J Thromb Thrombolysis (2017) 43:550–561
DOI 10.1007/s11239-017-1482-4

Reduced anticoagulation variability in patients on warfarin monitored with Fiix-prothrombin time associates with reduced thromboembolism: The Fiix-trial

Alma Rut Oskarsdóttir[1] · Brynja R. Gudmundsdottir[1] · Olafur S. Indridason[1] · Sigrun H. Lund[2] · David O. Arnar[1] · Einar S. Bjornsson[1,2] · Magnus K. Magnusson[1,2] · Hulda M. Jensdottir[1] · Brynjar Vidarsson[1] · Charles W. Francis[3] · Pall T. Onundarson[1,2]

Published online: 18 February 2017

Abstract Fiix-prothrombin time (Fiix-PT) differs from traditional PT in being affected by reduced factor (F) II or FX only. In the randomized controlled Fiix-trial, patients on warfarin monitored with Fiix-PT (Fiix-warfarin patients) had fewer thromboembolisms (TE), similar major bleeding (MB) and more stable anticoagulation than patients monitored with PT (PT-warfarin patients). In the current Fiix-trial report we analyzed how reduced anticoagulation variability during Fiix-PT monitoring was reflected in patients with TE or bleeding. Data from 1143 randomized patients was used. We analyzed the groups for anticoagulation intensity (time within target range; TTR), international normalized ratio (INR) variability (variance growth rate B_1; VGR) and dose adjustment frequency. We assessed how these parameters associated with clinically relevant vascular events (CRVE), ie TE or MB or clinically relevant non-MB. TTR was highest in Fiix-warfarin patients without CRVE (median 82%;IQR 72–91) and lowest in PT-warfarin patients with TE (62%;56–81). VGR was lowest in Fiix-warfarin patients without CRVE (median VGR B_1 0.17; 95% CI 0.08–0.38) and with TE (0.20;0.07–0.26) and highest in PT-warfarin patients with TE (0.50;0.27–0.90) or MB (0.59;0.07–1.36). The mean annual dose adjustment frequency was lowest in Fiix-warfarin patients with TE (mean 5.4;95% CI 3.9–7.3) and without CRVE (mean 6.0; 5.8–6.2) and highest in PT-warfarin patients with TE (14.2;12.2–16.3). Frequent dose changes predicted MB in both study arms. Compared to patients monitored with PT, high anticoagulation stability in Fiix-warfarin patients coincided with their low TE rate. Those with bleeding had high variability irrespective of monitoring method. Thus, although further improvements are needed to reduce bleeding, stabilization of anticoagulation by Fiix-PT monitoring associates with reduced TE.

Keywords Prothrombin time · Oral anticoagulants · Monitoring · Warfarin · Fiix · INR

Introduction

Anticoagulation with vitamin K antagonists (VKA) relies on controlling their inhibition of the γ-carboxylation of vitamin K dependent coagulation factors (F) [1]. For 65 years this has been accomplished by measuring the Quick prothrombin time (PT) [2] or the variant Owren´s PT [3], both being equally affected by reduced activity of any of FII, FVII or FX. Using a calibrator, PT results are converted into an internationally normalized PT ratio (INR, PT-INR) [4] but INR variability, often ascribed to food and drug interactions, remains a problem. Variability causes frequent dose adjustments and repeated testing and low time within therapeutic target range (TTR) and anticoagulation variability associates with unfavorable clinical outcome [5, 6]. In the short term, INR variability may be mainly caused by rapid fluctuations in FVII activity that has a very short half-life of 4–6 h. However, evidence suggests

Electronic supplementary material The online version of this article (doi:10.1007/s11239-017-1482-4) contains supplementary material, which is available to authorized users.

✉ Pall T. Onundarson
pallt@landspitali.is

[1] Department of Laboratory Hematology, Landspitali National University Hospital of Iceland, K-building, Hringbraut, 101 Reykjavik, Iceland

[2] Faculty of Medicine, University of Iceland, Reykjavik, Iceland

[3] University of Rochester Medical Center, Rochester, NY, USA

that reduced FVII has little influence on the antithrombotic effect that depends mainly on controlled reduction of the longer half-life FII or FX activity or their combined reduction [7, 8]. The new Fiix-prothrombin time (Fiix-PT) was developed to circumvent measuring the influence of FVII and to monitor only reductions in FII and FX during VKA treatment in order to stabilize the anticoagulant effect [8].

The mixed population Fiix-trial confirmed its prespecified hypotheses that monitoring warfarin with Fiix-PT based normalized ratio (Fiix-INR, Fiix-NR) would stabilize anticoagulation, reduce testing, reduce dose adjustments and lead to at least non-inferior clinical outcomes compared to high-TTR standard PT-INR monitoring. Furthermore, there was a larger TE reduction in the Fiix-monitoring arm than expected, from 2.3% annually in controls to 1.2% with Fiix-INR monitoring or by 48% (RR 0.52; 95% CI 0.26–1.13, $P_{non\text{-}inferiority} < 0.0001$, $P_{superiority} = 0.0890$ but the trial was not powered to demonstrate superiority). Major bleeding was not increased in the Fiix-PT arm [9]. As a lower incidence of TE only became apparent beyond 6 months of Fiix-INR monitoring when the TE event curves started diverging, a separate post-hoc analysis of the primary efficacy endpoint was also reported after excluding the first 6 months. In that secondary analysis the TE reduction was significanly superior to standard monitoring (1.1% vs. 2.2%; P = 0·03 for superiority) [9]. A similar delayed efficacy improvement was previously observed with ximelagatran versus warfarin [10]. A meta-analysis comparing outcome of Fiix-trial atrial fibrillation (AF) patients to that of PT-INR monitored controls in four major direct oral anticoagulant (DOAC) trials in AF demonstrated a statistically significant 49% reduction in total TE and a 37% non-significant reduction in major bleeding [11].

In the current Fiix-trial report, the aim was to assess how the reduced anticoagulation variability observed in patients monitored with Fiix-PT was reflected in patients with thrombotic or bleeding events.

