Come misurare?

Casi clinici dal mondo reale

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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)

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.**

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.**

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

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

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

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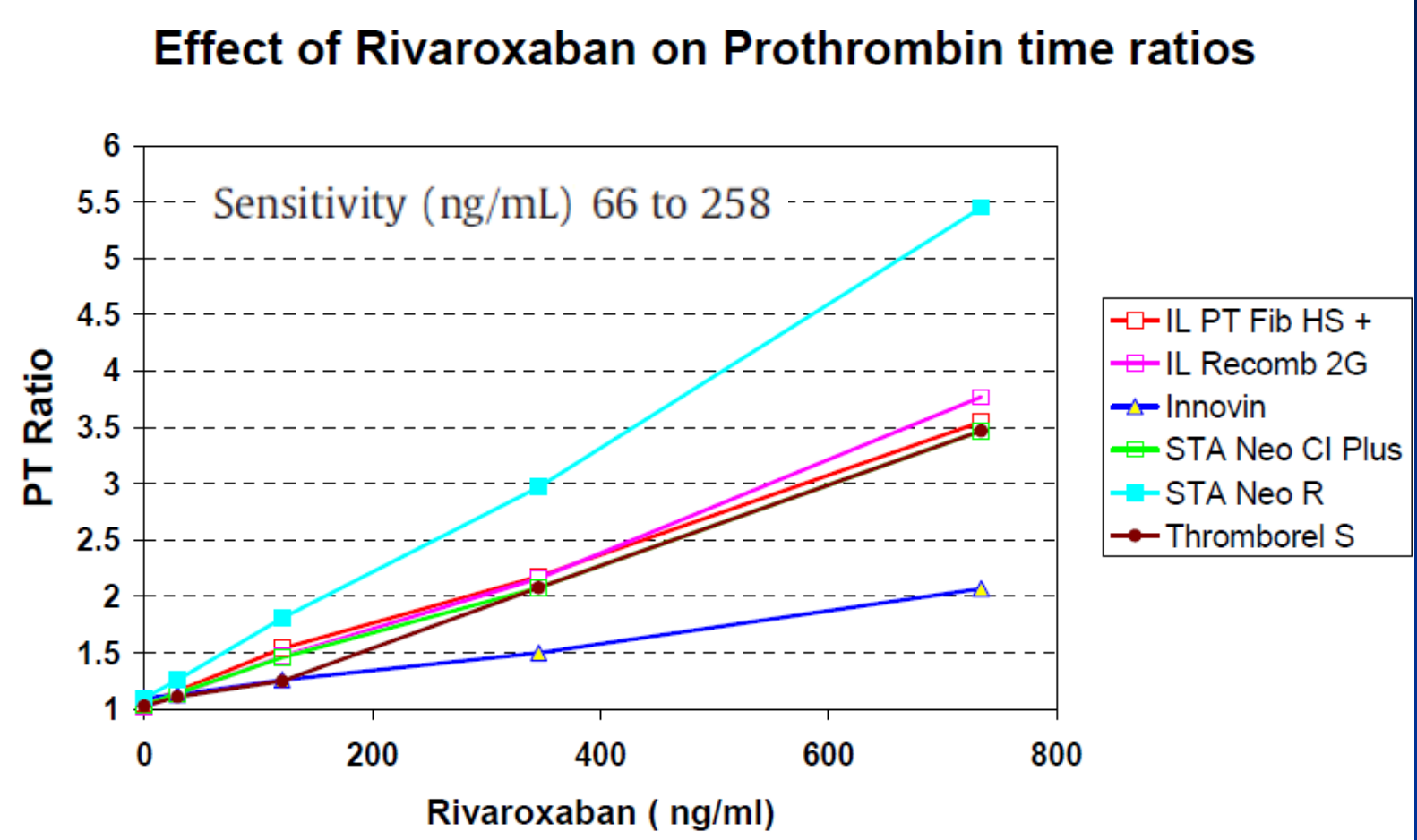
RIVAROXABAN SUPPLEMENTARY EXERCISE September 2014

