Exploring adverse drug reactions associated with the use of direct oral anticoagulants

From greater fundamental knowledge
To better clinical prevention

Anne-Laure Sennesael

Septembre 2018

Thèse présentée en vue de l'obtention du grade de docteur
en sciences biomédicales et pharmaceutiques

Secteur des sciences de la santé

« Chaque difficulté rencontrée doit être
l'occasion d'un nouveau progrès. »

Pierre de Coubertin

TABLE OF CONTENTS

JURY MEMBERS

SUPERVISORS

Promoter: Anne SPINEWINE (Louvain Drug Research Institute, CHU UCL Namur, Université catholique de Louvain, Brussels, Belgium)

Co-promoter: Jean-Michel DOGNÉ (Département de Pharmacie, Université de Namur, Namur, Belgium)

JURY MEMBERS

President: Françoise VAN BAMBEKE (Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium)

Isabelle GOUIN-THIBAULT (Laboratoire d'Hématologie, Centre Hospitalier Universitaire, Rennes, France)

Isabel SPRIET (Department of Pharmaceutical and Pharmacological Sciences, Katholieke Universiteit Leuven, Leuven, Belgium)

Olivia DALLEUR (Louvain Drug Research Institute, Cliniques Universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium)

Laure ELENS (Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium)

Philippe HAINAUT (Cliniques Universitaires Saint-Luc, Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Brussels, Belgium)

Peter VERHAMME (Department of Cardiovascular Sciences, Katholieke Universiteit Leuven, Leuven, Belgium)

ACKNOWLEDGEMENTS

Au moment de conclure ces 4 années de recherche, je tiens tout d'abord à exprimer ma profonde reconnaissance envers ma promotrice, le professeure Anne Spinewine. Merci pour la confiance que tu m'as accordée dès notre première rencontre en juillet 2012. Cela m'a ouvert des horizons nouveaux et m'a permis de m'épanouir tant sur le plan professionnel que personnel. Merci pour ta disponibilité, ta rigueur et ton esprit critique, qui à chaque fois me font progresser. Je souhaite également remercier mon co-promoteur, le professeur Jean-Michel Dogné, pour son regard éclairé sur le sujet en évolution constante de l'anticoagulation orale.

Je remercie les membres de mon comité d'accompagnement, les professeurs Françoise Van Bambeke, Olivia Dalleur, Laure Elens, Philippe Hainaut et Peter Verhamme, pour leurs conseils avisés tout au long de ce projet. Un merci particulier à Laure Elens pour m'avoir guidée dans l'univers de la pharmacogénomique. Merci également aux membres extérieurs du jury, les professeurs Isabel Spriet et Isabelle Gouin-Thibault, pour leur lecture critique de ma thèse et leurs commentaires qui ont amélioré mon manuscrit.

Menant un projet de recherche interdisciplinaire, j'ai eu la chance de côtoyer de nombreuses équipes.

Je remercie l'équipe du CLIP pour les moments passés ensemble : Sophie Marien, Pauline Anrys, Stefanie Thevelin et Catherine Pétein pour le partage de bureau, de rires et de doutes, ainsi que Séverine Henrard pour les délires statistiques.

Merci aux membres du NTHC et de l'université de Namur pour ces années de précieuse collaboration : Jonathan Douxfils, François Mullier et Sarah Lessire pour les échanges passionnés sur la mesure des AOD, Valérie Mathieux et Bérangère Devalet pour le temps consacré aux évaluations, Christian Chatelain pour la relecture du MAI, ainsi que Lionel Pochet, Christelle Vancraeynest et Romain Siriez pour la gestion de la LC-MS/MS.

Je tiens à remercier chaleureusement l'équipe du LTAP pour l'accueil incroyable qu'ils m'ont réservé. Un merci tout particulier à Nadtha Panin pour sa gentillesse et ses explications claires concernant la culture cellulaire, ainsi qu'à Vincent Haufroid pour son expertise et ses précieux conseils.

Je pense également à une équipe qui m'est chère, celle de la pharmacie de Mont-Godinne. Merci pour vos encouragements lors de chacune de mes venues. Un immense merci à Anne-Sophie Larock pour m'avoir initiée au suivi des patients AOD et pour la fructueuse collaboration que nous avons depuis lors. Merci aussi à Barbara Sneyers pour son implication dans la réalisation de la revue systématique.

Merci à toutes les personnes qui ont contribué à ce projet de recherche : Bruno Krug, pour sa bonne humeur même lorsque nous avions plusieurs milliers de références à analyser, Franck Verschuren et Xavier Muschart pour m'avoir fait découvrir le monde des urgences, Benoit Boland pour les discussions gériatriques, mais aussi les patients et professionnels de santé ayant participé à notre étude clinique, Kaoutar Abdellaoui, Jean-Benoît le Polain de Waroux, Sébastien Marchandise, Patrice Laloux, Isabelle Aujoulat, Martin McGarry, Thomas Exner, Martin Wiesen, Justine Baudar, Maïté Guldenpfennig, Nicolas Dauguet…

Mes remerciements vont également à mes coéquipiers de l'Enjambée et du Brussels Triathlon Club. Les nombreux entraînements que nous avons partagés ont été des bouffées d'oxygène indispensables à la réalisation de cette thèse, et m'ont appris à ne jamais rien lâcher.

Merci à mes deux compères, Charlotte Declaye et Laura Soumoy, pour leurs encouragements ainsi que leur amitié précieuse qui résiste à la distance et aux années. Un grand merci aussi aux colocataires de la rue Longue qui m'ont « supportée » au quotidien.

Pour terminer, j'adresse mes plus sincères remerciements à ma famille pour son soutien inconditionnel durant cette thèse. Merci pour votre aide lorsque les échéances se rapprochaient, pour les moments de répit passés à recharger mes batteries. Un merci particulier à ma maman, dont les talents en coaching ont été mis à rude épreuve pendant ces 4 années.

SUMMARY

Direct oral anticoagulants (DOAC) have transformed the landscape of oral anticoagulant therapy, with the promise of allegedly safer and more convenient drugs. However, serious adverse drug reactions (ADR) have been frequently described in DOAC patients, including thrombotic and bleeding events. This interdisciplinary project aimed to contribute to a better understanding, management and prevention of ADR related to the use of DOAC.

First a prospective study demonstrated that over half of DOAC-related ADR were potentially preventable, mainly due to prescribing and adherence issues. Contributing factors such as discontinuity of care or the lack of knowledge were identified through qualitative research methods. Then, highly variable DOAC plasma levels were observed in bleeding patients, underlining the usefulness of DOAC measurement for managing reversal or urgent procedures. An original coagulation assay was evaluated in that context, and proved to be suitable provided that further improvements are made. Finally, several genetic polymorphisms of the gene coding for ABCB1 (also called P-glycoprotein) were investigated *in vitro,* but did not influence the transport of rivaroxaban. Therefore, they are unlikely to contribute to the inter-individual variability in rivaroxaban levels.

These findings pave the way for designing and implementing risk-minimization strategies in order to prevent DOAC-related ADR. Interventions focusing on prescribing, patient follow-up and continuity of care should help improve DOAC management in clinical practice. Evidence about computerized decision supports systems (CDSS) for DOAC prescribing was reviewed as a first step in this direction. Our findings highlighted that the scope of CDSS should evolve to tackle the misuse of DOAC. Systems were overall well designed, improving practitioner performance in 9 of 15 trials. However, future work is needed to improve the relevance and accuracy of notifications, and to consider implementation outcomes.

ABBREVIATIONS

ADR	Adverse drug reaction
AF	Atrial fibrillation
aPTT	Activated partial thromboplastin time
BID	Twice-daily
BMI	Body Mass Index
CDSS	Computerized clinical decision support system
CV	Coefficient of variation
DE	Dabigatran etexilate
DOAC	Direct oral anticoagulants
dRVVT	Dilute Russell's viper venom time
DVT	Deep vein thrombosis
ED	Emergency department
FDA	Food and Drug Administration
GI	Gastro-intestinal
GP	General practitioner
HCP	Health care professional
INR	International normalized ratio
IP	Individual plasma
LMWH	Low molecular weight heparin
LOD	Limit of detection
LOQ	Limit of quantification
MAI	Medication Appropriateness Index
MDR1	Multidrug resistance 1
NNH	Number needed to harm
NNT	Number needed to treat
NPP	Normal pooled plasma
NPV	Negative predictive value
NSAID	Nonsteroidal anti-inflammatory drugs
NVAF	Non-valvular atrial fibrillation
OD	Once-daily
PBS	Phosphate buffer saline
PE	Pulmonary embolism
P-gp	P-glycoprotein
POC	Point-of-care
PopPK	Population pharmacokinetic
PPP	Platelet-poor plasma

ABBREVIATIONS

PT	Prothrombin time
RCT	Randomized controlled trial
SNP	Single nucleotide polymorphism
SNRI	Serotonin-norepinephrine reuptake inhibitors
SSRI	Selective serotonin reuptake inhibitors
TDM	Therapeutic Drug Monitoring
TIA	Transient ischemic attack
VKA	Vitamin K antagonists
VTE	Venous thromboembolism

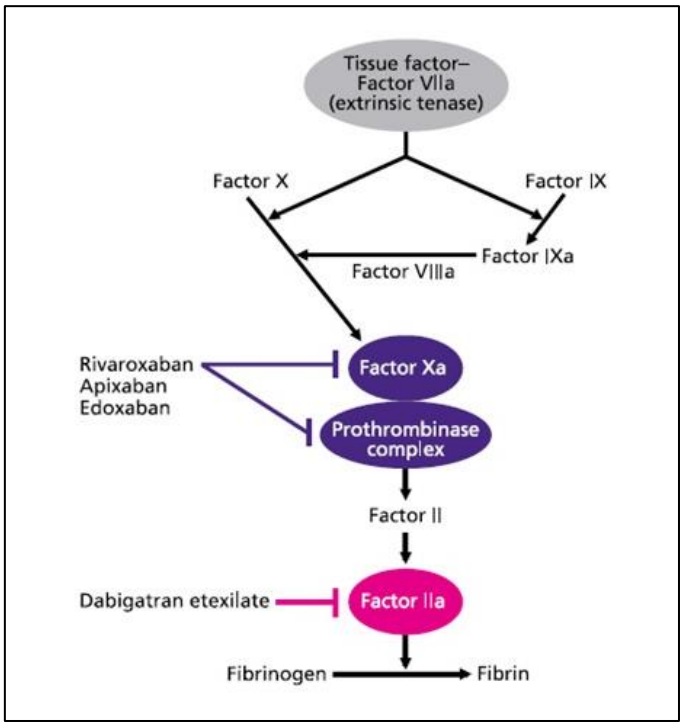
Introduction

I. General introduction

1) Indications and characteristics of direct oral anticoagulants

Direct oral anticoagulants (DOAC) have broadened the landscape of oral anticoagulant therapy for almost a decade. They were first approved for the prevention of venous thromboembolism (VTE) after hip or knee replacement surgery. Since 2011, they have also been indicated for stroke prevention in patients with non-valvular atrial fibrillation (NVAF) as well as for the treatment and secondary prevention of VTE[1, 2]. In 2016, guidelines issued by the European Society of Cardiology or the American College of Chest Physicians recommended DOAC over vitamin K antagonists (VKA) in eligible patients[3, 4].

Figure 1: overview of the coagulation cascade



Adapted from Turpie & Esmon, 2011[5]

Four DOAC are currently used in clinical practice: three direct factor Xa inhibitors (apixaban, edoxaban and rivaroxaban) and a direct factor IIa (thrombin) inhibitor (dabigatran etexilate). They specifically and reversibly bind to the free or clot-bound forms of their respective targets, without relying on antithrombin to mediate their effect[6-8] (Figure 1). As a result, they prevent the amplification of the coagulation cascade and thrombus formation[9]. Edoxaban was not available in Belgium when this research project started in 2014. Therefore, the present thesis work focused on the clinical use of apixaban, dabigatran etexilate (DE) and rivaroxaban.

DOAC share common characteristics such as predictable dose responses, fast onsets of action and short half-lives. However, they differ in several respects making it challenging to select the most appropriate oral anticoagulant therapy for each individual patient[2, 10, 11]. For instance, renal elimination of the unchanged drug ranges from 27 % for apixaban to 80 % for dabigatran. DOAC pharmacokinetic properties and dosage regimen for stroke prevention in NVAF are compared in Table 1.

Table 1: DOAC pharmacokinetic properties and dosage regimen in NVAF[1, 6, 7, 12-19]

	APIXABAN	DABIGATRAN	EDOXABAN	RIVAROXABAN
Brand name	Eliquis®	Praxaxa®	Lixiana®	Xarelto®
Target	Factor Xa	Factor IIa	Factor Xa	Factor Xa
Prodrug	No	Yes (dabigatran etexilate)	No	No
Bioavailability	50 % Independant of food intake	3-7 %, pH sensitive Independant of food intake	62 % Minimal effect of food	66 % (without food) Almost 100 % (with food)
T_{MAX}	3-4 h	0.5-2 h	1-2 h	2-4 h
Protein binding	87 %	35 %	55 %	92-95 %
Renal excretion*	27 %	80 %	50 %	35 % (active secretion 30 %)
Metabolism	CYP3A4/5 (25 %)	20 % active glucuronide conjugates	CYP3A4/5 (<4 %)	CYP3A4/5 (18 %) CYP2J2 (14 %)
Drug transport	P-gp and BCRP	P-gp (dabigatran etexilate)	P-gp	P-gp and BCRP
Half-life	8-15 h (healthy individuals)	12-14 h (healthy individuals)	10-14 h (healthy individuals)	5-9 h (healthy individuals) 11-13 h (elderly)
Dosing in NVAF**	5 mg twice a day	150 mg twice a day	60 mg once a day	20 mg once a day
Dose reduction	2.5 mg twice a day If CrCl 15-29 ml/min or ≥ 2 risk factors: ≥ 80 years, ≤ 60 kg or serum creatinine ≥ 1.5 mg/dl	110 mg twice a day If > 80 years or verapamil To consider if 75-80 years or CrCl 30-50 ml/min	30 mg once a day If CrCl 15-50 ml/min, ≤ 60 kg, ciclosporin, dronedarone, erythromycin or ketoconazole	15 mg once a day If CrCl 15-49 ml/min
Concomitant use not recommended	Ketoconazole, itraconazole, voriconazole, posaconazole, HIV protease inhibitors, rifampicin, St John's wort, phenytoin, carbamazepine	Ketoconazole[$], itraconazole[$], HIV protease inhibitors, dronedarone[$], ciclosporin[$], tacrolimus, rifampicin, St John's wort, phenytoin, carbamazepine	HIV protease inhibitors, St John's wort	Ketoconazole, itraconazole, voriconazole, posaconazole, HIV protease inhibitors, rifampicin, St John's wort, phenytoin, carbamazepine

BCRP: breast cancer resistance protein, NVAF: non-valvular atrial fibrillation, P-gp: P-glycoprotein, Tmax: time to reach peak concentrations
* unchanged drug, ** Dosage regimen approved in the European Union, [$] concomitant use contra-indicated

2) Trends in DOAC prescribing in Belgium

The number of patients treated with an oral anticoagulant has doubled in the last decade in Belgium, to reach nearly 300,000 patients in 2016[20]. The database Intego, which contains information from Flemish general practices, has shown an increase in the prevalence of atrial fibrillation over the same period. Moreover, the proportion of patients with an oral anticoagulant started in the first year after their diagnosis rose from 16% in 2002 to 45% in 2014[21].

DOAC prescribing has increased since their reimbursement in 2012, while the number of VKA-treated patients has dropped to a lesser extent. Between 2012 and 2014, 60% of patients starting chronic oral anticoagulation received DOAC as first choice. In 2015, numbers of patients prescribed DOAC or VKA were similar. Since then, the proportion of DOAC-treated patients is higher than for VKA[20].

In 2016, 50% of DOAC-treated patients received rivaroxaban, while 30% and 20% were prescribed apixaban and DE respectively[20]. The prescription of reduced DOAC doses has been frequent since their arrival on the market: 58% for DE, 42% for rivaroxaban and 24% for apixaban[21]. According to the Belgian InterMutualistic Agency database, the first DOAC prescription was performed by the general practitioner (GP) for 61% of patients. However, these figures do not take into account cardiologist's recommendations to GP[21].

The rise in oral anticoagulant prescribing and the higher cost of DOAC (about ten times that of VKA) have led to a significant increase in healthcare reimbursed expenditure. In 2016, 123 million euros were spent for the reimbursement of oral anticoagulants, including 117 million for DOAC[20]. Apixaban, DE and rivaroxaban were among the top 20 of drugs involved in health expenditure for ambulatory care[22].

II. Benefits and advances

1) Convenience for clinicians and patients

DOAC have been developed to overcome the drawbacks associated with the clinical use of VKA. Given their direct inhibition of coagulation factors, DOAC act independently of vitamin K reserves. They also present fewer food and drug interactions compared to VKA[17, 23]. As a result, DOAC have a more predictable dose response allowing a fixed-dose regimen. They do not require routine laboratory monitoring for dose adjustments[23]. Recommended dosing regimens are nevertheless based on clinical characteristics (weight, age, renal function) and some co-medications.

An additional benefit of DOAC is their short onset of action, with a maximum effect observed between 1 and 4 h after drug intake[6, 7, 24]. This is in sharp contrast with the 48-hour to 72-hour delay required for depletion of vitamin-K dependent coagulation factors after VKA administration[25]. Their offset of action is also rapid, with half-lives around 12 hours. Therefore, DOAC do not require bridging with low molecular weight heparin (LMWH) when they are initiated or discontinued in a short perioperative period[15, 26]. This is a notable feature as anticoagulation bridge therapy was shown to be associated with an increased risk of bleeding[27-29].

Treatment satisfaction was investigated in patients with atrial fibrillation or venous thromboembolism. Overall, patients taking DOAC, whether switched from VKA or not, showed a lower perceived burden of anticoagulant treatment compared to VKA[30-33]. However, the absence of regular laboratory monitoring appears to have a limited impact on patients' preferences[34, 35]. It could even be a source of concern for patients regarding the occurrence of thrombotic or bleeding complications[36].

2) Improved efficacy or safety outcomes

The efficacy and safety of DOAC for stroke prevention in NVAF was compared with warfarin in randomized controlled trials (RCT). According to pooled meta-analysis data, DOAC reduced the risk of stroke or systemic embolism by 19 %, all-cause mortality by 10 % and intracranial hemorrhage by 52 %[37, 38]. However, the risk of gastrointestinal bleeding was higher, driven by dabigatran 150 mg and rivaroxaban. A detailed analysis for individual DOAC is provided in Table 2, including numbers needed to treat and harm (NNT, NNH).

The generalization of RCT results is a major concern, as less than half of real-life patients with AF would have been included in DOAC pivotal trials[39]. Data derived from observational studies have nevertheless become numerous in the last years. They were gathered in several meta-analyses, confirming the favorable risk-benefit profile of DOAC in the setting of atrial fibrillation (Table 2)[40-42].

Table 2: DOAC efficacy and safety outcomes (vs VKA) in patients with NVAF[37, 38, 40-43]

		Stroke/SE	All-cause mortality	Major bleeding	IC bleeding	GI bleeding
Apixaban*	RCT	↓ 303[a]	↓ 238[a]	↓ 104[a]	↓ 213[a]	= N/A
	OBS	↓	↓	↓	↓	↓
Dabigatran 110 mg	RCT	= N/A	= N/A	↓ 154[a]	↓ 196[a]	= N/A
	OBS	= *	↓	↓	↓	=
Dabigatran 150 mg	RCT	↓ 172[a]	= N/A	= N/A	↓ 227[a]	↑ 204[b]
	OBS	= *	↓	↓	↓	↑
Rivaroxaban*	RCT	= N/A	= N/A	= N/A	↓ 500[a]	↑ 101[b]
	OBS	=	=	=	↓	↑
Edoxaban 30 mg	RCT	= N/A	↓ 182[a]	↓ 56[a]	↓ 170[a]	↓ 244[a]
Edoxaban 60 mg	RCT	= N/A	= N/A	↓ 148[a]	↓ 218[a]	↑ 358[b]

* Standard dose or any dose, [a] NNT, [b] NNH

IC: intracranial, GI: gastrointestinal, N/A: not applicable, NNH: number needed to harm, NNT: number needed to treat, NVAF: non-valvular atrial fibrillation, OBS: observational studies, RCT: randomized controlled trial, SE: systemic embolism

No meta-analysis of observational studies available for edoxaban

In patients with acute pulmonary embolism (PE) or deep vein thrombosis (DVT), RCT findings revealed a similar efficacy of DOAC vs. standard therapy to prevent VTE recurrence. The risk of major and intracranial bleeding were significantly reduced[44, 45]. Observational studies have confirmed that DOAC were as effective as VKA in this indication, with a similar risk of major bleeding[46-48].

3) Do DOAC decrease underuse and patient non-adherence?

Although oral anticoagulants are strongly recommended in AF patients at high risk of stroke, their underuse is a common issue[3]. As an example, the international GARFIELD-AF registry highlighted that 38 % of patients with $CHADS_2$ score ≥ 2 did not receive anticoagulant therapy[49]. Main reasons reported by physicians included patient refusal, advanced age or concerns over bleeding risk, falls risk and compliance[49, 50].

