Simposio SISET/SIPMEL/SIBIOC

"Le sfide diagnostiche del laboratorio di coagulazione"

Come e perché misurare i farmaci anticoagulanti

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Anticoagulanti orali diretti: il laboratorio

- Non necessario il monitoraggio nella pratica clinica quotidiana
- Utile un controllo di laboratorio in alcune particolari situazioni:
  - Presenza di eventi avversi (emorragia o trombosi)
  - Chirurgia o manovre invasive (in emergenza e non)
  - Trombolisi
  - Sovradosaggio
  - Pazienti sotto- o sovra-peso, pazienti molto anziani
  - Insufficienza renale e/o alter. funzionalità epatica
  - Sospetta o nota interazione con altri farmaci
  - Uso di antidoti

Anticoagulanti orali diretti: il laboratorio

Test globali:

- PT (Rivaroxaban, Apixaban, Edoxaban)
- aPTT (Dabigatran)

Test specifici:

- Dosaggio cromogenico attività anti Xa (Rivaroxaban, Apixaban, Edoxaban)
- Tempo trombina diluito, Ecarin clotting time, dosaggio cromogenico attività anti IIa (Dabigatran)

Caso 1 - 1

- Donna di 65 anni in seguito a visita cardiologica ambulatoriale riceve diagnosi di fibrillazione atriale (FA) con indicazione a terapia anticoagulante
- Normale funzionalità renale, inizia somministrazione di Dabigatran 150 mg x 2

- Dopo 1 mese di terapia il cardiologo prescrive PT e aPTT; quest'ultimo test risulta 3.6 ratio
- Il Dabigatran viene sospeso. Dopo 4 giorni l'aPTT viene ripetuto e risulta 2.4 ratio (funzionalità renale normale)
- Riscontro di un aPTT normale 2 anni prima, non eseguiti PT e aPTT prima di iniziare il Dabigatran

Caso 1 - 2

- Richieste indagini appropriate al Laboratorio Specialistico di Coagulazione, eseguite 10 giorni dopo
- In sede di colloquio prima del prelievo risulta che in un controllo di routine eseguito 6 mesi prima l'aPTT era 2.60 ratio
- LAC fortemente positivo e marcato aumento del livello degli ACA e anti GPI

- La paziente con fibrillazione atriale e fenomeno LAC positivo non ha eseguito terapia anticoagulante per almeno un mese

Caso 2 - 1

- Uomo di 47 anni (peso Kg 78), ricoverato in terapia intensiva cardiologica per embolia polmonare non emodinamica e TVP prossimale (ilio-femorale) arto inferiore SN
- Riceve il primo giorno enoxaparina sodica 8000 UI sc 2 volte al dì
- Dal giorno successivo inizia trattamento con Rivaroxaban 15 mg 2 volte al dì
- Dopo 2 giorni in esami di routine il PT risulta 1.25 ratio

Caso 2 - 2

- Dosaggio del Rivaroxaban eseguito dopo 2 giorni (data la non urgenza)
- Il risultato del dosaggio dell'effetto anticoagulante del Rivaroxaban nel campione ricevuto dal Laboratorio era di 210 ng/ml (test cromogenico anti-Xa)

- Dal giorno dell'esecuzione del prelievo da inviare al Laboratorio, era stata aggiunta dal cardiologo terapia con enoxaparina sodica 4000 UI sc 2 volte al dì

POOR RELIABILITY OF COAGULATION SCREENING TEST IN PATIENTS TREATED WITH DIRECT ORAL ANTICOAGULANTS: RESULTS FROM A MULTICENTER MULTIPLATFORM OBSERVATIONAL STUDY

Sophie Testa[*], Cristina Legnani[†], Armando Tripodi[‡], Oriana Paoletti[*], Vittorio Pengo[§], Rosanna Abbate[¶], Claudia Dellanoce[*], Laura Bassi[*], Paolo Carraro[#], Michela Cini[†], Rita Paniccia[¶], Daniela Poli[¶], Gualtiero Palareti[**] for the START-Laboratory Register

Journal of Thrombosis and Haemostasis, **14**: 1–8

This is a prospective observational multicenter study in patients with NVAF treated with dabigatran, rivaroxaban or apixaban and was approved by the ethical committee of the general hospital of Cremona. Four large Italian Anticoagulation Clinics [Bologna (A), Cremona (B), Padua (C) and Florence (D)], affiliated with the Italian Federation of Anticoagulation Clinics (FCSA) and engaged in the Start Register (Survey on anTicoagulated pAtients RegisTer) (www.start-register.org), were asked to join the collaborative study by collecting plasma from patients treated with DOAC.

Aims. To evaluate correlation, responsiveness and variability of PT and aPTT vs DOAC anticoagulant levels measured with specific coagulation tests performed with different platforms in 4 anticoagulation clinics.

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Journal of Thrombosis and Haemostasis, **14**: 1–8

Table 1 Patients and number of samples, instruments, reagents, prothrombin time (PT) and activated partial thromboplastin time (APTT) upper limits of normal range used in the four anticoagulation clinics

	Clinic A	Clinic B	Clinic C	Clinic D
Dabigatran (patient no./sample no.)	47/94	70/158	89/178	25/50
Rivaroxaban (patient no./sample no.)	72/144	108/216	61/122	23/46
Apixaban (patient no./sample no.)	30/60	91/182	–	10/20
Coagulometer	STA compact (Stago)	STA-R (Stago)	CA 7000 (Sysmex)	ACL TOP 700 (Werfen)
Reagents				
PT	Recombiplastin (Werfen)	Neoplastin (Stago)	Innovin (Siemens)	Recombiplastin (Werfen)
APTT	Actin (Siemens)	PTT (Stago)	Actin-FS (Siemens)	SynthASil (Werfen)
Dabigatran	Thrombin Siemens	Thrombin Stago	Hyphen Hemoclot	Hyphen Hemoclot
Rivaroxaban	Liquid Anti-Xa Stago	Liquid Anti-Xa Stago	Hyphen DiXal	Hyphen DiXal
Apixaban	Liquid Anti-Xa Stago	Liquid Anti-Xa Stago	–	Technochrome anti-Xa Kit
Calibrators				
Dabigatran	Hyphen Biomed	Hyphen Biomed	Hyphen Biomed	Hyphen Biomed
Rivaroxaban	Calibrator Stago	Calibrator Stago	Biophen Rivaroxaban	Hyphen Biomed
Apixaban	Calibrator Stago	Calibrator Stago	–	Technoview Apixaban
PT upper limit of normal range	< 1.20	< 1.20	< 1.13	< 1.20
APTT upper limit of normal range	< 1.25	< 1.22	< 1.30	< 1.27

