophlebitis. Secondary efficacy outcomes included non-thromboembolism and non-haemorrhagic related deaths. Safety outcomes were major bleeding and a composite of major bleeding and other clinically relevant non-major bleeding occurring in the per-protocol population. Major bleeding was defined according to the International Society on Thrombosis and Haemostasis criteria—ie, bleeding leading to hospital admission, transfusion of at least two units of packed red cells, or bleeding into a closed compartment such as the cranium or pericardium.[13] We defined other clinically relevant non-major bleeding as overt bleeding not meeting the criteria for major bleeding but associated with medical intervention, unscheduled

	Fiix-PT group		PT group		Relative risk (95% CI)	p value for total events†
	n	Percentage per patient observation year*	n	Percentage per patient observation year*		
Primary endpoints						
Efficacy						
Primary outcome population	573	..	575	..		
Total observation years	828	100%	835	100%		
Fatal and first non-fatal thromboembolism including myocardial infarction	10 (1)	1·21% (0·12)	19 (3)	2·28% (0·36%)	0·52 (0·25–1·13)	<0·0001
Cerebral infarction or transient ischaemic attacks	9 (0)	1·09%	14 (1)	1·68% (0·12%)	0·65 (0·28–1·48)	0·0002
Cerebral infarction	7 (0)	0·85%	11 (0)	1·31%	0·64 (0·25–1·64)	0·0002
Transient ischaemic attack	2 (0)	0·24%	3 (0)	0·36%	0·67 (0·11–3·99)	0·0001
Myocardial infarction	1 (1)	0·12%	3 (2)	0·36% (0·24)	0·33 (0·03–3·21)	<0·0001
Peripheral arterial occlusion	0	0	1 (0)	0·12% (0)	..	..
Venous thromboembolism	0	0	1 (0)	0·12% (0)	..	..
Safety endpoints						
Per-protocol population	571	..	573	..	..	..
Total observation years	771	100%	786	100%	..	..
First major bleeding	17 (1)	2·20% (0·13%)	20 (3)	2·5% (0·38%)	0·85 (0·45–1·61)	0·0034
Gastrointestinal	12 (1)	1·56% (0·13%)	10 (0)	1·27% (0)	1·2 (0·52–2·76)	0·0093
Intracranial	2 (0)	0·26% (0)	5 (1)	0·64% (0·13%)	0·4 (0·08–2·06)	<0·0001
Intracerebral	1 (0)	0·13% (0)	3 (1)	0·38% (0·13%)	0·33 (0·03–3·21)	<0·0001
Other major bleeding	3 (0)	0·39% (0)	4 (2)	0·51% (0·25%)	0·75 (0·17–3·35)	0·0002
Non-major clinically relevant bleeding						
All (including repeated)	118	14·25%	135	16·16%	0·88 (0·71–1·09)	0·0140
First non-major clinically relevant bleeding	87	10·51%	95	11·38%	0·92 (0·7–1·2)	0·0379
Minor bleeding	279	36·70%	301	36·05%	0·93 (0·83–1·04)	0·0185
Secondary endpoints						
Death from any cause	12	1·45%	16	1·92%	0·75 (0·36–1·58)	0·0008
Non-vascular death	10	1·21%	10	1·20%	1 (0·42–2·39)	0·0027
Composite major vascular events	27 (2)	3·50% (0·26%)	39 (6)	4·96% (0·76%)	0·69 (0·43–1·12)	0·0006

Non-inferiority analysis of total major events occurring during days 1–720 from randomisation. Fatal events are shown in parentheses. Efficacy of the monitoring method is assessed based on intention-to-monitor analysis, but safety of monitoring method is based on actual time on warfarin including a 5-day washout period after warfarin discontinuation (per-protocol population). Fiix-PT=Fiix-prothrombin time. PT=prothrombin time. *Percentage with event per patient observation year. †p value by Farrington–Manning test of non-inferiority with a non-inferiority margin of 0·025.

