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# The need for an adapted initiation nomogram during Fiix prothrombin time monitoring of warfarin

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**Abstract**
The new Fiix prothrombin time (Fiix-PT) and its derived Fiix-normalized ratio (Fiix-NR) is affected only by reductions in coagulation factors (F) II and X, the two factors responsible for the antithrombotic effect of vitamin K antagonists (VKA). Due to insensitivity to reductions in the short half-life FVII, the Fiix-NR rises later than standard PT-INR during warfarin initiation. To describe a warfarin initiation nomogram adapted for monitoring with Fiix-NR, anticoagulation development was assessed during use of standard PT-INR based initiation nomogram and after adapting the initiation nomogram for Fiix-NR monitoring. Normalized ratios were retrospectively assessed in consecutive warfarin naïve patients during their first 60 days of warfarin intake for one year prior to (PT-INR period) and for one year after replacing the PT-INR with the Fiix-NR (Fiix-NR period). The INR target was NR 2.0–3.0. We evaluated 160 patients monitored with PT-INR and dosed with the PT-nomogram, 57 monitored with Fiix-INR but dosed with PT-nomogram, and 163 Fiix-NR monitored patients dosed using a new Fiix nomogram. Mean PT-INR over 2.0 was reached on day 7 during the PT-period and remained around 2.5 thereafter. When the PT-nomogram continued in use during Fiix-monitoring significantly more patients became over-anticoagulated during days 11–29. After the nomogram was modified to respond to rising Fiix-NR with larger initial dose reduction, the mean Fiix-NR reached over 2 on day 8–9 and remained around 2.5 thereafter. When warfarin is monitored with Fiix-NR, an adjusted dosing nomogram should be used during initiation to prevent early overanticoagulation.

## Highlights

- The Fiix prothrombin time (Fiix-PT) is a new warfarin monitoring test being commercialized that is only affected by reduced factors II and/or X and reduces the long-term variability of warfarin anticoagulation and improves clinical outcome.
- During monitoring of warfarin initiation with Fiix-PT we found that a modified dosing method was needed to prevent early warfarin overanticoagulation.
- A successful modification of the initiation nomogram is described that we recommend if Fiix-NR monitoring of warfarin is used. However, modifications of the dosing algorithm are not needed during long-term Fiix-warfarin anticoagulation.

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## Introduction

The Fiix prothrombin time (Fiix-PT, Fiix-test) is a new modified PT that is affected only by reductions in the vitamin K dependent (VKD) factors (F) II and X. It differs from the Owren´s type PT in not being affected by reduced FVII and from the Quick type PT in not being affected by reductions in FVII or the non-VKD factors V or fibrinogen [1]. This has been achieved by mixing in a specially made factor II and X deficient plasma that corrects the prediluted test sample for all factor deficiencies other than those of factors II and X [1].

As, the Fiix-PT is only affected by the two VKD factors that are mainly responsible for the antithrombotic effect of vitamin K antagonists (VKA) [2] and which are the main cause of reduced thrombin generation in patients taking vitamin K antagonists (VKA), [3, 4] the Fiix-PT reflects the anticoagulation level of warfarin accurately at all times. This is in contrast to the traditional PT which is also markedly affected by

reduced FVII, in particular recently after dose adjustments as the half-life of factor VII is markedly shorter than that of factors II and X. In support of this, the Fiix-PT has been shown to reflect automated thrombin generation ex vivo better than the standard PT does [4]. A Fiix-normalized ratio (Fiix-NR) can be calculated based on the Fiix-PT ratio in a manner similar to the traditional PT based international normalized ratio (INR, PT-INR) using standards traceable to the WHO international sensitivity index (ISI) standards. However, whereas the Fiix-NR is similar to the PT-INR during stable therapy, the results may differ significantly during initiation and following recent dose changes when fast occuring reductions in factor VII mainly drive the standard PT-INR prolongation but do not affect the Fiix-NR [1].

