Workshop

The role of PK and PD measurements in the use of Direct Oral Anticoagulants (DOACs)

Session 3: The analytical part

Measuring the concentration of the direct thrombin inhibitor (dabigatran etexilate)

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Conflicts of interest

Research Support/P.I.	**No relevant conflicts of interest to declare**
Employee	**No relevant conflicts of interest to declare**
Consultant	**No relevant conflicts of interest to declare**
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Speakers Bureau	**No relevant conflicts of interest to declare**
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Scientific Advisory Board	**No relevant conflicts of interest to declare**

Objectives

1. Why and when to monitor patients on dabigatran etexilate?

2. What are the performances of the **tests** available today for the **measurement/estimation** of plasma concentrations in **clinical routine (accuracy, precision, sensitivity, specificity, linearity, available external control, standardization, calibration and availability)**.

Absence of therapeutic range

Absence of therapeutic range,

But expected plasma concentration or

on-therapy range

Table III. Expected plasma concentrations of Oral Direct Inhibitors.

Drug	Dose	Peak levels mean and range	Trough levels mean and range	References
Apixaban	2·5 mg bd	0·062 mg/l (CV 37%)	0·021 mg/l (CV 17%)	Frost *et al* (2013)
Apixaban	5 mg bd	0·128 mg/l (CV 10%)	0·050 mg/l (CV 20%)	Frost *et al* (2013)
Dabigatran	150 mg bd	0·184 mg/l (95% CI 0·064–0·443)	0·090 mg/l (0·031–0·225)	Van Ryn *et al* (2010)
Rivaroxaban	10 mg od	0·125 mg/l (0·091–0·195)	0·009 mg/l (0·001–0·038)	Mueck *et al* (2008)
Rivaroxaban	20 mg od	0·223 mg/l (0·16–0·36)	0·022 mg/l (0·004–0·096)	Mueck *et al* (2008)

CV, coefficient of variation; 95% CI, 95% confidence interval.

