

REVIEW

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Replacement of traditional prothrombin time monitoring with the new Fiix prothrombin time increases the efficacy of warfarin without increasing bleeding. A review article

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Abstract

The antithrombotic effect of vitamin K antagonists (VKA) depends on controlled lowering of the activity of factors (F) II and X whereas reductions in FVII and FIX play little role. PT-INR based monitoring, however, is highly influenced by FVII, which has the shortest half-life of vitamin K-dependent coagulation factors. Hence, variability in the anticoagulant effect of VKA may be partly secondary to an inherent flaw of the traditional monitoring test itself. The Fiix prothrombin time (Fiix-PT) is a novel test that is only sensitive to reductions in FII and FX and is intended to stabilize the VKA effect. Two clinical studies have now demonstrated that when warfarin is monitored with the Fiix-PT based normalized ratio (Fiix-NR) instead of PT-INR, anticoagulation is stabilized and less testing and fewer dose adjustments are needed. Furthermore, the relative risk of thromboembolism was reduced by 50–56% in these studies without an increase in major bleeding.

Keywords: Warfarin, Oral anticoagulation, Monitoring, Fiix, Prothrombin time

Introduction

From their advent, 70 years ago, the effect of vitamin K antagonists (VKAs) has been monitored by measuring the prothrombin time (PT), either Quick or Owren type [1, 2], that equally reflect reductions in vitamin K-dependent (VKD) coagulation factors (F) II, VII or X but not FIX [3, 4] to effectively prevent and treat thromboembolism (TE) [5, 6]. However, the pharmacodynamic effect of PT-monitored warfarin (hereafter referred to as PT-warfarin) is quite variable in many patients. International standardization of PT ratios for the purpose of

VKA monitoring, leading to the international normalized ratio (INR, hereafter referred to as PT-INR) [7], has not reduced intra-individual anticoagulation variability that is mainly blamed on food and drug interactions and patient non-adherence.

In this article, we argue that the reasons for using the PT to monitor VKAs are primarily historical. Based on knowledge that accumulated after the development of the PT, it can be questioned whether the most relevant anticoagulant effect of VKAs has been monitored throughout the decades of their use. This article reviews recent data suggesting that the PT-INR suboptimally reflects the anticoagulant effect of warfarin and that anticoagulation outcomes can be improved considerably by monitoring a different effect, namely only the influence of FII and FX [3, 8, 9] while ignoring FVII and FIX.

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Efficacy and safety of current oral anticoagulants

In accordance with recent clinical guidelines [10, 11], the unmonitored newer direct oral anticoagulants (DOACs) have increasingly replaced warfarin as first-line anticoagulants in non-valvular atrial fibrillation (AF) and venous thromboembolism (VTE). In addition to convenience, this practice-shift is mainly based on pharmaceutical industry initiated clinical trials that have concluded that DOACs are at least similarly effective as warfarin that is dosed based on traditional PT-warfarin monitoring and that DOACs carry lower risk of intracranial hemorrhage [12, 13]. However, PT-warfarin has been found to be more effective and safer than DOACs in high thrombogenic-risk patients with mechanical heart valves [14] or triple-positive antiphospholipid antibody syndrome [15–17] and possibly as well following anterior wall myocardial infarction [18]. If PT-warfarin has advantages over DOACs in high-risk patients, why not also in patients that are at lower risk? Poor warfarin management, i.e. low time within target INR range (TTR), associates with high risk of TE, bleeding and mortality [5, 19–21]. Is it possible that poor PT-warfarin management or other quality issues in the control groups of the DOAC trials (e.g. low TTR) influenced the study outcomes? Some published data suggest so [22–24]. Furthermore, two real-world practice studies involving 130,911 and 196,061 patients have suggested that PT-warfarin may actually be more effective in clinical practice than are the DOACs. The authors of those studies suggest that the benefit of reduced intracranial bleeding with DOACs has been overemphasized as it may be at the cost of more thromboembolism due to lower anticoagulation level [25, 26].

There is little argument that DOACs have the major advantage of not needing to be routinely monitored. However, warfarin patients with very stable INR control can safely go up to 12-weeks between PT-INR tests [27]. Therefore, if the effectiveness and stability of warfarin could be further improved then further comparative effectiveness studies would at least seem warranted.