Methods

Study population, conduct and approvals

This is a secondary analysis of the investigator initiated Fiix-trial, a single-center, double-blind non-inferiority randomized controlled clinical trial (RCT) conducted at Landspítali - the National University Hospital of Iceland in Reykjavik, Iceland from March 1st 2012 to February 28 2014. In the Fiix-trial, patients 18 years and older receiving or starting short- or long-term warfarin therapy with an INR target value of 2.0–3.0 were randomized in a 1:1 ratio to either a research arm monitored with Fiix-PT (Fiix arm, Fiix-warfarin patients) or a control arm monitored with standard Quick PT (PT arm, PT-warfarin patients). Both arms were dosed based on a protocol and software algorithm designed for monitoring with PT-INR, using the DAWN® anticoagulation software (4-S, Penrith, England) assisted dosing with a maximum recommended 6 weeks interval between monitoring tests [9]. Heparin or low-molecular weight bridging was applied only during periods of surgery or hospitalization when patients were excluded temporarily from the study [9]. 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 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. Both tests used Neoplastin (Diagnostica Stago, Asnieres, France) and an 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 a blinded R-INR to dosing staff (nurses, biomedical scientists, and physicians). The dosing staff, patients and members of the clinical event adjudication committee that assessed and classified clinical events were blinded to the test origin of the reported INR. The trial was conducted according to the Helsinki protocol with all appropriate approvals and primary clinical results have been published [9]. The Fiix-trial was registered at http://www.clinicaltrials.gov as NCT01565239. A consort flow diagram is shown as an online supplement.

Procedures

The Fiix arm and the PT arm of the Fiix-trial were divided into subgroups based on occurrence or absence of clinically relevant vascular events (CRVE). The CRVE groups were thromboembolism (TE) or clinically relevant bleedings (CRB), either major bleedings (MB) or in the absence of MB the first non-major CRB [9]. MB was defined according to the International Society on Thrombosis and Haemostasis (ISTH) criteria [12]. TE events had to be objectively verified by treating physicians and imaging and were classified as non-fatal or fatal arterial TE (ATE) or venous TE (VTE), including myocardial infarction (MI), peripheral arterial embolism, cerebral infarction and transient ischemic attacks (TIA) [9]. Patients with major events (TE, MB) were censored at the time of event. For patients with non-major clinically relevant bleeding only time until their first event was used to assess surrogate parameters. In those

with no event the whole study period as defined above was used. Hence, for each subgroup we evaluated, before the occurrence of the CRVE, monitoring test numbers, test intervals, dosing intervals and dose, percent time within target range (TTR) by the method of Rosendaal [6, 13], and the variance growth rate (VGR) as an indicator of INR or Fiix-INR variability [6]. Due to a 3.5-month laboratory INR calibration problem during the study, which may have led to unnecessary and aberrant dose reductions in the Fiix arm as described in the Fiix-trial initial publication [9], this period was excluded from both study arms in the current analysis leading to a shorter observation interval than in the initial publication. Also, this lead to one patient from the Fiix arm and four from the PT arm being excluded from the current analysis, resulting in 1143 participants instead of 1148 in the initial publication.

Calculations and statistical analysis

All subgroup analyzes reported here were done based on an intention-to-monitor (ITM) analysis, i.e., all TE and bleeding events and results were included from the day of enrolment until 5 days after final discontinuation of warfarin or study completion, regardless of temporary discontinuations of therapy during the study. The number of monitoring tests, number of observation days and number of days between monitoring tests were counted in each patient and the annual test frequency rate for each patient was calculated. The number of dose changes were counted for each patient and the number of annual dose changes in each patient were calculated as well as dose changes per monitoring test for each patient. To calculate TTR and VGR, only patients with three or more monitoring tests were included in the calculations and, hence, 28 patients were excluded; 12 in the Fiix arm (3 Fiix event, 9 Fiix no event) and 16 in the PT arm (3 PT event, 13 PT no event). INR variability was calculated using VGR formula B_1 [6].

Formula B_1 (INR variation between adjacent INR tests):

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

In these formulas, n refers to the number of INR measurements, i to individual INR result, τ to time in weeks between the present and previous INR measurement.

The non-parametric Mann–Whitney test was used to compare continuous data between two groups and the Kruskal–Wallis test (ANOVA) for more than two groups. The Fisher exact test or the Chi square tests were used to compare categorical data. Differences in rates were compared using Poisson regression using the corresponding numerator as an offset. All P values less than 0.05 are considered statistically significant. All statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA) and R (R Foundation for Statistical Computing, Vienna, Austria).

Results

Study population and treatment description

This subgroup analysis is based on 22,525 monitoring tests from 1143 patients; 11,026 from 572 patients monitored with Fiix-PT and 11,499 from 571 patients monitored with PT (Table 1). The median individual observation time was 1.4 years. In the current analysis 73% had nonvalvular AF and 23% VTE. No patients were lost to follow-up.

During the study, 115 patients in the Fiix arm (20%) developed CRVE (112 with three or more monitoring tests) and 457 had no CRVE (448 with three or more monitoring tests). In the PT arm, 132 patients (23%) developed CRVE (129 with three or more monitoring tests) and 439 had no CRVE (426 with three or more monitoring tests). The CRVE included 29 major events (TE 10, MB 19) in the Fiix arm and 40 (TE 19, MB 21) in the PT arm. Non-major CRB occurred in 86 and 92, respectively. The TE reduction in the Fiix-arm was statistically non-inferior ($P_{\mathrm{non-inferiority}} = 0.0002$). Patients in the event groups were older and had higher blood pressure than those in the no event groups. AF patients with CRVE also had higher CHA_2DS_2-VASC risk score (Table 1).

Anticoagulation at time of major vascular events

Fiix-INR and PT-INR measurements were available from the time of all TE and MB events in both study arms (Fig. 1). Fiix-warfarin patients with TE had significantly lower median Fiix-INR (1.8) prior to the event than those with no events (2.5, P = 0.0024). PT-warfarin patients also had significantly lower median PT-INR (1.9) prior to TE event than those without events (2.5, P = 0.0059). Fiix-warfarin patients with MB had higher median Fiix-INR than those without events (3.2 vs. 2.5, P = 0.0467) whereas the median PT-INR in PT-warfarin patients with MB was not elevated (2.5) compared to those without major events (P = 0.5123). Nonetheless, major bleeding occurred in both arms over a spectrum of low and high INRs.