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



Valori attesi (Rivaroxaban 20 mg od): valle = 12-137 ng/ml; picco: 184-343 ng/ml

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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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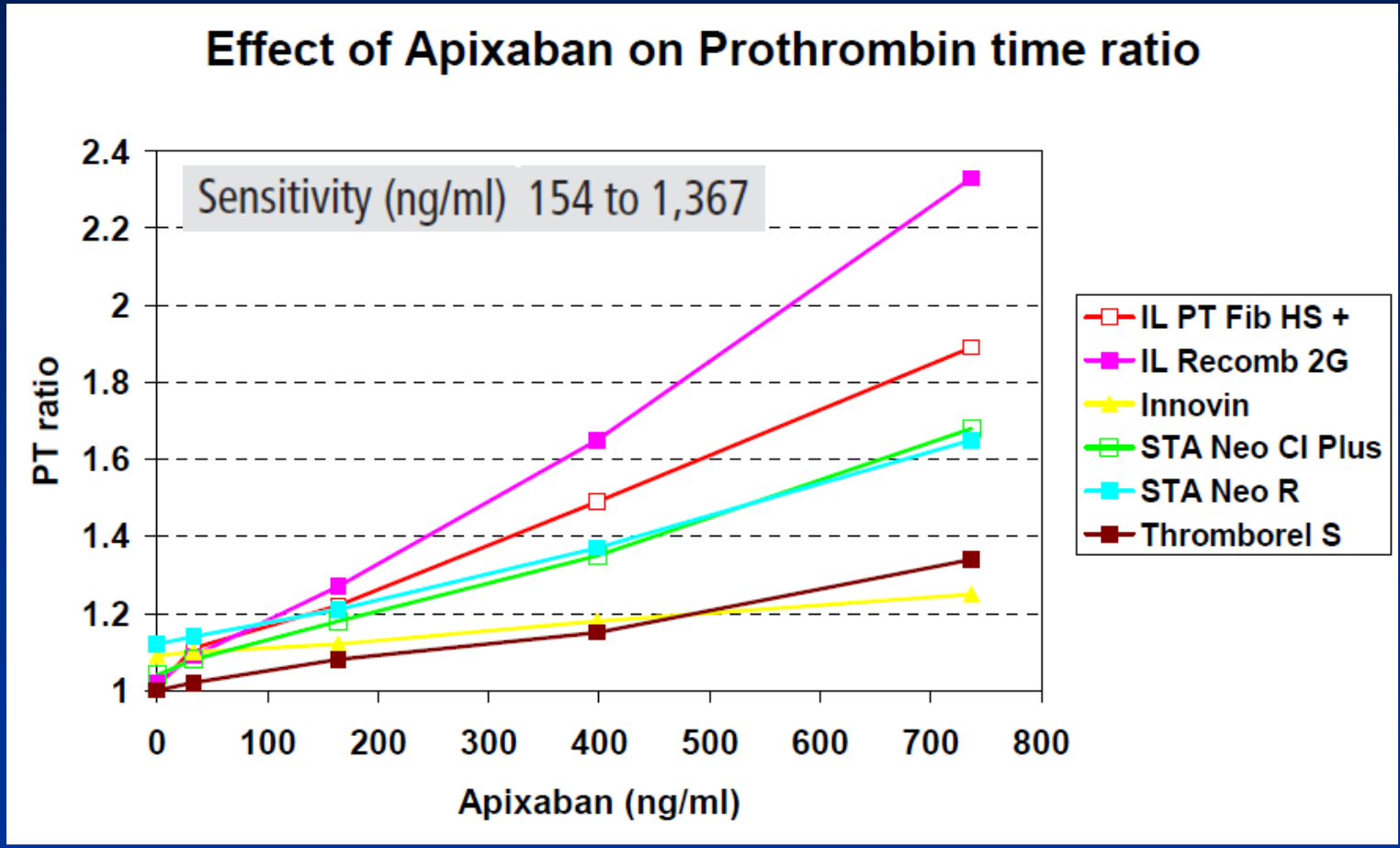
APIXABAN SUPPLEMENTARY EXERCISE September 2014

