INR fluctuation:

a reflection of treatment inadequacy - or of prothrombin time inadequacy?

2011
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Chief, Dept. of Laboratory Hematology and Coagulation Disorders,
Landspitali, Reykjavik, Iceland

The trouble with warfarin

Bad press – rat poison

FDA
"BLACK BOX" WARNING

"Warfarin sodium can cause major or fatal bleeding. Bleeding is more likely to occur during the starting period and with a higher dose (resulting in a higher INR)…"

ED VISITS FOR ADE´S

- Estimated 701, 547 ADEs per year.
- 17% require hospitalization
- Insulin (8%) or **warfarin (6%):** implicated in 14% of ADEs treated in ED.

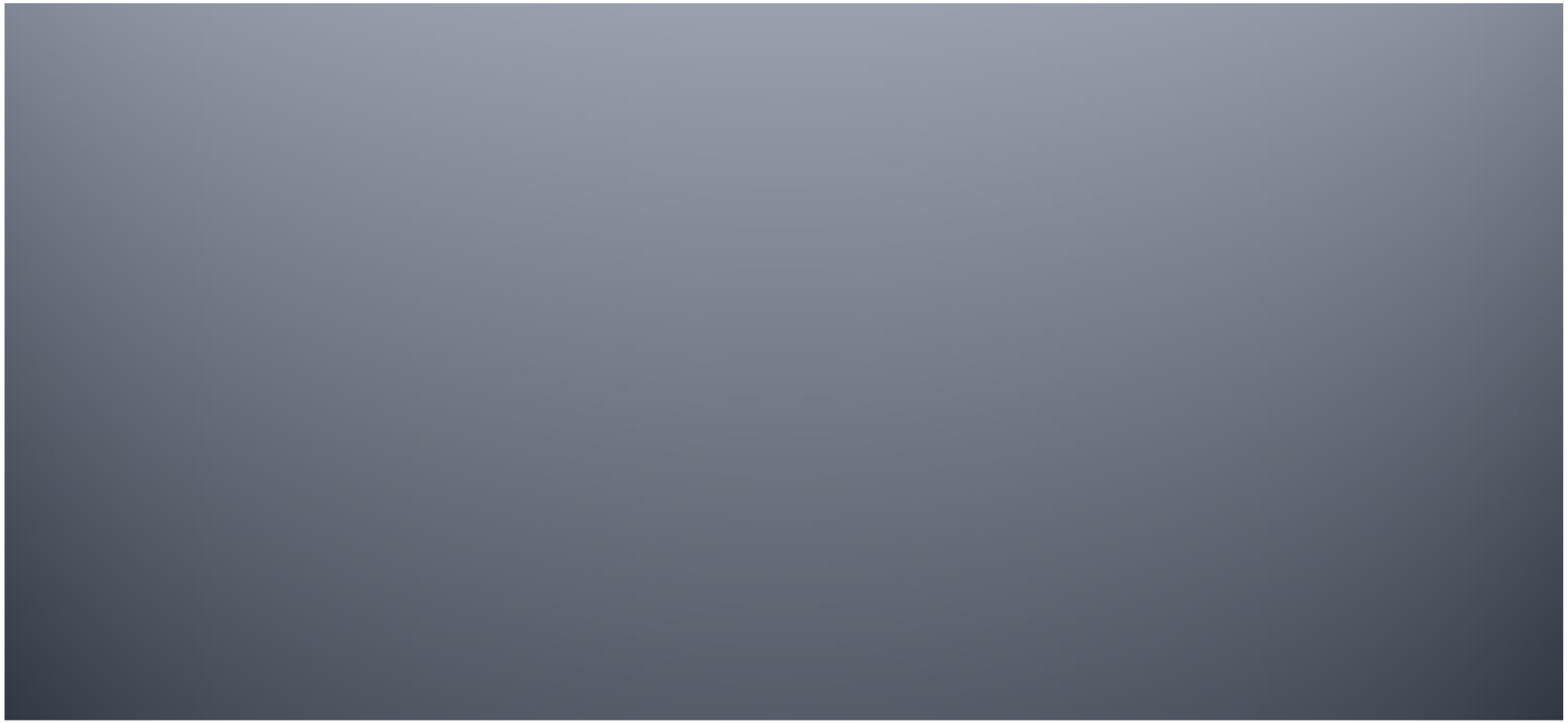
(JAMA 2006; 296. 1858-1866)

Warfarin/coumarins
a love-hate relationship

- **Hate**
  - Slow onset of effect
  - Variable dose
    - Mutations affecting metabolism and dose size
  - INR fluctuates in many patients leading to frequent dose adjustments
  - Serious bleeding complications
  - Needlestick
  - Work!

- **Love**
  - Well studied and effective
  - Controllable dose by monitoring
    - Therapeutic window well delineated – and can be adjusted to personal needs
    - Standardized
  - Easily reversed

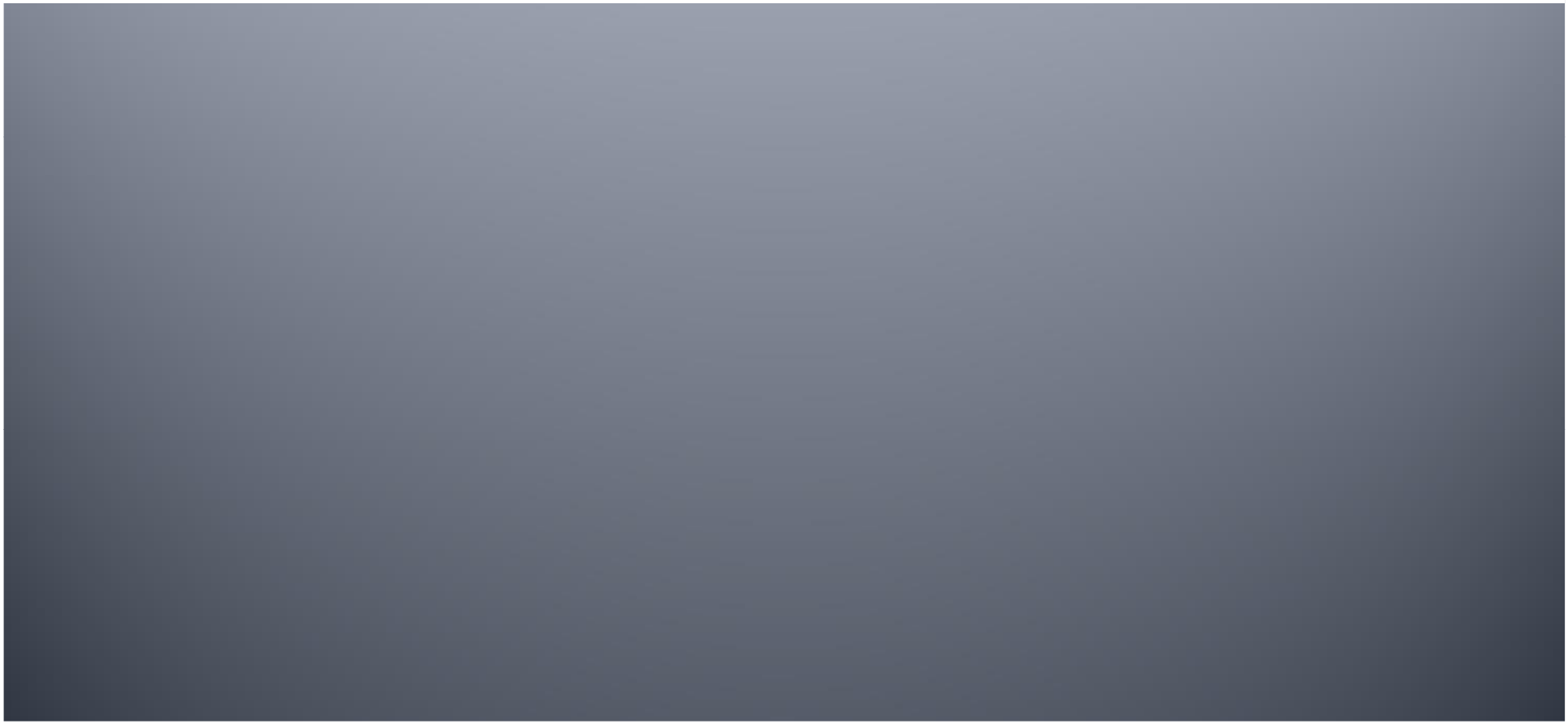
We have a dream of an ideal anticoagulant



The Ideal Anticoagulant

	Warfarin	Dabigatran	Rivaroxaban
Low cost (daily cost)	√ (<$2)	- ($6)	- (?)
Predictable anti-thrombotic effect	-	√?	√?
Patients rarely bleed	-	-	-
Oral	√	√	√
Quick onset of action	-	√	√
Once daily dosing	√	-	+/-
Titratability - individualization	√		
Liver excretion	√	-	-
Stable effect	-	√	√
No monitoring	-	√ ?	√ ?
Little food and drug interactions	-	√	√
Immediate reversal with antidote	√	-	-
Compliance can be confirmed	√	-	-
Non-hemostatic side effects	rare	common	…

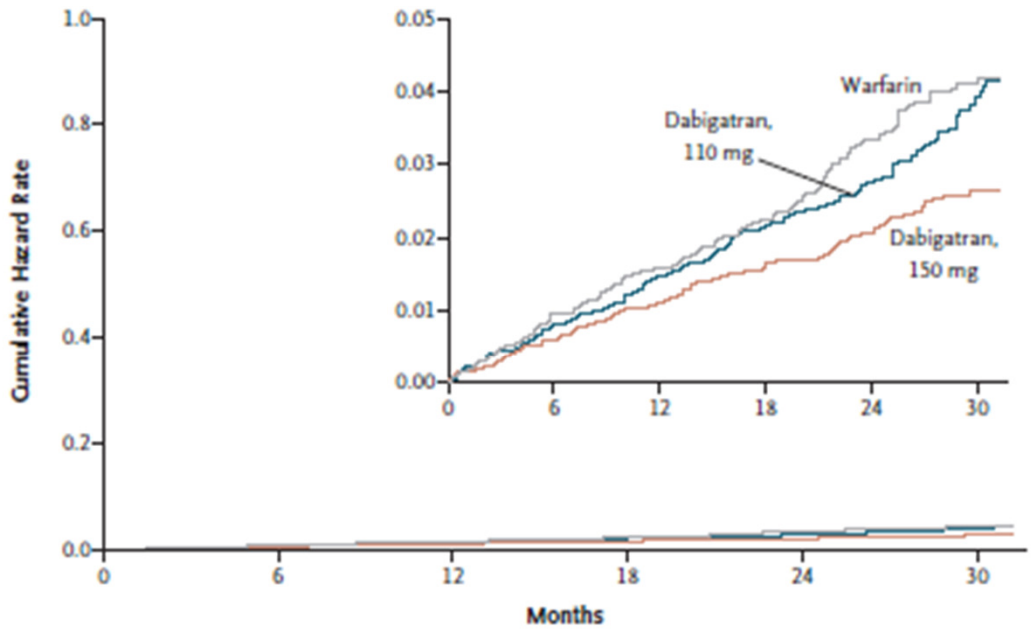
Are the new oral agents better than warfarin?



Dabigatran versus Warfarin in Patients with Atrial Fibrillation

Stuart J. Connolly, et al and the RE-LY Steering Committee and Investigators

N Engl J Med 2009; 361:1139-1151
September 17, 2009



No. at Risk

Warfarin	6022	5862	5718	4593	2890	1322
Dabigatran, 110 mg	6015	5862	5710	4593	2945	1385
Dabigatran, 150 mg	6076	5939	5779	4682	3044	1429

Figure 1. Cumulative Hazard Rates for the Primary Outcome of Stroke or Systemic Embolism, According to Treatment Group.