In the investigator initiated randomized and blinded Fiix-trial published in 2015, we observed a statistically non-inferior 50% reduction in thromboembolism (TE) without increased bleeding when warfarin was monitored with Fiix-NR compared to high quality Quick PT-INR monitoring [5]. The reduction in TE was statistically superior during long-term treatment in two secondary analyses of the Fiix-trial [5, 6] and this was at least in part related to reduced anticoagulation variability and reduced dose manipulations [7]. The Fiix-PT is easily automated and is now being commercialized under the premise that it can improve vitamin K antagonist (VKA) treatment outcome if it replaces the standard Quick PT [8] or the Owren´s PT (also known as P&P-test or prothrombin kompleks (PK) test in Scandinavia), the latter PT-variant being used mainly in the Nordic countries, Holland and Japan [9].

A standard PT based dosing nomogram was used for Fiix-NR monitoring during the Fiix-trial. Due to its promising results we replaced the Owren´s type PT-INR with Fiix-NR in our routine warfarin management practice on July 1st 2016. For the first few months, the same low-dose warfarin initiation nomogram continued in use as previously for PT-INR monitoring. However, it soon became evident that this method led to early over-anticoagulation in a number of Fiix-warfarin patients and there was concern that this could lead to increased bleeding during initiation. Consequently we adapted the initiation nomogram to take better into account the slower rise in Fiix-NR during initiation. We, however, did not modify the maintainance dosing nomogram as its efficacy and safety had been shown in the Fiix-trial. The purpose of this report is to stress the need for a modified initiation nomogram if PT-INR monitoring of warfarin is replaced with Fiix-NR monitoring.

## Methods

### Patients

We retrospectively assessed the laboratory evidence of evolving anticoagulation during the first 60 days of low-dose warfarin initiation in consecutively referred warfarin naïve patients being monitored by either the PT (PT-INR, PT-warfarin patients) or the Fiix-PT (Fiix-NR, Fiix-warfarin patients). This was done by reviewing the DAWN anticoagulation management software® records (Dawn Clinical Software, 4-S, Penrith, England) and the hospital laboratory information system normalized ratios over a two year period. All patients included in the analysis had a normalized ratio target range of 2.0–3.0.

During July 1st 2015 to June 30th 2016 (the "PT-INR period"), PT-warfarin patients were started on warfarin and monitored with the international normalized ratio that was calculated based on the Owren´s type prothrombin time (INR, PT-INR). After July 1st 2016 all patients have been monitored with the new Fiix-NR (the "Fiix-NR period"). During the whole INR period and during months +1 to +3 of the Fiix-NR monitoring period, dosing was based on a warfarin initiation nomogram designed for low-dose PT-warfarin initiation (PT-INR monitoring, PT nomogram). After that, from month +4 and on, we used a new adapted initiation nomogram, the Fiix nomogram, in order to make the dosing staff respond differently to the slower response of the Fiix-NR.

## Warfarin initiation nomograms

Patients younger than 65 were started on 6 mg daily from day 1 and those 65 and older received 4 mg daily. A monitoring test was planned on day 4 (day 3–5 in practice). Subsequent dose adjustments followed the nomograms shown in Table 1. Although we use the DAWN anticoagulation management software for routine maintainance phase monitoring we do not use its dosing algorithms during initiation until a likely dose range has been identified.

## Clotting times and normalized ratio calculation and assessment

Both the PT and the Fiix-PT tests were done at the central coagulation laboratory using venous citrated platelet poor plasma as previously described [1, 7]. The automated STA-R Evolution coagulation analyser (Diagnostica Stago, Asnieres, France) was used for both tests. For the PT we used the Owren´s PT (SPA reagent, Diagnostica Stago, Asnieres, France), and for the Fiix-PT we used Neoplastin (Diagnostica Stago, Asnieres, France) with added factor II and factor X depleted plasma [1]. In-house standardisation of the thromboplastin sensitivity was done for the separate monitoring tests in the following manner:

**Table 1** Standard prothrombin time (PT-INR) initiation nomogram and new Fiix prothrombin time (Fiix-NR) initiation nomogram

| Starting dose on day 1 | Normalized ratio on day 4 | Warfarin dose; PT-INR nomogram (percent change) | Warfarin dose; adapted Fiix-NR nomogram (percent change)[a] |
|---|---|---|---|
| Up to 65 year old patient; initial dose 6 mg daily | < 1.3 | + 50 | + 33 |
| | 1.3 | 0 | 0 |
| | 1.4–1.5 | 0 | − 25 |
| | 1.6–1.7 | 0 | − 50 |
| | 1.8–2.5 | − 50 | − 67 to − 75 |
| | > 2.5 | − 67 | Dose skipped and adapted[b] |
| Over 65 year old patient; initial dose 4 mg daily | < 1.3 | + 33 | + 25 |
| | 1.3 | 0 | 0 |
| | 1.4–1.5 | 0 | − 25 |
| | 1.6–1.7 | 0 | − 50 |
| | 1.8–2.5 | − 50 | - 67 to - 75 |
| | > 2.5 | − 67 | Dose skipped and adapted** |

[a]The Fiix normalized ratio (Fiix-NR) responds slower than the PT-INR due to its insensitivity to factor VII reductions

[b]Dose usually skipped for 1–2 days and dose then reduced based on rate of rise of the normalized ratio

## Prothrombin time standardization

A local international sensitivity index (ISI) standardization of the SPA reagent thromboplastin was done using the STA-R instrument according to the procedure for PT standardisation provided by the Danish Institute for External Quality Assurance in Health Care (DEKS, Glostrup, Denmark) using provided standards traceable to the WHO international standards [10, 11]. In short, prothrombin times were measured in three provided plasma standards with standardized PT-INR of 1.0, 2.26 and 3.50. The local ISI for the SPA is the slope of the calibrator curve calculated by orthogonal regression of the log of local PT seconds vs the log of the provided INR (see www.deks.dk/kmov/pakvedlæ.html). The PT-INR in an anticoagulated patient´s sample is subsequently calculated = [PT clotting time (patient)/median PT clotting time of the normal population]$^{ISI}$.

## Fiix prothrombin time standardization

The Fiix-NR is not an internationally standardized test yet but for our purposes we have used the DEKS standards (above) as "primary standards" to obtain the Fiix-PT clotting time and a Fiix calibration curve. We subsequently found that the clotting times of in-house normal samples and samples from two highly stable warfarin patients (within target range and no dose adjustments for 10 months straight) with locally calibrated PT-INR fitted the clotting time curve obtained with the DEKS standards perfectly. Hence, we deemed the DEKS standards to be adequate for Fiix-INR determinations. Based on the DEKS standards we assigned a "Fiix sensitivity index" to the Neoplastin and then calculated a Fiix normalized ratio in patient samples by calculating the [Fiix-PT clotting time in patient test sample)/ median Fiix-PT clotting time of the normal population]$^{FiixSI}$ As a quality check we correlated the standardized Fiix-NR to the standardized PT-INR in anticoagulated samples obtained from 197 warfarin patient samples and found the slope to be acceptable; PT-INR = 1.02 × Fiix-PT − 0.06, $R^2 = 0.92$ (graph not shown). As the Fiix-test is insensitive to reduced FVII, the Fiix-NR will differ from the PT-INR during periods when FVII is low, e.g. during early initiation or shortly after dose changes. The Fiix-NR, however, does correlate well with the PT-INR during stable anticoagulation [1].

## Statistical analysis

Normalized ratios are shown as means ± 1 standard deviations (SD) of pooled results from samples drawn over consecutive 3-day intervals during initiation (days 1–3, 4–6, 7–9 etc.) as previously [4]. We calculated Students t to compare normalized ratios during initiation. In order to make it easier to compare the variance in normalized ratio we chose to use identical size patient groups that were dosed using the old INR based nomogram vs the new Fiix-NR based dosing nomogram. In order to achieve this, we randomly selected 160 patients from the 280 new patients started during the PT-INR period. All other new patients were included. The selection was based on sequential dates of birth (DD-MM-YYYY) and, thus, no skewing of age or gender was possible.

## Results and discussion

During the PT-INR monitoring period data was analyzed from 160 patients (86 male/74 female, median age 69 (mean 66; range 17–91). During the first Fiix-NR period

using PT-INR initiation nomogram there were 57 patients, 34 males/23 females, age 69 (68;30–85). During the second Fiix-NR period using the Fiix-NR adapted nomogram there were 161 patients (81 males/80 females, age 68 (65;17–95).

