DOCTOR OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES

Optimization of the perioperative management of direct oral anticoagulants

Lessire, Sarah

Award date:
2016

Awarding institution:
University of Namur

OPTIMIZATION OF THE PERIOPERATIVE MANAGEMENT OF DIRECT ORAL ANTICOAGULANTS

Sarah LESSIRE

Thesis submitted in fulfilment of the requirements for the degree of Doctor in Pharmaceutical and Biomedical Sciences

2016

Faculté de Médecine

Département de Pharmacie

Rue de Bruxelles, 61

5000 Namur

Belgique

Jury:

Professor Jean-Michel Dogné (Promoter, UNamur)

Professor Maximilien Gourdin (Co-promoter, UCL)

Professor Nathalie Caron (UNamur)

Professor Bernard Chatelain (UCL)

Professor Brigitte Ickx (ULB)

Professor Charles-Marc Samama (Université de Paris Descartes)

The research described in this thesis was conducted at:

- the CHU UCL NAMUR, Université catholique de Louvain, Department of Anesthesia (Professor Edith Collard)
- the CHU UCL NAMUR, Université catholique de Louvain, Laboratory of Hematology and Hemostasis (Professor François Mullier, Professor Bernard Chatelain)
- the University of Namur, Department of Pharmacy (Professor Jean-Michel Dogné)

Graphisme de couverture : © Presses universitaires de Namur

Rempart de la Vierge, 13
B - 5000 Namur (Belgique)

Imprimé en Belgique
ISBN : 978-2-87037-960-8
Dépôt légal: D/2016/1881/44

« Peut-être ne saurez-vous jamais quel a été le résultat de ce que vous avez fait. Mais, si vous ne faites rien, il n'y aura jamais de résultat. »

Le Mahatma Gandhi (1869-1948)

« Pour s'améliorer, il faut changer. Donc, pour être parfait, il faut avoir changé souvent. »

Winston Churchill (1874-1965)

Remerciements

Cette thèse est l'aboutissement d'un travail de groupe, et j'ai eu la chance d'avoir été entourée par une équipe compétente et bienveillante.

Tout d'abord mon promoteur de thèse, le Professeur Jean-Michel Dogné qui m'a encadrée par son expertise exceptionnelle. Je le remercie sincèrement pour tous les bons conseils, les encouragements et la bienveillance qu'il a manifestés à mon égard.

Je remercie sincèrement mon co-promoteur de thèse, le Professeur Maximilien Gourdin, qui m'a poussée à réaliser une thèse et qui m'a encadrée avec bienveillance et patience tout le long de ce travail. En tant que Directeur aux Affaires Académiques du CHU UCL Namur, son travail est indispensable à nos ambitions de recherche.
Merci aussi d'être un voisin de bureau avec qui je peux débattre sur de nombreux sujets et parfois refaire un peu le monde.

Je garde une profonde admiration pour le laboratoire d'Hémostase et d'Hématologie dirigé par les Professeurs Bernard Chatelain et François Mullier, qui font un travail remarquable de recherche dans différents sujets touchant à l'hématologie ou l'hémostase.
J'ai par moment regretté de ne pas avoir choisi la biologie clinique comme spécialité, tellement j'ai trouvé leur travail passionnant. Leur plateforme est incroyable et ils bénéficient d'un personnel de pointe, qui m'a beaucoup encadrée dans mes différentes manipulations. Merci à Justine, Maïté, Françoise, Célia et Nicolas pour m'avoir aidée dans mes différents travaux de recherche et de nous permettre d'envisager la suite. Merci aussi à tous les autres technologues de laboratoire Nicole, Alex, Nicolas, Sébastien, Adrien, Javier et Jeanine pour leur accueil et leur gentillesse.

Merci à tous les membres du département de Pharmacie de l'Université de Namur qui m'ont aidée dans les différents projets et qui ont réalisé des manipulations pour nos travaux. Merci au pharmacien PhD Jonathan Douxfils qui m'a fait bénéficier de sa rigueur et de ses nombreux points de vue enrichissants. Merci au Professeur Lionel Pochet et à Christelle Vancraeynest pour tout leur apport au niveau de la spectrométrie de masse.

Merci à Philippe Devel, Valentine Minet, Damien Gheldof pour leurs encouragements.

Je remercie le Professeur Nathalie Caron, présidente du comité d'encadrement de ma thèse pour ses encouragements et sa bienveillance.

Je remercie le Professeur Brigitte Ickx et le Professeur Charles-Marc Samama, membres extérieurs du jury de ma thèse. Leurs conclusions et encouragements sur ce travail sont précieux et me donnent l'énergie pour poursuivre les recherches ensemble au sein du Groupe d'Intérêt en Hémostase Périopératoire présidé par le Professeur Pierre Albaladejo.

Je remercie le Professeur Paul Hjemdahl et son équipe de la Karolinska Institute (Suède) pour leur collaboration dans nos travaux et la réalisation de la spectrométrie de masse des échantillons contenant du dabigatran.

Je remercie sincèrement Monsieur Jean Amiral (HYPHEN BioMed®), Messieurs David Courtois et Pierre Lammens (Diagnostica Stago®) et Monsieur Kris Van Assche (Nodia®) pour leur collaboration et les échanges réalisés en vue d'améliorer la performance des tests spécifiques mesurant les anticoagulants directs oraux.

Merci à tous les membres du Namur Thrombosis and Hemostasis Center, grâce à qui nous pouvons organiser des symposiums annuels et élaborer des projets de recherche ensemble.

Je remercie le Namur Research Institute for Life Sciences (NARILIS) et son assistante exécutive, PhD en sciences biomédicales, Mme Virginie van Scherpenzeel Thim, pour toute la plateforme de recherche qu'ils exposent et leurs bourses de voyage.

Je remercie le Dr Elizabeth Wager pour son enseignement de qualité sur l'écriture scientifique organisé par les masterclasses de l'European Society of Anaesthesiology en novembre 2013. Nous avons par la suite pu bénéficier de ses cours au sein du CHU UCL Namur via l'appui de l'Unité de Support Scientifique en octobre 2014, puis via NARILIS l'année d'après. Elle a perfectionné l'anglais d'un grand nombre de nos manuscrits. Son travail et sa pédagogie sont exceptionnels.

Je remercie sincèrement le Professeur Bosly et tous les donateurs de la Fondation Mont-Godinne pour le soutien financier de la recherche au sein du CHU UCL Namur. Votre rôle est fondamental dans la concrétisation de nos travaux de recherche. Merci à Carine Mahieux et Karine Rocchetti pour leur assistance dans l'obtention des bourses de recherche.

Je remercie le Professeur Yves Poullet, recteur de l'Université de Namur ainsi que le Professeur Yves Poumay, doyen de la Faculté de Médecine, pour avoir permis de m'inscrire dans leur école doctorale et de m'avoir soutenue pour ma défense de thèse.

Je remercie tous les membres du Conseil Médical du CHU UCL Namur - site Godinne qui ont veillé à ce que je sois bien reçue et encouragée dans mes démarches académiques.

Je remercie de tout cœur ma chef de service le Professeur Edith Collard, qui a cru dès le départ en moi et m'a énormément soutenue dans tout ce que j'entreprenais. C'est une pédagogue hors du commun et je lui souhaite une belle continuité vu qu'elle vient de remettre les clés de sa succession au Professeur Philippe Dubois, que je remercie également pour tous ces encouragements et sa bienveillance dans mon parcours au sein du service d'Anesthésie du CHU UCL Namur - site Godinne.

Je remercie également le Professeur Louis De Cannière, chef de département de Médecine Aiguë et des services Médico-Techniques et chef de service des Urgences du site Godinne, pour ses nombreux encouragements.

Un grand merci à tous mes collègues d'anesthésie pour m'avoir accueillie chaleureusement et encouragée dans mes différents projets.
Une pensée toute particulière à ma collègue, le Docteur Anne-Sophie Dincq, avec qui j'ai énormément travaillé sur les différents projets de ma thèse. J'ai apprécié à tout point de vue son optimisme, sa simplicité, son grand sens de l'organisation et sa rigueur. Sa présence a été précieuse à mes côtés.

Je remercie les secrétaires Sandra Panella, Isabelle Sohet et Néri Paredes pour être des vrais moteurs… mais aussi des psychologues, des oreilles attentives à nos moments de détresse, avec toujours un mot bienveillant et philosophique pour la suite…

Je remercie également tous mes collègues avec qui j'ai pu partager des avis ou réfléchir ensemble à de nouvelles procédures : le Professeur Christian Chatelain, les docteurs Bérangère Devalet, Valérie Mathieux et Jean-Baptiste Nicolas, les pharmaciennes cliniciennes Anne-Sophie Larock, Anne-Laure Sennesael et le Professeur Anne Spinewine, le service de cardiologie, le service des urgences, …

Je remercie tous les employés de l'Unité de Support Scientifique du CHU UCL Namur, qui font un travail remarquable pour organiser les réunions de recherche, assurer la comptabilité de nos travaux, mais aussi pour nous fournir nos articles indispensables à la rédaction de nos manuscrits. Je remercie le Professeur Jacques Jamart et Monsieur Benoît Bihin pour leur aide statistique dans nos différents travaux. Je remercie Monsieur Christian Deneffe pour tout le support informatique qu'il m'a apporté. C'est une personne passionnée par son métier et très dévouée aux autres.

Je remercie chaleureusement tout le personnel du bloc opératoire, des différents départements de médecine et du laboratoire de biologie clinique pour nous avoir permis de récolter et de traiter les échantillons nécessaires pour nos recherches.

Je remercie tous les patients qui ont accepté de rentrer dans nos études et ont permis de faire aboutir nos recherches. Nous espérons que leur contribution amènera à améliorer leur prise en charge.

Je remercie aussi toutes les personnes qui m'ont marquée pendant mes études et ma thèse, par leur sens du professionnalisme, leur exigence dans leur métier, leur humanité et leur pédagogie… Ils sont très nombreux, aussi bien des professeurs tel que le Professeur M. De Kock, que des médecins ou soignants de différentes spécialités et divers horizons, des étudiants et assistants en formation…

J’ai le plaisir maintenant de remercier toutes les personnes qui ont passé le plus de temps à me supporter dans tous les sens du terme…

En premier lieu, mon mari François et ma fille Eva, qui m’ont soutenue tout le long de ce parcours. Votre présence et votre amour sont deux éléments indispensables à mon épanouissement. Merci d’avoir toléré ces moments plus difficiles, où j’étais moins présente et moins agréable dans la vie de tous les jours. Je pense encore affectueusement à ma fille de 3 ans, qui se manifestait en toute discrétion auprès de moi, lorsque les week-ends je rattrapais mon retard dans l’écriture de mon manuscrit. Mais également à mon mari, qui grâce à sa stabilité et sa confiance en moi, font de lui un pilier fondamental dans ma vie. J’ai beaucoup appris par son expérience dans la recherche et il m’a encadrée de façon remarquable… un vrai coach de la vie… mais surtout un mari exceptionnel et très patient...

Je tiens à remercier mes parents pour leur amour inconditionnel et les idéaux qui ont forgé leur vie et en partie façonné la mienne…
La passion pour leur métier et leur devise de ne pas exiger de leurs enfants d’être les meilleurs, mais que ceux-ci fassent tout simplement du mieux qu’ils peuvent, m’ont toujours encouragée à ne pas choisir la facilité et de m’investir intensément dans mes choix de vie. Ils m’ont témoigné de nombreuses fois leur grand sens de l’humanité en manifestant de l’empathie pour leur prochain, un sens du partage et de l’accueil des personnes plus isolées et démunies. Merci d’avoir marqué mon chemin de votre belle empreinte…
Je remercie aussi ma sœur, Judith et sa famille, pour tout l’amour et la confiance qu’ils m’ont témoignés tout au long de ma vie. Notre complicité est essentielle à mon bien-être.
Je remercie également ma belle-famille pour leurs nombreux encouragements et leur dévouement.

J’ai une pensée émue pour ma grand-mère maternelle et ma tante Marielle, qui nous ont quittés toutes les deux ces trois derniers mois. Elles ont été très présentes dans ma vie d’étudiante…

Je remercie mes ami(e)s de longue date, qui ont partagé différentes étapes de ma vie et apporté un soutien indispensable à mon bien-être. Eva, Sandra, Katrin, Aurore, Virginie, Isabelle, Nounou, Simona, Sofia, Rita, Dunja, Mieke, Ludivine, Sandrine, Cecilia, Emily, Julia, Murielle, Parvesh, François, Mathieu, Catherine, Delphine, Pascaline, Sylou et Hélène…

Je pense encore avoir omis de nombreuses personnes dans mes remerciements, néanmoins, elles auront laissé de belles marques sur mon chemin et je les remercie sincèrement pour cela…

Par ailleurs je terminerai par une citation d'Annelou Dupuis qui me tient à cœur :

« A la fin de la vie, nos succès perdent leur importance.
Seules nos amitiés comptent. »

Table of contents

ii. *Case series showing the discrepancy of dabigatran plasma concentrations estimation with the different coagulation assays in a perioperative context.*

iii. *Comparison of the performance of chromogenic assays specifically developed for the measurement of low rivaroxaban concentrations with the reference LC-MS/MS method. Impact of heparin bridging on their performance.*

III. Results

PART 1

Is Thrombin Time useful for the assessment of dabigatran concentration? An in vitro and ex vivo study

PART 2

Estimation of dabigatran plasma concentrations in the perioperative setting: An ex vivo study using dedicated coagulation assays

PART 3

Use of appropriate coagulation assays in peri-procedural contexts for patients on dabigatran etexilate: a case series

PART 4

Estimation of rivaroxaban plasma concentrations in the perioperative setting in patients with or without heparin bridging

IV. Discussion and Perspectives

A. Why, when and how to assess the anticoagulant effect of DOACs in a perioperative setting

i. The preoperative assessment for elective invasive procedures

ii. Emergencies

iii. Outside the perioperative or emergency setting

B. Perspectives for laboratory tests

C. Perspectives in the perioperative management and implication of laboratory tests

i. Timing for neuraxial anaesthesia

ii. Heparin-bridging therapy

- *Which patients may benefit from heparin bridging when anticoagulant therapy is interrupted?*
- *Who are the patients with high thrombo-embolic risk?*
- *What is the best heparin-bridging regimen?*

List of abbreviations

ACCP: American College of Chest Physicians

ACS: Acute coronary syndrome

AF: Atrial fibrillation

APTT: Activated partial thromboplastin time

ARISTOTLE: Apixaban for Reduction of Stroke and Other Thromboembolic Complications in Atrial Fibrillation

ASA: Acetylsalicylic acid

ASRA: American Society of Regional Anesthesia and Pain Medecin

AT: Anti-thrombin

AUC: Area under the curve

BAL: Broncho-alveolar lavage

Bid: Bis In Die (Latin: Twice a Day)

Caps: capsule

CG equation: Cockcroft-Gault (C-G) equation

CI: Confidence Interval

Cmax: Peak concentration

Cmin: Lowest concentration

CrCl: Creatinine clearance

Ctrough: Trough concentration

CV%: Coefficient of variation expressed in percentage

CYP3A4: Cytochrome P450 isozyme 3A4

DE: Dabigatran etexilate

DiXaI: Biophen®Direct Factor Xa Inhibitors

DOAC: Direct Oral Anticoagulant

DPT: Dilute prothrombin time

DRVVT: dilute Russell's Viper Venom Time

DTT: Diluted thrombin time

DVT: Deep vein thrombosis

ECA: Ecarin chromogenic assay

ECA-II: STA®-ECA-II

ECT: Ecarin clotting time

EMA: European Medicines Agency

ESI: Electrospray ionization

Fab: Fragment Antigen Binding

FDA: Food and Drug Administration

GIHP: French Group of Perioperative Hemostasis/ Groupe d'Intérêt en Hémostase Périopératoire

HTI: Hemoclot Thombin Inhibitors®

ICU: Intensive care unit

INR: International Normalized Ratio

IQR: Interquartil range

LC-MS/MS: Liquid chromatography coupled with a tandem mass-spectrometry

LMWH: Low molecular weight heparin

LOD: Limit of detection

LOQ: Limit of quantification

MBE: Major bleeding events

MRM: Multiple reaction monitoring

NIH/ml: National Institutes of Health (NIH) units per ml

NPP: Normal pool plasma

NSAID: Nonsteroidal anti-inflammatory drug

NVAF: Non-valvular atrial fibrillation

OD: Optical density

OR: Odd Ratio

ORBIT-AF: Outcomes Registry for Better Informed Treatment of Atrial Fibrillation

OTC: Optimal thrombin concentration

PCC: Prothrombin complex concentrate

PCTL: Percentiles

PE: Pulmonary embolism

P-gp: P-glycoprotein

PiCT: Prothrombinase-induced clotting time

PPP: Platelet-poor-plasma

PT: Prothrombin time

Qd: Quaque die (Latin: Once a day)

Reverse-AD: Reversal Effects of Idarucizumab on Active Dabigatran

RSD: Relative standard deviation

S: Seconds

SD: Standard deviation

SmPC: Summary of Products Characteristics

SPAF: stroke prevention in atrial fibrillation

SSE: Stroke and systemic embolism

TAT: Turnaround time

TE: Thromboembolism

TEG: Thromboelastography

TEM: Thromboelastometry

TFPI: Tissue factor pathway inhibitor

TGA: Thrombin generation assay

THR: Total hip replacement

TKR: Total knee replacement

TI: Temporary interruption

TMAX: Limit of measurement of thrombin time

Tmax: Time to reach peak concentration

TT: Thrombin time

UFH: Unfractionated heparin

ULM: Upper limit of measurement

UPLC: Ultra Performance Liquid Chromatography

VKAs: Vitamin K antagonists

VTE: Venous thromboembolism

Summary

Direct oral anticoagulants (DOACs) (i.e dabigatran, rivaroxaban, apixaban and edoxaban) are used for the prevention and treatment of thrombotic events including the treatment and prevention of recurrent venous thromboembolism (VTE) and stroke prevention in patients with non-valvular atrial fibrillation (NVAF). Annually, approximately 10-15 % of these patients will require DOAC interruption before an elective or invasive procedure.

Laboratory testing is not recommended routinely, as these drugs have a rapid onset and offset of action, a short half-life, predictable pharmacokinetics and a large on-therapy range.

However, many frail patients (i.e. low body weight, several drug interferences) who receive DOACs in real-life were initially excluded from the clinical phase III trials. In addition, a link between dabigatran or edoxaban concentration and clinical events (venous thromboembolism or major bleeding) was demonstrated. Multicenter studies have shown a high interindividual variability with DOACs in real-life patients that cannot account only for the renal function.

Furthermore, there is no agreement between the different proposals that aimed at guiding the best timing to surgery.

Therefore, DOAC monitoring has become progressively a part of the perioperative management of DOACs, especially in emergencies.

Initial perioperative proposals suggested that routine coagulation assays like the activated partial thromboplastin time (aPTT) or the prothrombin time (PT) could assess residual plasma concentrations of DOACs. Nevertheless, these assays give only qualitative informations, as their responsiveness is coagulometer and reagent dependent and as they are not sensitive enough to exclude clinically relevant DOAC concentrations.

Thrombin time (TT) is the most sensitive coagulation test for dabigatran. However, its methodology is not standardized between the laboratories and its performance is reagent dependent. Therefore, its use has been restricted to exclude clinically relevant concentration of dabigatran in a perioperative context.

Specific coagulation assays were proposed to allow quantitative measurements of DOACs. These include the diluted TT (dTT), the ecarin clotting time (ECT), or the ecarin chromogenic assay (ECA) for dabigatran, and chromogenic anti-FXa assays for rivaroxaban, apixaban, and edoxaban. They have been largely validated in several publications outside the perioperative context. These tests showed a limit of quantitation with standard methodology and calibrators around 30 and 50 ng/ml, which was not accurate enough for the perioperative context. Indeed, the safety threshold proposed for DOAC plasma concentrations before an invasive procedure carrying a high bleeding risk was set at 30ng/ml.

Therefore, the primary objectives of this work were:

1) To optimize and validate the use of thrombin time in the perioperative management of patients on dabigatran etexilate.
2) To compare the performance of coagulation tests, specifically developed for the measurement of low plasma concentrations of dabigatran and rivaroxaban with the reference LC-MS/MS method.
3) To study the interference of heparin bridging on the performance of these specific coagulation assays.

Our in vitro results demonstrated the influence of thrombin concentration, thrombin origin and the type of coagulometer on the measurement of thrombin time. To use thrombin time in a perioperative context, we optimized the thrombin concentration on two coagulometers. In addition, we illustrated the lack of stability of plasma samples and suggested measuring TT within 2 hours of sampling in plasma containing dabigatran.

We confirmed in an ex vivo pilot study performed on 24 plasma samples from patients treated with dabigatran etexilate, that an optimised TT may be useful in assessing low dabigatran concentrations (< 50 ng/ml), especially for laboratories that do not have access to specific assays. However, clinicians need to consider the different variables affecting TT (i.e. heparin bridging, inflammatory syndrome, fibrin/fibrinogen degradation products).

In the second ex vivo study performed on 33 plasma samples from patients treated with dabigatran etexilate, we showed that two coagulation tests specifically developed for the measurements of low dabigatran plasma concentrations (the Hemoclot Thrombin Inhibitors® LOW (HTI LOW) from Hyphen BioMed®, Neuville-sur-Oise, France and the STA®-ECA II (ECA-II) from Diagnostica Stago®, Asnière-sur-Seine, France), performed well with dabigatran plasma concentrations < 50 ng/ml, and thus should be preferred to the standard procedure of HTI in this range of concentrations.
When TT is available before a dabigatran level is requested, then it can guide the laboratory in choosing the more accurate coagulation tests (i.e. HTI or HTI LOW/ECA-II) in order to avoid unnecessary costs. The added value of this strategy was illustrated in a case-series of patients monitored in a perioperative context.

In the last ex vivo study performed on 79 patients, we demonstrated that specific procedures with adapted methodology and calibrators (i.e. Biophen®DiXaI LOW and STA®-Liquid Anti-Xa) should be preferred for the estimation of rivaroxaban concentrations below 50 ng/ml. These methods are sensitive to low molecular weight heparins (LMWHs) and may consequently be influenced with residual LMWH found even 24 h after the last administration.

The development of a chromogenic anti-Xa assay accurate for low rivaroxaban concentrations and insensitive to heparins is required to optimize the perioperative management of patients at high risk of thrombosis that receive a bridging therapy with heparin before an elective procedure.

In conclusion, DOACs monitoring may be necessary to manage some patients in a perioperative or emergent context.

Accurate specific assays with an adapted methodology and calibrators for low DOACs concentrations may guide the clinicians for the optimal management of these patients.

Despite attractive pharmacokinetic properties, the high interindividual variability of DOACs plasma concentration support that their pre-procedural interruption should not be based only on their respective half-life, but also on residual drug concentrations. Further perioperative studies using DOAC monitoring with accurate laboratory tests are needed to validate a unique and safe peri-procedural management.

Résumé

Les agents anticoagulants oraux directs (AODs) (dabigatran, rivaroxaban, apixaban et edoxaban) sont utilisés pour la prévention et le traitement des événements thromboemboliques, incluant le traitement et la prévention de la maladie thromboembolique veineuse (MTEV) et la prévention des accidents vasculaires cérébraux chez les patients avec fibrillation auriculaire non valvulaire (FANV). Chaque année, environ 10-15 % des patients vont nécessiter une interruption de leur AOD avant une procédure élective ou invasive.

Les tests de laboratoire ne sont pas recommandés en routine, étant donné que ces médicaments ont un temps d'action et un temps d'arrêt rapides, une demi-vie courte, une pharmacocinétique prévisible et un large éventail de concentrations plasmatiques sous traitement.

Cependant, beaucoup de patients fragiles (ex: faible poids corporel, plusieurs interactions médicamenteuses) qui reçoivent un AOD dans notre pratique clinique, ont été exclus des études cliniques de phase III. De plus, un lien a été démontré entre les concentrations en dabigatran ou edoxaban, et des événements cliniques (MTEV ou saignement majeur). Des études multicentriques ont montré une importante variabilité interindividuelle des concentrations plasmatiques en AODs chez les patients traités et qui ne peut être expliquée uniquement par la fonction rénale.

De plus, il n'y a pas de consensus entre les recommandations visant à guider le meilleur timing d'arrêt des AODs avant une chirurgie.

Pour ces raisons, le monitoring des AODs a pris progressivement sa place dans la gestion périopératoire des AODs, et en particulier celle des urgences.

Les premières propositions périopératoires suggéraient l'utilisation des tests de coagulation de routine tels que le temps de céphaline activée (TCA) ou le temps de prothrombine (TP) pour évaluer les concentrations plasmatiques résiduelles des AODs.

Cependant, ces tests ne donnent qu'une information qualitative étant donné que leur réponse dépend du coagulomètre et du réactif et qu'ils ne sont pas assez sensibles pour exclure des concentrations en AODs cliniquement relevantes.

Le temps de thrombine (TT) est par contre le test de coagulation le plus sensible au dabigatran. Cependant, sa méthodologie n'est pas standardisée entre les laboratoires et sa performance dépend du réactif utilisé. De ce fait, son utilisation en péri-opératoire a été limitée à exclure les concentrations cliniquement relevantes de dabigatran.

Des tests de coagulation spécifiques ont été proposés pour permettre de quantifier les AODs. Ces tests incluent le temps de thrombine dilué (dTT), le temps de coagulation à base d'écarine (ECT), ou le test chromogénique à base d'écarine pour le dabigatran, et les tests chromogéniques anti-Xa pour le rivaroxaban, l'apixaban et l'edoxaban.

Ces tests ont été largement validés dans plusieurs publications en dehors du contexte péri-opératoire. Cependant, ces tests ont montré une limite de quantification entre 30 et 50 ng/ml avec une méthodologie et des calibrateurs standard, ce qui est insuffisamment exact pour guider des décisions péri-opératoires. En effet, le seuil de concentrations plasmatiques qui a été proposé avant une procédure invasive à haut risque de saignement est de 30 ng/ml.

Dès lors, les objectifs primaires de ce travail de thèse furent:

1) Optimiser et valider l'utilisation du temps de thrombine dans la gestion péri-opératoire des patients sous dabigatran etexilate.
2) Comparer la performance des tests de coagulation, spécifiquement développés pour la mesure des faibles concentrations plasmatiques en dabigatran et rivaroxaban, avec la méthode de référence (LC-MS/MS).
3) Etudier l'interférence d'un relais par héparine sur la performance de ces tests de coagulation spécifiques.

Nos résultats in vitro ont démontré l'influence de la concentration en thrombine, de l'origine de la thrombine et du type de coagulomètre sur le temps de thrombine.

Pour utiliser le TT dans un contexte péri-opératoire, nous avons optimisé la concentration en thrombine sur deux coagulomètres. De plus, nous avons

illustré le manque de stabilité des échantillons plasmatiques et suggéré de mesurer le temps de thrombine sur les échantillons contenant du dabigatran endéans les 2 heures après prélèvement. Nous avons confirmé dans une étude pilote ex vivo réalisée sur 24 échantillons plasmatiques de patients traités par dabigatran etexilate, qu'un temps de thrombine optimisé peut être utilisé pour évaluer les faibles concentrations en dabigatran (< 50 ng/ml), en particulier pour les laboratoires n'ayant pas accès aux tests spécifiques. Cependant, les cliniciens doivent prendre en compte les différentes variables affectant le TT (ex: relais par héparine, syndrome inflammatoire, produits de dégradation de la fibrine et du fibrinogène).

Dans la seconde étude ex vivo réalisée sur 33 échantillons de patients traités par dabigatran étexilate, nous avons montré que les deux tests de coagulation spécifiquement développés pour estimer les concentrations plasmatiques basses en dabigatran (le Hemoclot Thrombin Inhibitors® LOW (HTI LOW) de Hyphen BioMed®, Neuville-sur-Oise, France et le STA®-ECA II (ECA-II) de Diagnostica Stago®, Asnière-sur-Seine, France), présentent de bonnes performances pour les concentrations plasmatiques < 50 ng/ml et devraient être préférés à la procédure standard de l'HTI pour cette gamme de concentration.

Lorsque le TT est accessible avant qu'une estimation du niveau d'anticoagulation par dabigatran ne soit demandée, alors il peut guider le

laboratoire dans le choix des tests de coagulation les plus exacts (HTI ou HTI LOW/ECA-II) afin d'éviter des coûts inutiles. La valeur ajoutée de cette stratégie a été illustrée dans une série de cas de patients suivis dans un contexte péri-opératoire.

Dans la dernière étude ex vivo réalisée sur 79 patients, nous avons démontré que les procédures spécifiques avec méthodologie et calibrateurs adaptés (ex: le Biophen®DiXaI LOW et le STA®-Liquid Anti-Xa) devraient être préférées pour l'estimation des concentrations en rivaroxaban < 50 ng/ml. Cependant, ces méthodes sont sensibles aux héparines de bas poids moléculaire (HBPMs) et peuvent être influencées par des concentrations résiduelles en HBPM retrouvées minimum 24h après leur dernière administration.

Le développement d'un test chromogénique anti-Xa pour les concentrations faibles en rivaroxaban et insensible aux héparines est nécessaire pour optimiser la gestion péri-opératoire des patients à haut risque thrombotique nécessitant un relais par héparine avant une procédure élective.

En conclusion, le monitoring des AODs peut s'avérer nécessaire pour une prise en charge optimale de certains patients dans un contexte péri-opératoire ou d'urgence. Des tests spécifiques exacts avec une méthodologie

et des calibrateurs adaptés pour les concentrations faibles en AODs, devraient guider les cliniciens en charge de ces patients.

Malgré des propriétés pharmacocinétiques attractives, la grande variabilité interindividuelle des concentrations plasmatiques en AODs supportent le fait que leur interruption pré-procédurale ne doit pas être basée uniquement sur leur demi-vie respective, mais aussi sur leurs concentrations plasmatiques résiduelles. De futures études péri-opératoires utilisant des tests de laboratoires exacts pour quantifier les AODs sont nécessaires pour valider une procédure péri-opératoire unique et sure.

I. Introduction

A. Overview of the main characteristics of the direct oral anticoagulants

Direct oral anticoagulants (DOACs) (i.e dabigatran, rivaroxaban, apixaban and edoxaban) are used in several indications for the prevention and treatment of thrombotic events including the treatment and prevention of recurrent venous thromboembolism (VTE) and stroke prevention in atrial fibrillation (SPAF). Pharmacokinetic properties, indications and dose regimens of DOACs in the European Union are summarized in Table 1 and Table 2. These agents are administered as either once-daily (*qd*) or twice-daily (*bid*) fixed-dose regimens, with dosage determined mainly by indication, age and/or creatinine clearance, body weight, and some co-medication use (depending on the agent).[1-4] As highlighted by data from clinical trials and case studies, all DOACs carry the risk of bleeding despite careful selection and patient management.

Bleeding events have been reported in all the major clinical trials comparing DOACs with other anticoagulants, despite regular monitoring of adverse events, strong medication adherence and careful patient selection.[5-9]

Large randomised trials comparing bleeding risks of different DOACs are not available as DOACs are always compared with warfarin, low molecular weight heparin (LMWH) and anti-platelet agents. When DOACs were prescribed at prophylactic doses in orthopaedic surgery, rates of bleeding were similar to LMWHs.[10] In patients with non-valvular atrial fibrillation

(NVAF) or VTE, a recent meta-analysis showed a lower rate of fatal bleeding and case-fatality of major bleeding events (MBE) with DOACs than with warfarin.[11] Another meta-analysis showed that patients with a creatinine clearance (CrCl) between 50-80 ml/min who received DOACs had significantly fewer MBE than those receiving Vitamin K antagonists (VKAs). For patients with CrCl <50ml/min, the difference in MBE was not statistically significant, but using indirect comparisons, apixaban was associated with significantly fewer MBE than other DOACs.[12]

Post-marketing surveillance registries provide real world data on the safety and efficacy of DOACs. The Dresden registry found that the routine safety profile of dabigatran etexilate (DE) was not worse than that reported in the RELY trial even if selection bias might exist.[13] The Danish national registry found a higher bleeding rate in previous VKA users than VKA naive patients.[14] Concerning rivaroxaban, large observational studies as well as prospective non-interventional studies showed that the major bleeding rate was generally consistent with registration trial results and that fatal bleeds were rare. [15,16,17] The MBE rate with rivaroxaban from the Dresden registry was lower than that for VKAs.[18]

Table 1: Summary of pharmacokinetic properties of direct oral anticoagulants in the European Union [19-28]

	Dabigatran	Rivaroxaban	Apixaban	Edoxaban
Target	Factor lla	Factor Xa	Factor Xa	Factor Xa
Prodrug	Yes	No	No	No
Tmax (h)	1.5 - 3.0	2.0 - 4.0	3.0 - 4.0	1.0-2.0
Distribution volume (L)	60 – 70	± 50	23	107
Half-life (h)	12 - 17	5 - 9: healthy individuals 11 - 13: elderly	8 - 15: healthy individuals	10-14
Bioavailability	3 - 7 % pH sensitive	2.5 mg to 10mg dose: 80-100% (fasting/fed) 15 - 20mg dose: 66% (fasting) +- 100% (fed)	± 50%	62%
Protein binding	35%	>90%	87%	55%
Metabolism	Conjugation	CYP-dependent and independent	CYP-dependent	CYP-dependent (<5%) and independent (<10%)

Active metabolites	Yes - glucuronide conjugates	No	No	Yes (<15%)
Elimination	80% renal	33% unchanged via the kidney	27% renal	50% renal
	20% bile (glucuronide conjugation)	66% metabolized in the liver into inactive metabolites eliminated via the kidney (50%) or the colon (50%)	73% through the liver while the residue is excreted by the hepatobiliary route	50% metabolism and biliary/intestinal excretion
Effects of food	Tmax delayed; Cmax & AUC unchanged	Tmax delayed; Cmax & AUC increased (15-20 mg)	Tmax delayed; Cmax & AUC unchanged	C max increased, but minimal effect on total exposure
CYP substrate	No	CYP3A4, CYP2J2	CYP3A4	CYP3A4 (<5%)
P-gp substrate	DE: Yes	Yes	Yes	Yes

Tmax: time to reach peak concentration; Cmax: peak concentration; AUC: air under the curve; CYP3A4: cytochrome P450 isozyme 3A4; P-gp: P-glycoprotein.

Table 2. Indication and dose regimens of direct oral anticoagulants in the European Union[29-52]

VTE Prophylaxis	• **220 mg/day** (2 caps 110 mg *qd*) **Cmax:** 71 (35 – 162) ng/ml (mean; 25-75 PCTL) **Cmin:** 22 (13 – 36) ng/ml (mean; 25–75 PCTL) • **150mg/day** (2 caps 75 mg *qd*) → if CrCl 30-50 ml/min, if >75 years, if verapamil, amiodarone and quinidine THR: 28-35 days TKR: 10 days	• **10 mg/day** (1 tablet 10 mg *qd*) **Cmax:** 125 (91 – 196) ng/ml (median; 5–95 PCTL) **Cmin:** 9 (1 - 38) ng/ml (median; 5–95 PCTL) THR: 5 weeks TKR: 2 weeks	• **5 mg/day** (1 tablet 2.5 mg *bid*) **Cmax:** 77 (41 – 146) ng/ml (median; 5–95 PCTL) **Cmin :** 51 (23– 109) ng/ml (median; 5–95 PCTL) THR: 32-38 days TKR: 10 days	× (EU)
Non-valvular atrial fibrillation	• **300 mg/day** (1 caps 150mg *bid*) **Cmax:** 175 (117-275) ng/ml (mean; 25-75 PCTL) **Cmin:** 91 (61-143-200) ng/ml (mean; 25-75-90 PCTL)	• **20 mg/day** (1 tablet 20 mg *qd*) **Cmax:** 249 (184 – 343) ng/ml (mean; 5–95 PCTL) **Cmin:** 44 (12 – 137) ng/ml (mean; 5–95 PCTL)	• **10 mg/day** (1 tablet 5 mg *bid*) **Cmax :** 171 (91 – 321) ng/ml (median; 5–95 PCTL) **Cmin :** 103 (41– 230) ng/ml (median; 5–95 PCTL	• **60mg/day** (1 tablet 60 mg *qd*) **Cmax :** +/-170 (165-195) ng/ml (median; IQR) **Cmin :** 36 (19 – 62) ng/ml (median; IQR)

Non-valvulal atrial fibrillation	• **220 mg/day** (1 capsule 110 mg *bid*) → if >80 y or verapamil	• **15mg/day** (1 tablet 15 mg *qd*) → if CrCl between 15-49 ml/min **Cmax:** 229 (178 – 313) ng/ml (mean; 5–95 PCTL) **Cmin:** 57 (18 – 136) ng/ml (mean; 5–95 PCTL)	• **5 mg/day** (1 tablet 2.5mg *bid*) → If at least 2 of the following conditions: ≥80ys, ≤60kg or serum creatinine ≥ 1,5 mg/dl; → Or if CrCl 15-29ml/min **Cmax :** 123 (69 – 221) ng/ml (median; 5–95 PCTL) **Cmin :** 79 (34– 162) ng/ml (median; 5–95 PCTL)	• **30mg/day** (1 tablet 30mg *qd*) → if CrCl between 15-50 ml/min, ≤ 60kg or concomitant use of dronedarone, erythromycin, ketoconazole, ciclosporin **Cmin :** 27 (15-45) ng/ml (median; IQR)
VTE treatment	• **300mg/day** (1 caps 150mg *bid*) after at least 5 days of parenteral anticoagulants **Cmax:** 175 (117 – 275) ng/ml (mean; 25-75 PCTL) **Cmin:** 60 (39 – 95- 146) ng/ml (mean; 25-75-90 PCTL) • **220 mg/day** (1 caps 110 mg *bid*) → if >80 y or verapamil	**Treatment phase:** • **30 mg/day** (1 tablet 15 mg *bid*) for 21 days → followed by • **20mg/day** (1 tablet 20 mg *qd*) for 3 to 6 months **Cmax:** 270 (189 – 419) ng/ml (mean; 5–95 PCTL) **Cmin:** 26 (6 – 87) ng/ml (mean; 5–95 PCTL)	**Treatment phase:** • **20mg/day** (2 tablet 5mg *bid*) for 7 days **Cmax :** 251 (111-572) ng/ml (median; 5–95 PCTL) **Cmin :** 120 (41– 335) ng/ml (median; 5–95 PCTL) → followed by • **10mg/day** (1 tablet 5mg *bid*) for 3 to 6 months	• **60mg/day** (1 tablet 60 mg *qd*) after at least 5 days of parenteral anticoagulant treatment. • **30mg/day** (1 tablet 30mg *qd*) → if CrCl between 15-50 ml/min, BW ≤ 60kg or concomitant use of dronedarone, erythromycin, ketoconazole, ciclosporin

		• **15mg/day** (1 tablet 15 mg *qd*) → if CrCl between 15-49 ml/min and the risk of bleeding outweighs the risk of recurrent DVT or PE	**Cmax :** 132 (59 – 302) ng/ml (median; 5–95 PCTL) **Cmin :** 63 (22– 177) ng/ml (median; 5–95 PCTL) <u>If high risk of recurrent DVT or PE:</u> • **5mg/day** (1 tablet 2.5mg *bid*) after 6-months treatment	
Prevention of athero-thrombotic events after ACS with elevated cardiac biomarkers	×	• **5 mg/day** (1 tablet 2.5 mg *bid*) with ASA (75-100 mg) or, ASA + clopidogrel (75 mg) or ticlopidine **Cmax:** 46 (28 – 70) ng/ml (median; 5–95 PCTL) **Cmin:** 17 (6 – 37) ng/ml (mediaan; 5–95 PCTL)	×	×

× Off-label; caps: capsule; qd: once daily; *bid*: twice daily; CrCl: creatinine clearance; DVT: Deep-vein thrombosis; PE: pulmonary embolism; THR: total hip replacement TKR: total knee replacement; ASA: acetylsalicylic acid; ACS: acute coronary syndrome, Cmax and Cmin: peak and trough concentrations providing from the clinical trials; PCTL: percentiles, IQR: interquartil range

B. Why was it necessary to optimize laboratory assays and perioperative management?

Dabigatran etexilate and rivaroxaban were firstly licensed for the prevention of VTE events in a postoperative context of some orthopaedic surgeries (total hip and knee surgery in the European Union). This indication has a different dose regimen (see Table 2) than the prevention of stroke in NVAF or VTE treatment.