Table 2: **Primary analysis of clinical outcome**

physician contact, temporary cessation of treatment, discomfort such as pain, or impairment of activities of daily life. We classified other bleeding as minor. We calculated composite major vascular events as the total incidence of both fatal and non-fatal thromboembolism events and major bleeding. Surrogate efficacy variables calculated in the per-protocol population included the number of tests in each study group, dose adjustment frequency, and the percentage of tests at defined ranges. We calculated the percentage of time that each individual patient spent within the INR target range using the Rosendaal formula.[14] We also calculated the variance growth rate as an indicator of INR variability between tests (B2 method).[15]

Statistical analysis

To calculate the non-inferiority margin, we used data from previous prospective studies[16–19] in patients with mixed indications for anticoagulation, with an INR target of 2–3, monitored and dosed by our anticoagulation management centre. On the basis of these studies, we used an expected 3% annual thromboembolism incidence and a non-inferiority margin of 2·5%. We regarded this 2·5% difference as clinically significant, yet within the 95% CI for event incidence in previous studies.[20] On the basis of these considerations, with two separate online calculators, we calculated the number of participants needed to show statistical non-inferiority of clinically important events for the test method with an 80% certainty per year of observation as being 576 patients in each group. A study time of 24 months was expected to allow for a delay in recruitment and potential dropouts.

The prespecified primary and post-hoc secondary analyses of efficacy were assessed in an intention-to-monitor population—ie, all events were counted from

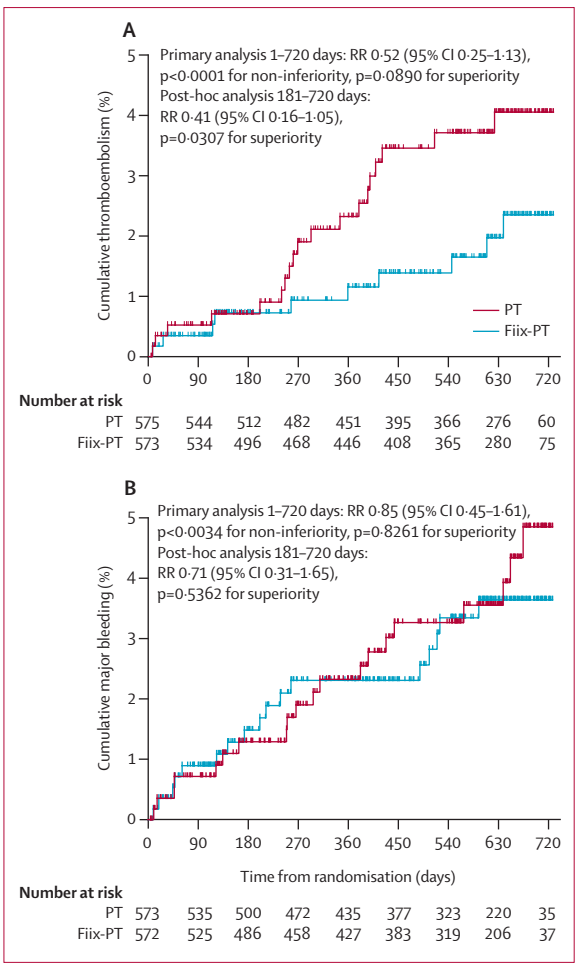

Figure 2: **Cumulative event incidences during days 1–720 for primary efficacy outcome thromboembolism (A) and major bleeding (B) in all patients monitored with Fiix-prothrombin time (Fiix-PT) versus quick prothrombin time (PT)**

Non-fatal and fatal events are shown. RR=relative risk. PT=prothrombin time.

the day of enrolment until 5 days after final discontinuation of warfarin or study completion. Patients with thromboembolism events were censored after the occurrence. We analysed safety outcomes in the per-protocol population—ie, we included bleeding events occurring from enrolment of warfarin-experienced patients or after two INRs fell within the target range after warfarin initiation in new patients and until 5 days after discontinuation or study completion, but we excluded periods of temporary discontinuation of warfarin or dose management outside the anticoagulation management centre from analysis (eg, during surgery, hospital admission, or precardioversion for atrial fibrillation with monitoring at fixed weekly intervals) until two INRs fell within the target range after restarting warfarin. Patients were censored after the first major bleed. We did calculations of surrogate variables in the per-protocol population.