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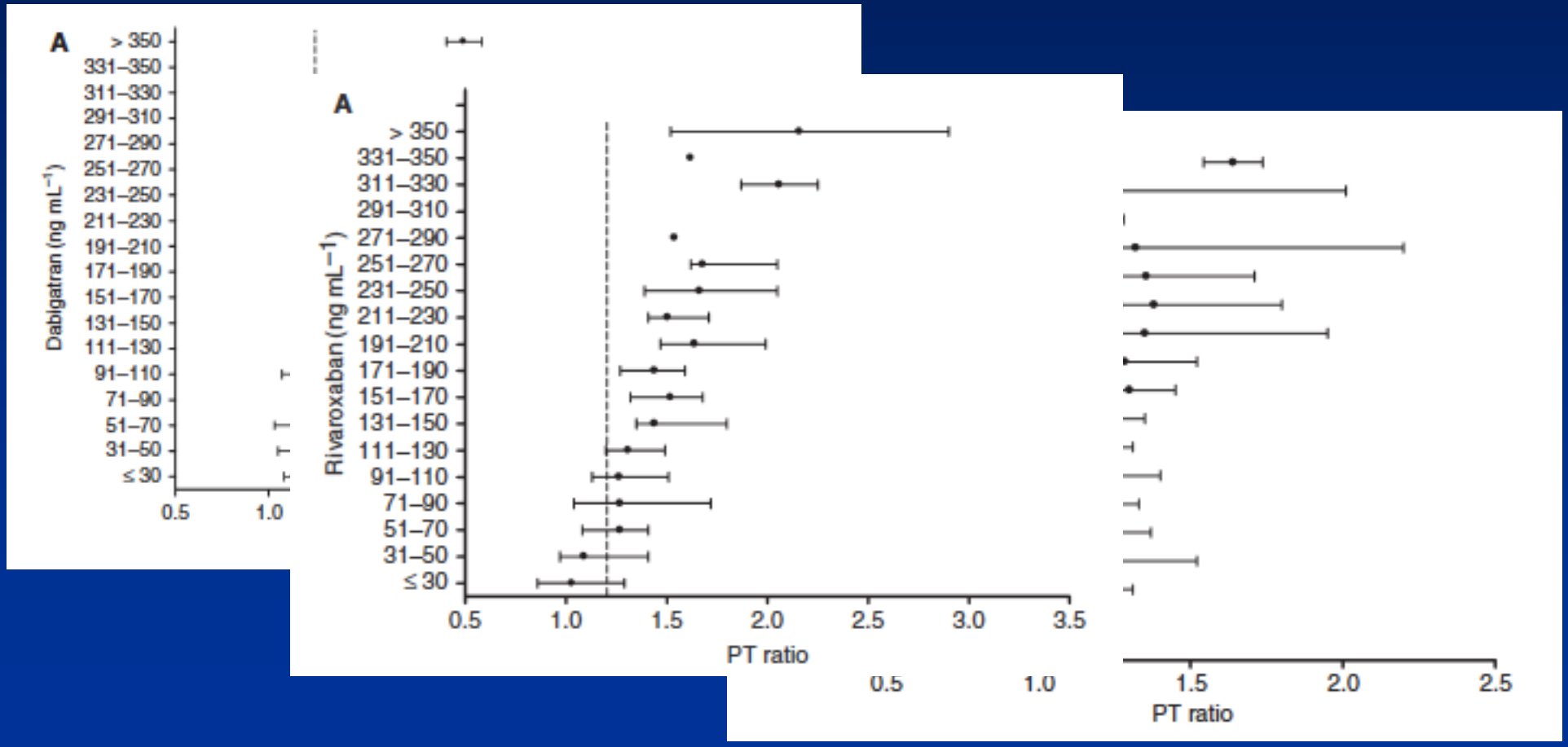
Journal of Thrombosis and Haemostasis, **14**: 1–8



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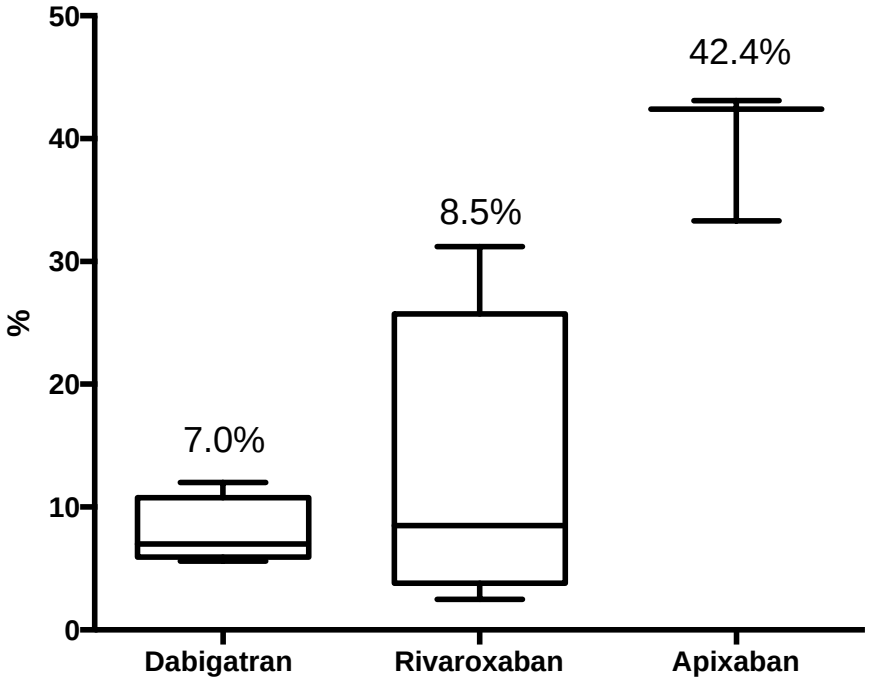
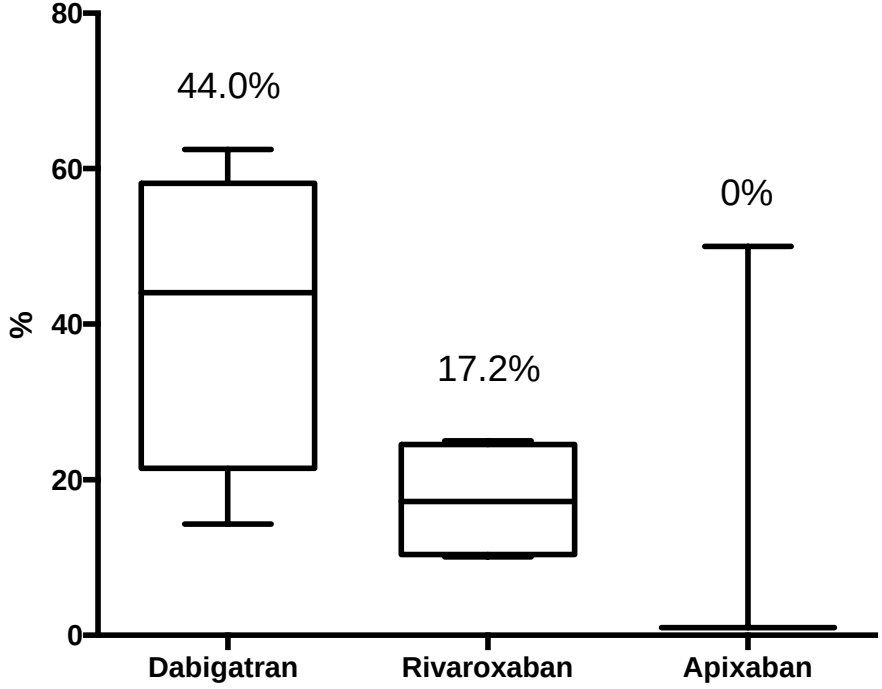
Journal of Thrombosis and Haemostasis, **14**: 1–8