Laboratory testing is not recommended routinely, as these drugs have a rapid onset and offset of action, short half-life, predictable pharmacokinetics and a large on-therapy range.

However, numerous publications came rapidly out to report haemorragic complications due to DOACs and pointed out the limit of the clinical phase III studies that allowed the license of the drug.

i. Limit of clinical phase III trials

A major criticism was that many frail patients who received DOACs in real-life were excluded from the clinical phase III trials. These patients were older, with lowerer body weight and had also several co-medications that interact with DOACs' metabolism. In an cross-sectionnel study conducted in 2010 including patients hospitalized for NVAF, 42% of these had at least one medication affecting P-glycoprotein.[53]

Sub-studies of these clinical phase III trials aimed at selecting specific clinical characteristics that increased the risk of bleeding. A reduced DOAC

dosage was recommended when specific medications with high drug-drug interaction and/or clinical characteristics based on age, renal clearance and body weight (see Table 2) were present.

Reilly *et al.* were the first to publish a link between adverse events and plasmatic concentrations of dabigatran. They described a cut off of dabigatran plasma concentrations inside of which patients had the lowest risk to present a thromboembolism (TE) or haemorragic event. As DOACs have a large therapeutic range, this analysis clearly demonstrated that high plasma concentrations of dabigatran could led to severe bleeding and suggested a follow up for specific patient populations such as frail patients or patients presenting haemorragic complications.

The debate around DOAC monitoring was intense, as a key strength of the marketing policy of these medications was the "no need of routine monitoring". Furthermore, reduced dosages were not validated according to specific plasma concentrations.

However, it rapidly became evident that coagulation testing could be of value in emergency situations to determine the presence or absence of DOACs, to plan the best timing of an invasive procedure since last DOAC administration, for the assessment of adherence, and to help evaluating the cause of ischemic stroke or bleeding.[54]

ii. Issues with routine coagulation assays

The DOACs have different effects on routine coagulation tests, such as the activated partial thromboplastin time (aPTT), the thrombin time (TT), and the prothrombin time (PT). Moreover, the responsiveness of these tests to the DOACs is coagulometer and reagent dependent [12,13].

Guidances based on routine tests were rapidly published as specific coagulation assays were not widely available and requested specialized laboratories and staff.

However, these proposals were not adequate as most of these routine tests were not sufficiently sensitive to measure quantitatively DOACs plasma concentration. Routine coagulation tests have a qualitative value that can inform about a range of DOACs plasma concentration.

For example, a normal aPTT was initially recommended to exclude the absence of a significant dabigatran effect.[54] However, normal aPTT do not systematically exclude dabigatran concentrations up to 60 ng/ml.

Cuker showed in a recent review that dabigatran prolonged the aPTT in a concentration-dependent manner in both ex vivo and in vitro studies.[55] The dose response was curvilinear, with a linear response up to a concentration of 200 to 300 ng/ml and then a flattening of the response for higher drug levels[56,57]. This relationship does not enable quantitative assessment of dabigatran levels, particularly at higher concentrations. Furthermore, the

sensitivity of dabigatran to commercial aPTT reagents differs widely, and therefore laboratories should perform dose-response studies using calibration standards to determine the sensitivity of their aPTT reagent and communicate the results to clinicians. When insensitive reagents are used, the aPTT may not be prolonged in the presence of typical on-therapy trough levels.

Thrombin time is the most sensitive coagulation test for dabigatran. However, it is not standardized between the laboratories and, depending on the reagent, dabigatran concentrations of as little as 25 and up to 150 ng/ml may exceed the limits of detection[56-59].

However, irrespectively of the reagent used, its normal range can exclude presence of clinically relevant concentrations of dabigatran. [55,60]

Both aPTT and TT suffer from several biological and clinical variables that need to be taken into account in the interpretation of the tests results.[60]

For direct anti-Xa agents like rivaroxaban and edoxaban, the PT is more prolonged than the aPTT in therapeutic ranges, but normal test results do not exclude a significant drug effect.[55]

Rivaroxaban and edoxaban prolonged the PT in spiked plasma in a concentration dependent and linear way.[61,62 63]

For rivaroxaban, typical trough (41 to 60 ng/ml) and peak (219 to 305 ng/ml) concentrations increased PT (in seconds) by 6% to 19% and 50% to

135%, respectively.[64-66] Thromboplastin reagents have variable sensitivities to rivaroxaban and edoxaban.[61,63,67]

An in vitro study showed that the interassay variability can be reduced by the use of an international sensitivity index specific for rivaroxaban, but not by conversion to an International Normalized Ratio (INR) used for the monitoring of VKA therapy.[68]

Laboratories should perform dose-response studies using calibration standards to determine the sensitivity of their particular PT method for rivaroxaban and edoxaban, and communicate the results to clinicians.[55]

For apixaban, the PT is not sensitive enough as a normal PT cannot exclude therapeutic ranges even at peak time, except for the reagent Triniclot PT Excel S®.[69] To detect rapidly high anticoagulant effect of apixaban in patients with bleeding complications and normal routine coagulation tests, some publications proposed the use of the dilute Russell's Viper Venom Time (dRVVT).[70]

There are no routine tests that have a comparable sensitivity for direct anti-Xa agents such as TT has for dabigatran. Therefore, laboratories need to use specific calibrated chromogenic assays to exclude clinically relevant presence of these agents.

When specific tests are not available, management of emergencies are based on routine coagulation tests, on the last timing of DOACs intake and on patient's renal clearance. The aim is to estimate roughly if the residual DOAC activity may potentially lead to bleeding or to guide the timing to emergent surgery. Using routine coagulation tests to follow the effect of bleeding treatment or antidotes administration is not accurate enough as normal ranges cannot exclude DOAC concentrations of 50-60 ng/ml (except TT for dabigatran). Furthermore, some studies on dabigatran with prothrombin complex administration did not show an improvement of aPTT,[71,72] and prolonged routine coagulation tests could also partly reflect an additional coagulopathy due to severe bleeding or polytrauma.[73]

Clinical trials using routine coagulation assays to screen for DOAC's presence need to provide detailed informations about the methodology and reagent used to allow potential comparison between their results and to enable some recommendations.

iii. Issues with specific tests for quantitative estimation of DOACs

To allow quantitative measurements of DOACs, the use of different specific coagulation assays have been largely validated in several publications.

These include the diluted TT (dTT), the ecarin clotting time (ECT), or the ecarin chromogenic assay (ECA) for dabigatran, and chromogenic anti-FXa assays for rivaroxaban, apixaban, and edoxaban.

On-therapy plasma concentration ranges of DOACs for VTE treatment and SPAF are presented in Table 2. The DOACs plasma concentration range varies according to the indication and dosing regimen. This needs to be taken into account when interpreting the test results. The elimination half-life of DOACs is relatively short compared with vitamin K antagonists. Therefore, the time of the last DOAC dose relative to blood sampling time must also be considered.

These specific assays are not widely available as it requested specialized laboratories and staff, and furthermore, its turnaround time (TAT) may be too long to guide rapidly clinicians who are faced to critical situations involving DOACs.

These tests showed a limit of detection with standard methodology and calibrators around 30 and 50 ng/ml, which is not accurate enough to guide experts' proposals based on DOACs plasma concentration.[74-79]

Furthermore, many specific assays (e.g. the dTT or most chromogenic assays for anti-Xa agents) are not specific to DOACs, as that they can falsely estimate higher DOACs plasma concentrations in the presence of heparin.

This interference is especially high with chromogenic assays using the same reagent for direct anti-Xa agents and heparins.

iv. Issues with different perioperative management proposals

An increasing number of patients are receiving a DOAC. In Belgium, the number has tripled in two years (i.e. 33000 patients in 2012 and 100 000 patients in 2014).[80]

Annually approximately 10-15% of such patients will require a DOAC interruption for an elective procedure.[81] Several reviews provided guidances regarding the perioperative management of patients on DOACs.[82-84] Their aim was to ensure mimimal to no anticoagulant effect at the time of the procedure, and to minimize the period without protection against thromboembolism.

Although this was an important step, especially for clinicians with a poor knowledge of these drugs, many proposals had a different strategy to guide the best timing to surgery.

The main approach to guide perioperative management in elective surgery was based on the elimination half-life of the drug, which is highly influenced by renal clearance in patients taking dabigatran. Despite drug-drug interactions that are frequently found in patients treated with NVAF, only few proposals add some extra delay to the surgery.[85] For rivaroxaban, there

is no guidance for patients with altered liver metabolism in the perioperative setting.

Many proposals integrated the bleeding risk of the surgery in the perioperative algorithm, allowing some low bleeding risk procedures to be realized with clinically significant residual anticoagulant effect of DOACs, without consequences on the patient's outcome.

For elective surgery, a good understanding of drug's elimination half-life may prevent DOAC monitoring. However, some patients may have unexpected health conditions that can prolong DOAC elimination, and a quantitative measurement of DOAC can be requested before a high bleeding risk procedure.

This is different for the emergency setting, where the estimation of DOACs plasma concentration has rapidly revealed to be crucial in guiding patients' management at a time where antidotes were not available, e.g. when an emergent surgery needed to be rapidly planned or the patient required aggressive transfusion of blood products.

The first proposals on DOACs plasma concentration in the emergency setting were provided by the *Groupe d'Intérêt en Hémostase Périopératoire* (GIHP). They proposed that the threshold of 30 ng/ml plasma concentration of dabigatran and rivaroxaban should provide adequate haemostasis for high bleeding risk procedures. If possible a delay to emergent surgery was

recommended, and a new estimation of DOACs plasma concentration was suggested before allowing the invasive procedure.[75-78,86] The threshold of 30 ng/ml was defined as the expected concentrations reached after three half-lives, when 87.5% of the Cmax has been eliminated, assuming that the mean Cmax is 215 and 175 ng/ml (Summary of Products Characteristics (SmPC) values for NVAF patients) for rivaroxaban and dabigatran, respectively. [79]

In the same idea, a report of the European Medecines Agency (EMA) recommended a dabigatran plasma concentration below 48 ng/ml before a surgery, which would correspond to 25% of the mean Cmax, reached after two half-lifes. [87]

It can be hazardous to consider only the elimination half-life of DOACs to estimate their residual concentration in emergency settings, as it does not take into account the high inter-individual variability of DOAC concentrations, which has recently been shown to have a poor correlation with patient's renal function, except for dabigatran concentrations at trough.[88]

However, the measurement of DOACs plasma concentration requires the most accurate laboratory tests for the suspected plasma range, which in turn depends on the clinical setting that need a monitoring (see Table 3). It is important to note that the proposed tests for each of the DOACs are subject to limitations with regard to their sensitivity, specificity, the experience

within the laboratory, the availability, the inter-reagent variability, the linearity of the response, as well as the current existence or absence of a known cut-off level for bleeding risks (see Table 3).

Accurate laboratory tests and exclusion of important biological or clinical interferences, may allow clinicians to take the best decision for the management of their patients.

This research project aimed at improving the use of some routine and specific coagulation assays frequently used in the perioperative context.

Table 3. Laboratory monitoring of direct oral anticoagulants

Drugs	Laboratory tests	Utility	Availability	Sensitivity/ Specificity	Dependence of the reagent	Linearity of the response
Dabigatran	**aPTT**	Limited: *Bleeding event/ Emergencies* *Reflects poorly the intensity of anticoagulation*	24/7 – all laboratories	± 100 ng/ml / No	Yes	No
	TT	Limited: *Exclude the presence of dabigatran in the peri-operative setting*	24/7 – all laboratories	Very sensitive - LOD 1ng/ml LOQ 2ng/ml / No	Yes	Depend on the reagent – until 50 ng/ml
	dTT **High and low calibrators**	*Bleeding or thrombotic event/ emergencies* *Accurate estimation of plasma concentrations (ng/ml)*	Requires trained staff – only in specialized laboratories	± 10 ng/ml / No	No	Yes
	ECT	Limited: *Standardisation and validation required*	Requires trained staff – only in specialized laboratories	± 15 ng/ml / No	Probably not but an inter-lot variability has been reported	Yes

Dabigatran	**ECA**	*Bleeding or thrombotic event/ emergencies* *Accurate estimation of plasma concentrations (ng/ml)*	Requires trained staff – only in specialized laboratories	± 10 ng/ml / No	No	No
Rivaroxaban/ (Edoxaban)	**PT**	Limited: *Bleeding event/ Emergencies* *Reflects poorly the intensity of anticoagulation*	24/7 – all laboratories	from ±100 to > 500 ng/ml (depending on the reagent) / No	Yes	Yes
Rivaroxaban / Apixaban / Edoxaban	**Chromogenic anti-Xa assays** **High and Low calibrators**	*Bleeding or thrombotic event/ emergencies* *Accurate estimation of plasma concentrations (ng/ml)*	Requires trained staff – only in specialized laboratories	± 10 ng/ml / Yes-No (depend on the anti-Xa assay)	No	Depends on the reagent
Dabigatran/ Rivaroxaban / Apixaban / Edoxaban	**LC-MS/MS**	*Not for emergencies* *Accurate estimation of plasma concentrations (ng/ml)*	Requires trained staff – only in specialized laboratories	LOD and LOQ around 1 and 3 ng/ml	Not applicable	Yes

Dabigatran/ Rivaroxaban / Apixaban / Edoxaban	**DRVV-T**	Partially proven: *Confirmation needed in plasma samples from patients treated with apixaban and edoxaban.* *Detection of apixaban in bleeding or thrombotic events/ emergencies.*	Only in specialized laboratories	± 100 to 200 ng/ml (depend on the reagent and the molecule) / No	Yes, but < to PT or aPTT	No

II. Objectives

Research questions

A. How can we improve the use of thrombin time in the perioperative management of patients on dabigatran etexilate?

B. Which specific assays are more accurate for the perioperative setting of patients on DOACs?

i. Comparison of the performance of coagulation tests specifically developed for the measurement of low plasma dabigatran concentrations with the reference LC-MS/MS method.

ii. Case series showing the discrepancy of dabigatran plasma concentrations estimation with the different coagulation assays in a perioperative context.

iii. Comparison of the performance of chromogenic assays specifically developed for the measurement of low rivaroxaban concentrations with the reference LC-MS/MS method. Impact of heparin bridging on their performance.

III. Results

Part 1

How can we improve the use of thrombin time in the perioperative management of patients on dabigatran etexilate?

Thrombosis Research 136 (2015) 693–696

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Correspondence

Is Thrombin Time useful for the assessment of dabigatran concentrations? An in vitro and ex vivo study

The stability of TT was compared using the Wilcoxon signed-rank test. We used Medcalc software version 6.0 for Windows® and GraphPad Prism® version 6.0 for Mac OSx®.

The study was approved by the Medical Ethical Committee of the CHU Dinant Godinne UcL Namur (BU3920096633). We prepared nor-

Is Thrombin Time useful for the assessment of dabigatran concentrations? An in vitro and ex vivo study

Sarah Lessire[1,2*], Jonathan Douxfils[2*], Justine Baudar[3], Nicolas Bailly[3], Anne-Sophie Dincq[1], Maximilien Gourdin[1], Jean-Michel Dogné[2], Bernard Chatelain[3], François Mullier[2,3]

[1] *Université catholique de Louvain, CHU UCL Namur, Namur Research Institute for Life Sciences (NARILIS), Namur Thrombosis and Hemostasis Center (NTHC), Department of Anesthesiology, Yvoir, Belgium*

[2] *University of Namur, Namur Research Institute for Life Sciences (NARILIS), Namur Thrombosis and Hemostasis Center (NTHC), Department of Pharmacy, Namur, Belgium*

[3] *Université catholique de Louvain, CHU UCL Namur, Namur Research Institute for Life Sciences (NARILIS), Namur Thrombosis and Hemostasis Center (NTHC), Hematology Laboratory, Yvoir, Belgium*

[*] Contributed equally

Guidance regarding dabigatran monitoring has been published[56,89], but the specific assays are not all targeted to low levels of dabigatran encountered in the preoperative setting. For example, the Hemoclot® Thrombin Inhibitor (HTI) and the ecarin chromogenic assay (ECA) have a limit of detection and quantification (LOD and LOQ) between 30 and 50 ng/ml [90-92]. In our recent study, a modified HTI (HTI LOW) and ECA (STA®-ECA II) adapted for low dabigatran concentrations showed better performances than conventional HTI to assess plasma dabigatran concentrations below 50 ng/ml [93], but these assays are not widely available. The activated partial thromboplastin time should not be used to assess dabigatran in plasma as it has only a modest correlation with dabigatran levels and is affected by factor deficiencies, lupus anticoagulant or elevated FVIII in inflammatory conditions [94].

Some recent publications have suggested that thrombin time (TT) could be useful to assess the presence of dabigatran to guide the peri-procedural management [55,57]. However, TT is affected by many variables [95] and there is a lack of standardization between laboratories [96].

Therefore, we decided to analyse TT following validation methods published by Marlar *et al.* and Chandler [97,98] in plasma samples

containing dabigatran. We assessed the TT of different spiked dabigatran concentrations with intra- and inter-assay precision, its stability in time (at 2, 4 and 24 hours) and at different temperatures (room temperature and 4°C), its LOD and LOQ for dabigatran, the linearity of the results, and finally, the sensitivity of TT to different spiked concentrations of unfractionated heparin (UFH) (Leo Pharma, Ballerup, Denmark) and enoxaparin (Clexane®, Sanofi-Aventis, Diegem, Belgium) compared to HTI. Afterwards, we analysed TT in 24 plasma samples from dabigatran treated patients expected to be in the low ranges.

The stability of TT was compared using the Wilcoxon signed-rank test. We used Medcalc software version 6.0 for Windows® and GraphPad Prism® version 6.0 for Mac OSx®.

The study was approved by the Medical Ethical Committee of the CHU Dinant Godinne UcL Namur (BU3920096633). We prepared normal pool plasma (NPP) and platelet-poor-plasma (PPP) samples and stored them as described previously [56]. Dabigatran was purchased from Alsachim® (Strasbourg, France) and spiked at increasing concentrations (0, 10, 20, 30, 40, 50, 100 and 200 ng/ml) in NPP as described previously [56]. These concentrations were designed to cover pre-procedural ranges (from 0 to 50 ng/ml) [86,99] and trough therapeutic

ranges (from 50 to 200 ng/ml). The limits of the trough therapeutic range represent the range in which ischemic stroke/systemic embolic events and major bleeding events are lowest [100]. Two thrombin reagents were tested (a bovine thrombin: HemosIL® TT, Instrumentation Laboratory, Lexington, KY, USA, and a human thrombin: STA®-Thrombin, Diagnostica Stago, Asnière, France) on two coagulometers (STA-R® Evolution and ACL TOP®700). We prepared 6 different concentrations of bovine thrombin (3.33, 3.75, 3.90, 4.05, 4.28 and 5.00 NIH/ml) and 4 different concentrations of human thrombin (1.36, 1.50, 1.76 and 2.00 NIH/ml). We defined the optimal thrombin concentration (OTC) for three arbitrarily selected conditions, as the minimal concentration that gave a reproducible TT:

- less than 120 seconds at 50 ng/ml;
- less than 300 seconds at 200 ng/ml;

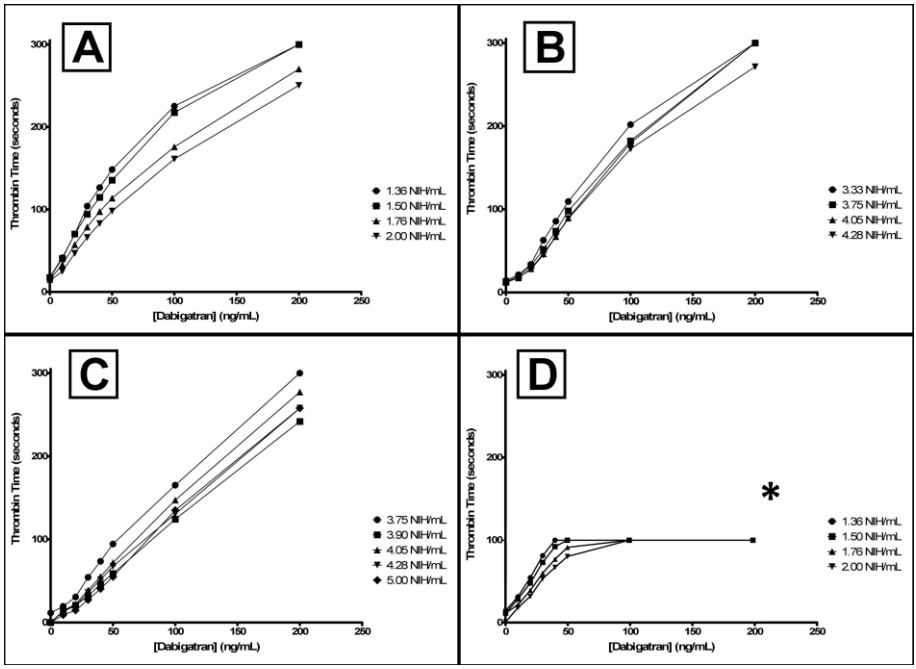
and higher than the minimum TT measurable with the coagulometer at 0 ng/ml of dabigatran (e.g. > 8 seconds for ACL TOP®).

We set TMAX at 300 seconds (instead of the recommended 120 seconds on STA-R® and 100 seconds on ACL TOP®) to allow measurement of dabigatran concentration up to 200 ng/ml. This was

technically not possible to achieve for the combination STA®-Thrombin on ACL TOP®.

As shown, in Fig.1. the OTCs were 1.76 NIH/ml for STA-R® with STA®-Thrombin, 4.28 NIH/ml for STA-R® with HemosIL® TT, 3.75 NIH/ml for ACL TOP® with HemosIL® TT and we found no OTC for ACL TOP® with STA®-Thrombin (TMAX = 100 seconds). The type of coagulometer (different mechanism of clot detection, i.e. mechanical (STA-R®) versus optical (ACL TOP®) clot detection) and, especially, the thrombin origin were two important variables. We decided afterwards to use a homogeneous system for the validation methods and chose two OTCs for each coagulometer (1.50 and 1.76 NIH/ml of STA®-Thrombin for STA-R®; 3.75 and 3.90 NIH/ml of HemosIL® TT for ACL TOP®). For STA®-Thrombin, the concentration recommended by the manufacturer is 1.50 NIH/ml. At this thrombin concentration, TT was sometimes > 120 seconds for dabigatran concentrations of 50 ng/ml, so that it failed to respect one of the OTC's arbitrary conditions, but for routine facilities we tested it further. For HemosIL® TT, the manufacturer's recommendations (1.9, 3.0 and 7.5 NIH/ml) were never optimal following our arbitrary definitions (preliminary tests not shown).

Fig 1. Optimization of thrombin time on different coagulometers and with different reagents



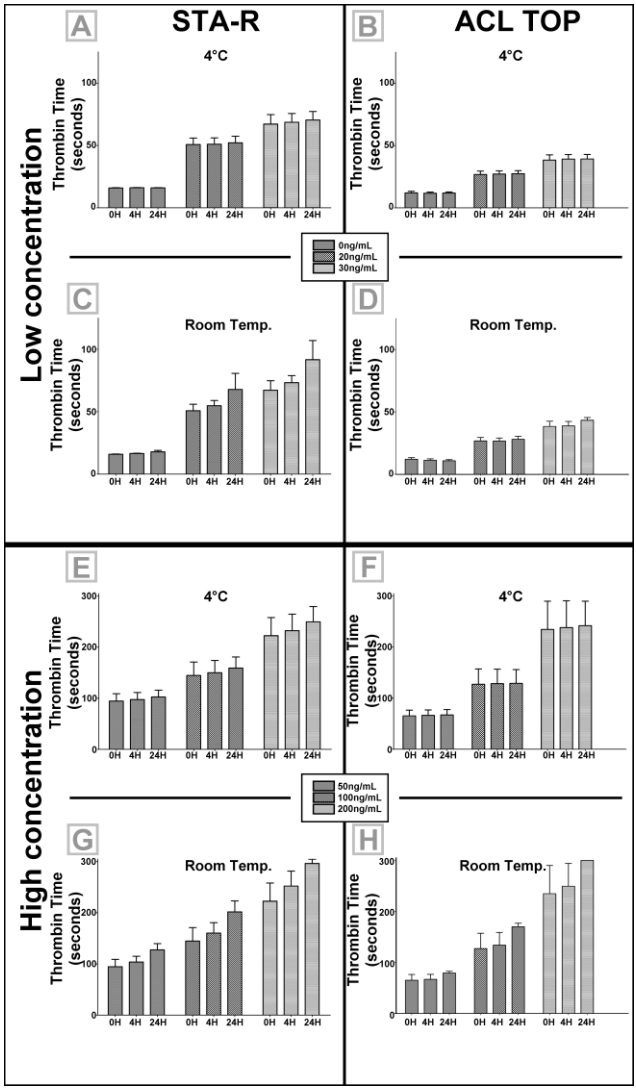
A) STA-R Evolution®, STA®-Thrombin; B) STA-R Evolution®, HemosIL® TT; C) ACL TOP®700, HemosIL® TT; D) ACL TOP®700, STA®-Thrombin. Thrombin times < 8 seconds were placed at zero in images C and D. Thrombin time > 300 seconds (A-C) or 100 seconds (D)* were set at 300 and 100 seconds, respectively.

Our repeatability experiments showed an acceptable variability (below 10% for intra-assays and below 12% for inter-assays) [97]. ACL TOP® with HemosIL® TT showed higher CV% than STA-R® with STA® Thrombin. This may be explained by the mechanism of clot detection. Mechanical clot detection (STA-R®) may be preferable to optical clot detection (ACL TOP®) due to its increased sensitivity [101] and greater precision [102].

Stability experiments were tested for one OTC on each coagulometer. A slight increase of TT appeared after 2 hours of storage at room temperature. For 1.50 NIH/ml on STA-R®, the stability tests showed a statistically significant increase in TT for all dabigatran concentrations when plasma samples were stored at room temperature for 4 or 24 hours. For plasma samples stored at 4°C, we observed statistically and clinically significant increases in TT for samples with dabigatran concentrations > 30 ng/ml that were stored for 24 hours.

For 3.75 NIH/ml on ACL TOP®, only plasma samples stored at room temperature showed a statistically and clinically significant increase in TT for dabigatran concentrations > 30 ng/ml and stored for 24 hours. The stability for the different dabigatran concentrations at room temperature and 4°C is shown in Fig. 2.

Fig 2. Stability of plasma samples containing low and high dabigatran concentrations, at 4°C and room temperature.



A, C, E and G correspond to the measurements of TT (mean + SD) on STA-R® Evolution with STA®-Thrombin at 1.50 NIH/ml. B, D, F and H correspond to the measurements of TT (mean + SD) on ACL TOP® 700 with HemosIL® TT at 3.75 NIH/ml.

This time-related increase in TT may be due to platelet and soluble coagulation factor activation [103]. These findings should be validated on samples from patients treated with dabigatran. We suggest to measure TT as soon as possible (within 2 hours of sampling) in plasma containing dabigatran. If this is not possible, samples should be rapidly stored at 4°C.

We tested TT and HTI in the presence of UFH and enoxaparin both in NPP at 0, 0.15, 0.30, 0.60, 0.90, 1.20, 1.50 and 2.00 UI/ml. Thrombin time was above the reference range at a concentration of 0.15 UI/ml of UFH, 0.30 UI/ml of enoxaparin whereas the HTI gave a false positive result at 0.60 UI/ml of UFH and 1.20 UI/ml of enoxaparin on STA-R®. The aim of this comparison was to show the differences in sensitivity of TT to two different heparins and to underline the limit of HTI in patients bridged with heparin. A commercially available heparinase can be added to the blood sample to detect the presence of heparin [104].

Limit of detection and LOQ were lower than 0.9 and 3.0 ng/ml respectively for STA®-Thrombin on STA-R®, and lower than 2.2 and 7.2 ng/ml for HemosIL® TT on ACL TOP®. As stated in previous publications [55,56], TT is a reliable test to exclude clinically relevant dabigatran presence in the blood.

Following the linear regressions presented in Table 1, TT measured with STA®-Thrombin concentration proposed by the manufacturer (1.5 NIH/ml) can also detect dabigatran concentrations < 50 ng/ml (R-square = 0.97). In addition, optimised HemosIL® TT on ACL TOP® is a reliable qualitative test for dabigatran concentrations up to 200 ng/ml (R-squares = 0.98), which is not the case for STA®-Thrombin on STA-R® (R-squares = 0.78 and 0.75 for 1.5 NIH/ml and 1.76 NIH/ml respectively).

Table 1. Linearity of thrombin time and dabigatran concentrations following optimized thrombin concentrations

	1.50 NIH/ml STA®-Thrombin STA-R® Evolution	**1.76 NIH/ml STA®-Thrombin STA-R® Evolution**	**3.75 NIH/ml HemosIL® TT ACL TOP®700**	**3.90 NIH/ml HemosIL® TT ACL TOP®700**
For dabigatran concentrations: 0, 30, 50, 100 and 200 ng/ml				
R-square	0.78	0.75	0.98	0.98
For dabigatran concentrations: 0, 30 and 50 ng/ml				
R-square	0.97	0.99	0.98	0.99

Therefore, we do not support the suggestion of Chin *et al.* [105] to use TT to assess trough dabigatran concentrations and adjust treatment doses. Furthermore, other studies are needed to validate the improvement in outcomes with dose adjustment related to plasma levels and patient characteristics [100,106].

In plasma samples from patients treated with dabigatran etexilate, TT was measured under routine conditions (1.50 NIH/ml of STA®-Thrombin on STA-R®). Most samples (12/13) with a TT < 120 seconds had a dabigatran concentration < 50 ng/ml (confirmed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)). The only exception was a sample with a free dabigatran of 76 ng/ml and a TT of 110.3 seconds, collected in a context of an inflammatory syndrome. Seven samples had a normal TT with a LC-MS/MS measuring maximum 4 ng/ml. One sample had a TT of 64.3 seconds and no dabigatran measured, but this patient was switched to therapeutic enoxaparin.

Most samples (10/11) with a TT ≥ 120 seconds had a dabigatran concentration > 50 ng/ml, except for one patient (free dabigatran: 28 ng/ml) who was admitted to the intensive care unit with a medical history suggesting presence of fibrin/fibrinogen degradation products.

These results emphasize the importance of taking into account the clinical context in the interpretation of TT.

In terms of limitations, the conclusions might not be generalizable to other coagulometers and our ex vivo results should be confirmed in a larger population.

We conclude that TT may be used as an alternative in assessing low dabigatran concentrations (< 50 ng/ml), especially for laboratories that do not have access to specific assays. However, clinicians also need to consider all the different variables affecting TT. Thrombin time should not be used to assess dabigatran concentrations in the normal therapeutic range.

Part 2

Comparison of the performance of two coagulation tests specifically developed for the measurement of low plasma dabigatran concentrations with the reference LC-MS/MS method.

Estimation of dabigatran plasma concentrations in the perioperative setting

An *ex vivo* study using dedicated coagulation assays

Jonathan Douxfils[1*]; Sarah Lessire[1,2*]; Anne-Sophie Dincq[2]; Paul Hjemdahl[3]; Yuko Rönquist-Nii[3]; Anton Pohanka[3]; Maximilien Gourdin[2]; Bernard Chatelain[4]; Jean-Michel Dogné[1]; François Mullier[1,4]

[1]Department of Pharmacy, Namur Thrombosis and Hemostasis Center (NTHC), Namur Research Institute for LIfe Sciences (NARILIS), University of Namur, Belgium; [2]Department of Anaesthesiology, Namur Thrombosis and Hemostasis Center (NTHC), Namur Research Institute for LIfe Sciences (NARILIS), CHU Dinant Godinne UcL Namur, Université Catholique de Louvain, Belgium; [3]Department of Clinical Pharmacology, Karolinska University Hospital and Clinical Pharmacology Unit, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden; [4]Haematology Laboratory, Namur Thrombosis and Hemostasis Center (NTHC), Namur Research Institute for LIfe Sciences (NARILIS), CHU Dinant Godinne UcL Namur, Université Catholique de Louvain, Belgium

Summary

The perioperative management of dabigatran is challenging, and recommendations based on activated partial thromboplastin time (aPTT) and thrombin time (TT) are unsatisfactory. Dedicated coagulation tests have limitations at plasma concentrations < 50 ng/ml. Therefore, a more sensitive test, which is available 24/7, is required. It was the aim of this study to investigate the performance of the Hemoclot Thrombin Inhibitors® LOW (HTI LOW) kit, a diluted thrombin time, and the STA® – ECA II (ECA-II) kit, a chromogenic variant of the ecarin clotting time, that were developed to measure low dabigatran concentrations, compared to reference dabigatran analysis by liquid chromatography tandem mass-spectrometry (LC-MS/MS). This study included 33 plasma samples from patients treated with dabigatran etexilate who had plasma concentrations < 200 ng/ml. HTI LOW and ECA-II were performed along with HTI, aPTT (STA®-C. K.Prest® and SynthasIL®) and TT (STA® – Thrombin). All procedures were performed according to recommendations by the manufacturers. Linear (or curvilinear) correlations and Bland-Altman analyses were calculated. For free dabigatran concentrations < 50 ng/ml, the R^2 of linear correlations were 0.69, 0.84 and 0.61, with HTI, HTI LOW and ECA-II, respectively. The R^2 for TT, STA®-C. K.Prest® and SynthasIL® were 0.67, 0.42 and 0.15. For HTI, HTI LOW and ECA-II, Bland-Altman analyses revealed mean differences of –6 ng/ml (95 %CI: –25–14 ng/ml), 1 ng/ml (95 %CI: –18–19 ng/ml) and –1 ng/ml (95 %CI: –25–23 ng/ml), demonstrating that tests dedicated to measuring low concentrations are more accurate than HTI. In conclusion, the use of HTI LOW or ECA-II to assess low plasma dabigatran concentrations is supported by our findings.

Keywords

Dabigatran, monitoring, perioperative, guidelines

Correspondence to:
Jonathan Douxfils
Department of Pharmacy
Namur Thrombosis and Hemostasis Center (NTHC)
Namur Research Institute for LIfe Sciences (NARILIS)
University of Namur, B-5000, Belgium
E-mail: jonathan.douxfils@unamur.be

* These authors contributed equally.

Received: September 30, 2014
Accepted after major revision: November 7, 2014
Epub ahead of print: December 18, 2014

http://dx.doi.org/10.1160/TH14-09-0808
Thromb Haemost 2015; 113: 862–869

Estimation of dabigatran plasma concentrations in the perioperative setting:

An ex vivo study using dedicated coagulation assays

Jonathan Douxfils[1*], Sarah Lessire[1,2*], Anne-Sophie Dincq[2], Paul Hjemdahl[3], Yuko Rönquist-Nii[3], Anton Pohanka[3], Maximilien Gourdin[2], Bernard Chatelain[4], Jean-Michel Dogné[1], François Mullier[1,4]

[1] University of Namur, Namur Research Institute for LIfe Sciences (NARILIS), Namur Thrombosis and Hemostasis Center (NTHC), Department of Pharmacy, Namur, Belgium

[2] Université catholique de Louvain, CHU UCL Namur, Namur Research Institute for LIfe Sciences (NARILIS), Namur Thrombosis and Hemostasis Center (NTHC), Department of Anaesthesiology, Yvoir, Belgium

[3] Karolinska University Hospital and Clinical Pharmacology Unit, Department of Clinical Pharmacology, Department of Medicine Solna, Karolinska Institutet, SE-171 76 Stockholm, Sweden.