Prothrombin time based VKA monitoring; history

The Quick-PT, invented in 1935 [1], in its original form mixed undiluted thromboplastin of rabbit brain origin and calcium chloride into citrated patient plasma and the ensuing clotting (“prothrombin”) time was measured. At the time, it was only known to be affected by a reduction in either of two then known coagulation factors, i.e., fibrinogen (FI) or prothrombin (FII), only the latter being a VKD factor. PT monitoring made VKA use possible in humans around 1950. Two new factors influencing the PT had then just been discovered, the non-VKD FV [28] and proconvertin (FVII) [2, 29]. For the purpose of VKA monitoring, Owren modified the

PT in order to eliminate the influence of reduced non-VKD factors (fibrinogen or FV) on the PT [2]. Two further VKD coagulation factors, FIX and FX, only the latter of which influences Quick PT and Owren PT, were described in the 1950s [30, 31]. Over the ensuing decades the common wisdom was that an equal reduction in all VKD factors was necessary for full VKA anticoagulation, although it was considered sufficient to monitor only reductions in three out of the four, namely FII, FVII and FX.

Thromboplastins from different sources have different “strengths”; a strong thromboplastin has a short clotting time and does not detect the VKA effect as accurately as the more “sensitive thromboplastins” that have longer clotting times. Due to major differences observed in PT ratios, obtained from the same test plasmas from patients managed with VKAs with different thromboplastins, PT-ratios were later standardized as international normalized ratios (INR, PT-INR) [7]. This finally made the anticoagulation level comparable between clinical laboratories despite use of different thromboplastins and instruments [7]. Nevertheless, the use of a sensitive thromboplastin is generally recommended for VKA monitoring. The implementation of PT-INR worldwide improved the efficacy, safety and comparability of VKA management but did not solve the problem of high intra-individual variability.

Target ranges and the therapeutic window

VKAs need monitoring of their anticoagulant effect. Although inconvenient, monitoring has benefits such as improving drug adherence [32] and providing the ability to identify food and drug interactions that remain obscure for the DOACs. Measuring a biological effect in blood has also led to an understanding of the relationship between PT-INR variability, TE and bleeding and it is universally accepted that VKAs have a narrow therapeutic window [5]. Over the decades, a consensus has been reached empirically on standard (2.0–3.0) and high intensity (2.5–3.5) PT-INR target ranges, the latter being used mainly for those with mechanical heart valves [5]. A low and in the authors’ opinion too narrow target range of 1.5–2.0 was shown to be less effective than a range of 2.0–3.0 in VTE [33]. Although a target range is a different concept from a therapeutic range, calculating the time that the PT-INR stays within specified target ranges (TTR) has turned out to be a useful surrogate measure of likely efficacy and safety during VKA therapy [19, 21]. Other informative variability measures include variance growth rate (VGR) [34–36], monitoring testing frequency, and dose change frequency [36, 37].

VKA dose

The daily dose of warfarin varies from 0.5 to 20 mg daily between patients and this causes problems during initiation [38]. The dose is influenced by hereditary metabolic differences in sensitivity to VKA action but the daily dose requirement is also influenced by vitamin K supply from food and colonic bacteria [5, 6, 38]. However, the likely dose range needed for each patient becomes evident during the first treatment weeks. Once the dose range is established the main difficulty becomes variable PT-INR that influences the TTR.

Factors adversely influencing the ability to maintain stable warfarin dosing include geographical and cultural differences (e.g. distance from clinical laboratories, health insurance issues, dietary practices) and patient factors such as drug interactions, genetic factors, female gender, age, presence of chronic disorders such as heart failure, variable vitamin K intake, high PT-INR target (≥ 3), sudden change in lifestyle and poor adherence. Factors that associate with high TTR and favorable clinical outcome include dosing by specialized staff, self-management using portable INR monitors, formal dosing algorithms, and use of dosing software [5, 6]. Finally, studies on the value of genotyping cytochrome P450 2C9 or the VKOR genes at the initiation of VKA therapy have yielded conflicting results and continue to be debated [38, 39]. Although predictive of dose, the usefulness of genotyping during long-term anticoagulation is unknown.

PT-INR variability

The variable anticoagulant effect of VKAs as indicated by the PT-INR may be the single most problematic feature of VKA management following the initiation phase.

PT-INR variability is assumed to correctly reflect anti-thrombotic effect variability and, therefore, leads to dose adjustments, repeat testing and further dose adjustments. Furthermore, variable PT-INR associates with occurrence of both TE and bleeding [34, 35, 37]. PT-INR variability is usually blamed on food and drug interactions or patient non-adherence [5, 6, 40]. The question has, however, been raised whether the perceived variability in the antithrombotic effect when measured as PT-INR could be partly due to the PT-test being affected by a factor that confounds anticoagulation assessment and dosing, namely FVII.