Several studies, including one in Belgium, have compared the prescribing of oral anticoagulants before and after the availability of DOAC. Results were encouraging with a decrease in the underuse among high-risk patients in the latter period[51-53]. The greatest convenience of DOAC and their substantially reduced risk of intracranial bleeding could be put forward to explain the findings. However, it may also be due to a greater awareness among providers of the importance of anticoagulation[54].

Adherence rates to medications are low in patients with chronic conditions, declining sharply after the first 6 months of therapy[55]. In VKA patients, non-adherence was the most frequent cause of labile INR, increasing the risk of thrombotic event, morbidity and mortality[56]. It was shown that 25-35 % of patients starting VKA will discontinue their treatment within the first year[56, 57].

Adherence to DOAC therapy is of even greater concern given their short offset of action and the lack of routine monitoring[15, 57]. In RCT, DOAC discontinuation rates were not different compared with warfarin[58]. Some observational studies have demonstrated higher discontinuation rates for rivaroxaban and DE than for VKA or apixaban[59-62]. Conversely, in other database analyses, DOAC patients had higher persistence rates compared to VKA[57, 63]. Non-adherence to DOAC was recently associated with a higher risk of stroke or VTE[64].

III. Pitfalls and challenges

1) Inappropriate use of DOAC in clinical practice

Despite the fixed-dose regimen and highest convenience of DOAC, current data suggest that ensuring their adequate use in routine practice remains a challenge. The appropriateness of DOAC prescribing has been assessed in both retrospective and prospective studies, with variable results depending on outcome definitions. Three studies used the Medication Appropriateness Index (MAI), an implicit tool made up of 10 criteria, for a comprehensive analysis of DOAC prescriptions[65]. They found at least one inappropriate criterion in 28 to 60 % of inpatients or outpatients[66-68]. The most frequent inappropriate criteria are further discussed below.

Indication of DOAC therapy

The use of DOAC for unapproved indications has been reported to range from 6 to 20 %[66, 68-70]. It included the prescription of DOAC for valvular atrial fibrillation, orthopedic indications other than hip or knee replacement surgery and VTE treatment when it had not been approved yet. Rates of off-label use may be expected to decrease with the ongoing extension of DOAC indications.

Appropriateness of dosing

Dose adjustments are required in DOAC patients, particularly based on renal function. The ORBIT-AF registry has shown that 13 % of DOAC patients were receiving an inappropriate dose according to U.S. labeling, with 10 % of patients being underdosed[71]. This is similar to other research findings, reporting 14-18 % of inappropriate DOAC doses[72, 73]. A Spanish prospective registry has recently demonstrated that one in three DOAC patients did not receive an adequate dose. However, broader criteria for dose reduction were considered[74].

In the above-mentioned studies, patients who were female, older, with multiple comorbidities or a high thromboembolic risk score were more likely to receive off-label DOAC doses[71-73]. The concomitant use of aspirin and nonsteroidal anti-inflammatory drugs (NSAID) were also risk factors for being prescribed an inadequate reduced dose[75].

Modalities of administration

Incorrect modalities of administration have been reported in 8-23 % of DOAC patients, including inadequate frequency of drug administration or variable time of intake[66-68, 76]. In particular, around one in 5 patients were taking rivaroxaban inappropriately without food[76]. Preclinical studies in healthy volunteers have shown a decrease in drug exposure of 39% when rivaroxaban 15 mg and 20 mg was taken under fasting conditions[12]. Although the clinical relevance of this food-drug interaction is not established, a case report described the recurrence of PE in a rivaroxaban-treated patient with irregular intake of meals[77]. Peak concentrations increased from 115 ng/ml to 318 ng/ml when taken with proper breakfast. In a Canadian survey, only half of HCP knew that rivaroxaban 15 and 20 mg should be taken together with food[78]. It highlights the need to strengthen HCP and patient education.

Impact on clinical outcomes

An analysis of bleeding events with DE has indicated that, in approximately 25 % of patients, prescribing errors contributed to the adverse event. Inadequate transitions from VKA therapy and contraindicated use in patients with severe renal impairment were observed[79]. In the same way, each inappropriate criterion of the MAI has been shown to double the odds of bleeding[67]. The prescription of a higher dose than recommended was associated with an increased risk of mortality and major bleeding. On the contrary, underdosing increased the risk of thromboembolic events[80, 81].

2) Inter-individual variability in real-life DOAC patients

Although DOAC have a predictable dose response, inter-individual variability in their plasma concentrations is a matter of concern. The one-size-fits-all regimen of DOAC was already questioned in pivotal RCT, as drug exposure was influenced by various clinical covariates including age, renal impairment and interacting drugs[82]. The observed DOAC plasma levels varied over a 6-fold to 11-fold range at trough, considering the 5th and the 95th percentiles (Table 3) [83].

Table 3: DOAC trough concentrations observed in phase 3 trials[6, 13, 14]

Dabigatran*	**Apixaban****	**Rivaroxaban****	**Edoxaban***
Stroke prevention in NVAF			
150 mg BID	5 mg BID	20 mg OD	60 mg OD
61-143 ng/ml	41-230 ng/ml	12-137 ng/ml	19-62 ng/ml
Treatment and secondary prevention of VTE			
150 mg BID	5 mg BID	20 mg OD	60 mg OD
61-143 ng/ml	22-177 ng/ml	6-87 ng/ml	10-39 ng/ml

BID: twice-daily, NVAF: non-valvular atrial fibrillation, OD: once-daily, VTE: venous thromboembolism
* 25-75th percentiles, ** 5-95th percentiles

Even greater between-patients variations have been described in routine clinical practice, due to multiple comorbidities and co-medications. Specifically, inter-individual variability in DOAC levels, expressed as coefficient of variation (CV), was found to range from 35 to 67 % at peak, and from 49 to 86 % at trough[84-87]. In 68 older patients with NVAF, dabigatran plasma concentrations at steady-state were measured between 9 and 720 ng/ml at peak, and between 2 and 594 ng/ml at trough[86]. Another prospective study revealed that 40 % of rivaroxaban plasma concentrations, measured during follow-up visits, were outside the 5th-95th percentiles described in phase 3 trials.[88]

Correlation with clinical outcomes

Secondary analyses of phase 3 studies have demonstrated that ischemic stroke and bleeding outcomes were correlated with DOAC plasma concentrations[83, 89, 90]. For example, in a sub-study of the RELY trial, dabigatran concentrations of 210 ng/ml at trough doubled the risk of major bleeding compared with median trough levels of 88 ng/ml [89]. Inversely, a relationship between low DOAC plasma levels measured in the first month of treatment and the occurrence of subsequent thromboembolic events has recently been highlighted [87].

Determinants of DOAC exposure

Real-life evidence has suggested that older or underweight (<50 kg) patients were more likely to present higher-than-expected DOAC concentrations[91, 92]. Renal impairment was also a significant determinant of increased plasma levels, either at steady-state or after DOAC discontinuation in a perioperative setting[86, 91, 93, 94]. In some studies, the concomitant use of amiodarone, an ABCB1 and CYP3A4 inhibitor, was associated with high DOAC levels[93, 94], while this effect did not reach significance in other analyses[86, 88].

In recent years, genetic variations have been suggested to explain high or low DOAC plasma levels in individual patients[95, 96]. Several studies have investigated single nucleotide polymorphisms (SNP) in genes encoding for drug-metabolizing enzymes (carboxylesterase 1, CES1; CYP3A5) or transporters (ABCB1, ABCG2). Their effects on DOAC exposure are reported in Table 4, with inconsistent results. In a genome-wide association study, the SNP rs2244613 in *CES1* gene, responsible for the conversion of DE to dabigatran, was associated with a lower risk of any bleeding (odds ratio (OR) 0.67 per minor allele) [97].

Table 4: Effect of genetic polymorphisms on DOAC plasma concentrations[97-103]

Gene	SNP		DOAC	Participants		MAF	Effect
CES1	rs2244613		dabigatran	1490 AF patients	Caucasian	0.18	**↓ trough concentrations**
				60 healthy males	Caucasian	0.19	No significant effect
	rs8192935		dabigatran	1490 AF patients	Caucasian	0.33	**↓ peak concentrations**
				97 AF patients	Caucasian	0.32	**↓ trough concentrations**
ABCB1	rs4148738		dabigatran	1490 AF patients	Caucasian	0.45	**↑ peak concentrations**
				97 AF patients	Caucasian	0.47	No significant effect
			apixaban	80 AF patients	Caucasian	0.47	**↑ peak concentrations**
				17 AF patients	Stroke	0.44	No significant effect
	rs1128503	1236C>T	apixaban	44 AF patients	Japanese	0.49	No significant effect
	rs2032582	2677G>T/A	apixaban	44 AF patients	Japanese	0.52	No significant effect
	rs1045642	3435C>T	apixaban	44 AF patients	Japanese	0.30	No significant effect
				17 AF patients	Stroke	0.44	No significant effect
			edoxaban	485 healthy ind.	≠ ethnic groups		No significant effect
	1236C>T-2677G>T-3435C>T		dabigatran	60 healthy males	Caucasian		No significant effect
			rivaroxaban	60 healthy males	Caucasian		No significant effect
ABCG2	rs2231142	421C>A	apixaban	44 AF patients	Japanese	0.33	**↑ trough concentrations**
CYP3A5	rs776746	CYP3A5*3	apixaban	44 AF patients	Japanese	0.78	**↑ trough concentrations**
				17 AF patients	Stroke	0.91	No significant effect

AF: atrial fibrillation, ind.: individuals, MAF: minor allele frequency, SNP: single nucleotide polymorphism

3) DOAC measurement in specific clinical situations

Variations in DOAC plasma levels have raised the question whether laboratory measurements could improve the risk-benefit profile of DOAC[83, 104-106]. Therapeutic drug monitoring (TDM) is currently not recommended for DOAC, as presented in Table 5. In particular, the definition of a therapeutic range for individual DOAC is a remaining issue as optimal plasma concentrations were shown to vary with clinical characteristics[83, 89]. As an example, at a dabigatran trough level of 100 ng/ml, the annual incidence of major bleeding was around 2.5 % and 8 % in patients aged 65 and 85 years respectively[89]. An alternative approach to interpret DOAC measurement may lie in considering the "on-therapy" ranges described during large clinical trials[107].

Table 5: criteria for drugs to be suitable for therapeutic drug monitoring

Criteria for TDM	Applicable to DOAC?	
Significant pharmacokinetic variability	**Yes**	No
Defined concentration-effect relationship	**Yes**	No
No direct measure of therapeutic effect	**Yes**	No
Evidence-based therapeutic range, relatively narrow considering the variability	Yes	**No**
Serious consequences in case of therapeutic failure	**Yes**	No
Suitable and available laboratory assay	**Yes**	No
TDM demonstrated to improve clinical outcomes	Yes	**No**

TDM: therapeutic drug monitoring
Adapted from Lucas & Donovan, 2013[108] and Mismetti & Laporte, 2010[109]

Assessing the individual response may nevertheless benefit patients with numerous risk factors for drug accumulation or therapeutic failure[110, 111]. In an emergency setting, laboratory measurements may be helpful in managing DOAC patients admitted with serious bleeding events. Especially, it could provide a basis to assess DOAC contribution to the event, as well as to warrant and monitor the administration of specific reversal agents[15, 112]. In addition, the exclusion of clinically significant DOAC levels appears useful before urgent invasive procedures or

thrombolysis[26]. Thresholds have been proposed to guide appropriate use of antidotes for DOAC reversal (i.e. idarucizumab for dabigatran, andexanet alpha for direct factor Xa inhibitors)[113].

Laboratory assays for DOAC measurement

The ideal DOAC laboratory assay should highly correlate with LC-MS/MS measurements across a broad range of plasma concentrations. Moreover, it should be sensitive enough to allow the detection of low but clinically relevant levels. This assay also needs to be available 24/7 and to provide results with a short turnaround time. Finally, using a unique assay for the measurement of all DOAC would be more convenient for clinicians[111].

None of the laboratory tests currently available in practice fulfill all of these criteria. Among routine coagulation assays, the activated partial thromboplastin time (aPTT) and the prothrombin time (PT) have been proposed to assess the anticoagulant intensity of dabigatran and rivaroxaban respectively. However, they weakly correlated with DOAC concentrations in patient plasma samples, and their sensitivity varied widely according to the reagent used[114-116]. As a consequence, aPTT and PT showed a poor performance to exclude clinically significant DOAC levels[117].

Specific assays have been recommended to estimate DOAC plasma concentrations: the diluted thrombin time or ecarin-based assays for dabigatran, and chromogenic anti-Xa assays for apixaban, edoxaban and rivaroxaban[107, 111]. Although they highly correlate with DOAC levels, these tests require specific calibrators and controls, increasing the turnaround time if not implemented in routine. Recently, the concept of reporting the inhibitory activity of anti-Xa drugs rather than drug concentration has been proposed[118]. It would suppress the need for specific calibration curves and allow the implementation of a universal and readily available anti-Xa assay.

The dilute Russell's viper venom time (dRVVT) has been suggested as an easy-to-perform biological assay to screen rapidly the anticoagulant effect of all DOAC[119, 120]. This coagulation assay is already used in clinical practice for the detection of lupus anticoagulants. It correlated well with dabigatran and rivaroxaban concentrations in patient plasma samples, and provided better performances than aPTT or PT[121, 122]. However, the dRVTT is not suitable for an accurate estimation of DOAC levels. The different laboratory assays for DOAC measurement are described in Table 6.

Table 6: Laboratory assays for DOAC measurement[107, 110, 111, 121, 123, 124]

Assay	DOAC	Interpretation	Characteristics	Availability	Reagent
		Routine coagulation assays			
aPTT	Dabigatran	Exclusion of above on-therapy levels if normal	Qualitative assessment Rapid TAT	24/7 All laboratories	Dependent
TT	Dabigatran	Exclusion of clinically relevant levels if normal	Qualitative assessment Rapid TAT, highly sensitive	24/7 All laboratories	Dependent
PT	Rivaroxaban Edoxaban	Exclusion of above on-therapy levels if normal (only at peak for edoxaban)	Qualitative assessment Rapid TAT	24/7 All laboratories	Dependent
		Specific assays			
dTT **ECA**	Dabigatran	Estimation of plasma levels Adapted procedure (< 50 ng/ml) and sensitivity to heparin for some tests	Quantitative assessment Calibrators and controls	Not in all laboratories	Independent
Chromogenic Anti-Xa	Rivaroxaban Apixaban Edoxaban	Estimation of plasma levels Adapted procedure (< 50 ng/ml) and sensitivity to heparin for some tests	Quantitative assessment Calibrators and controls	Not in all laboratories	Independent
		Other laboratory assays			
LC-MS/MS	All DOAC	Measurement of plasma levels = gold-standard	Quantitative assessment Calibrators and controls Validation	Specialized laboratories	Not applicable
dRVVT	Dabigatran Rivaroxaban	Exclusion of above on-therapy levels if normal	Qualitative assessment Rapid TAT	Not in all laboratories	Dependent (< PT/aPTT)

aPTT: activated partial thromboplastin time, dRVVT: diluted Russell's viper venom time, dTT: dilute thrombin time, ECA: ecarin chromogenic assay, LC-MS/MS: liquid chromatography–tandem mass spectrometry, PT: prothrombin time, TAT: turnaround time, TT: thrombin time

IV. Adverse drug reactions in DOAC patients

Serious adverse drug reactions (ADR) have been extensively described in the literature for patients taking DOAC, including thrombotic and bleeding events[125-128]. Spontaneous safety reports related to the use of DOAC have also been frequent worldwide since their arrival on the market[129]. Dabigatran was for instance the most frequent suspect drug in safety problems reported to the US Food and Drug Administration (FDA) for the year 2012[130].

1) Risk factors for experiencing ADR

The inappropriate use of DOAC and patient non-adherence were previously shown in the present thesis to influence the occurrence of thrombotic and bleeding events. Several other risk factors for adverse events have been highlighted. First, registries and retrospective cohort studies have demonstrated a higher risk of major or gastro-intestinal bleeding in older DOAC patients[131-134]. Patients experiencing major bleeding also suffered more frequently from impaired renal function[132, 135]. As an example, every 1 ml/min absolute decrease in creatinine clearance was shown to increase the risk of major bleeding by 2%[136].

Patients with extreme body weight (<50 kg or >120 kg) were poorly represented in DOAC phase 3 trials[137]. In two real-life studies, having a low body mass index (BMI) was associated with a higher incidence of major bleeding[138, 139]. Although cases of thrombotic events have been reported in obese patients, there is actually no evidence that high BMI are associated with a decreased DOAC efficacy[140, 141]. On the contrary, better on-treatment rates of clinical outcomes were observed in obese patients despite their higher cardiovascular risk factors[142, 143].

Finally, drug interactions with DOAC are a serious matter of concern. Combination therapy with aspirin significantly increased the risk of major bleeding in pivotal trials and real-life studies[134, 144]. As an example, spontaneous vitreous hemorrhage

were described in 3 patients who were taking rivaroxaban and an additional antithrombotic[125]. Regarding pharmacokinetics interactions, the concomitant use of simvastatin (a potent P-gp inhibitor) was associated with a higher bleeding risk in patients taking DE[145]. A recent retrospective cohort study has also described the influence of concurrent medications on the adjusted incidence rates of major bleeding[146]. However, we observed methodological limitations in the approach used by the authors. They were compiled in the following letter to the Editor.

Drug interactions with non-vitamin K oral anticoagulants
Letter to the Editor

Sennesael AL, Henrard S, Spinewine A
JAMA 2018; 319:829.

To the Editor

An increased risk of major bleeding was found for the concurrent use of NOACs and amiodarone, fluconazole, rifampin, and phenytoin[146]. Rifampin and phenytoin are not inhibitors of P-glycoprotein and CYP3A4, as stated by the authors, but instead are strong inducers[147]. Rifampin has been shown to decrease plasma levels of apixaban, dabigatran, and rivaroxaban by more than 50% in healthy volunteers[148]. Therefore, the finding that rifampin and phenytoin were associated with an increased risk of major bleeding was unexpected. Moreover, results were not consistent across the different NOACs and sites of bleeding. For instance, the concomitant use of rifampin was associated with a significantly reduced risk of intracranial hemorrhage.

These findings raise questions about the risk of bias and confounding. When comparing patients taking another drug concurrently with a NOAC or a NOAC alone,

other studied medications were not considered and could have confounded the results, especially as they included both inhibitors and inducers of P-glycoprotein and CYP3A4. These medications should have been included in the calculation of the propensity scores. Also, we would like to know how the inverse probability of treatment weighting (IPTW) was taken into account in the models. In the Supplement (eTables 2A to 2L), after the use of the IPTW, the numbers of patients were similar in the 2 groups (with and without a concurrent drug), and remained unchanged in the group of patients taking a drug concurrently with a NOAC. To our knowledge, this should not be the case, as using the IPTW takes all patients into account and provides a pseudopopulation in which the number of patients is weighted.

Although this cohort study highlights the importance of drug-drug interactions in patients treated with NOACs, we believe that strong recommendations cannot be made on the basis of its results. The authors found, for instance, that clarithromycin was associated with a reduced risk of major bleeding. However, serious bleeding events and high rivaroxaban levels have been reported following the addition of this drug[128]. A comprehensive bleeding risk assessment, including review of drug-drug interactions, renal function monitoring, and close patient follow-up, remains essential to improve the safety of NOACs.

2) Associated outcomes

In 2013-2014, rivaroxaban and dabigatran were among the top ten drugs implicated in emergency department admissions in older patients in the United States[149]. Several studies have investigated hospitalizations due to major bleeding in DOAC patients[150-153]. Gastrointestinal and intracranial hemorrhage were the most frequent ADR, experienced by 46-62 % and 19-22 % of the patients respectively. The mean length of stay varied between 7 and 11 days. Despite the lack of specific reversal agent when studies were conducted, in-hospital mortality following major or intracranial bleeding was lower for DOAC patients compared to VKA[150, 154]. Death at 30 days occurred in 9-13 % of DOAC patients admitted to the hospital for major bleeding[150, 151].

In the following clinical case, we described the emergency department admission for epistaxis of an older patient taking rivaroxaban. It provided an opportunity to discuss several risk factors of major bleeding and to highlight simple and inexpensive means to reduce the risk of harm in DOAC patients.

Optimizing the safe use of direct oral anticoagulants in older patients: a teachable moment

Sennesael AL, Dogné JM, Spinewine A
JAMA Intern Med. 2015; 175:1608-9

Story from the Front Lines

An 86-year-old woman (weighing 55 kg) presented to the emergency department with persistent epistaxis. She had a history of atrial fibrillation and had been taking rivaroxaban, 20 mg, once daily, for stroke prevention for 1 year. She also had a history of peripheral arterial disease and had been taking aspirin, 80 mg, once daily for primary prevention for 9 months. Her medical history also included a

bioprosthetic heart valve replacement 4 years before presentation. On admission, her creatinine clearance was 21 mL/min according to the Cockroft-Gault equation; hemoglobin, 9.4g/dL; and prothrombin time Quick value, 30% (normal range, 75%-100%).