Anticoagulation variability prior to vascular events irrespective of monitoring method

Table 2 demonstrates that patients with CRVE had more variable control than patients with an uneventful course.

Table 1 Patient characteristics

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

Table 1 (continued)

	Fiix arm CRVE	Fiix arm No event	PT arm CRVE	PT arm No event	*ANOVA P* value[a]
Any other drugs	113 (98.3)	412 (90.2)	119 (90.2)	406 (92.5)	0.0317

Patients with and without clinically relevant vascular events (CRVE) according to monitoring method with either Fiix-prothrombin time (Fiix arm) or standard Quick prothrombin time (PT arm)

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

Major bleeding, other non-major clinically relevant bleeding or thromboembolism

[a]ANOVA comparison of the four groups; Kruskal–Wallis test for continuous data, P-values < 0.05 are considered significant

[b]IQR denotes interquartile (25–75%) range

[c]The CHA_2DS_2-VASC risk score indicates the risk of thromboembolic events in AF patients only

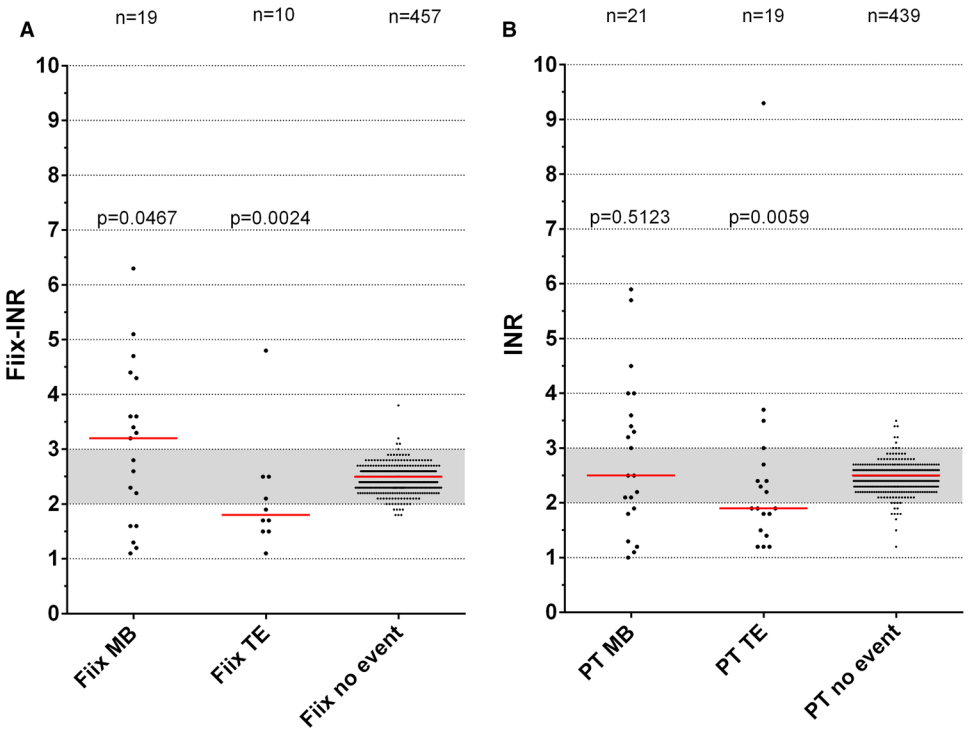

Fig. 1 INR at the time of major events and prior INR variability. The INR values at the time of major bleedings and thromboembolism compared with the average anticoagulation of each patient in the no event groups. *MB* major bleeding, *TE* thromboembolic event. The *dotplots* display the distribution of the values, the *red line* is the median, and the *gray zone* is the therapeutic target range. P-values by Mann–Whitney test are shown compared to the no event groups. **a** Fiix arm, **b** PT arm

This was manifested by significantly more frequent monitoring tests, shorter time between tests, fewer tests within target range and lower TTR. Patients with CRVE also had more dose adjustments than those with an uneventful course.

Fiix-warfarin and PT-warfarin intensity and variability in relation to vascular event occurrence

Fiix-warfarin and PT-warfarin anticoagulation variability in relation to occurrence or absence of CRVE is shown in Tables 3 and 4.

Number of monitoring tests and test intervals

Patients that experienced CRVE had significantly shorter intervals between monitoring tests than those without (21 vs. 23 in the Fiix arm and 19 vs. 24 in the PT arm), indicating instability. Consequently, they also had more annual monitoring tests, i.e. 18 versus 16, respectively, in the Fiix-arm (P = 0.0180) and 19 versus 16 in the PT-arm (P < 0.0001). The CRVE groups also had fewer tests within therapeutic range than the no event groups (61% vs. 67% in the Fiix arm and 59% vs. 64% in the PT arm, P < 0.0001 within both arms).

Table 2 Test numbers, intervals and dosing parameters in relation to clinically relevant vascular events (CRVE) irrespective of monitoring method

	CRVE	No event	P-value[a]
Number	247	896	n.a
Age	74 (66–79)	71 (63–78)	0.0021
Observation days per patient	507 (277–573)	508 (323–582)	0.2456
Test number and intervals			
Monitoring tests—n	5307	17,218	n.a
Annual tests per patient –	18.5 (18.0–19.0)	16.1 (15.8–16.3)	<0.0001
Days between monitoring tests	20 (15–27)	24 (17–32)	<0.0001
Tests within defined Fiix-INR or INR ranges			
2–3—n (%)	3185 (60)	11,238 (65)	<0.0001
<2—n (%)	1163 (22)	3385 (20)	0.0004
>3—n (%)	959 (18)	2595 (15)	<0.0001
Percent time within target range[b]	77 (64–85)	81 (70–89)	<0.0001
Dosing			
Daily warfarin dose in mg	4.5 (3.2–6.2)	4.7 (3.4–6.5)	0.2022
Annual dose changes in each patient	7.9 (7.6–8.3)	6.3 (6.2–6.5)	<0.0001

Results are shown as median (interquartile range) unless otherwise noted

Major bleeding, other non-major clinically relevant bleeding or thromboembolism

[a]Rates were estimated with Poisson regression using the number of years as numerator, the Mann–Whitney test was used for other continuous data and Chi square test with Yates correction for categorical data. P-values < 0.05 are considered significant

[b]Rosendaal method

Time in range

Patients in both study arms that suffered from CRVE had lower median TTR than those without events; 79% versus 82% in the Fiix arm (P = 0.0627) and 76% versus 80% in the PT-arm (P = 0.0006), see Table 3. The median TTR was particularly low in PT-warfarin patients that suffered from thromboembolism (TTR 62%).