See Online for appendix

We compared continuous data with the Mann–Whitney test and categorical data with χ^2 or Fisher's exact tests. We used the Farrington–Manning test of non-inferiority to calculate non-inferiority of the efficacy and safety endpoints[21,22] and calculated relative risk (RR) with a 95% CI. We generated Kaplan-Meier curves to show events occurring over time and compared them with the Breslow–Gehan–Wilcoxon test for superiority assessment. We did a subgroup analysis for the largest subgroup of non-valvular atrial fibrillation. We regarded p of less than 0·05 as significant. We did statistical analyses with GraphPad Prism version 6.0 and R version 3.1.1 (July 10, 2014).

This study is registered with ClinicalTrials.gov, number NCT01565239.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. PTO, CWF, OSI, SJJ, HMJ, SHL, and BRG had access to the data. All authors were responsible for the final decision to submit this report for publication.

Results

Between March 1, 2012, and Feb 28, 2014, we enrolled 1156 patients (figure 1). After exclusion of four patients in each group, 573 patients were monitored with Fiix-INR and 575 with PT-INR. The total intention-to-monitor analysis observation time was 828 patient-years in the Fiix-PT group and 835 patient-years in the PT group with a median follow-up of 1·7 years (IQR 1·1–1·9) per person in both groups. In the Fiix-PT group, 440 patients were followed up for more than 1 year, comprising 766 of the observation years; in the PT group, 450 patients were followed up for more than 1 year, comprising 773 observation years. Baseline characteristics did not differ between the groups (table 1), although minor numerical differences were reported. The average CHA_2DS_2-VASC scores were similar in patients with atrial fibrillation in both groups, mean 2·9 (SD 1·6) and median 3·0 (IQR 2–4) in the Fiix-PT group and 3·0 (SD 1·6) and 3·0 (IQR 2–4) in the control group, supporting a similar thromboembolism risk in both study groups. The number of participants who discontinued warfarin, switched to direct oral anticoagulants, or discontinued from the study for other reasons did not differ between groups. No patients were lost to follow-up (appendix p 3).

In the primary analysis of thromboembolism events occurring during days 1–720, thromboembolism occurred in ten patients in the Fiix-PT group (incidence of 1·2% per patient-year) versus 19 (2·3% per patient-year) in the PT group (RR 0·52, 95% CI 0·25–1·13, $p_{non\text{-}inferiority}<0·0001$, $p_{superiority}=0·0890$; 17 *vs* 20 events, p=0·9285 for superiority; table 2, figure 2). The occurrence of ischaemic strokes (including transient ischaemic attack) alone or myocardial infarction alone was also non-inferior (table 2). In a secondary analysis, that was not prespecified but based on

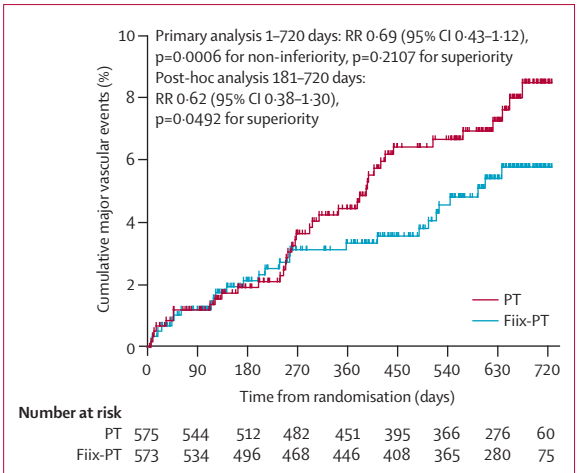

Figure 3: **Composite fatal and non-fatal major vascular events in all patients monitored with either Fiix-prothrombin time (Fiix-PT) or quick prothrombin time (PT; control group)**
Thromboembolic events are assessed based on intention-to-monitor analysis and major bleeding based on per-protocol (on-treatment) analysis. The primary analysis is for days 1–720 and a secondary analysis was done for days 181–720 when only warfarin-experienced long-term patients are observed. RR=relative risk. PT=prothrombin time. INR=international normalised ratio.