Dabigatran versus Warfarin in Patients with Atrial Fibrillation

Stuart J. Connolly, et al. The RE-LY study

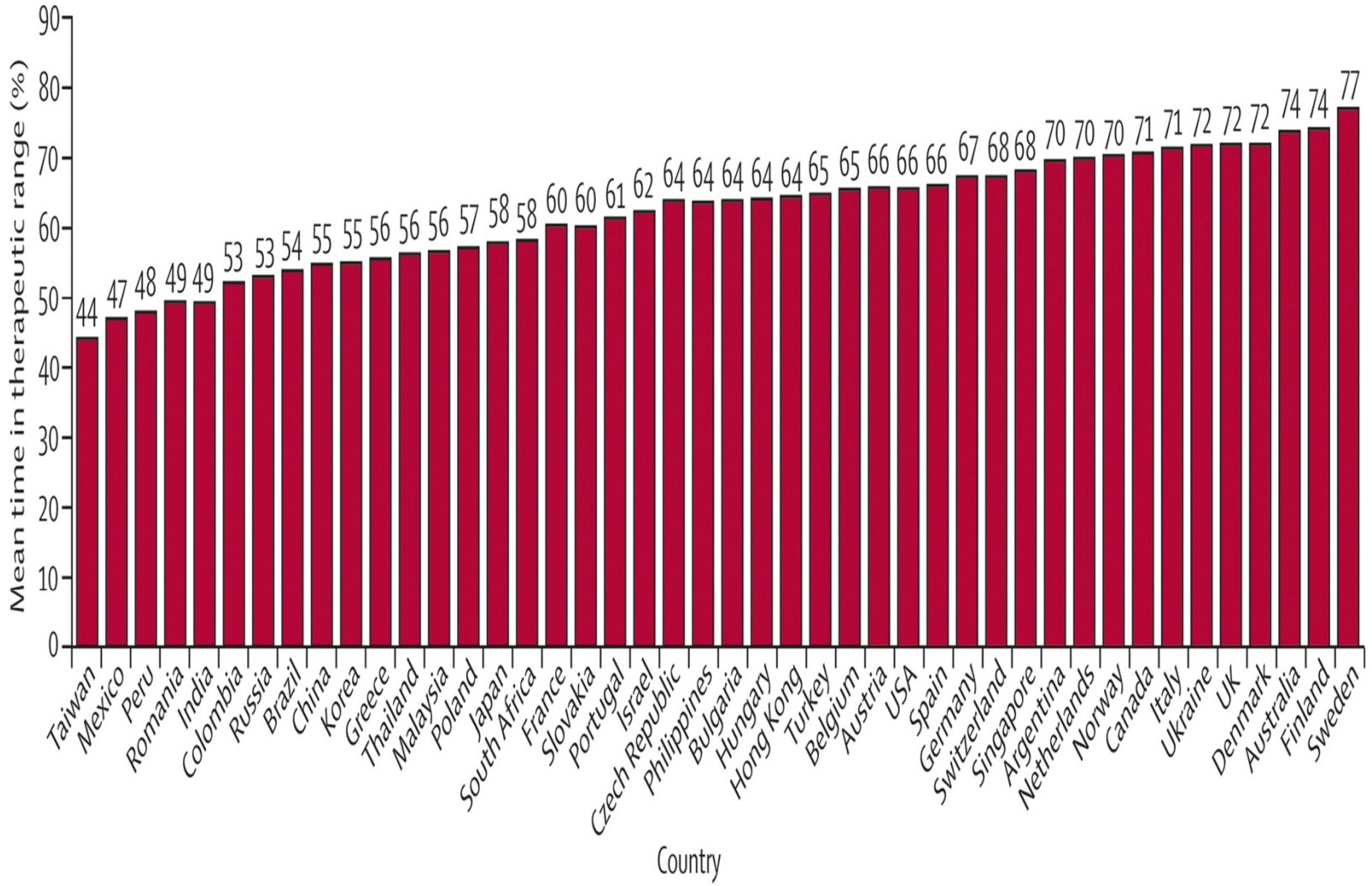
N Engl J Med 2009; 361:1139-1151September 17, 2009

	Warfarin	Dabigatran 110 mg bid	Dabigatran 150 mg bid
Major bleeding	3.36%	2.71%**	3.11% (ns)
Hemorrhagic stroke	0.36%	0.12%***	0.10%***
Annual mortality	4.13%	3.75% (ns)	3.64%(*)

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

Lars Wallentin et al on behalf of the RE-LY investigators

The Lancet, Volume 376, Issue 9745, 18-24 September 2010, Pages 975-983



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

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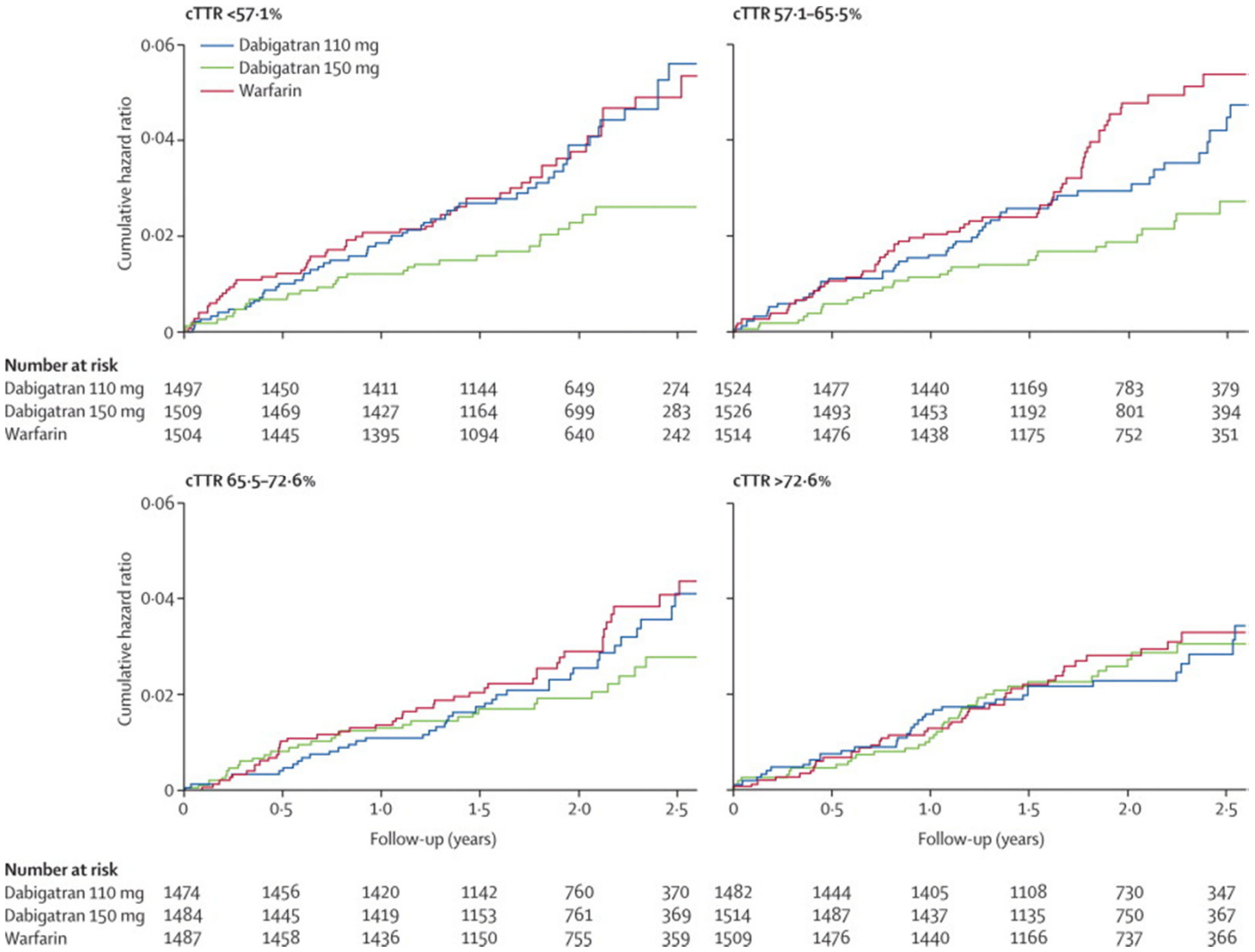
- Time to primary outcome in each quartile of centre's mean time in therapeutic range
- cTTR=centre's mean time in therapeutic range.



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

Lars Wallentin ProfMDa, , et al . The RE-LY investigators
The Lancet, Volume 376, Issue 9745, 18-24 September 2010, Pages 975-983

- Time to major bleeding event in each quartile of centre's mean time in therapeutic range
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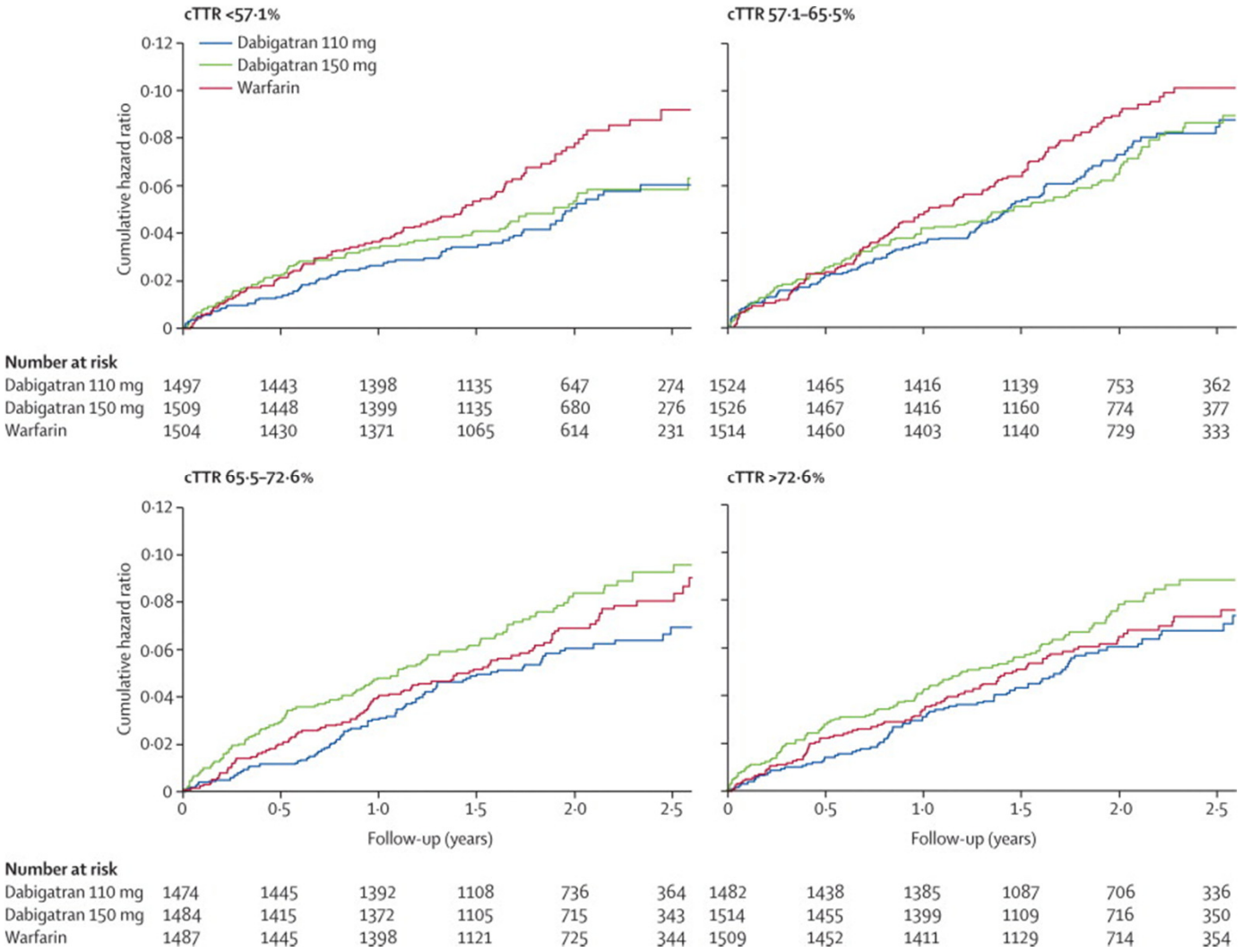