Anticoagulation variability (INR variance growth rate; VGR)

The between-test INR variability was calculated using the VGR_{B1} formula and detailed results are shown in Table 4. The VGR was consistently lower in the Fiix-arm. In both arms, the variability was significantly higher in the event groups than in the no event groups with the exception of Fiix-warfarin patients with TE which did not demonstrate significantly increased variability (VGR 0.20) and in PT-warfarin patients with MB where the apparent higher variability (VGR 0.59) was no statistically significantly so. Variability was 2.5 fold higher in the 19 PT-warfarin patients with TE than in the ten Fiix-warfarin patients suffering TE (0.50 vs. 0.20, P = 0.0035). The variability was also higher in PT-warfarin patients with no events than in the corresponding Fiix-warfarin patients (0.21 vs. 0.17, P = 0.0163).

Dose and dose adjustment frequency

The median daily warfarin dose in mg within each monitoring arm did not differ according to presence or absence of CRVE, ranging from 4.5 to 4.8 mg. However, in both Fiix-warfarin and PT-warfarin patients the event groups needed more frequent annual dose changes, 7.3 and 8.5, than those without events (6.0 and 6.6; P < 0.0001), respectively. The dose change frequency was significantly lower with Fiix-warfarin (P ≤ 0.0002). This was particularly evident in those with TE; PT-warfarin patients with TE had 2.6 times more frequent annual dose adjustments (14.2) than Fiix-warfarin patients with TE (5.4) (P < 0.0001). The dose change frequency was also higher in PT-warfarin patients (8.0) than in Fiix-warfarin patients (7.4) with clinically relevant bleeding (including MB) (P = 0.0075) and was higher in those with major bleeding. However, in those with major bleeding there was higher annual dose change frequency with Fiix-warfarin (11.3) than with PT-warfarin (8.9)(P = 0.0394).

Discussion

Anticoagulation was more stable during monitoring with Fiix-PT and, paradoxically, Fiix-warfarin patients with TE had anticoagulation variability similar to no-event patients, contrary to PT-warfarin patients with TE who had the

Table 3 Test numbers and testing intervals in relation to vascular events and monitoring method

	Fiix arm CRVE	Fiix arm No event	P-value[a] Within Fiix arm	PT arm CRVE	PT arm No event	P-value Within PT arm	P-value Fiix CRVE versus PT CRVE	P-value Fiix no event versus PT no event
Patient number (% of all in each arm)	115 (20)	457 (80)		132 (23)	439 (77)			
Test number and intervals								
Number of monitoring tests –n	2382	8644	–	2925	8574	–	–	–
Number of tests within defined Fiix-INR or INR ranges								
2–3—n (%)	1457 (61)	5770 (67)	<0.0001	1728 (59)	5468 (64)	<0.0001	0.1291	<0.0001
<2—n (%)	515 (22)	1581 (18)	0.0003	648 (22)	1804 (21)	0.2137	0.6644	<0.0001
>3—n (%)	410 (17)	1293 (15)	0.0077	549 (19)	1302 (15)	<0.0001	0.1527	0.6927
Annual tests per patient (mean ± 95% CI)	18.0 (17.3–18.7)	16.0 (15.6–16.3)	<0.0001	19.0 (18.2–19.7)	16.2 (15.8–16.6)	<0.0001	0.0698	0.3932
Days between monitoring tests in each patient	21 (15–28)	23 (17–32)	0.0166	19 (15–25)	24 (189 – 32)	<0.0001	0.1586	0.5347

Warfarin patients monitored with Fiix-prothrombin time (Fiix arm) or prothrombin time (PT arm) analyzed in relation to presence or absence of major and non-major clinically relevant vascular events (CRVE)

Results are shown as median (interquartile range) unless otherwise noted

Major bleeding, other non-major clinically relevant bleeding and thromboembolism

[a]Rates were estimated with Poisson regression using the number of years as numerator, Mann–Whitney test was used for other continuous data and Chi square test with Yates correction for categorical data. P-values < 0.05 are considered significant

highest observed variability leading to very instable dosing. However, this finding coincided with the previously reported lower annual rate of TE during Fiix-PT monitoring (1.2%) than during standard monitoring (2.3%) [9, 14] suggesting that improved stability affected the TE rate favorably. With either monitoring method, clinically relevant bleeding or MB alone associated with highly variable anticoagulation measured either as high VGR or high annual dose change frequency. The TTR data was consistent with the variability measures but at a lower significance level.

High anticoagulation intensity (high TTR) lowers the incidence of thromboembolism and bleeding [5, 13, 15, 16] but geographical (or cultural) differences in VKA management lead to different intensity at different sites as confirmed in recent direct oral anticoagulant (DOAC) trials in NVAF [17–19]. Additionally, many conditions have been associated with variable VKA anticoagulation including age <70 years [20, 21], female gender [20], multiple chronic disorders [20, 21], a high PT-INR target (≥3) [22], sudden physical activity increase [23, 24] and low vitamin K intake [25] which concomitant daily low dose vitamin K supplementation can overcome [26]. Patient nonadherence including from alcoholism causes instability of any anticoagulant [27, 28] and instable VKA anticoagulation also leads to inconvenience and cost due to more frequent testing and dose adjustments. Various methods have successfully improved the stability of VKA anticoagulation, including dosing by specialized staff at anticoagulation management centers, self-monitoring [5], dosing algorithms and dosing software [29]. Genotyping cytochrome P450 or the VKOR gene at the initiation of VKA therapy has not been shown to improve control in the short term [30–32] but usefulness of genotyping during long-term anticoagulation is unknown [1].