The PT-INR is similarly affected by reduced concentrations of FII, FVII or FX (Fig. 1A) [3]. The very short half-life of FVII (4–6 h) leads to this particular factor having a major influence on the PT-INR in the short term, e.g. during initiation, after dose changes, and when dose changes are made repeatedly within short time intervals. As further discussed later, it should be noted that during stable PT-warfarin management the percent normal activity levels of FII, FVII, FIX and FX differ considerably, i.e. FII 27 (95% range 15–40), FVII 48 (18–79), FIX 61 (32–89) and FX 15 [11–19] [41]. The activity range variation is higher for FVII and FIX which may reflect their shorter half-lives, 4–6 h and 21–30 h, respectively, than those of factors II and X, 42–72 h and 27–48 h, respectively.

The antithrombotic effect of warfarin

The assumed need to reduce all VKD coagulation factors similarly to obtain a full anticoagulant effect of VKAs has been questioned for decades. First, in the 1980's Furie and Furie published studies suggesting that monitoring the native prothrombin antigen was a more

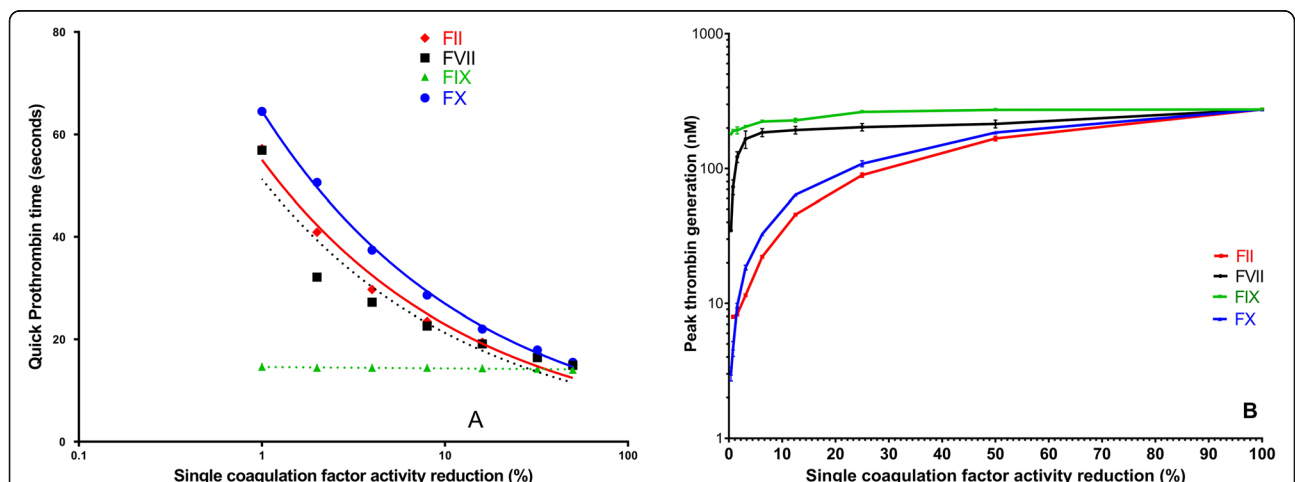


Fig. 1 Influence of selective reduction in vitamin K dependent factors, a single factor at a time while others remain within normal range, on the undiluted Quick prothrombin time (Neoplastin with ISI 1.3, Stago, Asnieres, France: panel A) and automated peak thrombin generation with clotting activated with a diluted thromboplastin (Neoplastin diluted 1:17,000: panel B) The figures are reproduced with permission from *Thrombosis Research* 2012;130:674–81 [3] and *Journal of Thrombosis and Haemostasis* 2016;15:131–9, respectively [41].

effective monitoring method of warfarin than the PT ratio which was, however, poorly standardized at the time [42, 43]. Second, in vitro experiments demonstrated that thrombin generation was linearly dependent on the prothrombin concentration at any activity level and also dependent on FX concentration at activity levels < 25–30% but almost independent of the activity of FVII and FIX unless their activity was markedly reduced to << 5% [8]. Third, animal experiments demonstrated that the antithrombotic effect of warfarin depends on reductions in FII and FX and that reductions in FVII and FIX have little role at concentrations expected during therapeutic VKA anticoagulation [9]. Finally, these conclusions were confirmed by in vitro experiments suggesting a similar effect of reductions in FII and FX but not of FVII or FIX on automated thrombin generation and ROTEM clot formation [3, 41]. Based on those experiments, at the activity levels of VKD factors present during maintenance phase warfarin anticoagulation [41] only FII (15–40 u/dL) and FX (11–19 u/dL) would be expected to significantly reduce thrombin generation in vitro (Fig. 1B).

Is the PT-INR partly to blame for PT-warfarin anticoagulation instability?