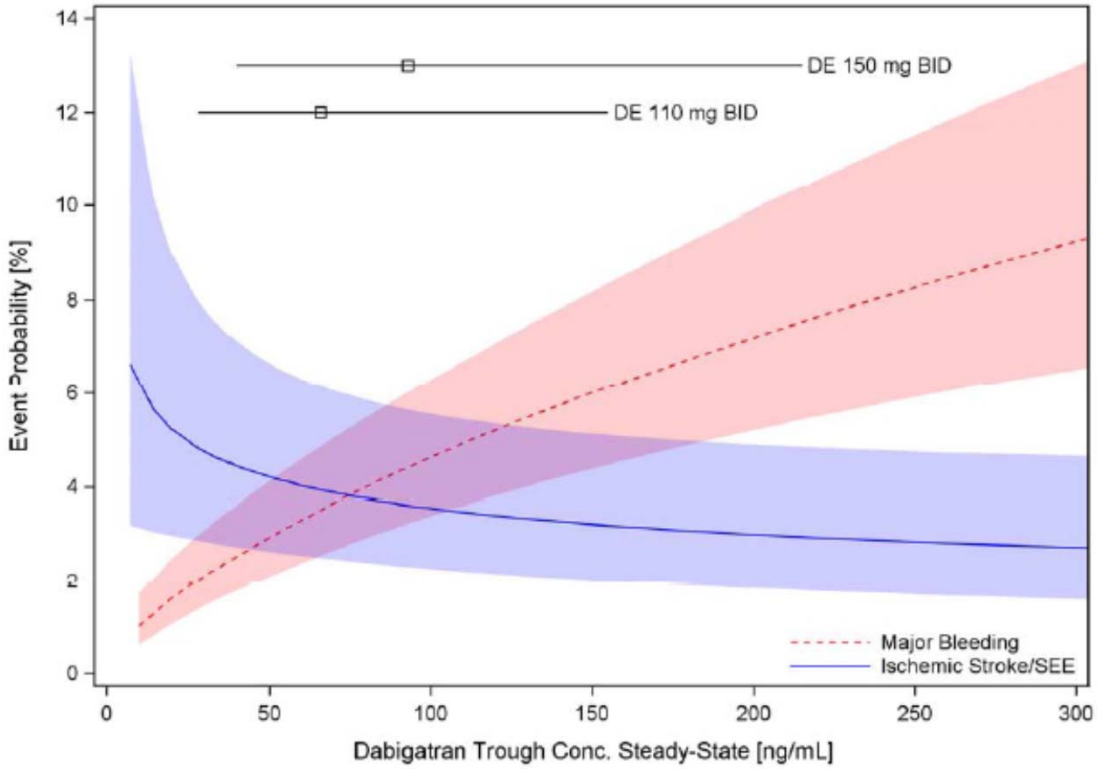
Kitchen S, *et al*. Br J Haematol. 2014

MONITORING?

WHY AND WHEN?

Why?

- Correlation between plasma concentrations and clinical events



Reilly PA *et al*. JACC. 2013.

When? Patients or situations requiring an assessment of the response

- Bleeding or recurrence of thrombosis
- Before an invasive procedure (elective or urgent surgery at risk of bleeding, thrombolysis)
- In patients with potential drug interactions that affect the pharmacokinetics of DOACs (P-gp)
- In patients with extreme body weight (< 50 or > 110 kg)
- In elderly patients (> 75 years of age) with renal failure
- In patients with genetic mutations (i.e., rs2244613 minor allele carriers → dabigatran etexilate only)
- In case of accumulating interfering factors
- Effect of Antidotes

Douxfils *et al.* Exp. Op. Drug. Saf. 2015.

MONITORING?

HOW?

Mass spectrometry : advantages and limitations of the gold standard method

Advantages	Limitations
specificity	labour-intensive preparation
sensitivity LOD around 1 ng/mL LOQ around 3 ng/mL	complexity of the technique
selectivity compared to coagulation-based assay	cost >< availability
reproducibility intra-assay precision < 6% inter-assay precision < 10%	Lack of standardization: in-house validation matrix effect - interference with plasma phospholipids - presence of other compounds removal of proteins interference by drug metabolites (either active or not)
accuracy	~~external quality control~~
	turn-around time

Douxfils J *et al.* Trends in Analytical Chemistry. Submitted

Evidence regarding laboratory measurement: a systematic review

TABLE 6 Suggestions for Laboratory Measurement of Non-Vitamin K Oral Anticoagulants

	Clinical Objective					
	Determine If Clinically Relevant Below On-Therapy Drug Levels Are Present		Estimate Drug Levels Within On-Therapy Range		Determine If Above On-Therapy Drug Levels Are Present	
Drug	Suggested Test	Interpretation	Suggested Test	Interpretation	Suggested Test	Interpretation
Dabigatran	TT	Normal TT likely excludes clinically relevant drug levels	Dilute TT, ECA, ECT	–	APTT, dilute TT, ECA, ECT	Normal APTT likely excludes excess drug levels; only dilute TT, ECA, and ECT are suitable for quantitation
Rivaroxaban	Anti-Xa	Normal anti-Xa activity likely excludes clinically relevant drug levels	Anti-Xa	–	Anti-Xa, PT	Normal PT likely excludes excess drug levels; only anti-Xa is suitable for quantitation
Apixaban	Anti-Xa	Normal anti-Xa activity likely excludes clinically relevant drug levels	Anti-Xa	–	Anti-Xa	–

Suggestions for laboratory measurement of the anticoagulant activity of dabigatran, rivaroxaban, and apixaban are based on the clinical objective. Typical on-therapy drug levels are shown in Table 1.
Abbreviations as in Table 2.

Cuker *et al.* J Am Coll Cardiol. 2014.

National and international recommendations

Table 3 Summary of national and international recommendations regarding urgent and routine assessment of DOACs.