During six decades of VKA use, the appropriateness of monitoring VKAs with the now 80 years old PT test that is affected by reduced factors I, II, V, VII or X has rarely been

Table 4 Assessment of anticoagulation intensity and variability in relation to different clinically vascular event occurrence

	Fiix arm Event	Fiix arm No event	P-value[a] Within Fiix arm	PT arm Event	PT arm No event	P-value Within PT arm	P-value Fiix event versus PT event	P-value Fiix no event versus PT no event
Percent time within target range (Rosendaal; median and IQR[b])								
Any clinically relevant vascular event	79 (68–86)	82 (72–91)	0.0627	76 (63–84)	80 (69–89)	0.0006	0.0332	0.0805
TTR thromboembolism only	81 (71–95)	82 (72–91)	0.7630	62 (56–81)	80 (69–89)	0.0012	0.0571	–
TTR total clinically relevant bleeding	79 (67–86)	82 (72–91)	0.0729	76 (64–84)	80 (69–89)	0.0297	0.1325	–
TTR major bleeding only	76 (55–85)	82 (72–91)	0.1385	77 (40–84)	80 (69–89)	0.0565	0.6536	–
INR fluctuation between tests (VGR-B_1[c]; median and IQR[b])								
Any clinically relevant vascular event	0.21 (0.10–0.48)	0.17 (0.08–0.38)	0.0444	0.34 (0.13–0.77)	0.21 (0.09–0.49)	0.0011	0.0098	0.0163
Thromboembolism	0.20 (0.07–0.26)	0.17 (0.08–0.38)	0.7106	0.50 (0.27–0.90)	0.21 (0.09–0.49)	0.0017	0.0035	–
Total clinically relevant bleeding	0.23 (0.12–0.53)	0.17 (0.08–0.38)	0.0043	0.31 (0.12–0.73)	0.21 (0.09–0.49)	0.0147	0.2391	–
Major bleeding only	0.31 (0.15–0.97)	0.17 (0.08–0.38)	0.0120	0.59 (0.07–1.36)	0.21 (0.09–0.49)	0.1248	0.9652	–
Annual dose changes (mean and 95% CI[d])								
Any clinically relevant vascular event	7.3 (6.8–7.7)	6.0 (5.8–6.2)	< 0.0001	8.5 (8.1-9.0)	6.6 (6.4–6.9)	< 0.0001	0.0002	<0.0001
Thromboembolism	5.4 (3.9–7.3)	6.0 (5.8–6.2)	0.4932	14.2 (12.2–16.3)	6.6 (6.4–6.9)	< 0.0001	< 0.0001	–
Total clinically relevant bleeding	7.4 (6.9–7.7)	6.0 (5.8–6.2)	< 0.0001	8.0 (7.5–8.4)	6.6 (6.4–6.9)	< 0.0001	0.0075	–

Table 4 (continued)

	Fiix arm Event	Fiix arm No event	P-value[a] Within Fiix arm	PT arm Event	PT arm No event	P-value Within PT arm	P-value Fiix event versus PT event	P-value Fiix no event versus PT no event
Major bleeding only	11.3 (9.6–13.3)	6.0 (5.8–6.2)	< 0.0001	8.9 (7.5–10.4)	6.6 (6.4–6.9)	0.0007	0.0394	–

Intensity is measured as TTR and variability as INR variance growth rate (INR-VGR) or dose change frequency in patients with and without clinically relevant bleeding events or thromboembolism, monitored with either Fiix-prothrombin time (Fiix arm) or prothrombin time (PT arm).

[a]Mann–Whitney test for continuous data except dose adjustments rates were estimated with Poisson regression using the number of years as numerator. P-values < 0.05 are considered significant

[b]IQR denotes interquartile (25–75%) range

[c]Variance growth rate (VGR) formula B_1

[d]95% confidence interval

questioned. This is somewhat surprising as experimental data suggests that the antithrombotic effect mainly depends on controlled reduction of FII and FX. FVII is probably not critical except at very low concentrations rarely observed during controlled VKA treatment [7, 8, 33]. The main previous attempt at alternative monitoring was probably monitoring the native prothrombin antigen using enzyme immunoassay [34, 35].

The new Fiix-PT was designed to measure only reduced FII or FX and circumvent measuring the influence of reduced FVII, fibrinogen and FV. It was hypothesized that monitoring with Fiix-PT would stabilize warfarin anticoagulation while leading to at least non-inferior clinical outcome. These hypotheses were tested in the randomized and blinded clinical Fiix-trial [9] which demonstrated that despite the PT-monitored control group having high anticoagulation intensity (TTR) similar to that at many other European centers [17, 18, 36], Fiix-PT monitored patients had even higher TTR as well as lower anticoagulation variability. In the primary analysis, Fiix-PT monitoring was clinically non-inferior in the primary analysis but in secondary analyses resulted in significant improvement in long-term annual TE incidence (1.1% vs. 2.2%) without increasing bleeding [9, 11]. A subsequent meta-analysis comparing outcome of atrial fibrillation (AF) patients participating in the Fiix trial to warfarin controls or DOAC treated patients in the four large DOAC trials in AF appears to confirm the findings [11]. In the meta-analysis, outcome of standard PT-warfarin monitored controls in the Fiix-trial was similar to that observed in PT-warfarin treated controls in the DOAC trials. However, with Fiix-warfarin (Fiix-PT monitored warfarin) in comparison to the standard PT-warfarin treated controls in the DOAC trials, there was a statistically significant 49% reduction in total TE (RR 0.51; 95% CI 0.26–0.99), a 34% reduction in composite major vascular events (0.66;0.43–1.00) and a 37% non-significant reduction in major bleeding (0.63; 0.37–1.07). The effect size of these reductions is larger than that of any of the DOACs compared to warfarin in AF trials.

The current subanalysis of Fiix-trial data adds that Fiix-warfarin patients with TE had similar anticoagulation stability as those without vascular events whereas their stability was markedly improved over that observed in PT-warfarin patients with TE, the latter having 2.6 fold higher dose change frequency and a twofold TE incidence. This finding may appear to be paradoxical as high instability is usually associated with TE but we interpret it as actually being a reflection of a true biological effect of improved stability with Fiix-warfarin, namely that near maximum achievable efficacy has been achieved with the more stable Fiix-warfarin (cf. 1.1% annual TE incidence with Fiix-warfarin vs. 2.2% with PT-warfarin in the Fiix-trial). Thus, the very instable doses as observed with PT-warfarin may be major determinants of a high TE rate, even more so than of bleeding, whereas low variability does not entirely eliminate TE. In the meta-analysis of Hart et al. it was found that active treatment with PT-warfarin reduced TE rate in non-valvular AF to about 36% of that observed in placebo treated controls [37]. Assuming at least similar efficacy in our PT-warfarin patients, Fiix-warfarin may have reduced TE in AF to about 18% of that expected in untreated patients, i.e., to about 1% annually as opposed to about 5% annually without anticoagulation. On the other hand, as the bleeding rate was similar and bleeding did associate with high variability in both trial arms, instable patients should be systematically identified in order to provide them with better or alternative care. Our results suggest that the easiest way to identify unstable patients may be to assess dose change frequency on a regular basis as our data suggest that patients needing more than monthly or even bimonthly dose adjustments are at increased risk.