[4] Université catholique de Louvain, CHU UCL Namur, Namur Research Institute for LIfe Sciences (NARILIS), Namur Thrombosis and Hemostasis Center (NTHC), Haematology Laboratory, Yvoir, Belgium

[*]contributed equally

ABSTRACT

Background

The perioperative management of dabigatran is challenging and recommendations based on activated partial thromboplastin time (aPTT) and thrombin time (TT) are unsatisfactory. Dedicated coagulation tests have limitations at plasma concentrations <50ng/ml. Therefore, a more sensitive test, which is available 24/7, is required.

Aim

To investigate the performance of the Hemoclot Thrombin Inhibitors® LOW (HTI LOW) kit, a diluted thrombin time, and the STA® - ECA II (ECA-II) kit, a chromogenic variant of the ecarin clotting time, that were developed to measure low dabigatran concentrations, compared to reference dabigatran analysis by liquid chromatography tandem mass-spectrometry (LC-MS/MS).

Materials

This study included 33 plasma samples from patients treated with dabigatran etexilate who had plasma concentrations <200ng/ml. HTI LOW and ECA-II were performed along with HTI, aPTT (STA®-C.K.Prest® and SynthasIL®) and TT (STA® - Thrombin). All procedures were performed according to recommendations by the

manufacturers. Linear (or curvilinear) correlations and Bland-Altman analyses were calculated.

Results and discussion

For free dabigatran concentrations <50ng/ml, the R^2 of linear correlations were 0.69, 0.84 and 0.61, with HTI, HTI LOW and ECA-II, respectively. The R^2 for TT, STA®-C.K.Prest® and SynthasIL® were 0.67, 0.42 and 0.15. For HTI, HTI LOW and ECA-II, Bland-Altman analyses revealed mean differences of -6ng/ml (95%CI: -25 – 14ng/ml), 1ng/ml (95%CI: -18 – 19ng/ml) and -1ng/ml (95%CI: -25 – 23ng/ml), demonstrating that tests dedicated to measuring low concentrations are more accurate than HTI.

Conclusions

The use of HTI LOW or ECA-II to assess low plasma dabigatran concentrations is supported by our findings.

INTRODUCTION

Pradaxa®, dabigatran etexilate, has received its market authorization for various indications worldwide and was developed and marketed to be used in fixed dose regimens without a need for regular monitoring. However, assessments of drug plasma levels are desired to ensure a safe use of the product in several specific situations [107]. In this context, the perioperative management of dabigatran is quite challenging especially in the absence of a specific reversal agent. During the two years of follow-up in the RE-LY study, approximately 25% of the patients underwent at least one invasive procedure. Compared with warfarin, dabigatran is associated with similar rate of perioperative bleeding and thrombotic complications, even among patients having major or urgent surgery [108]. However, in patients who had treatment withdrawn > 3 days before the intervention dabigatran was associated with more bleeds, perhaps at least in part due to accumulation of the drug in patients with poor renal function. Furthermore, the equality of outcomes should be viewed in relation to how warfarin-related bleeds or risk of bleeding were handled in the study. Thus, vitamin K was given perioperatively to fewer than 5% of warfarin-treated patients. Fresh-frozen plasma was given to 2%, and prothrombin complex concentrate treatment was apparently not used at all. The comparison

of bleeding problems might have been less favourable for dabigatran if patients taking warfarin would more often have received treatments that are recommended by expert opinion and guidelines [109]. This underlines the necessity to implement risk minimization strategies in the perioperative management of dabigatran in absence of specific antidote, especially for high-risk surgery, e.g. major interventions or neurosurgery.

In the initial assessment report of the European Medicines Agency (EMA), the marketing authorization holder informed that a dabigatran concentration below 48 ng/ml is equivalent to elimination of at least 75% of dabigatran and should be reached before invasive intervention [110]. The "Groupe d'Intérêt en Hémostase Périopératoire (GIHP)" is much more conservative and put the threshold at 30 ng/ml [86]. A diluted Thrombin Time (dTT, i.e. the Hemoclot Thrombin Inhibitors®, Hyphen BioMed, Neuville-Sur-Oise, France) has been proposed for accurate determinations of dabigatran in plasma, but this test was not intended for measurements of low levels of dabigatran [111]. Other studies have assessed the performance of this test, but also of a chromogenic variant of the well-established ecarin clotting time [112]. Both tests had lower limits of quantitation between 30 and 50 ng/ml [89-

[92]. Therefore, a specific laboratory test, which is accurate in the low concentration range and available 24/7, is highly requested. So far, most routine coagulation tests for assessments of dabigatran concentrations or effects in the lower concentration range have been too insensitive. This limits their usefulness in the perioperative setting, and the use of the undiluted thrombin time test (TT) is currently presented as an alternative due to its very high sensitivity to dabigatran [56,95,105]. This approach, although interesting, has some limitations because TT is affected by several analytical and biological variables making its standardization difficult [113].

In this study, we investigated the performance of two coagulation tests specifically developed for measurements of low plasma dabigatran concentrations (the Hemoclot Thrombin Inhibitors® LOW (HTI LOW), a diluted thrombin time (Hyphen BioMed®, Neuville-sur-Oise, France) and the STA®-ECA II (ECA-II), a chromogenic variant of the ecarin clotting time (Diagnostica Stago®, Asnière-sur-Seine, France)) and to compare their results to direct measurements by a reference liquid chromatography coupled with a tandem mass-spectrometry (LC-MS/MS) method [89]. We also assessed the performances of the standard procedure of HTI (Hyphen BioMed®) and the activated

partial thromboplastin time (aPTT), which are recommended in the EMA documents [110,114], as well as of the TT.

MATERIALS AND METHODS

The study was performed in accordance with the Declaration of Helsinki and was approved by the Medical Ethics Committee of the Centre Hospitalier Universitaire Dinant Godinne UcL Namur in Yvoir, Belgium. Written informed consent was obtained from each donor.

Clinical samples and normal pooled plasma

Thirty-three plasma samples from patients treated with dabigatran etexilate for stroke prevention in non-valvular atrial fibrillation were included in the study. Blood was taken by venipuncture in the antecubital vein and collected into 0.109 M sodium citrate (9:1 v/v) tubes (Venosafe®, Terumo, Heverlee, Belgium) using a 21-gauge needle (Terumo). Platelet-poor plasma (PPP) was obtained from the supernatant fraction after double centrifugation for 15 minutes at 1500g at room temperature. Afterwards, plasma was aliquoted and frozen at –80°C without delay. Frozen plasma samples aliquots were thawed and heated to 37°C for at least 5 minutes just before experiments. Plasma samples were taken randomly and included in

this study after a first assessment of plasma dabigatran concentrations using the HTI to select samples with plasma concentrations <200 ng/ml. The exact plasma level of dabigatran was measured using a reference LC-MS/MS method, as described below [89].

Normal pooled plasma (NPP) was obtained from the PPP of 27 healthy individuals which was prepared as for the clinical samples. The exclusion criteria for the healthy individuals were: thrombotic and/or haemorrhagic events, pregnancy, and use of antiplatelet and/or anticoagulant medication and/or drugs potentially affecting platelet and/or coagulation factor functions during two weeks prior to sampling.

Liquid chromatography coupled with tandem mass spectrometry

This method has been described previously [89]. Briefly, plasma concentrations of free dabigatran were determined after sample preparation by protein precipitation of 50 µL citrated plasma with 150 µL acetonitrile containing dabigatran-d3 as an internal standard. The calibration curve for dabigatran in plasma was linear over the range 1-400 ng/ml and the limit of detection (LOD) was estimated to be <0.5 ng/ml. Validation experiments with three levels of control samples (8.1, 202 and 393 ng/ml) on three different occasions (six

determinations per concentration), showed an inter- and intra-assay precision between 6.0% and 9.2% and between -0.9% and 3.6%, respectively.

Coagulation assays

For the ordinary HTI assay, a diluted thrombin time (dTT), the tested plasma was diluted 1:8 in Owren-Koller® buffer. Fifty µL of diluted plasma were mixed with 100 µL of NPP and were incubated during 240 seconds. One hundred µL of highly purified human thrombin, in the α-form pre-incubated at 37°C was then added to start the reaction. The procedure was calibrated with a 3-calibrators kit at 35, 240 and 470 ng/ml (Hyphen BioMed®). For HTI LOW, the methodology of the test was the same (i.e. it used the same reagent than HTI) except that 1) the dilution of the sample was reduced to 1:2 compared to 1:8 for the normal procedure, and that 2) specific calibrators (and controls) at lower concentrations (0, 57 and 110 ng/ml) were required (Hyphen BioMed®) to establish a new calibration curve. All calibrations were defined by linear correlation.

For ECA-II, the tested plasma was diluted 1:5 in Owren-Koller® buffer. Fifty µL of diluted plasma were mixed with 140 µL of prothrombin and then 70 µL of chromogenic substrate were added and

incubated during 240 seconds. Seventy µL of ecarin, pre-incubated at 37°C, was then added to start the reaction. Optical density was followed during 40 seconds (from 70 to 110 seconds after ecarin addition) and the result was expressed as the difference of OD/min. The procedure was calibrated with a 5-calibrator kit at 0, 50, 96, 177 and 240 ng/ml (Diagnostica Stago®) and results were fitted by an exponential decay equation model. For results above 230 ng/ml, the ECA-II proposed an automatic re-dilution of the sample (1:10 instead of 1:5). Thrombin Time (TT), using STA®-Thrombin at 1.5 NIH units/ml, with an upper limit of measurement (ULM, i.e. the maximal clotting time for which the coagulometer provides a numerical result) extended to 300 seconds (Diagnostica Stago®; NPP baseline clotting time: 16.8 seconds; standard deviation: 0.3 seconds; local acceptable range: 13.5 – 20.5 seconds) and the activated partial thromboplastin time (aPTT), using STA®-C.K.Prest® (Diagnostica Stago®; NPP baseline clotting time: 30.3 seconds; standard deviation: 0.2 seconds; acceptable range: 26.4 – 37.6 seconds) and SynthasIL® (Instrumentation Laboratory®; NPP baseline clotting time: 31.4 seconds; standard deviation: 0.2 seconds; local acceptable range 26.0 – 38.0 seconds) reagents were also measured to compare the performance of each of these tests in presence of low concentrations

of dabigatran.All procedures were performed on a STA-R Evolution® coagulometer (Diagnostica Stago®), according to the recommendations of the manufacturers.

Statistical analyses

Statistical analyses were performed using GraphPad Prism version 5.00 (GraphPad Software, San Diego California, USA, www.graphpad.com) for Windows. Results for coagulation tests expressed in ng/ml were compared to the LC-MS/MS method by linear regression. Bland-Altman analyses were also performed and were calculated as follows: Difference (A-B)/average, where A was the result of the corresponding coagulation test and B, the result of the LC-MS/MS.

Thrombin time showed a linear regression while aPTT is better fitted by a curvilinear (first-order) relation described by the following model: Y=Y0 + (Plateau-Y0)*(1-exp(-K*x)).

Sensitivity was defined as the concentration in dabigatran in the original sample required to double the clotting time or halve the optical density per minute (OD/min). For the coagulation assays, the lower limit of detection (LOD) and the lower limit of quantitation (LOQ) were calculated as follows: LOD = [(3*standard deviation of Y0)/ slope] and LOQ = [(10*standard deviation of Y0/ slope]. The

standard deviation of Y0 was calculated by running the tests 10 times with NPP.

RESULTS

The plasma concentration range of free dabigatran was from 0 to 200 ng/ml according to LC-MS/MS measurements. ►**Table 1** summarises data on plasma drug concentrations according to the time elapsed since dabigatran etexilate administration. Among these samples, 17 were between 0 and 50 ng/ml. For assays that express results in ng/ml (i.e. HTI, HTI LOW and ECA-II) the linear correlation parameters and the Bland-Altman analyses versus exact plasma concentrations are provided in ►**Table 2** and represented in ►**Figure 1** for concentrations between 0 and 200 ng/ml. A stratification for results between 0 and 50 ng/ml is also provided in the same table and figure. ►**Figure 2** shows the results for TT and aPTT.

Table 1: Median plasma drug concentrations according to the time elapsed since dabigatran etexilate administration.

	LC-MS/MS (ng/ml)	HTI (ng/ml)	HTI LOW (ng/ml)	STA®-ECA-II (ng/ml)	STA®-Thrombin (s)	SynthasIL® (s)	STA®-C.K.PREST® (s)
	Time elapsed since the last dose: 0 hours† (n=2)						
Median	0	0	0	0	18.8	31.7	32.5
Range (min – max)	0 to 0	0 to 0	0 to 0	0 to 0	18.6 to 19.0	31.5 to 31.8	27.5 to 37.5
	Time elapsed since the last dose: 2 hours (n=4)						
Median	133	122	138	153	229.2	56.8	52.9
Range (min –max)	65 to 185	64 to 191	87 to 168	77 to 204	18.1 to M > MMax‡	32.6 to 59.3	27.5 to 56.9
	Time elapsed since the last dose: 3 hours (n=4)						
Median	125	119	132	146	235.4	51.1	48.3
Range (min – max)	49 to 200	68 to 194	79 to 179	77 to 161	215.5 to 292.5	42.1 to 57.7	42.2 to 57.1
	Time elapsed since the last dose: 12 hours (n=5)						
Median	76	46	76	83	157.8	48.2	42.9
Range (min – max)	52 to 88	22 to 83	61 to 103	65 to 118	103.3 to 233.5	38.3 to 56.5	38.7 to 54.6

	Time elapsed since the last dose: 20 hours (n=8)						
Median	41	17	42	34	91.7	39.5	40.4
Range (min – max)	0 to 76	0 to 56	0 to 95	0 to 83	19.9 to 189.2	24.8 to 52.5	24.2 to 46.5
	Time elapsed since the last dose: Unknown (n=10)						
Median	9	0	1	8	26.5	32.0	28.7
Range (min – max)	0 to 40	0 to 30	0 to 41	0 to 37	16.7 to 80.2	21.9 to 55.2	22.7 to 35.5

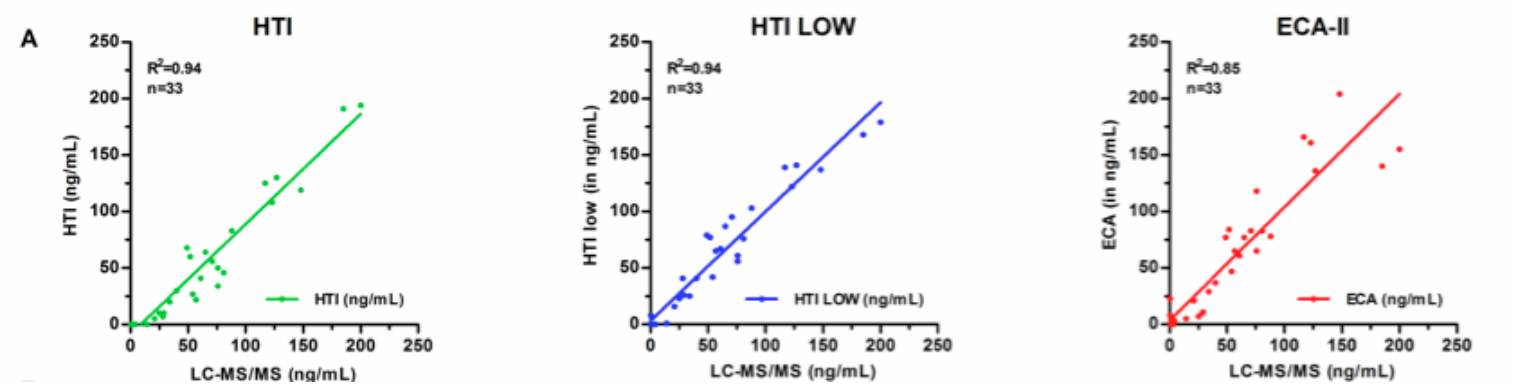
Table 1. Two hours and 3 hours correspond to the expected C_{max} of the drug while 12 hours corresponds to the C_{min}. Plasma samples taken more than 20 hours after the last intake of dabigatran etexilate were grouped together. For 11 samples, the exact time elapsed since the last intake was unknown.

[†] *0 hours correspond to baseline value (before the first intake of dabigatran etexilate)*

[‡] *M>MMax means that the upper limit of measurement (ULM) of the test (i.e. 300 seconds) was exceeded*

HTI: Hemoclot Thrombin Inhibitors®; LC-MS/MS: Liquid chromatography coupled with tandem mass-spectrometry

Figure 1. Results of the linear correlation (A) and the Bland-Altman analyses for the entire concentration range (B) for HTI, HTI LOW and ECA-II compared to LC-MS/MS.



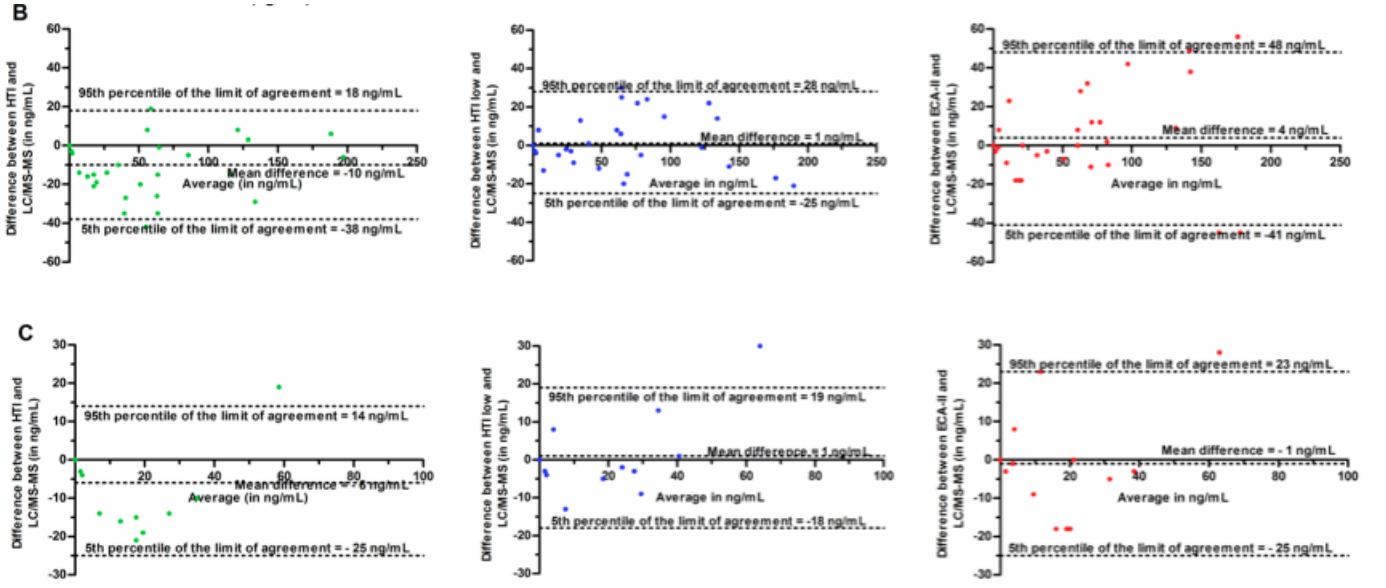


Fig 1. Bland-Altman analyses were also extracted for concentration specifically <50 ng/ml (C). Bland-Altman analyses were calculated as follow: Difference (A-B)/average, where A was the result of the corresponding coagulation test and B, the result of the LC-MS/MS. *HTI: Hemoclot Thrombin Inhibitors® - HTI LOW: Hemoclot Thrombin Inhibitors® - ECA-II: STA®-ECA II*

Linear and curvilinear correlations

For concentrations between 0 and 200 ng/ml, the R^2 of the linear correlations were 0.94, 0.94 and 0.85 for HTI, HTI LOW and ECA-II, respectively. For STA®-Thrombin, the R^2 of the linear correlation was 0.79 and the clotting time ranged from 16.7 to 292.5 seconds. For aPTT, the R^2 were 0.62 and 0.43 for STA®-C.K.Prest® and SynthasIL®, respectively. The clotting times ranged from 22.7 to 57.1 seconds for STA®-C.K.Prest® and from 21.9 to 59.3 seconds for SynthasIL®.

Figure 2

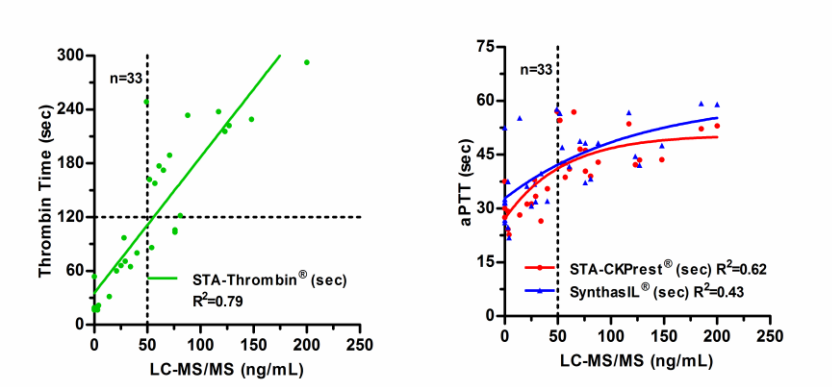


Fig 2. Results of the linear correlation for TT (STA®-Thrombin at 1.5 NIH/ml; NPP baseline clotting time: 16.8 seconds; standard deviation: 0.3 seconds; acceptable range: 13.5 – 20.5 seconds) and the curvilinear correlation for aPTT (STA®-C.K.Prest®; NPP baseline clotting time: 30.3 seconds; standard deviation: 0.2 seconds; acceptable range: 26.4 – 37.6 seconds and SynthasIL®; NPP baseline clotting time: 31.4 seconds; standard deviation: 0.2 seconds; acceptable range 26.0 – 38.0 seconds), respectively.

For concentration below 50 ng/ml, the R^2 of the linear correlations were 0.69, 0.84 and 0.61 for HTI, HTI LOW and ECA-II, respectively. The R^2 of the linear correlation for TT was 0.67 and the longest clotting time for this range of concentrations was 248.7 seconds for a sample with 49 ng/ml. For aPTT, linear correlation R^2 values of 0.42 and 0.15 were found for STA®-C.K.Prest® and SynthasIL®, respectively. The highest clotting times in this concentration range were 57.1 and 57.7 seconds for STA®-C.K.Prest® and SynthasIL® for the sample with a plasma concentration of 49 ng/ml.

Bland-Altman analyses

For concentration between 0 and 200 ng/ml, the Bland-Altman analysis showed a mean difference of -10 ng/ml (95%CI: -38 to 18 ng/ml), 1 ng/ml (95%CI: -25 to 28 ng/ml) and 4 ng/ml (95%CI:-41 to 48 ng/ml) for HTI, HTI LOW and ECA-II, respectively.

For concentration below 50 ng/ml, the Bland-Altman analysis showed a mean difference of -6 ng/ml (95%CI: -25 to 14 ng/ml), 1 ng/ml (95%CI: -18 to 19 ng/ml) and -1 ng/ml (95%CI:-25 to 23 ng/ml) for HTI, HTI LOW and ECA-II, respectively.

Table 2. Summary of results obtained with the different analyses as related to direct measurements of dabigatran in plasma by LC-MS/MS.

	HTI	HTI LOW	STA®-ECA-II	STA®-Thrombin
	0 to 200 ng/ml (n=33)			
R-square	0.94	0.94	0.85	0.79
slope	0.9748	0.9663	1.002	
95% CI	*(0.8803 to 1.069)*	*(0.8773 to 1.055)*	*(0.8490 to 1.154)*	
intercept	-8.633	3.204	3.635	
95% CI	*(-15.93 to -1.336)*	*(-3.672 to 10.08)*	*(-8.159 to 15.43)*	
Bland-Altman (in ng/ml)	-10	1	4	
95% CI	(-38 to 18)	(-25 to 28)	(-41 to 48)	
	0 to 50 ng/ml (n=17)			
R-square	0.69	0.84	0.61	0.67
slope	0.8740	1.219	0.9403	
95% CI	*(0.5516 to 1.197)*	*(0.9268 to 1.511)*	*(0.5302 to 1.351)*	
intercept	-4.03	-2.629	-0.2403	
95% CI	*(-11.60 to 3.00)*	*(-8.994 to 3.736)*	*(-9.182 to 8.701)*	
Bland-Altman (in ng/ml)	-6	1	-1	
95% CI	*(-25 to 14)*	*(-18 to 19)*	*(-25 to 23)*	

Table 2. Results are given for the linear regression (R^2, slope, intercept) and the Bland-Altman analyses. Results are also stratified for dabigatran concentrations up to 200 ng/ml and <50 ng/ml.

Sensitivity and Limits of Detection/Quantitation

The concentrations of dabigatran in the sample required to double the clotting time of HTI, HTI LOW, TT, aPTT (STA®-C.K.Prest®) and aPTT (SynthasIL®) were 298, 67, 6, 585 and 551 ng/ml, respectively. For ECA-II, the concentration of dabigatran required to halve the DO/min was 102 ng/ml. The LOD and LOQ were 8 and 25, respectively, for HTI compared to 2 and 7 ng/ml for HTI LOW. For ECA-II the entire calibration curve is not defined by a linear correlation but by a second order polynomial relation. Therefore, we took only the 3 first points of the calibration curve, i.e. 0, 50 and 96 ng/ml, to calculate the LOD and LOQ for ECA-II. These 3 points were well defined by a linear correlation model ($R^2 = 1.00$), thus. The LOD and LOQ for ECA-II in this concentration range were 14 and 46 ng/ml while for TT, the LOD and LOQ were 1 and 2 ng/ml, respectively.

DISCUSSION

Currently, there is no clear consensus regarding the perioperative management of patients treated with dabigatran etexilate, especially not among patients with impaired renal function in whom longer time periods are required to eliminate the drug [115]. While several authors recommend the use of a dTT, such as HTI, or an aPTT in the perioperative context [54,116], their insufficient sensitivity [89-92] limits their utility in patients with low plasma levels of dabigatran [100,113]. The inaccuracy of HTI at low

concentrations may not change the clinical outcomes in invasive interventions with low bleeding risk. However, in connection with high-risk surgery, such as neurosurgery, a more accurate assessment may be needed and risk minimization strategies should be provided.

Thrombin time is an alternative in this context due to its very high sensitivity towards dabigatran [117]. However, it is influenced by several biological and analytical variables [57]. Thus, up to now, anaesthetists, surgeons and doctors involved in critical care units are so far ill-equipped to cope with situations requiring accurate evaluations of residual dabigatran effects and/or plasma concentrations and a specific laboratory test which is accurate in the low concentration range and available 24/7 is highly requested.

Performance of dedicated coagulation tests compared to global ones

Our results show that HTI LOW performs better than HTI and ECA-II in the concentration range assessed in this study, i.e. from 0 to 200 ng/ml. It provides a steeper correlation compared with ECA-II and a lower systematic difference compared to LC-MS/MS with a narrower 95% confidence interval (►**Table 2**). However, ECA-II provides less systematic deviation than HTI (4 ng/ml versus -10 ng/ml) for assessments of plasma concentrations in the 0-200 ng/ml range. For concentrations below 50 ng/ml, HTI LOW correlates better with LC-MS/MS than HTI or ECA-II, which

were equivalent at these low concentrations. Regarding the systematic deviation, both HTI LOW and ECA-II performed very well with a small preference for HTI LOW due to a narrower 95% confidence interval.

Regarding sensitivity, HTI LOW was the most sensitive assay followed by ECA-II and HTI. Surprisingly, ECA-II did not perform better than previously shown for the "non-dedicated" methodology [91,92]. However, both HTI LOW and ECA-II reflect plasma dabigatran concentrations <50 ng/ml reasonably well with a slight preference for HTI LOW due to narrower confidence intervals. However, HTI LOW requires a new calibration curve and a new set of controls which further increases the turn-around time in an emergency situation. This is not required for ECA-II which directly introduces a calibration curve for the low concentrations and an automatic re-dilution of the sample if the result exceeds 230 ng/ml. However, if the test is already calibrated on the coagulometer, our proposal for sample management (see below) can reduce the turn-around time by choosing directly the right procedure to use between HTI and HTI LOW.

As expected, the lowest LOD and LOQ were obtained with TT, i.e. 1 and 2 ng/ml, respectively. With the normal procedure (with an upper limit of measurement (ULM) of 120 seconds with STA®-Thrombin at 1.5 NIH units/ml on a STA-R Evolution® coagulometer), concentrations above 50 ng/ml frequently exceed the ULM making the evaluation of the degree of

anticoagulation impossible. For example, in our study, one sample with 49 ng/ml dabigatran gave a TT of 248.7 seconds while in another sample, TT reached 86.2 seconds for a plasma concentration of dabigatran of 54 ng/ml (► **Figure 2)**. Thus, thrombin time may be less useful than dedicated assays to provide estimates of dabigatran effects. Initial recommendations on the peri-procedural management of patients taking dabigatran etexilate stated that a normal aPTT indicated the absence of a significant dabigatran effect. It was also recommended that surgery should be delayed for ≥1 half-life after the last dose of dabigatran or until the aPTT is normal or "near normal" [54]. However, aPTT reacts differently depending on reagents, coagulometers and their combinations, and a normal aPTT may be found in the presence of therapeutic dabigatran concentrations making these recommendations too simplistic [56,118,119]. In the present study, one sample was within the normal range of the STA-C.K.Prest® (35.5 seconds; normal range: 26.4 to 37.6 seconds) and SynthasIL® (32.1 seconds; normal range: 26 to 38 seconds), despite a dabigatran concentration of 40 ng/ml. Two samples exhibited normal Synthasil® and STA®-C.K.Prest® values despite concentrations of 76 and 34 ng/ml, respectively. Thus, the aPTT alone is not reliable and decisions based on aPTT should be avoided. Dedicated coagulation tests should be preferably used to guide the perioperative management. In the absence of dedicated coagulation tests, the aPTT may be used but, only concomitantly with the TT, as proposed previously [57,117].

Proposal for sample management

The laboratory can be guided by TT regarding which dedicated coagulation assay to use. We propose the following approach for the rational management of plasma samples from patients on dabigatran etexilate: For TT that exceeds the ULM (i.e. a TT < 120 seconds or ± 6-times the upper limit of normal on a STA-R Evolution® coagulometer using the recommendations of the manufacturer), we propose the use of the standard HTI assay which has demonstrate a sufficient accuracy in estimating plasma concentrations of dabigatran above 50 ng/ml [89,91,92,111]. Following our experiments, if TT is between the baseline clotting time and the ULM, a dedicated method should be employed. The HTI LOW or the ECA-II assays both provide good estimates of the plasma concentrations but HTI LOW seems to be more accurate thanks to narrower confidence interval. This simple paradigm may avoid unnecessary costs and ensure the best estimation of the plasma concentrations when required, e.g., in the perioperative setting.

Bridging therapy? The advantage of generating meizothrombin

A recent prospective and non-interventional registry revealed that heparin bridging did not reduce cardiovascular events in the perioperative context but lead to a significantly higher rate of major bleedings. However, the use of heparin bridging increased with the severity of the procedure (30% of patients in this registry received heparin bridging compared with 16% in the

RE-LY analysis). The authors conclude that patients at cardiovascular risk who need to undergo major procedures may benefit from heparin bridging [120]. In case of heparin bridging, the anticoagulant effect is transiently affected by low molecular weight heparins to the effect of dabigatran. Despite being less accurate than HTI LOW, one of the main advantage of ecarin based assays is that in case of switching or bridging therapy, meizothrombin, i.e. the intermediate product released by ecarin from prothrombin, is unaffected by the presence of heparin and derivatives. This allows an accurate assessment of the direct thrombin inhibitor in plasma. This was demonstrated in one of our patients who was bridged from dabigatran etexilate to enoxaparin. While LC-MS/MS and ECA-II revealed no residual dabigatran in plasma (0 ng/ml) the HTI LOW assay yielded a dabigatran plasma concentration of 8 ng/ml. The TT and the SynthasIL® were 53.9 and 52.9 seconds, respectively. Thus, HTI LOW may be influenced by the presence of heparin or LMWH and laboratory tests should be interpreted in conjunction with the clinical history. Another advantage of the ECA-II method is that the user must not choose between a “LOW” or a “normal” procedure since the test is intended to perform both by itself on a Stago® platform. However, this is at the expense of higher reagent consumption.

LIMITATIONS

Our study has some limitations. First, the LC-MS/MS method proposed in this study only measured free dabigatran and does not include the conjugated form. However, the latter only contributes approximately to 20% of the total dabigatran in plasma and is most likely of minimal importance at these low concentrations of dabigatran [113,121]. The second limitation concerns the low number of samples assessed that did not allow us to provide strong recommendations on whether HTI LOW or ECA-II should be used at the lowest plasma concentrations. Nevertheless, Bland-Altman analyses revealed that both HTI LOW and ECA-II perform better than HTI in the concentration range tested in this study and that they should be preferred to the standard procedure of HTI for evaluations of assess plasma concentrations between 0 and 50 ng/ml. This single center pilot study with a small patient material was not designed and powered to address clinical outcomes. Consequently, the implementation of a second-tier assay for dabigatran testing in routine practice should be further investigated to confirm clinical benefits in a large, multicentric study.

CONCLUSIONS

So far, TT has been the only functional test sufficiently sensitive to guide the management of patients in the perioperative context since both the Hemoclot Thrombin Inhibitors® (HTI) and the Ecarin Chromogenic Assay (ECA) showed weak performances when assessing low dabigatran concentrations. Measurements of such low plasma concentrations may have clinical implications for high-risk surgery and dedicated coagulation tests are therefore required. The HTI LOW and the ECA-II assays, specifically adapted to evaluate low dabigatran concentrations, performed well with dabigatran concentrations in plasma <50 ng/ml. We therefore recommended the use of HTI LOW or ECA-II to assess the plasma concentrations when they are suspected to be low, such as those encountered in the perioperative context. HTI LOW seems to be more accurate due to a narrower confidence interval but this should be confirmed in a larger study. However, screening of such samples by TT may be useful when choosing dedicated coagulation tests in order to avoid unnecessary costs and ensure the best estimation of dabigatran plasma concentrations, when required. The clinical benefit of such a procedure should be confirmed in a large multicentric study that addresses its impact on clinical outcomes.

EXTRA TABLE

What is known about this topic?
• The perioperative management of dabigatran is quite challenging especially in the absence of a specific reversal agent and possibilities to evaluate the intensity of the treatment are therefore valuable. • The use of dTT or an aPTT has been recommended in the perioperative context but their insufficient sensitivity limits their utility in patients with low plasma levels of dabigatran. • Thrombin time is an alternative in this context due to its very high sensitivity but it is influenced by several biological and analytical variables.
What does this paper add?
• The performance of two coagulation tests specifically developed for measurements of low plasma dabigatran concentrations (the Hemoclot Thrombin Inhibitors® LOW (HTI LOW) (Hyphen BioMed®, Neuville-sur-Oise, France) and the STA®-ECA II (ECA-II) (Diagnostica Stago®, Asnière-sur-Seine, France) has been assessed and compare to direct measurements by a reference LC-MS/MS method**.** • The HTI LOW and the ECA-II assays, specifically adapted to evaluate low dabigatran concentrations, performed well with dabigatran concentrations in plasma <50 ng/ml. • Screening by the thrombin time test may be useful in choosing the dedicated coagulation tests (i.e. HTI or HTI LOW/ECA-II) in order to avoid unnecessary costs.

Part 3

Case series showing the discrepancy of dabigatran plasma concentrations estimation with the different coagulation assays in a perioperative context.

The manuscript is currently under rewiewing in the journal Blood Coagulation & Fibrinolysis

Appropriate coagulation assays in peri-procedural contexts for patients on dabigatran etexilate: a case series

Lessire Sarah[1,2], Douxfils Jonathan[2], Larock Anne-Sophie[3], Mullier François[4]

[1] *Université catholique de Louvain, CHU UCL Namur, Namur Thrombosis and Hemostasis Center (NTHC), NAmur Research Institute for LIfe Sciences (NARILIS), Department of Anesthesiology, Yvoir, Belgium*

[2] *Université de Namur, Namur Thrombosis and Hemostasis Center (NTHC), NAmur Research Institute for LIfe Sciences (NARILIS), Department of Pharmacy, Namur, Belgium*

[3] *Université catholique de Louvain, CHU UCL Namur, Namur Thrombosis and Hemostasis Center (NTHC), NAmur Research Institute for LIfe Sciences (NARILIS), Department of Pharmacy, Yvoir, Belgium*

[4] *Université catholique de Louvain, CHU UCL Namur, Namur Thrombosis and Hemostasis Center (NTHC), NAmur Research Institute for LIfe Sciences (NARILIS), Haematology Laboratory, Yvoir, Belgium*

ABSTRACT

Introduction

Management of emergencies and invasive procedures in patients on dabigatran etexilate may request a ponctual assessment of dabigatran plasma concentrations.

Methods

Two dedicated coagulation assays for low dabigatran concentrations (the Hemoclot Thrombin Inhibitor® with low calibrators and the STA®-ECA II) and a standard methodology (the Hemoclot Thrombin Inhibitor® with standard calibrators) have been used to assess residual dabigatran plasma concentrations in three patients in a peri-procedural context. The results were compared with the reference method liquid chromatography coupled with a tandem mass-spectrometry. We raised the difference in residual dabigatran plasma concentrations' estimation depending on the coagulation assay and on the different perioperative management available in the literature.

Results

The Hemoclot Thrombin Inhibitor® with low calibrators and the STA®-ECA II assays were more accurate for low dabigatran plasma concentrations than the Hemoclot Thrombin Inhibitor® with standard calibrators. The different periprocedural management may lead to large differences in residual dabigatran concentrations at the time of the procedure.

Conclusions

This case-series illustrates the interest of using appropriate coagulation assays for the expected range of dabigatran plasma concentration in situations requiring accurate measurements (e.g. serious bleeding, urgent procedure at high risk of bleeding). Furthermore, this case-series highlights the need for a unique periprocedural management policy for patients on dabigatran etexilate.