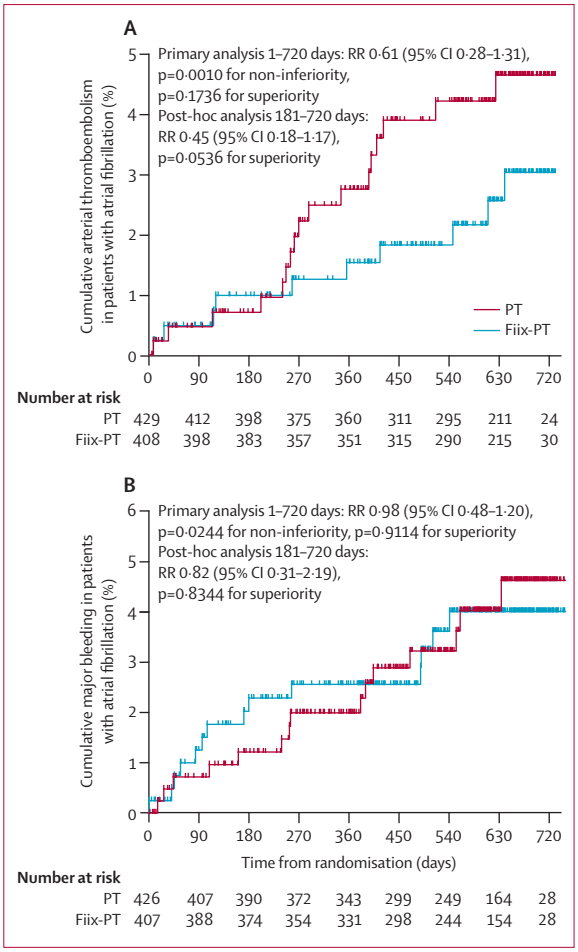

Figure 4: **Cumulative event incidences during days 1–720 for primary efficacy outcome (A) and major bleeding (B) in the subgroup of patients with atrial fibrillation monitored with Fiix-prothrombin time (Fiix-PT) versus quick prothrombin time (PT)**
Non-fatal and fatal events are shown. RR=relative risk. PT=prothrombin time. INR=international normalised ratio.

the observation that a difference emerged after 6 months, we excluded the first 6 months after randomisation from analysis. In this analysis, Fiix-PT monitoring led to a significant long-term reduction in thromboembolism (figure 2A), six versus 15 cases (1·1% *vs* 2·2% per patient-year; RR 0·41, 0·16–1·05, p=0·0307 for superiority).

Major bleeding was analysed in the per-protocol population and was similar between the Fiix-PT group and the PT group (table 2, figure 2B). Unlike thromboembolism, the incidence was consistent and showed no divergence after 6 months. An intention-to-monitor analysis did not change the outcome (19 *vs* 21 events, p=0·9090 for superiority; data not shown). Despite the small number of events, Fiix-PT was also non-inferior to PT in the frequency of gastrointestinal bleeding, intracranial haemorrhage, intracerebral haemorrhage, and other major bleeding (table 2). Incidences of clinically relevant non-major bleeding events and minor bleeding events with Fiix-PT were similar to the incidences observed with PT (table 2). The incidence of composite major bleeding and non-major clinically relevant bleeding was also non-inferior in the Fiix-PT group (p=0·0351; appendix p 5).

12 patients died in the Fiix-PT group (1·45% per patient-year) versus 16 (1·92% per patient-year) in the control group (table 2). Composite major vascular events occurred in 27 patients in the Fiix group compared with 39 controls (table 2, figure 3). In the secondary post-hoc analysis beyond 6 months of Fiix-PT monitoring, significantly fewer major vascular events occurred in the Fiix-PT than in the PT group (p=0·0492 for superiority; figure 3). However, incidence of combined composite major vascular events and deaths from any cause did not differ significantly between groups (p=0·0537 for superiority; appendix p 6).