Several limitations of the present analysis should be considered. First, the trial size limited the ability to interpret clinical outcomes in subgroups. Instead we analyzed

surrogate outcome markers of intensity and stability in relation to monitoring method and whether these markers showed consistency with occurrence of clinically relevant vascular events. Secondly, the Fiix trial was designed to show clinical non-inferiority which it did in the primary efficacy analysis. The effect size for efficacy improvement, nevertheless, was high (RR 0.52, $P_{non\text{-}inferiority} < 0.0001$) albeit statistically not superior ($P_{superiority} < 0.09$). Given the effect size, these results could be false negative consequent to insufficent statistical power of the trial. Therefore, we stress the post-hoc secondary analysis finding of improved efficacy of Fiix-warfarin in the long-term (RR 0.41, $P_{superiority} = 0.03$) [9] and that results of the surrogate outcome markers described in the current study are consistent with the clinical outcomes. Our meta-analysis of patients with AF only also supports the notion of superiority of Fiix-warfarin over standard warfarin monitoring [11]. Unfortunately, a superiority trial at least quadruple the size of the Fiix-trial would be needed to confirm these results and this is far beyond the means of independent investigators without strong financial sponsors. Third, a single center trial such as ours cannot provide data on how the new test would affect different dosing practices around the globe, e.g., manual dosing methods by private physicians vs centralized management. Nevertheless, a single center trial does have certain strengths when testing for a proof of a new concept, i.e. totally identical management of both observation arms except for the active arm being monitored using a test that is not affected by the VKA influenced FVII. Fourth, it should be pointed out that the management protocol that was used in the Fiix-trial was designed for monitoring with the PT and not the Fiix-PT. Although it remains to be shown, we suggest that further improvements in stability may be possible once the current management protocol has been adapted for the Fiix-PT. In support of this, our recent data suggests that the Fiix-PT stabilizes warfarin anticoagulation early and that it also more accurately reflects thrombin generation than the standard PT does [38]. Finally, it is possible that ignoring FVII levels may have detrimental influence in some cases. We think this is unlikely, however, as FVII never reached dangerously low leves or levels that affected thrombin generation during VKA in our fiix-trial setting with either monitoring method. Therefore, we contend that during high quality warfarin management FVII has very little role in preventing TE events and that only during extreme overanticoagulation FVII levels are likely to decrease to levels that would risk spontaneous bleeding.

Conclusions

Anticoagulation variability and dose-adjustment need in Fiix-warfarin patients with TE was similar to that observed in event-free patients. On the other hand variability was significantly increased in standard PT-INR monitored patients with TE. Decreased variability coincided with 50% fewer TE events in Fiix-warfarin patients. The results therefore suggest that that Fiix-warfarin not only is more stable but also improves efficacy over that attainable during high quality standard PT-monitoring of warfarin. Thus, although further improvements may be needed to reduce bleeding, stabilization of anticoagulation by Fiix-PT monitoring improves warfarin as an anticoagulant.

Addendum

All the data is original and has not been previously published. No identifiable patient data is included.

- Alma Rut Oskarsdóttir BS, graduate medical student: Analyzed data and wrote first manuscript draft.
- Brynja R. Guðmundsdottir MS,: Designed trial, administered laboratory, analyzed data and edited manuscript. Brynja is a co-inventor of the Fiix-prothrombin time and holds shares in Fiix Diagnostics Ltd, see above.
- Sigrun H. Lund PhD, statistical consultant. Analyzed data. She had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
- Olafur S. Indridason MD MPH. Statistical consultant, designed trial protocol, analyzed data and edited manuscript.
- David O. Arnar MD PhD. Member of adjudication committee, edited manuscript.
- Einar S. Bjornsson MD PhD. Member of adjudication committee, edited manuscript.
- Magnus K. Magnusson MD. Member of adjudication committee, edited manuscript.
- Hulda M. Jensdottir BS graduate medical student, Landspitali The National University Hospital of Iceland, Reykjavik, Iceland. Analyzed data.
- Brynjar Vidarsson, MD. Member of safety committee, edited manuscript.
- Charles W. Francis MD, Designed trial protocol, analyzed data and edited manuscript.
- Pall T. Onundarson MD, study chair: Designed trial protocol, administered trial, analyzed data and co-wrote manuscript. He had full access to all data and takes responsibility for the data and the accuracy of the data analysis. Pall is a co-inventor of the Fiix-prothrombin time and holds shares in Fiix Diagnostics Ltd, a start-up company that has obtained a patent for the Fiix-PT. The Fiix Diagnostics Ltd is owned by Pall T. Onundarson, Brynja R. Gudmundsdottir, the Landspitali University Hospital and the University of Iceland.

Brynja Laxdal RN, Gunnhildur Magnusdottir RN and Tinna Halldorsdottir RN were paid employees responsible for data collection and their work is highly appreciated as is the practical guidance of Halla Arnardottir RN, and the coagulation laboratory staff; Kristin A Einarsdottir BS, Oddny Olafsdottir BS, and Loic Letertre BS.

Acknowledgements The Fiix trial was partially supported by grants from Innovation Center Iceland, University of Iceland Science Fund, Landspitali Science Fund, The University of Iceland Science Fund and Actavis Inc. The funding sources had no involvement in the design, conduct, analysis or publication of the trial data and had no access to trial data. Pall T. Onundarson and Brynja R. Gudmundsdottir are co-inventors of the Fiix-prothrombin time test that is owned by Fiix Diagnostics Ltd and which has been issued a patent in USA, China and the EU with patent pending status in other areas.