New Oral Anticoagulants:

Rivaroxaban 2010

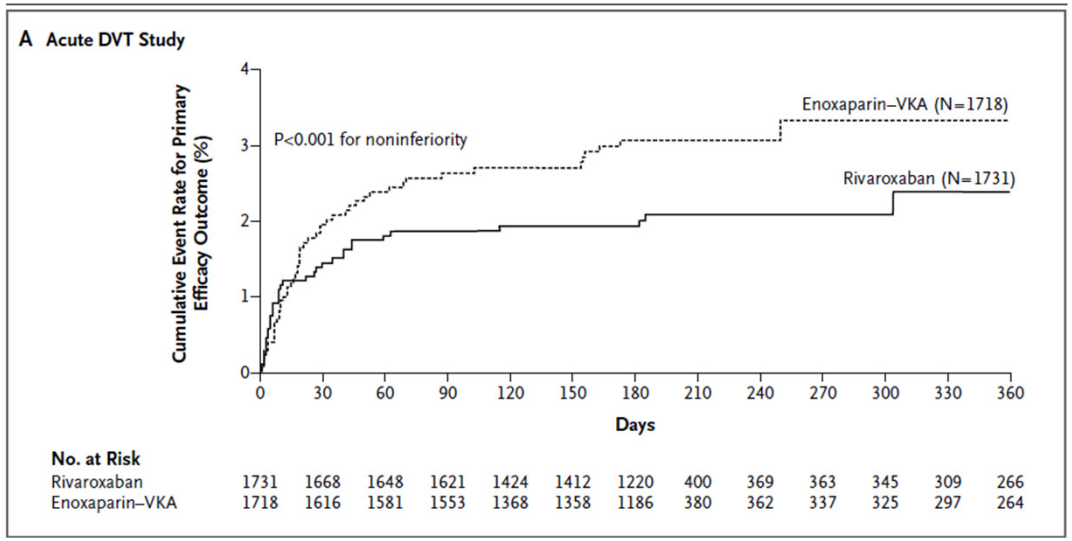
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

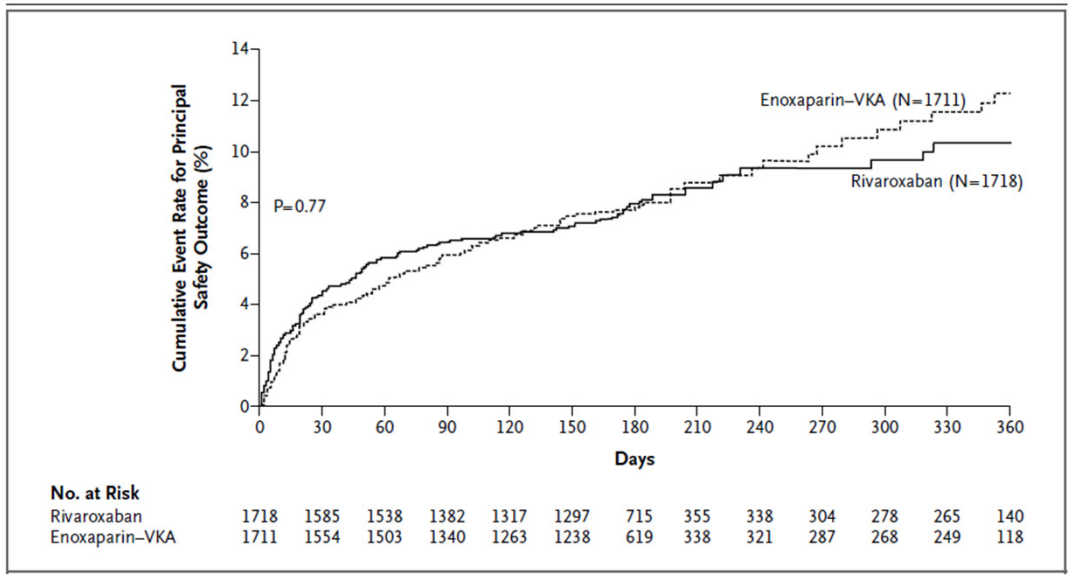
Oral Rivaroxaban for Symptomatic Venous Thromboembolism

The EINSTEIN Investigators*

Primary efficacy



Safety Outcome



A Acute DVT Study
Rivaroxaban (N=1731)
Enoxaparin–VKA (N=1718)
P<0.001 for noninferiority
Rivaroxaban
Enoxaparin–VKA
No. at Risk
Days
Cumulative Rate of Efficacy Outcome (%)

- **Event free survival**
  - 97 vs 97.9% event free survival for 360 days

The NEW ENGLAND JOURNAL of MEDICINE

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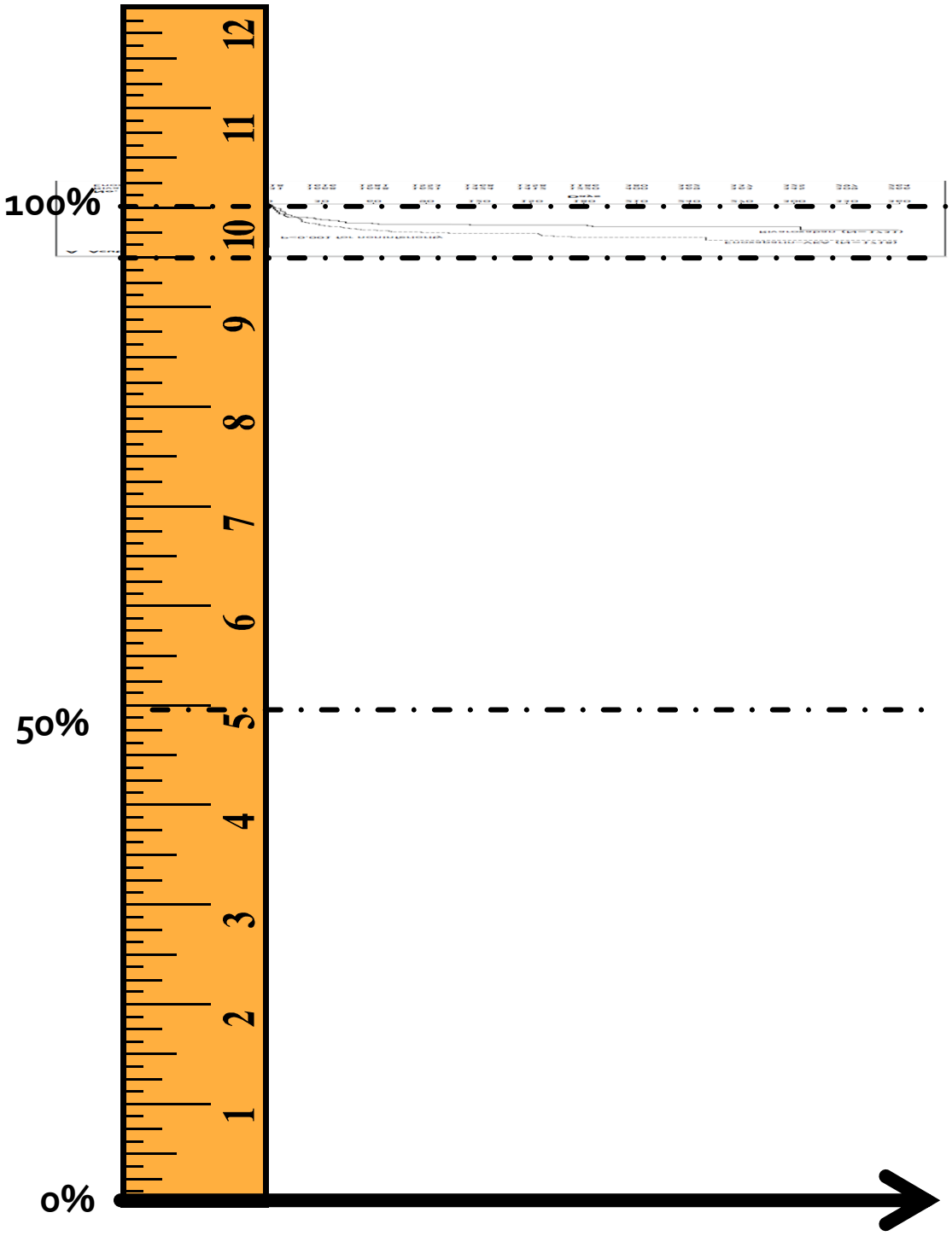
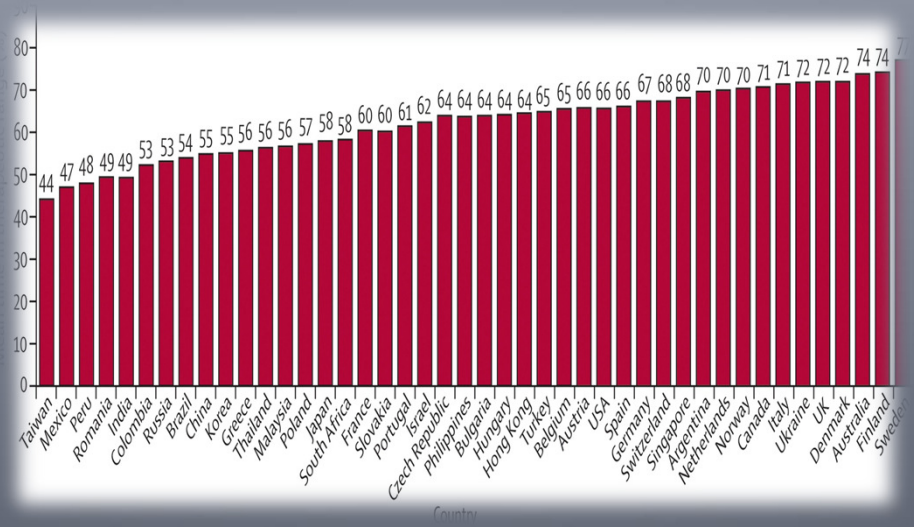


Pall´s Conclusion:

- No health benefit of dabigatran over adequate warfarin treatment
  - if TTR is >65%
- Hardly a relevant difference with rivaroxaban
- Efficacy and safety benefit of new agents over warfarin may be limited to centers with poorer management of warfarin
- Other side effects, antidote and prize must be an issue
- Warfarin is not yet moribund

Can treatment with warfarin be improved?