Introduction

With the growing number of patients treated with direct oral anticoagulants (DOACs), clinicians are increasingly confronted with challenging situations where an estimation of their anticoagulant effect may be required. [78,122]

In the last 5 years, the impact and clinical relevance of the anticoagulant effect of DOACs on several coagulation assays have been reported. [55,61,62,66,93,123-127] For dabigatran, the activated partial thromboplastin time (aPTT) and the thrombin time (TT) are routine coagulation tests that may be used to estimate dabigatran plasma level, while more specific coagulation assays such as the diluted thrombin time (dTT) or ecarin-based assays such as the ecarin chromogenic assay (ECA) are used to provide accurate measurements of dabigatran plasma concentrations. [55,125]

However, the aPTT can be normal in the presence of therapeutic concentrations of dabigatran [89,91,92,128] and is not accurate at high dabigatran concentrations. [95] Inversely, the TT is very sensitive to dabigatran and therefore useful to exclude its presence at clinically relevant concentrations before invasive procedures. [55] However, its lack of standardisation makes it unsuitable for quantitative measurements. [60] Moreover, both aPTT and TT are not specific to dabigatran and may be influenced by pre-analytical and analytical variables.

Of the commercially available specific tests, dTTs such the Hemoclot® Thrombin Inhibitor (HTI) (Hyphen BioMed®, Neuville-sur-Oise, France) or

ecarin-based assays such STA® ECA II (ECA II) (Diagnostica Stago®, Asnière-sur-Seine, France) are validated for the quantitative measurement of dabigatran plasma concentrations. However, the HTI with standard calibrators and the preceding version of ECA II have previously shown a limit of quantitation between 30 and 50 ng/ml [89-92] precluding accurate measurements of the low plasma concentrations often encountered in the perioperative setting after dabigatran has been stopped [79]. To date, the gold standard method for measuring such low concentrations is liquid chromatography coupled with a tandem mass-spectrometry (LC-MS/MS) [56,89,129] but this is available only in highly specialized centers and not in routine practice.

Therefore, improved specific assays, i.e. the HTI with low calibrators (HTI LOW) and the ECA II, have been developed to cover this range. We demonstrated in an earlier ex-vivo analytical study using 33 plasma samples from patients treated with dabigatran etexilate (DE), that the estimation of low dabigatran concentrations was more accurate with the adapted methodologies than with the conventional HTI for dabigatran concentrations below 50 ng/ml. [93]

For some challenging situations requiring a measurement of dabigatran plasma concentration, we use the HTI LOW and ECA II methodologies systematically when the plasma range is expected to be low.

This case series describe three peri-procedural settings of patients on DE with a serious bleeding or requiring the planning of an urgent procedure, and for whom the anticoagulant effect of dabigatran was monitored. Through these three illustrations, we first aimed at highlighting the potential impact on patients' management according to the difference in residual plasma concentrations obtained with the specific tests HTI, HTI LOW and ECA II. Secondly, we aimed at demonstrating that the different periprocedural recommendations proposed in the current literature lead to significant different residual plasma concentrations at the suggested time of the invasive procedure.

Methods

The study was performed in accordance with the Declaration of Helsinki and was approved by the Medical Ethics Committee of the CHU UCL Namur in Yvoir, Belgium (number B039201524384). All patients provided written informed consent. We selected three patients for whom a coagulation screening was realized at different times before and after an urgent invasive procedure.

For urgent dabigatran plasma concentration measurement, only the standard method HTI was realized when on-therapy range was suspected (e.g. prolonged routine coagulation assays aPTT and TT > 120 s). Some blood samples were collected in the follow-up of these patients for observational

studies, without impacting patients' management. In these samples, impact of dabigatran on coagulation were estimated with routine coagulation assays (aPTT and TT) and plasma concentrations were measured using specific tests (conventional HTI, HTI LOW and ECA-II). All these procedures were performed on a STA-R Evolution® coagulometer (Diagnostica Stago®) following the manufacturer's recommendations.

To compare our results with the reference method, the samples were sent for LC-MS/MS measurements to the Department of Clinical Pharmacology, Karolinska University Hospital and Clinical Pharmacology Unit, Stockholm, Sweden.

Diluted Thrombin Time

For the conventional method of the Hemoclot® Thrombin Inhibitor (HTI), the tested plasma was diluted in a 1:8 ratio in Owren-Koller® buffer with an incubation time of 240 s. Purified human thrombin, in the α-form, was added to start the reaction. Calibration was performed using a commercial kit of 3 standards at concentrations of 35, 240 and 470 ng/ml from Hyphen BioMed®. For the low procedure, the methodology was the same except that the initial dilution was reduced (1:2) and a new calibration curve using commercially available calibrators from the same manufacturer, with lower concentrations (0, 57 and 110 ng/ml) was used.[93]

Chromogenic assay

For the STA®-ECA-II, the tested plasma was diluted in a 1:5 ratio in Owren-Koller® buffer and mixed with prothrombin and a chromogenic substrate. After an incubation time of 240 s, purified ecarin was added to start the reaction. The optical density (OD) was assessed for 40 s (from 70 to 110 s) and the result was expressed in OD/min. The procedure was calibrated with a 5-level calibration kit at concentrations of 0, 50, 96, 177 and 240 ng/ml (Diagnostica Stago®). If the estimated plasma concentration exceeded 230 ng/ml, as measured by the test, the STA®-ECA-II procedure automatically re-diluted the sample by increasing the initial dilution step (1:10 instead of 1:5).

Routine coagulation assays

For the TT, we used the STA®-Thrombin, which contains human thrombin at a concentration of 1.5 NIH/ml (Diagnostica Stago®; baseline clotting time between 13.5 – 20.5 s; higher limit of measurement of 120 s) on a STA-R Evolution® coagulometer (Diagnostica Stago®).

For the aPTT, we used the STA®-C.K.Prest® (Diagnostica Stago®; baseline clotting time between 26.4 – 37.6 s) on a STA-R Evolution® coagulometer (Diagnostica Stago®).

All tests were performed according to the manufacturers' recommendations using appropriate controls.

Results

Case description 1

A 65-year-old man weighing 79.5 kg was admitted to the emergency department with left lower lobe pneumonia. His medical history included hypertension, hypercholesterolemia, previous smoking, ischemic heart disease with angioplasty, and ablation for atrial flutter. He was receiving amiodarone 200 mg/day for 5 days/week, DE 150 mg twice a day (bid), molsidomine, quinapril with hydrochlorothiazide, acetylsalicylic acid (80 mg) and rosuvastatin (10 mg). He was hospitalized and treated with amoxicillin clavulanate. Clarithromycin was introduced three days later. Dabigatran plasma concentration was measured to exclude supratherapeutic levels, as both amiodarone and clarithromycin are strong inhibitors of P-glycoprotein. The Cockcroft-Gault (C-G) equation gave a creatinine clearance (CrCl) of 82 ml/min and the dabigatran trough concentration (22 hours after the last intake - see **Table 1**: patient 1 - sample 1) estimated with HTI was 66 ng/ml, which is not associated with an increased risk of major bleeding. This assertion is based on a substudy of the RELY trial, in which a trough plasma dabigatran concentration of 210 ng/ml doubled the rate of major bleeding compared with median concentrations of 88 ng/ml, measured 10-16 h after the last dabigatran intake (for 150 mg of DE bid).[100]

The cardiologist advised that anticoagulation should be continued as the patient had had several episodes of atrial fibrillation (AF) after his ablation.

As his oxygen requirement increased despite treatment, broncho-alveolar lavage (BAL) by fibroscopy was performed to test for the presence of Legionella antigen. The BAL was carried out 4 hours after DE intake and the fibroscopy revealed an alveolar hemorrhage. Afterwards, the patient mentioned that he had coughed up blood before the fibroscopy. Anticoagulants were stopped and the patient received rapidly intravenous four-factor prothrombin complex concentrate (PCC) 50 UI/kg. Unfortunately, the clinicians did not request a dabigatran monitoring before fibroscopy or PCC infusion. However, the first sample realized the day before did not show supra-therapeutic dabigatran concentrations. Furthermore, the patient was symptomatic before the procedure and with acute deterioration of its respiratory function, the clinicians decided to decrease rapidly the anticoagulant effect of DE as the diagnosis of alveolar hemorrhage came short after peak time. In order to assess the decrease in anticoagulant level after PCC infusion, further estimations of dabigatran plasma concentration were realized as illustrated in **Table 1**. The Legionella antigen test was positive and the patient was admitted to the intensive care unit (ICU) two days later with acute respiratory distress syndrome. He was discharged from hospital three months later.

Case description 2

A 77-year-old man weighing 77.0 kg attended the emergency department because of bradycardia, discomfort with dizziness, and a short loss of

consciousness. His medical history included hypertension, type 2 diabetes, previous smoking, dyslipidemia and cardioversion for AF one month before. He was receiving DE 150 mg bid, sotalol 40 mg bid, ramipril, gliquidone, furosemide and simvastatin. He had been receiving clarithromycin, but had stopped the day before admission. The patient was conscious. An electrocardiogram showed a third degree atrioventricular block with a heart rate of 20 beats/min and ventricular escape rhythm.

His CrCl was 71 ml/min according to the C-G equation. HTI indicated 360 ng/ml of dabigatran 6 hours after the last intake. In the RE-LY trial, the 90th percentile of dabigatran plasma concentration at peak time was 383 ng/ml measured at 1 to 3 hours after last oral intake [19,100]. The patient was paced with external patches during his transfer to ICU. As the external patches were not sufficient, the patient received cardiac massage intermittent compression and had to be intubated and sedated for his comfort. He received 25 IU/kg of PCC before the placement of an internal pacemaker through an echo-guided femoral access with chest X-ray control. The following day, the cardiologist decided to place a permanent pacemaker. As the patient had such a high peak plasma concentration, dabigatran levels were monitored to ensure elimination before surgery (see **Table 1**: patient 2 - samples 2-4). Unfortunately, the patient developed a respiratory tract infection after 2 days, postponing the procedure 6 days later. The patient was discharged from ICU the day after pacemaker insertion.

Case description 3

A 76-year-old woman weighing 62.0 kg was hospitalized for a femoral neck fracture. Her medical history revealed AF with left atrial appendix thrombosis, severe aortic stenosis, sub-dural hematomas within the past year and 10 years before, type 2 diabetes, hypertension, hypercholesterolemia, smoking, and chronic obstructive pulmonary disease. She was taking DE 110 mg bid, amiodarone, metformin, aldactazine, pantoprazole (20 mg) and ezetimibe (10 mg). Her CrCl on the day of admission was 75 ml/min according to the C-G equation. Surgery was delayed for 5 days after the last intake of DE, because of DE anticoagulation and operating room availability. Because of a high CHA2DS2-VASc score (6/9), the patient was bridged with enoxaparin 60 mg bid the second day after her admission. As HTI and HTI LOW can be falsely elevated in the presence of enoxaparin, dabigatran measurements were made before the next enoxaparin administration (see **Table 1**: patient 3 – samples 2-4).

Table 1: Coagulation screening in three patients on dabigatran etexilate in a peri-procedural setting

	Time after last intake of dabigatran (hours)	Time after PCC trans-fusions (hours)	HTI (ng/ml)	HTI LOW (ng/ml)	ECA II (ng/ml)	LC-MS/MS (ng/ml)	aPTT (s)	aPTT ratio	TT (s)	Fib (mg/dl)
Patient 1										
Sample 1	22	/	66	/	/	/	46.0	1.5	>120	1,129
Sample 2	24	16	19	/	/	50	39.8	1.3	79.1	1,382
Sample 3	50	42	0	0	4	4	33.5	1.1	20.9	1,005
Patient 2										
Sample 1	5	/	360	/	/	/	52.7	1.7	>120	415
Sample 2	27	20	102	/	/	/	26.4	0.8	>120	396
Sample 3	44	37	34	75	62	65	33.3	1.1	>120	470
Sample 4	54	47	/	/	/	/	27.4	0.9	73.1	558
Patient 3										
Sample 1	18	/	/	/	/	/	50.1	1.7	>120	355
Sample 2	40	/	27	60	61	67	38.0	1.3	>120	/
Sample 3	84	/	6	0	0	10	27.5	0.9	29.5	/
Sample 4	138	/	0	0	0	4	31.5	1.0	19	737

Table 1. HTI: Hemoclot Thrombin Inhibitor®, HTI LOW: Hemoclot Thrombin Inhibitor® LOW, ECA II: Ecarin Chromogenic Assay II®
LCMS-MS: Liquid chromatography coupled with a tandem mass-spectrometry
aPTT: activated partial thromboplastin time (normal range: 26.4 – 37.6 s), TT: Thrombin Time (normal range: 13.5 – 20.5 s)
Fib: fibrinogen (normal range: 180-400 mg/dl), PCC: Prothrombin complex concentrate, /: coagulation assays not asked

Discussion

Issues with non standardized periprocedural managements…

The threshold for plasma concentrations of dabigatran allowing safe hemostasis for procedures carrying a high risk of bleeding has not been studied in clinical trials, but is currently based on expert opinion. The French Group of Perioperative Hemostasis (GIHP) has proposed a cut-off value of 30 ng/ml [86] which corresponds to the expected plasma DOAC concentration reached after 3 half-lives, when 87.5 % of the assumed Cmax (175 ng/ml) [100] has been eliminated. This threshold is now also proposed by the International Society of Thrombosis and Hemostasis. [130] Based on this threshold, conventional tests, such as HTI, do not provide sufficient accuracy compared with specific methods for assessing low concentrations. In patients 2 and 3 (who had measurements taken 40 to 44 hours after stopping dabigatran), values obtained via HTI LOW and ECA-II were closer to the LC-MS/MS values than those obtained with HTI, which underestimates dabigatran concentrations. These differences in dabigatran measurements might influence decisions about perioperative management for procedures carrying a high risk of bleeding by allowing surgery for patients who actually have therapeutically relevant dabigatran plasma concentrations (in our cases, 65 and 67 ng/ml in patients 2 and 3, respectively).

Considering another type of perioperative management, based on the timing of last dabigatran intake according to a procedure's bleeding risk and the patient's renal function, as proposed by Schulman *et al.* [131], these three patients would have undergone low risk procedures with residual dabigatran plasma concentrations ranging from 66 to 120 ng/ml.

On the same basis, for a procedure carrying a high risk of bleeding (for which a CrCl >50 ml/min at 48 hours after stopping dabigatran is recommended), patients 2 and 3 would have had residual dabigatran plasma concentrations of around 60 ng/ml.

A sub-analysis of the prospective study on the perioperative management of patients on dabigatran was conducted by Douketis *et al.* It analyzed the residual anticoagulant effect of dabigatran found in plasma from patients the day of the elective invasive procedure using the HTI and a validated LC-MS/MS method. [132] Blood samples were frozen and laboratory tests were performed later, ensuring that results of the coagulation tests and the LC-MS/MS did not influence the perioperative management of these patients. The conventional HTI used in the study of Douketis *et al*. estimated that 13% of the patients had a residual dabigatran concentrations > 20 ng/ml, which corresponded to the limit of detection of the test, as described by the authors. The LC-MS/MS measured around 16% over 20 ng/ml. The TT,

which demonstrated a high sensitivity towards dabigatran [56,60,93], was higher than the upper limit of the normal range (20 to 30 s, as described by the authors) in 43% of these blood samples. These results strengthened the hypothesis that a longer period of interruption might be required before high bleeding-risk procedures. [132] In addition, their results underlined the difference with the conventional HTI method in detecting concentration > 20 ng/ml compared with LC-MS/MS (13% versus 16%, respectively), suggesting that conventional HTI is not optimal to assess low dabigatran concentrations. These data are in agreement with a recent multicenter study showing that waiting 48 hours after the last dose did not guarantee residual plasma concentrations ≤ 30 ng/ml, as 14% of patients did not achieve this cut-off value. [79]

Another issue with the different proposals for perioperative management are the differences in the definition of high and low bleeding risk procedures. For example, Schulman *et al.* consider pacemaker placement to carry a high risk of bleeding because of the risk of pocket hematoma [131]; while Heidbuchel *et al.* consider it to have a low risk of bleeding. [24] Obviously, these different categorizations influence the timing of surgery, especially in patients with poor renal function. This was confirmed in a recent multicenter study where the CrCl showed significant correlations with dabigatran at trough concentrations. [88] In addition, Douketis *et al.* showed that the

proportion of patients with dabigatran levels > 20 ng/ml in the high bleeding risk procedure arm was doubled in patients with a CrCl between 30 and 50 ml/min compared with patients with a CrCl > 50 ml/min. However, due to the small number of bleeding outcomes, they could not perform any clinically meaningful correlation analyses between the clinical outcomes and the pre-procedural residual anticoagulant effect.

How can DOAC measurements improve the periprocedural management?

Periprocedural estimation of dabigatran plasma concentration will be most of the time realized in emergency settings carrying a high risk of bleeding, or when a patient presents an unexpected impaired renal function before a high bleeding procedure.

Elective periprocedural management of patients treated with DE does not require routine dabigatran monitoring. However, a cautious assessment of the procedures' bleeding risk and patients' renal function is required to determine a safe period of DE interruption.

In this case-series, some measurements of dabigatran concentration had no impact on their management. However, measurements realized for observational studies enable us to confirm the results of our previous studies [60,93] and to raise concern about current perioperative recommendations. Indeed, Testa *et al*. demonstrated high intra-inter variability of DOAC plasma concentrations in patients on DOACs. They warn clinicians about

higher than expected circulating dabigatran levels in clinical conditions such as surgery or invasive procedures. [88] Along the same lines, experts in regional anesthesia recommand further research studies including DOAC periprocedural estimation to validate a safe DOAC interruption before procedures at high bleeding risk, such as neuraxial anesthesia. [133]

Such research studies strengthen decisions concerning DOACs perioperative management. Indeed, the recent update on periprocedural management published by Spyropoulos *et al*.[134] proposes a longer interruption of DOAC than the one proposed by Schulman *et al.*[131] (i.e. DE interruption before procedures carrying a high bleeding risk in patients with ClCr > 50 ml/min at day 3 instead of day 2).

How to deal with heparin bridging in patients treated with dabigatran etexilate…

The HTI and HTI LOW are not specific to dabigatran. Spiked unfractionated heparin (UFH) and enoxaparin at 0.60 IU/ml and 1.20 IU/ml respectively, gave false positive results with HTI, according to previous data. [60] Thus, for patients bridged with low molecular weight heparins (LMWH) when dabigatran is interrupted, the HTI should be realized just before the next administration of LMWH and care should be taken in the interpretation of the result. Importantly, the HTI LOW will have increased sensitivity towards heparins due to lower dilution step, as set in the procedure. The ECA-II

assay has the advantage to be specific to direct thrombin inhibitors and effective in assessing low concentrations. Therefore, it should be preferred to estimate preoperative dabigatran plasma concentrations in bridged patients. [93]

Decision tree for urgent dabigatran monitoring in different clinical settings

Monitoring the anticoagulant effect of dabigatran has a clear added value when it can guide patients' management, e.g. in case of bleeding or thrombotic events or in case of emergencies with high bleeding risk.

For example, recent guidelines for the administration of idarucizumab [130], the high-costly antidote of DE, are partially based on dabigatran plasma concentrations. As the threshold to consider administration of idaricuzimab is 50 ng/ml for serious bleeding and 30 ng/ml for urgent invasive procedures carrying a high risk of bleeding, decision to administer the antidote should be guided with accurate coagulation assays. Conventional coagulation tests are not appropriate in this context. Interestingly, in this case-series, the difference in dabigatran measurements of dedicated assays for low concentrations (HTI LOW and ECA II) and standard methodology (HTI) can strongly influence the management of serious bleeding. Indeed, the plasma concentrations measured with standard HTI (in patient 1 - sample 2, patients 2 and 3 – sample 2) would not suggest to use the antidote, as the ones measured with HTI LOW and ECA II (confirmed with LCMS-MS)

would suggest to use the antidote. As yet, the benefit of idarucizumab has not been proved on patient outcomes. However, clinicians need to rely on accurate tools to guide antidote's administration.

Table 2 proposes an algorithm of the laboratory tests to use in relation to the different clinical contexts that may require dabigatran monitoring.

Nowadays, clinicians sometimes have to make do with routine coagulation assays to estimate the residual anticoagulant effect of dabigatran. The use of sensitive aPTT reagents and an optimized TT (i.e. a TT using a thrombin concentration able to detect dabigatran plasma concentrations up to 50 ng/ml within its limits of analytical range) can provide qualitative estimation of dabigatran plasma levels. Moreover, both routine tests can guide the laboratory in choosing the most accurate coagulation assays to measure the expected dabigatran plasma concentration.

Although expert's raise concerns of the urgent need to ensure access to specific coagulations assays for DOAC monitoring in emergency settings, these are not always available within an appropriate turnaround time. Efforts should be performed by laboratories and manufacturers to reduce the turnaroun time of specific assays (i.e by reducing the time of centrifugation and/or by reducing the time required to reconstitute controls and reagents) and make the specific assays widely available. [135]

Table 2: Appropriate coagulation assays to use in clinical situations that may require a monitoring of dabigatran

<table>
<tr><th>Emergencies
that cannot be delayed
or
need to be rapidly
plannified</th><th>Periprocedural
bleeding events</th><th>Suspicion of insufficient
perioperative arrest before
a high bleeding risk
procedure</th></tr>
<tr><td colspan="2">If TT < 120 s and/or normal aPTT :
Use HTI LOW or ECA II</td><td rowspan="2">If TT < 120 s
and/or normal aPTT :

Use HTI LOW or
ECAII</td></tr>
<tr><td colspan="2">If TT > 120 s and prolonged aPTT :

Use HTI or ECA II</td></tr>
<tr><td colspan="3">In case of heparin bridging :
Use ECA II</td></tr>
</table>

Table 2. TT: Thrombin Time, aPTT: activated partial thromboplastin time, HTI: Hemoclot® Thrombin Inhibitor from Hyphen BioMed® ECA II: ecarin chromogenic assay from from Diagnostica Stago®, DE: dabigatran etexilate.

Our laboratory uses a TT with optimized conditions (STA®-Thrombin, 1.5 NIH/ml; baseline clotting time between 13.5 – 20.5 s; higher limit of measurement of 120 s) on a STA-R Evolution® coagulometer (Diagnostica Stago®), allowing a measurement of TT within its analytical range for spiked dabigatran concentration in normal pool plasma between 0 and 50 ng/ml.

Conclusions

The optimal time to stop a direct oral anticoagulant to minimize thromboembolic or bleeding complications remains unclear. [136] We need further prospective studies to standardize an optimal periprocedural management policy for patients on DE and to validate a safe cut-off of plasma concentration for procedures carrying a high risk of bleeding. When patients require a point measurement of dabigatran plasma concentration, laboratories should use the coagulation assay that has the best performance for the expected concentration ranges and patients' specific clinical context.

Part 4

Comparison of the performance of chromogenic assays specifically developed for the measurement of low rivaroxaban concentrations with the reference LC-MS/MS method.
Impact of heparin bridging on their performance.

The manuscript has been accepted in September 2016 in the journal Clinical and Applied Thrombosis/Hemostis

Estimation of rivaroxaban plasma concentrations in the perioperative setting in patients with or without heparin bridging

Authors:

Sarah Lessire[*†], Jonathan Douxfils[†], Lionel Pochet[†], Anne-Sophie Dincq[*], Anne-Sophie Larock[‡], Maximilien Gourdin[*], Jean-Michel Dogné[†], Bernard Chatelain[§], François Mullier[§]

[*] *Université catholique de Louvain, CHU UCL Namur, Namur Thrombosis and Hemostasis Center (NTHC), NAmur Research Institute for LIfe Sciences (NARILIS), Department of Anesthesiology, Yvoir, Belgium*

[†] *University of Namur, Namur Thrombosis and Hemostasis Center (NTHC), NAmur Research Institute for LIfe Sciences (NARILIS), Department of Pharmacy, Namur, Belgium*

[‡] *Université catholique de Louvain, CHU UCL Namur, Namur Thrombosis and Hemostasis Center (NTHC), NAmur Research Institute for LIfe Sciences (NARILIS), Department of Pharmacy, Yvoir, Belgium*

[§] *Université catholique de Louvain, CHU UCL Namur, Namur Thrombosis and Hemostasis Center (NTHC), NAmur Research Institute for LIfe Sciences (NARILIS), Haematology Laboratory, Yvoir, Belgium*

ABSTRACT

Introduction

Estimation of residual rivaroxaban plasma concentrations may be requested before invasive procedures and some patients at high thrombo-embolic risk will have a bridging therapy with heparins when rivaroxaban is interrupted.

The objective of this study was to assess the performance of the STA®-Liquid Anti-Xa assay (STA®LAX) and the low and normal procedures of the Biophen®Direct Factor Xa Inhibitors (DiXaI) assay, in patients with and without bridging with low-molecular weight heparins (LMWH).

Material and Methods

Seventy-nine blood samples were collected from 77 patients on rivaroxaban at C_{TROUGH} or before an invasive procedure. Rivaroxaban plasma concentrations were estimated using Biophen®DiXaI, Biophen®DiXaI LOW and STA®LAX and compared with liquid chromatography coupled with mass spectrometry (LC-MS/MS) measurements. Stratifications were performed according to heparin bridging.

Results

The Biophen®DiXaI LOW and STA®LAX showed better correlation with LC-MS/MS measurements than Biophen®DiXaI in patients not bridged with LMWH (R: 0.97, 0.96 and 0.91, respectively). However, the performance of Biophen®DiXaI LOW and STA®LAX decreased when residual LMWH

activity was present (R: 0.18 and 0.19 respectively) demonstrating that these tests are not specific to rivaroxaban.

Conclusions

In patients not bridged with LMWH, we suggest to use the Biophen®DiXaI LOW and STA®LAX for the estimation of rivaroxaban concentrations <50 ng/ml. These results should be confirmed on a larger cohort of patients. Patients bridged with LMWH have inaccurate estimates of low levels of rivaroxaban and the three assays studied should not be used to estimate if it is safe to perform a procedure.

Introduction

The management of rivaroxaban in the perioperative context requires specific expertise in both clinical and laboratory aspects [137]. In the ROCKET AF trial, treatment interruptions occurred in 33% of participants, and 45% of the interruptions were in patients treated with rivaroxaban. Approximately 8% of these patients were bridged, and a low molecular weight heparin (LMWH) was used in 98% of these patients. Surgical procedures accounted for 39% of the treatment interruptions in the rivaroxaban arm [138]. A similar pattern was also reported from the Dresden registry in which 27% of the participants underwent a surgical procedure, of whom 30% were bridged with a LMWH after the pre-procedural interruption of their direct oral anticoagulant (DOAC)[120]. The median duration of DOAC interruption before surgery was 2 days[120]. However, a prospective observational study on the periprocedural management of patients treated with dabigatran etexilate or rivaroxaban showed that 2 days of interruption may not be sufficient to eliminate the entire residual effect of these drugs. Indeed, in 14% of patients, although DOACs had been discontinued for 48 h, DOAC concentrations remained above 30 ng/ml which was defined as the safe threshold for invasive procedures[79]. Thus, some situations will require an assessment of the residual effect of rivaroxaban to guide therapeutic decisions, for example, before selecting fibrinolytic treatment in a patient on rivaroxaban presenting with an acute ischemic stroke[139].

Specific coagulation assays are now available that can reliably estimate rivaroxaban plasma concentrations within its therapeutic range [55,61,66,140,141]. Nevertheless, as shown for dabigatran [142], two previous studies have demonstrated the need for coagulation assays sensitive to low (<30 ng/ml) plasma concentrations of rivaroxaban [143,144]. These studies used blood samples collected in real-life conditions, but none of them dealt with the problem of measuring plasma rivaroxaban concentrations in patients bridged with heparins when rivaroxaban is stopped.

Some tests used to estimate the plasma concentration of rivaroxaban are also used to estimate heparin concentrations by applying appropriate calibrators.[70] The STA®-Liquid Anti-Xa assay from Diagnostica Stago® (Diagnostica Stago®, Asnière, France) is a global anti-Xa assay designed to estimate low and on-therapy plasma concentrations of rivaroxaban and to measure LMWH anti-Xa activity. As a consequence, the presence of heparin when estimating rivaroxaban concentrations might affect the result and vice versa.

To avoid this interference, Samama *et al.* have developed an optimized chromogenic assay (Biophen®Direct Factor Xa Inhibitors (DiXaI) from Hyphen BioMed®, Neuville-Sur-Oise, France). This test uses a specific buffer allowing the measurement of the sole anti-Xa activity from rivaroxaban even in the presence of heparins or fondaparinux [145]. Its limit of quantitation (LOQ) of around 50 ng/ml is not appropriate for the

perioperative setting, and an adaptation of the procedure is proposed to allow the estimation of lower rivaroxaban plasma concentrations.

We hypothesized that low residual heparin plasma concentrations found the day of the invasive procedure may interfere with some assays used to quantify rivaroxaban.[70]

The objective of this study was to assess the performance (limit of detection, limit of quantitation and accuracy) of the low and the high procedure of the Biophen®DiXaI assay, and the STA®-Liquid Anti-Xa assay from Diagnostica Stago® (Diagnostica Stago®, Asnière, France), using real-life samples from patients with or without bridging with LMWH.

Materials and methods

The study was performed in accordance with the Declaration of Helsinki and was approved by the Medical Ethics Committee of the CHU UCL Namur, Yvoir, Belgium. Written informed consent was obtained from each donor.

Clinical samples

Eighty-six plasma samples from 84 patients on rivaroxaban were collected from April 2014 until the end of July 2015 in the CHU UCL Namur, Yvoir, Belgium.

The inclusion criteria were patients receiving rivaroxaban for secondary prevention of thrombo-embolic events in atrial fibrillation or treatment of

venous thromboembolism, and collection of blood samples at trough concentrations or just before an invasive procedure.

The only exclusion criterion was the absence of result for one of the three chromogenic assays. Seven blood samples were excluded from the study, which was realized on the remaining 79 blood samples collected from 77 patients.

We aimed at collecting at least thirty samples from patients bridged and not bridged with LMWH. Blood was taken by venipuncture in the antecubital vein using a 21-gauge needle (Terumo™) or through a peripheral venous catheter (BD Insyte-W®, 18 or 16-gauge) and collected into 0.109 M sodium citrate (9:1v/v) tubes (Venosafe®, Terumo™, Heverlee, Belgium).

Platelet-poor plasma (PPP) was obtained from the supernatant fraction after double centrifugation at 1,500 G for 15 min at room temperature. Samples were then aliquoted and frozen immediately at -80°C. Plasma sample aliquots were thawed and heated to 37°C for at least 5 min before running the experiments.

We measured the creatinine clearance with the Cockcroft-Gault equation, using the actual body weight.

Distribution of collected blood samples following LMWH presence and last administration

Some blood samples came from patients bridged with LMWH after

cessation of rivaroxaban. As residual heparin might interfere with the estimation of plasma concentrations of rivaroxaban by anti-Xa assays, we divided patients into three groups (➢**Figure 1**).

- **Group A** represents the patients who were not bridged with LMWH (n=31). These blood samples were collected from patients at C_{TROUGH} (n=28; timing of last administration between 21 and 48 h) outside a perioperative context or within a perioperative context without the use of heparin bridging (n=3; timing of last administration between 42 and 72 h).

- **Group B** represents the patients who were bridged with LMWH (n=48; timing of last administration between 65 and 158 h).

- **Group C** was a subdivision of group B and included patients bridged with therapeutic LMWH whose last administration of LMWH was ≥ 24 hours ago (n=26). The group C was designed to analyze the effect of low LMWH levels (last administration of LMWH was ≥ 24 hours) on rivaroxaban estimation.

This stratification of the time interval between blood sampling and last LMWH administration is based on the international recommendations for safe hemostasis before an invasive procedure (e.g. neuraxial anesthesia), when therapeutic LWMH bridging is established preoperatively [84,146,147]. Therapeutic LMWH was defined as enoxaparin 1 mg/kg twice a day *(bid)* or nadroparin calcium 171 international units (IU) anti-Xa/kg once a day *(qd)*.

Figure 1. Distribution of the collected blood samples following LMWH presence and last administration.

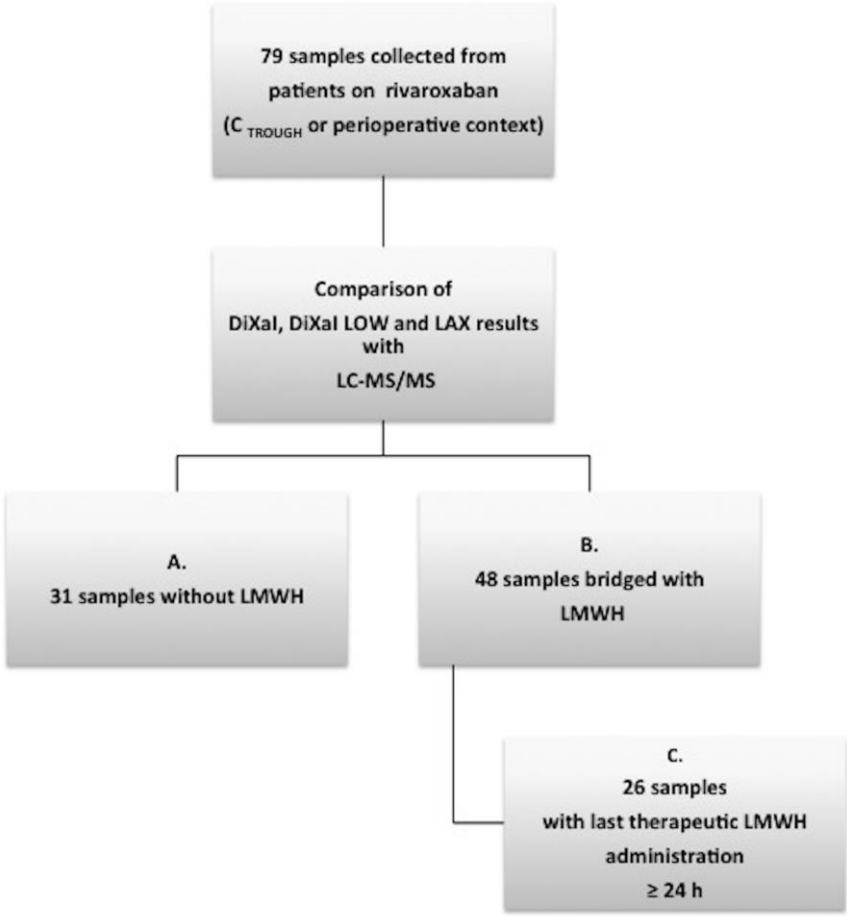


Fig 1. The 79 blood samples included in the analyses were collected from patients receiving rivaroxaban at trough concentrations (C_{TROUGH}) or before an invasive procedure. For these samples, an estimation of rivaroxaban plasma concentrations with three chromogenic assays (Biophen®DiXaI, Biophen®DiXaI LOW and STA®LAX) was performed and compared with measurements obtained by LC-MS/MS. Stratifications were performed according to heparin bridging.

Estimation of the anti-Xa activity of LMWH administered ≥ 24h ago

To assess the impact of residual anti-Xa activity from LMWH on the estimation of rivaroxaban concentrations, samples from **Group C** were used (n=26). In these samples, the residual rivaroxaban plasma concentrations were below the LOQ of the LC-MS/MS (i.e. 1.0 ng/ml). Thus, residual LMWH anti-Xa activity can be measured without interference from the anti-Xa activity of any residual rivaroxaban.

Normal pooled plasma

Normal pooled plasma (NPP) was obtained from the platelet poor plasma (PPP) of 49 healthy individuals. The exclusion criteria for the recruitment of healthy individuals were: thrombotic or hemorrhagic events, pregnancy, or use of antiplatelet, anticoagulant agent or any drug potentially affecting platelet or coagulation factors within two weeks of blood sampling. Blood was taken by venipuncture in the antecubital vein using a 21-gauge needle (Terumo™). The sample preparation steps were the same as for the clinical samples.

Chromogenic anti-Xa assays

Biophen®Direct Factor Xa Inhibitors. We incubated 200 µl of diluted plasma (1:50 with the Tris-NaCl-EDTA buffer at pH 7.85) with 75 µl of human FXa (Hyphen BioMed) for 120 s at 37°C, then 75 µl of a specific FXa substrate [CS-11(65)] (Hyphen BioMed) were added to start the

reaction on a STA-R®Evolution coagulometer (Diagnostica Stago). The concentrations of calibrators for the normal therapeutic range of rivaroxaban (Biophen®Rivaroxaban Plasma Calibrator, Hyphen BioMed) were 50, 250 and 500 ng/ml in the initial samples after reconstitution.

The low procedure (Biophen®DiXaI LOW) was the same as the normal one except that plasma was diluted 1:8 in the buffer and the calibration was performed with standards for low plasma concentrations of rivaroxaban (Biophen®Rivaroxaban Calibrator Low). The standard rivaroxaban concentrations were 0, 52 and 110 ng/ml.

STA®-Liquid Anti-Xa. We mixed 30 µl of diluted plasma (1:4 in STA® - Owren-Koller) with 150 µl of chromogenic substrate (CBS 02.44 consisting of MAPA-glycine-arginyl-p-nitroanilide, HCl) and incubated this for 240 s. We then added 150 µl of bovine FXa (pre-warmed at 37°C), starting the reaction on a STA-R®Evolution coagulometer (Diagnostica Stago). The concentrations of calibrators (STA®-Rivaroxaban Calibrator, Diagnostica Stago) were 0, 94, 236 and 472 ng/ml in the initial samples after reconstitution.

Anti-Xa measurement of LMWH. The anti-Xa activity of LMWH was measured with the Biophen®Heparin Liquid Reagent Technology (LRT) assay (Hyphen BioMed). We mixed 50 µl of diluted plasma (1:2 in STA®Owren-Koller) with 125 µL of pre-warmed specific chromogenic substrate of factor Xa (SXa-11) and incubated this for 240 s at 37°C. We

then added 125 µl of bovine FXa (pre-warmed at 37 °C) and measured the color development. Results were expressed in OD/min. Calibration curves were realized with Biophen®Heparin Calibrators (Hyphen BioMed) (5 plasma samples with LMWH concentrations ranging from 0.0 to 1.6 IU/ml). The LOQ of the anti-Xa activity is 0.05 UI/ml, according to the manufacturer.