Results of chromogenic anti–factor Xa assay showed that her plasma concentration of rivaroxaban was supratherapeutic, and so treatment with rivaroxaban was discontinued. Two days later, the rivaroxaban concentration was still within the therapeutic range, highlighting slow drug elimination. A medication history performed by the clinical pharmacist revealed that the patient had been halving her rivaroxaban tablet intake for 2 months because of recurrent nosebleeds.

Given the patient's condition (e.g., renal failure and low weight), the team decided to switch her anticoagulant, and the patient was discharged with a prescription of acenocoumarol. In addition her aspirin treatment was discontinued.

A teachable moment

This case highlights the challenge of ensuring appropriate and safe use of direct oral anticoagulants (DOACs) in frail older patients. Prescription of DOACs is increasing rapidly, partly owing to claims made for their convenience of use for clinicians and patients. Nevertheless, cautious and conservative prescribing should remain a golden rule[155]. A vitamin K antagonist (VKA) often remains more suitable, as was the case for the present patient. Moreover, in this instance, the addition of antiplatelet therapy for stable vascular disease was inappropriate.

Renal insufficiency and older age are important factors contributing to bleeding, as illustrated in the present case. In an analysis of bleeding events with dabigatran[79], two-thirds of patients were older than 80 years, and nearly 60% of those had moderate or severe renal impairment. Reports of major hemorrhages have led regulatory agencies to warn clinicians of the dangers of prescribing DOACs in older

patients with renal failure. Because DOACs are partially excreted by the kidneys, dose reductions are recommended in the event of renal impairment. In the present case, the prescribed dose of rivaroxaban should have been reduced to 15 mg/d, as recommended for creatinine clearances ranging from 15 to 50 mL/min. Use of the Cockroft-Gault equation is advised to estimate renal function because this is the formula used in clinical trials. Because patients with severe renal impairment have been excluded from phase 3 studies, DOACS should be avoided in this population. The same applies to patients with extreme body weights.

Addition of aspirin to a regimen requires a careful assessment in patients treated with anticoagulants. In an outpatient registry described by Steinberg et al[156], combination therapy increased the risk of major bleeding by 50% compared with anticoagulant use alone, from 1.8% to 3.0% at 6-month follow-up. Combination therapy is considered appropriate for up to 12 months after acute coronary syndromes, percutaneous coronary interventions, or stenting procedures[157]. However, in cases of stable vascular disease, the use of an anticoagulant alone is recommended, given the lack of substantial benefit and the increased bleeding risk of combination therapy. The exception is for patients with mechanical heart valves. In these cases, aspirin plus oral anticoagulant can be appropriate, but DOACs are not recommended, and so only a VKA should be used[157]. Steinberg et al[156] found that 35% of patients with an anticoagulant prescribed for atrial fibrillation also received aspirin, with no accepted indication in 40% of cases. In our patient's case, discontinuation of aspirin treatment could have reduced the risk of bleeding.

Although routine laboratory monitoring of patients taking a DOAC is not required, close follow-up is essential, particularly in the elderly population. Follow-up visits allow clinicians to reassess renal function and evaluate patient adherence, understanding of therapy, and adverse events. In the present case, the patient intentionally decreased her intake of rivaroxaban owing to recurrent nosebleeds

over a relatively long period (i.e. 2 months). Performing a regular medication review would have led to the earlier reconsideration of the anticoagulant treatment.

The management of bleeding in patients taking DOACs is complicated by the current lack of a specific antidote and the absence of a rapid biological assay. Although DOACs do not require close therapeutic monitoring, screening the anticoagulation status remains useful in clinical situations such as bleeding or renal impairment. Measurement of the international normalized ratio is not recommended for monitoring DOACs. A normal prothrombin time virtually excludes supratherapeutic rivaroxaban levels, whereas only anti–factor Xa chromogenic assays are currently suitable for estimating rivaroxaban concentrations[158].

Even though DOACs present several advantages compared with VKAs, their use remains challenging in clinical practice, especially in frail older patients. In this context, a conservative strategy may be more valuable[155]. This kind of “less is more” approach includes avoiding prescribing DOACs rather than VKAs without clear, compelling, evidence-based reasons; regularly reassessing renal function; monitoring for adverse effects; and reappraising aspirin prescription.

REFERENCES

1. Harder S, Graff J. Novel oral anticoagulants: clinical pharmacology, indications and practical considerations. Eur J Clin Pharmacol 2013; 69: 1617-33.
2. Czuprynska J, Patel JP, Arya R. Current challenges and future prospects in oral anticoagulant therapy. Br J Haematol 2017: 10.1111/bjh.14714.
3. Kirchhof P, Benussi S, Kotecha D, et al. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. Europace 2016; 18: 1609-78.
4. Jain A, Cifu AS. Antithrombotic therapy for venous thromboembolic disease. JAMA 2017; 317: 2008-9.
5. Turpie AG, Esmon C. Venous and arterial thrombosis--pathogenesis and the rationale for anticoagulation. Thromb Haemost 2011; 105: 586-96.
6. Mueck W, Stampfuss J, Kubitza D, Becka M. Clinical pharmacokinetic and pharmacodynamic profile of rivaroxaban. Clin Pharmacokinet 2014; 53: 1-16.
7. Stangier J. Clinical pharmacokinetics and pharmacodynamics of the oral direct thrombin inhibitor dabigatran etexilate. Clin Pharmacokinet 2008; 47: 285-95.
8. Eriksson BI, Quinlan DJ, Weitz JI. Comparative pharmacodynamics and pharmacokinetics of oral direct thrombin and factor xa inhibitors in development. Clin Pharmacokinet 2009; 48: 1-22.
9. Ansell J. Factor Xa or thrombin: is factor Xa a better target? J Thromb Haemost 2007; 5 Suppl 1: 60-4.
10. Weitz JI, Eikelboom J. Incorporating edoxaban into the choice of anticoagulants for atrial fibrillation. Thromb Haemost 2016; 115: 257-70.
11. Schaefer JK, McBane RD, Wysokinski WE. How to choose appropriate direct oral anticoagulant for patient with nonvalvular atrial fibrillation. Ann Hematol 2016; 95: 437-49.
12. Xarelto - Summary of Product Characteristics. European Medicines Agency; 2017 [Accessed 23 March 2018]. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000944/WC500057108.pdf.
13. Pradaxa - Summary of Product Characteristics. European Medicines Agency; 2017 [Accessed 23 March 2018]. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000829/WC500041059.pdf.
14. Eliquis - Summary of Product Characteristics. European Medicines Agency; 2017 [Accessed 23 March 2018]. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002148/WC500107728.pdf.
15. Steffel J, Verhamme P, Potpara TS, et al. The 2018 European Heart Rhythm Association Practical Guide on the use of non-vitamin K antagonist oral anticoagulants in patients with atrial fibrillation. Eur Heart J 2018; 39: 1330-93.
16. Interactions médicamenteuses, cytochromes P450 et P-glycoprotéine (Pgp). Hôpitaux Universitaires de Genève; 2017 [Accessed 23 March 2018]. Available at: https://www.hug-ge.ch/sites/interhug/files/structures/pharmacologie_et_toxicologie_cliniques/a5_cytochromes_6_2.pdf.
17. Hellwig T, Gulseth M. Pharmacokinetic and pharmacodynamic drug interactions with new oral anticoagulants: what do they mean for patients with atrial fibrillation? Ann Pharmacother 2013; 47: 1478-87.

18. Parasrampuria DA, Truitt KE. Pharmacokinetics and Pharmacodynamics of Edoxaban, a Non-Vitamin K Antagonist Oral Anticoagulant that Inhibits Clotting Factor Xa. Clin Pharmacokinet 2016; 55: 641-55.
19. Lixiana - Summary of Product Characteristics. European Medicines Agency; 2017 [1st August 2018]. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002629/WC500189045.pdf.
20. Réunion de consensus: usage rationnel des anticoagulants oraux en cas de fibrillation auriculaire et en cas de thromboembolie veineuse. INAMI, 2017.
21. Van Brabandt H, San Miguel L, Fairon N, et al. Anticoagulants in non-valvular atrial fibrillation. Health Technology Assessment (HTA). Brussels: Belgian Health Care Knowledge Centre (KCE), 2016. KCE Reports 279. D/2016/10.273/101.
22. Infosport. Le TOP 25 des principes actifs dans les dépenses du secteur ambulatoire de l'assurance soins de santé en 2016. INAMI, 2017. D/2017/0401/23.
23. Baglin T. Clinical use of new oral anticoagulant drugs: dabigatran and rivaroxaban. Br J Haematol 2013; 163: 160-7.
24. Frost CE, Song Y, Shenker A, et al. Effects of age and sex on the single-dose pharmacokinetics and pharmacodynamics of apixaban. Clin Pharmacokinet 2015; 54: 651-62.
25. Mega JL, Simon T. Pharmacology of antithrombotic drugs: an assessment of oral antiplatelet and anticoagulant treatments. Lancet 2015; 386: 281-91.
26. Dubois V, Dincq AS, Douxfils J, et al. Perioperative management of patients on direct oral anticoagulants. Thromb J 2017; 15: 14.
27. Douketis JD, Spyropoulos AC, Kaatz S, et al. Perioperative Bridging Anticoagulation in Patients with Atrial Fibrillation. N Engl J Med 2015; 373: 823-33.
28. Bouillon K, Bertrand M, Boudali L, Ducimetiere P, Dray-Spira R, Zureik M. Short-Term Risk of Bleeding During Heparin Bridging at Initiation of Vitamin K Antagonist Therapy in More Than 90 000 Patients With Nonvalvular Atrial Fibrillation Managed in Outpatient Care. J Am Heart Assoc 2016; 5.
29. Garwood CL, Korkis B, Grande D, Hanni C, Morin A, Moser LR. Anticoagulation Bridge Therapy in Patients with Atrial Fibrillation: Recent Updates Providing a Rebalance of Risk and Benefit. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy 2017; 37: 712-24.
30. Suarez Fernandez C, Castilla-Guerra L, Cantero Hinojosa J, et al. Satisfaction with oral anticoagulants in patients with atrial fibrillation. Patient Prefer Adherence 2018; 12: 267-74.
31. Benzimra M, Bonnamour B, Duracinsky M, et al. Real-life experience of quality of life, treatment satisfaction, and adherence in patients receiving oral anticoagulants for atrial fibrillation. Patient Prefer Adherence 2018; 12: 79-87.
32. Hanon O, Chaussade E, Gueranger P, Gruson E, Bonan S, Gay A. Patient-Reported Treatment Satisfaction with Rivaroxaban for Stroke Prevention in Atrial Fibrillation. A French Observational Study, the SAFARI Study. PLoS One 2016; 11: e0166218.
33. Keita I, Aubin-Auger I, Lalanne C, et al. Assessment of quality of life, satisfaction with anticoagulation therapy, and adherence to treatment in patients receiving long-course vitamin K antagonists or direct oral anticoagulants for venous thromboembolism. Patient Prefer Adherence 2017; 11: 1625-34.
34. Bottger B, Thate-Waschke IM, Bauersachs R, Kohlmann T, Wilke T. Preferences for anticoagulation therapy in atrial fibrillation: the patients' view. J Thromb Thrombolysis 2015; 40: 406-15.

35. Lutsey PL, Horvath KJ, Fullam L, et al. Anticoagulant Preferences and Concerns among Venous Thromboembolism Patients. Thromb Haemost 2018; 118: 553-61.

36. Wilke T, Bauer S, Mueller S, Kohlmann T, Bauersachs R. Patient Preferences for Oral Anticoagulation Therapy in Atrial Fibrillation: A Systematic Literature Review. Patient 2017; 10: 17-37.

37. Ruff CT, Giugliano RP, Braunwald E, et al. Comparison of the efficacy and safety of new oral anticoagulants with warfarin in patients with atrial fibrillation: a meta-analysis of randomised trials. Lancet 2014; 383: 955-62.

38. Lopez-Lopez JA, Sterne JAC, Thom HHZ, et al. Oral anticoagulants for prevention of stroke in atrial fibrillation: systematic review, network meta-analysis, and cost effectiveness analysis. Bmj 2017; 359: j5058.

39. Desmaele S, Steurbaut S, Cornu P, Brouns R, Dupont AG. Clinical trials with direct oral anticoagulants for stroke prevention in atrial fibrillation: how representative are they for real life patients? Eur J Clin Pharmacol 2016; 72: 1125-34.

40. Ntaios G, Papavasileiou V, Makaritsis K, Vemmos K, Michel P, Lip GYH. Real-World Setting Comparison of Nonvitamin-K Antagonist Oral Anticoagulants Versus Vitamin-K Antagonists for Stroke Prevention in Atrial Fibrillation: A Systematic Review and Meta-Analysis. Stroke 2017; 48: 2494-503.

41. Carmo J, Moscoso Costa F, Ferreira J, Mendes M. Dabigatran in real-world atrial fibrillation. Meta-analysis of observational comparison studies with vitamin K antagonists. Thromb Haemost 2016; 116: 754-63.

42. Proietti M, Romanazzi I, Romiti GF, Farcomeni A, Lip GYH. Real-World Use of Apixaban for Stroke Prevention in Atrial Fibrillation: A Systematic Review and Meta-Analysis. Stroke 2018; 49: 98-106.

43. Fibrillation auriculaire: prévention des événements thromboemboliques. Farmaka, 2014.

44. van Es N, Coppens M, Schulman S, Middeldorp S, Buller HR. Direct oral anticoagulants compared with vitamin K antagonists for acute venous thromboembolism: evidence from phase 3 trials. Blood 2014; 124: 1968-75.

45. Gomez-Outes A, Terleira-Fernandez AI, Lecumberri R, Suarez-Gea ML, Vargas-Castrillon E. Direct oral anticoagulants in the treatment of acute venous thromboembolism: a systematic review and meta-analysis. Thromb Res 2014; 134: 774-82.

46. Jun M, Lix LM, Durand M, et al. Comparative safety of direct oral anticoagulants and warfarin in venous thromboembolism: multicentre, population based, observational study. Bmj 2017; 359: j4323.

47. Sindet-Pedersen C, Langtved Pallisgaard J, Staerk L, et al. Comparative safety and effectiveness of rivaroxaban versus VKAs in patients with venous thromboembolism. A Danish nationwide registry-based study. Thromb Haemost 2017; 117: 1182-91.

48. 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: e12-21.

49. Kakkar AK, Mueller I, Bassand JP, et al. Risk profiles and antithrombotic treatment of patients newly diagnosed with atrial fibrillation at risk of stroke: perspectives from the international, observational, prospective GARFIELD registry. PLoS One 2013; 8: e63479.

50. Pugh D, Pugh J, Mead GE. Attitudes of physicians regarding anticoagulation for atrial fibrillation: a systematic review. Age Ageing 2011; 40: 675-83.

51. Henrard S, Vandenabeele C, Marien S, Boland B, Dalleur O. Underuse of Anticoagulation in Older Patients with Atrial Fibrillation and CHADS2 Score >/= 2: Are We Doing Better Since the Marketing of Direct Oral Anticoagulants? Drugs Aging 2017; 34: 841-50.
52. Admassie E, Chalmers L, Bereznicki LR. Changes in Oral Anticoagulant Prescribing for Stroke Prevention in Patients With Atrial Fibrillation. Am J Cardiol 2017; 120: 1133-8.
53. Huisman MV, Rothman KJ, Paquette M, et al. The Changing Landscape for Stroke Prevention in AF: Findings From the GLORIA-AF Registry Phase 2. J Am Coll Cardiol 2017; 69: 777-85.
54. Palomaki A, Mustonen P, Hartikainen JE, et al. Underuse of anticoagulation in stroke patients with atrial fibrillation--the FibStroke Study. Eur J Neurol 2016; 23: 133-9.
55. Osterberg L, Blaschke T. Adherence to medication. N Engl J Med 2005; 353: 487-97.
56. Suryanarayan D, Schulman S. When the rubber meets the road: adherence and persistence with non-vitamin K antagonist oral anticoagulants and old oral anticoagulants in the real world-a problem or a myth ? Semin Thromb Hemost 2014; 40: 852-9.
57. Raparelli V, Proietti M, Cangemi R, Lip GY, Lane DA, Basili S. Adherence to oral anticoagulant therapy in patients with atrial fibrillation. Focus on non-vitamin K antagonist oral anticoagulants. Thromb Haemost 2017; 117: 209-18.
58. Chatterjee S, Sardar P, Giri JS, Ghosh J, Mukherjee D. Treatment discontinuations with new oral agents for long-term anticoagulation: insights from a meta-analysis of 18 randomized trials including 101,801 patients. Mayo Clin Proc 2014; 89: 896-907.
59. Collings SL, Vannier-Moreau V, Johnson ME, et al. Initiation and continuation of oral anticoagulant prescriptions for stroke prevention in non-valvular atrial fibrillation: A cohort study in primary care in France. Arch Cardiovasc Dis 2018; 111: 370-9.
60. Forslund T, Wettermark B, Hjemdahl P. Comparison of treatment persistence with different oral anticoagulants in patients with atrial fibrillation. Eur J Clin Pharmacol 2016; 72: 329-38.
61. Johnson ME, Lefevre C, Collings SL, et al. Early real-world evidence of persistence on oral anticoagulants for stroke prevention in non-valvular atrial fibrillation: a cohort study in UK primary care. BMJ Open 2016; 6: e011471.
62. Maura G, Billionnet C, Alla F, Gagne JJ, Pariente A. Comparison of Treatment Persistence with Dabigatran or Rivaroxaban versus Vitamin K Antagonist Oral Anticoagulants in Atrial Fibrillation Patients: A Competing Risk Analysis in the French National Health Care Databases. Pharmacotherapy 2018; 38: 6-18.
63. Beyer-Westendorf J, Ehlken B, Evers T. Real-world persistence and adherence to oral anticoagulation for stroke risk reduction in patients with atrial fibrillation. Europace 2016; 18: 1150-7.
64. Deshpande CG, Kogut S, Laforge R, Willey C. Impact of medication adherence on risk of ischemic stroke, major bleeding and deep vein thrombosis in atrial fibrillation patients using novel oral anticoagulants. Curr Med Res Opin 2018: 1-8.
65. Hanlon JT, Schmader KE, Samsa GP, et al. A method for assessing drug therapy appropriateness. J Clin Epidemiol 1992; 45: 1045-51.
66. Larock AS, Mullier F, Sennesael AL, et al. Appropriateness of prescribing dabigatran etexilate and rivaroxaban in patients with nonvalvular atrial fibrillation: a prospective study. Ann Pharmacother 2014; 48: 1258-68.
67. Whitworth MM, Haase KK, Fike DS, Bharadwaj RM, Young RB, MacLaughlin EJ. Utilization and prescribing patterns of direct oral anticoagulants. Int J Gen Med 2017; 10: 87-94.

68. Basaran O, Filiz Basaran N, Cekic EG, et al. PRescriptiOn PattERns of Oral Anticoagulants in Nonvalvular Atrial Fibrillation (PROPER study). Clin Appl Thromb Hemost 2017; 23: 384-91.
69. Chowdhry U, Jacques A, Karovitch A, Giguere P, Nguyen ML. Appropriateness of Dabigatran and Rivaroxaban Prescribing for Hospital Inpatients. Can J Hosp Pharm 2016; 69: 194-201.
70. Carley B, Griesbach S, Larson T, Krueger K. Assessment of dabigatran utilization and prescribing patterns for atrial fibrillation in a physician group practice setting. Am J Cardiol 2014; 113: 650-4.
71. Steinberg BA, Shrader P, Thomas L, et al. Off-Label Dosing of Non-Vitamin K Antagonist Oral Anticoagulants and Adverse Outcomes: The ORBIT-AF II Registry. J Am Coll Cardiol 2016; 68: 2597-604.
72. Howard M, Lipshutz A, Roess B, et al. Identification of risk factors for inappropriate and suboptimal initiation of direct oral anticoagulants. J Thromb Thrombolysis 2017; 43: 149-56.
73. Shrestha S, Baser O, Kwong WJ. Effect of Renal Function on Dosing of Non-Vitamin K Antagonist Direct Oral Anticoagulants Among Patients With Nonvalvular Atrial Fibrillation. Ann Pharmacother 2018; 52: 147-53.
74. Ruiz Ortiz M, Muniz J, Rana Miguez P, et al. Inappropriate doses of direct oral anticoagulants in real-world clinical practice: prevalence and associated factors. A subanalysis of the FANTASIIA Registry. Europace 2017.
75. McAlister FA, Garrison S, Kosowan L, Ezekowitz JA, Singer A. Use of Direct Oral Anticoagulants in Canadian Primary Care Practice 2010-2015: A Cohort Study From the Canadian Primary Care Sentinel Surveillance Network. J Am Heart Assoc 2018; 7.
76. Simon J, Hawes E, Deyo Z, Bryant Shilliday B. Evaluation of prescribing and patient use of target-specific oral anticoagulants in the outpatient setting. J Clin Pharm Ther 2015.
77. Beyer-Westendorf J, Siegert G. Of men and meals. J Thromb Haemost 2015; 13: 943-5.
78. Piran S, Schulman S, Panju M, Pai M. Oral anticoagulant dosing, administration, and storage: a cross-sectional survey of Canadian health care providers. J Thromb Thrombolysis 2018; 45: 180-5.
79. Harper P, Young L, Merriman E. Bleeding risk with dabigatran in the frail elderly. N Engl J Med 2012; 366: 864-6.
80. Steinberg BA, Shrader P, Pieper K, et al. Frequency and Outcomes of Reduced Dose Non-Vitamin K Antagonist Anticoagulants: Results From ORBIT-AF II (The Outcomes Registry for Better Informed Treatment of Atrial Fibrillation II). J Am Heart Assoc 2018; 7.
81. Yao X, Shah ND, Sangaralingham LR, Gersh BJ, Noseworthy PA. Non-Vitamin K Antagonist Oral Anticoagulant Dosing in Patients With Atrial Fibrillation and Renal Dysfunction. J Am Coll Cardiol 2017; 69: 2779-90.
82. Gong IY, Kim RB. Importance of pharmacokinetic profile and variability as determinants of dose and response to dabigatran, rivaroxaban, and apixaban. Can J Cardiol 2013; 29: S24-33.
83. Eikelboom JW, Quinlan DJ, Hirsh J, Connolly SJ, Weitz JI. Laboratory Monitoring of Non-Vitamin K Antagonist Oral Anticoagulant Use in Patients With Atrial Fibrillation: A Review. JAMA Cardiol 2017; 2: 566-74.
84. 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. Thromb Res 2016; 137: 178-83.
85. Chan NC, Coppens M, Hirsh J, et al. Real-world variability in dabigatran levels in patients with atrial fibrillation. J Thromb Haemost 2015; 13: 353-9.