	Screening		Drug level assessment	
	Anti-FIIa drugs	Anti-FXa drugs	Anti-FIIa drugs	Anti-FXa drugs
ISTH [41]	APTT	PT	dTT[a]	Anti-FXa assay
ACCP [42]	TT	None	ECT	Anti-FXa assay
ESC [43]	APTT	PT[b]	dTT[a] or ECT	Anti-FXa assay[b]
EHRA [44]	APTT	PT[c]	dTT[a] or ECT	Anti-FXa assay[c]
Australian Consensus Document [45]	N/A[d]	N/A[d]	dTT[d]	Anti-FXa assay[d]
Government of South Australia [46]	APTT or TT	N/A	dTT[a]	N/A
ASTH [47]	APTT (and TT)	PT[c]	dTT[a]	Anti-FXa assay[c]
FCSA, SIMeL, SIBioC, and CISMEL [48]	APTT	PT	dTT[a] or ECT	Anti-FXa assay
BCSH [49]	APTT	PT	dTT[a] or ECT	Anti-FXa assay
SSTH [50]	APTT	N/A	dTT[a] or ECT	N/A

Lippi G. CCLM. 2014.

International survey – perioperative management of DOACs

1

Table 2
Tests used to assess coagulation status in a bleeding patient treated with a new oral anticoagulant

Monitoring	Dabigatran	Apixaban	Rivaroxaban
aPTT	36 (32.1)	27 (24.1)	26 (23.2)
PT	19 (17)	29 (25.9)	32 (28.6)
INR	22 (19.6)	23 (20.5)	22 (19.6)
ACT	11 (9.8)	8 (7.1)	8 (7.1)
ECT	28 (25.0)	5 (4.5)	6 (5.3)
Factor Xa activity	16 (14.3)	**42 (37.5)**	**47 (41.9)**
Thrombin time	**35 (31.2)**	13 (11.6)	12 (10.7)
Diluted thrombin time (Hemoclot)	14 (12.5)	2 (1.8)	2 (1.8)
None	**33 (29.5)**	**34 (30.4)**	32 (28.6)

Heterogeneity of tests used in the perioperative management of DOACs

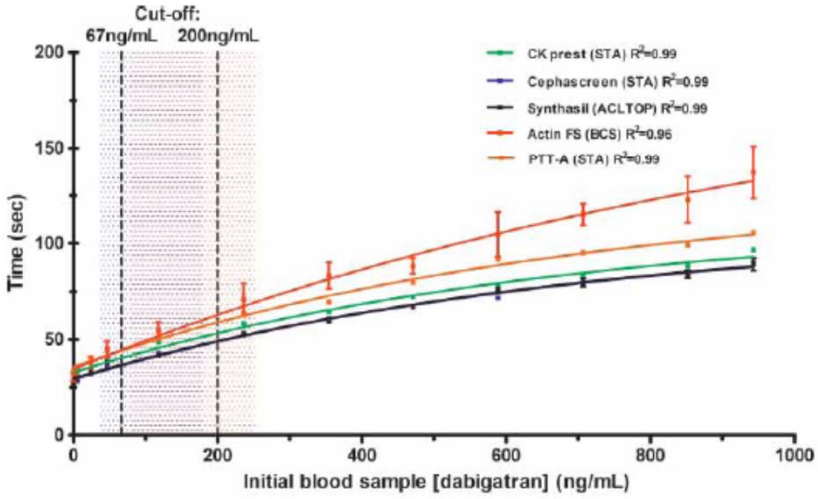
Faraoni *et al.* Clin Lab Med. 2014.

Slide 12

1 Faut préciser l'année où elle s'est faite, il me semble que cest 2012, depuis plus de publis sur les tests bio sont sortis...
Sarah Lessire, 18/11/2015

Dabigatran and aPTT

- Dabigatran
  - aPTT: in vitro study



B) In atrial fibrillation (AF): dabigatrain etexilate 150 mg bid		
Reagent	Clotting time corresponding to a risk a bleeding in AF at C_{trough} (i.e. 200 ng/ml) (15)	
	Sec	Ratio
Actin FS®	62.5	2.06
Cephascreen®	48.6	1.77
CKPrest®	53.0	1.74
PTT-A®	58.6	1.77
Synthasil®	49.0	1.78
Hemoclot Thrombin Inhibitor®	54.7	1.64

Adapted from Douxfils J, Mullier F *et al.* Thromb Haemost. 2012

Table 2: Coagulation test thresholds at trough that may be associated with an increased risk of bleeding.