Liquid chromatography coupled with tandem mass spectrometry

All chemical reagents and solvents were used as obtained from commercial sources (Sigma Aldrich, Acros, Biosolve). Rivaroxaban and [$^{13}C_6$]-rivaroxaban-d5 were purchased from Alsachim. The LC-MS/MS method was adapted from the procedures described by Douxfils *et al*. [62] and Rohde *et al.* [148]. All LC-MS/MS measurements were carried out on an Ultra Performance Liquid Chromatography (UPLC) (Acquity H-Class system, Waters) coupled to a tandem-quadrupole mass spectrometer (Xevo TQ-S, Waters). Chromatographic separation was achieved using a Waters Cortecs® UPLC® C18 column (2.1 x 100 mm, 1.6 µm) at a temperature of 40°C and a flow rate of 500 µl/min. The mobile phase was composed of ammonium formate buffer (10 mM, pH 3) (A) and acetonitrile (B). Gradient started at 20% B and linearly ramped up to 55% B in 4 min. The proportion of B was then set to 90 % for 1 min before returning to 20% B. All experiments were carried out with an injection volume of 2 µl, the autosampler was set to 10°C. The MS/MS detection was done with

electrospray ionization (ESI) in the positive mode and multiple reaction monitoring (MRM). The capillary voltage was set to 0.5 kV, the source block temperature was 150°C and the nitrogen desolvation gas was heated to 600°C with a flow rate of 1000 l/h. Four transitions were monitored: 436.00>145.05 with cone voltage at 50.0 V and collision energy at 30.0 ev (rivaroxaban, quantitative ion product); 436.00>231.20 with cone voltage at 50.0 V and collision energy at 20.0 ev (rivaroxaban, qualitative ion product); 442.00>145.05 with cone voltage at 50.0 V and collision energy at 30.0 ev ([$^{13}C_6$]-rivaroxaban, quantitative ion product); 442.00>237.20 with cone voltage at 50.0 V and collision energy at 20.0 ev ([$^{13}C_6$]-rivaroxaban, qualitative ion product). The dwell time was set to 0.038 sec. Preparation of the sample was realized according the following procedure: 50 µl of plasma was added to an Ostro^{TM} plate (Waters); 350 µl of 1% formic acid containing internal standard (2.0 ng/ml) was mixed with each sample for protein precipitation. The plate was then placed on a vacuum manifold (Waters) and the samples were collected in a Waters® 2 ml square collection plate. Weighted (1/x) linear calibration was done using calibration standards prepared by spiking blank plasma at 1, 3, 6, 10, 50, 125, 250 and 500 ng/ml. Validation standards were prepared by spiking blank plasma at 1, 3, 9, 200, 400 ng/ml. Validation with the QCs on three different days showed repeatability relative standard deviation (RSD) below 5.0%, an intermediate precision RSD below 9.8 % and a relative bias below 11.1 %. The limit of

detection (LOD) and the LOQ were 0.5 and 1.0 ng/ml, respectively. The method was validated according to FDA Guidelines [149].

Statistical analyses

GraphPad Prism® version 6.0c for MacOSx (GraphPad Software, San Diego, CA, USA, www.graphpad.com) was used for the statistical analyses. Due to the fact that little is known about the combined impact of Xa-Inhibitors and LMWHs on Xa-Assays, this study is of exploratory nature and not formally powered. Results for the rivaroxaban anti-Xa assays (Biophen®DiXaI, Biophen®DiXaI LOW and STA®LAX) and those obtained using LC-MS/MS were compared by Spearman correlation, linear regression and Bland-Altman analyses. The coefficients of correlation of group A were compared with those of group B and C. A p-value lower than 0.05 was considered as statistically significant. Bland-Altman was calculated as the difference (A-B)/average, where A was the result of the corresponding coagulation test and B, the result of the LC-MS/MS. Limits of agreement of the Bland-Altman analyses were calculated as the mean difference – or + 1.96*standard deviation for the 5^{th} and the 95^{th} limit of agreement, respectively. The lower limit of detection (LOD) was calculated as [(3*standard deviation of Y0)/ slope] and the lower limit of quantitation (LOQ) was calculated as [(10*standard deviation of Y0/ slope]. The standard deviation of Y0 was calculated by running the tests 10 times with NPP. For STA®LAX, the calibration curve is better defined by a second

order polynomial relation. Therefore, the three first points of the calibration curve (value of the intrapolation: 8.6 ng/ml, 85.2 ng/ml and 233.9 ng/ml) that can be fitted by a linear regression ($R^2 \geq 0{,}98$) were taken to calculate the LOD and LOQ.

Results

Plasma concentrations of rivaroxaban in the blood samples ranged from 0.0 ng/ml to 108.2 ng/ml according to LC-MS/MS. ➢**Table 1** shows the statistical characteristics of the rivaroxaban plasma concentrations of the different groups. All the patients had a creatinine clearance estimated with the Cockcroft-Gault equation above 50 ml/min.

Spearman and linear correlations

For **Group A**, the Spearman r was 0.91, 0.97 and 0.96 for Biophen®DiXaI, Biophen®DiXaI LOW and STA®LAX, respectively. The linear regression gave an R-square of 0.82, 0.94 and 0.95 for Biophen®DiXaI, Biophen®DiXaI LOW and STA®LAX, respectively (➢**Table 1**).

The difference in correlation with the LC-MS/MS measurement of Biophen®DiXaI LOW and Biophen®DiXaI was clinically significant (95% confidence interval (CI) of Spearman coefficient (r): 0.81 to 0.96 vs 0.94 to 0.99, respectively (**Table 1**). For STA®LAX and Biophen®DiXaI, this difference tended towards significance (95% CI of Spearman r: 0.92 to 0.98

vs 0.81 to 0.96, respectively).

For Groups B and C, the Spearman r and the linear regression showed only a weak correlation between chromogenic assays and LC-MS/MS measurements.

For Biophen®DiXaI, Biophen®DiXaI LOW and STA®LAX, the coefficients of correlation obtained with group A are significantly higher than the coefficients of correlation obtained with group B (e.g for Biophen®DiXaI the 95% CI was 0.81 to 0.96 for group A vs -0.03 to 0.52 for group B). The same results were obtained when comparing the coefficients of correlation of groups A and C **(➢Table 1).**

Bland-Altman analyses

➢**Figure 2** and ➢**Table 1** present the Bland-Altman analyses.

In the absence of bridging (group A), the mean difference between specific assays and LC-MS/MS was less than 10 ng/ml.

In bridged patients (group B), the mean difference between Biophen®DiXaI LOW, STA®LAX and LC-MS/MS increased (from 4.7ng/ml to 13.5ng/ml for Biophen®DiXaI LOW and from -1.5 ng/ml to 20.9 ng/ml for STA®LAX) whereas it remains similar from Biophen®DiXaI (8.8 ng/ml for group A, 12.4 ng/ml for group B).

Table 1. Statistical characteristics, Spearman correlation, linear regression and Bland-Altman analyses of the rivaroxaban plasma concentrations in the different groups.

	Biophen®DiXaI	Biophen®DiXaI LOW	STA®-Liquid Anti-Xa
Group A: Patients not bridged (n=31)			
Range of rivaroxaban plasma concentration (ng/ml)	2.1 to 108.2		
median (ng/ml)	32.6		
interquartile range (ng/ml)	13.4 to 71.30		
Bland-Altman			
mean bias (ng/ml)	8.8	4.7	-1.5
SD (ng/ml)	16.8	8.4	10.7
95% limits of agreement (ng/ml)	-24.1 to 41.8	-11.8 to 21.2	-22.4 to 19.5
R square	0.82	0.94	0.95
Spearman *r*	0.91	0.97	0.96
95% CI	0.81 to 0.96	0.94 to 0.99	0.92 to 0.98
Group B: Patients bridged with LMWHs (n=48)			
Range of rivaroxaban plasma concentration (ng/ml)	< 1.0 to 10.1		
median (ng/ml)	1.0		
interquartile range (ng/ml)	0.0 to 1.0		

Bland-Altman *mean bias (ng/ml)*	12.4	13.5	20.9
SD (ng/ml)	9.7	14.5	12.6
95% limits of agreement (ng/ml)	-6.6 to 31.5	-14.8 to 41.8	-3.7 to 45.5
R square	0.05	0.01	0.00
Spearman *r*	0.26	0.18	0.19
95% CI	-0.03 to 0.52	-0.11 to 0.45	-0.11 to 0.45
Group C: Patients bridged with therapeutic LMWH with a last administration of LMWH ≥ 24 hours before (subgroup of group B) (n=26)			
Range of rivaroxaban plasma concentration (ng/ml) *median (ng/ml)* *interquartile range (ng/ml)*	< 1.0 to 10.1 0.5 0.0 to 1.0		
Bland-Altman *mean bias (ng/ml)*	11.4	10.2	17.6
SD (ng/ml)	10.4	7.0	6.6
95% limits of agreement (ng/ml)	-8.9 to 31.7	-3.6 to 24.0	4.7 to 30.6
R square	0.01	0.10	0.10
Spearman *r*	0.04	0.25	0.29
95% CI	-0.37 to 0.43	-0.16 to 0.59	-0.12 to 0.62

Table 1. Bland-Altman was calculated as the difference (A-B)/average, where A was the result of the corresponding coagulation test and B, the result of the LC-MS/MS. Limits of agreement of the Bland-Altman analyses were calculated as the mean difference – or + 1.96*standard deviation for the 5th and the 95th limit of agreement, respectively.

Figure 2. Bland-Altman analyses of the estimation of rivaroxaban plasma concentrations of Biophen®DiXaI, Biophen®DiXaI LOW and STA®LAX with LC-MS/MS measurements.

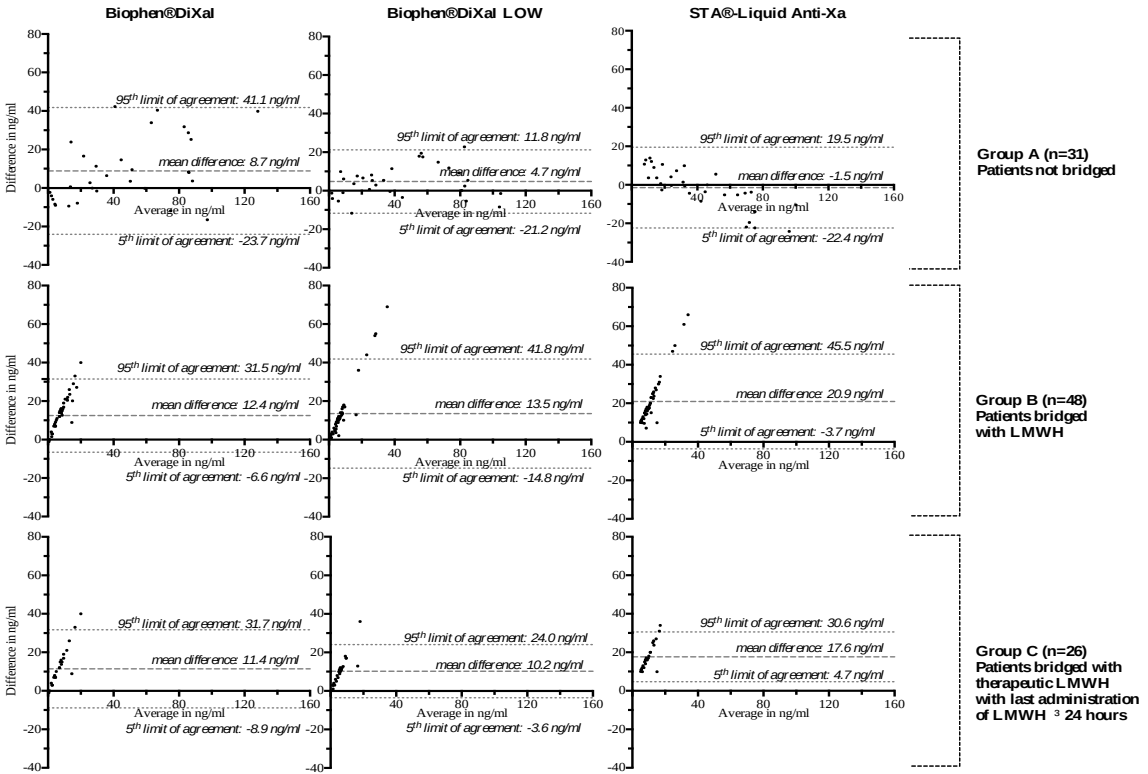


Figure 3. Influence of LMWHs on the estimation of rivaroxaban plasma concentration with the three chromogenic anti-Xa assays.

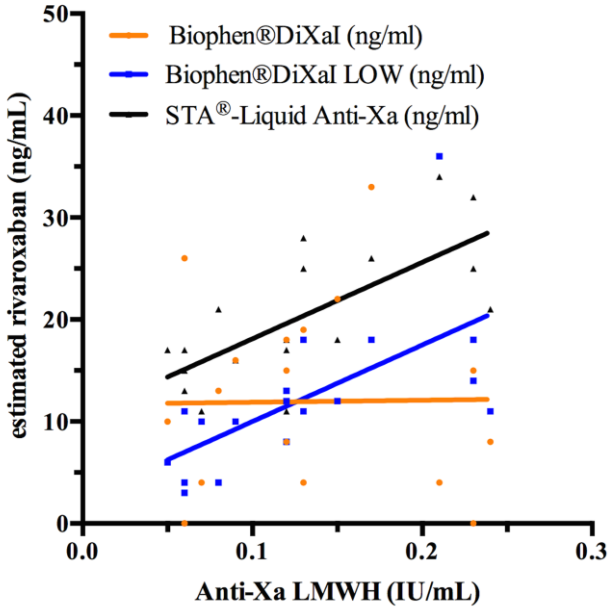


Fig 3. Biophen®DiXaI LOW and STA®LAX are influenced by the presence of LMWH. Up to 0.12 UI/ml, Biophen®DiXaI LOW performs better than Biophen®DiXaI but above this threshold, the overestimation of the Biophen®DiXaI LOW is higher than that of Biophen®DiXaI which is not influenced by LMWH at these concentrations. Samples with LMWH concentrations below the LOQ of the heparin assay were not included.

Limit of detection and quantitation

The LOD and LOQ were 16 and 53 ng/ml respectively for Biophen®DiXaI, 4 and 12 ng/ml for Biophen®DiXaI LOW and 11 and 36 ng/ml for STA®LAX.

Interference of the anti-Xa activity of LMWH administered ≥ 24h ago with Biophen®DiXaI, Biophen®DiXaI LOW and STA®LAX

Among patients in **Group C**, the residual LMWH activity could not be estimated in 4 samples because of insufficient plasma. For the remaining 22 samples, the LMWH activity was below the LOQ of the anti-Xa assay (i.e. Biophen®Heparin LRT) in 4 samples, and between 0.05 UI/ml and 0.25 UI/ml in 18 samples. ➢**Figure 3** shows the impact of residual LMWH anti-Xa activity on the estimation of rivaroxaban in these 18 samples. The correlation was non-significant for Biophen®DiXaI (Spearman r: 0.09; 95% CI: -0.41 to 0.55) but was significant for Biophen®DiXaI LOW (Spearman r: 0.76; 95% CI: 0.44 to 0.91) and STA®LAX (Spearman r: 0.73; 95% CI: 0.39 to 0.90).

Discussion

These results demonstrate the value of the low version of the Biophen®DiXaI assay (Biophen®DiXaI LOW) for the estimation of low

rivaroxaban concentrations. Due to its higher sensitivity compared with the standard procedure, the LOD and LOQ of Biophen®DiXaI LOW were roughly 4-times lower than those of the standard procedure, allowing accurate measurements of plasma concentrations <50 ng/ml, which is a limitation of the standard Biophen®DiXaI [62]. We found that STA®LAX was also reliable for the estimation of rivaroxaban plasma concentrations at C_{TROUGH}. However, and importantly, agreement with LC-MS/MS depended on the presence of LMWH. Indeed, the performance of Biophen®DiXaI LOW and STA®LAX decreased when residual LMWH activity was present, demonstrating that these tests are not specific to the direct factor Xa inhibitors (➤**Table 1**, ➤**Figure 2&3**). To our knowledge, this study is the first that enables the assessment of the performance of chromogenic anti-Xa assays with high- and low- procedures in blood samples containing both residual rivaroxaban and LMWH.

However, some limitations concerning this study need to be discussed. First, the range of rivaroxaban plasma concentration in patients bridged with LMWH (group B and C) was quite limited (from 0 to 10 ng/ml) and was also below the LOQ of the more sensitive chromogenic anti-Xa assay, the Biophen®DiXaI LOW. Samples from patients bridged with LMWH in the 10 to 50 ng/ml rivaroxaban concentration range would have been needed to assess the performance of the specific chromogenic tests in the concentration

range for which they are designed. However, due to the design of our study, which followed the initial perioperative management protocol proposed by the French Working Group on Perioperative Haemostasis (Groupe d'Intérêt en Hémostase Périopératoire - GIHP [86]), patients stopped their rivaroxaban well before the invasive procedure with high bleeding risks, resulting in very low residual rivaroxaban concentrations at the time of the intervention compared with samples recruited at C_{TROUGH} in patients outside the perioperative context (➢**Table 1**). Furthermore, the number of samples recruited in group A could only suggest a tendency to a statistical significant difference between Biophen®DiXaI and STA®LAX. Therefore, these findings need to be confirmed in a larger cohort, preferably within a multicenter study also using coagulometers from different manufacturers since it is recognized that this may affect the sensitivity [150]. According to the previous findings of Harenberg *et al.*, a similar study should also be performed with other chromogenic assays available on the market to analyze the inter-assay variability and reduce it using a mathematical modeling [140]. Lastly, our conclusions cannot be extrapolated to blood samples of patients receiving prophylactic bridging, where 12 h discontinuation of LMWH is considered sufficient to undergo an invasive procedure with appropriate hemostatic conditions.

Report of previous findings

Mani *et al*. have already published the interest of using low calibrators for the measurement of low rivaroxaban concentrations with three other chromogenic anti-FXa assays (COAMATIC®Heparin from Chromogenix, Instrumentation Laboratory, and Technochrom®anti-Xa with and without exogenous anti-thrombin (AT) from Diagnostica Stago®). The LOQ was decreased from 25 ng/ml to 10 ng/ml, when the low calibrators were used instead of the high calibrators. Therefore, they recommended to re-run plasma samples with the low calibrators when rivaroxaban was estimated < 25 ng/ml with the high calibrators. They demonstrated also that anti-Xa assays including exogenous AT were unsuitable for the measurement of rivaroxaban levels as AT falsely elevates rivaroxaban plasma concentrations. This was explained by the ability of AT in excess to complex residual factor Xa. The *ex vivo* samples came all from patients who had received rivaroxaban 10 mg once daily for postoperative prevention of deep venous thromboembolism (DVT) after elective hip or knee replacement, so none had residual heparin [143].

Königsbrügge *et al.* have also used high and low commercial standards of rivaroxaban to calibrate the Biophen®DiXaI and Biophen®Heparin LRT, two chromogenic anti-Xa assays which are and aren't, respectively, specific to rivaroxaban. For the low procedure of the Biophen®DiXaI, they used a different dilution of the plasma samples in the buffer (1:20 instead of 1:8 in

our methodology). They demonstrated that the use of the low calibrators improved the LOD, the LOQ, the mean difference and the standard deviation (in ng/ml) of the two anti-Xa assays compared with high calibrators, for rivaroxaban concentrations below 102 ng/ml [144]. For the Biophen®DiXaI, the LOD and LOQ decreased from 35 ng/ml and 107 ng/ml to 9 ng/ml and 28 ng/ml, respectively. For the Biophen®LRT assay, the LOD and LOQ decreased from 49 ng/ml and 149 ng/ml to 2 ng/ml and 6 ng/ml, respectively. All their samples came from patients treated with rivaroxaban without LMWH bridging. Therefore, we cannot evaluate if the difference in their methodology of the Biophen®DiXaI LOW (dilution 1:20) would have less interference with LMWH than our methodology (dilution 1/8). We can only observe an expected decrease in the LOD and LOQ with the dilution 1:8 compared with the dilution 1:20 (i.e. 4 and 12 ng/ml compared with 9 and 28 ng/ml, respectively). The dilution 1:8 might be more appropriate in the perioperative setting, as some experts group recommend reaching a rivaroxaban plasma concentration ≤ 30 ng/ml before an emergency procedure with high bleeding risk [86].

Proposal for sample management

Low concentrations of rivaroxaban can only be assessed properly with chromogenic anti-Xa assays [61,62,137,151]. However, most of these anti-Xa assays have a LOQ around 30 to 50 ng/ml, making them unreliable for assessing concentrations below the LOQ. Measurements of residual

rivaroxaban concentrations in the perioperative setting are currently recommended before emergencies that cannot be delayed (e.g. thrombolysis in the setting of an acute cerebral thrombosis) or emergencies with high bleeding risks. If a patient is suspected to have an insufficient preoperative interruption of rivaroxaban (e.g. a clinical context with suspected supra-therapeutic plasma concentration), the measurement of rivaroxaban is arguable, especially if the invasive procedure is at high bleeding risk [78].

Without LMWH bridging, the Biophen®DiXaI LOW showed a better correlation than Biophen®DiXaI with the LC-MS/MS measurement (➢ **Table 1**). This difference of correlation with LC-MS/MS between STA®LAX and Biophen®DiXaI tended toward significance. However, one of the advantages of the STA®LAX is its wide application range since the operator does not have to choose between a low and a normal procedure, simplifying the management of samples for the estimation of residual rivaroxaban concentrations. Therefore, we suggest that Biophen®DiXaI LOW and STA®LAX may be used for the estimation of low rivaroxaban, e.g. when rivaroxaban has been stopped for more than 24 h.

The problem of bridging therapy

Recent publications demonstrated the increase risk of major bleedings without a decrease of thrombotic events in patients group bridged with heparins during the perioperative interruption of oral anticoagulants [120,152-154].

The most recent updated guidelines recommend to bridge only patient who are at high risk of thromboembolic events, especially as those were underrepresented in clinical trials analyzing the benefit or harm of bridging therapies (e.g. the BRIDGE clinical trial) [155-157].

With the exception of the Biophen®DiXaI and its specific buffer, LMWH anti-Xa activity leads to an overestimation of the rivaroxaban concentration [145]. We expected that the low procedure of the Biophen®DiXaI would also have this specificity since it is directly derived from the Biophen®DiXaI assay. Unfortunately, this study reveals that in the absence of any rivaroxaban effect, increasing concentrations of residual LMWH overestimated rivaroxaban plasma concentrations measured by Biophen®DiXaI LOW (➢**Figure 2**&**3**). From our data, above 0.12 IU/ml of LMWH, there is no benefit from the low procedure of Biophen®DiXaI (➢ **Figure 3**). These findings may be explained by the fact that, in order to improve the sensitivity, the LOD and the LOQ, the sample is less diluted in the high ionic strength buffer which is less apt to inhibit the catalytic action of LMWHs. The overestimation of rivaroxaban by STA®LAX was predictable as it is not specific for rivaroxaban, however it had no advantage compared with Biophen®DiXaI even in samples with 0.05 IU/ml of LMWH. Therefore, both Biophen®DiXaI LOW and STA®LAX are not suitable for the measurement or the exclusion of residual rivaroxaban

concentrations in bridged patients. The standard Biophen®DiXaI was, as expected, not influenced by the presence of LMWH, even at therapeutic LMWH concentrations (data not shown), but its lack of precision at low levels of rivaroxaban precludes its use for the accurate estimation of residual rivaroxaban in this concentration range.

Some manufacturers have proposed the use of a global anti-Xa plasma activity calibrated with heparin. However, these assays are affected by a high inter-assay variability and the absence of correlation between anti-Xa concentration and heparin anti-Xa IU/ml, except for the low anti-Xa concentrations.[158,159] In case of a patient initially on rivaroxaban and bridged with LMWH, the result will reflect the activity of residual rivaroxaban and LMWH. These assays are thus suitable to exclude presence of anti-Xa activity but should not be used to quantify direct anti-Xa activity or to predict the bleeding risk of the patients receiving LMWH after rivaroxaban suspension.

Perspectives

This study represents real-life conditions, without routine access to LC-MS/MS. Thus, a chromogenic anti-Xa assay that is not sensitive to heparin and is more accurate than Biophen®DiXaI for the low concentrations of rivaroxaban should be developed. Our findings should help to improve the performance of these dedicated tests in the presence of heparins. These results suggest that both STA®LAX and Biophen®DiXaI LOW could be tested with a high ionic strength buffer which, in the case of Biophen®DiXaI LOW, should be six times more concentrated than the current buffer to recover the specificity of Biophen®DiXaI. Secondly, as suggested by Asmis *et al.*, the use of a heparin-neutralizing agent such as polybrene or heparinase, could be tested to evaluate if it improves the specificity to rivaroxaban [141]. Meanwhile, clinicians need to inform their laboratory about the timing and dose of the last LMWH and rivaroxaban administration, so that the most reliable test can be used depending on the patient's specific situation and the clinical question.

Conclusion

This study confirms previous data and recommendations about the use of low methodologies to estimate low rivaroxaban concentrations. However, the main point is that it highlights important limitations of the chromogenic

tests currently available for the management of patients bridged with LMWHs. While the low procedures may be preferred for the estimation of rivaroxaban concentrations below 50 ng/ml, these methods are sensitive to LMWHs and may be influenced even 24 h after of the last LMWH administration. Currently clinicians and laboratories are ill-equipped to assess bridged patients when a reliable estimate of the residual rivaroxaban concentration is required. Even if bridging therapies with heparins will decrease in the near future, there will still be patients at high thromboembolic risks who will benefit from it. Therefore, the development of a chromogenic anti-Xa assay accurate at low rivaroxaban concentrations and insensitive to heparins is required to optimize their perioperative management.

ADDENDUM

Disclosures

The authors have no relevant conflicts of interest to disclose.

Consent for publication

All the studies were performed in accordance with the Declaration of Helsinki and were approved by the Medical Ethics Committee of the Centre Hospitalier Universitaire (CHU) Dinant Godinne UcL Namur in Yvoir, Belgium (number B039201524384).

Funding

Sarah Lessire received a research grant from the Fondation Mont Godinne to complete her thesis.

Aknowledgements

Is Thrombin Time useful for the assessment of dabigatran concentrations? An in vitro and ex vico study

The authors thank Dr Elizabeth Wager for language editing.

The authors thank Prof. Edith Collard for her logistic support.

Estimation of dabigatran plasma concentrations in the perioperative setting: An ex vivo study using dedicated coagulation assays

The authors thank Mrs. Justine Baudar, Mr. Philippe Devel and Mr. Sébastien Walbrecq for their contributions to this work.

The authors thank Hyphen BioMed® and Diagnostica Stago® for supplies of reagents.

Use of appropriate coagulation assays in peri-procedural contexts for patients on dabigatran etexilate: a case series

The authors thank Dr Elizabeth Wager for language editing.

The authors thank Pr Jean-Michel Dogné, Dr Anne-Sophie Dincq, Pr Maximilien Gourdin, and Dr Gisèle Maury for reviewing the manuscript, Prof. Edith Collard for her logistic support, and Dr Gisèle Maury and Mrs Anne-Laure Sennesael for contributing to the collection of clinical data.

The authors thank Mrs Justine Baudar, Mrs Françoise Biot and Mrs Maité Guldenpfennig for laboratory testing.

The authors thank Pr Paul Hjemdahl, Andreas Engström and Yuko Rönquist from the Department of Clinical Pharmacology, Karolinska University Hospital and Clinical Pharmacology Unit, Stockholm, Sweden, for providing LC-MSMS.

Estimation of rivaroxaban plasma concentrations in the perioperative setting in patients with or without heparin bridging

The authors thank Dr. Elizabeth Wager for language editing and Benoît Bihin for reviewing the statistical section of our manuscript.

The authors thank Justine Baudar, Maïté Guldenpfennig, Françoise Biot, Celia Devroye, Christelle Vancraeynest and Charlotte Tordoir for their technical support and Professor Edith Collard for her logistic support.

IV. Discussion and perspectives

A. Why, when and how to assess the anticoagulant effect of DOACs in a perioperative setting

i. The preoperative assessment for elective invasive procedures

Throughout this thesis, the clinical utility of accurate DOAC measurement could be tested in several situations encompassing the perioperative management of patients treated with DOACs.

First of all, optimizing the TT in our institution enabled us to use the TT outside the actual recommended situation which is to confirm the absence of dabigatran when a TT is within its normal range. Despite TT had a good correlation of variation on both coagulometers for concentrations up to 50 ng/ml, we do not recommend to use it as a quantitative tool but either as a qualitative tool. This is due to the impact of the clinical and biological variables on TT that we cannot exclude with certainties, especially in emergent situations. However, knowing that the range of dabigatran plasma concentrations is below 50 ng/ml when TT is below 120 s (with STA®-Thrombin, 1.5 NIH/ml on a STA-R Evolution® coagulometer (Diagnostica Stago®), can guide the laboratory in estimating the range of dabigatran concentrations and in choosing the more accurate coagulation assay for its range of plasma concentrations.

Another way to use TT in a preoperative assessment is to estimate if the patient has a good elimination of dabigatran. Knowing his last intake

(e.g. the morning of the preoperative assessment) and having a TT below 120 s can guarantee that the patient is not accumulating dabigatran. If the TT would be higher than 120 s, clinicians can only estimate a dabigatran concentration probably over 50 ng/ml. Therefore, a sensitive aPTT reagent would give further informations. If the aPTT was normal, the dabigatran range would be probably close to 50-60 ng/ml, as these concentrations can show a normal aPTT in real life. If the aPTT was increased, only a specific coagulation assay would estimate accurately the plasma concentration of dabigatran.

A TT > 120 s after several days of dabigatran arrest can also indicate that the patient is within a supratherapeutic range, when other interferences with TT have previously been excluded.

A well-optimised TT is a coagulation assay that can give useful informations about patient's pharmacokinetics when it is related to its timing of last drug intake.

In our institution we take profit of the preoperative assessment to spot patients that could be in supra therapeutic concentrations. Following a careful anamnesis of patient's medical history, we aimed at detecting patients that have DOACs prescribed in an off-label use (e.g. incorrect doses), or whom clinical characteristics may enter the patient in a subpopulation that are at increased risk of bleeding. [160] In these cases, a point measurement of the DOACs plasma concentration is prescribed

the day of the preoperative assessment. Except for emergencies, this allows to have a result within a week and to contact the patient and the prescriber if the estimation is sub- or supra-therapeutic.[161] However, in situations where the plasma concentration is outside the on-therapy range, a second analysis at peak and trough should confirm the first statement. Indeed, a recent multicentric study showed a wide inter-individual variability for dabigatran, rivaroxaban and apixaban at peak (mean CV = 46%) and at trough (mean CV= 63%) that could not be accounted for the CrCl only. The correlation with the renal function was poor, except for dabigatran plasma concentrations at trough. The mean CV% of intra-individual variability was 36.6% at trough and 34.0% at peak. [88]

Except in a context of adverse event (thrombosis or bleeding), cautious should be advised before changing the dose, as currently there are no recommendations based on DOACs plasma concentration, except for some sub-populations (i.e. extreme obese patients).[162]

The estimation of DOACs plasma concentration during a preoperative assessment in patients at risk of being in a supra-therapeutic anticoagulant level allows, if needed, to adapt the time of DOAC's interruption before an invasive procedure.

ii. Emergencies

Emergencies that cannot be delayed, with a recent DOAC intake or with a severe bleeding, are examples of indication for a measurement of DOACs residual plasma concentrations to guide patient's management.

Even if rapidly available (on site), it takes still 45 minutes to 1 hour before a specific laboratory is able to deliver a result, as these specific reagents are not daily prepared. A new calibration is only required when the lot of reagents changes or the control is not in the validated range. However, the TAT for DOACs plasma concentration estimation is limited with the timing of reconstitution of reagents and controls, which currently takes 30 minutes. A decrease in the TAT could be obtained when specific procedures are in place for emergencies with severe bleeding, such as for example, warning the laboratory about the type of DOAC before samples are collected, bypassing login and re-labelling of blood samples,[163] or reducing the time of blood samples centrifugation[164] or reagent/control's reconstitution and analysis. However, this needs to be validated in an adequate study.

This has already been evaluated for standard coagulation assays where an emergency haemorrhage panel (prothrombin time, fibrinogen, platelet count, haematocrit) was used for transfusion decisions on bleeding patients and where the TAT was reduced to less than 20 minutes.[165]

For patients presenting a TE on DOAC therapy, as for example an acute ischemic stroke, a rapid TAT for specific coagulation assays can optimize the management of these patients in allowing a thrombolysis within the 3 hours of the beginning of the event, when residual DOAC plasma concentration is low.

The question of which threshold of DOACs plasma concentration is safe for a thrombolysis or whether an antidote should be administered before a thrombolysis of an acute ischemic stroke remains currently unanswered. Mechanical thrombectomy is an alternative to intravenous thrombolysis. However, every cerebral embolism cannot benefit from this technique, and furthermore, this requires an extra organisation to get the interventional radiologist and anaesthetist rapidly ready for the thrombectomy. Every minute counts in this critical situation and therefore improving the TAT of the specific laboratory tests may speed up the access to different treatment option of acute ischemic strokes. Furthermore, even if a thrombolysis cannot be administered, having the initial DOACs plasma concentration can help in the follow up of the patient, e.g. when a haemorragic component due to an important cerebral infarct appears after the ischemic stroke. It can also assess the level of anticoagulation when the TE happened, which is of interest for post-marketing reports and further management of the patient's

anticoagulation (e.g. TE with a correct anticoagulation level or TE because of poor drug adherence).

Recent guidances commented the decision to administer thrombolytic therapy based on the last intake of DOAC, which is an arbitrary recommendation that has not yet been tested.[24] However, this information can be missing (e.g. unconscious patients). Furthermore, with the high inter-individual variability of DOACs plasma concentrations[88] it can be hazardous to perform a thrombolysis only guided by the rough estimation of the last intake.

Therefore, the very sensitive TT has the advantage to rapidly inform about dabigatran's presence. Yet, no residual plasma concentration is validated before a thrombolysis and an elevated TT requires a specific test accurate in low plasma concentrations to manage emergencies, especially if the TT is not optimized. Optimizing the TT inside each laboratory would give rapidly a rough estimation of dabigatran's plasma concentration, as it would have a limit of measurement around a predefined dabigatran concentration. However, care should be advised in emergency settings where the TT can be falsely reduced or prolonged.

A recent study analysed the predictive value of PT, aPTT and TT to detect DOAC concentrations of 30, 50 and 100 ng/ml in an emergency setting (abstract published during the last International Society of Thrombosis and Haemostasis (ISTH) congress in May 2016). They used

the same TT as in our study (i.e. the reagent STA®-Thrombin, Diagnostica Stago, Asnière, France), which had a negative predictive value (NPV) of 100% to exclude clinically relevant concentrations of dabigatran (≥ 30 ng/ml) in emergency situations (813 samples collected in 660 patients).[166]

For the direct anti-Xa agents, a similar sensitive routine test is not available, and even a sensitive reagent of PT cannot exclude clinically relevant concentrations of these drugs. Indeed, in the previous described study using sensitive reagents of PT and aPTT, a combined normal PT and aPTT excluded rivaroxaban concentrations ≥100 ng/ml in 100% of patients, but not ≥50 ng/ml. For apixaban, the NPV did not exceed 70.6% for concentrations of 100 ng/ml.[166]

If a center decides to thrombolyse only patients with no residual effect of anti-Xa agents, a specific coagulation assay accurate in the low plasma concentrations is required.

Kepplinger J *et al.* published recently their decision making protocol based on routine and specific coagulation assays. Their higher threshold of DOACs plasma concentration before a thrombolysis should be validated in a large clinical trial. [139]

It is thus essential to validate an acute stroke management based on several steps:

1. Which DOAC, timing of last intake, warn rapidly the laboratory (Which coagulation test to use? Improve the TAT for standard and specific coagulation assays)
2. Haemorrhagic component at the cerebral scanner (antidote?)
3. Is a mechanical thrombo-embolectomy feasible?
4. Is a thrombolysis feasible? Centers should fix a threshold for residual DOAC concentrations allowing thrombolysis and validate the safety of their procedure.

The laboratory tests should not delay a mechanical thrombo-embolectomy. The rapid measurement of initial anticoagulant level may authorize the addition of a thrombolysis to the procedure, which can be useful if mechanical thrombo-embolectomy fails.

iii. Outside the perioperative or emergency setting

In our recent publication "Minimisation of Bleeding Risks Due to Direct Oral Anticoagulants"[9], which is an updated version of our previous manuscript "Preventive Strategies Against Bleeding Due to Nonvitamin K Antagonist Oral Anticoagulants"[160], we focused on several clinical characteristics or situations (e.g. dehydration, loss of weight) that might

increase the plasmatic concentrations of DOACs in patients outside a perioperative context and who are chronically on DOACs.