86. Chaussade E, Hanon O, Boully C, et al. Real-Life Peak and Trough Dabigatran Plasma Measurements over Time in Hospitalized Geriatric Patients with Atrial Fibrillation. J Nutr Health Aging 2018; 22: 165-73.

87. Testa S, Paoletti O, Legnani C, et al. Low drug levels and thrombotic complications in high-risk atrial fibrillation patients treated with direct oral anticoagulants. J Thromb Haemost 2018; 16: 842-8.

88. Gulilat M, Tang A, Gryn SE, et al. Interpatient Variation in Rivaroxaban and Apixaban Plasma Concentrations in Routine Care. Can J Cardiol 2017; 33: 1036-43.

89. 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). J Am Coll Cardiol 2014; 63: 321-8.

90. 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: 2288-95.

91. Rottenstreich A, Zacks N, Kleinstern G, et al. Direct-acting oral anticoagulant drug level monitoring in clinical patient management. J Thromb Thrombolysis 2018; 45: 543-9.

92. Arachchillage D, Reynolds R, Devey T, Maclean R, Kitchen S, van Veen JJ. Effect of extremes of body weight on drug level in patient treated with standard dose of rivaroxaban for venous thromboembolism; real life experience. Thromb Res 2016; 147: 32-5.

93. Godier A, Dincq AS, Martin AC, et al. Predictors of pre-procedural concentrations of direct oral anticoagulants: a prospective multicentre study. Eur Heart J 2017; 38: 2431-9.

94. Kaserer A, Schedler A, Jetter A, et al. Risk Factors for Higher-than-Expected Residual Rivaroxaban Plasma Concentrations in Real-Life Patients. Thromb Haemost 2018; 118: 808-17.

95. Douros A, Schlemm L, Bolbrinker J, Ebinger M, Kreutz R. Insufficient anticoagulation with dabigatran in a patient with short bowel syndrome. Thromb Haemost 2014; 112: 419-20.

96. Ing Lorenzini K, Daali Y, Fontana P, Desmeules J, Samer C. Rivaroxaban-Induced Hemorrhage Associated with ABCB1 Genetic Defect. Front Pharmacol 2016; 7: 494.

97. Pare G, Eriksson N, Lehr T, et al. Genetic determinants of dabigatran plasma levels and their relation to bleeding. Circulation 2013; 127: 1404-12.

98. Gouin-Thibault I, Delavenne X, Blanchard A, et al. Interindividual variability in dabigatran and rivaroxaban exposure: contribution of ABCB1 genetic polymorphisms and interaction with clarithromycin. J Thromb Haemost 2017; 15: 273-83.

99. Dimatteo C, D'Andrea G, Vecchione G, et al. Pharmacogenetics of dabigatran etexilate interindividual variability. Thromb Res 2016; 144: 1-5.

100. Dimatteo C, D'Andrea G, Vecchione G, et al. ABCB1 SNP rs4148738 modulation of apixaban interindividual variability. Thromb Res 2016; 145: 24-6.

101. Ueshima S, Hira D, Fujii R, et al. Impact of ABCB1, ABCG2, and CYP3A5 polymorphisms on plasma trough concentrations of apixaban in Japanese patients with atrial fibrillation. Pharmacogenet Genomics 2017; 27: 329-36.

102. Kryukov AV, Sychev DA, Andreev DA, et al. Influence of ABCB1 and CYP3A5 gene polymorphisms on pharmacokinetics of apixaban in patients with atrial fibrillation and acute stroke. Pharmgenomics Pers Med 2018; 11: 43-9.

103. Vandell AG, Lee J, Shi M, Rubets I, Brown KS, Walker JR. An integrated pharmacokinetic/pharmacogenomic analysis of ABCB1 and SLCO1B1 polymorphisms on edoxaban exposure. Pharmacogenomics J 2018; 18: 153-9.
104. Douxfils J, Mullier F, Dogne JM. Dose tailoring of dabigatran etexilate: obvious or excessive? Expert Opin Drug Saf 2015; 14: 1283-9.
105. Powell JR. Are new oral anticoagulant dosing recommendations optimal for all patients? Jama 2015; 313: 1013-4.
106. Safouris A, Triantafyllou N, Parissis J, Tsivgoulis G. The case for dosing dabigatran: how tailoring dose to patient renal function, weight and age could improve the benefit-risk ratio. Ther Adv Neurol Disord 2015; 8: 245-54.
107. Douxfils J, Ageno W, Samama CM, et al. Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians. J Thromb Haemost 2018; 16: 209-19.
108. Lucas C, Donovan P. 'Just a repeat' - When drug monitoring is indicated. Aust Fam Physician 2013; 42: 18-22.
109. Mismetti P, Laporte S. New oral antithrombotics: a need for laboratory monitoring. For. J Thromb Haemost 2010; 8: 621-6.
110. Douxfils J, Gosselin RC. Laboratory Assessment of Direct Oral Anticoagulants. Semin Thromb Hemost 2017; 43: 277-90.
111. Cuker A. Laboratory measurement of the non-vitamin K antagonist oral anticoagulants: selecting the optimal assay based on drug, assay availability, and clinical indication. J Thromb Thrombolysis 2016; 41: 241-7.
112. 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. Arch Cardiovasc Dis 2013; 106: 382-93.
113. 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. J Thromb Haemost 2016; 14: 623-7.
114. 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. Thromb Haemost 2013; 110: 543-9.
115. 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. Thromb Haemost 2013; 110: 723-31.
116. Van Blerk M, Bailleul E, Chatelain B, et al. Influence of dabigatran and rivaroxaban on routine coagulation assays. A nationwide Belgian survey. Thromb Haemost 2015; 113: 154-64.
117. Jabet A, Stepanian A, Golmard JL, et al. Are Screening Tests Reliable to Rule Out Direct Oral Anticoagulant Plasma Levels at Various Thresholds (30, 50, or 100 ng/mL) in Emergency Situations? Chest 2018; 153: 288-90.
118. van Pelt LJ, Lukens MV, Testa S, Chatelain B, Douxfils J, Mullier F. The DaXa-inhibition assay: A concept for a readily available, universal aXa assay that measures the direct inhibitory effect of all anti-Xa drugs. Thrombosis Research 2018; 168: 63-6.
119. Exner T, Ellwood L, Rubie J, Barancewicz A. Testing for new oral anticoagulants with LA-resistant Russells viper venom reagents. An in vitro study. Thromb Haemost 2013; 109: 762-5.

120. Colombini MP, Derogis P, de Aranda VF, de Campos Guerra JC, Hamerschlak N, Mangueira CLP. Comparison of different laboratory tests in the evaluation of hemorrhagic risk of patients using rivaroxaban in the critical care setting: diagnostic accuracy study. Thromb J 2017; 15: 21.
121. Douxfils J, Chatelain B, Hjemdahl P, et al. Does the Russell Viper Venom time test provide a rapid estimation of the intensity of oral anticoagulation? A cohort study. Thromb Res 2015; 135: 852-60.
122. Pratt J, Crispin P. Screening test for direct oral anticoagulants with the dilute Russell viper venom time. Eur J Haematol 2018; 100: 567-74.
123. Lessire S, Douxfils J, Pochet L, et al. Estimation of Rivaroxaban Plasma Concentrations in the Perioperative Setting in Patients With or Without Heparin Bridging. Clin Appl Thromb Hemost 2018; 24: 129-38.
124. 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. Thromb Haemost 2015; 113: 862-9.
125. Jun JH, Hwang JC. Association of rivaroxaban anticoagulation and spontaneous vitreous hemorrhage. JAMA Ophthalmol 2015; 133: 1184-6.
126. Wychowski MK, Kouides PA. Dabigatran-induced gastrointestinal bleeding in an elderly patient with moderate renal impairment. Ann Pharmacother 2012; 46: e10.
127. Legrand M, Mateo J, Aribaud A, et al. The use of dabigatran in elderly patients. Arch Intern Med 2011; 171: 1285-6.
128. Fralick M, Juurlink DN, Marras T. Bleeding associated with coadministration of rivaroxaban and clarithromycin. Cmaj 2016; 188: 669-72.
129. Monaco L, Biagi C, Conti V, et al. Safety profile of the direct oral anticoagulants: an analysis of the WHO database of adverse drug reactions. Br J Clin Pharmacol 2017; 83: 1532-43.
130. Moore TJ, Cohen MR, Furberg CD. Anticoagulants the leading reported drug risk in 2012. QuarterWatch 2012 Quarter 4. 2012.
131. 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: 63-8.
132. 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: 955-62.
133. Abraham NS, Noseworthy PA, Yao X, Sangaralingham LR, Shah ND. Gastrointestinal Safety of Direct Oral Anticoagulants: A Large Population-Based Study. Gastroenterology 2017; 152: 1014-22 e1.
134. Hernandez I, Baik SH, Pinera A, Zhang Y. Risk of bleeding with dabigatran in atrial fibrillation. JAMA Intern Med 2015; 175: 18-24.
135. 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. Eur Heart J 2016; 37: 1145-53.
136. Becattini C, Giustozzi M, Ranalli MG, et al. Variation of renal function over time is associated with major bleeding in patients treated with direct oral anticoagulants for atrial fibrillation. J Thromb Haemost 2018; 16: 833-41.

137. 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. J Thromb Haemost 2016; 14: 1308-13.

138. Park CS, Choi EK, Kim HM, Lee SR, Cha MJ, Oh S. Increased risk of major bleeding in underweight patients with atrial fibrillation who were prescribed non-vitamin K antagonist oral anticoagulants. Heart Rhythm 2017; 14: 501-7.

139. Lee CH, Lin TY, Chang SH, et al. Body mass index is an independent predictor of major bleeding in non-valvular atrial fibrillation patients taking dabigatran. Int J Cardiol 2017; 228: 771-8.

140. Breuer L, Ringwald J, Schwab S, Kohrmann M. Ischemic stroke in an obese patient receiving dabigatran. N Engl J Med 2013; 368: 2440-2.

141. Tittl L, Endig S, Marten S, Reitter A, Beyer-Westendorf I, Beyer-Westendorf J. Impact of BMI on clinical outcomes of NOAC therapy in daily care - Results of the prospective Dresden NOAC Registry (NCT01588119). Int J Cardiol 2018; 262: 85-91.

142. Balla SR, Cyr DD, Lokhnygina Y, et al. Relation of Risk of Stroke in Patients With Atrial Fibrillation to Body Mass Index (from Patients Treated With Rivaroxaban and Warfarin in the Rivaroxaban Once Daily Oral Direct Factor Xa Inhibition Compared with Vitamin K Antagonism for Prevention of Stroke and Embolism Trial in Atrial Fibrillation Trial). Am J Cardiol 2017; 119: 1989-96.

143. Sandhu RK, Ezekowitz J, Andersson U, et al. The 'obesity paradox' in atrial fibrillation: observations from the ARISTOTLE (Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation) trial. Eur Heart J 2016; 37: 2869-78.

144. Shah R, Hellkamp A, Lokhnygina Y, et al. Use of concomitant aspirin in patients with atrial fibrillation: Findings from the ROCKET AF trial. Am Heart J 2016; 179: 77-86.

145. Antoniou T, Macdonald EM, Yao Z, et al. Association between statin use and ischemic stroke or major hemorrhage in patients taking dabigatran for atrial fibrillation. Cmaj 2017; 189: E4-e10.

146. Chang S, Chou I, Yeh Y, et al. Association between use of non–vitamin k oral anticoagulants with and without concurrent medications and risk of major bleeding in nonvalvular atrial fibrillation. JAMA 2017; 318: 1250-9.

147. Di Minno A, Frigerio B, Spadarella G, et al. Old and new oral anticoagulants: Food, herbal medicines and drug interactions. Blood Rev 2017; 31: 193-203.

148. 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 2015; 17: 1467-507.

149. Shehab N, Lovegrove MC, Geller AI, Rose KO, Weidle NJ, Budnitz DS. US emergency department visits for outpatient adverse drug events, 2013-2014. JAMA 2016; 316: 2115-25.

150. Xu Y, Schulman S, Dowlatshahi D, et al. Direct Oral Anticoagulant- or Warfarin-Related Major Bleeding: Characteristics, Reversal Strategies, and Outcomes From a Multicenter Observational Study. Chest 2017; 152: 81-91.

151. Becattini C, Franco L, Beyer-Westendorf J, et al. Major bleeding with vitamin K antagonists or direct oral anticoagulants in real-life. Int J Cardiol 2017; 227: 261-6.

152. Bouget J, Oger E. Emergency admissions for major haemorrhage associated with direct oral anticoagulants. Thrombosis Research 2015; 136: 1190-4.

153. Milling TJ, Fromm C, Ganetsky M, Pallin DJ, Cong J, Singer AJ. Management of Major Bleeding Events in Patients Treated With Dabigatran for Nonvalvular Atrial Fibrillation: A Retrospective, Multicenter Review. Ann Emerg Med 2017; 69: 531-40.

154. Inohara T, Xian Y, Liang L, et al. Association of Intracerebral Hemorrhage Among Patients Taking Non-Vitamin K Antagonist vs Vitamin K Antagonist Oral Anticoagulants With In-Hospital Mortality. JAMA 2018; 319: 463-73.

155. Schiff GD, Galanter WL, Duhig J, Lodolce AE, Koronkowski MJ, Lambert BL. Principles of conservative prescribing. Arch Intern Med 2011; 171: 1433-40.

156. Steinberg BA, Kim S, Piccini JP, et al. Use and associated risks of concomitant aspirin therapy with oral anticoagulation in patients with atrial fibrillation: insights from the Outcomes Registry for Better Informed Treatment of Atrial Fibrillation (ORBIT-AF) Registry. Circulation 2013; 128: 721-8.

157. Lip GY, Windecker S, Huber K, et al. Management of antithrombotic therapy in atrial fibrillation patients presenting with acute coronary syndrome and/or undergoing percutaneous coronary or valve interventions: a joint consensus document of the European Society of Cardiology Working Group on Thrombosis, European Heart Rhythm Association (EHRA), European Association of Percutaneous Cardiovascular Interventions (EAPCI) and European Association of Acute Cardiac Care (ACCA) endorsed by the Heart Rhythm Society (HRS) and Asia-Pacific Heart Rhythm Society (APHRS). Eur Heart J 2014; 35: 3155-79.

158. Cuker A, Siegal DM, Crowther MA, Garcia DA. Laboratory measurement of the anticoagulant activity of the non-vitamin K oral anticoagulants. J Am Coll Cardiol 2014; 64: 1128-39.

Aim and objectives

Direct oral anticoagulants (DOAC) are increasingly used in clinical practice, accounting for a significant portion of health care spending. Like any anticoagulant therapy, they are essential but high-risk medications. In recent years, serious adverse drug reactions (ADR) related to the use of DOAC have been described, including thrombotic and bleeding events. However, underlying reasons for the occurrence of ADR in patients taking DOAC remain poorly understood.

The overarching aim of the present thesis was to contribute to a better understanding, management and prevention of patient safety issues related to the use of DOAC. Specifically, we developed an interdisciplinary approach combining both fundamental and clinical research. The following research questions were addressed: 1) How preventable are serious ADR associated with the use of DOAC? 2) How suitable is an original assay for screening the intensity of anticoagulation in DOAC patients? 3) Can *ABCB1* polymorphisms help explain the inter-individual variability in DOAC levels? 4) Do computerized clinical decision support systems (CDSS) improve the prescribing of oral anticoagulants?

Table 1: detailed research objectives

Chapter	Specific objectives
I	To determine the preventability of serious ADR associated with the use of DOAC To explore contributing factors to preventable and serious ADRs To compare results with vitamin K antagonists (VKA)
II.1	To assess the correlation with DOAC plasma concentrations in clinical samples To evaluate the assay performance to rule out clinically relevant DOAC levels To determine the assay sensitivity to VKA plasma samples and heparins
II.2	To describe rivaroxaban levels in 10 patients admitted for bleeding events To discuss clinical, medication and genetic characteristics contributing to bleeding
III	To evaluate the *in vitro* effect of *ABCB1* single nucleotide polymorphisms (1236C>T-2677G>T-3435C>T and 1199G>A) on the transport activity towards rivaroxaban
IV	To review the impact of CDSS for the prescribing of oral anticoagulants To describe CDSS characteristics associated with success

ADR: adverse drug reaction, CDSS: computerized clinical decision support system, DOAC: direct oral anticoagulant, VKA: vitamin K antagonist

This research project will contribute to fill in important knowledge gaps regarding the clinical use of DOAC, particularly in the fields of medication errors, laboratory measurement, pharmacogenomics, and health information technology. Our findings will pave the way for designing and implementing risk-minimization strategies in order to reduce preventable hospital admissions in patients treated with DOAC.

Chapter I

Preventability of serious ADR associated with the use of DOAC

Preventability of serious thromboembolic and bleeding events related to the use of direct oral anticoagulants and vitamin K antagonists: a prospective study

Anne-Laure Sennesael, Anne-Sophie Larock, Bérangère Devalet, Valérie Mathieux, Franck Verschuren, Xavier Muschart, Olivia Dalleur, Jean-Michel Dogné, Anne Spinewine

British Journal of Clinical Pharmacology 2018; 84:1544-56

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Serious adverse drug reactions (ADRs) have frequently been reported in patients taking direct oral anticoagulants (DOACs).
- Less is known about the preventability of serious ADRs related to the use of DOACs. Moreover, underlying causes of medication errors have not been specifically explored.

WHAT THIS STUDY ADDS

- More than half of both DOAC- and VKA-associated serious ADRs were deemed potentially preventable.
- We identified contributing factors to DOAC-related ADRs, such as drug selection, patient education or communication.
- These results should help target interventions more effectively to improve the use of DOACs in primary and secondary care.

Abstract

AIMS: To determine the preventability of serious adverse drug reactions (ADR) related to the use of direct oral anticoagulants (DOAC), and to explore contributing factors to preventable ADRs. Results were compared with vitamin K antagonists (VKA).

METHODS: We conducted a prospective observational study in the emergency departments of two teaching hospitals from July 2015 to January 2016. Patients admitted with a thrombotic or bleeding event while under DOAC or VKA were included. Four independent reviewers assessed causality, seriousness and preventability of ADRs, using pilot-tested scales. For cases of serious and potentially preventable ADRs, we performed semi-structured interviews with general practitioners to identify contributing factors to ADRs. The primary outcome was the proportion of serious ADRs that were potentially preventable.

RESULTS: The analysis included 46 DOAC and 43 VKA patients (median age 79 years). Gastro-intestinal (n=34) and intracranial (n=16) bleedings were the most frequent ADRs. 53% of DOAC- and 61% of VKA-related serious ADRs were deemed potentially preventable. Prescribing issues and inadequate monitoring were frequent for DOAC and VKA respectively. We identified many causes of preventable ADRs that applied to all oral anticoagulants, such as pharmacodynamics drug interactions and lack of communication.

CONCLUSIONS: More than half of serious ADRs were potentially preventable for both DOACs and VKAs. Interventions focusing on prescribing, patient education and continuity of care should help improve the use of DOACs in practice.

Introduction

For many years vitamin K antagonists (VKA) have been the single therapy option for oral anticoagulation. Their efficacy is well established for several indications. For instance warfarin use decreases the risk of stroke by 64% in atrial fibrillation[1]. Nevertheless VKAs are amongst the most frequent drugs associated with adverse drug reactions (ADR) in the inpatient as well as in the outpatient settings[2-5]. Bleedings are the main ADRs experienced by VKA-treated patients, contributing to the large underuse of oral anticoagulants (around 50% in older people)[6].

Direct oral anticoagulants (DOACs) were developed in response to the need for oral anticoagulants that are more convenient for clinicians and patients. Four DOACs are currently used in clinical practice: three direct factor Xa inhibitors (apixaban, edoxaban, rivaroxaban) and one direct thrombin inhibitor (dabigatran etexilate). They present several advantages compared with VKAs: predictable dose responses, fewer interactions with medications and food, no need for frequent laboratory monitoring, and a lower risk of intracranial bleeding[7, 8].