In a non-prespecified subgroup analysis of patients with atrial fibrillation, ten arterial thromboembolism events (1·63% per patient-year) occurred in the Fiix-PT group versus 17 (2·7% events per patient-year) in the PT group (RR 0·62, 95% CI 0·29–1·33, $p_{non\text{-}inferiority}$=0·0010, $p_{superiority}$=0·1763; figure 4A). In the secondary analysis of thromboembolism beyond the first 6 months in participants with atrial fibrillation, six events occurred in the Fiix-PT group (1·4% per patient-year) versus 14 (3·2% per patient year) in the PT group (RR 0·45, 95% CI 0·18–1·17, $p_{superiority}$=0·0536). Major bleeding occurred in 14 participants with atrial fibrillation in the Fiix-PT group (2·5% per patient-year) and 15 (2·5% per patient-year) in the PT group (RR 0·98, 0·48–2·0, $p_{non\text{-}inferiority}$=0·0244; figure 4B).

	Fiix-PT group (n=571), 771 patient-years	PT group (n=573), 786 patient-years	p value
Number of monitoring tests			
Total	11 231	11 719	..
Days 1–180	4204	4258	..
Days 181–720	7027	7461	..
Number of tests within defined INR ranges			
INR 2–3	7817 (69·6%)	7924 (67·6%)	0·0011
INR <2	1538 (13·6%)	1931 (16·5%)	<0·0001
INR >3	1876 (16·7%)	1864 (15·9%)	0·1051
Days between monitoring tests			
Days 1–180	19 (12–28)	20 (13–28)	0·37
Days 181–720	22 (14–38)	21 (14–35)	<0·0001
Dose changes per monitoring test in each patient			
Days 1–180	33% (17–50)	38% (17–55)	0·0249
Days 181–720	33% (20–48)	38% (22–50)	0·0250
Days 181–360	33% (17–57)	43% (20–60)	0·0410
Days 361–540	33% (0–50)	32% (0–54)	0·96
Days 541–720	11% (0–33)	20% (0–47)	0·0178
Number of dose changes per patient per year			
Days 1–180	5·3 (2·2–10·9)	6·5 (2·3–11·7)	0·0716
Days 181–720	4·1 (2·0–7·1)	5·0 (2·2–8·0)	0·0101
TTR of each patient			
Days 1–180	85% (71–96)	81% (68–93)	0·0133
Days 181–360	85% (72–98)	80% (66–94)	0·0002
Days 361–540	80% (65–96)	81% (69–96)	0·3373
Days 541–720	87% (66–100)	79% (61–96)	0·0043
Median VGR	0·14	0·18	0·0003
VGR, patients with major events	0·21	0·28	0·2684
VGR, patients without major events	0·14	0·17	0·0008
Daily warfarin dose (mg)	4·7 (3·3–6·3)	4·7 (3·3–6·3)	0·20

Data are median (IQR), unless otherwise stated. Per-protocol (on-treatment) analysis of the safety of monitoring method. Only patients that have three or more tests were included in the TTR interval calculation and warfarin initiation periods were excluded from the TTR analysis until two INRs fell within target range. Fiix-PT=Fiix-prothrombin time. PT=prothrombin time. NA=not applicable. INR=international normalised ratio. TTR=time within target range by Rosendaal method. VGR=variance growth rate by B2 method—ie, between test variance in INR.

Table 3: **Surrogate outcome variables**

Dose change frequency was reduced with Fiix-PT monitoring, particularly long term (ie, after day 180, p=0·0101), with no significant differences in the first 6 months; however, the median daily warfarin dose was identical in both groups, 4·7 mg (IQR 3·3–6·3; table 3). With long-term Fiix-PT monitoring (180–720 days), monitoring tests were reduced by 5·8%. More monitoring tests were within the target range in the Fiix-PT group than in the control group and fewer tests with an INR less than 2·0 occurred in the Fiix-PT group (table 3). The median percent time in range in the control group was 81%, 80%, 81%, and 79% during four consecutive 6-month observation periods, whereas in the Fiix-monitoring group the median percent time in range was 85%, 85%, 80%, and 87%, respectively. INR fluctuation measured as variance growth rate was significantly higher in the PT group than in the Fiix-PT group (table 3). Patients with major events had a higher variance growth rate than did those without major events (table 3).