Based on the discussion in the previous sections the following issue emerges: The PT-INR that has been the basis of all therapeutic VKA use for seven decades is highly sensitive to reductions of a VKD coagulation factor (FVII) that has little role in bringing about the required antithrombotic effect. Furthermore, due to FVII's much shorter half-life than that of VKD factors II and X, it is a major cause of short-term variability in the measured PT-INR effect, even within a day. If only FII and X reductions matter, monitoring FVII has little meaningful role but confounds assessment as it exaggerates food and drug interactions and day to day variations in the drug effect.

The Fiix prothrombin time and Fiix normalized ratio (Fiix-NR)

Experimental basis

Our group (PTO, BRG) hypothesized that by ignoring FVII in addition to FIX and measuring only the influence of FII and FX reductions during warfarin monitoring, anticoagulation variability could be decreased, potentially to a degree favorably affecting the efficacy and/or safety of VKA management. In turn a new modified PT was designed that is only sensitive to reductions in FII or FX, called Fiix-PT (pronounced “fix PT”) [3]. The new Fiix-test is not affected by reduced fibrinogen, FV or FVII but only by reductions in factors FII or FX as Fiix-deficient plasma is mixed into diluted test plasma, thereby normalizing all factor levels except those of FII and FX [3, 41]. Other coagulation activators can also be used but when thromboplastin is used to initiate clotting, the Fiix test principle can also be achieved

by adding FII, FIX and FX deficient plasma to the test sample and bioequivalent results will be obtained, namely measuring only the influence of FII and FX. A Fiix normalized ratio (Fiix-NR) can then be calculated based on the Fiix-PT ratio in a manner identical to the traditional PT based international normalized ratio (INR, PT-INR) using standards traceable to the WHO international sensitivity index (ISI) standards [7, 44].

The Quick-PT and the Owren's PT have previously been shown to correlate excellently with each other [4]. In samples drawn during stable warfarin management, the Fiix-NR has been shown to correlate well with both the Quick-PT based INR ($R^2 = 0.91x$; $y = 0.91 + 0.20$; 49 samples) and the Owren's PT based INR ($R^2 = 0.92$; $y = 0.95x + 0.05$; 60 samples). These previously unpublished data from the study of Gudmundsdottir BR et al. 2012 [3] are shown in Fig. 2 A and B. On the other hand, during warfarin initiation and when factor VII is low for any reason the PT-INR and the Fiix-NR may diverge as illustrated in Fig. 3 [3].

Fiixing warfarin management

The Fiix trial

To test the hypothesis, in an investigator initiated single-center double-blind randomized non-inferiority clinical trial, named the Fiix-trial [35] mostly warfarin-experienced patients with typical mixed indications for anticoagulation were randomized to either Fiix-NR monitoring (Neoplastin and Fiix-deficient plasma, $n = 573$) or standard Quick PT-INR monitoring (Neoplastin, $n = 575$). Fiix-NR or PT-INR (depending on blinded assignment) measured in the central laboratory was reported as a blinded “research INR” to patients, dosing staff and event adjudicators. After a median follow-up of 1.7 years, Fiix-NR anticoagulation variability (variance growth rate) [34], was reduced, TTR was higher (84 vs 80%), and testing and dose adjustments were reduced. Furthermore, a 48% statistically non-inferior reduction was observed in thromboembolic events in Fiix-warfarin patients compared to the PT-warfarin controls (Fig. 4A). As reduced TE became evident only 180 days after the laboratory switched to Fiix-NR monitoring, a post-hoc analysis was performed after excluding the first 180 days and then a statistically superior 59% reduction in TE was observed ($P_{\text{superiority}} = 0.0307$). Despite improved efficacy, major bleeding (MB) was not increased (2.3% per person year in both study arms). With Fiix monitoring, dose change frequency and INR variability were reduced and TTR increased as well, all statistically significantly so. Overall, the Fiix-trial confirmed the hypothesis that the PT-INR is a source of warfarin anticoagulation variability. Further analysis confirmed that higher variability associated with adverse outcomes [37]. One interpretation of these results is that Fiix-warfarin patients have a more consistent anticoagulation level than PT-warfarin patients leading