Test (trough value)	Indication	
	pVTEp orthopaedic surgery	SPAF and DVT/PE
dTT [ng/mL]	> 67	> 200
ECT [x-fold upper limit of normal]	No data	> 3
aPTT [x-fold upper limit of normal]	> 1.3	> 2
INR	Should not be performed	Should not be performed

EU-SmPC – Pradaxa available on the EMA website

Dabigatran and aPTT

APTT is not reliable:

- not sensitive enough to dabigatran
- not possible to estimate dabigatran concentration
- normal aPTT cannot exclude the presence of dabigatran
- multiple other biological variable interfering with aPTT

Douxfils J, Mullier F *et al.* Thromb Haemost. 2012

Dabigatran and dedicated coagulation assays

Ecarin clotting time

- measures the activity of anti-thrombin agents
- absence of standardized procedure/kit for the measurement of dabigatran
- not recommended

Douxfils J *et al.* BioMed Res. Int. 2014

Dedicated coagulation assays for dabigatran: External quality control: UK NEQAS SURVEY October 2014

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DABIGATRAN/RIVAROXABAN/APIXABAN ASSAYS

SUPPLEMENTARY EXERCISE

OCTOBER 2014

This exercise comprised 3 samples for each assay.

Samples **S14:11, S14:12** and **S14:13** were provided for Dabigatran assay.
S14:11 was distributed in an earlier exercise for screening tests, labelled S12:02. This was a normal plasma, spiked with Dabigatran. In this earlier exercise, sample S12:02 was estimated to contain 30ng/ml Dabigatran, based on results from 12 centres.
S14:12 was distributed in an earlier exercise for screening tests, labelled S12:01. This was a normal plasma, containing no Dabigatran. In this earlier exercise, sample S12:01 was estimated to contain 0ng/ml Dabigatran, based on results from 12 centres.
S14:13 was distributed in an earlier exercise for screening tests, labelled S12:03. This was a normal plasma, spiked with Dabigatran. In this earlier exercise, sample S12:03 was estimated to contain 155ng/ml Dabigatran, based on results from 12 centres.

External quality control: UK NEQAS SURVEY October 2014

- 10 different methods were employed to assay dabigatran.
- 27/34 centres reporting source of calibrator for dabigatran assays used Hyphen calibrators.
- High CVs were observed for the two samples with lower levels of dabigatran.
- Limits of detection for these assays of around 30ng/ml have previously been reported.
- Between center agreement was better for sample S14:13 (median concentration 158.5ng/ml, CV 19.1%).

Dabigatran Assays

Sample	n	Median (ng/ml)	Range (ng/ml)	CV (%)
S14:11	45*	34.0	5.0-75.09	38.7
S14:12	34**	0.0	0.0-40.0	185.9
S14:13	49	158.5	80.0-249.6	19.1

** - a further 4 centres reported results as "<"*
*** - a further 15 centres reported results as "<"*

External quality control: UK NEQAS SURVEY October 2014

Sample S14:12 Method	n*	Median (ng/ml)	Range (ng/ml)	CV (%)
Biophen Chromogenic Iia assay	1	0.0	-	-
Biophen DTI	1	<32	-	-
Ecarin chromogenic assay: source not stated	1	11.3	-	-
HemosIL Direct thrombin inhibitor assay	5	0.0	0.0-1.18	-
Hyphen Biomed Hemoclot	22	22.0	0.0-40.0	172.1
In house Dilute thrombin time (Siemens)	1	<30	-	-
Nadia Chromogenic assay	1	<35	-	-
Roche Dilute Thrombin time	1	1.0	-	-
Siemens DTI assay	2	4.25	0.0-8.5	-
Stago ECAII Ecarin assay	2	7.5	0.0-15.0	-
Overall	**34***	**0.0**	**0.0-40.0**	**185.8**