16 months into the study, an ISI calibration issue occurred, causing the median Fiix-INR to be reported 0·2 points higher than previously despite control samples being within limits, whereas the PT-INR did not change. This issue would have mainly affected the analysis during days 450–630 of observation. A corresponding temporary lowering of times in range in the Fiix group but not the PT group was observed and might have led to unneeded and aberrant dose reductions in the Fiix-PT group. The difficulty was identified to coincide with the receipt of a new order of the DEKS ISI calibrator. When the old batch DEKS calibrator could be obtained again and used to recalibrate ISI, the median Fiix-INR returned to the previous levels, and a higher time in the range returned. The appendix (p 4) shows reanalysed data from table 3 after exclusion of R-INRs done during this calibration period.

Discussion

Warfarin monitoring with Fiix-PT (Fiix-INR) stabilised and increased the intensity of anticoagulation in a well managed typical anticoagulation management centre patient population with an INR target of 2–3. This resulted in fewer thromboembolisms per year in patients monitored with Fiix-PT than in patients monitored with standard PT, without an increase in bleeding. Additionally, although not a prespecified analysis, we noted significantly fewer thromboembolisms, with a low bleeding incidence in patients who were monitored long term with Fiix-PT, compared with those monitored by PT-INR, although this was based on a small number of events.

Warfarin anticoagulation instability from food and drug interactions and patient non-compliance has long been regarded to contribute to adverse events during warfarin management.[1] On the basis of our results, however, an additional source of INR fluctuation emerges—namely, a confounding side-effect of the PT. The PT is equally sensitive to reduction in the activity of factor II, factor VII, or factor X.[8] However, the long half-life factor II and factor X and not the short half-life factor VII mainly affect clot and thrombus formation.[8,9,23] By replacement of PT with Fiix-PT, the confounding effect of the fluctuating factor VII in the test sample on INR is eliminated, which improves stability. However, as evident from our study, a special Fiix-INR calibrator will probably be needed.

An increased time in range was evident in the Fiix-PT group during the first 6 months, but the long-term clinical benefit only emerged subsequently. Reduced thromboembolism in the Fiix-PT group was associated with proportionally more tests in range and fewer tests below range (table 3), whereas the proportion with an increased INR with Fiix-PT was similar to PT-INR, the similar proportion above target range possibly partly

explaining similar, albeit low, bleeding incidence in both study groups. The significantly lower variance growth rate in the Fiix-PT group than the PT group suggests less variability of anticoagulation monitored with the Fiix-INR. Although why the clinical effect of improved anticoagulation stability only becomes evident after 6 months of Fiix-PT monitoring is unclear, we suggest that this could be explained by the lower variance growth rate in the Fiix-PT group because a high variance growth rate (unstable INR) has been retrospectively shown to be predictive of clinical events 3–6 months later.[15] Alternatively, other long-term effects such as effects on the vessel wall might be implicated. Our trial might be the first prospective study to show the effect of variance growth rate on clinical events in patients receiving wafarin.

Large multicentre clinical trials have suggested that unmonitored direct oral anticoagulants are clinically non-inferior or even superior to warfarin in both non-valvular atrial fibrillation[12,24–26] and in venous thromboembolism.[27–29] However, the benefit of direct oral anticoagulants in atrial fibrillation has been reported mainly at study sites[24,30,31] that did not maintain a high time within target range in patients in the warfarin control group and this inevitably exaggerates the reported benefit of direct oral anticoagulants. Our subgroup analysis of patients with non-valvular atrial fibrillation (75% of the study population) raises the question of whether management of patients with atrial fibrillation on warfarin with Fiix-PT instead of PT has the potential to improve long-term outcomes in atrial fibrillation even further than that achievable with unmonitored direct oral anticoagulants. In the patients with atrial fibrillation in this study, Fiix-PT led to a long-term reduction after 6 months in arterial thromboembolism (combined ischaemic strokes, transient ischaemic attacks, other arterial embolism, and myocardial infarction). In the direct oral anticoagulant trials,[12,24–26] the change in arterial thromboembolism (including myocardial infarction but excluding transient ischaemic attacks) in the active study groups ranged from +11% to –19% compared with outcome during only moderately well managed warfarin monitored with PT with time within target range in the 58–65% range. Notably, the absolute incidence of total arterial thromboembolism in our patients with atrial fibrillation monitored with Fiix-PT was lower than in the direct oral anticoagulation studies and the low major bleeding incidence, including intracranial haemorrhage incidence, compared favourably with direct oral anticoagulants. Improved stability of warfarin management with Fiix-PT, with resulting improved long-term outcome and a low bleeding incidence could therefore lead to less impetus to switch patients to the unmonitored and more expensive direct oral anticoagulants. Although unmonitored direct oral anticoagulants might be convenient to use, the efficacy, safety, reversibility, titratability, and affordability must all be taken into account. These new drugs also do not yet have antidotes for emergency reversal and might not be straightforward in elderly patients and in those with reduced kidney function.