Compliance with Ethical Standards

Conflict of interest No other authors have conflicting interests related to this work.

Ethical standards All procedures performed were in accordance with the ethical standards of the National Bioethics Committee of the Republic of Iceland and with the 1964 Helsinki declaration and its later amendments.

Informed consent Informed consent was obtained from all participants included in the study.

References

1. Furie B (2013) Do pharmacogenetics have a role in the dosing of vitamin K antagonists? N Engl J Med 369(24):2345–2346. doi:10.1056/NEJMe1313682
2. Quick AJ, Stanley-Brown M, Bancroft FW (1935) A study of the coagulation defect in hemophilia and in jaundice. Am J Med Sci 190:501–511
3. Owren PA, Aas K (1951) The control of dicumarol therapy and the quantitative determination of prothrombin and proconvertin. Scand J Clin. Lab Invest 3:201–208
4. Kirkwood TB (1983) Calibration of reference thromboplastins and standardisation of the prothrombin time ratio. Thromb Haemost 49(3):238–244
5. Ageno W, Gallus AS, Wittkowsky A, Crowther M, Hylek EM, Palareti G, American College of Chest P (2012) Oral anticoagulant therapy: antithrombotic therapy and prevention of thrombosis, 9th ed: American college of chest physicians evidence-based clinical practice guidelines. Chest 141 (2 Suppl):e44S–88 S. doi:10.1378/chest.11-2292
6. Ibrahim S, Jespersen J, Poller L, European, Action, on, Anticoagulation (2013) The clinical evaluation of International Normalized Ratio variability and control in conventional oral anticoagulant administration by use of the variance growth rate. J Thromb Haemost 11(8):1540–1546. doi:10.1111/jth.12322
7. Zivelin A, Rao LV, Rapaport SI (1993) Mechanism of the anticoagulant effect of warfarin as evaluated in rabbits by selective depression of individual procoagulant vitamin K-dependent clotting factors. J Clin Invest 92:2131–2140
8. Gudmundsdottir BR, Francis CW, Bjornsdottir AM, Nellbring M, Onundarson PT (2012) Critical role of factors II and X during coumarin anticoagulation and their combined measurement with a new Fiix-prothrombin time. Thromb Res 130(4):674–681
9. Onundarson PT, Francis CW, Indridason OS, Arnar DO, Bjornsson ES, Magnusson MK, Juliusson SJ, Jensdottir HM, Vidarsson B, Gunnarsson PS, Lund SH, Gudmundsdottir BR (2015) Fiix-prothrombin time versus standard prothrombin time for monitoring of warfarin anticoagulation: a single centre, double-blind, randomised, non-inferiority trial. Lancet Haematol 2(6):e231–e240
10. Olsson SB, Executive Steering Committee of the SIIII (2003) Stroke prevention with the oral direct thrombin inhibitor ximelagatran compared with warfarin in patients with non-valvular atrial fibrillation (SPORTIF III): randomised controlled trial. The Lancet 362(9397):1691–1698
11. Onundarson PT, Arnar DO, Lund SH, Gudmundsdottir BR, Francis CW, Indridason OS (2016) Fiix-prothrombin time monitoring improves warfarin anticoagulation outcome in atrial fibrillation: a systematic review of randomized trials comparing Fiix-warfarin or direct oral anticoagulants to standard PT-warfarin. International journal of laboratory hematology 38(Suppl 1):78–90. doi:10.1111/ijlh.12537
12. Schulman S, Kearon C, Subcommittee on Control of Anticoagulation of the Scientific Standardization Committee of the International Society on Thrombosis and Haemostasis (2005) Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. J Thromb Haemost 3 (4):692–694. doi:10.1111/j.1538-7836.2005.01204.x
13. Rosendaal FR, Cannegieter SC, van der Meer FJ, Briet E (1993) A method to determine the optimal intensity of oral anticoagulant therapy. Thromb Haemost 69:236–239
14. Lippi G, Favaloro EJ (2015) Laboratory monitoring of warfarin in the era of direct oral anticoagulants (editorial comment). Lancet Hematol 2(6):e223–e224
15. Wan Y, Heneghan C, Perera R, Roberts N, Hollowell J, Glasziou P, Bankhead C, Xu Y (2008) Anticoagulation control and prediction of adverse events in patients with atrial fibrillation: a systematic review. Circulation 1 (2):84–91. doi:10.1161/CIRCOUTCOMES.108.796185
16. Björck F, Renlund H, Lip GYH, Wester P, Svensson PJ, Själander A (2016) Outcomes in a warfarin-treated population with atrial fibrillation. JAMA Cardiol 1(2):172. doi:10.1001/jamacardio.2016.0199
17. Wallentin L, Yusuf S, Ezekowitz MD, Alings M, Flather M, Franzosi MG, Pais P, Dans A, Eikelboom J, Oldgren J, Pogue J, Reilly PA, Yang S, Connolly SJ, investigators R-L (2010) Efficacy and safety of dabigatran compared with warfarin at different levels of international normalised ratio control for stroke prevention in atrial fibrillation: an analysis of the RE-LY trial. The Lancet 376(9745):975–983. doi:10.1016/S0140-6736(10)61194-4
18. Wallentin L, Lopes RD, Hanna M, Thomas L, Hellkamp A, Nepal S, Hylek EM, Al-Khatib SM, Alexander JH, Alings M, Amerena J, Ansell J, Aylward P, Bartunek J, Commerford P, De Caterina R, Erol C, Harjola VP, Held C, Horowitz JD, Huber K, Husted S, Keltai M, Lanas F, Lisheng L, McMurray JJ, Oh BH, Rosenqvist M, Ruzyllo W, Steg PG, Vinereanu D, Xavier D, Granger CB, Investigators AfRiSOTEiAF (2013) Efficacy and safety of apixaban compared with warfarin at different levels of predicted international normalized ratio control for stroke prevention in atrial fibrillation. Circulation 127(22):2166–2176. doi:10.1161/CIRCULATIONAHA.112.142158
19. Ruff CT, Giugliano RP, Braunwald E, Hoffman EB, Deenadayalu N, Ezekowitz MD, Camm AJ, Weitz JI, Lewis BS, Parkhomenko A, Yamashita T, Antman EM (2014) Comparison of the efficacy and safety of new oral anticoagulants with warfarin in patients with atrial fibrillation: a meta-analysis of