** - *a further 15 centres reported results as "<"*

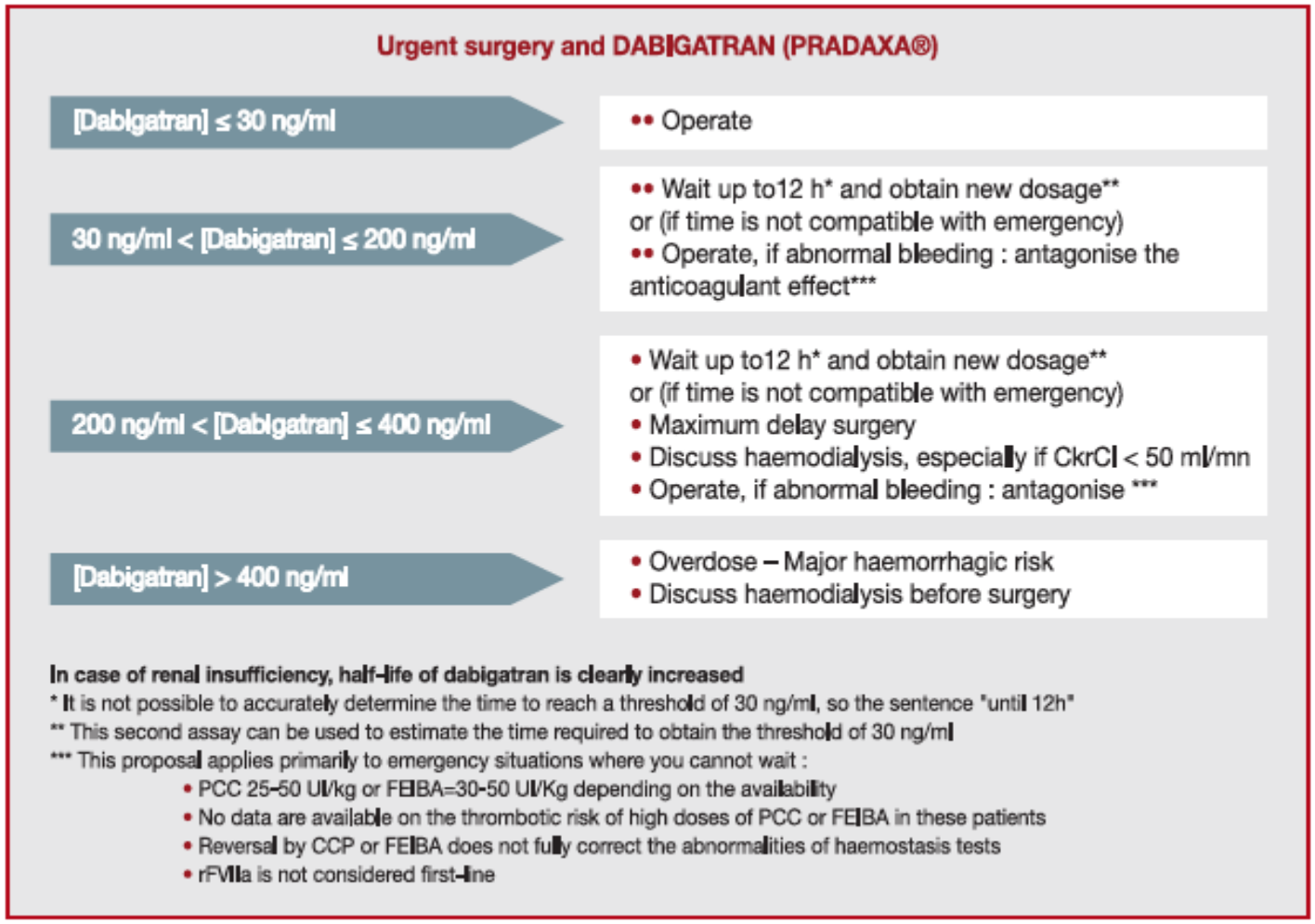
3 CE marked assays (new: Technoview ® (Technoclone))