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



Valori attesi (Apixaban 5 mg bid): valle = 22-352 ng/ml; picco: 52-486 ng/ml

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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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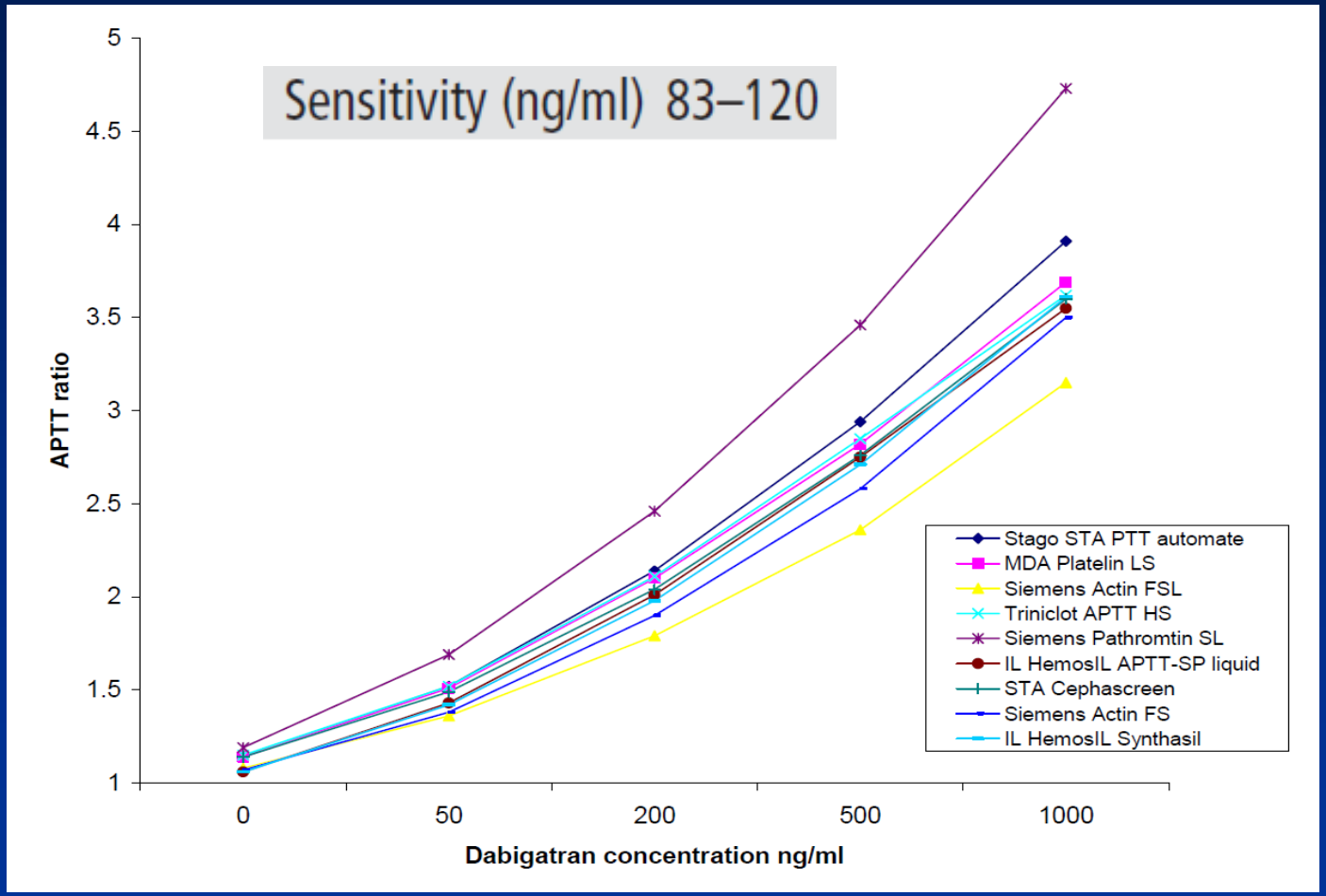
DABIGATRAN SUPPLEMENTARY EXERCISE APRIL 2012

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



Valori attesi (Dabigatran 150 mg bid): valle = 40-215 ng/ml; picco: 74-383 ng/ml

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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: PT - aPTT

- Risultati reagente dipendenti
- Poco sensibili
  = a volte normali in presenza di concentrazioni rilevanti del farmaco
- Poco specifici
  = spesso alterati per altre cause

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)

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

SUPPLEMENTARY EXERCISE

OCTOBER 2014

Dabigatran Assays

Sample	n	Median (ng/ml)	Range (ng/ml)	CV (%)
S14:11	45*	34.0	5.0-75.09	38.7
S14:13	49	158.5	80.0-249.6	19.1

Rivaroxaban Assays

Sample	n	Median (ng/ml)	Range (ng/ml)	CV (%)
S14:15	53**	37.0	12.9-80.0	37.8
S14:16	55	140.3	94.0-473.0	33.7