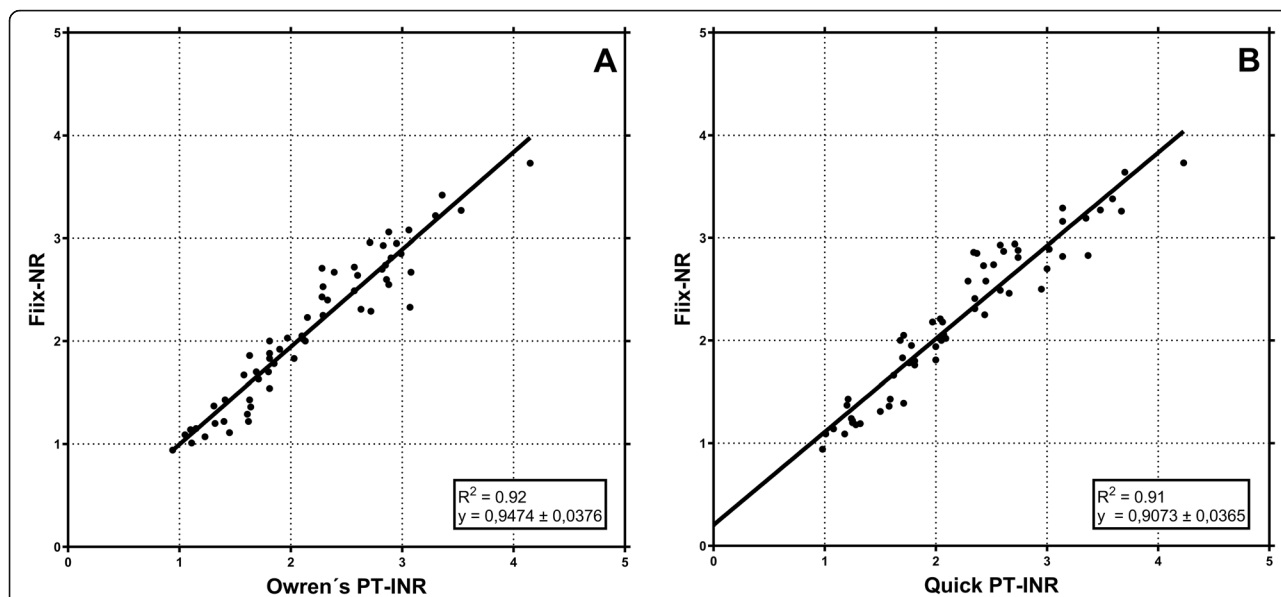


Fig. 2 Correlation of Fiix-normalized ratio with undiluted Owren's PT-INR (SPA, Stago, Asnieres, France: upper panel) or undiluted Quick PT-INR (Neoplastin, Stago, Asnieres, France: lower panel) in samples from patients on stable warfarin therapy. Previously unpublished data from the study described in *Thrombosis Research* 2012;130:674–81 [3].

to reduced TE but that reduced variability at the same time may prevent a simultaneous increased bleeding risk.

Meta-analysis comparing Fiix-warfarin to PT-warfarin and DOACs in non-valvular atrial fibrillation

Using meta-analytic methods [45], outcomes of Fiix-trial patients with non-valvular AF monitored with PT-warfarin ($n = 427$) or Fiix-warfarin ($n = 406$) were

compared to the outcomes of patients with non-valvular AF on PT-warfarin ($n = 29,272$) or on rivaroxaban, apixaban, edoxaban, or dabigatran ($n = 42,411$) in the pivotal pharmaceutical company initiated RCTs [46–49]. The reported adverse events' incidence of PT-warfarin control groups in all included trials was similar. This analysis found a statistically significant 49% reduction in composite stroke, systemic embolism or myocardial