Most of the assays are useful for normal and high concentrations

But have **lower limits of quantitation between 30 and 50 ng/mL**

Stangier J *et al.* Blood Coagul Fibrin 2012; Lange U *et al.* Pathophysiology of Haemostasis and Thrombosis 2003; Gosselin RC *et al.* Ann Pharmacother 2013; Douxfils J *et al.* Thromb Haemost 2013, Hawes EM *et al.* J Thromb Haemost 2013; Antovic JP *et al.* Eur J Clin Pharmacol 2013; Douxfils J, Mullier F *et al.* Thromb Haemost 2012; van Ryn J *et al.* Thromb Haemost 2010; Skeppholm M. *et al.* Thromb Res 2014

Low plasma concentrations of dabigatran in the perioperative management of patients



EMA EPAR: 48 ng/ml

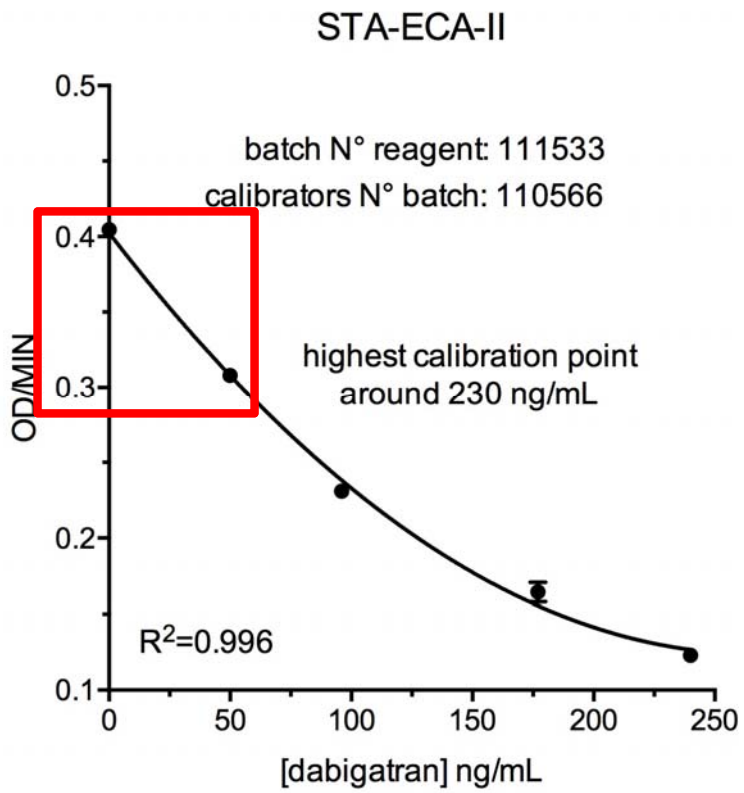
Pernod *et al.* Arch Cardiovasc Dis 2013