Volume 352, Issue 9139, 7 November 1998, Pages 1505-1509

Multicentre randomised study of computerised anticoagulant dosage multicentre randomised study of computerised anticoagulant dosage

L Poller ProfDSc[a], CR Shiach MD[b], PK MacCallum MD[c], AM Johansen MD[d], AM Münster MD[e], A Magalhães MD[f], J Jespersen DSc[e] and on behalf of the European Concerted Action on Anticoagulation

Background

We compared the benefits of computer-generated anticoagulant dosing with traditional dosing decided by experienced medical staff in achieving target international normalised ratios (INRs).

Methods

In five European centres we randomly assigned 285 patients in the stabilisation period and stabilised patients to the computer-generated-dose group (n=137) or traditional-dose group (n=148). Centres had a specialist interest in oral anticoagulation but no previous experience with computer-generated dosing. The computer program calculated doses and times to next visit. Our main endpoint was time spent in target INR range (Rosendaal method).

Findings

The mean time within target INR range for all patients and all ranges was 63·3% (SD 28·0) of days in the computer-generated-dose group compared with 53·2% (27·7) in the traditional-dose group.

Interpretation

The computer program gave better INR control than the experienced medical staff

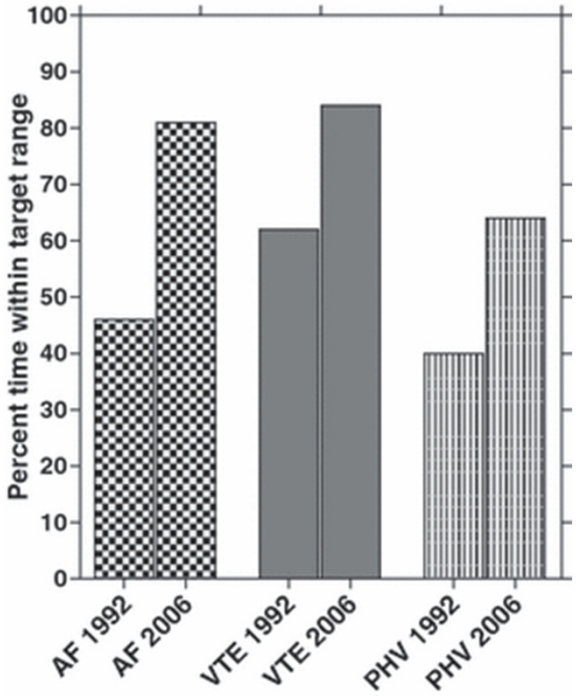
Quality of warfarin management

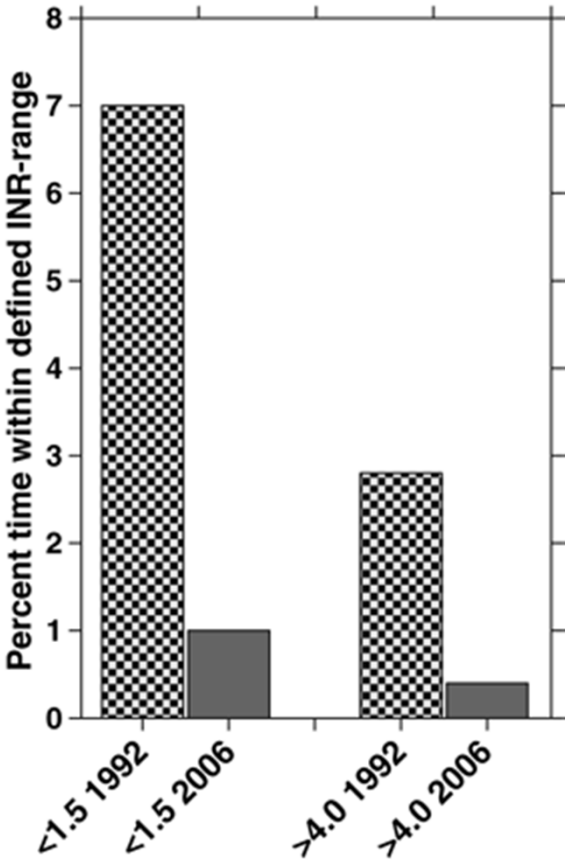
DAWN® based AMC improves time in range compared to cardiologist dosing

INT J LAB HEMATOL. 2008;30(5):382-9.

WARFARIN ANTICOAGULATION INTENSITY IN SPECIALIST-BASED AND IN COMPUTER-ASSISTED DOSING PRACTICE.

ONUNDARSON PT, EINARSDOTTIR KA, GUDMUNDSDOTTIR BR.





What are the current major limitations of warfarin?

- Dose size at initiation
- Fluctuating INR:
  - frequent dose changes
  - frequent monitoring
- Fluctuating INR is caused by:
  - Food and drugs
  - Prothrombin time?

Coumarin effect and it´s monitoring

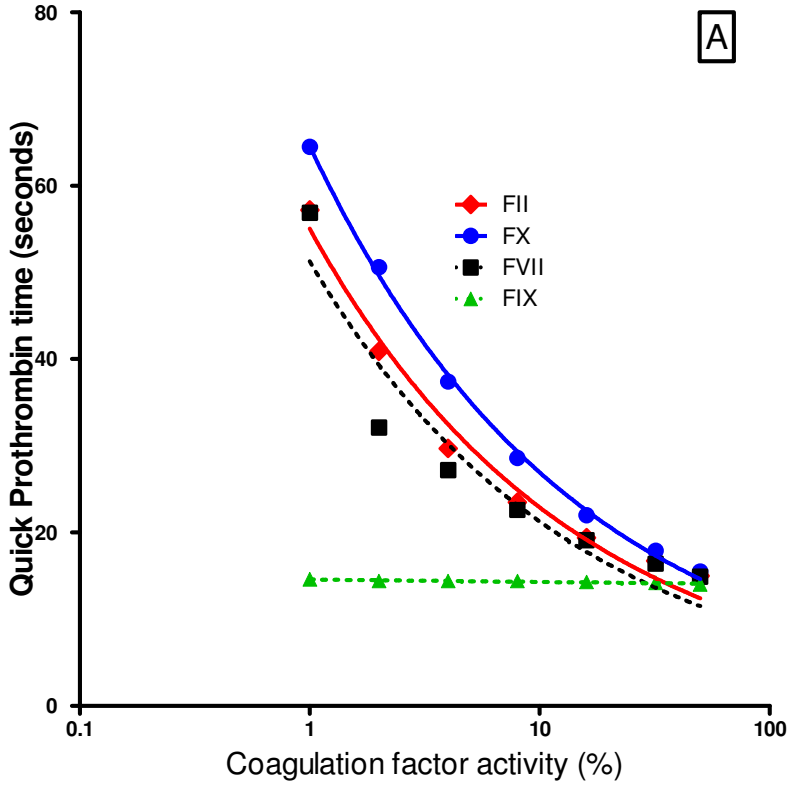
- Vitamin K inhibition
- Gamma carboxylation of ii, vii, ix and x prevented
- $T_{1/2}$
- "Common wisdom"
  - Reduction in all VKD factors is equally important for the anticoagulant effect of coumarins
- Confounding effect of factor VII during initiation
  - „Must reduce all vit K dependent factors to be anticoagulated"

1935-1984:

Presumption: ii, vii, ix and x contribute equally to antithrombotic effect

Prothrombin times

- measure activity of II, VII and X (not IX)
- the clotting times obtained are equally sensitive to reduction in coagulation factors II, VII or X.



Monitoring tests

ARMAND QUICK

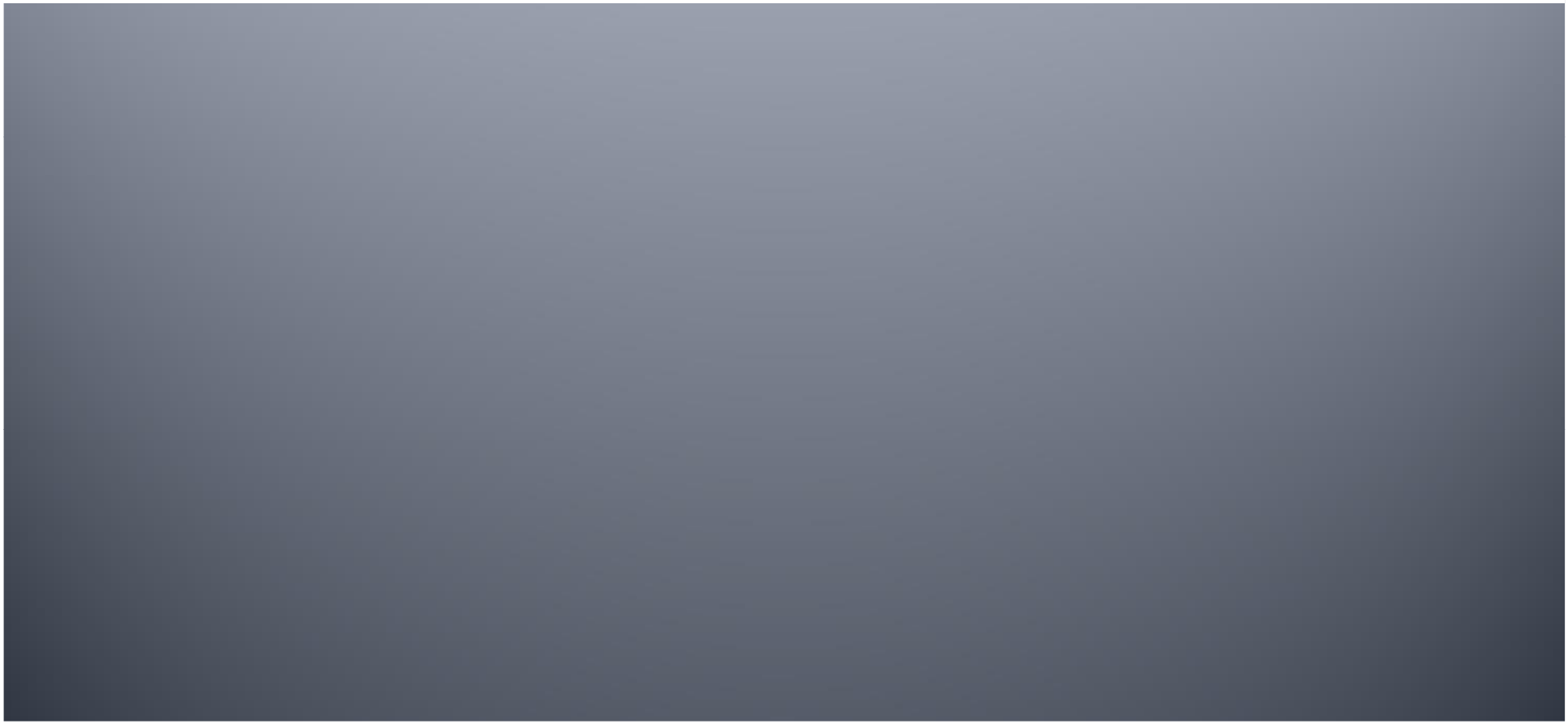
- **PT** (1935)
  - sensitive to reduced factors
    - ii, vii, x
    - i (fibrinogen), v

PAUL A. OWREN

- **Owren PT (P&P test) (1951)**
  - diluted test sample
  - adsorbed bovine plasma mixed with patient plasma
  - sensitive to reduced factors
    - ii, vii, x
    - Not i, v
- Thrombotest (1959)
  - ii, vii, ix and x

Do individual VKD factors influence clot formation *in vivo* equally?

Shouting in the desert 1984-2011…



Blood. 1984 Aug;64(2):445-51.

Comparison of the native prothrombin antigen and the prothrombin time for monitoring oral anticoagulant therapy.