% di aPTT/PT normali per concentrazione dei farmaci > 50 ng/ml



% di aPTT/PT alterati per concentrazione dei farmaci £ 50 ng/ml



bjh guideline

Measurement of non-coumarin anticoagulants and their effects on tests of Haemostasis: Guidance from the British Committee for Standards in Haematology

Steve Kitchen,[1] Elaine Gray,[2] Ian Mackie,[3] Trevor Baglin[4] and Mike Makris[1,5] on behalf of the BCSH committee

British Journal of Haematology, 2014, **166**, 830–841

Direct Thrombin Inhibitors (DTIs)

Recommendations

- **Dilute thrombin-based assays, ecarin-based assays or chromogenic anti-IIa assays (in the absence of heparin) are suitable for determination of plasma concentrations of dabigatran.**
- **Assays to determine anticoagulant concentration should be calibrated with drug-specific calibrators.**
- **Prothrombin time (PT) and activated partial thromboplastin time (APTT) should not be used to measure the plasma concentration of dabigatran.**

- **The APTT can be used with most reagents for a crude estimate of the relative intensity of anticoagulation due to dabigatran but some patients with therapeutic concentrations will have a normal APTT.**

Direct factor Xa inhibitors

Recommendation

- **Anti-Xa chromogenic assays should be used to determine plasma concentration of direct FXa inhibitors.**
- **Product-specific calibrator should be used and results should be expressed in mass concentration.**
- **PT and APTT should not be used to measure the plasma concentration of Xa inhibitors.**

- **The PT or APTT can be used with most reagents for a crude estimation of the relative intensity of anticoagulation due to rivaroxaban but some patients with therapeutic concentrations will have a normal PT or APTT.**

Misura dell'attività degli anticoagulanti orali diretti: PT - aPTT

Vantaggi
• Semplici • Rapidi • Poco costosi • Bassi CV% • Disponibili in tutti i labs

Svantaggi
• Poco sensibili (a volte normali in presenza di concentrazioni rilevanti del farmaco) • Poco specifici (spesso alterati per altre cause) • Risultati reagente dipendenti

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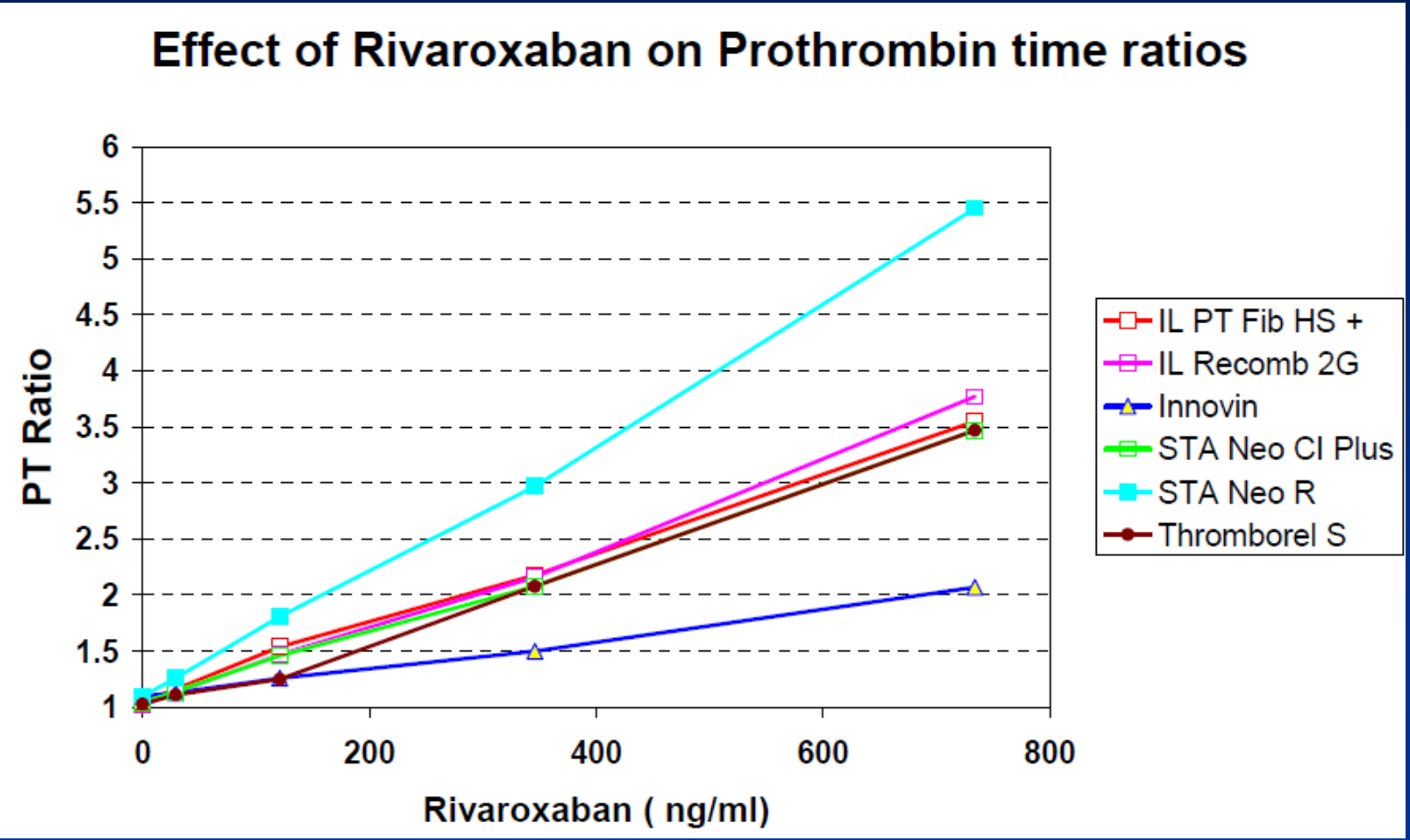
UK NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEMES

RIVAROXABAN SUPPLEMENTARY EXERCISE September 2014

Plot of median PT ratio against Rivaroxaban concentration for reagents used by >20 centres



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UK NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEMES

RIVAROXABAN SUPPLEMENTARY EXERCISE September 2014

Plot of median PT ratio against Rivaroxaban concentration for reagents used by >20 centres

Table 5. S14:04 (246 ng/ml)	n	Median Ratio	CV (%)
IL HemosIL PT-FIB HS Plus	31	2.18	12.9%
IL HemosIL Recombiplastin 2G	210	2.16	8.8%
Siemens Innovin	292	1.5	7.1%
Siemens Thromborel S	22	1.60	5.6%
STA Neoplastin CI Plus	43	2.08	12.5%
STA Neoplastin R	38	2.97	4.1%

Rivaroxaban 246 ng/ml => PT ratio 1.50 – 2.97

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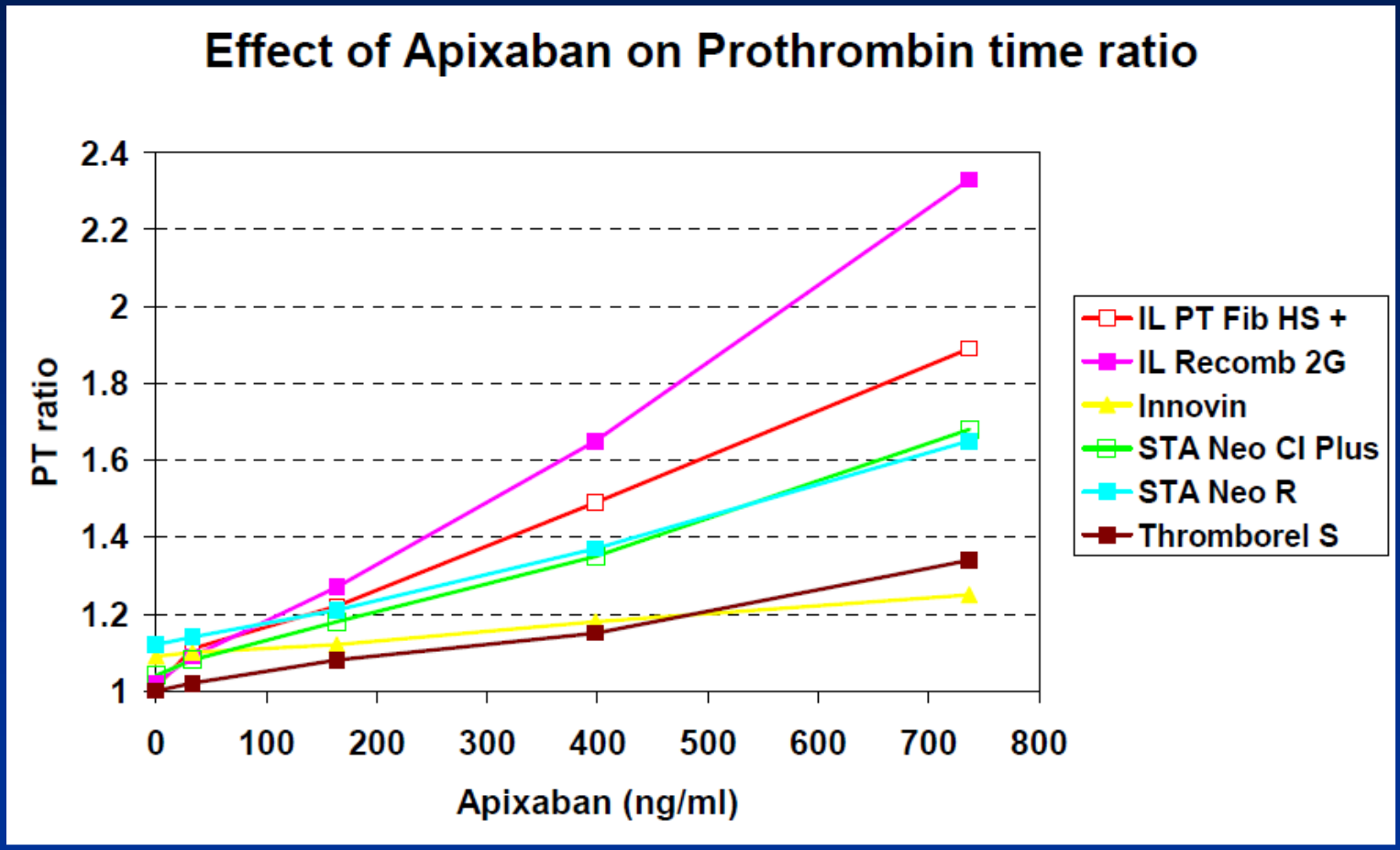
APIXABAN SUPPLEMENTARY EXERCISE September 2014

Plot of median PT ratio against Apixaban concentration for reagents used by >20 centres



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APIXABAN SUPPLEMENTARY EXERCISE September 2014

Plot of median PT ratio against Apixaban concentration for reagents used by >20 centres

Table 4. S14:08 (164 ng/ml Apixaban)	n	Median Ratio	CV (%)
IL HemosIL PT-FIB HS Plus	31	1.22	8.8%
IL HemosIL Recombiplastin 2G	210	1.27	8.6%
Siemens Innovin	292	1.12	5.6%
Siemens Thromborel S	22	1.08	7.9%
STA Neoplastin CI Plus	43	1.18	4.9%
STA Neoplastin R	38	1.21	4.6%