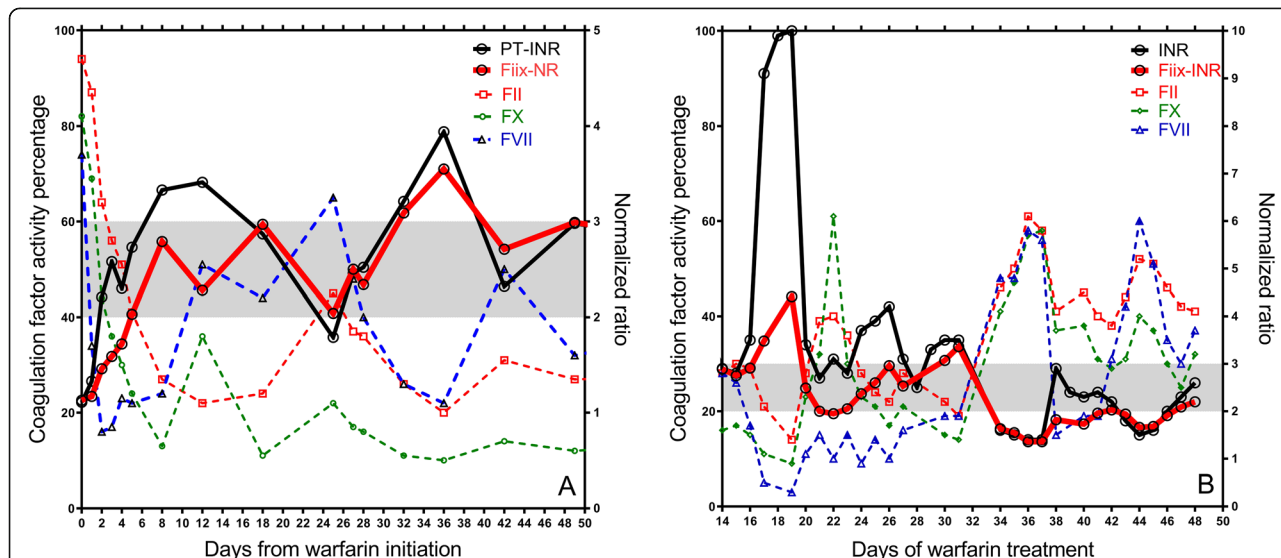
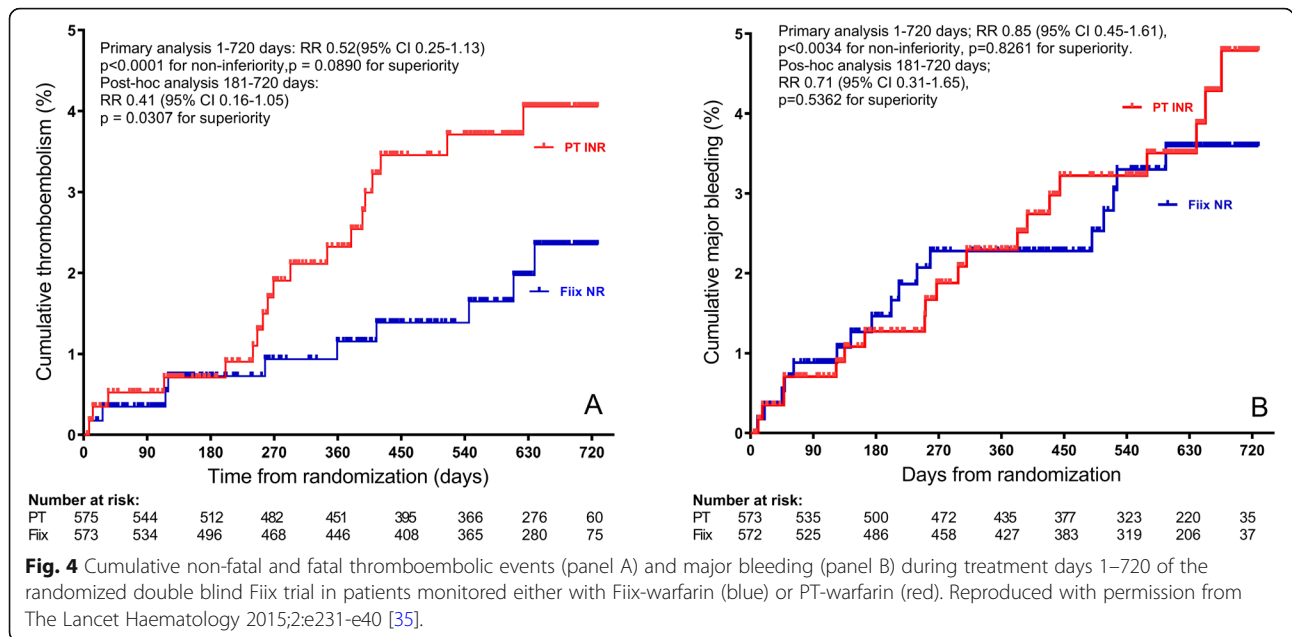


Fig. 3 Examples of differences in PT-INR and Fiix-NR during warfarin initiation when factor VII is fluctuating due to dose changes. Both the PT-INR and Fiix-NR were measured using undiluted Neoplastin CI Plus with ISI 1.3 (Diagnostica Stago, Asnieres, France) but in the Fiix-NR measurement factor II and X deficient plasma is added to diluted test plasma prior to addition of Neoplastin. Reproduced with permission from *Thrombosis Research* 2012;130:674–81 [3].



infarction (MI) in Fiix-warfarin patients when compared to outcome with PT-warfarin control patients in these trials. MB was numerically 37% lower but this finding was non-significant. Point estimates suggested that Fiix-warfarin might also compare favorably to the pooled clinical outcome with the combined DOAC drugs with a 39% reduction in composite stroke or systemic embolism (SSE) or MI and 24% fewer major bleeds but due to limited power these findings were non-significant.