randomised trials. The Lancet 383(9921):955–962. doi:10.1016/S0140-6736(13)62343-0
20. Witt DM, Delate T, Clark NP, Martell C, Tran T, Crowther MA, Garcia DA, Ageno W, Hylek EM (2009) Outcomes and predictors of very stable INR control during chronic anticoagulation therapy. Blood 114(5):952–957. doi:10.1182/blood-2009-02-207928
21. Witt DM, Delate T, Clark NP, Martell C, Tran T, Crowther MA, Garcia DA, Ageno W, Hylek EM (2010) Twelve-month outcomes and predictors of very stable INR control in prevalent warfarin users. J Thromb Haemost 8:744–749
22. Onundarson PT, Einarsdottir KA, Gudmundsdottir BR (2008) Warfarin anticoagulation intensity in specialist-based and in computer-assisted dosing practice. Int J Lab Hematol 30(5):382–389
23. Lenz TL, Lenz NJ, Faulkner MA (2004) Potential interactions between exercise and drug therapy. Sports Med 34(5):293–296
24. Shibata Y, Hashimoto H, Kurata C, Ohno R, Kazui T, Takinami M (1998) Influence of physical activity on warfarin therapy. Thromb Haemost 80(1):203–204
25. Sconce E, Khan T, Mason J, Noble F, Wynne H, Kamali F (2005) Patients with unstable control have a poorer dietary intake of vitamin K compared to patients with stable control of anticoagulation. Thromb Haemost 93(5):872–875. doi:10.1267/THRO05050872
26. Sconce E, Avery P, Wynne H, Kamali F (2007) Vitamin K supplementation can improve stability of anticoagulation for patients with unexplained variability in response to warfarin. Blood 109(6):2419–2423
27. Kimmel SE, Chen Z, Price M, Parker CS, Metlay JP, Christie JD, Brensinger CM, Newcomb CW, Samaha FF, Gross R (2007) The influence of patient adherence on anticoagulation control with warfarin: results from the International Normalized Ratio Adherence and Genetics (IN-RANGE) Study. Arch Intern Med 167(3):229–235. doi:10.1001/archinte.167.3.229
28. Platt AB, Localio AR, Brensinger CM, Cruess DG, Christie JD, Gross R, Parker CS, Price M, Metlay JP, Cohen A, Newcomb CW, Strom BL, Laskin MS, Kimmel SE (2008) Risk factors for nonadherence to warfarin: results from the IN-RANGE study. Pharmacoepidemiol Drug Saf 17(9):853–860. doi:10.1002/pds.1556
29. Poller L, Shiach CR, MacCallum PK, Johansen AM, Munster AM, Magalhaes A, Jespersen J (1998) Multicentre randomised study of computerised anticoagulant dosage. European concerted action on anticoagulation. The Lancet 352(9139):1505–1509
30. Pirmohamed M, Burnside G, Eriksson N, Jorgensen AL, Toh CH, Nicholson T, Kesteven P, Christersson C, Wahlstrom B, Stafberg C, Zhang JE, Leathart JB, Kohnke H, Maitland-van der Zee AH, Williamson PR, Daly AK, Avery P, Kamali F, Wadelius M, Group E-P (2013) A randomized trial of genotype-guided dosing of warfarin. N Engl J Med 369(24):2294–2303. doi:10.1056/NEJMoa1311386
31. Kimmel SE, French B, Kasner SE, Johnson JA, Anderson JL, Gage BF, Rosenberg YD, Eby CS, Madigan RA, McBane RB, Abdel-Rahman SZ, Stevens SM, Yale S, Mohler ER 3rd, Fang MC, Shah V, Horenstein RB, Limdi NA, Muldowney JA 3rd, Gujral J, Delafontaine P, Desnick RJ, Ortel TL, Billett HH, Pendleton RC, Geller NL, Halperin JL, Goldhaber SZ, Caldwell MD, Califf RM, Ellenberg JH, Investigators C (2013) A pharmacogenetic versus a clinical algorithm for warfarin dosing. N Engl J Med 369(24):2283–2293. doi:10.1056/NEJMoa1310669
32. Verhoef TI, Ragia G, de Boer A, Barallon R, Kolovou G, Kolovou V, Konstantinides S, Le Cessie S, Maltezos E, van der Meer FJ, Redekop WK, Remkes M, Rosendaal FR, van Schie RM, Tavridou A, Tziakas D, Wadelius M, Manolopoulos VG, Maitland-van der Zee AH, Group E-P (2013) A randomized trial of genotype-guided dosing of acenocoumarol and phenprocoumon. N Engl J Med 369(24):2304–2312. doi:10.1056/NEJMoa1311388
33. Xi M, Beguin S, Hemker HC (1989) The relative importance of the factors II, VII, IX and X for the prothrombinase activity in plasma of orally anticoagulated patients. Thromb Haemost 62:788–791
34. Furie B, Liebman HA, Blanchard RA, Coleman MS, Kruger SF, Furie BC (1984) Comparison of the native prothrombin antigen and the prothrombin time for monitoring oral anticoagulant therapy. Blood 64:445–451
35. Furie B, Diuguid CF, Jacobs M, Diuguid DL, Furie BC (1990) Randomized prospective trial comparing the native prothrombin antigen with the prothrombin time for monitoring oral anticoagulant therapy. Blood 75:344–349
36. Le Heuzey JY, Ammentorp B, Darius H, De Caterina R, Schilling RJ, Schmitt J, Zamorano JL, Kirchhof P (2014) Differences among western European countries in anticoagulation management of atrial fibrillation. Data from the PREFER IN AF registry. Thromb Haemost 111(5):833–841. doi:10.1160/TH13-12-1007
37. Hart RG, Benavente O, McBride R, Pearce LA (1999) Antithrombotic therapy to prevent stroke in patients with atrial fibrillation: a meta-analysis. Ann Intern Med 131(7):492–501
38. Jonsson PI, Letertre LR, Juliusson SJ, Gudmundsdottir BR, Francis CW, Onundarson PT (2017) During warfarin induction the anticoagulation level is better reflected by the Fiix-prothrombin time than by the standard prothrombin time. J Thromb Haemost 15:131–139. doi:10.1111/jth.13549