However, serious ADRs have been extensively reported for patients taking DOACs, including thrombotic and bleeding events[9-12]. In 2013-2014, rivaroxaban and dabigatran etexilate were among the top ten drugs implicated in emergency department admissions in older patients in the United States[13]. More importantly, previous studies revealed that some of these ADRs may be caused by medication errors[14, 15]. This suggests that ensuring safe use of DOACs in clinical practice remains a challenge.

In this prospective study, we assessed the preventability of serious ADRs related to the use of DOACs, and compared results with VKAs. Furthermore, to our knowledge, the underlying causes of medication errors with oral anticoagulants have not been specifically explored using qualitative research approaches, neither for DOACs nor VKAs. Therefore, a second objective was to get a better

understanding of contributing factors to preventable ADRs associated with the use of oral anticoagulants.

Methods

Setting and participants

We conducted a prospective observational cohort study on patients admitted between July 2015 and January 2016 in two teaching hospitals in Belgium (a 1000-bed hospital in an urban setting and a 450-bed hospital in a rural area). Patients aged ≥18 years, taking DOAC or VKA and presenting at the emergency department (ED) with a thromboembolic or a bleeding event were eligible for inclusion. Patients already discharged at the time of identification were excluded from the study. Thromboembolic events included ischemic stroke, transient ischemic attack (TIA), systemic embolism, deep vein thrombosis (DVT) and pulmonary embolism (PE). Patients admitted with anaemia and suspected digestive haemorrhage were also included if additional investigations were carried out. The study was approved by the Ethics Committees of the Cliniques Universitaires Saint-Luc (Brussels, Belgium) and the CHU UCL Namur (Yvoir, Belgium). Written informed consent was obtained from each patient. The study was registered with clinicaltrials.gov (NCT02720328).

Data collection

Patients were identified within 24h of their admission to the ED (72h for the weekend). Every morning, the list of patients admitted to the ED the day before was screened for thromboembolic or bleeding events. Charts were reviewed to identify patients treated with an oral anticoagulant. We also asked ED physicians to inform the investigators when one of their patients met inclusion criteria. A clinical pharmacist (ALS or ASL) then performed a comprehensive medication history with patients (and/or relatives). A standardized form was used to collect sociodemographic, medical and medication data, along with information about

anticoagulation, adverse events, drug management and adherence. Electronic medical records were also reviewed. When necessary, the general practitioner (GP), the pharmacist or relatives were contacted to gather supplementary information. In July 2016, patient's charts were examined and patients contacted by phone if needed to assess 3-month mortality.

Outcome measurements

The primary outcome was the proportion of serious adverse drug reactions (ADRs) related to the use of DOAC or VKA that were potentially preventable. According to the European Medicines Agency definitions, we considered ADR as "*a response to a medicinal product which is noxious and unintended"* and we defined a serious ADR as an event that *"results in death, is life-threatening, requires in-patient hospitalization or results in persistent or significant disability or incapacity*" [16]. ADRs resulting from medication errors were deemed preventable. Secondary outcomes included analysis of anticoagulant prescribing, stages of medication process at which medication errors occurred, contribution of ADRs to hospital admission, duration of hospitalization and 3-month mortality. Factors contributing to preventable ADRs were analysed in a qualitative approach.

Analysis of anticoagulant prescribing

The Medication Appropriateness Index (MAI) was used for an in-depth analysis of DOAC and VKA prescriptions at the time of admission. This tool was developed to assess prescribing in older patients according to 10 criteria (indication, choice, dosage, administration (modalities and practicability), drug-drug interaction, drug-disease interaction, duplication, duration, cost-effectiveness)[17]. Explicit instructions were given for each criterion, defining what is considered appropriate [A], moderately appropriate [B] or inappropriate [C]. We updated a form previously developed in a pilot study to assess DOAC prescribing[18] (Appendix A). A new form was designed to analyse VKA prescription, based on summary of product

characteristics, literature review and guidelines (Appendix B). The MAI criterion of cost-effectiveness was not included. Members of a multidisciplinary team working in the field of thrombosis and haemostasis reviewed instructions of both tools. Two pharmacists (ALS and AS) applied independently the new form on the first ten VKA-treated patients included in the study. They discussed disagreements and the tool was improved accordingly.

Characteristics of adverse events

Two pharmacists (ALS and ASL) and two clinical haematologists (BD and VM) evaluated independently causality, seriousness and preventability of adverse events, using pilot-tested tools. For each patient, a summary of the clinical case was sent alternately to three of the four reviewers. Adverse events without an absolute agreement were discussed once a month with all reviewers. For bleeding events, causality (i.e. likelihood that a medication is the cause of an observed adverse event) was assessed using the Naranjo ADR probability scale[19]. The relationship between an inadequate therapy and a thromboembolic event was evaluated using the Therapeutic Failure Questionnaire[20]. Possible, probable and certain adverse events were considered as ADRs. Serious ADRs were then classified into unavoidable, potentially preventable and preventable ADRs using Hallas criteria[21]. For (potentially) preventable ADRs, we identified stages of medication process at which medication errors occurred (i.e. prescribing, transcribing - that is copying medication orders before sending them to the pharmacy, dispensing, administration or monitoring). DOAC doses not adapted to renal function were considered as prescribing issues, unless clear evidence pointed out that the initial DOAC dose was appropriate and that renal function was not properly monitored. In the latter case, the stage of medication process involved in medication error was monitoring. The contribution of serious ADRs to hospital admission was assessed according to definitions proposed by Hallas and colleagues[21]. All the tools were first pilot-tested on 5 clinical cases by three experienced clinical pharmacists (ALS, OD

and AS). Disagreements and imprecisions were highlighted so that the different tools were improved. Scales and definitions are presented in Appendix C.

Statistical analysis

Due to the observational nature of the study, we did not calculate a minimum sample size. However, to have a good balance between the labour-intensive character of the qualitative part and the achievement of reliable quantitative results, we were willing to have at least 40 patients with a serious ADR in each group (DOAC and VKA)[22, 23]. We selected a 1:1 DOAC:VKA sample to explore to the same extent the factors contributing to DOAC- and VKA-related ADRs in the qualitative part of the study. Patient enrolment was therefore followed and temporary stopped in one group if there were more patients included in this group. Outcome measurements were compared in a descriptive way between DOAC- and VKA-treated patients.

Factors contributing to preventable ADRs

For cases of serious and (potentially) preventable ADRs, we performed semi-structured interviews with general practitioners (GP) of the patients to identify contributing factors to ADRs. This approach called “qualitative research” is frequently used in patient safety research to reach comprehensive understanding of an issue[24]. GPs were contacted by phone after patient discharge. They were informed about the voluntary nature of their participation and data confidentiality. Interviews were held at the practice site of GPs agreeing to participate. We used a guide with specific, open-ended questions that was pilot-tested with three GPs. The subjects covered were: difficulties encountered for prescribing and monitoring of oral anticoagulants, personal understanding of anticoagulant-associated adverse events (in general and for the patient included), and means of improvement of the use of oral anticoagulants. The interviews lasted between 30 and 45 minutes. Each participant provided informed consent.

Analysis of the interviews

Interviews were recorded and transcribed *verbatim*. Themes and categories were identified inductively by ALS and discussed with a second researcher (KA) until an agreement was reached. Categorization was refined according to the framework for analysing risk and safety in clinical medicine[25]. The adopted coding frame was then applied to all transcripts, using the software QSR NVivo 10. We organized two focus groups of 90 minutes to present results of the analysis to different health care professionals (i.e. cardiologist, GP, pharmacist, geriatrician, and haematologist) and patients. Participants were asked whether they agree or disagree with identified themes. A final report was drawn up accordingly, and checked by AS.

Results

Study population

Eighty-nine patients admitted to the emergency department were included in the analysis (46 DOAC- and 43 VKA-treated patients), as presented in figure 1. Median age was 79 years. Fifty-four percent were male, and 79% were living at home. The most frequent comorbidities were atrial fibrillation (79%), hypertension (73%) and coronary artery disease (33%). On admission, 45% of the patients had impaired renal function. Seventy-six percent used at least 5 daily drugs. Sociodemographic, clinical and medication data are presented separately for DOAC and VKA in table 1.

Figure 1: Flow chart of the inclusion process.
DOAC: direct oral anticoagulant, LMWH: low molecular weight heparin, VKA: vitamin K antagonist.

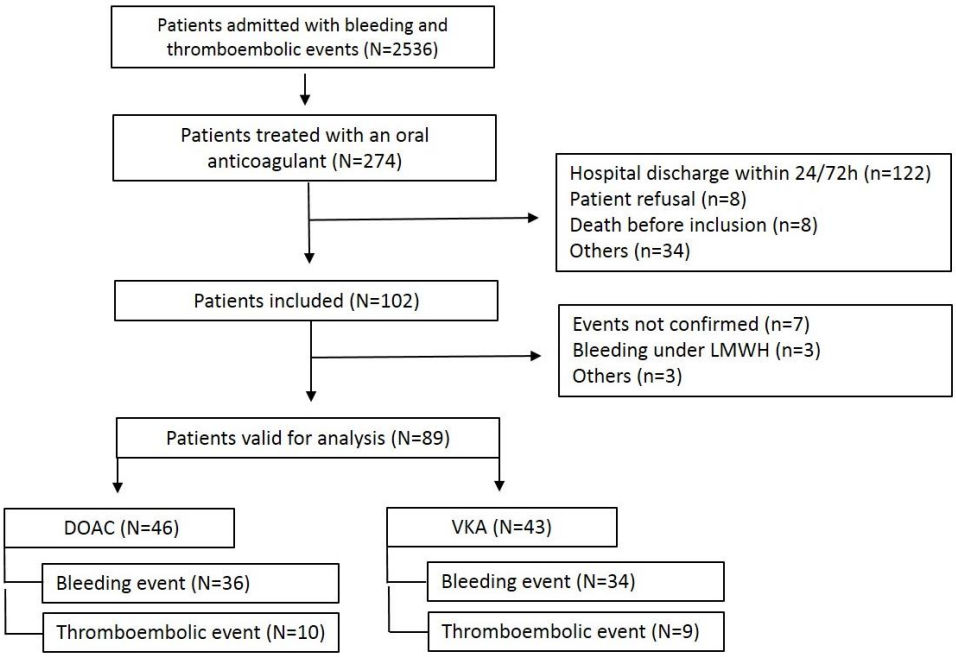


Table 1: Demographic, clinical and medication characteristics of DOAC- and VKA-treated patients admitted to the emergency department with a thromboembolic or bleeding event

	TOTAL (N=89)	DOAC (N=46)	VKA (N=43)
Age (years; median [IQ range])	79 [72-87]	80 [73-87]	78 [70-85]
Age ≥ 75 years, n [%]	58 [65]	32 [70]	26 [61]
Gender (male), n [%]	48 [54]	24 [52]	24 [56]
Weight (kg; median [IQ range])	72 [63-83]	70 [63-80]	77 [66-85]
BMI (kg/m^2; mean ± SD)	26 ± 5	25 ± 4	27 ± 6
Place of residence (n [%])			
Home	70 [79]	37 [80]	33 [77]
Nursing home	12 [13]	6 [13]	6 [14]

Table 1: Demographic, clinical and medication characteristics of DOAC- and VKA-treated patients admitted to the emergency department with a thromboembolic or bleeding event (continued)

	TOTAL (N=89)	DOAC (N=46)	VKA (N=43)
Creatinine clearance (CG) (n [%]) *			
≥ 30 to < 50 ml/min	32 [36]	20 [44]	12 [28]
≥ 15 to < 30 ml/min	8 [9]	3 [7]	5 [12]
Comorbidities, n [%]			
Atrial fibrillation	70 [79]	40 [87]	30 [70]
Hypertension	65 [73]	35 [76]	30 [70]
Coronary artery disease	29 [33]	13 [28]	16 [37]
Prior stroke or TIA	27 [30]	16 [35]	11 [26]
Venous thromboembolism	21 [24]	10 [22]	11 [26]
Diabetes	18 [20]	10 [22]	8 [19]
Heart failure	14 [16]	6 [13]	8 [19]
Peripheral artery disease	13 [15]	9 [20]	4 [9]
Myocardial infarction	10 [11]	4 [9]	6 [14]
Medications, n [%]			
Number of drugs (median [IQ range])	7 [5-9]	7 [5-8]	7 [5-9]
≥ 5 medications/day	68 [76]	36 [78]	32 [74]
NSAIDs	4 [4]	3 [7]	1 [2]
Antiplatelet therapy	27 [30]	9 [20]	18 [42]
SSRIs	13 [15]	6 [13]	7 [16]
Amiodarone	22 [25]	15 [33]	7 [16]
Diuretics	39 [44]	19 [41]	20 [47]
Proton Pump Inhibitors	35 [39]	17 [37]	18 [42]
Use of the oral anticoagulant, n [%]			
< 30 days	5 [6]	2 [5]	3 [7]
1 month – 1 year	21 [25]	15 [35]	6 [15]
> 1 year	58 [69]	26 [60]	32 [78]

BMI: body mass index, CG: Cockroft-Gault, IQ: interquartile, NSAID: nonsteroidal anti-inflammatory drugs, SD: standard deviation, SSRI: selective serotonin reuptake inhibitors, TIA: transient ischemic attack. * based on creatinine serum level on admission

Anticoagulation

Rivaroxaban was the most prescribed DOAC (n=29), followed by apixaban (n=9) and dabigatran etexilate (n=8). VKA-treated patients were taking acenocoumarol (n=40), phenprocoumone (n=2) and warfarin (n=1). The main indications of treatment were non-valvular atrial fibrillation (85% for DOAC, 56% for VKA) and treatment and secondary prevention of venous thromboembolism (15% for DOAC, 19% for VKA)*. Cardiologists initiated oral anticoagulants for 46% of the patients, while the general practitioner first prescribed in 12% of the cases. Most patients had been treated for more than 1 year (60% and 78% for DOAC and VKA respectively), as shown in table 1. Only 2 DOAC- and 3 VKA-treated patients were within their first month of treatment. Twenty patients under DOAC had been taking VKA previously (43%). Main reasons for switching from VKA to DOAC included unstable INR results (n=5), greater comfort (n=4) and bleeding events (n=2). One of the 20 patients was admitted to the ED within 30 days after drug transition.

Characteristics of adverse events

We observed 19 thromboembolic events (21%) and 70 bleeding events (79%). Characteristics of adverse events and management of bleeding are presented in table 2. Gastro-intestinal bleedings were the most frequent adverse events (n=34), followed by intracranial bleeding (n=16). Twelve patients presented with a haemoglobin level ≤ 8 g/dl. Concerning VKA-treated patients, the INR value (mean ± SD) on admission was 2.0 ± 0.7 for thromboembolic events (n=9) and 3.6 ± 2.2 for bleeding events (n=34). Thromboembolic events were recurrent in 12 of the 19 patients (63%), while 14 of the 70 patients with bleeding (20%) already had a previous episode of major bleeding. Among the 32 patients with gastro-intestinal bleeding events, 12 (7 DOAC, 5 VKA) were taking proton pump inhibitors concomitantly (38%).

* Adapted from the published version

Table 2: description of thromboembolic and bleeding events and management of bleeding according to the class of oral anticoagulant (DOAC and VKA)

	TOTAL (N=89)	DOAC (N=46)	VKA (N=43)
Thromboembolic events, n (%)			
Ischemic stroke	10 (11)	6 (13)	4 (9)
Transient Ischemic Attack	3 (3)	1 (2)	2 (5)
Systemic embolism	3 (3)	2 (4)	1 (2)
DVT/PE	3 (3)	1 (2)	2 (5)
Bleeding events, n (%) *			
Upper GI bleeding	21 (24)	11 (24)	10 (23)
Lower GI bleeding	13 (15)	10 (22)	3 (7)
Intracranial bleeding	16 (18)	9 (20)	7 (16)
Hematoma	12 (13)	4 (9)	8 (19)
Hematuria	10 (11)	4 (9)	6 (14)
Epistaxis	3 (3)	2 (4)	1 (2)
Management of bleeding, n (%)			
RBC transfusion	23 (26)	11 (24)	12 (28)
Fresh Frozen Plasma	1 (1)	0 (0)	1 (2)
Vitamin K	15 (17)	0 (0)	15 (35)
Prothrombin Complex Conc.	14 (16)	4 (9)	10 (23)

DVT: deep vein thrombosis, GI: gastro-intestinal, PE: pulmonary embolism, RBC: red blood cell. * As patients could have several sites of bleeding on admission, the percentages in square brackets may not add up to 100%.

Table 3: Prevalence and examples of inappropriate ratings for each criterion of the Medication Appropriateness Index (MAI), according to the anticoagulant therapy (DOAC or VKA)

	DOAC (N=46)	VKA (N=43)	Examples
Inappropriate ratings, n [%]			
Indication	0 [0]	0 [0]	
Choice	12 [26]	6 [14]	*DOAC*: body weight <50 kg or >110 kg, poor adherence, severe renal failure; *VKA:* history of labile INR
Dosage	8 [17]	22 [51]	*DOAC*: dosage too high/low, apixaban or dabigatran etexilate taken once daily; *VKA*: INR values out of range (difference > 0.5)
Administration (modalities)	13 [28]	3 [7]	*DOAC:* rivaroxaban ≥ 15mg without meals; *VKA:* variable time of intake
Administration (practicability)	2 [4]	1 [2]	*DOAC*: evening dose of dabigatran etexilate forgotten; *VKA*: INR measurements not performed
Drug-drug interactions	22 [48]	20 [47]	*DOAC:* + LMWH*, +* amiodarone and/or diltiazem and bleeding; *VKA: +* LMWH (INR in the therapeutic range)*,* + antibiotics and changes of INR, *ALL*: + antiplatelets, SSRIs, NSAIDs and bleeding
Drug-disease interactions	12 [26]	4 [9]	*DOAC*: moderate (dabigatran etexilate) and severe renal failure, *VKA*: esophageal varices, *ALL*: alcohol abuse
Duplication	1 [2]	1 [2]	*ALL*: + LMWH
Duration	2 [4]	4 [9]	*ALL*: no reevaluation of anticoagulation after pulmonary embolism

INR: International Normalized Ratio, LMWH: low molecular weight heparin, MAI: Medication Appropriateness Index, NSAID: non-steroidal anti-inflammatory drugs, SSRI: selective serotonin reuptake inhibitors

Analysis of anticoagulant prescribing

Analyses of DOAC and VKA prescriptions according to the Medication Appropriateness Index (MAI) are presented in table 3. Inappropriate ratings concerning drug-drug interactions were frequent, both for DOACs and VKAs[†]. Pharmacodynamics interactions were identified in 13 DOAC and 17 VKA patients admitted for bleeding, among which two were strictly contraindicated (concurrent use of DOAC and low molecular weight heparin (LMWH), or VKA (therapeutic INR) and LMWH). Twelve DOAC patients presented potential pharmacokinetics interactions with amiodarone and/or diltiazem. For 3 VKA patients, the recent introduction of an antibiotic increased the INR value. Two VKA patients experienced INR modification following the administration of either amiodarone or anion-exchange resin.

Assessment of adverse events

For the 46 patients taking DOAC, 38 adverse events were evaluated as serious ADRs. Among these, 20 ADRs (53%) were considered to be (potentially) preventable. Prescribing was the main stage of medication process involved in medication error (n=16), followed by compliance (n=5). Concerning the 43 VKA-treated patients, 41 adverse events were evaluated as serious ADRs. Twenty-five of these ADRs (61%) were (potentially) preventable. For VKAs, monitoring (n=16) and prescribing (n=14) were the main stages of medication process involved in medication errors. Details on the assessment of adverse events (causality, seriousness and preventability) are presented in table 4, while table 5 shows some examples of serious and (potentially) preventable ADRs. The contribution of ADRs

[†] According to MAI instructions, an inappropriate rating was assigned in case of drug-drug interaction with a clinical sign of toxicity. The intake of the concomitant drug, although a significant risk factor, did not automatically lead to a preventable assessment (e.g. aspirin after an acute coronary syndrome, amiodarone). The preventability of ADRs was evaluated in a further step by 4 independent reviewers.

to admission was dominant for 84% and 78% of patients taking DOAC and VKA respectively. Mean duration of hospitalization was 7 ± 9 days for DOAC and 12 ± 19 days for VKA patients. At 90 days, mortality was 7% (3/45) for DOAC and 14% (6/43) for VKA patients.