Furie B, Liebman HA, Blanchard RA, Coleman MS, Kruger SF, Furie BC.

Table 1. Native Prothrombin, Abnormal Prothrombin, and Prothrombin Time Index in Subpopulations of Patients Treated With Warfarin

	Native Prothrombin (μg/mL)*	Abnormal Prothrombin (μg/mL)	Prothrombin Time Index
All patient samples	26.4 (1.5–173)	36 (0–144)	1.88 (1–8.4)
Patients with complications			
Bleeding (n = 13)	6.2 (1.5–12)	68.7 (28–144)	2.75 (1.8–5.3)
Thrombotic (n = 7)	30.6 (14–45)	75.2 (32–118)	2.0 (1.4–3.1)
Normal subjects	108 ± 19	0	1.0

*Mean (range).

*Mean (range).

The native prothrombin antigen correlated with the occurrence of complications in 95% of samples. **Of 13 samples from patients with bleeding complications, 13/13 (100%) had a native prothrombin of 12 micrograms/mL or lower. Of seven samples from patients with thromboembolic complications, 6/7 (86%) had a native prothrombin of 24 micrograms/mL or greater**. By comparison, a prothrombin time index of 1.5 to 2.5, 1.5 to 2.2, 1.5 to 2.0, or 1.3 to 1.8 identified 6/20 (30%), 9/20 (45%), 11/20 (55%), or 12/20 (60%) patients at risk, respectively.

TARGET RANGE 12-24 ug/mL

Randomized prospective trial comparing the native prothrombin antigen with the prothrombin time for monitoring oral anticoagulant therapy

Furie B, Diuguid CF, Jacobs M, Diuguid DL, Furie BC, New England Medical Center, Boston

Blood. 1990 Jan 15;75(2):344-9

Abstract

Patients with indications for anticoagulation were randomized to be monitored by the native prothrombin antigen (therapeutic range, 12 to 24 micrograms/mL) or the prothrombin time index (therapeutic range, 1.5 to 2.0).

Of the prothrombin time group (N = 80), seven (8.8%) had bleeding or thrombotic complications, with a complication rate of 9.5%/patient-year.

In the native prothrombin antigen group (N = 76), one subject (1.3%) had a bleeding complication. The complication rate per patient-year was 1.5%.

These results indicate an 85% reduction in the complication rate of the native prothrombin antigen group compared with the complication rate of the prothrombin time group. This difference is statistically significant by the Fisher exact test (P = .037) and by Kaplan Meier survival analysis (P = .040).

This study suggests that the use of the native prothrombin antigen assay has the potential to decrease the complications associated with anticoagulation therapy with warfarin sodium.

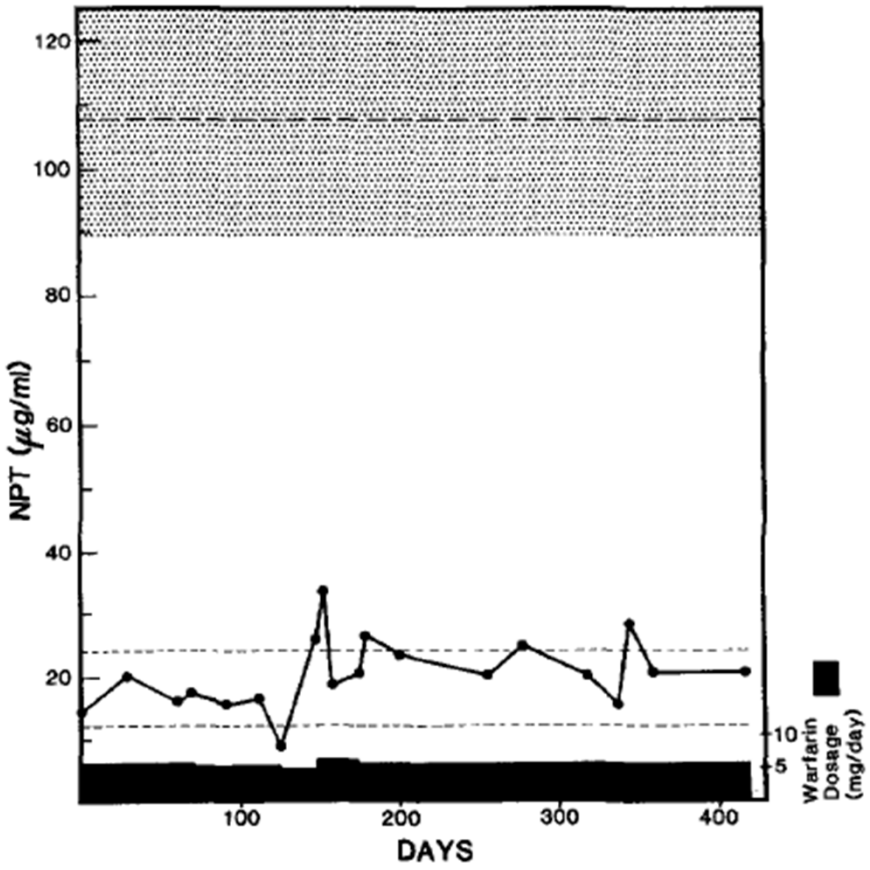


Fig 1. Monitoring of warfarin dosage with the native prothrombin antigen. A patient on chronic warfarin therapy because of aortic and mitral prosthetic valves was evaluated with the NPT assay, and the warfarin dose adjusted accordingly. The goal was to maintain the NPT at 18 μg/mL, within the therapeutic range of 12 to 24 μg/mL. The NPT in normal subjects is 108 ± 19 μg/mL.

Thromb Haemost. 1989 Sep 29;62(2):788-91.

The relative importance of the factors II, VII, IX and X for the prothrombinase activity in plasma of orally anticoagulated patients.

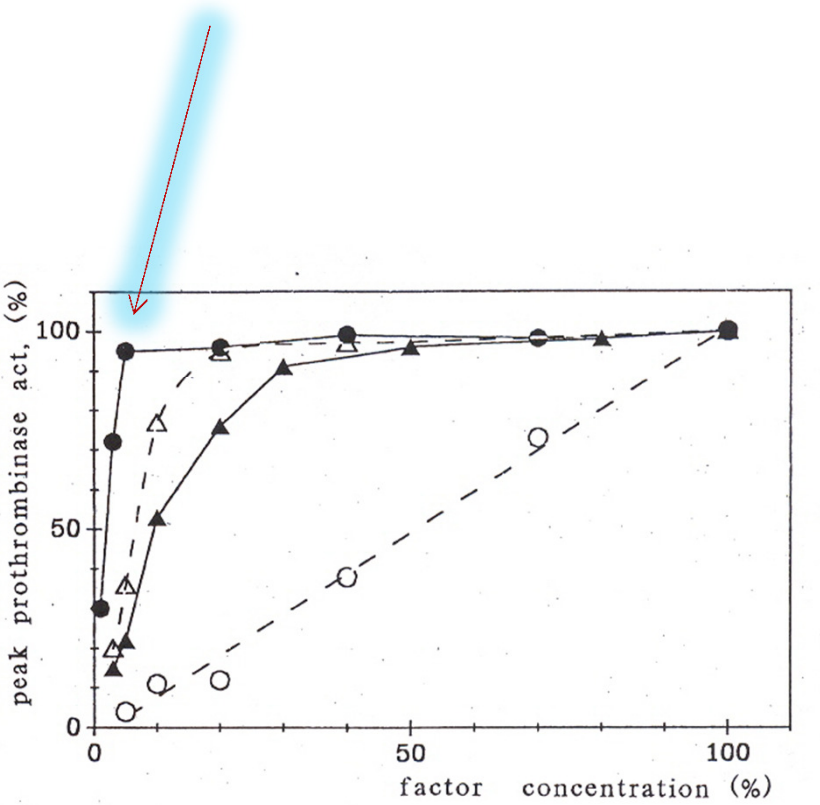
Xi M, Béguin S, Hemker HC.
Department of Biochemistry, University of Limburg, Maastricht, The Netherlands.

- The individual importance of each of the four vitamin K-dependent clotting factors on the generation of prothrombinase activity in the plasma of orally anticoagulated patients has been investigated. **Addition of purified factors VII, IX or X to plasma from deeply anticoagulated patients (International Normalized Ratio 2.8-4.8) did not influence the amount of prothrombinase activity or the amount of thrombin formed. Only the prothrombin level in the plasma determines the course of thrombin generation.**

- Addition of increasing amounts of purified factor II, VII, IX or X to plasmas deficient in respectively factor II, VII, IX or X showed that the prothrombinase activity increases linearly with the concentration of factor II added and that the concentration below which the factors VII, IX and X start to have a measurable effect on prothrombinase activity are **5%, 20%, and 30%, respectively**. Half maximal prothrombinase activity was found at about 1% factor VII, 5% factor IX and 8% factor X respectively.

- From these observations we conclude **that primarily the variation in factor II level determines thrombin generation and hence presumably the antithrombotic effect of oral anticoagulant therapy.** It therefore seems likely that, for the control of oral anticoagulant therapy, tests that reflect factor II activity would be suitable.

S2238



thrombin generation curves. ○: factor II; ●: factor VII; △: factor IX; ▲: factor X

Circulation. 1993 Aug;88(2):454-60.

Comparison of native prothrombin antigen with the prothrombin time for monitoring oral anticoagulant prophylaxis.

Kornberg A, Francis CW, Pellegrini VD Jr, Gabriel KR, Marder VJ.
Department of Medicine, University of Rochester School of Medicine and Dentistry

These results indicate that the NPA concentration more accurately reflects the antithrombotic effect of warfarin than does prothrombin time and may be superior in monitoring prophylactic oral anticoagulation.

J. Clin. Invest. 92(5): 2131-2140 (1993). doi:10.1172/JCI116814.

Mechanism of the anticoagulant effect of warfarin as evaluated in rabbits by selective depression of individual procoagulant vitamin K-dependent clotting factors.

A Zivelin, L V Rao and S I Rapaport

Department of Medicine, University of California, San Diego 92093.

- Immunodepletion of plasma factor X or prothrombin, but not of factor VII or factor IX, protected otherwise normal rabbits against tissue factor-induced coagulation.
- Next, we determined the effect upon the protection in warfarin-treated rabbits of selectively restoring factor X or prothrombin before infusing tissue factor. When either factor was selectively restored, warfarin's protective effect was abolished. Moreover, selective restoration of prothrombin sensitized warfarin-treated rabbits to coagulation more severe than observed in nontreated control rabbits.
- One may extrapolate from these data that depression of both factor X and prothrombin are required for warfarin's clinical antithrombotic efficacy and that depression of plasma prothrombin is particularly important.