A mean PT-INR of over 2.0 was reached on average on day 7 during the INR period, and it remained close to 2.5 thereafter (Fig. 1A). The relatively fast rise in PT-INR is due to fast occuring reductions in the short half-life factor VII rather than a true anithrombotic effect which isn´t present until few days later when factors II and X are reduced as well [4, 12–14]. For the first 3 months of the Fiix-NR period the PT-INR nomogram remained in use unchanged despite the monitoring method now being the Fiix-NR. During this period a mean Fiix-NR over 2.0 was reached on days 8–9. However, subsequently a higher number of warfarin naïve patients became overanticoagulated when monitored with the Fiix-PT but dosed based on the PT-nomogram than during the traditional PT-monitoring based initiation (Fig. 1B). Thus, the mean Fiix-NR reached as high as 3.0 on days 17 and 29 and some patients had considerably higher Fiix-NR. The mean Fiix-NR during PT-nomogram monitoring was also consistently higher from day 17 to day 32 (Fig. 1B). The reason for this was that the PT-nomogram failed to respond with adequate dose reductions to a slowly rising Fiix-NR, the Fiix-NR being solely dependent on reductions in the long half-life factors II and X. Using the PT based nomogram led to the dose reductions that were too small and too slow. Thus, the traditional PT-INR initiation algorithm proved not to be suitable when Fiix-NR was used for monitoring during treatment start. Consequently, the initiation nomogram was modified in order to respond with a larger dose reduction depending on the magnitude of the early Fiix-NR rise and the modified nomogram is described in Table 1. This successfully led to the mean Fiix-NR hitting the Fiix-NR range of over 2 on day 9–10 and remaining at about 2.5 for the remainder of the 60 day initiation period (Fig. 1A).

Factor VII has a very short half-life of only 4–6 h and influences the PT-INR as much as reductions in FII or FX do [1]. However, the degree of reductions in FVII observed during long-term warfarin treatment are neither important for the efficacy nor the safety of warfarin management [1–4]. In our opinion FVII reductions simply confound warfarin dose management through influence on the PT-INR [2, 4, 5]. The Fiix-PT was invented based on the hypothesis