Apixaban 164 ng/ml => PT ratio 1.08 – 1.27

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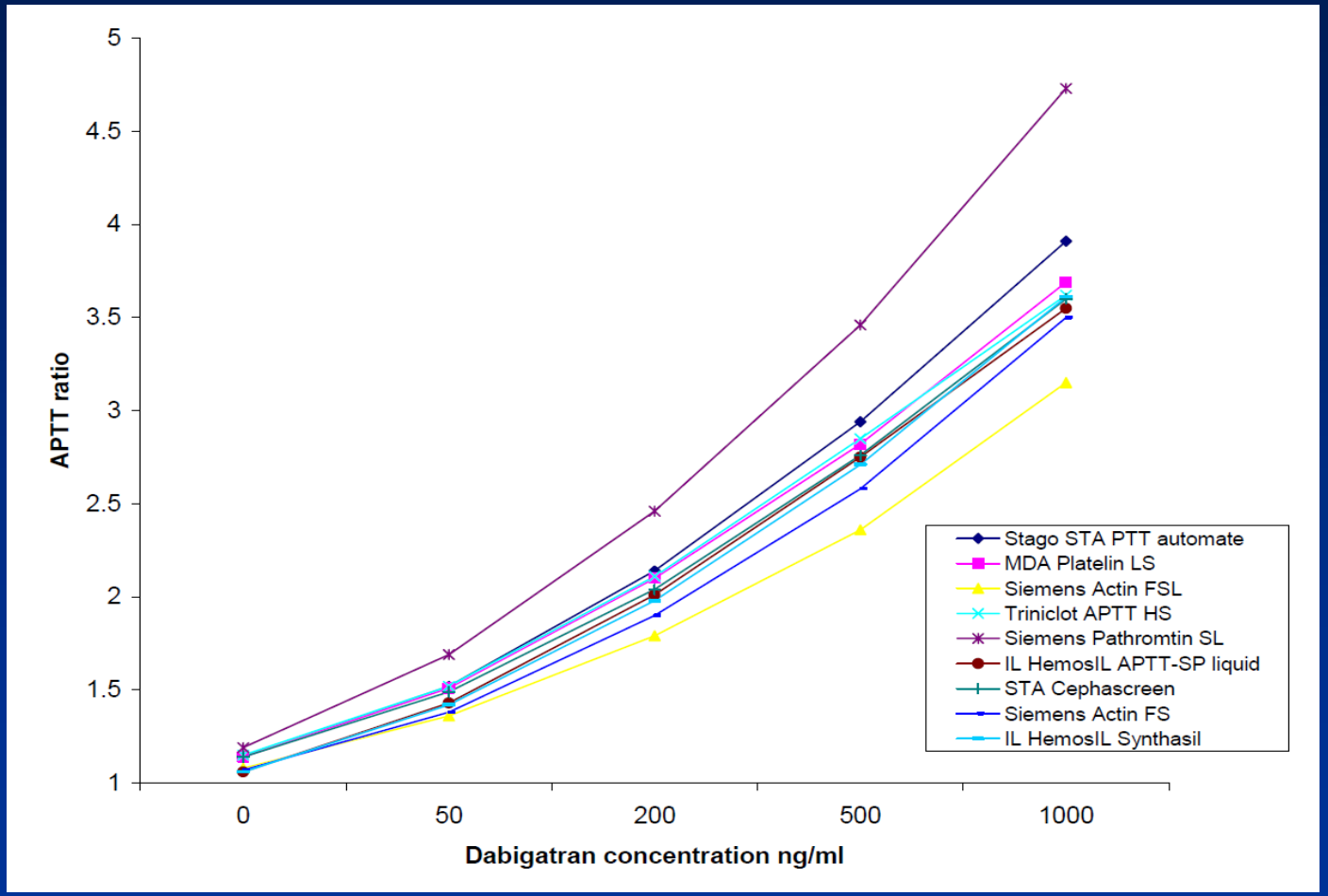
UK NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEMES

DABIGATRAN SUPPLEMENTARY EXERCISE APRIL 2012

Plot of median APTT ratio against Dabigatran concentration for reagents used by >10 centres



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UK NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEMES

DABIGATRAN SUPPLEMENTARY EXERCISE APRIL 2012

Plot of median APTT ratio against Dabigatran concentration for reagents used by >10 centres

Table 10. S12:03 (155ng/ml)	n	Median Ratio	CV (%)
IL HemosIL APTT-SP liquid	32	2.01	6.2
IL HemosIL Synthasil	188	1.98	5.8
MDA Platelin LS	14	2.10	4.0
Siemens Actin FS	176	1.90	5.2
Siemens Actin FSL	15	1.79	8.1
Siemens Pathromtin SL	23	2.46	4.0
STA Cephascreen	43	2.04	4.0
Stago STA PTT automate	12	2.14	4.2
Triniclot APTT HS	20	2.11	7.5

Dabigatran 155 ng/ml => aPTT ratio 1.79 – 2.46

Misura dell'attività degli anticoagulanti orali diretti: Test specifici

- Dosaggio cromogenico attività anti Xa (Rivaroxaban, Apixaban, Edoxaban)

- Tempo trombina diluito, Ecarin clotting time, dosaggio cromogenico attività anti IIa (Dabigatran)

Comparison of calibrated chromogenic anti-Xa assay and PT tests with LC-MS/MS for the therapeutic monitoring of patients treated with rivaroxaban

Thromb Haemost 2013; 110: 723–731

Jonathan Douxfils[1*] ; Anne Tamigniau[2*] ; Bernard Chatelain[3] ; Christian Chatelain[4] ; Pierre Wallemacq[2] ; François Mullier[1, 3#] ; Jean-Michel Dogné[1']

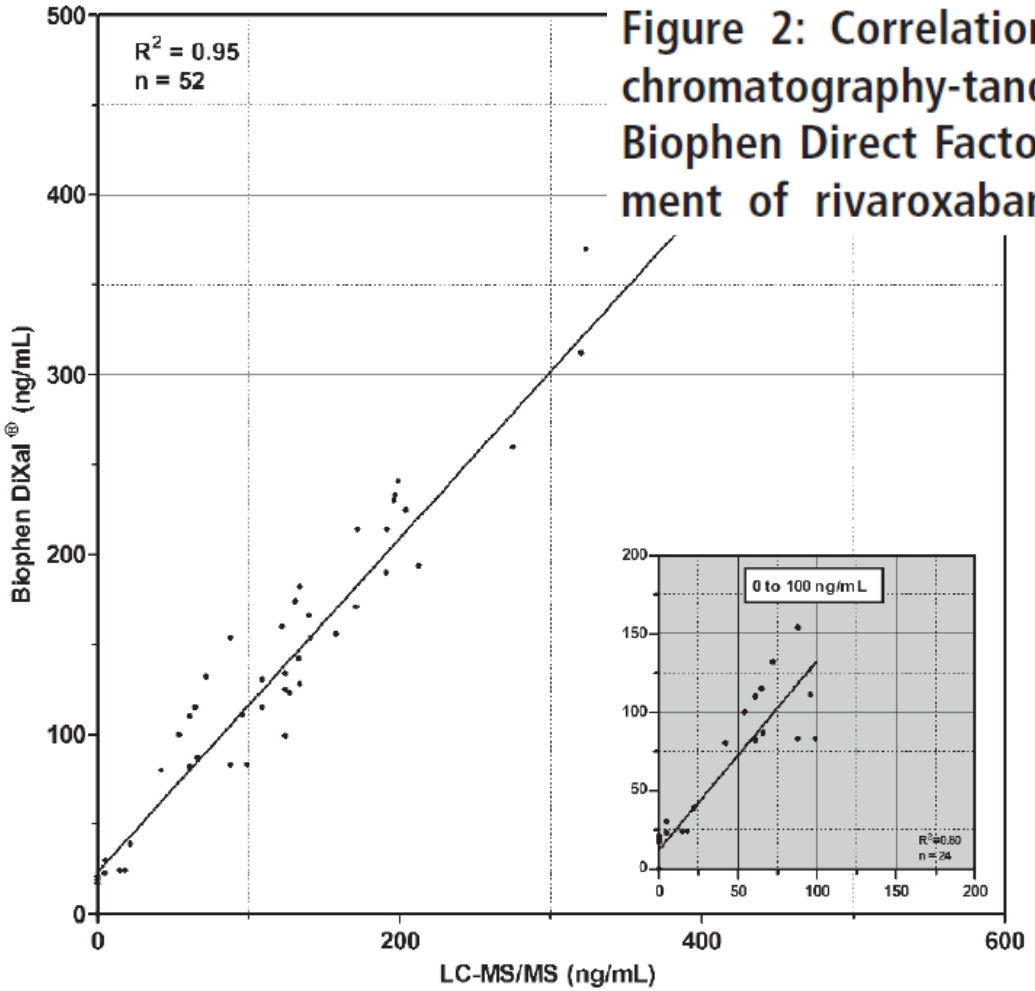


Figure 2: Correlation and Bland-Altman analysis between liquid chromatography-tandem mass spectrometry (LC-MS/MS) and the Biophen Direct Factor Xa Inhibitor® (DiXal) assay for the measurement of rivaroxaban concentrations in patient plasma samples.