Fiix in real world practice

After completing the Fiix-trial in February 2014, all warfarin patients at the Reykjavik center were switched back to the prior Owren’s type PT-INR monitoring. All were then transitioned to Fiix-NR monitoring beginning on July 1st 2016. The effect of the transition was then retrospectively assessed [36]. This real-world single-cohort interrupted time series study assessed the incidence of TE and MB in 2667 patients and compared Fiix-NR to Owren’s PT-INR. Incidence was assessed at monthly intervals during 12 months prior to and 18 months after laboratory switching to Fiix-NR monitoring. Using two-segmented regression, a breakpoint in total TE monthly incidence was seen six months after the change, followed by a 56% reduction in total TE incidence (from 2.82 to 1.23% per patient year, $P = 0.019$; number needed to treat to prevent one TE = 63). After excluding this 6-month transition period, there was no difference in MB between the 12-month Fiix-period (2.3%) and the 12-month PT-period (2.7%, $p = 0.25$). A separate analysis showed that Fiix-monitoring significantly reduced testing, dose adjustments and normalized ratio variability by about one third and increased TTR as well albeit to a

minor degree ($P = 0.0157$). A further analysis shown in Fig. 5 demonstrated that the relative risk of suffering any TE, was reduced to 0.45 (0.27–0.75) during Fiix-NR compared to PT-INR monitoring and even more in patients treated long-term. The relative risk of suffering major bleeding was not significantly reduced in that analysis (0.80 (0.53–1.21).

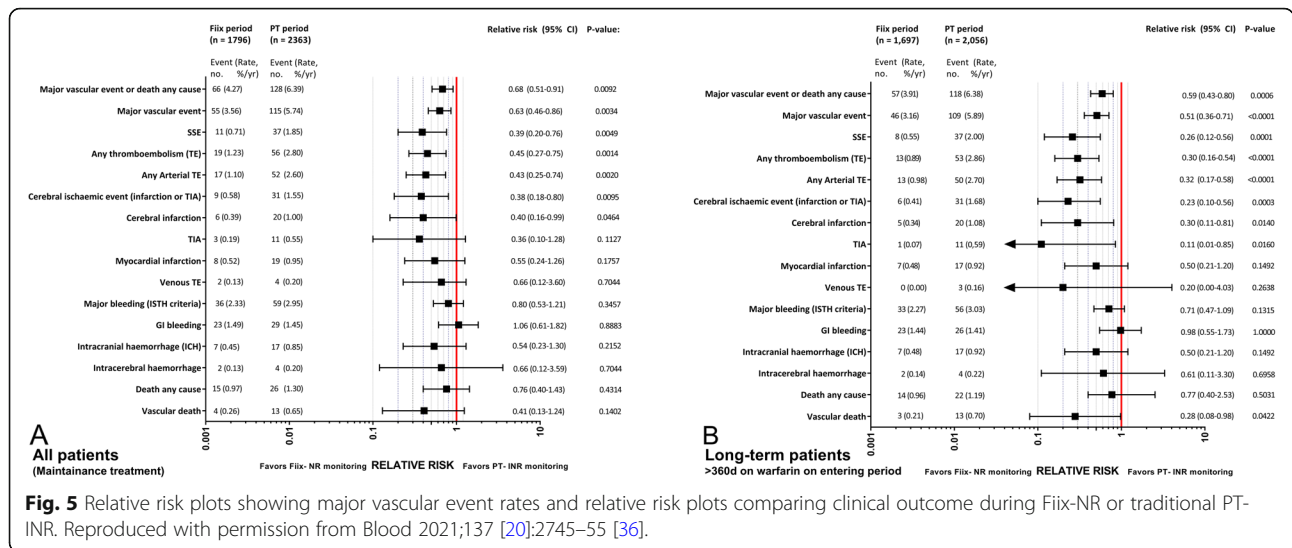
The results of this pre-post study were similar to the Fiix-trial results. Together the two clinical studies suggest that Fiix-NR monitoring is an improvement over monitoring with two standard but different thromboplastin reagents, namely Neoplastin (Quick PT-INR, rabbit brain, ISI 1.3) and the SPA reagent (Owren’s PT-INR, rabbit brain, ISI 1.1).

Fiix-warfarin initiation dose protocol

During warfarin initiation, Fiix-NR changes will be observed later than most physicians might predict based on their experience with managing FVII sensitive PT-INRs. This can lead to inaccurate warfarin dose adjustments and early over-anticoagulation. Therefore, a modified initiation algorithm must be used (Table 1) that prevents early over-anticoagulation during Fiix-warfarin initiation [44]. However, during maintenance Fiix-warfarin treatment, the dosing algorithm designed for PT-warfarin patients is appropriate as this did associate with favorable outcomes in our studies [35, 36].