Table 4: Assessment of causality, seriousness and preventability of anticoagulant-related adverse events by four independent reviewers using pilot-tested scales

	TOTAL	DOAC	VKA
Causality of adverse events, n [%][a]	**N = 89**	**N = 46**	**N = 43**
Improbable	7 [8]	5 [11]	2 [5]
Possible ADR	51 [57]	28 [61]	23 [53]
Probable ADR	31 [35]	13 [28]	18 [42]
Serious ADR, n [%][b]	**N = 79**	**N = 38**	**N = 41**
Not preventable	34 [43]	18 [47]	16 [39]
Potentially preventable	24 [30]	8 [21]	16 [39]
Preventable[c]	21 [27]	12 [32]	9 [22]
Reasons for a (potentially) preventable assessment, n [%] *	**N = 45**	**N = 20**	**N = 25**
PD drug-drug interaction	13 [29]	4 [20]	9 [36]
Choice of drug	10 [22]	7 [35]	3 [12]
Compliance issue	10 [22]	5 [25]	5 [20]
Inappropriate monitoring	10 [22]	0 [0]	10 [40]
No indication	7 [16]	3 [15]	4 [16]
Inappropriate dose	4 [9]	4 [20]	0 [0]
Lack of communication	3 [7]	0 [0]	3 [12]
Wrong perioperative management	2 [4]	0 [0]	2 [8]
Omission of a preventive drug	2 [4]	2 [10]	0 [0]
PK drug-drug interaction	1 [2]	0 [0]	1 [4]

ADR: adverse drug reaction, PD: pharmacodynamics, PK: pharmacokinetics. * As several reasons for a (potentially) preventable assessment could be noticed, the percentages in square brackets may not add up to 100%. [a] According to the Naranjo scale or the Therapeutic Failure Questionnaire; [b] According to the European Medicines Agency definition of seriousness; [c] According to the Hallas criteria.

Table 5: Examples of two DOAC- and two VKA-treated patients admitted to the emergency department for a serious and preventable ADR

Clinical case	Assessment	Stage	Reasons of preventable assessment
A 93-year-old man living in nursing home, under **rivaroxaban** for atrial fibrillation, who was hospitalized for hip replacement surgery. At discharge, he received both LMWH and rivaroxaban. He was admitted for lower GI bleeding.	Preventable	Prescribing	The concomitant use of DOAC and LMWH is contraindicated given the increased bleeding risk.
A 61-year-old man, under **dabigatran etexilate** for atrial fibrillation. He was admitted for renal infarction. Dabigatran etexilate had not been taken for 10 days because of lack of prescription.	Preventable	Compliance	The patient did not refill DOAC prescription.
A 61-year-old man, under **acenocoumarol** for mesenteric venous thrombosis. He was admitted for upper GI bleeding, with an INR>8. Ibuprofen had been added 10 days before for knee pain. INR measurements were not performed regularly.	Preventable	Prescribing, monitoring	NSAIDs are not recommended in patients taking oral anticoagulants. Acenocoumarol was not reassessed 18 months after the acute event. INR monitoring was inadequate.
An 80-year-old man living in nursing home, under **acenocoumarol** for atrial fibrillation. He was admitted for anemia (Hb 6.7 g/dl). He had been suffering from melena for 4 months.	Potentially preventable	Monitoring	Melena had been present for several months but was not highlighted before ED admission.

ED: emergency department, GI: gastro-intestinal, Hb: hemoglobin, INR: International Normalized Ratio, LMWH: low molecular weight heparin, NSAID: nonsteroidal anti-inflammatory drugs

Factors contributing to preventable ADRs

Twenty-one general practitioners (GPs) were interviewed between September 2015 and June 2016 (9 female, 12 male)[‡]. Nine participants had more than 30 years of experience in general medicine, while 5 had been practicing for less than 10 years. Factors contributing to preventable ADRs were classified into five main categories: the patient, the health care professional (HCP), the task, communication and the work environment. Categories and subcategories are described below, and illustrative verbatim quotes are presented in Table 6.

The patient

Patient non-adherence was frequently reported for both DOACs and VKAs, as a result of carelessness, forgetfulness or lack of understanding. Some patients did not take their treatment deliberately, because they were scared of or experienced side effects. GPs mentioned the poor drug management of community-dwelling older patients. For VKAs, adherence issues also applied to therapeutic monitoring. One participant related the case of a DOAC patient for whom non-adherence remained undetected and led to adverse event.

Patient characteristics were perceived to contribute to ADRs, such as falls, cognitive disorders or acute infections. The main concern for DOAC-patients was about renal failure, especially in the frail elderly. Patients often did not think of reporting an adverse event or the intake of an over-the-counter medication to their GP.

The health care professional

Some GPs felt uncomfortable with DOAC therapy, because of a lack of knowledge. GPs appeared not to question cardiologist's recommendations with regard to anticoagulation, considering that specialists do not make mistakes. The same was

[‡] Adapted from the published version

true for the reassessment of aspirin prescription. The lack of awareness of anticoagulation risks among nursing home staff was also highlighted.

Opinions differed about GP's knowledge of their DOAC-treated patients. Two GPs estimated that they provide them poorer quality care compared to VKA-treated patients, because of a lack of familiarity. Medical history of patients who have changed family physician seemed to be frequently incomplete, preventing treatment review.

The task

Two main tasks were addressed during the interviews: prescribing and patient monitoring. GPs seemed to prescribe only one drug among each class of oral anticoagulants, with which they are more familiar. Initiation of VKA therapy remained highly intuitive, possibly leading to overdose. Some participants acknowledged that they did not think about reassessing long-term prescribed VKA.

Drug-drug interactions were perceived as a risk factor for ADRs, both for DOAC and VKA. The addition of new medications by several HCP without control of interactions was highlighted. Some GPs prescribed NSAIDs deliberately, in the absence of alternative. GPs considered the community pharmacist as a key player in avoiding the dispensation of medications interacting with oral anticoagulants.

In terms of monitoring, management of out-of-range INR values was found to be complicated in primary care. VKA dose adjustments seemed to be based on physician experience. GPs considered that there was no specific follow-up for DOAC patients, with the exception of renal function monitoring. However, DOAC dose was not always adapted in case of moderate renal impairment. The higher risk of unnoticed adverse events was highlighted for DOACs, because of the lack of regular visits. Perioperative management of DOACs and VKAs was seen as a high-risk period for medication errors, as illustrated by several GPs.

Communication

Communication issues with secondary care were discussed by half of the GPs, such as the lack of accessibility of hospital physicians. Poor information transfer at hospital discharge was frequently reported, including incorrect hospitalization reports or the lack of explanation concerning VKA treatment modalities (e.g. history of INR values, resumption). GPs regretted that anticoagulant therapy was often modified during hospitalization without being informed, resulting in complicated consultations when the patient returns home.

The communication of INR values in primary care was also at high-risk. The example of an INR value misunderstood over the phone, leading to patient ADR, was among others reported. Patients seemed often to request prescriptions by telephone, preventing regular follow-up in DOAC patients.

Work environment

Workloads of GPs and specialists were both mentioned as causes of ADRs, preventing close monitoring, treatment review or adequate information sharing. The long time frame to get results of INR measurements in primary care was also highlighted. Consultation cost seemed to play a role in monitoring non-adherence, even if the patient is later partly reimbursed. Concerning DOACs, the absence of dedicated consultations was perceived as a factor of low GP involvement in anticoagulant treatment.

The galenic formulation of acenocoumarol (i.e. small unscored tablets) was considered inappropriate to low-dose intake in elderly patients. Some GPs regretted that DOACs packages were for 3 months of treatment, preventing regular contact with the patient. Several GPs felt that pharmaceutical industry conveyed a comforting image of DOACS. The banalization of anticoagulation may have led to decreased vigilance of patients and HCP.

Table 6: Illustrative verbatim quotes from semi-structured interviews performed with general practitioners to identify contributing factors to oral anticoagulant- related adverse drug reactions

Category	Illustrative verbatim quote
Patient	Intentional non-adherence
	"I sometimes have people on oral anticoagulants who experience hematuria. So you have people on two doses a day who only want to take one. With all the consequences... It seems that for 12 hours they are anticoagulated, and for 12 hours they are not." [GP4]
	Unintentional non-adherence
	"To come back to that patient who had been on Sintrom [acenocoumarol] and then moved on to DOAC, I didn't think of checking regularly with him whether he understood the usefulness of his drugs and whether he followed his treatment correctly. It took an acute episode to make me realize that the patient was no longer able to manage his medications." [GP7]
	Complex condition
	"With elderly people who don't drink very much, take diuretics, take an ACE inhibitor, and then have a fever and don't drink for three days, we are called after three days. When we monitor renal function, we get some serious surprises." [GP1]
Health care professional	Drug knowledge
	"I have some fears, because I don't have enough knowledge. There is a huge amount you need to learn about these anticoagulants....So with those, I manage less, I manage less. Because I don't know enough about those products." [GP17]
	"And I had a serious problem two weeks ago. I was on duty on a Friday night. I was called at 12:30-1:00 am by a nursing home because an elderly person had fallen. So I asked the caregiver, 'Are there any anticoagulants?' That's the first thing I ask. 'Ah, I don't think so, I don't know.'" [GP20]
	Knowledge of the patient
	"There was a patient who came to my office one day because he came to live near here and he said, 'Well, I have to take Marcoumar [phenprocoumon] because I have had two pulmonary embolisms. I was told that was a lifelong treatment.' But I don't have much information about him." [GP3]

Table 6: Illustrative verbatim quotes from semi-structured interviews performed with general practitioners to identify contributing factors to oral anticoagulant- related adverse drug reactions (continued)

Category	Illustrative verbatim quote
Task	Drug selection
	"My brain has absorbed one [new molecule] and I use that one because I know it and know how to use it. I'm not going to start mixing the others." [GP11]
	Drug-drug interactions
	"But [interactions] happen. [The patient] comes back from a specialized consultation where she received an antibiotic, and Sintrom [acenocoumarol] was not considered." [GP18]
	"But there are some anti-inflammatory drugs that are, well, clearly not recommended, and yet I'm very unsure what to do. So sometimes I pretend to forget...an ibuprofen simply disappears." [GP21]
	Patient monitoring
	"It's very instinctive [dose adjustment]. It's rather unscientific and very instinctive. But I think it's kind of instinctive for everyone. Do we decrease by 1? Do we increase by 1? Do we halve? Do we make 3/4?" [GP11]
	"There is no follow-up [with DOACs]. We check for kidney function and things like that, but there is no particular follow-up. Well, there is less follow-up, anyway." [GP15]
	Perioperative management
	"[The patient] had to have a knee prosthesis. So, he was switched to fraxiparin, and when he was discharged from hospital, they let him out with a prophylactic dose of fraxiparin, not a therapeutic dose. He had a minor stroke, fortunately minimal, but he did have a minor stroke." [GP3]
Communi-cation	Primary-secondary care communication
	"When I have to contact a cardiologist in a hospital setting, sometimes I spend three quarters of an hour with the phone and music; sometimes I have one or two consultations while trying to get through on the phone, so it's very unpleasant for me." [GP17]
	"A patient whose treatment is changed without warning the general practitioner, I don't find that...especially because we have a particular treatment in mind and it is actually no longer the same." [GP15]

Table 6: Illustrative verbatim quotes from semi-structured interviews performed with general practitioners to identify contributing factors to oral anticoagulant- related adverse drug reactions (continued)

Category	Illustrative verbatim quote
Communi-cation	Primary-secondary care communication "Because we also see, for example, people who are discharged from hospital with a discharge letter [for the general practitioner] about medication, dosage, and all that. And they are also given a document with patient instructions about the treatment. And in that document, it's not the same." [GP9]
	GP-patient communication "And then I see someone come in who doesn't even suspect that she had a hemoglobin level of 7, and who came because she needed Xarelto [rivaroxaban], which I had refused to prescribe on the phone." [GP18]
Work environment	Workload "As I told you, it takes discipline, that's all. But for that, you need time. Because people with lots of patients don't have the time to follow the [INR] rates of all their patients and that could be a problem." [GP3]
	Consultation costs "I had a patient who lost his BIM status [increased reimbursement for health care]. He used to come every month for INR monitoring, as regular as clockwork. He lost his status and I've only seen him twice this year. Not even twice: I saw him once…Why? Because now he has to pay for his consultation. He will be reimbursed afterwards, OK. But..." [GP11]
	Lack of specific consultation "When they come for something else, and then in the list of prescriptions there is this drug [the anticoagulant] in addition, I'm not involved in the same way as when they come specifically for their anticoagulation." [GP1]
	Banalization of anticoagulation "With DOACs, the idea was sold to us that they were reliable and there wouldn't be any problem with them, to the extent that if you are not kind of cautious and vigilant as a doctor, there's a danger you won't carry out those blood tests every three months." [GP4]

Discussion

In this study, more than half of both DOAC- and VKA-associated serious ADRs were deemed potentially preventable. Prescribing issues were frequent for DOACs, while monitoring was the main stage of medication process involved for VKAs. We identified other contributing factors to ADRs that were common to both classes of oral anticoagulants, such as pharmacodynamics drug interactions or communication.

Patient characteristics were similar to previous studies describing DOAC-associated bleeding events[26]. Older age and renal impairment are two risk factors for experiencing major bleeding events, as shown in a registry of rivaroxaban patients[27]. Polypharmacy, which was present in 76% of our patients, was previously reported to be a determinant of higher bleeding risk and preventable medication-related hospital admissions[28, 29]. Therefore, interventions to improve anticoagulation management should primarily focus on these "high-risk" patients. Hypertension is an important reversible bleeding risk factor in patients taking oral anticoagulants[30]. Although it was a frequent comorbidity in our population, only one DOAC patient with intracranial bleeding had severe uncontrolled hypertension.

Patients were mainly taking rivaroxaban and acenocoumarol, in accordance with the anticoagulant prescribing pattern in Belgium. Nearly half of DOAC patients previously received VKA treatment, with similar proportions observed in pivotal trials and prospective registries[31, 32]. Patients seemed to be switched to the same extent because of labile INR or for convenience. In guidelines on atrial fibrillation, INR stability and patient preference were both emphasized when considering DOAC in patients already receiving VKA[30, 33].

Our finding that gastrointestinal (GI) bleedings were the most frequent DOAC-related ADRs is consistent with phase 3 and real-world trials[34, 35]. The higher rate of lower GI bleeding in DOAC compared to VKA patients was described in a

multicentric observational study[36]. The presence of active anticoagulant substances in the gut of DOAC patients has emerged as a possible explanation[37]. Guidelines and expert opinions suggest gastro-protective agents in anticoagulated patients with a history of peptic ulcer or GI bleeding, as well as in patients taking antiplatelet drugs[38, 39]. In our patients admitted with GI bleeding, 40% of those who were not receiving gastro-protective agents had a history of peptic ulcer or were taking antiplatelet therapy.

To our knowledge, this is the first study to assess the preventability of DOAC-related ADRs. We used a mixed-method design, combining a prospective cohort study and semi-structured interviews to get a better understanding of this patient safety issue. The analysis took into consideration initial or transition phases of anticoagulant therapy, as they were shown to entail a higher risk for bleeding or thromboembolic events[31, 40]. Previous studies reported high preventability rates for adverse events associated to the use of warfarin in outpatients (49%), or any anticoagulant in hospitalized patients (70%)[41, 42]. Given the fixed-dose regimen of DOACs, a lower rate of preventable ADRs could have been hypothesized. However, our results showed comparable preventability between DOACs and VKAs, with rates in the range of previous estimates. This observation may be explained by the fact that many causes of preventable ADRs apply to all oral anticoagulants, as revealed through interviews.

Inappropriate prescribing of DOACs was well described in previous observational studies[15, 18]. Several reports highlighted the occurrence of bleeding events in DOAC patients for whom both drug and dose selections were judged inadequate[43, 44]. In older patients for instance, DOACs should be avoided in case of severe renal impairment while a reduced dose is sometimes recommended for creatinine clearances below 50 ml/min[45]. Our results showed that choosing the most appropriate oral anticoagulant, tailored to patient characteristics, has become a new challenge for clinicians. Management of drug-drug interactions remains also

difficult in DOAC patients. Combination therapy with aspirin represents a serious matter of concern, as it often has no indication and increases bleeding risk by 50%[46, 47]. Moreover, the frequent concomitant use of P-gp inhibitors (e.g. amiodarone, simvastatin) was associated with increased DOAC levels and bleeding risk[35, 48]. To improve DOAC prescribing, interventions combining education and informatics tools should be promoted[49]. The role of community pharmacist in detecting anticoagulant-related problems has also been demonstrated[50].

Patient-related factors were often quoted during the interviews, both for older and younger patients, emphasizing the need to reinforce the education of anticoagulated patients. In a European survey, less than a quarter of DOAC patients were aware of kidney function monitoring or that some medications had to be avoided[51]. Providing patients with repeated information and suitable education material should reduce misunderstanding or underreporting of adverse events. Another area for improvement relates to DOAC management in nursing homes. Adverse events associated to the use of warfarin were previously demonstrated to be common in this setting, and often preventable[52]. We observed a limited awareness of nursing home staff about DOACs, which should be addressed.

Our findings suggested that clinical follow-up of DOAC patients was neglected compared to VKA patients, due to the lack of regular therapeutic monitoring. Consultations entirely dedicated to DOAC management should be planned at least every 3 months, including assessment of compliance, side effects and drug interactions[38, 53]. This specific follow-up can be performed by general practitioners (GP), provided that they receive appropriate training[54]. Patients may also benefit from follow-up care in dedicated anticoagulation clinics. However, this should be carried out in close collaboration with the GP.

In this study, communication was an important contributing factor to ADRs regardless of the class of oral anticoagulants. We observed poor communication

between healthcare providers, or between patients and healthcare providers. A previous root-cause analysis showed that communication was the second cause of adverse events in patients taking warfarin[55]. Especially, transitions of care are critical periods for the occurrence of medication errors. The low availability of discharge reports and lack of direct communication were previously characterized[56]. This issue takes on greater significance with high-risk medications like oral anticoagulants.

Clinical outcomes were consistent with previous findings. Two multicentric cohort studies reported a lower 30-day mortality with DOACs compared to VKAs, after major bleeding and in the absence of specific DOAC reversal agents[36, 57]. However, in the present study, thromboembolic events were also considered and sample size was small. Idarucizumab was used for one patient taking dabigatran, experiencing major bleeding. We were not allowed to evaluate the cases of 8 patients who died before inclusion in the study. Mortality data have therefore been underestimated.

Our study presents several limitations. First, patients already discharged home at the time of identification were not included. However, these ADRs were not expected to be serious. Second, our study did not consider ADRs associated to the underuse of oral anticoagulants (i.e. thromboembolic events occurring while anticoagulation was indicated but not prescribed). Finally, only clinical pharmacists and haematologists participated in the assessment of adverse events. Conversely, only GPs opinions were explored during the interviews. We did not calculate the inter-rater reliability of scales, but all tools were pilot-tested and improved accordingly.

In conclusion, ADRs related to the use of oral anticoagulants are still largely preventable despite the use of DOACs. Interventions focusing on prescribing, patient education and continuity of care should be designed to help improve DOAC management in clinical practice.

Acknowledgements

AL Sennesael is a Research Fellow of the Fonds National de la Recherche Scientifique (FNRS). The authors would like to thank the emergency departments of the Cliniques Universitaires Saint-Luc and CHU UCL Namur; the 3 general practitioners who contributed to the pilot testing of the interview guide; the 21 general practitioners who shared their experience during interviews; Kaoutar Abdellaoui for her contribution to the qualitative analysis; the healthcare professionals and patients who participated in focus groups; Christian Chatelain, Jean-Benoît le Polain de Waroux, Sébastien Marchandise, Patrice Laloux and Isabelle Aujoulat for their advice; and Martin McGarry for help with translation of quotes from French.

Conflict of Interest

Franck Verschuren has received fees from Boehringer Ingelheim.

Author Contributions

ALS, AS, JMD, OD designed the research study. ALS wrote the first draft of the manuscript and the final version. All the co-authors were responsible for the review of the manuscript. ALS, ASL, FV and XM contributed to data collection. ALS, ASL, BD and VM evaluated clinical cases.

REFERENCES

1. Freedman B, Potpara TS, Lip GY. Stroke prevention in atrial fibrillation. Lancet 2016; 388: 806-17.
2. Howard RL, Avery AJ, Slavenburg S, et al. Which drugs cause preventable admissions to hospital? A systematic review. Br J Clin Pharmacol 2007; 63: 136-47.
3. Aljadhey H, Mahmoud MA, Mayet A, et al. Incidence of adverse drug events in an academic hospital: a prospective cohort study. Int J Qual Health Care 2013; 25: 648-55.
4. Budnitz DS, Lovegrove MC, Shehab N, Richards CL. Emergency hospitalizations for adverse drug events in older Americans. N Engl J Med 2011; 365: 2002-12.
5. Gurwitz JH, Field TS, Harrold LR, et al. Incidence and preventability of adverse drug events among older persons in the ambulatory setting. Jama 2003; 289: 1107-16.
6. Desai Y, El-Chami MF, Leon AR, Merchant FM. Management of Atrial Fibrillation in Elderly Adults. J Am Geriatr Soc 2017; 65: 185-93.
7. Baglin T. Clinical use of new oral anticoagulant drugs: dabigatran and rivaroxaban. Br J Haematol 2013; 163: 160-7.
8. Weitz JI, Eikelboom J. Incorporating edoxaban into the choice of anticoagulants for atrial fibrillation. Thromb Haemost 2016; 115: 257-70.
9. Breuer L, Ringwald J, Schwab S, Kohrmann M. Ischemic stroke in an obese patient receiving dabigatran. N Engl J Med 2013; 368: 2440-2.
10. Legrand M, Mateo J, Aribaud A, et al. The use of dabigatran in elderly patients. Arch Intern Med 2011; 171: 1285-6.
11. Caliskan F, Akdemir HU, Nurata H, Akdemir N, Basara G, Yavuz Y. Rivaroxaban-induced severe diffuse intracerebral hemorrhage. Am J Emerg Med 2015; 33: 475.e1-5.
12. Jun JH, Hwang JC. Association of rivaroxaban anticoagulation and spontaneous vitreous hemorrhage. JAMA Ophthalmol 2015; 133: 1184-6.
13. Shehab N, Lovegrove MC, Geller AI, Rose KO, Weidle NJ, Budnitz DS. US emergency department visits for outpatient adverse drug events, 2013-2014. JAMA 2016; 316: 2115-25.
14. Harper P, Young L, Merriman E. Bleeding risk with dabigatran in the frail elderly. N Engl J Med 2012; 366: 864-6.
15. Whitworth MM, Haase KK, Fike DS, Bharadwaj RM, Young RB, MacLaughlin EJ. Utilization and prescribing patterns of direct oral anticoagulants. Int J Gen Med 2017; 10: 87-94.
16. Guideline on good pharmacovigilance practices (GVP). Annex I - Definitions. European Medicines Agency; [cited 2016 2 December]. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/05/WC500143294.pdf.
17. Hanlon JT, Schmader KE, Samsa GP, et al. A method for assessing drug therapy appropriateness. J Clin Epidemiol 1992; 45: 1045-51.
18. Larock AS, Mullier F, Sennesael AL, et al. Appropriateness of prescribing dabigatran etexilate and rivaroxaban in patients with nonvalvular atrial fibrillation: a prospective study. Ann Pharmacother 2014; 48: 1258-68.
19. Naranjo CA, Busto U, Sellers EM, et al. A method for estimating the probability of adverse drug reactions. Clin Pharmacol Ther 1981; 30: 239-45.