Clinical evaluation of laboratory methods to monitor apixaban treatment in patients with atrial fibrillation

Mika Skeppholm [a,*], Fadiea Al-Aieshy [b], Maria Berndtsson [c], Faris Al-Khalili [d], Yuko Rönquist-Nii [e], Lisbeth Söderblom [c], Annika Y. Östlund [e], Anton Pohanka [e], Jovan Antovic [c], Rickard E. Malmström

Thrombosis Research 136 (2015) 148–153

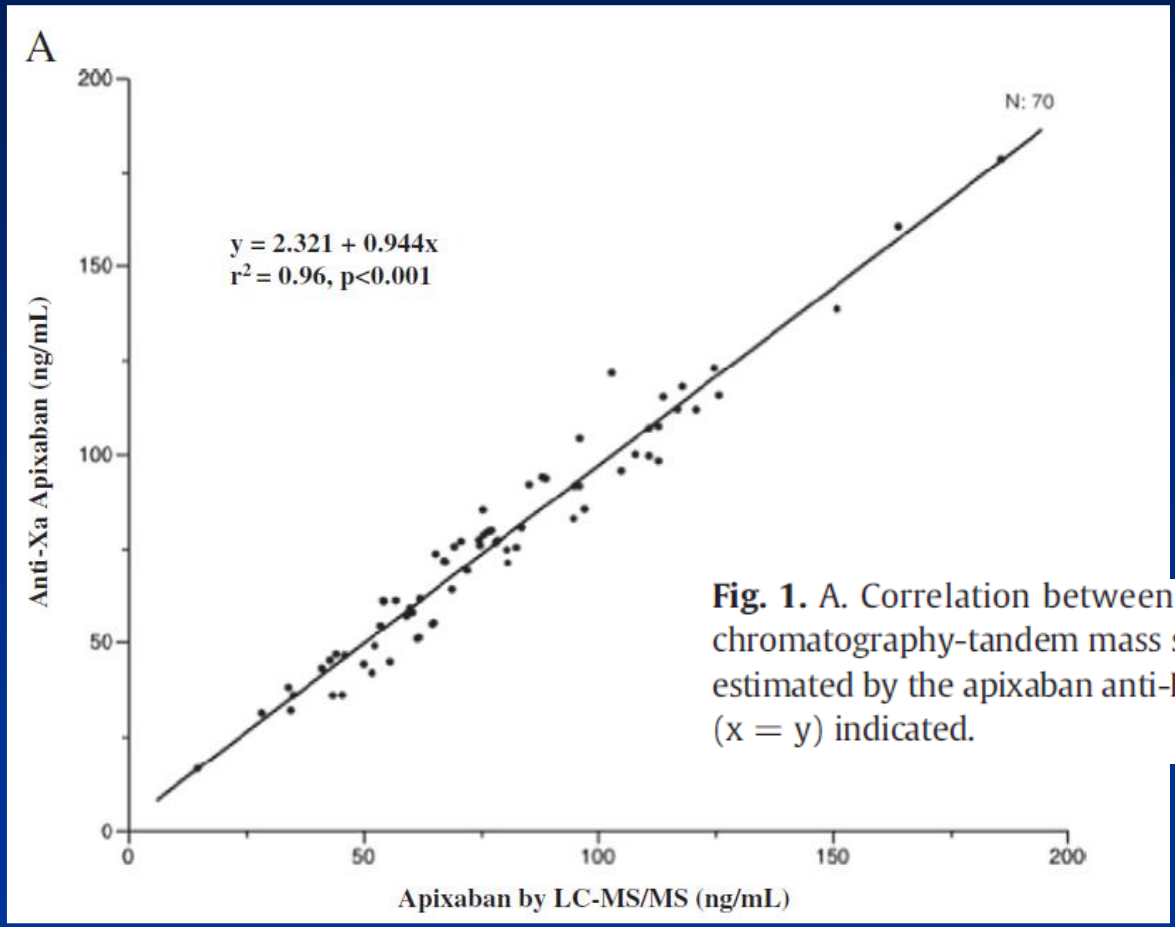
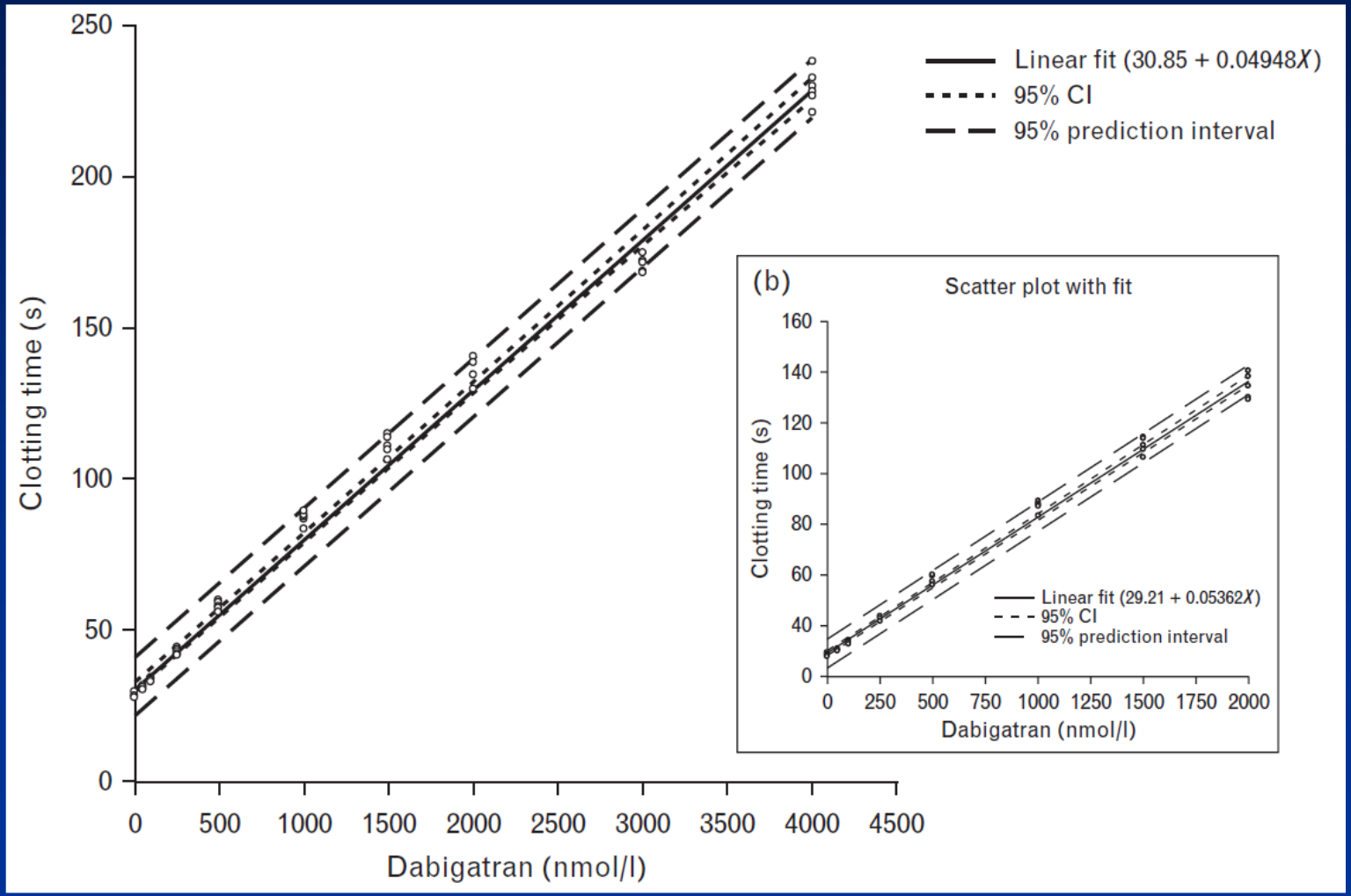