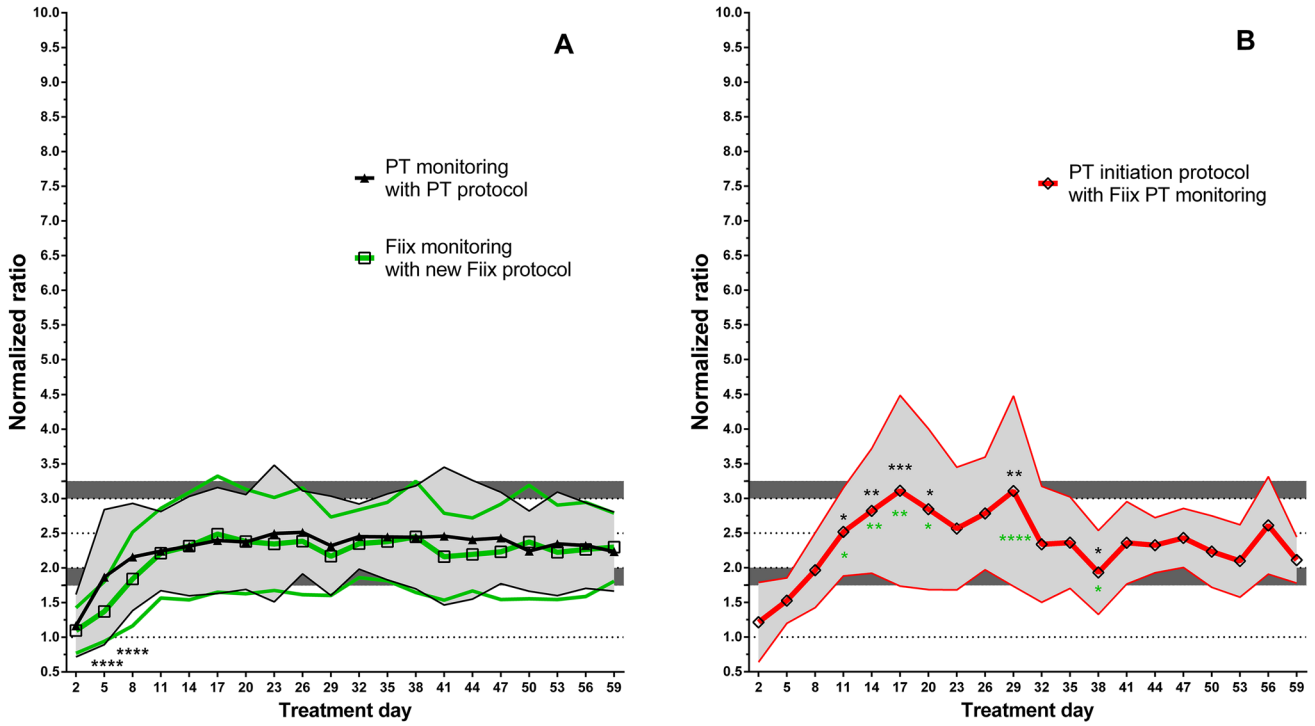


**Fig. 1** Anticoagulation development during first 60 days of warfarin management. Patients were monitored with PT-INR and dosed based on a PT-INR based initiation nomogram (n = 160, triangles in panel A), monitored with Fiix-NR but dosed based on the PT-based nomogram (n = 57, diamonds in panel B), or monitored with the Fiix-NR and dosed based on the adapted Fiix-NR based nomogram (n = 163, open boxes in panel A). Normalized ratios for each three consecutive days were pooled (days 1–3, 4–6, etc.) and results are shown as means of each 3 day interval (symbols and line) and one standard deviation. The dark shaded horisontal lines show normalized ratios 1.5–2.0 and 3.0–3.5. The asterisks in panel B reflect statistically significant differences compared to PT monitoring (black asterisks) and Fiix-monitoring adapted protocol (green asterisks). *P* is shown as *< 0.05, **< 0.01, ***< 0.001 and ****< 0.0001

that using a test that ignores FVII and only is affected by the two factors mainly responsible for the VKA effect, namely FII and FX, [2] would at all times reflect the antithrombotic effect more accurately than the traditional PT does. Thrombin generation studies support that notion and suggest that the antithrombotic effect of warfarin is present once factors II and X are low enough to cause a prolongation of the Fiix-NR to about 1.7–1.8 or more [3, 4]. Therefore, in contrast to the standard PT-INR, the true antithrombotic effect is always accurately predicted based on the Fiix-NR prolongation.

The lack of influence of early occuring reductions in FVII on the Fiix-NR was expected to lead to a slower responding normalized ratio than that observed with PT-INR monitoring and, potentially, a risk of overanticoagulation during Fiix-warfarin initiation. However, we did not observe this during the randomized Fiix-trial, most likely as relatively few new patients were included in the trial [5]. Our current data from clinical practice, however, demonstrates that overanticoagulation does indeed occur. Furthermore, we now provide a new modified initiation method, the adapted Fiix initiation nomogram, which corrects the issue. By inference, the modification likely minimizes the risk of bleeding during Fiix-warfarin initiation but this has not been studied as many more naïve patients would need to be included. Finally, we have made no attempt to modify the maintainance dose nomogram as the existing nomogram led to a major improvement in long-term efficacy in the Fiix-trial [5].

In conclusion, we recommend that if warfarin initiation is monitored with Fiix- instead of PT-INR an adjusted dosing nomogram such as the one suggested in Table 1 should be applied to prevent early over-anticoagulation.

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## Compliance with ethical standards

**Conflict of interest** Pall T. Onundarson and Brynja R. Gudmundsdottir are the co-inventors of the Fiix prothrombin time. Together with the University of Iceland and the Landspital National University Hospital they are owners of Fiix Diagnostics LLC which holds a patent for the Fiix-PT.

**Ethical approval** The study was approved by the Landspitali University Hospital science ethics committee (25/2017) and followed the guidelines of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was not obtained as this was a retrospective analysis approved by the hospital ethical committee which waives the need for consent for this type of non-interventional retrospective study.

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