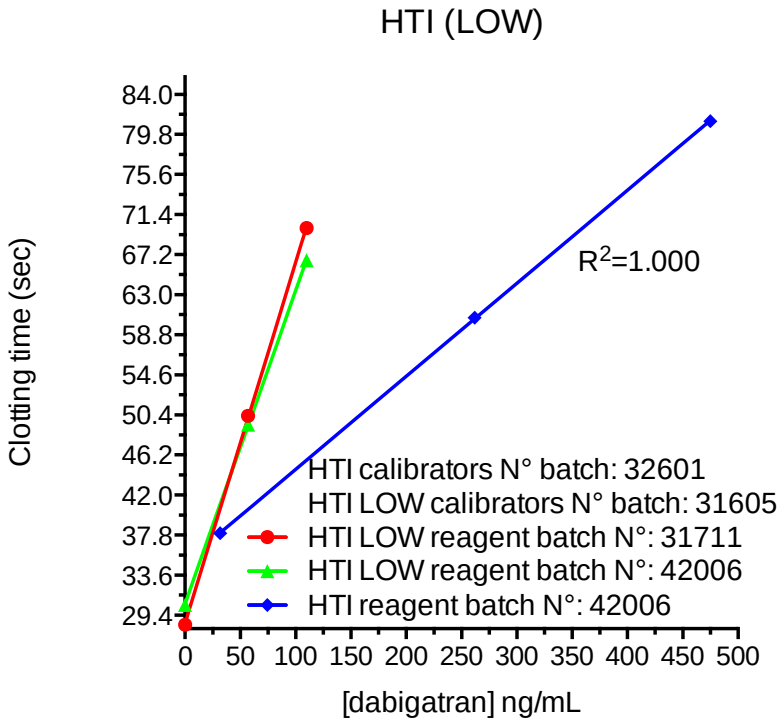
TT may be useful for low concentrations

- Often used in clinical practice even if it is not recommended
- Normal TT excludes clinical relevant dabigatran level
- A prolonged TT is not always associated with a concentration higher than 30 ng/ml → some anesthesiologists/surgeons may uselessly delay an invasive procedure
- Lack of standardisation and specificity (heparin bridging, inflammatory syndrome, fibrin degradation products…)
- Several biological and analytical variables:
  - Thrombin origin
  - Concentration of thrombin
  - Lot to lot reagent differences
  - Instrumentation/Storage

Lessire S, Douxfils J *et al.* Thromb Res 2015; Douxfils J, Lessire S *et al.* Thromb Haemost. 2015; Chin *et al.* Br J Clin Pharmacol 2014.

New specific tests are available for low dabigatran concentrations





Good accuracy in the low concentration range
HemosIL Direct Thrombin Inhibitor also accurate (UKNEQAS 2014)

Douxfils J, Lessire S, *et al.* Thromb Haemost. 2015.

Unsolved issues (1)

- Improvement of the precision in the low concentration range
- How to calibrate the assays? (home-made calibrators vs manufacturers calibrators): a reference international calibrator is required
- When to measure the drug level at Cmax (2-3-4h?)
- Data about sample stability are lacking

Unsolved issues (2): Catheter ablation

- During catheter ablation of AF, it's recommended to achieve and maintain an activated clotting time (ACT) of 300 to 400 seconds in order to reduce the risk of systemic thromboembolism.
- Uninterrupted oral anticoagulation has become the standard protocol
- However the ACT is affected by a lot of pre-analytical and analytical variables.
- Target ACT has not yet been re-determined for the peri-procedural use of DOACs for AF ablation.

Calkins H et al. J Interv Card Electrophysiol 2012
Jobes DR, et al. Anesth Analg 1989
Jude B, et al Ann Fr Anesth Reanim 2004
Douxfils et al Thrombosis Journal 2014
Nagao et al. Intern. Med. 2015

Conclusion: Key messages (1)

- **Recommendations of the SmPC are not optimal:**
  - **aPTT should not be used except for screening a bleeding patient on dabigatran etexilate (the current threshold associated with a risk of bleeding should be adapted)**
  - **Normal APTT and/or PT don't exclude presence of therapeutic levels: to be added**
  - **Normal TT excludes clinical relevant drug level: to be added**
  - **dTT and ECA should be preferred**
  - **ECT is not recommended**
  - **Importance of delay between last administration and sampling**

Conclusion: Key messages (2)

- **Perspectives**
  - **Improvement of coagulation tests in the perioperative setting**
  - **Development of an universal coagulation test for bleeding on DOACs**
  - **Development of point of care devices**
  - **Development of a reference international calibrator**
  - **Validation of the target ACT for the peri-procedural use of DOACs for AF ablation**

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