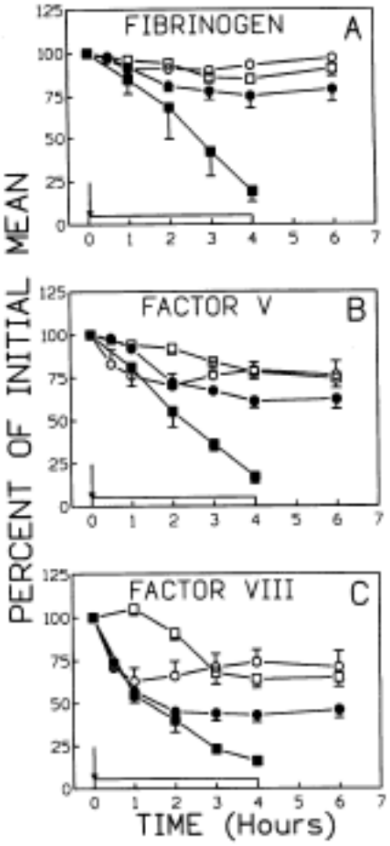


Figure 7. Evidence that selective immunodepression of plasma prothrombin activity to levels comparable to those obtained in high responders given warfarin protects against TF-induced intravascular coagulation as monitored by consumption of fibrinogen (*A*), factor V (*B*), and factor VIII (*C*). (●) Rabbits given antiprothrombin IgG and infused with TF ($n = 6$); (□) warfarin-treated rabbits infused with TF ($n = 7$); (■) control nontreated rabbits infused with TF ($n = 3$); (○) control rabbits given antiprothrombin IgG and infused with saline ($n = 4$). Values are mean percent (±SEM) of mean initial values before the IgG injection (↓), or before the infusion. The infusion period is indicated by the bar. At the end of the TF infusion the *P* values for differences between means for anti-II/TF and warfarin/TF animals were: for fibrinogen, $P = 0.2$; for factor V, $P = 0.012$; for factor VIII, $P = 0.008$. The corresponding *P* values for differences between means of anti-II/TF animals and control/TF animals were: for fibrinogen, $P = 0.002$; for factor V, $P < 0.0005$; for factor VIII, $P = 0.003$.

Prothrombin restoration in warfarin treated rabbits

Zivelin et al JCI 1993

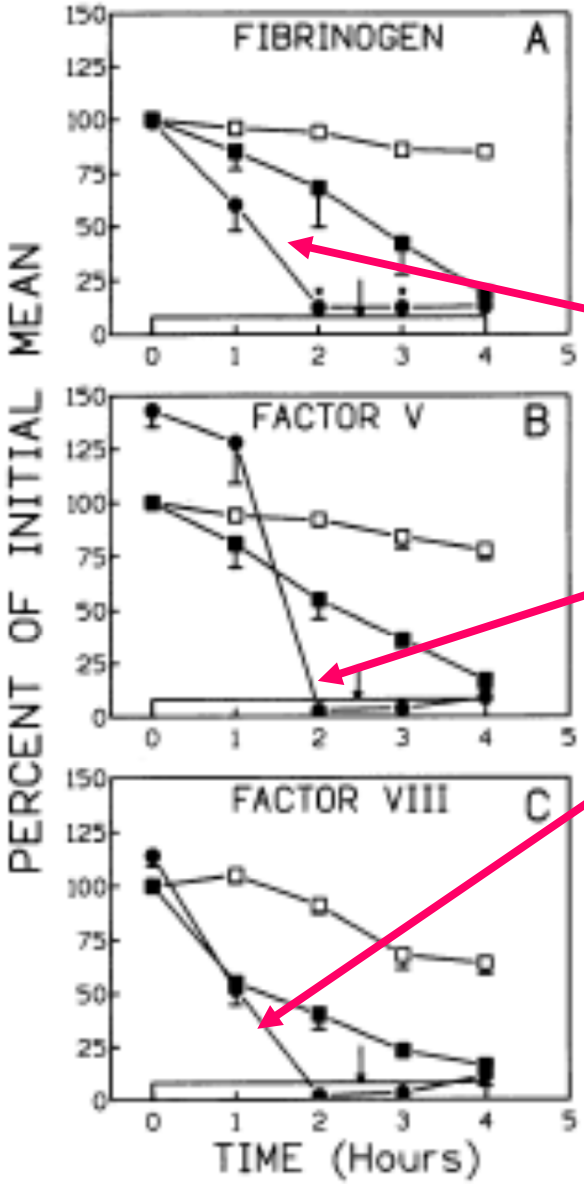


Figure 9. Evidence that selective restoration of prothrombin [redacted] sensitizes the rabbits to extensive TF-induced intravascular coagulation as monitored by consumption of fibrinogen (*A*), factor V (*B*), and factor VIII (*C*). Values are mean (±SEM) percent of the mean values before the injection of human prothrombin or before the beginning of the infusion in the other groups. All rabbits were infused with TF. (●) Warfarin-treated rabbits with restored prothrombin ($n = 4$); (■) control nontreated rabbits ($n = 3$); (□) warfarin-treated rabbits not given prothrombin ($n = 7$). The infusion period for the control groups is indicated by the bar. The arrow denotes the end of the mean infusion period for the experimental group. The asterisk denotes an unmeasurable fibrinogen level of < 25 mg/dl.

- Restoration of prothrombin led to consumption of fibrinogen, factor V and factor VIII

Oral Anticoagulation Thresholds

KE Brummel, SG Paradis, RF Branda, KG Mann
(Circulation 2001;104:2311-17)

- "Patients with similar INRs show significant individual variability* in their tissue factor coagulation response**, suggesting different risks…"

*Intra-individual, inter-individual
**including clotting time, formation of TAT complexes

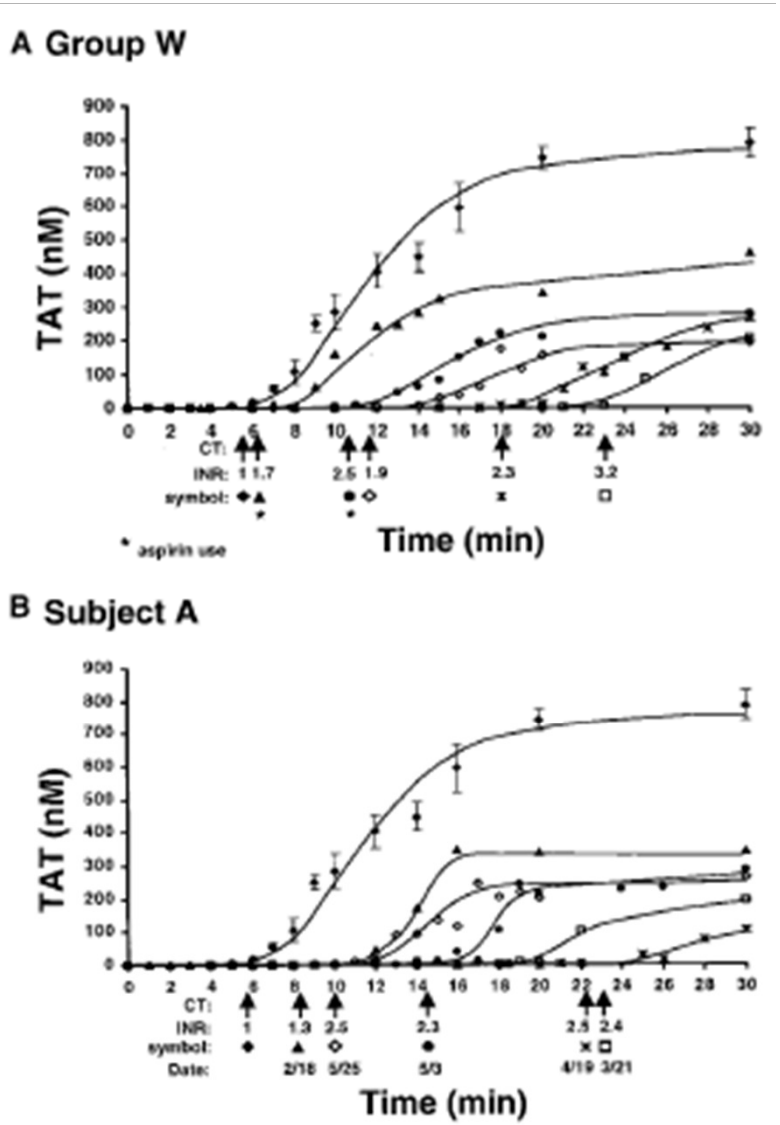


Figure 1. Thrombin generation determined from TAT complex formation. A, Selected TAT (nmol/L) vs time (min) profiles for group W. Control curve (♦) was generated from 5 age-related men. Visual CT (arrow ♦CT) was 5.7±0.3 minutes. Five individuals are depicted: subjects 1 (◇), 2 (□), 3 (▲), 5 (✕), and 6 (●). B, Five draws of subject A are depicted with date (month/day) of blood draw shown: A(3) (▲), A(7) (□), A(8) (✕), A(9) (●), and A(10) (◇). Visual CTs (arrow) and INR are depicted under each graph with corresponding symbols. *Aspirin use.

So…

- Prior evidence suggests that measuring factors II or X may better indicate clottability and anticoagulation in patients on warfarin than the prothrombin time does which included factor VII

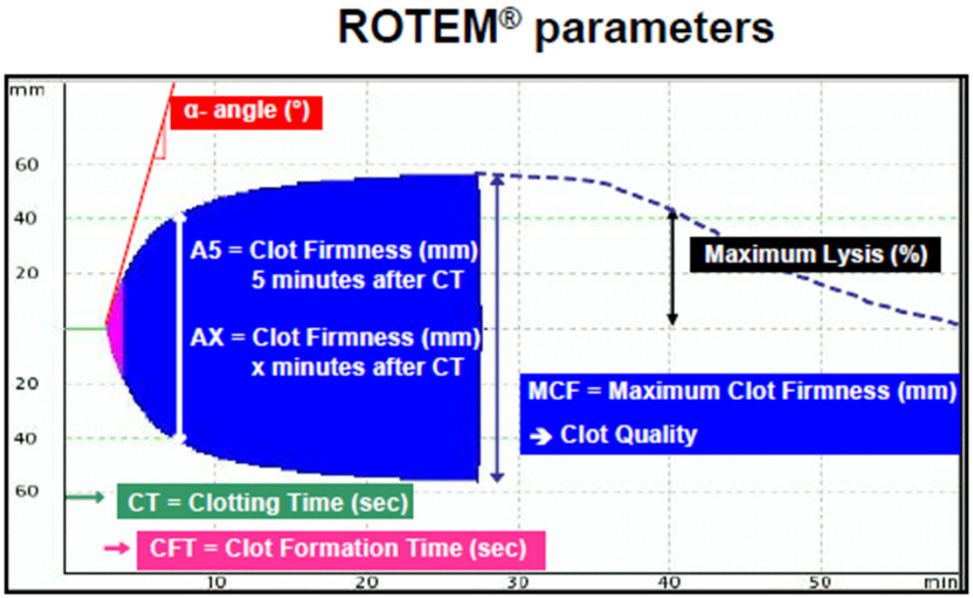
ROTEM STUDY

Rotational Thromboelastometry®

Gudmundsdottir BR, Francis CW and Onundarson PT, submitted 2011

ROTEM parameters

- Whole blood (300 µl, anticoagulated with citrate) is placed into the disposable cuvette using an electronic pipette.
- A disposable pin is attached to a shaft which is connected with a thin spring (the equivalent to Hartert's torsion wire in thrombelastography) and slowly oscillates back and forth.
- The signal of the pin suspended in the blood sample is transmitted via an optical detector system. The test is started by adding appropriate reagents.
- The instrument measures and graphically displays the changes in elasticity at all stages of the developing and resolving clot. The typical test temperature is 37°C, but different temperatures can be selected, e.g. for patients with hypothermia[8].
- In contrast to thrombelastography with its pendulum-like principle, the design of the TEM viscoelastic detection system (figure 1) makes it quite robust and insensitive against mechanical shocks or vibrations.