20. Kaiser RM, Schmader KE, Pieper CF, Lindblad CI, Ruby CM, Hanlon JT. Therapeutic failure-related hospitalisations in the frail elderly. Drugs Aging 2006; 23: 579-86.
21. Hallas J, Harvald B, Gram LF, et al. Drug related hospital admissions: the role of definitions and intensity of data collection, and the possibility of prevention. J Intern Med 1990; 228: 83-90.
22. Rojas-Velandia C, Ruiz-Garzon J, Moscoso-Alcina JC, et al. Characterization of adverse drug reactions causing admission to an intensive care unit. Br J Clin Pharmacol 2017; 83: 1134-40.
23. Franceschi M, Scarcelli C, Niro V, et al. Prevalence, clinical features and avoidability of adverse drug reactions as cause of admission to a geriatric unit: a prospective study of 1756 patients. Drug Saf 2008; 31: 545-56.
24. Dean B, Schachter M, Vincent C, Barber N. Causes of prescribing errors in hospital inpatients: a prospective study. Lancet 2002; 359: 1373-8.
25. Vincent C, Taylor-Adams S, Stanhope N. Framework for analysing risk and safety in clinical medicine. Bmj 1998; 316: 1154-7.
26. Bouget J, Oger E. Emergency admissions for major haemorrhage associated with direct oral anticoagulants. Thrombosis Research 2015; 136: 1190-4.
27. 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: 955-62.
28. Piccini JP, Hellkamp AS, Washam JB, et al. Polypharmacy and the Efficacy and Safety of Rivaroxaban Versus Warfarin in the Prevention of Stroke in Patients With Nonvalvular Atrial Fibrillation. Circulation 2016; 133: 352-60.
29. Leendertse AJ, Egberts AC, Stoker LJ, van den Bemt PM. Frequency of and risk factors for preventable medication-related hospital admissions in the Netherlands. Arch Intern Med 2008; 168: 1890-6.
30. Kirchhof P, Benussi S, Kotecha D, et al. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. Europace 2016; 18: 1609-78.
31. Mahaffey KW, Wojdyla D, Hankey GJ, et al. Clinical outcomes with rivaroxaban in patients transitioned from vitamin K antagonist therapy: a subgroup analysis of a randomized trial. Ann Intern Med 2013; 158: 861-8.
32. 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. Eur Heart J 2016; 37: 1145-53.
33. January CT, Wann LS, Alpert JS, et al. 2014 AHA/ACC/HRS guideline for the management of patients with atrial fibrillation: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the Heart Rhythm Society. J Am Coll Cardiol 2014; 64: e1-76.
34. Piccini JP, Garg J, Patel MR, et al. Management of major bleeding events in patients treated with rivaroxaban vs. warfarin: results from the ROCKET AF trial. Eur Heart J 2014; 35: 1873-80.
35. Steffel J, Giugliano RP, Braunwald E, et al. Edoxaban vs. warfarin in patients with atrial fibrillation on amiodarone: a subgroup analysis of the ENGAGE AF-TIMI 48 trial. Eur Heart J 2015; 36: 2239-45.
36. Xu Y, Schulman S, Dowlatshahi D, et al. Direct Oral Anticoagulant- or Warfarin-Related Major Bleeding: Characteristics, Reversal Strategies, and Outcomes From a Multicenter Observational Study. Chest 2017; 152: 81-91.

37. Sherwood MW, Nessel CC, Hellkamp AS, et al. Gastrointestinal Bleeding in Patients With Atrial Fibrillation Treated With Rivaroxaban or Warfarin: ROCKET AF Trial. J Am Coll Cardiol 2015; 66: 2271-81.
38. 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 2015; 17: 1467-507.
39. Bhatt DL, Scheiman J, Abraham NS, et al. ACCF/ACG/AHA 2008 expert consensus document on reducing the gastrointestinal risks of antiplatelet therapy and NSAID use: a report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents. Circulation 2008; 118: 1894-909.
40. Hylek EM, Evans-Molina C, Shea C, Henault LE, Regan S. Major hemorrhage and tolerability of warfarin in the first year of therapy among elderly patients with atrial fibrillation. Circulation 2007; 115: 2689-96.
41. Perrone V, Conti V, Venegoni M, et al. Seriousness, preventability, and burden impact of reported adverse drug reactions in Lombardy emergency departments: a retrospective 2-year characterization. Clinicoecon Outcomes Res 2014; 6: 505-14.
42. Piazza G, Nguyen TN, Cios D, et al. Anticoagulation-associated adverse drug events. Am J Med 2011; 124: 1136-42.
43. Sennesael AL, Dogne JM, Spinewine A. Optimizing the Safe Use of Direct Oral Anticoagulants in Older Patients: A Teachable Moment. JAMA Intern Med 2015; 175: 1608-9.
44. Escobar C, Barrios V. Dabigatran and bleeding risk: the importance of a correct prescription. J Emerg Med 2014; 46: 831-2.
45. American Geriatrics Society 2015 Updated Beers Criteria for Potentially Inappropriate Medication Use in Older Adults. J Am Geriatr Soc 2015; 63: 2227-46.
46. Shah R, Hellkamp A, Lokhnygina Y, et al. Use of concomitant aspirin in patients with atrial fibrillation: Findings from the ROCKET AF trial. Am Heart J 2016; 179: 77-86.
47. Xu H, Ruff CT, Giugliano RP, et al. Concomitant Use of Single Antiplatelet Therapy With Edoxaban or Warfarin in Patients With Atrial Fibrillation: Analysis From the ENGAGE AF-TIMI48 Trial. J Am Heart Assoc 2016; 5.
48. Antoniou T, Macdonald EM, Yao Z, et al. Association between statin use and ischemic stroke or major hemorrhage in patients taking dabigatran for atrial fibrillation. Cmaj 2017; 189: E4-e10.
49. Dreischulte T, Donnan P, Grant A, Hapca A, McCowan C, Guthrie B. Safer Prescribing--A Trial of Education, Informatics, and Financial Incentives. N Engl J Med 2016; 374: 1053-64.
50. Desmaele S, De Wulf I, Dupont AG, Steurbaut S. Pharmacists' role in handling problems with prescriptions for antithrombotic medication in Belgian community pharmacies. Int J Clin Pharm 2015; 37: 656-68.
51. Amara W, Larsen TB, Sciaraffia E, et al. Patients' attitude and knowledge about oral anticoagulation therapy: results of a self-assessment survey in patients with atrial fibrillation conducted by the European Heart Rhythm Association. Europace 2016; 18: 151-5.
52. Gurwitz JH, Field TS, Radford MJ, et al. The safety of warfarin therapy in the nursing home setting. Am J Med 2007; 120: 539-44.
53. Gladstone DJ, Geerts WH, Douketis J, Ivers N, Healey JS, Leblanc K. How to Monitor Patients Receiving Direct Oral Anticoagulants for Stroke Prevention in Atrial Fibrillation: A Practice Tool Endorsed by Thrombosis Canada, the Canadian Stroke Consortium, the Canadian Cardiovascular

Pharmacists Network, and the Canadian Cardiovascular Society. Ann Intern Med 2015; 163: 382-5.

54. Heidbuchel H, Berti D, Campos M, et al. Implementation of non-vitamin K antagonist oral anticoagulants in daily practice: the need for comprehensive education for professionals and patients. Thromb J 2015; 13: 22.

55. Graves CM, Haymart B, Kline-Rogers E, et al. Root Cause Analysis of Adverse Events in an Outpatient Anticoagulation Management Consortium. Jt Comm J Qual Patient Saf 2017; 43: 299-307.

56. Kripalani S, LeFevre F, Phillips CO, Williams MV, Basaviah P, Baker DW. Deficits in communication and information transfer between hospital-based and primary care physicians: implications for patient safety and continuity of care. Jama 2007; 297: 831-41.

57. Becattini C, Franco L, Beyer-Westendorf J, et al. Major bleeding with vitamin K antagonists or direct oral anticoagulants in real-life. Int J Cardiol 2017; 227: 261-6.

Appendix 1: Medication Appropriateness Index: Summary of criteria and instructions

1. DOACs

Criterion		Instructions (summary)
Indication	A	Valid indication (e.g. NVAF in patients with CHA_2DS_2-VASC score ≥ 2, VTE)
	B	Acceptable indication (e.g. NVAF in male patients with CHA_2DS_2-VASC score = 1)
	C	Off-label use (e.g. mechanical heart valve, moderate or severe mitral stenosis)
Choice	A	DOAC is the preferred choice: patient preference, labile INR with VKA, recurrent stroke/VTE on VKA, resistance to VKA, poor venous access or monitoring difficult to manage (and patient compliance)
	B	Another anticoagulant could have been a first choice: VKA not tested or switch from VKA to DOAC without compelling reasons, moderate renal impairment (DE), swallowing disorders (DE)
	C	DOAC was not the preferred choice: severe renal impairment, poor compliance, extreme body weight (< 50 kg or > 110 kg), severe hepatic impairment, active cancer, no reimbursement
Dosage	A	Daily dose is consistent with SmPC recommendations.
	B	Daily dose is consistent with SmPC recommendations, but a specific assay showed under/above on-therapy plasma concentrations.
	C	Daily dose is inconsistent with SmPC recommendations.
Modalities of administration (correct)	A	Modalities of intake are correct (e.g. rivaroxaban 15mg or 20 mg taken with meal, every day at the same hour)
	B	Modalities of intake are not optimal: DE taken without meal in patients suffering from dyspepsia
	C	Modalities of intake are not correct (e.g. variable time of intake, opening of DE capsules)
Modalities of administration (practical)	A	Patient has no difficulty taking the drug.
	C	Patient presents some difficulties (e.g. twice daily intake in patients who forget evening doses)

1. DOACs (continued)

Criterion		Instructions (summary)
Drug-drug interaction (DDI)	A	There is no DDI.
	B	Potential PK or PD DDI without clinical or biological sign of toxicity: concomitant use of P-gp/CYP3A4 substrate, inhibitor or inducer, NSAID, antiplatelet therapy, SSRI. The drug interactions table of Hôpitaux Universitaires de Genève (HUG, 2014) was used to identify PK DDI.
	C	Clinically significant DDI: concomitant use not recommended or contraindicated (e.g. protease inhibitors), or DDI with clinical or biological sign of toxicity (strong P-gp or CYP3A4 inhibitors or inducers, NSAID, antiplatelet therapy, SSRI).
Drug-disease interaction	A	There is no drug-disease interaction.
	B	Potential drug-disease interaction without clinical or biological sign of toxicity (e.g. thrombocytopenia, severe uncontrolled hypertension, alcohol abuse)
	C	Clinically significant drug-disease interaction: DOAC not recommended (e.g. child Pugh B cirrhosis, disease with high risk of bleeding) or interaction with clinical or biological sign of toxicity
Duplication	A	DOAC is the only anticoagulant.
	B	Concomitant anticoagulant use in case of a switch from DOAC to VKA
	C	Another anticoagulant is used (e.g. concomitant use of LMWH)
Duration	A	Treatment duration is consistent with SmPC recommendations and guidelines.
	C	Treatment duration is inconsistent with SmPC recommendations and guidelines (e.g. long term anticoagulant therapy for a unique VTE episode with an identified temporary risk factor)

DDI: drug-drug interaction, DE: dabigatran etexilate, INR: International Normalized Ratio, LMWH: low molecular weight heparin, NSAID: non-steroidal anti-inflammatory drugs, NVAF: non-valvular atrial fibrillation, P-gp: P-glycoprotein, PD: pharmacodynamic, PK: pharmacokinetic, SmPC: summary of product characteristics, SSRI: selective serotonin reuptake inhibitors, VTE: venous thromboembolism

2. VKAs

Criterion		Instructions (summary)
Indication	A	Valid indication (e.g. AF in patients with CHA_2DS_2-VASC score ≥ 2, VTE, mechanical heart valves)
	B	Acceptable indication (e.g. AF in male patients with CHA_2DS_2-VASC score = 1)
	C	No indication (e.g. AF in patients with CHA_2DS_2-VASC score = 0 (male) or 1 (female))
Choice	A	VKA is the preferred choice: patient preference, severe renal impairment, prosthetic heart valve, poor compliance, extreme body weight (< 50 kg or > 110 kg), DOAC not reimbursed
	B	Another anticoagulant could have been a first choice: DOAC not tested and no contraindications
	C	VKA is inappropriate: labile INR, recurrent stroke/VTE on VKA, resistance to VKA, poor venous access or monitoring difficult to manage (and patient compliance), severe hepatic impairment
Dosage	A	INR on admission is within the target range.
	B	INR on admission is outside the target range (difference ≤ 0.5). Previous INR was within the target
	C	range. INR on admission is outside the target range (difference > 0.5).
Modalities of administration (correct)	A	Modalities of intake are correct: VKA taken once-daily in the evening, every day at the same hour
	B	Modalities of intake are not optimal: VKA taken in the morning
	C	Modalities of intake are not correct: variable time of intake
Modalities of administration (practical)	A	Patient has no difficulty taking the drug.
	C	Patient presents some difficulties (e.g. halving VKA tablets)

2. VKAs (continued)

Criterion		Instructions (summary)
Drug-drug interaction (DDI)	A	There is no DDI.
	B	Potential PK and/or PD DDI without clinical or biological sign of toxicity. The list of potential interacting drugs was drawn up from SmPC, databases, review articles and the drug interactions table of Hôpitaux Universitaires de Genève (HUG, 2014).
	C	Clinically significant DDI: recent addition of a drug with potential pK DDI and modification of INR, or PD DDI with sign of bleeding event (NSAID, antiplatelet therapy, SSRI).
Drug-disease interaction	A	There is no drug-disease interaction.
	B	Potential drug-disease interactions without clinical or biological sign of toxicity (e.g. thrombocytopenia, severe uncontrolled hypertension, alcohol abuse)
	C	Clinically significant drug-disease interaction: VKA not recommended (e.g. disease with high risk of bleeding) or interaction with clinical or biological sign of toxicity
Duplication	A	VKA is the only anticoagulant.
	B	Concomitant anticoagulant use in case of a switch from LMWH to VKA, or DOAC to VKA
	C	Another anticoagulant is used (e.g. concomitant use of LMWH and therapeutic INR)
Duration	A	Treatment duration is consistent with SmPC recommendations and guidelines.
	C	Treatment duration is inconsistent with SmPC recommendations and guidelines (e.g. long term anticoagulant therapy for a unique VTE episode with an identified temporary risk factor)

AF: atrial fibrillation, DDI: drug-drug interaction, INR: International Normalized Ratio, LMWH: low molecular weight heparin, NSAID: non-steroidal anti-inflammatory drugs, PD: pharmacodynamic, PK: pharmacokinetic, SmPC: summary of product characteristics, SSRI: selective serotonin reuptake inhibitors, VTE: venous thromboembolism

Appendix 2: criteria, scales and definitions used to assess adverse events

Naranjo ADR probability scale for causality of bleeding events

		Yes	No
1.	Are there previous conclusive reports on this reaction?	+1	0
2.	Did the adverse event appear after the suspected drug was administered?	+2	-1
3.	Did the adverse reaction improve when the drug was discontinued or a specific antagonist was administered?	+1	0
4.	Did the adverse reaction reappear when the drug was readministered?	+2	-1
5.	Are there alternatives causes (other than the drug) that could on their own have caused the reaction?	-1	+2
6.	Did the reaction reappear when a placebo was given?	-1	+1
7.	Was the drug detected in the blood in concentrations known to be toxic?	+1	0
8.	Was the reaction more severe when the dose was increased, or less severe when the dose was decreased?	+1	0
9.	Did the patient have a similar reaction to the same or similar drugs in any previous exposure?	+1	0
10.	Was the adverse event confirmed by any objective evidence?	+1	0

Not applicable = 0
Score ≤ 0: improbable, 1-4: possible, 5-8: probable, ≥ 9: certain

Therapeutic failure questionnaire for causality of thromboembolic events

		Yes	No
1.	Was drug therapy omitted for the condition?	+1	0
2.	Did the condition improve with drug therapy?	+2	-1
3.	Was the drug prescribed at too low a dose by the physician or taken as too low a dose by the patient?	+1	0
4.	Was the drug detected in the blood in sub therapeutic concentrations?	+1	0
5.	Was there a drug-drug interaction that interfered with the effectiveness of the drug prescribed for the condition?	+1	0
6.	Did the condition improve with an increase in dose or worsen with a decreased dose?	+2	0

Therapeutic failure questionnaire for causality of thromboembolic events (continued)

		Yes	No
7.	Are there conclusive reports of efficacy for a drug for the condition?	+1	0
8.	Are there alternatives causes that on their own could have caused the condition?	-1	+2
9.	Did the patient have the same condition related to absent or inadequate drug therapy previously?	+1	0
10.	Was therapeutic failure confirmed by objective evidence?	+1	0

Not applicable = 0
Score ≤ 0: improbable, 1-3: possible, 4-7: probable, ≥ 8: certain

Hallas criteria for preventability

Definitely avoidable	The drug event was due to a drug treatment procedure inconsistent with present-day knowledge of good medical practice or was clearly unrealistic, taking the known circumstances into account.
Possibly avoidable	The prescription was not erroneous, but the drug event could have been avoided by an effort exceeding the obligatory demands.
Not avoidable	The drug event could not have been avoided by any reasonable means, or it was an unpredictable event in the course of a treatment fully in accordance with good medical practice.
Unevaluable	The data for rating could not be obtained or the evidence was conflicting.

Hallas criteria for contribution of ADR to hospital admission

Dominant	The suspected symptoms were the main reason for admission, and no other symptoms contributed significantly.
Partly contributing	The suspected symptoms played a substantial role in admission, but other factors also contributed significantly.
Less important	The suspected symptoms played a minor or uncertain role, and the patient would probably have been admitted without them.
Not contributing	Other symptoms/circumstances were the main reason for hospitalization.

Chapter II

Measurement of the intensity of anticoagulation in DOAC patients

An optimized dRVVT-based assay to estimate the intensity of anticoagulation in patients treated with direct oral anticoagulants

Anne-Laure Sennesael, Thomas Exner, Bernard Chatelain, Sarah Lessire, Anne-Sophie Larock, Christelle Vancraeynest, Lionel Pochet, Jean-Michel Dogné, Anne Spinewine, François Mullier*, Jonathan Douxfils*

* Contributed equally

Thrombosis Research 2017; 157:29-37

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Measurement of the anticoagulant intensity of DOACs remains useful in common clinical situations such as major bleeding or emergency surgery.
- A unique and easy-to-perform biological assay to screen rapidly the anticoagulant effect of all DOAC is still lacking.
- The dilute Russell's Viper Venom Time (dRVVT) has been suggested for DOAC measurement. It correlated well with dabigatran and rivaroxaban concentrations in patient plasma samples.

WHAT THIS STUDY ADDS

- An optimized, liquid-stable dRVVT-based assay (DRVV-DOAC) correlated well with plasma concentrations of apixaban, dabigatran and rivaroxaban.
- Mixing samples with normal pooled plasma improved correlation parameters but reduced sensitivity of DRVV-DOAC assay.
- Normal DRVV-DOAC results ruled out clinically significant concentrations of rivaroxaban and dabigatran. This rapid assay could be a useful screening test in an emergency setting.

Abstract

BACKGROUND: The dilute Russell's Viper Venom Time (dRVVT) has been suggested for the assessment of the intensity of anticoagulation of all direct oral anticoagulants (DOACs). This study aimed to compare the performance of an optimized liquid-stable dRVVT-based DOAC assay (DRVV-DOAC) on clinical samples before and after mixing these with normal pooled plasma (NPP).