Several publications described sub-groups for which the dose tailering could be based on their individual DOACs plasma concentration.[106]

Currently, clinical characteristics guide the dose tailoring to keep a patient within the wide therapeutic range of DOACs. As no routine monitoring was realized during the phase III clinical trials, it is difficult to give strong evidence that adapting the dosage to DOACs plasma concentrations is safe. However, Reilly and collaborators were the first to demonstrate a correlation between ischemic strokes and bleeding outcomes with dabigatran plasma concentrations. Age was the most important covariate. A main limitation of the study conclusions was the lack of dabigatran plasma concentration measurements at the moment of the advert event.[100]

However, the marketing authorization holder of DE (Boehringer Ingelheim Gmbh) has since filed a patent describing a safety profil using monitoring of DE in a patient suffering from NVAF.[61] For dabigatran etexilate 150 mg bid, the company found that plasma concentrations at trough (i.e. 12 h after the last intake) between 90 and 140 ng/ml after one week of DE intake, provided the best benefit risk ratio between prevention of stroke and occurrence of bleedings.[5] Following that DE plasma concentration is below, within or higher than the recommended

plasma concentration, the company proposes different dose adaptations. The simulation found in this dose adjustement, a comparable risk of SSE with a significant reduction of major bleeding.[167] This highlight the interest of screening a plasma level of anticoagulation that improves the benefit risk ratio of DOACs.[106]

In the clinical practice, physicians are regularly confronted to patients with brutal changes in their physiological conditions or medications. There are many examples supporting the usefulness of measuring DOACs plasma concentration in some routine settings. We had recently a patient on dabigatran who had a laryngectomy for several years. He presented difficulties in swallowing the capsule of dabigatran and tried to crush the capsule with its tongue to swallow it more easily. Opening dabigatran's capsule can increase its bioavailability up to 75% instead of 6-8%, putting the patient at risk to be chronically in supratherapeutic plasma concentrations. A dabigatran plasma concentration within high therapeutic ranges would guide the clinicians in prescribing another oral anticoagulant that may be safer for the patient (e.g. rivaroxaban or apixaban which can be safely administered crushed through a nasogastric tube).[168,169]

Another issue is the lack of available data on the extremes of weight and the number of patients in these extremes. The available pharmaco-kinetic and –dynamic evidence suggests decreased drug exposures,

reduced peak concentrations and shorter half-lives with increasing weight, which raises concerns about underdosing this population.[161] Current guidances from the SSC of the ISTH do not recommend the use of DOACs in patients with a BMI of >40 kg/m^2 or a weight of >120 kg. If DOACs are used in this population, the SSC suggest measuring drug-specific peak and trough level (anti-FXa for apixaban, edoxaban, and rivaroxaban; ecarin time or dilute thrombin time with appropriate calibrators for dabigatran; or mass spectrometry drug level for any of the DOACs). If the anticoagulant level is in the on-therapy range, continuation of the DOAC seems reasonable. However, if the level is found to be below the expected range, they suggest changing to a VKA rather than adjusting the dose of the DOAC.

The off-label use of DOACs is not unusual too. For apixaban for example, the dose reduction criterions of 2.5 mg *bid* of apixaban were chosen because pharmacokinetic modelling identified them as predictors of increased apixaban exposure when combined. In the Apixaban for Reduction of Stroke and Other Thromboembolic Complications in Atrial Fibrillation (ARISTOTLE) trial, only 4.7% patients of the apixaban group were concerned by the dose reduction. However, the current global prescription data revealed the use of the lower dose of apixaban in at least 25% of patients.[170] The inappropriate use of the lower dose of apixaban may reflect concern about the risk for bleeding, but this can put

patients in an increased risk for stroke. Indeed, a recent sub-study from the ARISTOTLE trial, showed that the 5 mg *bid* dose of apixaban was safe, efficacious, and appropriate for patients with only 1 dose-reduction criterion. Even if patients with atrial fibrillation and 1 dose-reduction criterion (isolated advanced age, low body weight, or renal dysfunction) (n=3,966) had a higher risk of stroke or systemic embolism (HR, 1.47; 95% CI, 1.20-1.81) and major bleeding than patients without dose reduction criterion (HR, 1.89; 95% CI, 1.62-2.20), both groups showed consistent benefits with the 5 mg twice daily dose of apixaban vs warfarin.[171]

Moreover, a recent observational study demonstrated, through an electronic questionnaire sent by email to prescribers, the lack of awareness of the nature of DOACs.[172] Indeed, on the 143 responders, 88%, 80% and 50% respectively recognised rivaroxaban, dabigatran and apixaban. But when a routine clinical situation was provided, only 13.5%, 17.5% and 16.8% respectively recognised that the hypothetical patient was anticoagulated and only 55 to 58% recognised that it was unsafe to proceed with an invasive procedure. These results indicate a significant risk for patient harm related to lack of knowledge about this new group of frequently used drugs. Additional education and training is required for prescribers and this concerns certainly the role and understanding of laboratory assays.[172]

B. Perspectives of laboratory tests

The performance of specific methodologies able to measure low plasma concentrations of dabigatran (the Hemoclot Thrombin Inhibitors® LOW (HTI LOW) from Hyphen BioMed®, Neuville-sur-Oise, France and the STA®-ECA II (ECA-II) from Diagnostica Stago®, Asnière-sur-Seine, France) and rivaroxaban (Biophen®Direct Factor Xa Inhibitors® LOW from Hyphen BioMed®, and STA®-Liquid Anti-Xa from Diagnostica Stago®) should be validated in a larger perioperative prospective cohort study, including emergencies and elective invasive procedures.

The same studies need to be performed for low plasma concentrations of apixaban and edoxaban in a perioperative setting.

A recent ex vivo study showed a better performance of a homemade dTT than the conventional HTI in the low to intermediate range of dabigatran plasma concentration (<100 ng/ml). They used the same reagent than their TT and calibrators from Hyphen BioMed® and homemade NPP (0 ng/ml of dabigatran).[173]

To improve the inter-laboratory precision, the development of an international reference calibrator and the use of specific calibrators for low DOAC concentrations should be advised. Indeed, in a recent European external quality control for normal to high concentration of dabigatran and rivaroxaban, the interlaboratory variation ranged from 5.0% to 27.4% for dabigatran and depended on the combination reagent-instrument.

Homemade dabigatran calibrators differed from commercially available calibrators. There are only few data about inter-laboratory precision in the low range of DOACs.[174]

For dabigatran, the STA®-ECA II assay can give accurate measurements for patients bridged with heparins with low residual DOAC plasma concentrations. However, as shown in our last study on rivaroxaban, this is not the case for the specific anti-Xa assays, as the specific Biophen®Direct Factor Xa Inhibitors (DiXaI) (from Hyphen BioMed®, Neuville-Sur-Oise, France) has a limit of detection of 30-50 ng/ml and its adapted methodology for low concentrations gives falsely increased measurements of rivaroxaban with small quantities of residual LMWH found at > 24 hours of last administration of therapeutic LMWH.

The recent published perioperative guidelines should drastically decrease the number of patients that need a bridging therapy. However, there will still be patients on rivaroxaban, apixaban or edoxaban that might receive a heparin bridge in a perioperative setting due to their high TE risk profil, prolonged fasting conditions, or even patients who require an initial LMWH treatment before starting edoxaban in the acute treatment of a VTE.

For this category of patients, laboratories need to adapt or develop assays specific for low plasma concentrations of direct anti-Xa agents that are not sensitive to other anticoagulants.

Gosselin *et al.* studied the potential utility of UFH or LMWH-calibrated heparin assays to screen the presence (or absence) of significant levels of FXa DOAC. Their anti-Xa assay data suggest that most LMWH- or UFH-calibrated methods can exclude significant levels of anti-Xa DOACs.

All tested LMWH-calibrated methods were able to detect the lowest concentration of drug (e.g. 21 ng/ml of edoxaban), and only in the AT-supplemented Berichrom UFH method, the concentration of 21 ng/ml of edoxaban fell below the LOQ.

Most coagulation analyzers have the capability to run the heparin assays, and FDA-approved heparin calibrators and controls are commercially available, which is not the case for direct anti-Xa agents.

Heparin assays are not specific and cannot distinguish between heparins and direct anti-Xa agents, but their use could assist clinicians in patient management in emergent situations, especially when the clinical history is not available.

Figure 1 illustrates the current performances of laboratory assays in the measurement of DOACS.

Figure 1. Current performances of laboratory assays in the measurement of DOACS[55,63,70,159]

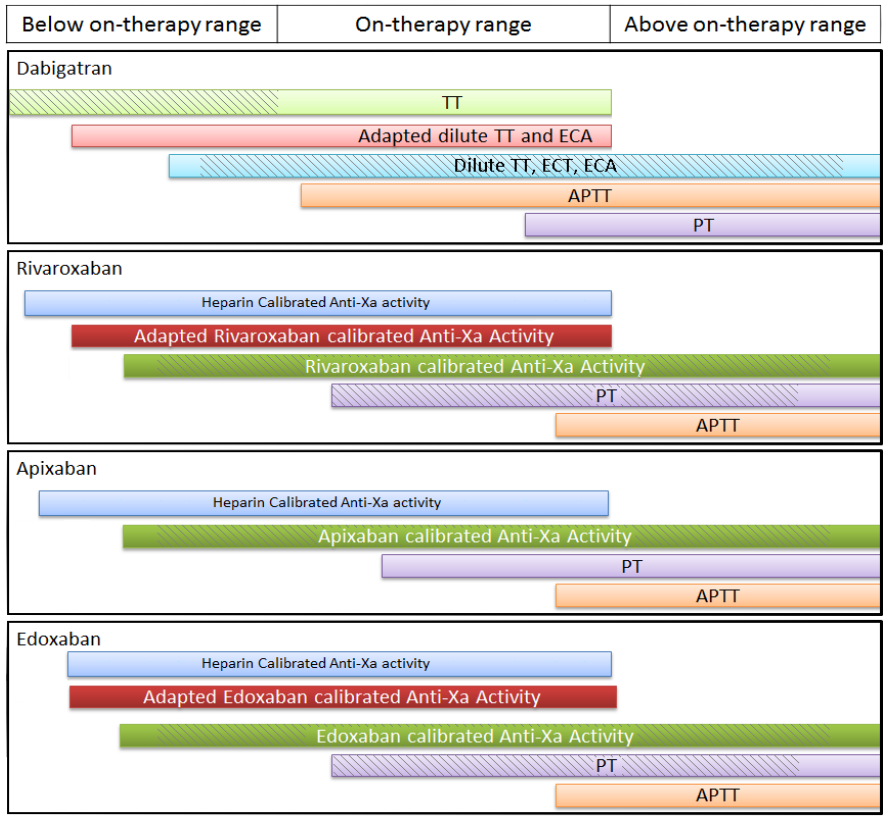


Fig 1. Sensitivity and Linearity of Coagulation Assays to Below, Within, and Above Typical On-therapy Concentrations of Dabigatran, Rivaroxaban, Apixaban and edoxaban. Horizontal bars correspond to the approximate range of detectability (i.e., sensitivity) of each assay to below, within, and above typical on-therapy concentrations of dabigatran, rivaroxaban, apixaban and edoxaban. Hatching corresponds to the linearity of each assay to below, within, and above typical on-therapy concentrations of dabigatran, rivaroxaban, apixaban and edoxaban. Ranges are approximations and may vary based on choice of reagent. Typical on-therapy drug levels are shown in Table 2. APTT = activated partial thromboplastin time; ECA = ecarin chromogenic assay; ECT = ecarin clotting time; PT = prothrombin time; TT = thrombin time.

Coagulation assays in emergency settings are the most efficient when their TAT is short. Point-of-care (POC) monitoring and other global assays have been tested for different DOACs.

A recent review described the limit and interest of these tests for each DOAC.[175] They concluded that for dabigatran, global assays (Thrombin generation assay (TGA), Prothrombinase induced clotting time (PiCT), Thromboelastography (TEG), Thromboelastometry (TEM), and Activated clotting time (ACT) lack standardization, are insufficiently studied and are not available in clinical practice. For rivaroxaban the dPT, DRVVT, modified PiCT and modified HepTest methods have no advantage over PT. Furthermore, the TGA, TEG and TEM are not routinely available and costly. However, they can be useful to assess the efficacy of reversal agents. For apixaban, the TGA can measure the intensity of apixaban anticoagulation and assess the effectiveness of reversal agents. For edoxaban, further studies of specialized and global tests are required.[175]

However, as previously discussed, it has more sense to give priority to research studies that improves the TAT of the specific assays measuring accurately low to high plasma concentrations of DOACs than to improve high costly POC monitoring that are less acurate and may have high intravariability in their plasma concentration estimation.[163]

Reducing the TAT of specific test to 20 minutes would supplant any POC monitoring.

Other research project including the monitoring of DOACs concerns the impact of DOACs on ACT measurements during catheter ablation of AF.

During this procedure, it is 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, and a target ACT has not yet been re-determined for the peri-procedural use of DOACs in AF ablation.[176-181]

C. Perspectives in the perioperative management and implication of laboratory tests

Outside the benefit of accurate tests for the measurements of DOACs, there is a clear need to standardize the peri-procedural management of patients on DOACs.

Even if in the last years the recent updated expert's recommendations showed more similarities, the timing of preoperative DOAC interruption is still varying and this is partly due to the definition of what is a low, moderate and high bleeding risk surgery.

For the GIHP, the high bleeding risk surgery integrates moderate and high risk bleeding procedures, which cannot be realized in patients with anticoagulation.[76] For the European Heart Rhythm Association practical guide, the low risk procedures have a low frequency of bleeding and/or minor impact of a bleeding and the high-risk procedures have a high frequency of bleeding and/or important clinical impact.[24,182]

Spyropoulos *et al.* categorize the high bleeding risk procedures by a two-day risk of major bleeding ≥ 2% and low procedures by a two-day risk of major bleeding < 2%. [134]

In any cases, there is sufficient evidence that some procedures may be realized on anticoagulant therapy (procedure with minimal risk), as for example superficial skin surgeries, parietal surgery, cataract surgery, minor dental procedures. It is sometimes suggested to avoid the dose of the

morning of the procedure to avoid peak concentrations during these procedures.

Pacemaker or cardioverter-defibrillator device implantation can be realized safely without stopping VKAs, but this should be confirmed for DOACs.[183-185]

However, some procedures are categorized in the high bleeding risk procedures or low bleeding risk procedures, depending on the experts' proposal. This discrepancy in the categorization of the bleeding risk influences the delay of arrest and thus has a clear impact on residual DOAC concentrations as shown in the case-series describing patients on dabigatran in a perioperative setting.

Taking the example of the neuraxial anaesthesia or intracranial neurosurgery, some experts define these procedures as other conventional high bleeding risk procedures[134,182], while others are more cautious and add an extra delay to DOAC interruption.[76]

DOAC resumption after high bleeding risk procedures are relatively similar: therapeutic doses of DOACs should be deferred for 24–72 h.[24,76,134,182] If necessary the use of a stepwise approach of bridging therapy with prophylactic dose of heparins can be considered for patient at high TE risks.[24,76,182] Spyropoulos *et al.* proposes rather a reduced dose of dabigatran (75 mg twice daily), rivaroxaban (10 mg once daily) or apixaban (2.5 mg twice daily) on the evening after surgery and on the following day (first

postoperative day) after surgery. They recommend bridging therapy with heparins only in patients who cannot tolerate oral medications.[134] If patients are at high TE risks and the haemostasis is not under control, mechanical VTE prophylaxis should be considered.

The GIHP recommend a bridge with heparins by the time an indwelling catheter is in place after neuraxial anaesthesia.[76]

For low bleeding risk surgery, some experts recommend to restart DOACs 6-8 hours after end of the surgery. Spyropoulos *et al.* recommend 24 hours before full dose of DOAC intake.

Planning a safe resumption of DOAC is essential as a premature re-initiation of heparin therapy (within 24 hours after the procedure) is an avoidable-independent predictor of major bleeding. [186]

Table 4 shows recent perioperative guidances.

Table 4. Resuming of recent perioperative management of DOACs[24,76,134,182]

DOAC			Dabigatran		Rivaroxaban - Apixaban		Edoxaban	
Bleeding risk of invasive procedure			**LOW Bleeding Risk**	**HIGH Bleeding Risk**	**LOW Bleeding Risk**	**HIGH Bleeding Risk**	**LOW Bleeding Risk**	**HIGH Bleeding Risk**
GIHP (Groupe d'Intérêt en Hémostase Péri-opératoire)	Preoperative interruption *No bridging (except patients with high risk of TE)*	CrCl ≥ 50 ml/min	Last dose ≥ 24h before surgery	Last dose 4 days before surgery	Last dose ≥ 24h before surgery	Last dose 3 days before surgery	Last dose ≥ 24h before surgery	Last dose 3 days before surgery
		CrCl > 30 ml/min		Last dose 5 days before surgery				
		For very high risk procedure (neuraxial anaesthesia)	Last dose 5 days before surgery					

<table>
<tr><td></td><td>Resumption after invasive procedure or surgery</td><td></td><td colspan="6">LOW Bleeding risk :
• resume minimum 6h after invasive procedure or surgery
HIGH Bleeding risk :
• prophylactic dose of LMWH, UFH or fondaparinux minimum 6h after invasive procedure or surgery if venous thromboprophylaxis is indicated
• therapeutic dose of DOACs when haemostasis is controlled (24-72 h)

For neuraxial anaesthesia with indwelling catheter:
• resumption with LMWH or UFH until indwelling catheter is out</td></tr>
<tr><td rowspan="6">Heidbuchel et al.</td><td rowspan="5">Preoperative interruption

No bridging</td><td>CrCl ≥ 80 ml/min</td><td>≥ 24h</td><td>≥ 48h</td><td>≥ 24h</td><td>≥ 48h</td><td>≥ 24h</td><td>≥ 48h</td></tr>
<tr><td>CrCl 50-80 ml/min</td><td>≥ 36h</td><td>≥ 72h</td><td>≥ 24h</td><td>≥ 48h</td><td>≥ 24h</td><td>≥ 48h</td></tr>
<tr><td>CrCl 30-50 ml/min</td><td>≥ 48h</td><td>≥ 96h</td><td>≥ 24h</td><td>≥ 48h</td><td>≥ 24h</td><td>≥ 48h</td></tr>
<tr><td>CrCl 15-30 ml/min</td><td>Not indicated</td><td>Not indicated</td><td>≥ 36h</td><td>≥ 48h</td><td>≥ 36h</td><td>≥ 48h</td></tr>
<tr><td>CrCl < 15 ml/min</td><td colspan="6">No official indication for use</td></tr>
<tr><td>Resumption after invasive procedure or surgery</td><td></td><td colspan="6">LOW Bleeding risk :
• DOACs 6-8h
HIGH Bleeding risk :
• LOW TE risk → resume DOACs 48-72h after procedure
• HIGH TE risk → prophylactic or intermediate dose of LMWH 6-8h after procedure, resume DOACs when haemostasis is controlled (48-72h)</td></tr>
</table>

Spyropoulos *et al.*	Preoperative interruption *No bridging (except patients with high risk of TE)*	CrCl > 50 ml/min	Last dose 2 days before surgery	Last dose 3 days before surgery	Last dose 2 days before surgery	Last dose 3 days before surgery	Last dose 2 days before surgery	Last dose 3 days before surgery
		CrCl 30-50 ml/min	Last dose 3 days before surgery	Last dose 4-5 days before surgery	Last dose 2 days before surgery	Last dose 3 days before surgery		
		CrCl 15-29 ml/min			Depends on patient and procedural factors	Depends on patient and procedural factors		
	Resumption after invasive procedure or surgery		LOW Bleeding risk : DOACs 24h HIGH Bleeding risk : • LOW TE risk → resume DOACs 48-72h after procedure • HIGH TE risk → consider a reduced dose of dabigatran (75 mg twice daily), rivaroxaban (10 mg once daily) or apixaban (2.5 mg twice daily) on the evening after surgery and on the following day (first postoperative day) after surgery.					

Surgical aptitudes influence the risk of bleeding of a procedure, as a surgeon of great ability and very cautious can balance some high risk bleeding surgery to lower bleeding risk surgery, and unfortunately the reverse holds true…

Nevertheless, once this categorization of bleeding risk done, the same recommendations of DOAC's interruption should be recommended. Some adaptations to patients at very high risk of thrombosis or bleeding should be proposed.

Outside the availability of experts' guidances in the literature, every institution should have an easy access to their peri-procedural management. Written documents should be available and a specific multidisciplinary team created to discuss some challenging cases concerning elective surgeries as well as different types of emergencies or adverse events due to DOACs.

The implementation of electronic supports may increase the adherence to the perioperative management of DOACs. In addition, regular educational support and contact with general practioners or prescribers should offer the potential to improve DOAC management.

In the following sub-chapters some perioperative pending issues are addressed with a section concerning DOAC monitoring.

i. Timing for neuraxial anaesthesia

Procedures as neuraxial anesthesia are considered as procedure with high bleeding risks. The overall incidences of spinal haematoma in patients receiving an epidural or spinal anaesthesia are estimated to be 1/220000 and 1/320000 patients, respectively. In the presence of risk factors (e.g. multiples attempts, spinal abnormalities, inherited or acquired coagulopathies, heparin administration), the bleeding risk can be increased up to a two orders of magnitude.[187,188]

Spinal haematomas need to be managed urgently to avoid irreversible damages.

These procedures require a complete haemostatic function and cautious in their perioperative management, especially when patients are on anticoagulant treatment. Alternatives in anaesthesia techniques should be proposed if uncertainties persist on the haemostatic conditions and no laboratory monitoring is available. However, as neuraxial techniques may improve post surgical outcome[189], a sufficient preoperative interruption of DOACs should be cautiously planned, avoiding unwanted report of interventions or change in anaesthetic management that could deprived a patient from a technique favourable to manage postoperative pain.

Different recommendations have been published these last years based on DOAC's estimated half-life.

Rosencher *et al.*[35] and Gogarten *et al.*[190] published guidances based on

DOAC's half-life in patients receiving a dose for VTE prevention post joint surgery. They consider an interruption of two half-lifes as sufficient before neuraxial anaesthesia. The authors assume that a stable clot is formed in 8 hours, and that the anticoagulant can be administered in a timing of *"8 hours minus its time to peak concentration (Tmax)" (*which is 1 to 4 hours for DOACs). They propose this timing for DOAC's resumption after catheter withdrawal, but this applies only for prophylactic doses of DOACs. The presence of bleeding during needle puncture and catheter placement should delay anticoagulant therapy post surgery for 24 h. [191]

Recent guidances have been published by the American Society of Regional Anesthesia and Pain Medecin[84] (ASRA) in collaboration with the European Society of Regional Anaesthesia and Pain Therapy, as well as other societies of Pain Medecine and Neuromodulation. They suggest a 5-elimination half-life interval between oral anticoagulants discontinuation and intermediate or high-risk pain procedures. This corresponds to an interruption of 4-5 days for dabigatran (6 days if end-stage renal disease), and 3 days for rivaroxaban and 3 to 5 days for apixaban, because of its wide pharmacokinetics variability.

They propose to restart DOACs administration 24 hours after the pain intervention, excepted if there is high risk of VTE. In this case, half of the usual dose can be given at 12-hours after the pain procedure and should be notified to the patient's treating physician(s).[84]

These guidances are similar to the recent updates of the perioperative management of DOACs written by the GIHP. They propose an interruption up to 5 days for DOACs to guarantee a normalized haemostasis (less than 3% of the anticoagulant concentration at peak time). [76]

Other experts' proposals have been published and the delay of interruption is based on the half-life of the medication and the renal function.

Heidbuchel *et al.* published recently a second update of the Europace guidances. They consider neuraxial anaesthesia as a conventional high bleeding risk procedure and for patients on DOACs with normal renal function (= CrCl > 80 ml/m) they suggest at least 48 hours of DOACs interruption. This delay increases for dabigatran proportionally to the decrease of CrCl (see Table 4). No recommendation is given for dabigatran interruption in patients with a CrCl < 30 ml/min, as it is not recommended for such renal impairment. For apixaban, edoxaban and rivaroxaban, the impact of the CrCl on half-life is lower and therefore they recommended at least 48 hours of DOAC interruption, except for CrCl below 15 ml/min where these DOACs are not recommended. [182]

However, 48 hours of arrest has been proved to be not sufficient to guarantee a normal haemostasis for all patients. Indeed, two multicentric studies with peri-procedural dosage of DOACs proved that around 15% of patients on dabigatran[192] or dabigatran and rivaroxaban[79], had over 20 ng/ml

and 30 ng/ml, respectively at 48 hours of DOACs arrest.

Spyropoulos *et al.* made a recent update of their peri-procedural management of DOACs. The delay of interruption is based on DOACs respective half-life and patients CrCl. They are almost similar to the Europace guidances, as they consider neuraxial anaesthesia as a conventional high bleeding risk surgery, but they propose a first delay of DOACs interruption for a CrCl below 50 ml/min (including here normal and slightly impaired renal function) of minimum 60 hours up to 72 hours. For decreased CrCl up to 30 ml/min they recommend a dabigatran interruption of 4 to 5 days. For patients with a CrCl of 15 to 30 ml/min treated with rivaroxaban and apixaban, the last dose of DOAC should be individualized on the basis of patient and procedural factors for bleeding and thrombosis. Interestingly, for edoxaban they only give a 60 to 72 hours DOACs interruption for patients with a CrCl >50 ml/min. No recommendations are given for edoxaban in patients with moderately impaired renal function. This is perhaps due to an increased renal metabolism of edoxaban, compared with apixaban and rivaroxaban (see Table 1), which can lead to uncertainties about half-life elimination of edoxaban with lower CrCl. A focused review of edoxaban clinical pharmacology showed that the lower dose (15mg) for severe renal impairment (CrCl between 15 and 30 ml/min) had similar plasma concentrations as reduced doses (30mg) for normal to mildly impaired renal function (CrCl > 50 ml/min). These plasma concentration

estimations do not show any drug accumulation with the adapted dose administration. However, it cannot guarantee that three days are sufficient to reach the recommended threshold in plasma concentration (i.e. 30 ng/ml) before a high bleeding risk procedure. Indeed, pharmacokinetic studies of edoxaban showed that patients with moderately impaired renal function had a decrease in total edoxaban clearance of 1/5 compared to mildly impaired renal function. For severely impaired renal function, the total edoxaban clearance decreased of ¼ of the clearance of mildly impaired renal function.[193]

Douketis *et al.* commented recently the last ASRA guidances.[132] They suggest that considering only the half-life of a DOAC is insufficient to determine the required interruption intervals before neuraxial anaesthesia. They put forward the need to define the ideal timing of interruption based on residual DOACs plasma concentration measured in the perioperative setting. The reply of Benzon *et al.* concluded with the need of conducting further research projects implementing DOAC perioperative measurements, to provide sufficient high quality evidences to guide perioperative DOACs interruption.[133]

We also commented this point of view in addressing the need to use accurate measurements for low DOACs plasma concentrations in such research

projects and in remembering the limit of using routine tests to conclude on the presence or absence of clinically relevant DOAC concentrations.[194] Indeed, as illustrated by our cases-series, conventional HTI measured dabigatran level < 30 ng/ml, as adapted methodologies for low concentrations of dabigatran (HTI LOW and ECA II) gave much closer results to LCMS/MS measurements (which were in the three cases > 30 ng/ml).

Observational studies that are realized with accurate perioperative measurements will validate a unique perioperative management of DOACs, with the best timing of interruption before neuraxial anaesthesia.

ii. Heparin-bridging therapy

As previously discussed, the issue of residual heparin interfering with the estimation of low concentrations of direct anti-Xa agents will decrease in the near future as new perioperative recommendations suggest to bridge only patients with high TE risk.

The administration of LMWH after arrest of oral anticoagulants was initially recommended to cover the periperative gap with insufficient anticoagulation. The 2012 ACCP Antithrombotic Therapy Guidelines recommended with a weak Grade 2C the use of heparin bridging (therapeutic doses of UFH or LMWH) in high TE risk patients. No recommendations on the use of heparin bridging therapy in moderate TE risk patients were made, but they suggested an individual assessment of patient-specific risk factors for bleeding and thrombosis to guide the decision to bridge or not.[156]

The recommendations of the European Society of Anaesthesiology (ESA) published in 2013[195] differed as they suggested that DOACs could be stopped 5 days before procedures requiring normal coagulation in patients treated with rivaroxaban, apixaban, edoxaban, and in patients treated with dabigatran and a CrCl higher than 50 ml/min. Bridging was not suggested for low risk low-risk patients (grade 1C), but well for high-risk patients including patients with AF and CHADS2 score > 2 (grade 2C).

In patients treated with dabigatran with a creatinine clearance between 30 and 50 ml/ min, they suggested 5 days of interruption before surgery with no

bridging therapy (grade 2C). Their approach of heparin bridging was cautious as they recommended starting LMWH or UFH only at day 3 before invasive procedures, avoiding the risk of overlapping high level of two different anticoagulants at day 4.[195] Their last guidances are currently available as a draft on the ESA website before definitive publication, and the bridging recommendations are hard to find.

In the last decades, LMWHs have largely replaced UFH because they are at least equally effective in prevention and treatment of VTEs and have the possibility of subcutaneous administration without the need of routine laboratory monitoring of the anticoagulant response. Moreover, LMWHs have a more predictable anticoagulant response and a longer half-life allowing once or twice daily administration. [196-200]

Over the past decade, the question of an effective and efficient peri-procedural bridge has been addressed in several standardized heparin-bridging regimens.[201-208]

In a meta-analysis published in 2012[153] analysing ATE and bleeding risks in patients receiving heparin bridging during interruption of vitamin K antagonists for elective procedures, thromboembolic events occurred in 0.9% of bridged patients (95% confidence interval [CI], 0.0.0-3.4) and 0.6% of non-bridged patients (95% CI, 0.0-1.2). In eight studies, the risk of thromboembolic events in bridged and non-bridged patients was the same (OR 0.80; 95% CI, 0.42-1.54). There was an increased risk of overall

bleeding in 13 studies (OR 5.40; 95% CI, 3.00-9.74) and major bleeding in 5 studies (OR 3.60; 95% CI, 1.52-8.50) in bridged patients compared with non-bridged patients. In a comparative study of full versus prophylactic/intermediate-dose low-molecular-weight heparin bridging, no difference in thromboembolic events was seen (OR 0.30; 95% CI, 0.04-2.09) but well an increased risk of overall bleeding (OR 2.28; 95% CI, 1.27-4.08) in the full heparin-bridging group. The need of a standardized perioperative protocol to compare bridged patients versus non-bridged patients was necessary to strengthen these results. [153]

The BRIDGE trial has recently confirmed the hypothesis that forgoing bridging anticoagulation was non-inferior to perioperative bridging with low-molecular-weight heparin for the prevention of arterial thromboembolism, and decreased the risk of major bleeding in patients on warfarin.

Indeed, there was a three-fold to four-fold increased risk of major bleeding with the use of therapeutic doses of heparin bridging, and no benefits in reducing the rate of TE events. [155]

These conclusions are validated for patients on warfarin. For other VKA, the timing of last intake should be adapted to each half-life and prospectively evaluated in a clinical trial.

Concerning the perioperative arrest of direct oral anticoagulants, several

observational studies have supported similar conclusions concerning the benefits of heparin bridging.

The DRESDEN registry was the first perioperative observational study including 595 patients who underwent 863 procedures (15.6% minimal, 74.3% minor, and 10.1% major procedures). Major cardiovascular events and major bleeding complications occurred in 1.0% (95% CI : 0.5–2.0) and in 1.2% (95% CI 0.6–2.1) of patients, respectively. Both events were highest after major procedures (4.6 and 8.0%, respectively). The use of heparin bridging significantly increased with the surgical risk (10.4, 27.8, and 74.7% for minimal, minor, and major surgery, respectively).

Multivariate analysis demonstrated that diabetes (OR 13.2) and major procedures (OR 7.3) were independent risk factors for cardiovascular events and that major procedures (OR 16.8) were an independent risk factor for major bleeding. Heparin bridging did not reduce cardiovascular events, but increased significantly the rate of major bleeding (2.7%) compared with no bridging (0.5%). However, when they assessed major and non-major procedures separately, they found that heparin bridging was not an independent risk factor for major bleeding.

They concluded that a short perioperative arrest (three days) of DOACs was safe without heparin bridging and that patients at cardiovascular risk undergoing major procedures may benefit from heparin bridging, but that bleeding risks need to be considered.

The Outcomes Registry for Better Informed Treatment of Atrial Fibrillation (ORBIT-AF) registry is a prospective, observational registry study of US outpatients with atrial fibrillation. They recorded incident temporary interruptions of oral anticoagulation for a procedure, including the use and type of bridging therapy. In a follow-up of 2 years, including 93% patients on warfarin and 7 % on DE, Steinberg *et al.* found a significant threefold to fourfold higher risk of clinically important major bleeding in bridged patients than in those who did not receive bridging anticoagulation (5.0% versus 1.3%; adjusted OR 3.84, P <0.001). Moreover, bridging anticoagulation did not seem to mitigate the risk of thromboembolic events, and a trend towards more adverse cardiovascular events was observed with bridging compared with no bridging (4.6% versus 2.5%; adjusted OR 1.62, P = 0.07). These findings occurred in bridged patients with nominally higher CHADS2 score (2.53 versus 2.34; P = 0.04) and CHA2DS2-VASc score (4.25 versus 4.03; P = 0.01) than non-bridged patients.[154]

For dabigatran, a sub-study of the RE-LY trial showed that bridging was used more during warfarin interruption than dabigatran interruption (27.5 % vs 15.4 %; p< 0.001). With dabigatran interruption, bridged patients had more major bleeding (6.5 % vs 1.8 %, p< 0.001) than those not bridged. Bridged and not bridged groups did not differ for any TE (1.2 % vs 0.6 %, p=0.16) and stroke and systemic embolism (SSE) (0.5 % vs 0.3 %, p=0.46). With warfarin interruption, bridged patients had more major bleeding (6.8 %

vs 1.6 %, p< 0.001) and any TE (1.8 % vs 0.3 %, p=0.007) than those not bridged. They did not differ for SSE (0.5 % vs 0.2 %, p=0.321).

The first prospective trial conducted by Schulman *et al.* proposed a timing of perioperative arrest depending on the renal function and the surgical risk of bleeding. There was no bridging with LMWH, except in postoperative situations where the patients were fasting or when an indwelling neuraxial catheter was in place. They included 541 patients in two years and had only one transient ischemic attack (0.2%; 95% CI, 0-0.5). They concluded that bridging was not necessary for a standardized arrest of dabigatran in a perioperative context.

For rivaroxaban, the ROCKET AF trial compared rivaroxaban (20 mg once daily) with warfarin for SPAF. Therefrom, 33% required temporary interruption (TI) of study drug, of whom 39.7% required a surgery/procedure. Participants with TI were similar to the overall ROCKET AF population in regard to baseline clinical characteristics. In most patients, rivaroxaban therapy was stopped at least 3 days preprocedure, and heparin bridging was used only in 6% (n=483). SSE and risk of major bleeding rates during the at-risk period were similar in rivaroxaban-treated and warfarin-treated participants (0.30% versus 0.41% per 30 days; hazard ratio [CI]=0.74 [0.36–1.50]; P =0.40) and (0.99% versus 0.79% per 30 days; hazard ratio [CI]=1.26 [0.80–2.00]; P =0.32), respectively. [138]

The 30-day rate of stroke or non-CNS embolism which was 0.36% is notable

compared with the overall SSE rate in the ROCKET AF trial of 2.2%/y.[50] When prorated over a 1-year period, this observed embolic event rates is consistent with other findings of patients interrupting anticoagulant therapy.[153] Patients enrolled in the ROCKET AF trial were considered as high risk, and the embolic event rate during TI can reflect the intrinsic susceptibility of these patients.

In the ARISTOTLE trial comparing apixaban (5 mg twice daily) with warfarin for SPAF, around 25% of patients required a procedure, wherefrom 62.5% had a TI. The rate of heparin bridging was only of 11.7% in both groups. During the 30 days postprocedure, stroke or systemic embolism occurred after procedures among apixaban-treated patients and warfarin-treated patients were 0.35% and 0.57%, resepctively. Major bleeding occurred in 1.62% procedures in the apixaban group and 1.93% in the warfarin group. The risk of death was similar with apixaban and warfarin (1.17% and 1.08% respectively).

Only 10.2% of the procedures were defined as major procedures. This limits the generalization of the conclusions of low ATE, death and major bleeding rates to all type of surgeries.

However, the conclusiveness of these trials should be considered cautiously. First, one of the main limitation of these studies is that most of these are observational studies where heparin bridging was left to the preference of the physicians. Secondly, patients at high TE risks are underrepresented in these trials (e.g. BRIDGE trial: only 3% of patients with CHADs2 score of 5-6), and therefore, these conclusions cannot be extended to these categories of patients.

- *Which patients may benefit from heparin bridging when anticoagulant therapy is interrupted?*

Chronic anticoagulation prevents arterial thromboembolism in patients with AF. Kaatz *et al.* showed that these patients undergoing an elective surgery or procedure appear to be at increased risk for perioperative stroke. Indeed, in a retrospective population-based study, the 30-day postoperative rate of stroke among 69 202 patients with AF was 1.8% compared with 0.6% among 2.4 million patients without AF.[209]

The clinical consequences of a thrombotic or bleeding event must be taken into consideration:

- mechanical heart valve thrombosis is fatal in 15% of patients
- embolic stroke results in death or major disability in 70% of patients
- VTE has a case-fatality rate of approximately 5%-9%
- major bleeding has a case-fatality rate of 8%-10%

The more severe clinical consequences of ATE, compared with major bleeding, will orient the physicians to opt for a strategy that incurs 3–10 more major bleeds to prevent one stroke, in theory.[210] However, heparin bridging has only sense when it can reduce the TE risk of the patients, and the BRIDGE trial could not prove this benefit in patients with low to moderate TE risks, as in both groups (non bridged and standardized heparin bridged patients) the overall TE risk was similar.

Patients with high TE risk are underrepresented in clinical trials, and therefore it is not possible to draw conclusions on the benefit of heparin-bridging when anticoagulants are interrupted for longer intervals.

As already discussed, some procedures can be safely realized on warfarin, as for example cardiac implantable electronic devices, as heparin bridging confers more bleeding risk than continuing warfarin.[211-213] However, more studies are needed to confirm the safety of continuing DOACs for this type of procedures[184], especially when there are combined with dual antiplatelet therapies. [214,215]

For high bleeding risk procedures, a prolonged DOAC interruption is required. High inter-variability of patient's plasma DOAC concentration has been reported in several publications[88,216], and therefore it is difficult to predict if a high TE risk patient will be sufficiently anticoagulated with a

prolonged DOAC interruption. As long as there is no evidence of the benefit or harm of heparin bridging in this population, the suggestion to bridge them should not disappear from the current perioperative proposals. To guide the best time to start the heparin bridging before an elective invasive procedure, a punctual measurement of DOACs plasma concentration during the preoperative assessment can give useful information in anticipating DOAC plasma elimination and avoiding an overlap of relevant levels of two different anticoagulants.