Rotational thromboelastometry (ROTEM) measures coagulation in more detail than the traditional clotting times

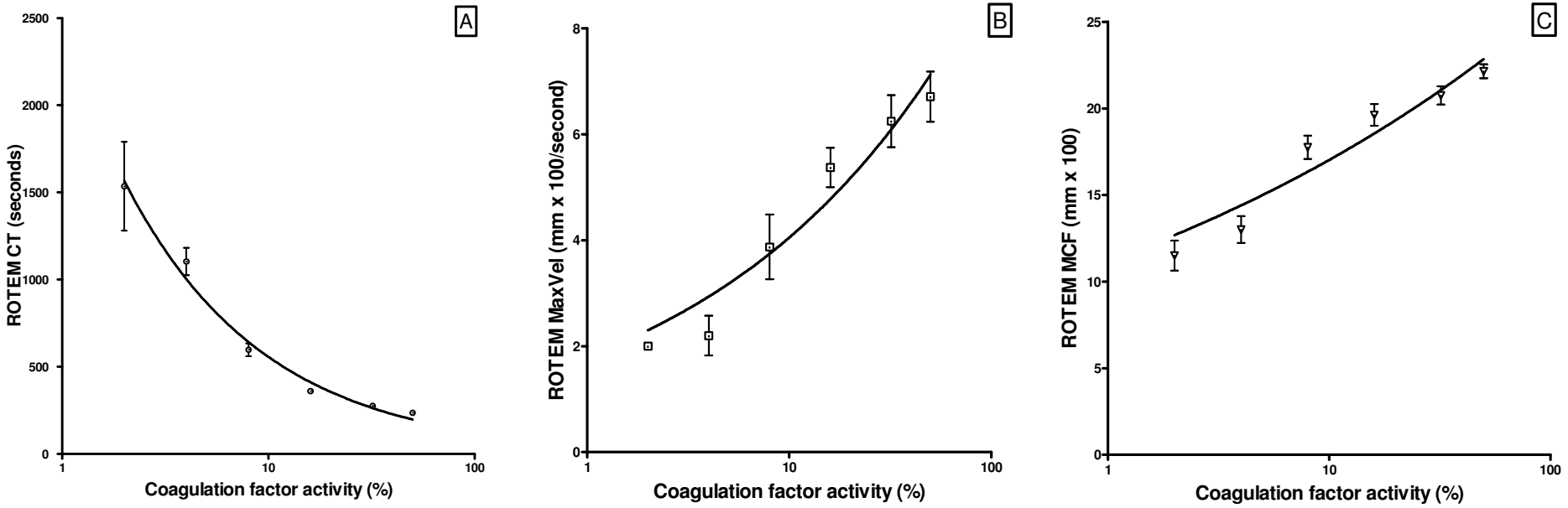
CT (initiation phase)
MaxVel (propagation phase)
MCF (stabilization phase)

Aim and Method

- We evaluated the effect of selective *in vitro* depression of individual vitamin K dependent (VKD) coagulation factors (and their restoration) on ROTEM
  - Coagulation activated in plasma by adding trace amount thromboplastin (1:17,000 dilution f.c.)

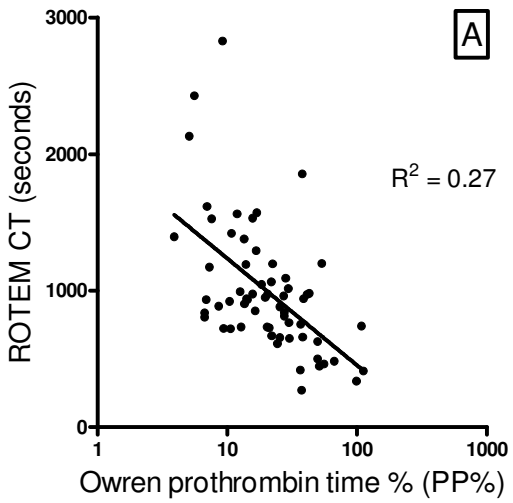
ROTEM and VKD coagulation factors

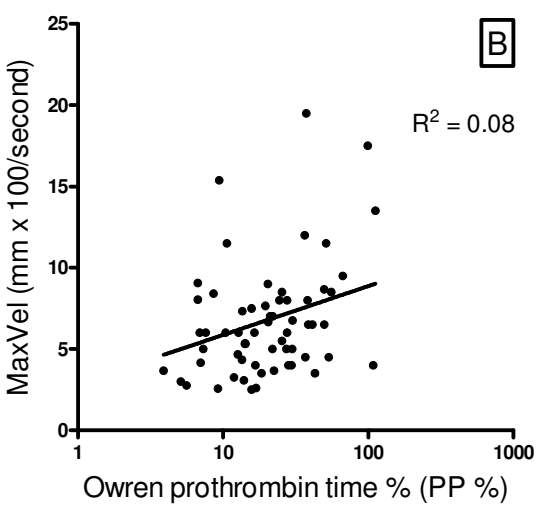
ROTEM parameters (mean, 1 SEM) in relation to equally lowered concentration of all vitamin K dependendent coagulation factors in platelet poor plasma.
Mixtures of adsorbed plasma and normal plasma

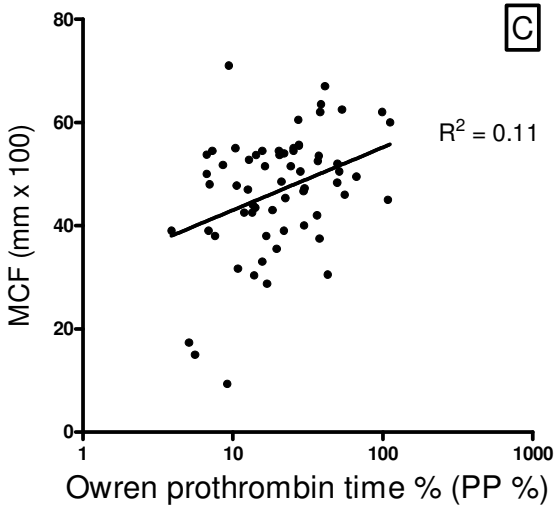


Gudmundsdottir BR, Francis CW and Onundarson PT, submitted 2011

ROTEM parameters in relation to PP percent coagulant activity in 65 samples from patients on stable anticoagulation with warfarin.

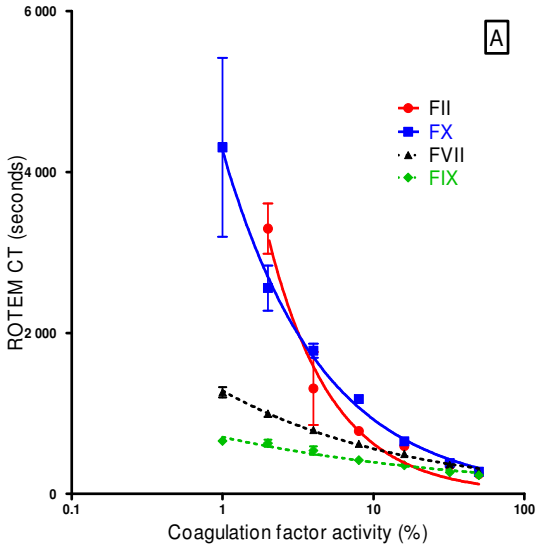


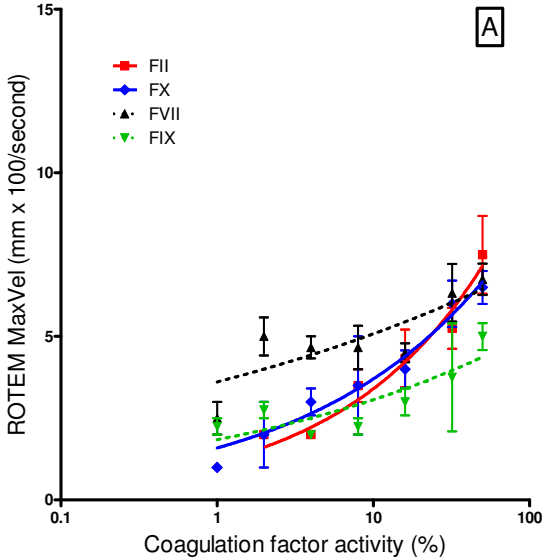


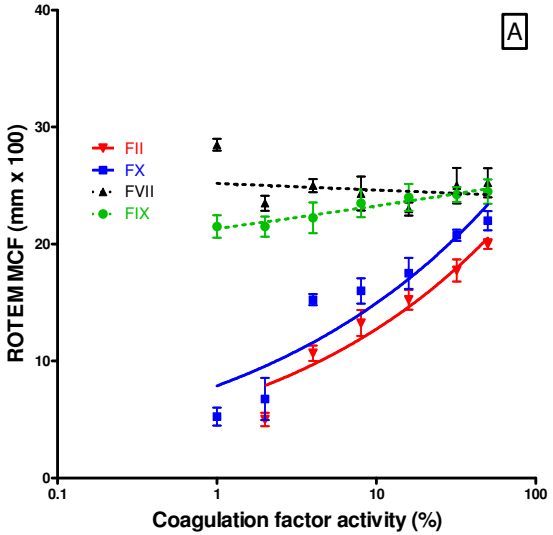


Gudmundsdottir BR, Francis CW and Onundarson PT, submitted 2011

ROTEM clotting time (CT), Maximum velocity (MaxVel) and maximum clot firmness (MCF) in relation to progressive reduction of individual vitamin K dependent coagulation factors in PPP







Gudmundsdottir BR, Francis CW and Onundarson PT, submitted 2011

Conclusions of ROTEM experiments

- ROTEM parameters correlated poorly with the Owren prothrombin time in anticoagulated patients´ plasma
- ROTEM clotting time (CT, initiation phase), ROTEM maximum velocity (Max Vel, propagation phase) ROTEM MCF (stabilization phase) were affected markedly more by mildly and moderately low concentrations of factors II or X than by identical FVII or factor IX concentrations
- FVII only mattered once << 5 U/dL

Hypothesis

- **Hypothesis: "The combined measurement of factors ii and x alone on fibrin formation reflects clotting better in anticoagulated patients on coumarins than the current PT based methods which include factors ii, vii and x"**

- The PT can be „Fiix"-ed

Fiix- prothrombin time

Stuart-prothrombin time

"Fiix – PT" (Stuart-prothrombin time)*

- Measurement of fibrin formation rate (clotting time) in coumarinized plasma that is made only deficient in factors II and X,
  - ie the clotting rate is in relation to deficiency in ii and x only
- **Fiix-method/ idea:** Addition of factor II and X deficient plasma to patient sample in order to correct for all other factor deficiencies
- Coagulation then activated by thromboplastin (eg. PT reagent)
  - Alternative test possibilities