Fig. 1. A. Correlation between apixaban plasma concentrations measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and concentrations indirectly estimated by the apixaban anti-FXa activity assay for all samples with the line of identity (x = y) indicated.

Using the HEMOCLOT direct thrombin inhibitor assay to determine plasma concentrations of dabigatran

Joachim Stangier[a] and Martin Feuring[b]

Blood Coagulation and Fibrinolysis 2012, 23:138–143



Comparison of calibrated dilute thrombin time and aPTT tests with LC-MS/MS for the therapeutic monitoring of patients treated with dabigatran etexilate

Thromb Haemost 2013; 110: 543–549

Jonathan Douxfils[1]; Jean-Michel Dogné[1]; François Mullier[1, 2]; Bernard Chatelain[2]; Yuko Rönquist-Nii[3]; Rickard E. Malmström[3]; Paul Hjemdahl[3]

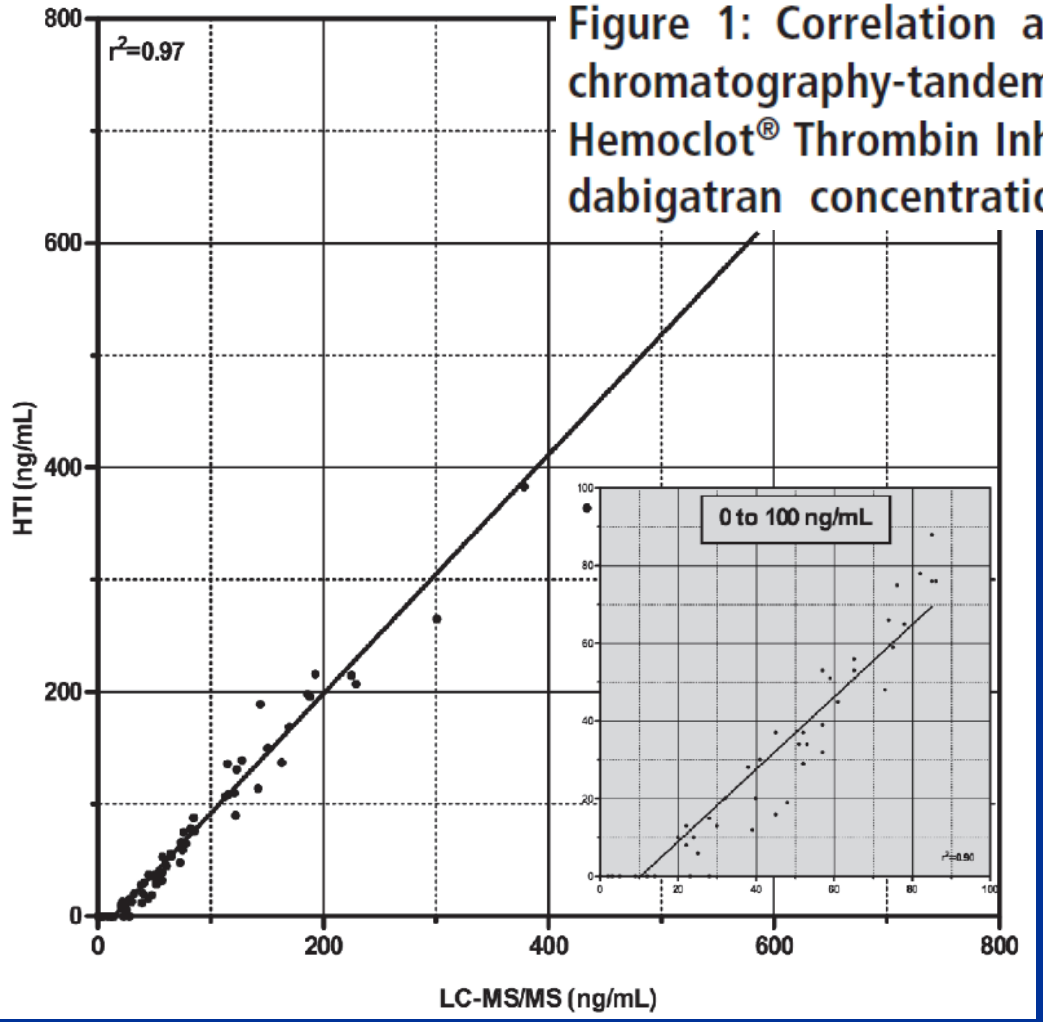
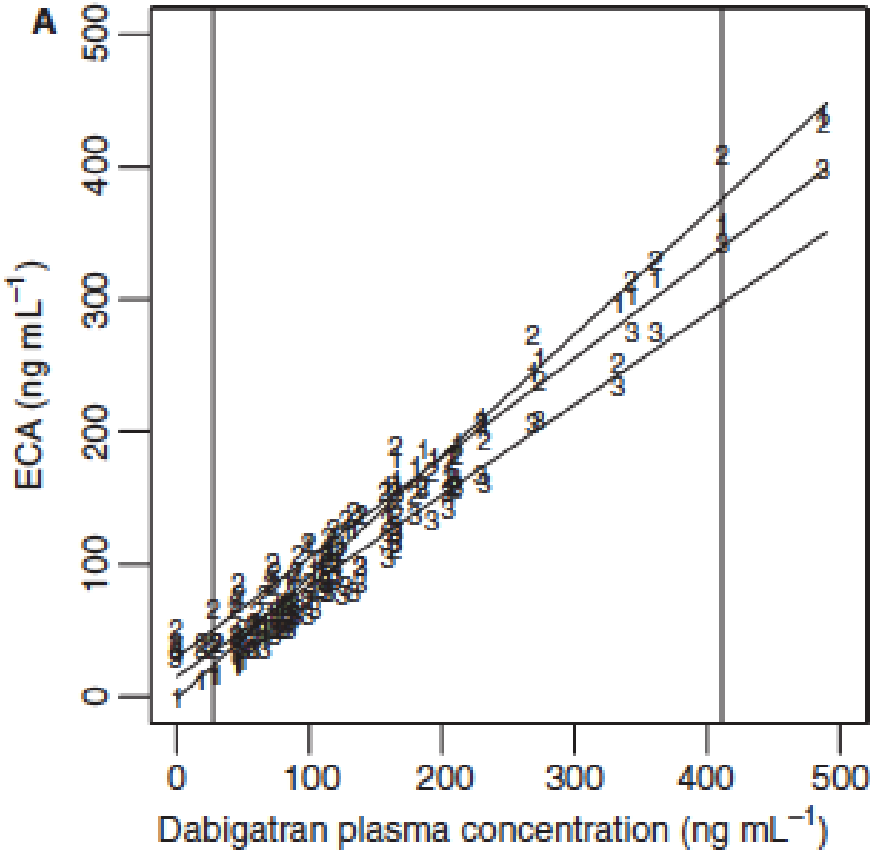