- *Who are the high TE risk patients?*

The 9th edition of the Perioperative management of Antithrombotic Therapy published by the American College of Chest Physicians categorized the different TE risks patients following **Table 5**.[156]

However, the question whether CHADs2 or CHA2Ds2-VAsc score are sufficiently sensitive to guide heparin-bridging recommendations in AF patients, or whether we should focus on specific biomarkers, past medical history of severe TE or repeated TE with underlying conditions increasing the risk of recurrent TE (active cancer[217], prolonged immobilisation) may lead to further research projects.

Table 5. Suggested Risk Stratification for Perioperative Thromboembolism[156]

(9th edition of the Perioperative management of Antithrombotic Therapy published by the American College of Chest Physicians)

	Indication for VKA Therapy		
Risk Stratum	Mechanical Heart Valve	Atrial Fibrillation	VTE
High[a]	• Any mitral valve prosthesis • Any caged-ball or tilting disc aortic valve prosthesis • Recent (within 6 mo) stroke or transient ischemic attack	• $CHADS_2$ score of 5 or 6 • Recent (within 3 mo) stroke or transient ischemic attack • Rheumatic valvular heart disease	• Recent (within 3 mo) VTE • Severe thrombophilia (eg, deficiency of protein C, protein S, or antithrombin; antiphospholipid antibodies; multiple abnormalities)
Moderate	• Bileaflet aortic valve prosthesis and one or more of the of following risk factors: atrial fibrillation, prior stroke or transient ischemic attack, hypertension, diabetes, congestive heart failure, age > 75 y	• $CHADS_2$ score of 3 or 4	• VTE within the past 3-12 mo • Nonsevere thrombophilia (eg, heterozygous factor V Leiden or prothrombin gene mutation) • Recurrent VTE • Active cancer (treated within 6 mo or palliative)
Low	• Bileaflet aortic valve prosthesis without atrial fibrillation and no other risk factors for stroke	• $CHADS_2$ score of 0 to 2 (assuming no prior stroke or transient ischemic attack)	• VTE > 12 mo previous and no other risk factors

$CHADS_2$ = congestive heart failure, hypertension, age ≥ 75 years, diabetes mellitus, and stroke or transient ischemic attack; VKA = vitamin K antagonist.
[a]High-risk patients may also include those with a prior stroke or transient ischemic attack occurring > 3 mo before the planned surgery and a $CHADS_2$ score < 5, those with prior thromboembolism during temporary interruption of VKAs, or those undergoing certain types of surgery associated with an increased risk for stroke or other thromboembolism (eg, cardiac valve replacement, carotid endarterectomy, major vascular surgery).

- *What is the best heparin-bridging regimen?*

Therapeutic bridging and early postoperative reintroduction[186] of heparin have been reported to increase major bleedings.

Heparin-bridging regimen should be adapted to the bleeding risk of the procedure and the TE risk of the patient.

Schulman *et al.* published a retrospective study of patients with mechanical heart valves (MHV) on long-term VKA receiving perioperative bridging with LMWH. Fifty two percent of the patients received prophylactic LMWH. Patients with mechanical mitral valves were more frequently bridged with therapeutic doses of LMWH (55 %) than those with mechanical aortic valves (44 %). The only major predictor for MBE was the bleeding risk of the surgery. Other variables, including age, sex, weight, renal function, pre-operative INR, concomitant treatment with antiplatelet agents or NSAIDs, and dose and timing of LMWH administration were not significantly associated with the development of MBE in this population. There was no TE event (cardiac valvular, arterial, or venous) recorded during 30 days after the invasive procedure/surgery in any of the patients.[218]

Whether a therapeutic or prophylactic regimen is more efficient with reduced risk of MBE needs to be evaluated in randomized clinical trials including specific populations that might benefit from heparin- bridging.

iii. Management with antidotes

Antidotes for DOACs have been developed these last years to respond to a tempered enthusiasm in the use of the anticoagulants among patients and physicians because of the perception of a lack of safety without available effective reversal strategies.[219]

- *When should antidotes be used?*

Proposals for the administration of antidotes are:

- Life threatening bleeding
- Bleeding in a critical organ or closed compartment
- Prolonged bleeding despite local haemostatic measures
- High risk of recurrent bleeding because of overdose or delayed clearance of DOACs
- Need for urgent intervention associated with a high risk of bleeding

Reversal is unlikely to be necessary when bleeding can be managed with local haemostatic measures, when bleeding has stopped or when interventions can be delayed to afford clearance of drugs, especially in patients with normal renal function. [130]

- *Which antidotes are available or ongoing clinical trials?*

1) Idarucizumab

For dabigatran[220], the antidote Idarucizumab is a Fragment Antigen Binding (Fab)-fragment of a humanised monoclonal antibody which directly binds

and inhibits dabigatran. Its affinity for dabigatran is around 350 times greater than the one of thrombin. The Fab-fragment neither bound to various clotting factors nor it had an effect on platelets. It has a very short half-life of 45 minutes as it is filtered and degraded by the glomerula (bound to dabigatran or free). The dabigatran is then eliminated with the urine.[220] In case of renal impairment the Idarucizumab-dabigatran complexes are less cleared, but the almost irreversible binding prevents any relevant rebinding of dabigatran to thrombin.

The efficacy and safety of Idarucizumab is currently being evaluated in a phase III Study of the Reversal Effects of Idarucizumab on Active Dabigatran (REVERSE-AD ; Clinical Trials.gov number NCT02104947) which aims to enrol 300 patients. The primary endpoint is the maximum percentage reversal of the anticoagulant effect of dabigatran within 4 hours of Idarucizumab administration, measured with a dTT or ECT.[130]

After publication of the first results of the phase III trial testing a 5g Idarucizumab infusion (two IV boluses of 2.5 g each administered overs 5-10 minutes no more than 15 minutes apart) in patients who experienced serious bleeding requiring reversal (group A= 51 patients) or who require emergency surgery that cannot be delayed for at least 8 hours (group B= 39 patients) in June 2015, the FDA and EMA have delivered expedited approval to have the antidote available in the clinical setting. However, its high cost and its potential to induce immunogenic issues should restrict its

use to the strict recommended indications. Furthermore, despite the clinical trial demonstrate the effect of this antidote to correct anticoagulation induced by DE, the benefit in terms of outcome and mortality has still not been proved. Whether it should be given before a fibrinolytic therapy in patients with acute ischemic stroke and relevant concentrations of dabigatran is still unknown. A recent case-report described the administration of Idaricuzimab before a thrombolysis in a patient with acute ischemic stroke and 90 ng/ml of dabigatran at admission. The authors reported that the patient developed further ischemia afterwards and could not conclude about the safety of Idarucizumab in such emergent setting.[221]

2) Andexanet alfa

For the direct anti-Xa inhibitors, the antidote Andexanet alfa is a modified, recombinant activated FXa (rFXa). It is catalytically inactive, but retains the ability to bind direct Fxa inhibitors as well as anti-thrombin activated by LMWH or fondaparinux. This antidote is currently in a phase III trial, the « Prospective, Open-Label Study of Andexanet Alfa in Patients Receiving a Factor Xa Inhibitor Who Have Acute Major Bleeding » (ClinicalTrials.gov number, NCT02329327). The dosing strategies used in the phase II clinical trials were different for Rivaroxaban (clinical trial ANNEXA-R) and Apixaban (clinical trial ANNEXA-A) due to pharmacokinetic and pharmacodynamic models. Bolus infusions resulted in a rapid decrease (within 2 to 5 minutes) in both anti-factor Xa and unbound plasma

concentrations of apixaban and rivaroxaban, but an increase in both measures could be detected within 15 minutes after completion of the bolus. Therefore, a 2-hour infusion was necessary to achieve sustained suppression of anti-factor Xa activity and plasma concentration of the anticoagulants.

In a phase II study were Andexanet alfa was given to healthy volunteers without anticoagulant, a transient reduction of tissue factor pathway inhibitor (TFPI), a slight increase of fibrinogen F1+2 fragments (prothrombin fragment 1.2, thrombin-antithrombin complexes)[130] and of D-dimer were observed, without associated thrombotic events. This phenomenon should be attenuated with the presence of oral FXa inhibitors as these last compete with TFPI for Andexanet binding. In the ANNEXA-A and ANNEXA-R studies, no serious adverse events nor thrombotic complications were recorded in both clinical trials including 101 patients receiving andexanet alfa.[219]

3) Aripazine

The third approach is the small molecule Aripazine, which antagonize the effects of all anticoagulants tested, except VKAs and argatroban.[220] It has a non-covalent binding to anticoagulants and in vitro studies showed no major interactions with other coagulation factors or albumin. In an in vivo study including healthy volunteers untreated or pre-treated with 60 mg of edoxaban, Aripazine did not induce serious adverse events and no procoagulant signal (measured by D-dimer, TFPI, prothrombin fragments

1.2). A single bolus of 300 mg of i.v. Aripazine normalised the whole blood clotting time in volunteers treated with 60 mg of edoxaban with a stable effect remaining 24 hours. Paradoxally, in a rabbit liver laceration model, there was a reduction in the blood loss induced with rivaroxaban with 30 mg/kg of Aripazine, and no effect on clotting assays. This might become an issue for the monitoring of the reversal therapy, especially as aripazine can reverse citrate.

Why monitoring the anticoagulant effect with antidote administration?

1) Guiding DOAC administration

Time of last DOAC intake and laboratory tests (including CrCl) can guide clinicians in administering the antidote.

However, antidote administration should not be delayed until laboratory tests results are available when patients have life-threatening bleeding such as an intracranial bleeding or patients requiring emergency surgery for life-threatening conditions such as ruptured aortic aneurysm. [130] In this situation, some rapidly available routine tests using sensitive agents to DOAC might inform clinicians about a qualitative anticoagulant effect of DOAC.

Indeed, an optimised TT and a sensitive reagent of aPTT can inform about residual dabigatran presence and a prolonged PT with a sensitive reagent can inform about clinically relevant presence of rivaroxaban and edoxaban.

However, routine tests can be prolonged due to induced coagulopathies caused by e.g. polytrauma or life threatening bleeding with aggressive fluid therapy.

Therefore, as already discussed, non-specific anti-Xa chromogenic assays are accurate enough to detect low and high plasma levels of direct anti-Xa agents.[70]

When severe bleeding patients are treated with antidotes and aggressive conventional therapy, aPTT, TT and PT cannot be used to monitor the reversal of DOACs.

Therefore, the residual anticoagulant effect can be accurately estimated with specific laboratory tests. Even if the antidote is administered before the availability of DOAC plasma estimation, having later on the initial concentrations may help to assess the efficient dosage of the antidote.

When laboratory tests can be realized on time, for patients with serious bleeding, a DOAC concentrations > 50 ng/ml is sufficiently high to warrant antidote administration, whereas in those requiring an urgent intervention associated with a high risk of bleeding, antidote administration should be considered if the drug concentration > 30 ng/ml.[130] However, for the last point, the administration of an antidote could be delayed into the operating room, if haemostatic conditions are unsatisfying during the procedure.

Levy *et al.* estimate that an antidote is unlikely to be necessary if the last dose of DOAC was taken 24 hours before, in patients with normal renal

function.[130] Blood samples of patients treated with rivaroxaban in our institution and collected at C_{TROUGH} (24 hours after last rivaroxaban administration) had commonly plasma concentrations > 50 ng/ml (confirmed with LCMS-MS). Therefore laboratory tests have a key role in the way we should administer the antidotes and some experts have expressed recently the urgent need to have DOAC monitoring widely available to manage emergencies and antidote administration.[135]

2) Follow-up after DOAC administration

In any case, laboratory tests will assess the effect of the antidote after its administration, and in this setting, the specific coagulation assays dedicated to low concentrations will have more accurate estimation of residual DOACs activity. This might be helpful to guarantee reasonable haemostatic conditions during a high risk bleeding surgery or to assess if a bleeding is still caused by significant DOAC concentrations to guide further bleeding management.

Furthermore, DOACs distribute within the intra- and extra-vascular compartment, and when DOACs has been removed or antagonised in the intravascular space, the extravascular compartment will redistribute the remaining DOACs concentration into the intravascular space. This concerns also the removing of dabigatran with haemodialysis.[220] In patients with impaired renal function, monitoring the reversal of Idarucizumab should be

realized after 24 hours to exclude dabigatran reapparance, especially if bleeding reoccurs lately.

Greinacher *et al.*[220] described well the potential issues with the development of immunogenicity due to antidote's use with DOACs and argue the need to monitor it.

For Idarucizumab, about 15% of normal individuals have natural antibodies binding the cleavage site of Fab fragments. Important is that there do not block the drug effect or cause cell alterations. A potential problem with pre-existing anti-Fab antibodies might be that the complexes of dabigatran-Idarucizumab and anti-Fab antibodies are too large to be filtered by the kidney and therefore may prolong the resistance to new doses of dabigatran. A further theoretical risk is the formation of anti-idiotype antibodies that bind to the variable region of an antibody and could thereby inactivate the dabigatran antidote in a further attempt to reverse dabigatran. Other problematic is that anti-idiotype antibodies can sterically mimick the original antigen of the idiotype antibody, thus in this case an anti-idiotype antibody against Idarucizumab may mimick sterically dabigatran and could become an endogenous thrombin inhibitor.

Concerning Andexanet alfa, a modified human protein presents always the risk to induce antibodies.[220]

These potential risks with the use of antidotes should be kept in mind in the follow-up of patients on DOACs.

V. Overall conclusions

Since the DOACs have been licensed, a lot of progress has been realized in the field of their perioperative management. Despite they were licensed without the need of routine monitoring, tricky clinical settings has lead to the development of accurate DOAC monitoring.

Our involvement in the improvement of the perioperative management of DOACs was to evaluate the potential of an optimised TT in the estimation of dabigatran plasma concentrations, and to assess the accuracy of specific laboratory assays developed for the estimation of low plasma concentrations of dabigratran and rivaroxaban. The results of these research studies have led to the development of an institutional strategy that enables the monitoring of DOACs in different clinical settings with the most accurate tests. Futur perioperative research projects need to confirm our results in larger cohorts and validate an institutional perioperative management. Further optimization of the performance and specificity of laboratory assays are needed for the monitoring of low plasma concentrations of direct anti-Xa agents. The role of DOACs monitoring is nowadays better understood and some experts raise concerns about the urgent need to have this monitoring widely available to manage life-threatening emergencies in patients treated with DOACs.[135] There are still improvements that can be realized to have a reduced TAT of DOACs monitoring in this setting. Therefore, the perioperative management of DOACs offers still challenges to work on.

VI. References

1. AG. BP. Xarelto® (rivaroxaban) Summary of Product Characteristics. 2015. [accessed 9 June 2016]. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000944/WC500057108.pdf
2. GmbH. BII. Pradaxa® (dabigatran etexilate) Summary of Product Characteristics. 2016.; [accessed 10 June 2016]. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000829/WC500041059.pdf.
3. Bristol-Myers Squibb P. Eliquis® (apixaban) Summary of Product Characteristics. 2016. . [accessed 10 June 2016]. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002148/WC500107728.pdf
4. GmbH. DSE. Lixiana® (edoxaban) Summary of Product Characteristics. 2016. . [accessed 10 June 2016]. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002629/WC500189045.pdf
5. Sholzberg M, Pavenski K, Shehata N, Cserti-Gazdewich C, Lin Y. Bleeding complications from the direct oral anticoagulants. *BMC Hematol.* 2015;15:18.
6. Southworth MR, Reichman ME, Unger EF. Dabigatran and postmarketing reports of bleeding. *N Engl J Med.* 2013;368(14):1272-1274.
7. Harenberg J, Marx S, Dahl OE, et al. Interpretation of endpoints in a network meta-analysis of new oral anticoagulants following total hip or total knee replacement surgery. *Thromb Haemost.* 2012;108(5):903-912.
8. Huisman MV, Rothman KJ, Paquette M, et al. Antithrombotic Treatment Patterns in Patients with Newly Diagnosed Nonvalvular Atrial Fibrillation: The GLORIA-AF Registry, Phase II. *The American journal of medicine.* 2015;128(12):1306-1313 e1301.
9. Vornicu O, Larock AS, Douxfils J, et al. Minimisation of Bleeding Risks Due to Direct Oral Anticoagulants
. *EMJ Hematol. 2016;4[1]:78-90.* 2016.
10. Riva N, Dentali F, Permunian ET, Ageno W. Major Bleeding and Case Fatality Rate with the Direct Oral Anticoagulants in Orthopedic Surgery: A Systematic Review and Meta-Analysis. *Seminars in thrombosis and hemostasis.* 2016;42(1):42-54.
11. Chai-Adisaksopha C, Hillis C, Isayama T, Lim W, Iorio A, Crowther M. Mortality outcomes in patients receiving direct oral anticoagulants: a systematic review and meta-analysis of randomized controlled trials. *Journal of thrombosis and haemostasis : JTH.* 2015;13(11):2012-2020.

12. Raccah BH, Perlman A, Danenberg HD, Pollak A, Muszkat M, Matok I. Major Bleeding and Hemorrhagic Stroke with Direct Oral Anticoagulants in Patients with Renal Failure: Systematic Review and Meta-Analysis of Randomized Trials. *Chest.* 2016.
13. Beyer-Westendorf J, Ebertz F, Forster K, et al. Effectiveness and safety of dabigatran therapy in daily-care patients with atrial fibrillation. Results from the Dresden NOAC Registry. *Thrombosis and haemostasis.* 2015;113(6):1247-1257.
14. Sorensen R, Gislason G, Torp-Pedersen C, et al. Dabigatran use in Danish atrial fibrillation patients in 2011: a nationwide study. *BMJ Open.* 2013;3(5).
15. Ageno W, Mantovani LG, Haas S, et al. Safety and effectiveness of oral rivaroxaban versus standard anticoagulation for the treatment of symptomatic deep-vein thrombosis (XALIA): an international, prospective, non-interventional study. *Lancet Haematol.* 2016;3(1):e12-21.
16. Camm AJ, Amarenco P, Haas S, et al. XANTUS: a real-world, prospective, observational study of patients treated with rivaroxaban for stroke prevention in atrial fibrillation. *European heart journal.* 2016;37(14):1145-1153.
17. Tamayo S, Frank Peacock W, Patel M, et al. Characterizing major bleeding in patients with nonvalvular atrial fibrillation: a pharmacovigilance study of 27 467 patients taking rivaroxaban. *Clin Cardiol.* 2015;38(2):63-68.
18. Beyer-Westendorf J, Forster K, Pannach S, et al. Rates, management, and outcome of rivaroxaban bleeding in daily care: results from the Dresden NOAC registry. *Blood.* 2014;124(6):955-962.
19. European Medicines Agency C. Pradaxa -EMEA/H/C/000829 - IB/0088 - Product information. First published: 15/12/2009 Last update: 09/12/2015; http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000829/WC500041059.pdf. Accessed 18/02/2016, 2016.
20. European Medicines Agency C. Eliquis -EMEA/H/C/002148 - R/0034 - Product Information. First published 20/06/2011 - Last update 12/02/2016; http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002148/WC500107728.pdf.
21. European Medicines Agency C. Lixiana -EMEA/H/C/002629 - IB/0002 - Product information. First published 03/07/2015 - Last update 18/11/2015; http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002629/WC500189045.pdf.

Accessed 18/02/2016, 2016.
22. Mueck W, Stampfuss J, Kubitza D, Becka M. Clinical pharmacokinetic and pharmacodynamic profile of rivaroxaban. *Clin Pharmacokinet.* 2014;53(1):1-16.
23. Dincq AS, Lessire S, Douxfils J, Dogne JM, Gourdin M, Mullier F. Management of non-vitamin K antagonist oral anticoagulants in the perioperative setting. *BioMed research international.* 2014;2014:385014.
24. Heidbuchel H, Verhamme P, Alings M, et al. Updated European Heart Rhythm Association Practical Guide on the use of non-vitamin K antagonist anticoagulants in patients with non-valvular atrial fibrillation. *Europace : European pacing, arrhythmias, and cardiac electrophysiology : journal of the working groups on cardiac pacing, arrhythmias, and cardiac cellular electrophysiology of the European Society of Cardiology.* 2015.
25. Frost C, Nepal S, Wang J, et al. Safety, pharmacokinetics and pharmacodynamics of multiple oral doses of apixaban, a factor Xa inhibitor, in healthy subjects. *Br J Clin Pharmacol.* 2013;76(5):776-786.
26. Frost C, Wang J, Nepal S, et al. Apixaban, an oral, direct factor Xa inhibitor: single dose safety, pharmacokinetics, pharmacodynamics and food effect in healthy subjects. *Br J Clin Pharmacol.* 2013;75(2):476-487.
27. Stangier J, Clemens A. Pharmacology, pharmacokinetics, and pharmacodynamics of dabigatran etexilate, an oral direct thrombin inhibitor. *Clin Appl Thromb Hemost.* 2009;15 Suppl 1:9S-16S.
28. Parasrampuria DA, Marbury T, Matsushima N, et al. Pharmacokinetics, safety, and tolerability of edoxaban in end-stage renal disease subjects undergoing haemodialysis. *Thrombosis and haemostasis.* 2015;113(4):719-727.
29. Ruff CT, Giugliano RP, Braunwald E, et al. Association between edoxaban dose, concentration, anti-Factor Xa activity, and outcomes: an analysis of data from the randomised, double-blind ENGAGE AF-TIMI 48 trial. *Lancet.* 2015;385(9984):2288-2295.
30. Investigators E, Bauersachs R, Berkowitz SD, et al. Oral rivaroxaban for symptomatic venous thromboembolism. *N Engl J Med.* 2010;363(26):2499-2510.
31. Connolly SJ, Ezekowitz MD, Yusuf S, et al. Dabigatran versus warfarin in patients with atrial fibrillation. *N Engl J Med.* 2009;361(12):1139-1151.
32. Eriksson BI, Borris LC, Friedman RJ, et al. Rivaroxaban versus enoxaparin for thromboprophylaxis after hip arthroplasty. *N Engl J Med.* 2008;358(26):2765-2775.

33. Eriksson BI, Borris LC, Dahl OE, et al. A once-daily, oral, direct Factor Xa inhibitor, rivaroxaban (BAY 59-7939), for thromboprophylaxis after total hip replacement. *Circulation.* 2006;114(22):2374-2381.
34. Eriksson BI, Dahl OE, Rosencher N, et al. Oral dabigatran etexilate vs. subcutaneous enoxaparin for the prevention of venous thromboembolism after total knee replacement: the RE-MODEL randomized trial. *J Thromb Haemost.* 2007;5(11):2178-2185.
35. Eriksson BI, Dahl OE, Rosencher N, et al. Dabigatran etexilate versus enoxaparin for prevention of venous thromboembolism after total hip replacement: a randomised, double-blind, non-inferiority trial. *The Lancet.* 2007;370(9591):949-956.
36. Mueck W, Eriksson BI, Bauer KA, et al. Population pharmacokinetics and pharmacodynamics of rivaroxaban--an oral, direct factor Xa inhibitor--in patients undergoing major orthopaedic surgery. *Clin Pharmacokinet.* 2008;47(3):203-216.
37. Eriksson BI, Quinlan DJ, Weitz JI. Comparative pharmacodynamics and pharmacokinetics of oral direct thrombin and factor xa inhibitors in development. *Clinical pharmacokinetics.* 2009;48(1):1-22.
38. Kakkar AK, Brenner B, Dahl OE, et al. Extended duration rivaroxaban versus short-term enoxaparin for the prevention of venous thromboembolism after total hip arthroplasty: a double-blind, randomised controlled trial. *The Lancet.* 2008;372(9632):31-39.
39. Lassen MR, Davidson BL, Gallus A, Pineo G, Ansell J, Deitchman D. The efficacy and safety of apixaban, an oral, direct factor Xa inhibitor, as thromboprophylaxis in patients following total knee replacement. *J Thromb Haemost.* 2007;5(12):2368-2375.
40. Lassen MR, Ageno W, Borris LC, et al. Rivaroxaban versus enoxaparin for thromboprophylaxis after total knee arthroplasty. *N Engl J Med.* 2008;358(26):2776-2786.
41. Lassen MR, Raskob GE, Gallus A, Pineo G, Chen D, Portman RJ. Apixaban or enoxaparin for thromboprophylaxis after knee replacement. *N Engl J Med.* 2009;361(6):594-604.
42. Turpie AG, Lassen MR, Davidson BL, et al. Rivaroxaban versus enoxaparin for thromboprophylaxis after total knee arthroplasty (RECORD4): a randomised trial. *Lancet.* 2009;373(9676):1673-1680.
43. Lassen MR, Raskob GE, Gallus A, et al. Apixaban versus enoxaparin for thromboprophylaxis after knee replacement (ADVANCE-2): a randomised double-blind trial. *Lancet.* 2010;375(9717):807-815.

44. Lassen MR, Gallus A, Raskob GE, et al. Apixaban versus enoxaparin for thromboprophylaxis after hip replacement. *N Engl J Med.* 2010;363(26):2487-2498.
45. Turpie AG, Lassen MR, Eriksson BI, et al. Rivaroxaban for the prevention of venous thromboembolism after hip or knee arthroplasty. Pooled analysis of four studies. *Thromb Haemost.* 2011;105(3):444-453.
46. Turpie AG, Haas S, Kreutz R, et al. A non-interventional comparison of rivaroxaban with standard of care for thromboprophylaxis after major orthopaedic surgery in 17,701 patients with propensity score adjustment. *Thromb Haemost.* 2014;111(1):94-102.
47. Mega JL. A new era for anticoagulation in atrial fibrillation. *N Engl J Med.* 2011;365(11):1052-1054.
48. Mega JL, Braunwald E, Wiviott SD, et al. Rivaroxaban in patients with a recent acute coronary syndrome. *N Engl J Med.* 2012;366(1):9-19.
49. Mega JL, Braunwald E, Murphy SA, et al. Rivaroxaban in patients stabilized after a ST-segment elevation myocardial infarction: results from the ATLAS ACS-2-TIMI-51 trial (Anti-Xa Therapy to Lower Cardiovascular Events in Addition to Standard Therapy in Subjects with Acute Coronary Syndrome-Thrombolysis In Myocardial Infarction-51). *J Am Coll Cardiol.* 2013;61(18):1853-1859.
50. Patel MR, Mahaffey KW, Garg J, et al. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. *N Engl J Med.* 2011;365(10):883-891.
51. Giugliano RP, Ruff CT, Braunwald E, et al. Edoxaban versus warfarin in patients with atrial fibrillation. *N Engl J Med.* 2013;369(22):2093-2104.
52. Frost CE ea. Clin Pharmacol Ther 2009;85 (suppl. 1):S34(PI-84).
53. Jungbauer L, Dobias C, Stollberger C, Weidinger F. The frequency of prescription of P-glycoprotein-affecting drugs in atrial fibrillation. *Journal of thrombosis and haemostasis : JTH.* 2010;8(9):2069-2070.
54. Weitz JI, Quinlan DJ, Eikelboom JW. Periprocedural management and approach to bleeding in patients taking dabigatran. *Circulation.* 2012;126(20):2428-2432.
55. Cuker A, Siegal DM, Crowther MA, Garcia DA. Laboratory measurement of the anticoagulant activity of the non-vitamin K oral anticoagulants. *Journal of the American College of Cardiology.* 2014;64(11):1128-1139.
56. Douxfils J, Mullier F, Robert S, Chatelain C, Chatelain B, Dogne JM. Impact of dabigatran on a large panel of routine or specific coagulation assays. Laboratory recommendations for monitoring of

dabigatran etexilate. *Thrombosis and haemostasis.* 2012;107(5):985-997.
57. Hapgood G, Butler J, Malan E, Chunilal S, Tran H. The effect of dabigatran on the activated partial thromboplastin time and thrombin time as determined by the Hemoclot thrombin inhibitor assay in patient plasma samples. *Thrombosis and haemostasis.* 2013;110(2):308-315.
58. Avecilla ST, Ferrell C, Chandler WL, Reyes M. Plasma-diluted thrombin time to measure dabigatran concentrations during dabigatran etexilate therapy. *Am J Clin Pathol.* 2012;137(4):572-574.
59. Dager WE, Gosselin RC, Kitchen S, Dwyre D. Dabigatran effects on the international normalized ratio, activated partial thromboplastin time, thrombin time, and fibrinogen: a multicenter, in vitro study. *The Annals of pharmacotherapy.* 2012;46(12):1627-1636.
60. Lessire S, Douxfils J, Baudar J, et al. Is Thrombin Time useful for the assessment of dabigatran concentrations? An in vitro and ex vivo study. *Thrombosis research.* 2015.
61. Douxfils J, Mullier F, Loosen C, Chatelain C, Chatelain B, Dogne JM. Assessment of the impact of rivaroxaban on coagulation assays: laboratory recommendations for the monitoring of rivaroxaban and review of the literature. *Thrombosis research.* 2012;130(6):956-966.
62. Douxfils J, Tamigniau A, Chatelain B, et al. Comparison of calibrated chromogenic anti-Xa assay and PT tests with LC-MS/MS for the therapeutic monitoring of patients treated with rivaroxaban. *Thrombosis and haemostasis.* 2013;110(4):723-731.
63. Douxfils J, Chatelain B, Chatelain C, Dogne JM, Mullier F. Edoxaban: Impact on routine and specific coagulation assays. A practical laboratory guide. *Thrombosis and haemostasis.* 2016;115(2):368-381.
64. Helin TA, Pakkanen A, Lassila R, Joutsi-Korhonen L. Laboratory assessment of novel oral anticoagulants: method suitability and variability between coagulation laboratories. *Clinical chemistry.* 2013;59(5):807-814.
65. Samama MM, Contant G, Spiro TE, et al. Evaluation of the prothrombin time for measuring rivaroxaban plasma concentrations using calibrators and controls: results of a multicenter field trial. *Clin Appl Thromb Hemost.* 2012;18(2):150-158.
66. Samama MM, Contant G, Spiro TE, et al. Evaluation of the anti-factor Xa chromogenic assay for the measurement of rivaroxaban plasma concentrations using calibrators and controls. *Thrombosis and haemostasis.* 2012;107(2):379-387.

67. Samama MM, Martinoli JL, LeFlem L, et al. Assessment of laboratory assays to measure rivaroxaban--an oral, direct factor Xa inhibitor. *Thrombosis and haemostasis.* 2010;103(4):815-825.
68. Tripodi A, Chantarangkul V, Guinet C, Samama MM. The International Normalized Ratio calibrated for rivaroxaban has the potential to normalize prothrombin time results for rivaroxaban-treated patients: results of an in vitro study. *J Thromb Haemost.* 2011;9(1):226-228.
69. Douxfils J, Chatelain C, Chatelain B, Dogne JM, Mullier F. Impact of apixaban on routine and specific coagulation assays: a practical laboratory guide. *Thromb Haemost.* 2013;110(2):283-294.
70. Gosselin R, Grant RP, Adcock DM. Comparison of the effect of the anti-Xa direct oral anticoagulants apixaban, edoxaban, and rivaroxaban on coagulation assays. *Int J Lab Hematol.* 2016.
71. van Ryn J, Schurer J, Kink-Eiband M, Clemens A. Reversal of dabigatran-induced bleeding by coagulation factor concentrates in a rat-tail bleeding model and lack of effect on assays of coagulation. *Anesthesiology.* 2014;120(6):1429-1440.
72. Honickel M, Treutler S, van Ryn J, Tillmann S, Rossaint R, Grottke O. Reversal of dabigatran anticoagulation ex vivo: Porcine study comparing prothrombin complex concentrates and idarucizumab. *Thrombosis and haemostasis.* 2015;113(4):728-740.
73. Carvalho M, Rodrigues A, Gomes M, et al. Interventional Algorithms for the Control of Coagulopathic Bleeding in Surgical, Trauma, and Postpartum Settings: Recommendations From the Share Network Group. *Clin Appl Thromb Hemost.* 2016;22(2):121-137.
74. Pernod G, Albaladejo P, Godier A, et al. [Management of major bleeding complications and emergency surgery in patients on long-term treatment with direct oral anticoagulants, thrombin or factor-Xa inhibitors. Proposals of the Working Group on Perioperative Haemostasis (GIHP) - March 2013]. *Ann Fr Anesth Reanim.* 2013;32(10):691-700.
75. Samama CM, Pernod G, Albaladejo P, Sie P, Groupe d'interet en hemostase p-o. [Perioperative management of new oral anticoagulants]. *Presse Med.* 2014;43(6 Pt 1):637-644.
76. Pierre Albaladejo FB, Normand Blais, Jean Philippe Collet, David Faraoni, Pierre Fontana, Anne Godier, Juan Llau, Dan Longrois, Patrick Mismetti, Emmanuel Marret, Nadia Rosencher, Stéphanie Roullet, Charles Marc Samama, Pierre Sié, Jean François Schved, Annick Steib, Sophie Susen et le Groupe d'Intérêt en Hémostase Périopératoire. . Pierre Albaladejo, Fanny Bonhomme, Normand Blais, Jean Philippe Collet, David Faraoni, Pierre Fontana, Anne Godier, Juan Llau, Dan Longrois, Patrick Mismetti, Emmanuel

Marret, Nadia Rosencher, Stéphanie Roullet, Charles Marc Samama, Pierre Sié, Jean François Schved, Annick Steib, Sophie Susen et le Groupe d'Intérêt en Hémostase Périopératoire. «Gestion des Anticoagulants Oraux Directs pour la chirurgie et les actes invasifs programmés : propositions réactualisées du Groupe d'Intérêt en hémostase préopératoire (GIHP)»http://sfar.org/wp-content/uploads/2015/09/Reactualisation-GIHP_AOD_actes-programmes_Septembre-20151.pdf. 2015; http://sfar.org/wp-content/uploads/2015/09/Reactualisation-GIHP_AOD_actes-programmes_Septembre-20151.pdf. Accessed 26/02/2016.

77. Sie P, Samama CM, Godier A, et al. [Surgery and invasive procedures in patients on long-term treatment with oral direct thrombin or factor Xa inhibitors]. *Ann Fr Anesth Reanim.* 2011;30(9):645-650.
78. Godier A, Gouin-Thibault I, Rosencher N, Albaladejo P, Groupe d'Interet en Hemostase P. [Management of direct oral anticoagulants for invasive procedures]. *J Mal Vasc.* 2015;40(3):173-181.
79. Godier A, Martin AC, Leblanc I, et al. Peri-procedural management of dabigatran and rivaroxaban: Duration of anticoagulant discontinuation and drug concentrations. *Thrombosis research.* 2015;136(4):763-768.
80. INAMI; Infospot: Antithrombotiques en pratique ambulatoire J-Fv-M-AahwrfbSi---fp.
81. Healey JS, Eikelboom J, Douketis J, et al. Periprocedural bleeding and thromboembolic events with dabigatran compared with warfarin: results from the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) randomized trial. *Circulation.* 2012;126(3):343-348.
82. Lai A, Davidson N, Galloway SW, Thachil J. Perioperative management of patients on new oral anticoagulants. *Br J Surg.* 2014.
83. Breuer G, Weiss DR, Ringwald J. 'New' direct oral anticoagulants in the perioperative setting. *Curr Opin Anaesthesiol.* 2014;27(4):409-419.
84. Narouze S, Benzon HT, Provenzano DA, et al. Interventional spine and pain procedures in patients on antiplatelet and anticoagulant medications: guidelines from the American Society of Regional Anesthesia and Pain Medicine, the European Society of Regional Anaesthesia and Pain Therapy, the American Academy of Pain Medicine, the International Neuromodulation Society, the North American Neuromodulation Society, and the World Institute of Pain. *Reg Anesth Pain Med.* 2015;40(3):182-212.
85. Faraoni D, Levy JH, Albaladejo P, Samama CM, Groupe d'Interet en Hemostase P. Updates in the perioperative and emergency

management of non-vitamin K antagonist oral anticoagulants. *Crit Care.* 2015;19:203.

86. Pernod G, Albaladejo P, Godier A, et al. Management of major bleeding complications and emergency surgery in patients on long-term treatment with direct oral anticoagulants, thrombin or factor-Xa inhibitors: proposals of the working group on perioperative haemostasis (GIHP) - March 2013. *Archives of cardiovascular diseases.* 2013;106(6-7):382-393.
87. European Medicine Agency. Pradaxa-H-C-829-X-13: EPAR - Assessment Report - Extension. 2011; http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000829/WC500110875.pdf. Accessed Feb 10, 2014.
88. Testa S, Tripodi A, Legnani C, et al. Plasma levels of direct oral anticoagulants in real life patients with atrial fibrillation: Results observed in four anticoagulation clinics. *Thrombosis research.* 2016;137:178-183.
89. Douxfils J, Dogne JM, Mullier F, et al. Comparison of calibrated dilute thrombin time and aPTT tests with LC-MS/MS for the therapeutic monitoring of patients treated with dabigatran etexilate. *Thrombosis and haemostasis.* 2013;110(3):543-549.
90. Gosselin RC, Dwyre DM, Dager WE. Measuring dabigatran concentrations using a chromogenic ecarin clotting time assay. *The Annals of pharmacotherapy.* 2013;47(12):1635-1640.
91. Antovic JP, Skeppholm M, Eintrei J, et al. Evaluation of coagulation assays versus LC-MS/MS for determinations of dabigatran concentrations in plasma. *European journal of clinical pharmacology.* 2013;69(11):1875-1881.
92. Hawes EM, Deal AM, Funk-Adcock D, et al. Performance of coagulation tests in patients on therapeutic doses of dabigatran: a cross-sectional pharmacodynamic study based on peak and trough plasma levels. *Journal of thrombosis and haemostasis : JTH.* 2013;11(8):1493-1502.
93. Douxfils J, Lessire S, Dincq AS, et al. Estimation of dabigatran plasma concentrations in the perioperative setting. An ex vivo study using dedicated coagulation assays. *Thrombosis and haemostasis.* 2014;113(3).
94. Stang L, Nahirniak S, Butcher K, Szkotak AJ. Dabigatran assessment in patients with acute complications using routine coagulation assays. *Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis.* 2014;25(5):426-434.