*Patent application has been filed for the Fiix-method

Owren´s PT (P&P) versus Fiix-PT

P&P test

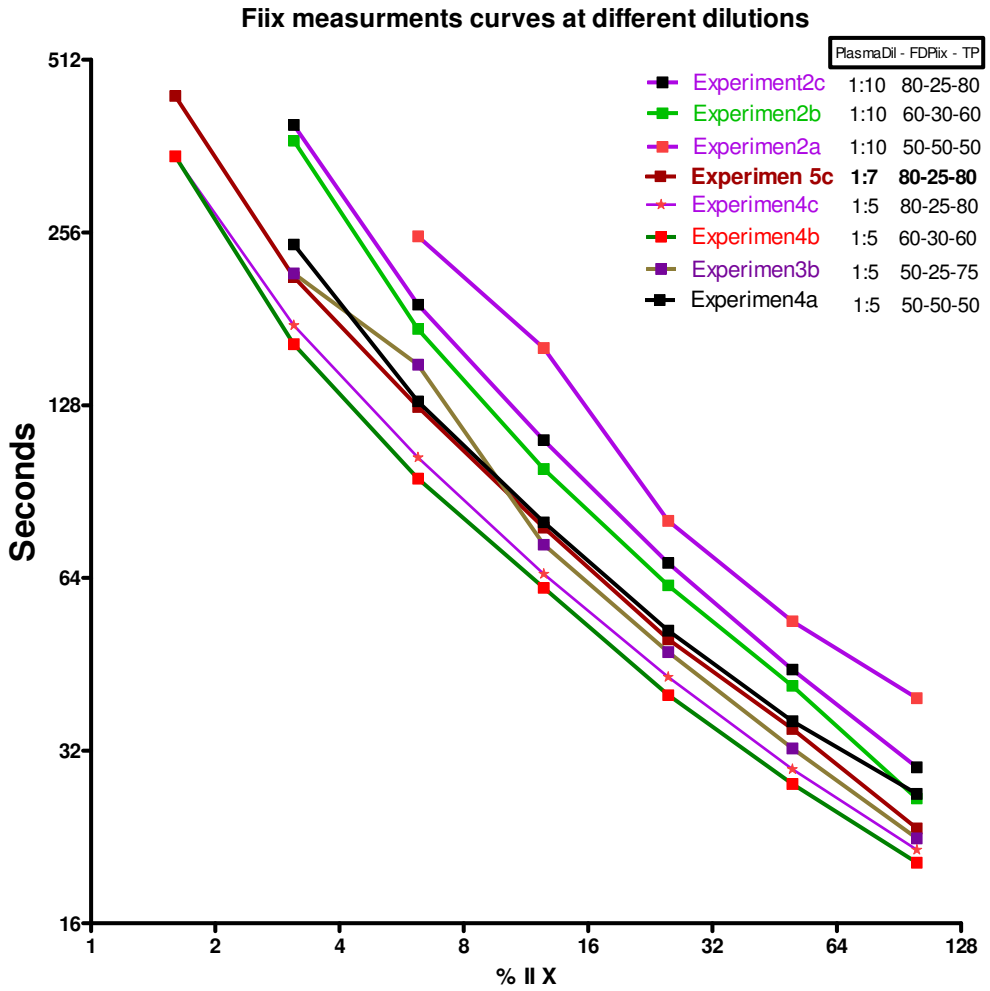
- Patient plasma dilution* 1/7
  - Volume 50 µl of dilution
- STA-SPA+ reagent
  - (Thromboplastin (rabbit brain) + adsorbed bovine plasma free of FII, VII, IX and X + $CaCl_2$)
  - Volume 100 µl

* Plasma dilution in Owren buffer

Fiix test

- Patient plasma 1/7 dilution*
  - Volume 80 µl of dilution
- Human plasma free of FII and FX
  - immunodepleted,
  - Volume 25 µl
- Thromboplastin
  - Neoplastin CI plus®
    - ie standard PT reagent from Stago (rabbit brain) reagent + $CaCl_2$
  - Volume 70 µl

Fiix-PT measurements using different dilutions and plasma ratios



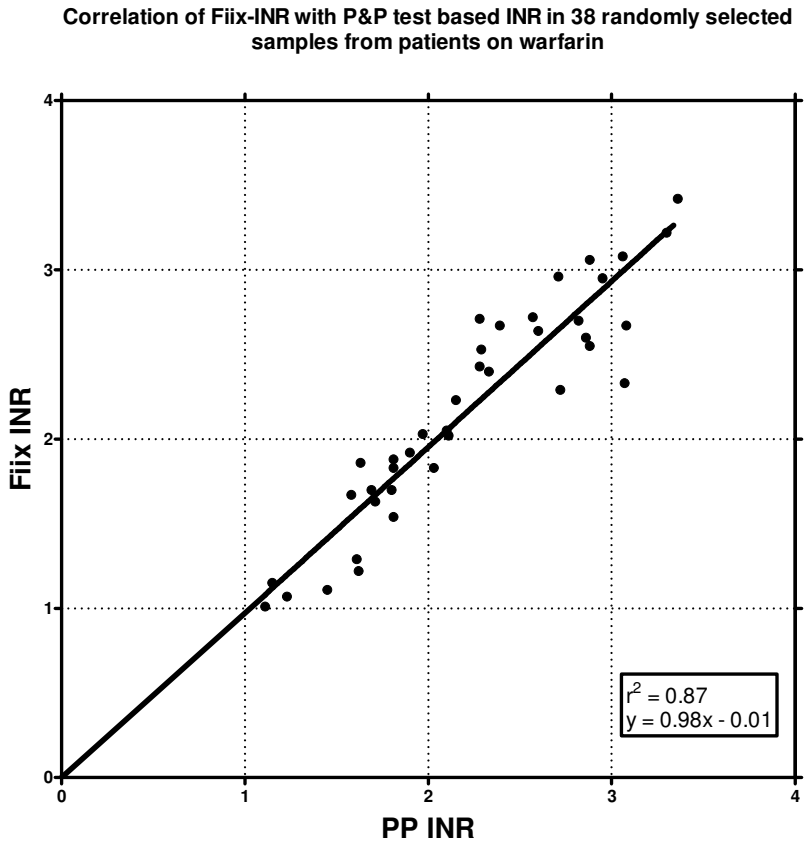
Gudmundsdottir BR, Francis CW and Onundarson PT, submitted 2011

Calculation

- In both tests, ISI is calculated (measured) from international standard (Decks 99) and INR calculated:

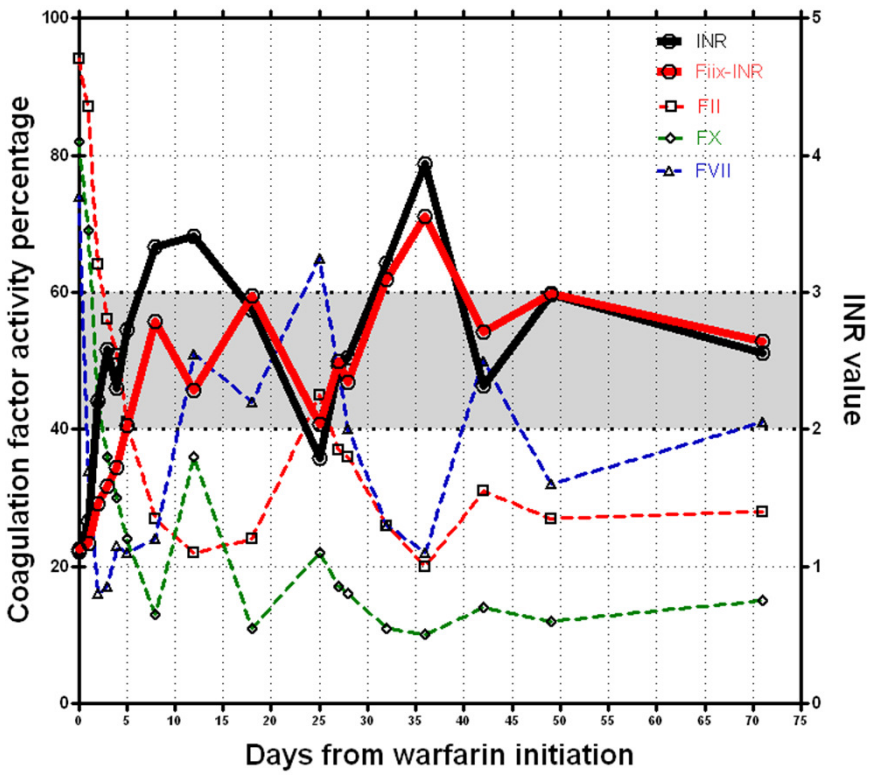
INR = (Patient´s clotting time/mean normal clotting time)^{ISI}

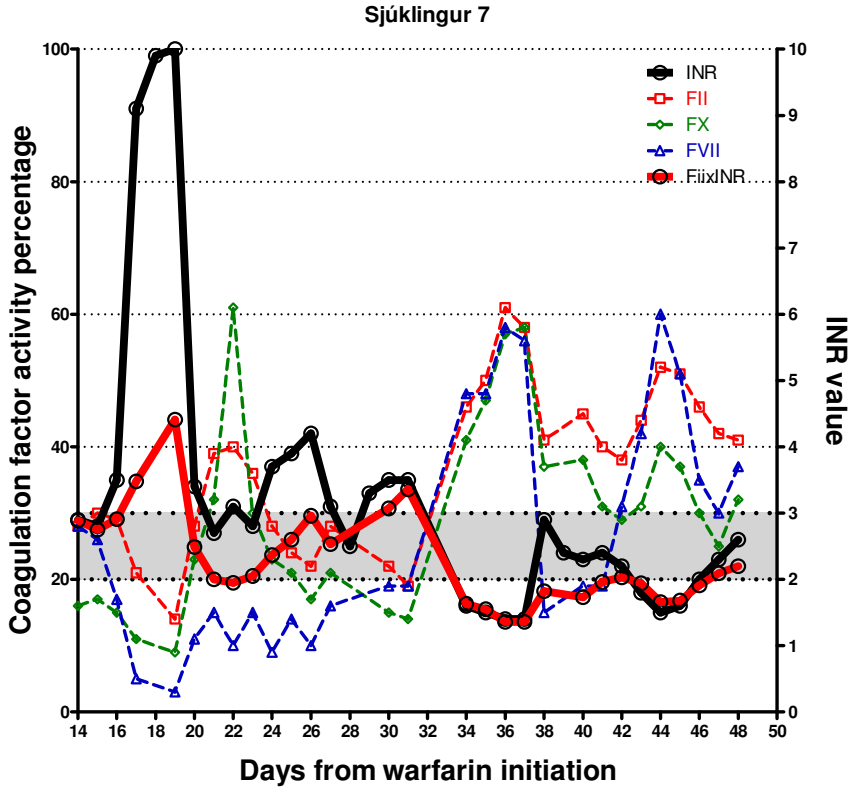
Fiix-"INR" (*Fiix-NR*) vs INR



Gudmundsdottir BR, Francis CW and Onundarson PT, submitted 2011

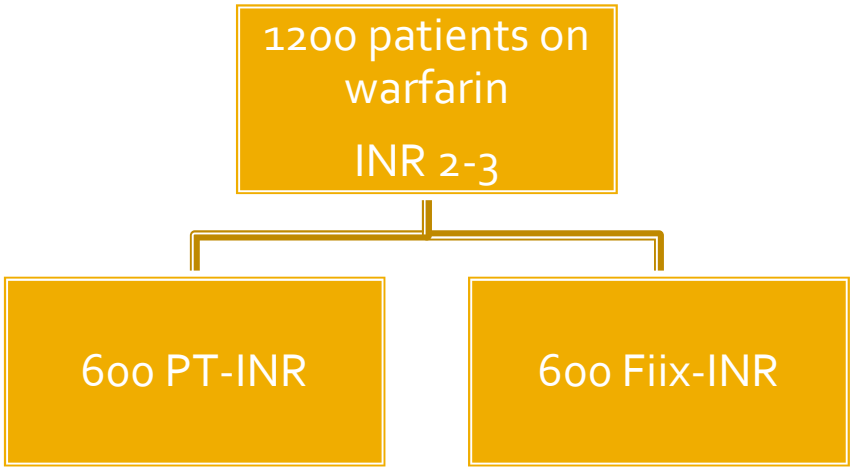
Sequential VKD- factor measurements in relation to the INR in two individuals





Gudmundsdottir BR, Francis CW and Onundarson PT, submitted 2011

Planned prospective randomized and blinded trial



- Single center (Landspitali Reykjavik)
- Dosing by DAWN and AMC staff based on blinded „INR"
- Followed for 1200 patients years
- Primary endpoints
  - TE
  - Bleeding events
- Surrogate endpoints:
  - TTR
  - number of tests

The End