Other possible uses of the Fiix test: the dilute Fiix-PT (dFiix-PT)

Measuring DOAC anticoagulant effect may have useful applications [50] but no single test can be used for this purpose [50]. A test using highly diluted thromboplastin,



the tissue thromboplastin inhibition test (a dilute PT (dPT) assay) used in the past for lupus anticoagulant detection was known to be sensitive to heparin, thrombin and anti-Xa inhibitors but not to pentasaccharide [51]. The sensitivity of both dPT and dilute Fiix-PT clotting times for the detection of different anticoagulants were tested using high dilutions of thromboplastin hypothesizing that the dFiix-PT might be less influenced by confounders. In short, the dFiix-PT at a single thromboplastin dilution could determine warfarin

normalized ratios and quantitative concentrations of dabigatran, rivaroxaban, apixaban, unfractionated heparin and enoxaparin but not of fondaparinux. The PT was less effective as two dilutions had to be used and the dPT ratio did not correlate well with the INR in warfarin patient samples [52].

Adoption of Fiix-PT into clinical practice

The Fiix test is already available as a CE marked product from a single manufacturer in Europe and can be easily

Table 1 Old PT-INR based initiation protocol and new initiation protocol modified for the slower responding Fiix prothrombin time (Fiix-NR). Modified from *Journal of Thrombosis and Thrombolysis* 2019;48:685–9 [44].

Starting daily dose on day 1	Normalized ratio on day 4	Warfarin dose; Old PT-INR initiation protocol (mg/day)	Warfarin dose; New adapted Fiix-NR initiation protocol (mg/day)*
< 65 year old; 4 mg daily			
	< 1.3	6	5
	1.3	4	4
	1.4–1.5	4	3
	1.6–1.7	4	2
	1.8–2.5	2	1.0–1.3
	> 2.5	1.3	Dose skipped and adapted**
≥65 year old; 6 mg daily			
	< 1.3	9	8
	1.3	6	6
	1.4–1.5	6	4.5
	1.6–1.7	6	3
	1.8–2.5	3	1.5–2.0
	> 2.5	2	Dose skipped and adapted**

*The Fiix normalized ratio (Fiix-NR) responds slower than the PT-INR due to insensitivity to factor VII reductions. ** Dose usually skipped for 1–2 days and dose then reduced based on rate of rise of the normalized ratio

automated. The additional Fiix-reagent (Fiix deficient plasma) adds somewhat to the cost of the reagent compared to traditional PT tests and this may initially be seen as an issue by laboratory directors. However, it must be considered that the benefit of Fiix-NR monitoring is to the patient, health insurance and society due to fewer serious events that have long-term consequences. A cost-benefit study will therefore be important along the way of introducing the Fiix concept to different stakeholders in health care.

Conclusion

The new Fiix test was based on the hypothesis that monitoring VKAs should focus on the two factors responsible for the antithrombotic effect, namely FII and FX, and that ignoring FVII (in addition to already traditionally ignoring FIX) is reasonable as VKA therapy rarely produces reduction in FVII and FIX activity sufficiently low to be associated with spontaneous bleeding. Two clinical studies now suggest that the hypothesis has merits but further study by independent investigators would be welcomed. As the Fiix-NR is insensitive to factor VII, the measured effect is more stable than was previously achievable and this leads to fewer dose-adjustments and, therefore, less variable warfarin anticoagulation. The TTR was improved as well in the high TTR populations studied, albeit less notable than the variability reduction. In future studies it will be important to assess how Fiix-monitored warfarin compares to clinical outcomes with DOAC drugs. Future studies could also investigate if a lower Fiix-NR target range could improve safety without much loss of efficacy. However, although these findings remain to be externally validated, Fiix-NR monitored warfarin appears to be an improved potentially practice-changing anticoagulant-monitoring test combination compared traditional PT-INR monitored warfarin.

Nomenclature

Fiix = factors II and X only (pronounced “fix”).

Fiix prothrombin time (Fiix-PT).

Fiix-NR = Fiix normalized ratio.

PT-warfarin = warfarin monitored with traditional PT-INR.

Fiix-warfarin = warfarin monitored with the new Fiix-NR.

VKA = vitamin K antagonists.

VKD = vitamin K dependent.

TTR = time within target INR range by Rosendaal method.

Authors' contributions

PTO wrote the first manuscript draft that was then critically edited by RP, DMW and BRG.

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Availability of data and materials

N.A.

Declarations

Ethics approval and consent to participate

All the quoted clinical work followed the principles of the Helsinki declaration and was approved by The National Bioethics Committee of Iceland.

Consent for publication

No personally identifiable data are reported.

Competing interests

The Fiix prothrombin time is a patented and trademarked property of Fiix diagnostics LLC, a start-up company owned by its inventors PTO and BRG together with The Landspítali National University Hospital and the University of Iceland. RP and DMW do not have COI related to this manuscript.

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