95. van Ryn J, Stangier J, Haertter S, et al. Dabigatran etexilate--a novel, reversible, oral direct thrombin inhibitor: interpretation of coagulation assays and reversal of anticoagulant activity. *Thrombosis and haemostasis.* 2010;103(6):1116-1127.
96. Flanders MM, Crist R, Rodgers GM. Comparison of five thrombin time reagents. *Clinical chemistry.* 2003;49(1):169-172.
97. Marlar RA, Gausman JN, Engel JW. Validation of hemostasis and coagulation assays: recommendations and guidelines. *Seminars in thrombosis and hemostasis.* 2014;40(2):186-194.
98. Chandler W. Initial evaluation of hemostasis: reagent and method selection. In: Kitchen S, Olson J, Prestion F, eds. *Quality in Laboratory Hemostasis and Thrombosis.* Vol 22013:95-104.
99. European Medicines Agency C. Pradaxa - EMEA/H/C/000829/X/13/G. . 2011; http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000829/WC500110875.pdf. Accessed 18/02/2016, 2016.
100. Reilly PA, Lehr T, Haertter S, et al. The effect of dabigatran plasma concentrations and patient characteristics on the frequency of ischemic stroke and major bleeding in atrial fibrillation patients: the RE-LY Trial (Randomized Evaluation of Long-Term Anticoagulation Therapy). *Journal of the American College of Cardiology.* 2014;63(4):321-328.
101. Mackie IJ, Kitchen S, Machin SJ, Lowe GD, Haemostasis, Thrombosis Task Force of the British Committee for Standards in H. Guidelines on fibrinogen assays. *British journal of haematology.* 2003;121(3):396-404.
102. Cunningham MT, Olson JD, Chandler WL, et al. External quality assurance of fibrinogen assays using normal plasma: results of the 2008 College of American Pathologists proficiency testing program in coagulation. *Archives of pathology & laboratory medicine.* 2012;136(7):789-795.
103. Ignjatovic V. Thrombin clotting time. *Methods in molecular biology.* 2013;992:131-138.
104. Inchiosa MA, Jr., Pothula S, Kubal K, Sanchala VT, Navarro I. Toward development of a point-of-care assay of enoxaparin anticoagulant activity in whole blood. *Journal of thrombosis and thrombolysis.* 2011;32(1):47-53.
105. Chin PK, Wright DF, Patterson DM, Doogue MP, Begg EJ. A proposal for dose-adjustment of dabigatran etexilate in atrial fibrillation guided by thrombin time. *British journal of clinical pharmacology.* 2014;78(3):599-609.

106. Douxfils J, Mullier F, Dogne JM. Dose tailoring of dabigatran etexilate: obvious or excessive? *Expert opinion on drug safety.* 2015:1-7.
107. Ten Cate H. New oral anticoagulants: discussion on monitoring and adherence should start now! *Thromb J.* 2013;11(1):8.
108. Healey JS, Eikelboom J, Douketis J, et al. Periprocedural bleeding and thromboembolic events with dabigatran compared with warfarin: results from the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) randomized trial. *Circulation.* 2012;126(3):343-348.
109. Hjemdahl P, Johnsson H, Wallen NH. Letter by Hjemdahl et al regarding article, "Periprocedural bleeding and thromboembolic events with dabigatran compared with warfarin: results from the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) Randomized Trial". *Circulation.* 2013;127(11):e505.
110. European Medicines Agency. Pradaxa - EMEA/H/C/000829/X/13/G. 2011; http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000829/WC500110875.pdf.
111. Stangier J, Feuring M. Using the HEMOCLOT direct thrombin inhibitor assay to determine plasma concentrations of dabigatran. *Blood Coagul Fibrinolysis.* 2012;23(2):138-143.
112. Lange U, Nowak G, Bucha E. Ecarin Chromogenic Assay - A New Method for Quantitative Determination of Direct Thrombin Inhibitors Like Hirudin. *Pathophysiology of Haemostasis and Thrombosis.* 2003;33(4):184-191.
113. Skeppholm M, Hjemdahl P, Antovic JP, et al. On the monitoring of dabigatran treatment in “real life” patients with atrial fibrillation. *Thrombosis research.* 2014.
114. European Medicines Agency. Pradaxa - Summary of Product Characteristics. 2014; http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000829/WC500041059.pdf. Accessed Jul 17, 2014.
115. Ferrandis R, Castillo J, de Andres J, et al. The perioperative management of new direct oral anticoagulants: a question without answers. *Thrombosis and haemostasis.* 2013;110(3):515-522.
116. Ortel TL. Perioperative management of patients on chronic antithrombotic therapy. *Blood.* 2012;120(24):4699-4705.
117. Hankey GJ. Unanswered questions and research priorities to optimise stroke prevention in atrial fibrillation with the new oral anticoagulants. *Thrombosis and haemostasis.* 2014;111(5):808-816.

118. Lindahl TL, Baghaei F, Blixter IF, et al. Effects of the oral, direct thrombin inhibitor dabigatran on five common coagulation assays. *Thromb Haemost.* 2011;105(2):371-378.
119. Van Blerk M, Bailleul E, Chatelain B, et al. Influence of dabigatran and rivaroxaban on routine coagulation assays. A nationwide Belgian survey. *Thrombosis and haemostasis.* 2014;112(6).
120. Beyer-Westendorf J, Gelbricht V, Forster K, et al. Peri-interventional management of novel oral anticoagulants in daily care: results from the prospective Dresden NOAC registry. *European heart journal.* 2014;35(28):1888-1896.
121. Stangier J, Rathgen K, Stahle H, Gansser D, Roth W. The pharmacokinetics, pharmacodynamics and tolerability of dabigatran etexilate, a new oral direct thrombin inhibitor, in healthy male subjects. *British journal of clinical pharmacology.* 2007;64(3):292-303.
122. Samama MM, Guinet C, Le Flem L. Do new oral anticoagulants require laboratory monitoring? The clinician point of view. *Thromb Res.* 2012;130 Suppl 1:S88-89.
123. Lindhoff-Last E, Samama MM, Ortel TL, Weitz JI, Spiro TE. Assays for measuring rivaroxaban: their suitability and limitations. *Ther Drug Monit.* 2010;32(6):673-679.
124. Samama MM. Coagulation Assays in Patients with New Oral Anticoagulants (NOACs): Why? When? *Drug Development Research.* 2013;74(8):582-586.
125. Tamigniau A, Douxfils J, Nicolas JB, et al. [Why, when and how to monitor new oral anticoagulants]. *Rev Med Suisse.* 2014;10(416):326-333.
126. Gouin-Thibault I, Flaujac C, Delavenne X, et al. Assessment of apixaban plasma levels by laboratory tests: suitability of three anti-Xa assays. A multicentre French GEHT study. *Thromb Haemost.* 2014;111(2):240-248.
127. Harenberg J, Kraemer S, Du S, et al. Determination of Direct Oral Anticoagulants from Human Serum Samples. *Semin Thromb Hemost.* 2013.
128. Testa S, Legnani C, Tripodi A, et al. Poor comparability of coagulation screening test with specific measurement in patients on direct oral anticoagulants: results from a multicenter/multiplatform study. *Journal of thrombosis and haemostasis : JTH.* 2016.
129. Skeppholm M, Hjemdahl P, Antovic JP, et al. On the monitoring of dabigatran treatment in "real life" patients with atrial fibrillation. *Thrombosis research.* 2014;134(4):783-789.
130. Levy JH, Ageno W, Chan NC, et al. When and how to use antidotes for the reversal of direct oral anticoagulants: guidance from the SSC

of the ISTH. *Journal of thrombosis and haemostasis : JTH.* 2016;14(3):623-627.
131. Schulman S, Carrier M, Lee AY, et al. Perioperative Management of Dabigatran: A Prospective Cohort Study. *Circulation.* 2015;132(3):167-173.
132. Douketis JD, Wang G, Chan N, et al. Effect of standardized perioperative dabigatran interruption on the residual anticoagulation effect at the time of surgery or procedure. *Journal of thrombosis and haemostasis : JTH.* 2016;14(1):89-97.
133. Benzon HT, Lindholm PF, Huntoon MA. Direct Oral Anticoagulants: Correlation of Laboratory Monitoring With Safe Interventional Pain Procedures. *Reg Anesth Pain Med.* 2016;41(2):123-124.
134. Spyropoulos AC, Al-Badri A, Sherwood MW, Douketis JD. Periprocedural Management of Patients on a Vitamin K Antagonist or a Direct Oral Anticoagulant Requiring an Elective Procedure or Surgery. *Journal of thrombosis and haemostasis : JTH.* 2016.
135. Weitz JI, Eikelboom JW. Urgent Need to Measure Effects of Direct Oral Anticoagulants. *Circulation.* 2016;134(3):186-188.
136. Daniels PR. Peri-procedural management of patients taking oral anticoagulants. *Bmj.* 2015;351:h2391.
137. Douxfils J, Mani H, Minet V, et al. Non-VKA Oral Anticoagulants: Accurate Measurement of Plasma Drug Concentrations. *Biomed Res Int.* 2014;2014:345138.
138. Sherwood MW, Douketis JD, Patel MR, et al. Outcomes of temporary interruption of rivaroxaban compared with warfarin in patients with nonvalvular atrial fibrillation: results from the rivaroxaban once daily, oral, direct factor Xa inhibition compared with vitamin K antagonism for prevention of stroke and embolism trial in atrial fibrillation (ROCKET AF). *Circulation.* 2014;129(18):1850-1859.
139. Kepplinger J, Prakapenia A, Barlinn K, et al. Standardized use of novel oral anticoagulants plasma level thresholds in a new thrombolysis decision making protocol. *Journal of thrombosis and thrombolysis.* 2015.
140. Harenberg J, Kramer R, Giese C, Marx S, Weiss C, Wehling M. Determination of rivaroxaban by different factor Xa specific chromogenic substrate assays: reduction of interassay variability. *J Thromb Thrombolysis.* 2011;32(3):267-271.
141. Asmis LM, Alberio L, Angelillo-Scherrer A, et al. Rivaroxaban: Quantification by anti-FXa assay and influence on coagulation tests: a study in 9 Swiss laboratories. *Thromb Res.* 2012;129(4):492-498.
142. Douxfils J, Lessire S, Dincq AS, et al. Estimation of dabigatran plasma concentrations in the perioperative setting. An ex vivo study

using dedicated coagulation assays. *Thrombosis and haemostasis.* 2015;113(4):862-869.
143. Mani H, Rohde G, Stratmann G, et al. Accurate determination of rivaroxaban levels requires different calibrator sets but not addition of antithrombin. *Thrombosis and haemostasis.* 2012;108(1):191-198.
144. Konigsbrugge O, Quehenberger P, Belik S, et al. Anti-coagulation assessment with prothrombin time and anti-Xa assays in real-world patients on treatment with rivaroxaban. *Annals of hematology.* 2015;94(9):1463-1471.
145. Samama MM, Amiral J, Guinet C, Perzborn E, Depasse F. An optimised, rapid chromogenic assay, specific for measuring direct factor Xa inhibitors (rivaroxaban) in plasma. *Thrombosis and haemostasis.* 2010;104(5):1078-1079.
146. Douketis JD, Woods K, Foster GA, Crowther MA. Bridging anticoagulation with low-molecular-weight heparin after interruption of warfarin therapy is associated with a residual anticoagulant effect prior to surgery. *Thrombosis and haemostasis.* 2005;94(3):528-531.
147. O'Donnell MJ, Kearon C, Johnson J, et al. Brief communication: Preoperative anticoagulant activity after bridging low-molecular-weight heparin for temporary interruption of warfarin. *Ann Intern Med.* 2007;146(3):184-187.
148. Rohde G. Determination of rivaroxaban--a novel, oral, direct Factor Xa inhibitor--in human plasma by high-performance liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2008;872(1-2):43-50.
149. Administration FaD. FDA Guidelines for Industry for Bioanalytical Method Validation. 2001.
150. Van Blerk M, Bailleul E, Chatelain B, et al. Influence of dabigatran and rivaroxaban on routine coagulation assays. A nationwide Belgian survey. *Thrombosis and haemostasis.* 2015;113(1):154-164.
151. Cuker A. Laboratory measurement of the non-vitamin K antagonist oral anticoagulants: selecting the optimal assay based on drug, assay availability, and clinical indication. *Journal of thrombosis and thrombolysis.* 2015.
152. Douketis JD, Healey JS, Brueckmann M, et al. Perioperative bridging anticoagulation during dabigatran or warfarin interruption among patients who had an elective surgery or procedure. Substudy of the RE-LY trial. *Thrombosis and haemostasis.* 2015;113(3):625-632.
153. Siegal D, Yudin J, Kaatz S, Douketis JD, Lim W, Spyropoulos AC. Periprocedural heparin bridging in patients receiving vitamin K

antagonists: systematic review and meta-analysis of bleeding and thromboembolic rates. *Circulation.* 2012;126(13):1630-1639.
154. Steinberg BA, Peterson ED, Kim S, et al. Use and outcomes associated with bridging during anticoagulation interruptions in patients with atrial fibrillation: findings from the Outcomes Registry for Better Informed Treatment of Atrial Fibrillation (ORBIT-AF). *Circulation.* 2015;131(5):488-494.
155. Douketis JD, Spyropoulos AC, Kaatz S, et al. Perioperative Bridging Anticoagulation in Patients with Atrial Fibrillation. *The New England journal of medicine.* 2015;373(9):823-833.
156. Douketis JD, Spyropoulos AC, Spencer FA, et al. Perioperative management of antithrombotic therapy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest.* 2012;141(2 Suppl):e326S-350S.
157. Mar PL, Familtsev D, Ezekowitz MD, Lakkireddy D, Gopinathannair R. Periprocedural management of anticoagulation in patients taking novel oral anticoagulants: Review of the literature and recommendations for specific populations and procedures. *Int J Cardiol.* 2016;202:578-585.
158. Adcock DM, Gosselin R. Direct Oral Anticoagulants (DOACs) in the Laboratory: 2015 Review. *Thrombosis research.* 2015;136(1):7-12.
159. Gosselin RC, Francart SJ, Hawes EM, Moll S, Dager WE, Adcock DM. Heparin-Calibrated Chromogenic Anti-Xa Activity Measurements in Patients Receiving Rivaroxaban: Can This Test Be Used to Quantify Drug Level? *The Annals of pharmacotherapy.* 2015;49(7):777-783.
160. Lessire S, Dincq AS, Douxfils J, et al. Preventive strategies against bleeding due to nonvitamin K antagonist oral anticoagulants. *BioMed research international.* 2014;2014:616405.
161. Martin K, Beyer-Westendorf J, Davidson BL, Huisman MV, Sandset PM, Moll S. Use of the direct oral anticoagulants in obese patients: guidance from the SSC of the ISTH. *Journal of thrombosis and haemostasis : JTH.* 2016;14(6):1308-1313.
162. Tripodi A, Chantarangkul V, Martinelli I, Bucciarelli P, Mannucci PM. A shortened activated partial thromboplastin time is associated with the risk of venous thromboembolism. *Blood.* 2004;104(12):3631-3634.
163. Chandler WL. Emergency assessment of hemostasis in the bleeding patient. *Int J Lab Hematol.* 2013;35(3):339-343.
164. Pollack CV, Jr., Reilly PA, Eikelboom J, et al. Idarucizumab for Dabigatran Reversal. *The New England journal of medicine.* 2015;373(6):511-520.

165. Chandler WL, Ferrell C, Trimble S, Moody S. Development of a rapid emergency hemorrhage panel. *Transfusion.* 2010;50(12):2547-2552.
166. Jabet A, Stepanian A, Golmard JL, et al. CA27: Can PT, aPTT and TT reliably be used to detect DOAC at clinically relevant concentrations in emergency situations? results from a 660-patient cohort study.; 2016.
167. Dugi K, Clemens A, Lehr T, Noack H, Inventors. Method for prevention of stroke. In. US 2013/0345262 A1. A61K 31/4439 ed. United States: 2013, 10. Boehringer Ingelheim International Gmbh. .
168. Moore KT KM, Vaidyanathan S, Sarich TC, Damaraju CV, and Fields LE Rivaroxaban Crushed Tablet Suspension Characteristics and Relative Bioavailability in Healthy Adults When Administered Orally or Via Nasogastric Tube. . *Pharmacotherapy.* 2014;Clinical Pharmacology in Drug Development 3(4) 321–32.
169. Song Y, Wang X, Perlstein I, et al. Relative Bioavailability of Apixaban Solution or Crushed Tablet Formulations Administered by Mouth or Nasogastric Tube in Healthy Subjects. *Clin Ther.* 2015;37(8):1703-1712.
170. Weitz JI, Eikelboom JW. Appropriate Apixaban Dosing: Prescribers Take Note. *JAMA Cardiol.* 2016.
171. Alexander JH, Andersson U, Lopes RD, et al. Apixaban 5 mg Twice Daily and Clinical Outcomes in Patients With Atrial Fibrillation and Advanced Age, Low Body Weight, or High Creatinine: A Secondary Analysis of a Randomized Clinical Trial. *JAMA Cardiol.* 2016.
172. Olaiya A, Lurie B, Watt B, McDonald L, Greaves M, Watson HG. An observational study of direct oral anticoagulant awareness indicating inadequate recognition with potential for patient harm. *Journal of thrombosis and haemostasis : JTH.* 2016.
173. Bozic-Mijovski M, Malmstrom RE, Malovrh P, et al. Diluted thrombin time reliably measures low to intermediate plasma dabigatran concentrations. *Ann Clin Biochem.* 2016;53(Pt 4):446-451.
174. Van Cott EM, Smock KJ, Chen D, Hsu P, Zantek ND, Meijer P. Testing for Dabigatran and Rivaroxaban by Clinical Laboratories. *Am J Hematol.* 2016.
175. Dale BJ, Chan NC, Eikelboom JW. Laboratory measurement of the direct oral anticoagulants. *British journal of haematology.* 2016;172(3):315-336.
176. Douxfils J, Tamigniau A, Chatelain B, Goffinet C, Dogne JM, Mullier F. Measurement of non-VKA oral anticoagulants versus classic ones: the appropriate use of hemostasis assays. *Thromb J.* 2014;12:24.

177. Calkins H, Kuck KH, Cappato R, et al. 2012 HRS/EHRA/ECAS Expert Consensus Statement on Catheter and Surgical Ablation of Atrial Fibrillation: recommendations for patient selection, procedural techniques, patient management and follow-up, definitions, endpoints, and research trial design. *Europace : European pacing, arrhythmias, and cardiac electrophysiology : journal of the working groups on cardiac pacing, arrhythmias, and cardiac cellular electrophysiology of the European Society of Cardiology.* 2012;14(4):528-606.
178. Jobes DR, Ellison N, Campbell FW. Limit(ation)s for ACT. *Anesthesia and analgesia.* 1989;69(1):142-144.
179. Nagao T, Inden Y, Shimano M, et al. Feasibility and safety of uninterrupted dabigatran therapy in patients undergoing ablation for atrial fibrillation. *Internal medicine.* 2015;54(10):1167-1173.
180. Jude B, Lasne D, Mouton C, et al. [Monitoring of heparin therapy during extracorporeal bypass: what are the remaining questions?]. *Ann Fr Anesth Reanim.* 2004;23(6):589-596.
181. Gunawardene M, Willems S, Schaffer B, et al. Influence of periprocedural anticoagulation strategies on complication rate and hospital stay in patients undergoing catheter ablation for persistent atrial fibrillation. *Clin Res Cardiol.* 2016.
182. Heidbuchel H, Verhamme P, Alings M, et al. Updated European Heart Rhythm Association practical guide on the use of non-vitamin-K antagonist anticoagulants in patients with non-valvular atrial fibrillation: Executive summary. *European heart journal.* 2016.
183. Kosiuk J, Koutalas E, Doering M, et al. Comparison of dabigatran and uninterrupted warfarin in patients with atrial fibrillation undergoing cardiac rhythm device implantations. Case-control study. *Circulation journal : official journal of the Japanese Circulation Society.* 2014;78(10):2402-2407.
184. Essebag V, Healey JS, Ayala-Paredes F, et al. Strategy of continued vs interrupted novel oral anticoagulant at time of device surgery in patients with moderate to high risk of arterial thromboembolic events: The BRUISE CONTROL-2 trial. *Am Heart J.* 2016;173:102-107.
185. Madan S, Muthusamy P, Mowers KL, et al. Safety of anticoagulation with uninterrupted warfarin vs. interrupted dabigatran in patients requiring an implantable cardiac device. *Cardiovasc Diagn Ther.* 2016;6(1):3-9.
186. Tafur AJ, McBane R, 2nd, Wysokinski WE, et al. Predictors of major bleeding in peri-procedural anticoagulation management. *Journal of thrombosis and haemostasis : JTH.* 2012;10(2):261-267.

187. Cappelleri G, Fanelli A. Use of direct oral anticoagulants with regional anesthesia in orthopedic patients. *J Clin Anesth.* 2016;32:224-235.
188. Bateman BT, Mhyre JM, Ehrenfeld J, et al. The risk and outcomes of epidural hematomas after perioperative and obstetric epidural catheterization: a report from the Multicenter Perioperative Outcomes Group Research Consortium. *Anesthesia and analgesia.* 2013;116(6):1380-1385.
189. Guay J, Choi P, Suresh S, Albert N, Kopp S, Pace NL. Neuraxial blockade for the prevention of postoperative mortality and major morbidity: an overview of Cochrane systematic reviews. *The Cochrane database of systematic reviews.* 2014(1):CD010108.
190. Gogarten W, Vandermeulen E, Van Aken H, Kozek S, Llau JV, Samama CM. Regional anaesthesia and antithrombotic agents: recommendations of the European Society of Anaesthesiology. *European journal of anaesthesiology.* 2010;27(12):999-1015.
191. Rosencher N, Bonnet MP, Sessler DI. Selected new antithrombotic agents and neuraxial anaesthesia for major orthopaedic surgery: management strategies. *Anaesthesia.* 2007;62(11):1154-1160.
192. Douketis JD, Wang G, Chan N, et al. Effect of Standardized Perioperative Dabigatran Interruption on Residual Anticoagulation Effect at the Time of Surgery or Procedure. *Journal of thrombosis and haemostasis : JTH.* 2015.
193. Yin OQ, Tetsuya K, Miller R. Edoxaban population pharmacokinetics and exposure-response analysis in patients with non-valvular atrial fibrillation. *European journal of clinical pharmacology.* 2014;70(11):1339-1351.
194. Lessire S, Dincq AS, Douxfils J, Mullier F. Periprocedural management of Direct Oral Anticoagulants should be guided by accurate laboratory tests. *Reg Anesth Pain Med.* 2016.
195. Kozek-Langenecker SA, Afshari A, Albaladejo P, et al. Management of severe perioperative bleeding: guidelines from the European Society of Anaesthesiology. *Eur J Anaesthesiol.* 2013;30(6):270-382.
196. Atiq F, van den Bemt PM, Leebeek FW, van Gelder T, Versmissen J. A systematic review on the accumulation of prophylactic dosages of low-molecular-weight heparins (LMWHs) in patients with renal insufficiency. *European journal of clinical pharmacology.* 2015;71(8):921-929.
197. Hirsh J, Warkentin TE, Shaughnessy SG, et al. Heparin and low-molecular-weight heparin: mechanisms of action, pharmacokinetics, dosing, monitoring, efficacy, and safety. *Chest.* 2001;119(1 Suppl):64S-94S.

198. Hirsh J, Anand SS, Halperin JL, Fuster V. Mechanism of action and pharmacology of unfractionated heparin. *Arteriosclerosis, thrombosis, and vascular biology.* 2001;21(7):1094-1096.
199. Samama MM, Gerotziafas GT. Comparative pharmacokinetics of LMWHs. *Seminars in thrombosis and hemostasis.* 2000;26 Suppl 1:31-38.
200. Collignon F, Frydman A, Caplain H, et al. Comparison of the pharmacokinetic profiles of three low molecular mass heparins--dalteparin, enoxaparin and nadroparin--administered subcutaneously in healthy volunteers (doses for prevention of thromboembolism). *Thrombosis and haemostasis.* 1995;73(4):630-640.
201. Douketis JD, Johnson JA, Turpie AG. Low-molecular-weight heparin as bridging anticoagulation during interruption of warfarin: assessment of a standardized periprocedural anticoagulation regimen. *Arch Intern Med.* 2004;164(12):1319-1326.
202. Dunn AS, Spyropoulos AC, Turpie AG. Bridging therapy in patients on long-term oral anticoagulants who require surgery: the Prospective Peri-operative Enoxaparin Cohort Trial (PROSPECT). *Journal of thrombosis and haemostasis : JTH.* 2007;5(11):2211-2218.
203. Spyropoulos AC, Turpie AG, Dunn AS, et al. Clinical outcomes with unfractionated heparin or low-molecular-weight heparin as bridging therapy in patients on long-term oral anticoagulants: the REGIMEN registry. *Journal of thrombosis and haemostasis : JTH.* 2006;4(6):1246-1252.
204. Pengo V, Cucchini U, Denas G, et al. Standardized low-molecular-weight heparin bridging regimen in outpatients on oral anticoagulants undergoing invasive procedure or surgery: an inception cohort management study. *Circulation.* 2009;119(22):2920-2927.
205. Malato A, Saccullo G, Lo Coco L, et al. Patients requiring interruption of long-term oral anticoagulant therapy: the use of fixed sub-therapeutic doses of low-molecular-weight heparin. *Journal of thrombosis and haemostasis : JTH.* 2010;8(1):107-113.
206. Jaffer AK, Ahmed M, Brotman DJ, et al. Low-molecular-weight-heparins as periprocedural anticoagulation for patients on long-term warfarin therapy: a standardized bridging therapy protocol. *Journal of thrombosis and thrombolysis.* 2005;20(1):11-16.
207. Kovacs MJ, Kearon C, Rodger M, et al. Single-arm study of bridging therapy with low-molecular-weight heparin for patients at risk of arterial embolism who require temporary interruption of warfarin. *Circulation.* 2004;110(12):1658-1663.
208. Spyropoulos AC. Bridging therapy and oral anticoagulation: current and future prospects. *Curr Opin Hematol.* 2010;17(5):444-449.

209. Kaatz S, Douketis JD, Zhou H, Gage BF, White RH. Risk of stroke after surgery in patients with and without chronic atrial fibrillation. *Journal of thrombosis and haemostasis : JTH.* 2010;8(5):884-890.
210. Spyropoulos AC, Douketis JD. How I treat anticoagulated patients undergoing an elective procedure or surgery. *Blood.* 2012;120(15):2954-2962.
211. Sant'anna RT, Leiria TL, Nascimento T, et al. Meta-analysis of continuous oral anticoagulants versus heparin bridging in patients undergoing CIED surgery: reappraisal after the BRUISE study. *Pacing Clin Electrophysiol.* 2015;38(4):417-423.
212. Birnie DH, Healey JS, Wells GA, et al. Pacemaker or defibrillator surgery without interruption of anticoagulation. *The New England journal of medicine.* 2013;368(22):2084-2093.
213. Du L, Zhang Y, Wang W, Hou Y. Perioperative anticoagulation management in patients on chronic oral anticoagulant therapy undergoing cardiac devices implantation: a meta-analysis. *Pacing Clin Electrophysiol.* 2014;37(11):1573-1586.
214. Rowley CP, Bernard ML, Brabham WW, et al. Safety of continuous anticoagulation with dabigatran during implantation of cardiac rhythm devices. *Am J Cardiol.* 2013;111(8):1165-1168.
215. Jennings JM, Robichaux R, McElderry HT, et al. Cardiovascular implantable electronic device implantation with uninterrupted dabigatran: comparison to uninterrupted warfarin. *J Cardiovasc Electrophysiol.* 2013;24(10):1125-1129.
216. Chan NC, Hirsh J, Ginsberg JS, Eikelboom JW. Real-world variability in dabigatran levels in patients with atrial fibrillation: reply. *Journal of thrombosis and haemostasis : JTH.* 2015;13(6):1168-1169.
217. Tafur AJ, Wysokinski WE, McBane RD, et al. Cancer effect on periprocedural thromboembolism and bleeding in anticoagulated patients. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO.* 2012;23(8):1998-2005.
218. Schulman JM, Majeed A, Mattsson E, Schulman S, Holmstrom M, Agren A. Strategies and outcomes of periprocedural bridging therapy with low-molecular-weight heparin in patients with mechanical heart valves. *Journal of thrombosis and thrombolysis.* 2015;40(4):430-436.
219. Connors JM. Antidote for Factor Xa Anticoagulants. *The New England journal of medicine.* 2015;373(25):2471-2472.
220. Greinacher A, Thiele T, Selleng K. Reversal of anticoagulants: an overview of current developments. *Thrombosis and haemostasis.* 2015;113(5):931-942.

221. Kafke W, Kraft P. Intravenous Thrombolysis after Reversal of Dabigatran by Idarucizumab: A Case Report. *Case Rep Neurol.* 2016;8(2):140-144.

Curriculum Vitae

Je suis née en Belgique, à Ottignies Louvain-La-Neuve, le 22 décembre 1980. Fille d'un médecin anesthésiste-réanimateur et d'un médecin biologiste, mon parcours fut quelque peu vallonné suite aux choix professionnels de mes parents. En effet, jusqu'à mes 11 ans, plusieurs allers-retours Belgique-Allemagne ont nécessité un sens de l'intégration rapide dans différents milieux, mais ces expériences m'ont permis de parler aisément la langue de Goethe et d'élargir très jeune mon entourage avec des amitiés de longue date au-delà des frontières belges.

Finissant mes humanités avec un tropisme prépondérant pour les mathématiques, mon empathie pour les autres m'a plutôt fait pousser les portes de la Faculté de Médecine de l'Université catholique de Louvain.

Indécise en doctorat sur le choix de ma future spécialité, j'ai suivi le conseil paternel de faire un stage à option en anesthésie, me permettant de découvrir une spécialité combinant un travail polyvalent, dans un secteur aigu, avec de multiples applications directes en physiologie.

Ce fut une très belle découverte qui m'a convaincue de passer le concours de spécialisation en avril 2006 dans le département d'Anesthésie-réanimation des Cliniques universitaires Saint-Luc de l'Université catholique de Louvain. Mes stages sur les cinq années de spécialisation m'ont permis de découvrir de nombreux secteurs dont celui de la chirurgie cardio-vasculaire et thoracique. Mon tropisme pour ce dernier m'a donné l'envie de passer 6 mois à l'hôpital NHS Papworth à Cambridge pour perfectionner ce secteur.

J'ai eu la chance d'intégrer en 2011 le service d'Anesthésie du Professeur Edith Collard au CHU UCL Namur - site Godinne. Je fus entourée d'une équipe polyvalente et bienveillante, qui m'a beaucoup soutenue dès mon arrivée dans mes différents projets. Sur conseil du Professeur Maximilien Gourdin, je me suis engagée dans un projet de thèse. Inscrite à l'école doctorale de la Faculté de Médecine de l'Université de Namur en octobre 2012, j'ai été encadrée par mon promoteur, le Professeur Jean-Michel Dogné du département de Pharmacie de l'Université de Namur, et par mon co-promoteur, le Professeur Maximilien Gourdin du département d'Anesthésie du CHU UCL Namur - site Godinne.

Le secteur périopératoire offrait de belles perspectives pour optimiser la prise en charge des patients sous anticoagulants directs oraux, et c'est dans ce domaine que nous avons élaboré un nouveau projet de thèse dont les travaux de recherche ont principalement été réalisés dans le laboratoire d'Hémostase du CHU UCL Namur, dirigé par les Professeurs Bernard Chatelain et François Mullier. Pour mener à bien ma thèse, j'ai pu bénéficier d'un mi-temps clinicien chercheur financé par la Fondation Mont-Godinne et le CHU UCL Namur de octobre 2013 à septembre 2016.

Ma collaboration avec de nombreux membres du personnel du laboratoire d'Hémostase, du service d'Hématologie, de la Pharmacie clinique, du département de Pharmacie de l'Université de Namur, ainsi que mes indispensables collègues anesthésistes, m'a permis d'aboutir concrètement dans ce travail de recherche.

Mariée depuis septembre 2014 à François Mullier, maman d'une petite fille Eva née en janvier 2013 et bientôt maman d'une petite Louise, je me réjouis des perspectives que ma situation familiale et professionnelle me réservent pour la suite.

♦♦♦♦♦♦♦♦♦

List of publications

Accepted manuscripts

1) **Estimation of rivaroxaban plasma concentrations in the perioperative setting in patients with or without heparin bridging**

   Lessire S, Douxfils J, Pochet L, Dincq AS, Larock AS, Gourdin M, Dogné JM, Chatelain B, Mullier F.

   Accepted - *S*eptember 2016 - Clin Appl Thromb Hemost (IF 1.97)

2) **Impact of the direct oral anticoagulants on the activated clotting time: interpretation and outstanding issues**
   Dincq AS*, **Lessire S***, Chatelain B, Gourdin M, Dogné, JM, Mullier F*, Douxfils J*.
   *Contributed equally
   Accepted - September2016 - J Cardiothorac Vasc Anesth (IF 1.52)

3) **Facing coagulation disorders after acute trauma**
   Mullier F, **Lessire S**, de Schoutheete JC, Bernard Chatelain B, Deneys V, Mathieux V, Hachimi Idrissi S, Dogné JM, Watelet JB, Gourdin M, Dincq AS.
   Accepted - July 2016 - B-ENT 2016 (IF 0.66)

Published manuscripts

1) **Periprocedural management of Direct Oral Anticoagulants should be guided by accurate laboratory tests - Letter to the Editor**

   Lessire S, Douxfils J, DIncq AS, Mullier F.

   Reg Anesth Pain Med (IF 3.46).

   September 28, 2016 - Volume Online First - Issue - ppg

   doi: 10.1097/AAP.0000000000000448

2) **Minimisation of bleeding risks due to oral anticoagulants**

Vornicu O, Larock AS, Douxfils J, Mullier F, Dubois V, Dogné JM, Gourdin M, **Lessire S***, Dincq AS*

*Contributed equally
EMJ Hematol. 2016;4[1]:78-90. June 2016

3) **Mass spectrometry in the therapeutic drug monitoring of direct oral anticoagulants. Useful or useless?**
Douxfils J, Pochet L, **Lessire S**, Vancraeynest C, Dogné JM, Mullier F.
Trends in Analytical Chemistry (2016) (IF 7.49)
doi: 10.1016/j.trac.2016.01.029

4) **Is Thrombin Time useful for the assessment of dabigatran concentrations? An in vitro and ex vivo study**
Lessire S, Douxfils J, Baudar J, Bailly N, Dincq AS, Gourdin M, Dogné JM, Chatelain B, Mullier F.
Thromb Res. 2015 Sep; 136(3):693-6. (IF 2.32)
doi: 10.1016/j.thromres.2015.07.018. Epub 2015 Jul 22.

5) **Double-lumen tubes for tracheostomized patients**
Dincq AS, **Lessire S**, Mayné A, Putz L.
J Cardiothorac Vasc Anesth. 2015 Jun;29(3):e35-6. (IF 1.52)
doi: 10.1053/j.jvca.2015.02.011. Epub 2015 Feb 7.

6) **Estimation of dabigatran plasma concentrations in the perioperative setting. An ex vivo study using dedicated coagulation assays**
Douxfils J*, **Lessire S***, Dincq AS, Hjemdahl P, Rönquist-Nii Y, Pohanka A, Gourdin M, Chatelain B, Dogné JM, Mullier F. *Contributed equally
Thromb Haemost. 2015 Apr; 113(4):862-9. (IF 5.26)
doi: 10.1160/TH14-09-0808. Epub 2014 Dec 18.

7) **Management of non-vitamin K antagonist oral anticoagulants in the perioperative setting**
Dincq AS*, **Lessire S***, Douxfils J, Dogné JM, Gourdin M, Mullier F. *Contributed equally
Biomed Res Int. 2014; 2014:385014. (IF 2.53)
doi: 10.1155/2014/385014. Epub 2014 Sep 3. Review.

8) **Preventive strategies against bleeding due to nonvitamin K antagonist oral anticoagulants**
Lessire S*, Dincq AS*, Douxfils J, Devalet B, Nicolas JB, Spinewine A, Larock AS, Dogné JM, Gourdin M, Mullier F. *Contributed equally
Biomed Res Int. 2014;2014:616405. (IF 2.53)
doi: 10.1155/2014/616405. Epub 2014 Jun 16.
Erratum in: Biomed Res Int. 2014;2014:347031.
Multiple author names corrected.

9) **Why, when and how to monitor new oral anticoagulants**
Tamigniau A, Douxfils J, Nicolas JB, Devalet B, Larock AS, Spinewine A, Dincq AS, **Lessire S**, Gourdin M, Watelet JB, Mathieux V, Chatelain C, Dogné JM, Chatelain B, Mullier F.
Rev Med Suisse. 2014 Feb 5;10(416):326-33. (IF 0.12)

10) **Rapid exclusion of the diagnosis of immune HIT by AcuStar HIT and heparin-induced multiple electrode aggregometry**
Minet V, Baudar J, Bailly N, Douxfils J, Laloy J, **Lessire S**, Gourdin M, Devalet B, Chatelain B, Dogné JM, Mullier F.
Thromb Res. 2014 Jun; 133(6):1074-8. (IF 2.45)
doi: 10.1016/j.thromres.2014.01.014. Epub 2014 Jan 18.

11) **L'arrêt Circulatoire en Hypothermie Profonde**
Lessire S, Arrowsmith JA, Klein A.
Le Praticien en Anesthésie Réanimation.
Vol 15 – N° 5 P 297-304 – octobre 2011.

Submitted manuscripts

1) **Appropriate coagulation assays in peri-procedural contexts for patients on dabigatran etexilate: a case series**
Lessire S, Douxfils J, Larock AS, Mullier F.
Under rewiewing – September 2016 - Blood Coagulation & Fibrinolysis (IF 1.24)

2) **A dRVVT-based assay to estimate the intensity of anticoagulation in patients treated with direct oral anticoagulants**
Sennesael A-L, Exner T, Chatelain B, **Lessire S**, Larock A-S, Vancraeynest C, Pochet L, Dogné J-M, Spinewine A, Mullier F*, Douxfils J*
*Contributed equally
Submitted – September 2016 - Thromb Haemost (IF 5.26)