Figure 1: Correlation and Bland-Altman analysis between liquid chromatography-tandem mass spectrometry (LC-MS/MS) and the Hemoclot® Thrombin Inhibitors (HTI) assay for the measurement of dabigatran concentrations in patient plasma samples.

Performance of coagulation tests in patients on therapeutic doses of dabigatran: a cross-sectional pharmacodynamic study based on peak and trough plasma levels

E. M. HAWES,* A. M. DEAL,† D. FUNK-ADCOCK,‡ R. GOSSELIN,§ C. JEANNERET,¶ A. M. COOK,** J. M. TAYLOR,‡ H. C. WHINNA,†† A. M. WINKLER‡‡ and S. MOLL§§

Journal of Thrombosis and Haemostasis, **11**: 1493–1502



The dilute thrombin time and chromogenic and clotting ecarin assays accurately identify therapeutic and supratherapeutic dabigatran levels.

Misura dell'attività degli anticoagulanti orali diretti: Test specifici

Vantaggi
• Specifici (risultati espressi in ng/ml) • Sensibili (< 10-20 ng/ml) • Risultati non reagente dipendenti • Dose/risposta lineare

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Journal of Thrombosis and Haemostasis, **14**: 1–8

Table 1 Patients and number of samples, instruments, reagents, prothrombin time (PT) and activated partial thromboplastin time (APTT) upper limits of normal range used in the four anticoagulation clinics

	Clinic A	Clinic B	Clinic C	Clinic D
Dabigatran (patient no./sample no.)	47/94	70/158	89/178	25/50
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Reagents				
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APTT	Actin (Siemens)	PTT (Stago)	Actin-FS (Siemens)	SynthASil (Werfen)
Dabigatran	Thrombin Siemens	Thrombin Stago	Hyphen Hemoclot	Hyphen Hemoclot
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Apixaban	Liquid Anti-Xa Stago	Liquid Anti-Xa Stago	–	Technochrome anti-Xa Kit
Calibrators				
Dabigatran	Hyphen Biomed	Hyphen Biomed	Hyphen Biomed	Hyphen Biomed
Rivaroxaban	Calibrator Stago	Calibrator Stago	Biophen Rivaroxaban	Hyphen Biomed
Apixaban	Calibrator Stago	Calibrator Stago	–	Technoview Apixaban
PT upper limit of normal range	< 1.20	< 1.20	< 1.13	< 1.20
APTT upper limit of normal range	< 1.25	< 1.22	< 1.30	< 1.27

LOD (ng/ml) dei principali metodi specifici per il dosaggio di Dabigatran, Rivaroxaban e Apixaban

Dabigatran	LOD	Rivaroxaban	LOD	Apixaban	LOD
Hyphen	10.5	Hyphen1	10.0	Hyphen1	8.3
		Hyphen2	6.4	Hyphen2	9.7
IL	0.7	IL	12.1	IL	3.3
Siemens	26.9	Siemens1	60.6	Siemens1	51.8
		Siemens2	9.2	Siemens2	9.8
Technoclone	24.5	Technoclone	5.5	Technoclone	16.8
Stago	10.3	Stago	14.8	Stago	15.2

LOD = limit of detection (mean of blank + 3SD of blank)

Misura dell'attività degli anticoagulanti orali diretti: Test specifici

Vantaggi	Svantaggi
• Specifici (risultati espressi in ng/ml) • Sensibili (< 10-20 ng/ml) • Risultati non reagente dipendenti • Dose/risposta lineare	• Costosi • Lunghi tempi di esecuzione • Complessi • Alti CV% • Non disponibili in tutti i labs

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DABIGATRAN/RIVAROXABAN/APIXABAN ASSAYS

PILOT PROGRAMME, EXERCISE 1

SEPTEMBER 2015

Dabigatran Assay

	DOAC 15:01				DOAC 15:02			
	n	Median	CV	Range	n	Median	CV	Range
Overall	**56**	**92.0**	**15.1**	**48-125**	**55**	**154.0**	**16.7**	**101-295**

Rivaroxaban Assay

	DOAC 15:03				DOAC 15:04			
	n	Median	CV	Range	n	Median	CV	Range
Overall	**68**	**140.7**	**55.1 (16.5)**	**60-827.6**	**68**	**140.0**	**18.2**	**88-249**

Apixaban Assay

	DOAC 15:05				DOAC 15:06			
	n	Median	CV	Range	n	Median	CV	Range
Overall	47	167.0	20.4	35.9-260	47	44.9	45.2	0-155.2

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CV% inter-metodo e inter-laboratorio (anno 2016)

Test	CV%	Test	CV%
INR	5.8	Fattore X	13.0
PT	6.1	Fattore VIII	14.0
aPTT	6.0	Fattore IX	14.2
Fibrinogeno	6.3	Fattore XI	8.6
Fattore II	10.5	Fattore XII	34.4
Fattore V	12.2	Fattore vW RiCof	46.2
Fattore VII	14.0	Fattore vW Ag	17.2

D-dimero non FEU	27.0	D-dimero FEU	26.1