Apixaban Assays

Sample	n	Median (ng/ml)	Range (ng/ml)	CV (%)
S14:18	24	45.55	20.8-69.49	22.2
S14:19	24	179.5	131.4-220.9	11.1

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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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Report on Survey 213

Survey distribution date: 21st July 2015

D-DIMER ASSAY

Table 10. Quantitative Results D-Dimer Kit	Total n	Median ng/ml	CV (%)	Range ng/ml
Overall (ng/ml) non-FEU	343	115	32.2 (34.5)	30.0 - 350.0
Overall (ng/ml) FEU	352	300	25.8 (38.2)	10.0 - 2030.0

Caso 1 - 1

- Donna di 65 anni in seguito a visita cardiologica ambulatoriale in un ospedale della provincia di Bologna, riceve diagnosi di fibrillazione atriale (FA) con indicazione a terapia anticoagulante
- Normale valore di creatinina, inizia mediante somministrazione di Dabigatran 150 mg x 2

Caso 1 - 2

- Dopo 1 mese di terapia il cardiologo prescrive PT e aPTT; quest'ultimo test risulta 3.6 ratio
- Preoccupati per un eccesso di rischio emorragico il Dabigatran viene sospeso. Dopo 4 giorni l'aPTT viene ripetuto insieme al dosaggio della creatinina
- L'aPTT risulta 2.4 ratio; creatinina normale
- Richieste indagini appropriate al Laboratorio Specialistico di Coagulazione
- Riscontro di un aPTT normale 2 anni prima, non eseguiti PT e aPTT prima di iniziare il Dabigatran

Caso 1 - 3

- Dopo ulteriori 10 giorni viene eseguito un prelievo presso nostro centro
- Al colloquio prima del prelievo vengono recuperati esami fatti 6 mesi in un controllo di routine, dai quali risulta un aPTT di 2.60 ratio
- Eseguito protocollo LAC e dosaggio Dabigatran

Caso 1 - 4

- LAC fortemente positivo
- Marcato aumento del livello degli ACA e anti GPI
- Dabigatran < 20 ng/ml

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

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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.

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

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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- 240 pazienti in terapia con Dabigatran
- Prelievi eseguiti a picco e a valle
- Dabigatran dosato con test specifici
  (dTT in tutti i centri)
- aPTT eseguito con:
  centro A = Actin (Siemens)
  centro B = PTT (Stago)
  centro C = Actin-FS (Siemens)
  centro D = Synthasil (Instrumentation Laboratory)

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aPTT alterato Dabigatran ≤ 50 ng/ml	37/86 (43.0%) 14.3% Centro A ---- 62.5% Centro C
aPTT alterato Dabigatran ≤ 30 ng/ml	8/32 (25.0%) 0% Centro A ---- 100% Centro C
aPTT normale Dabigatran > 50 ng/ml	34/394 (8.6%) 5.6% Centro B ---- 12.0% Centro D

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

Caso 2 - 2

- Dopo 2 giorni in esami di routine il PT risulta 1.25 ratio
- Preoccupati per il valore ai limiti della norma i colleghi telefonano al Laboratorio Specialistico di Coagulazione
- Viene inviato un campione di sangue per il dosaggio del farmaco, nonostante non ci fossero evidenti motivi per eseguirlo

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RIVAROXABAN SUPPLEMENTARY EXERCISE September 2014

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

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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

Table 5. S14:04 (246 ng/ml)	Median Ratio	Min ratio	Max ratio
Siemens Thromborel S	1.60	1.23	1.80

Caso 2 - 3

- 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

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- 264 pazienti in terapia con Rivaroxaban
- Prelievi eseguiti a picco e a valle
- Rivaroxaban dosato con test specifici
  (anti-Xa in tutti i centri)
- PT eseguito con:
  centro A = Recombiplastin (Instrumentation Laboratory)
  centro B = Neoplastin (Stago)
  centro C = Innovin (Siemens)
  centro D = Recombiplastin (Instrumentation Laboratory)

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PT normale Rivaroxaban > 50 ng/ml	46/329 (14.0%) 2.5% Centro B ---- 31.2% Centro D
PT normale Rivaroxaban > 100 ng/ml	14/244 (5.7%) 0% Centro B ---- 16.9% Centro D
PT alterato Rivaroxaban ≤ 50 ng/ml	26/199 (13.1%) 10.1% Centro A ---- 25.0% Centro C