Our trial compared in a randomised and masked manner the incidence of thromboembolism and bleeding in 1148 patients treated with warfarin monitored by two different tests at a single medical centre. We regard the masking to have been successful because the dosing staff could not decipher which monitoring group a patient was assigned to. A larger confirmatory multicentre study would, however, be preferred because such a trial could provide increased statistical power and the ability to do further subgroup analyses that would increase the generalisability of the results. The setting of our trial, nevertheless, was a typical anticoagulated population at one specialised academic medical centre applying specialised anticoagulation software that ensured that treatment and follow-up between the two groups was identical. The only difference was the absence of the effect of factor VII in the test sample on monitoring in the Fiix-PT group. Despite not detecting factor VII in the Fiix-PT group, anticoagulation stability was improved, thromboembolism was reduced in the long term and bleeding incidence remained low. Because we investigated only few warfarin-naive patients, conclusions should not be made from this study on clinical outcome differences in this group. Patients in the Fiix-PT group were dosed by a protocol that was designed for the fluctuating PT-INR. Therefore, dosing staff, on the basis of their PT-INR management experience, might have responded to the masked Fiix-INR with unnecessary dose adjustments that might have caused a bias in favour of the PT group. Hence, even further improvements could possibly be achieved with the Fiix-INR than those reported in this trial. Although minor differences were noted in patient characteristics, mainly indications for warfarin, they are probably to be expected in a study of this size. Also, although slightly fewer patients had atrial fibrillation in the Fiix-PT group than in the PT-INR group, somewhat more had a high-risk CHA_2DS_2-VASC score in the Fiix-PT group.

Finally, an unexpected Fiix-INR calibration issue occurred 16 months into the study that might have caused an erroneous dose reduction that would not have been in favour of an improved outcome in the active Fiix-PT group. Figure 3 might indicate that the composite major vascular event incidence increased in the Fiix-PT group during this period (mainly days 450–630) because the event curves seem to converge then diverge again once the issue was corrected. The issue was not reported with PT-INR calibration, which the calibrator is designed for. Hence, different calibrators will probably be needed to standardise the Fiix-INR.

We conclude that monitoring warfarin with Fiix-PT improves the stability of warfarin management and is clinically at least non-inferior to PT-INR. Furthermore, a post-hoc analysis suggests that thromboembolism is

reduced during long-term treatment when standard INR monitoring is replaced with Fiix-INR.

Contributors

PTO chaired the study. PTO, CWF, OSI, and BRG designed the trial. PTO, CWF, OSI, SJJ, SHL, HMJ, and BRG analysed the data. PTO, CWF, OSI, and BRG interpreted the data. PTO wrote the report draft. CWF, OSI, DOA, ESB, MKM, SJJ, HMJ, BV, PSG, and BRG edited the report. OSI, BV, and PSG were members of the data safety and monitoring board. DOA, ESB, and MKM were adjudication committee members. SHL was the statistician, consultant, and responsible for statistical calculations. OSI was also responsible for statistical calculations.

Declaration of interests

PTO and BRG together with the Landspitali and University of Iceland have applied for a patent (patent pending) for the Fiix-prothrombin time invention. All other authors declare no competing interests.

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