METHODS: Forty-one apixaban, 25 dabigatran, 56 rivaroxaban and 49 vitamin K antagonists (VKAs) plasma samples were included for retrospective analysis. Plasma DOAC concentrations were determined by liquid chromatography coupled with tandem mass-spectrometry. INR was determined for all VKA samples. DRVV-DOAC was performed with an original ready-to-use reagent (Haematex Research^{tm}) where plasma samples were tested neat and in a 1:1 mix with NPP.

RESULTS: Plasma concentrations ranged from 1 to 406 ng/ml for apixaban, 0 to 386 ng/ml for dabigatran and 0 to 719 ng/ml for rivaroxaban. INR ranged from 2.2 to 6.1. DRVV-DOAC correlated well with plasma concentrations (r^2=0.70, 0.94, 0.63 (non-mixed procedure) and 0.77, 0.97, 0.86 (mixed procedure) for apixaban, dabigatran and rivaroxaban, respectively). DRVV-DOAC measurements in the normal range ruled out dabigatran and rivaroxaban concentrations above 30 and 50 ng/ml, but performance was lower for apixaban. DRVV-DOAC was sensitive to VKA samples but poorly reflected INR values. When VKA samples were mixed with NPP, DRVV-DOAC measurements decreased to values close to baseline clotting time.

CONCLUSIONS: DRVV-DOAC is a quick method which showed increased sensitivity compared with other phospholipid-rich dRVVT reagents already investigated. Mixing samples with NPP improved the specificity but reduced sensitivity, especially for apixaban.

Introduction

Given their predictable dose response, direct oral anticoagulants (DOACs) do not require frequent laboratory monitoring and dose adjustment, as required for vitamin K antagonists (VKAs)[1]. However, measurement of the anticoagulant intensity remains useful in common clinical situations such as major bleeding and emergency surgery[2, 3]. Assessment of the individual response may also benefit to patients with extreme body weight, renal impairment or drug-drug interactions[4, 5].

Among routine coagulation assays, the activated partial thromboplastin time (aPTT) has been proposed to assess the anticoagulant intensity of dabigatran. The same was proposed with the prothrombin time (PT) for rivaroxaban, whereas no conventional test can be recommended for apixaban[3, 6, 7]. However, in patient plasma samples, aPTT and PT did not correlate adequately with DOACs concentrations, precluding their use for drug quantitation[8-10]. Prolongation of clotting times also depends on the reagent[11]. Thus, specific assays have been recommended to estimate DOACs concentrations: calibrated ecarin-based assays or calibrated diluted thrombin time for dabigatran, and calibrated chromogenic anti-Xa assays for apixaban and rivaroxaban[3, 7]. Although these tests correlate highly with DOAC concentrations, they require specific calibrators and controls and their turn-around time, if not implemented in routine, may limit their use in emergency situations. Consequently, a unique and easy-to-perform biological assay is still needed to screen rapidly the anticoagulant effect of all DOACs[12].

The dilute Russell's Viper Venom Time (dRVVT) has recently been suggested for DOACs measurement[13]. This coagulation test is currently used in clinical practice to detect lupus anticoagulants in the context of antiphospholipid syndrome. In patient plasma samples, dRVVT correlated well with dabigatran and rivaroxaban concentrations[14, 15] and provided better performances than aPTT or PT[14, 16]. For apixaban, a previous study demonstrated that the concentration required to affect

lupus anticoagulant screening is higher than for rivaroxaban and edoxaban, suggesting a lower sensitivity of dRVVT testing towards apixaban[17]. Moreover, some drawbacks have been highlighted, such as the inter-individual variability of phospholipid poor reagents and the lack of liquid-stable products. The reconstitution step increases the turnaround time (i.e. an extra 30 minutes), leads to product waste (i.e. stability of 3 days for reconstituted reagents in coagulometer, 7 days at 4°C) and can result in reconstitution errors (i.e. errors due to pipetting or contaminating water). Thus, even if promising, conventional dRVVT is not considered suitable for the urgent assessment of the intensity of anticoagulation of DOACs.

The present study aimed to compare the performance of an optimized liquid-stable dRVVT-based DOAC assay (DRVV-DOAC, Haematex Research™) on clinical samples before and after mixing these with normal pooled plasma (NPP). We also investigated the sensitivity of the DRVV-DOAC assay to VKA plasma samples and heparins.

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). Hospitalized and ambulatory patients have been recruited at the CHU UCL Namur. Written informed consent was obtained from each donor.

Clinical samples

Forty-one apixaban, 25 dabigatran, 56 rivaroxaban and 49 VKA plasma samples from patients were included in the study for retrospective analysis. Blood was taken by antecubital venipuncture and collected into 0.109 M sodium citrate (9:1 v/v) tubes (Venosafe®, Terumo, Belgium) using a 21-gauge needle (Terumo). Platelet-poor plasma (PPP) was obtained from the supernatant fraction after double

centrifugation for 15 min at 1500g at room temperature. Afterwards, PPP was aliquoted and frozen at -80°C without delay. Frozen plasma samples aliquots were thawed by heating to 37°C for at least 5 min just before experiments.

Patients were taking DOAC mainly for stroke prevention in non-valvular atrial fibrillation (NVAF) (n= 41 for apixaban, 18 for dabigatran etexilate (DE) and 31 for rivaroxaban). Other indications were the secondary prevention of venous thromboembolism (n= 1 for DE and 22 for rivaroxaban) and the prevention of venous thromboembolism after knee replacement surgery (n= 6 for DE). For 3 rivaroxaban samples, treatment indication was unknown. When scheduled, plasma samples were collected just before the next pill intake (at C_{trough}, i.e. 12 hours after the last intake for DE and apixaban and 24 hours for rivaroxaban) and 2 to 3 hours after the pill intake (at expected C_{max}). For other samples, blood was collected randomly or the delay since the last administration was unknown. VKA samples were collected in a random fashion within the routine laboratory. None of these patients had a history or were suffering from lupus anticoagulant.

Normal pooled plasma

Normal pooled plasma (NPP) was obtained from the PPP of 60 healthy individuals, which was prepared as described above for the clinical samples. Exclusion criteria consisted of thrombotic and/or haemorrhagic events, pregnancy, and use of antiplatelet and/or anticoagulant therapy and/or drugs potentially affecting platelet and/or coagulation factor functions during two weeks prior to sampling. Twenty different individual plasmas (IP) were also collected from this cohort in order to test the inter-individual variability of the DRVV-DOAC.

Home-made calibrators

Home-made calibrators were made from pure powder (>99% purity) of the active substances. Rivaroxaban, dabigatran and apixaban powders were generous gifts

from Bayer A.G. (Leverkusen, Germany), Boehringer-Ingelheim GmbH (Ingelheim am Rhein, Germany) and Bristol-Myers Squibb GmbH (München, Germany), respectively. Stock solutions at 1 mg/mL in DMSO (plus HCl 5% for dabigatran) were prepared and then diluted in phosphate-buffered saline (PBS) without Ca^{2+} and Mg^{2+}. For each active substance, six working solutions were prepared by mixing a 10-times concentrated solution with NPP in order to give final concentrations ranging from 0 to 1000 ng/ml. The final DMSO concentration in plasma was ≤ 0.1% (v/v) which does not influence most coagulation tests (own data).

Testing solutions of heparins

Unfractionated heparin (UFH) was purchased at Leo Pharma A/S (Ballerup, Denmark) and low molecular weight heparin (enoxaparin) was purchased at Sanofi Winthrop Industrie (Floirac, France). Stock solution of UFH and LMWH were at 5000 U/mL and 10000 IU/mL, respectively and were then diluted in phosphate-buffered saline (PBS) without Ca^{2+} and Mg^{2+}. UFH and LMWH were finally spiked in NPP at the following concentrations: 0.5 UI/ml, 1.0 UI/ml and 2.0 UI/ml.

Dilute Russell's Viper Venom time – based DOAC assay

DRVV-DOAC assay (X9211) is developed by Haematex Research™ (Hornsby, Australia). The test reagent is a modified phospholipid-rich, liquid-stable, ready-to-use dRVV reagent. Compared with reagents used in practice for lupus anticoagulant testing, phospholipids, Russell's viper venom enzymes and ionic components were modified to enhance the sensitivity to DOACs. The test is sensitive mainly to FV, less so for FII and FX and not at all to other factors. It is stable for 1 year stored at 4°C. The normal procedure consisted of one hundred µl of tested plasma, pre-incubated at 37°C for 240 seconds, mixed with 100 µl of DRVV-DOAC reagent which started the reaction. The acquisition time was extended to 300 seconds. In order to reduce the inter-individual variability, a mixing procedure was proposed which consists of mixing 50 µl of tested plasma with 50 µl of NPP. The mix was incubated at 37°C

during 240 seconds. The addition of 100 µl of DRVV-DOAC reagent started the reaction. Results were given in seconds, as ratios (versus NPP), and in ng/ml (after calibration). The method was performed on a STA-R Evolution® coagulometer (Diagnostica Stago, Asnières, France).

International Normalized Ratio (INR)

For VKA samples, the INR was determined using RecombiPlasTin 2G® (Werfen, Lexington, USA). RecombiPlasTin 2G® was performed on an ACL-TOP® analyser according to the recommendations of the manufacturer.

Liquid chromatography coupled with tandem mass spectrometry

Measurements of dabigatran plasma concentrations were performed as previously described [18]. For apixaban and rivaroxaban, the methods were adapted from the procedures previously described [14, 19]. Plasma concentrations were determined using liquid chromatography coupled with tandem mass spectrometry after sample preparation by protein precipitation of 50 µl citrated plasma with 350 µl of acetonitrile formic acid 1% containing [^{13}C,^{2}H7]-apixaban or [^{13}C6]-rivaroxaban-d5 as internal standards. Apixaban, [^{13}C,^{2}H7]-apixaban, rivaroxaban and [^{13}C6]-rivaroxaban-d5 were purchased from Alsachim (Strasbourg, France). An aliquot (2µl) of the final extract was injected into the LC-MS/MS system. Separation of the analytes was achieved on a Waters Cortecs® UPLC® C_{18} column (1.6 µm, 2.1 x 100 mm), using a gradient run with mobile phase A (formic acid 0.1% in H_2O for apixaban, 10mM ammonium formate adjusted to pH 3 with formic acid for rivaroxaban) and mobile phase B (acetonitrile). The analytes were detected using a Waters Xevo TQ-S mass spectrometer operating in positive electrospray ionization (ESI) mode utilizing multiple reaction monitoring (MRM) for the transitions 460.15>443.10 m/z (apixaban, quantitative ion product), 460.15>199.10 (apixaban, qualitative ion product), 468.20>451.20 ([^{13}C,^{2}H7]-apixaban, quantitative ion product), 468.20>199.10 ([^{13}C,^{2}H7]-apixaban, qualitative ion product). The

following transitions were monitored for rivaroxaban: 436.00>145.05 (rivaroxaban, quantitative ion product); 436.00>231.20 (rivaroxaban, qualitative ion product); 442.00>145.05 ([^{13}C6]-rivaroxaban, quantitative ion product); 442.00>237.20 ([^{13}C6]-rivaroxaban, qualitative ion product). The calibration curves for apixaban and rivaroxaban in plasma were linear over the range 1-400 ng/ml and the limits of detection (LOD) were 1.3 ng/ml for apixaban and 0.5 ng/ml for rivaroxaban. Validation experiments with five levels of control samples ranging from 1 to 400 ng/ml on five different days (three for rivaroxaban) showed repeatability relative standard deviation (RSD) below 2.4% and 5.0%, an intermediate precision RSD below 5.5 % and 9.8% and a relative bias below 2.3% and 11.1% for apixaban and rivaroxaban respectively. The method was validated according to FDA Guidelines.

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 6.00 for Windows (GraphPad Software, San Diego California, USA, www.graphpad.com). The intra- and inter-assays variability, expressed in coefficient of variation, was assessed by running 10 replicates of the NPP as well as 10 replicates of low (30 ng/ml) and high (300 ng/ml) concentrations of each drug. For the calibration curves, each home-made standard was run 3-times and the mean value (± standard deviation) was calculated. Three different lots were used to assess batch-to-batch variability, expressed in coefficient of variation. Measurements of the calibrated DRVV-DOAC assay were compared with LC-MS/MS method by Spearman correlation analysis and by linear regression. Bland-Altman analyses were also performed. Sensitivity was defined as the concentration in DOAC required to double the clotting time of the NPP. ROC curve analyses were performed to identify the best cut-off to exclude above on-therapy concentrations at trough. We considered the 95th percentile of apixaban and rivaroxaban trough levels for NVAF (i.e. 230 ng/ml and 137 ng/ml respectively), as it was the main indication of treatment [20, 21]. For dabigatran, we considered the 90th percentile of trough levels

(i.e. 200 ng/ml), according to the current Pradaxa® EU-SmPC [22]. We also examined if DRVV-DOAC measurements in the normal range (expressed as ratio) can rule out DOAC concentrations above 30 ng/ml and 50 ng/ml. The normal range was defined as the mean clotting time of the 20 IP ± 2*standard deviation. Calibration of DRVV-DOAC assay and correlation between DRVV-DOAC measurements (expressed in seconds and ratio) and plasma concentrations were best described by a linear regression for apixaban. A curvilinear (first-order) relation was more suitable for dabigatran and rivaroxaban, as described by the following model: Y=Y0 + (Plateau-Y0)*(1-exp(-K*x)). The limit of detection (LOD) and the limit of quantitation (LOQ) of DRVV-DOAC were calculated as follow, based on the calibration curve obtained with the spiked samples: LOD = [(3*standard deviation of Y0)/slope] and LOQ = [(10*standard deviation of Y0)/slope].

Results

Assay performances

The inter-individual variability, tested on 20 IP, was 7.0% and 3.3% for the non-mixed and the mixed tests, respectively. Results expressed as ratios ranged from 0.88 to 1.16 (mean ± 2 SD) for both tests. Reproducibility in NPP and in plasmas containing low and high DOAC concentrations, sensitivity and limits of detection and quantitation of DRVV-DOAC are summarised in Table 1 for each drug. Batch-to-batch variability varied from 4.5 to 10.8 % for the non-mixed test, and from 3.3 to 6.9 % for the mixed test.

Table 1: Summary of intra- and inter-assay variability, sensitivity, limits of detection and quantification of DRVV-DOAC. Intra- and inter-assay variability was tested in plasmas containing low (30 ng/ml) and high (300 ng/ml) DOAC concentrations. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated as follow, based on the calibration curve obtained with the spiked samples: LOD = [(3*standard deviation of Y0)/slope] and LOQ = [(10*standard deviation of Y0)/slope].

		Apixaban		Dabigatran		Rivaroxaban	
		DRVV-DOAC	DRVV-DOAC mix	DRVV-DOAC	DRVV-DOAC mix	DRVV-DOAC	DRVV-DOAC mix
Intra-assay variability (%)	NPP	1.0	/	1.0	/	1.0	/
Intra-assay variability (%)	Low	1.1	3.1	1.2	1.2	3.1	1.5
	High	1.0	1.7	1.1	2.1	2.7	1.0
Inter-assay variability (%)	Low	2.5	1.1	1.6	1.4	1.6	1.8
	High	2.0	3.4	2.4	1.1	3.4	1.3
Sensitivity (ng/ml)		334	691	99	194	101	219
Limit of detection (ng/ml)		55	52	14	12	15	15
Limit of quantification (ng/ml)		183	176	50	41	53	51

Patients' plasma sample concentrations and INR values

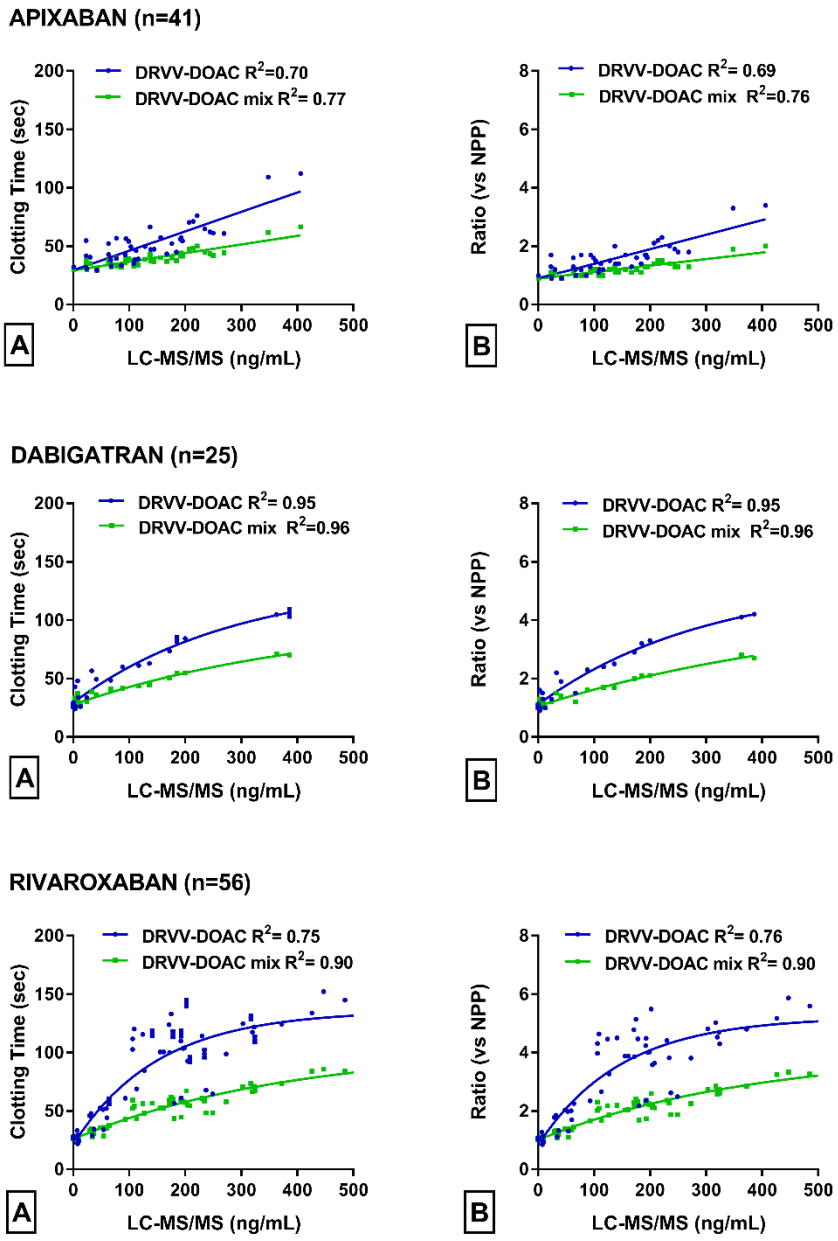
The patients' plasma sample concentrations ranged from 1 to 406 ng/ml for apixaban, 0 to 386 ng/ml for dabigatran and 0 to 719 ng/ml for rivaroxaban, according to LC-MS/MS measurements. INR varied from 2.2 to 6.1 within the VKA samples' group. Mean plasma drug concentrations according to the delay since the last pill intake are described in Supplementary data – Table 1.

Correlations between DRVV-DOAC and plasma drug concentrations/INR

Apixaban: The DRVV-DOAC assay yielded a linear concentration-dependent prolongation of clotting time (Fig 1). The r^2-values for linear regression were 0.70 and 0.69 when results were expressed in seconds or as ratios respectively. These parameters increased to 0.77 and 0.76 when plasma samples were mixed with NPP. The Spearman correlation between DRVV-DOAC results expressed in ng/ml and LC-MS/MS had coefficients of 0.75 and 0.81 for the non-mixed and the mixed tests respectively. The linear regression parameters and Bland-Altman analysis are provided in Table 2 and represented in Fig 2 and 3. The Bland-Altman analysis showed a mean difference of 60 ng/ml (95% CI: -162 to 281 ng/l) for the non-mixed test and 2 ng/ml (95% CI: -169 to 172 ng/ml) for the mixed test.

Dabigatran: The DRVV-DOAC assay yielded a curvilinear concentration-dependent prolongation of clotting time (Fig 1). The first-order equation gave r^2-values of 0.95 for the non-mixed test and 0.96 for the mixed test, whether the results were expressed in seconds or as ratios. The Spearman correlation between DRVV-DOAC results expressed in ng/ml and LC-MS/MS had coefficients of 0.85 and 0.86 for the non-mixed and the mixed tests respectively. The linear regression parameters and Bland-Altman analysis are provided in Table 2 and represented in Fig 2 and 3. The Bland-Altman analysis showed a mean difference of 23 ng/ml (95% CI: -67 to 113 ng/ml) for the non-mixed test and 26 ng/ml (95% CI: -68 to 120 ng/ml) for the mixed test.

Figure 1: Correlation between DRVV-DOAC measurements and plasma concentrations of apixaban, dabigatran and rivaroxaban in clinical samples. Fig. 1A and 1B show results expressed in seconds and as ratios respectively. The blue circles represent the non-mixed assay, while the green squares represent the mixed procedure.



<u>Figure 2:</u> Correlation between calibrated DRVV-DOAC assay and plasma concentrations of apixaban, dabigatran and rivaroxaban in clinical samples. Fig. 2A shows results for the non-mixed procedure, while Fig. 2B shows results for the mixed procedure.

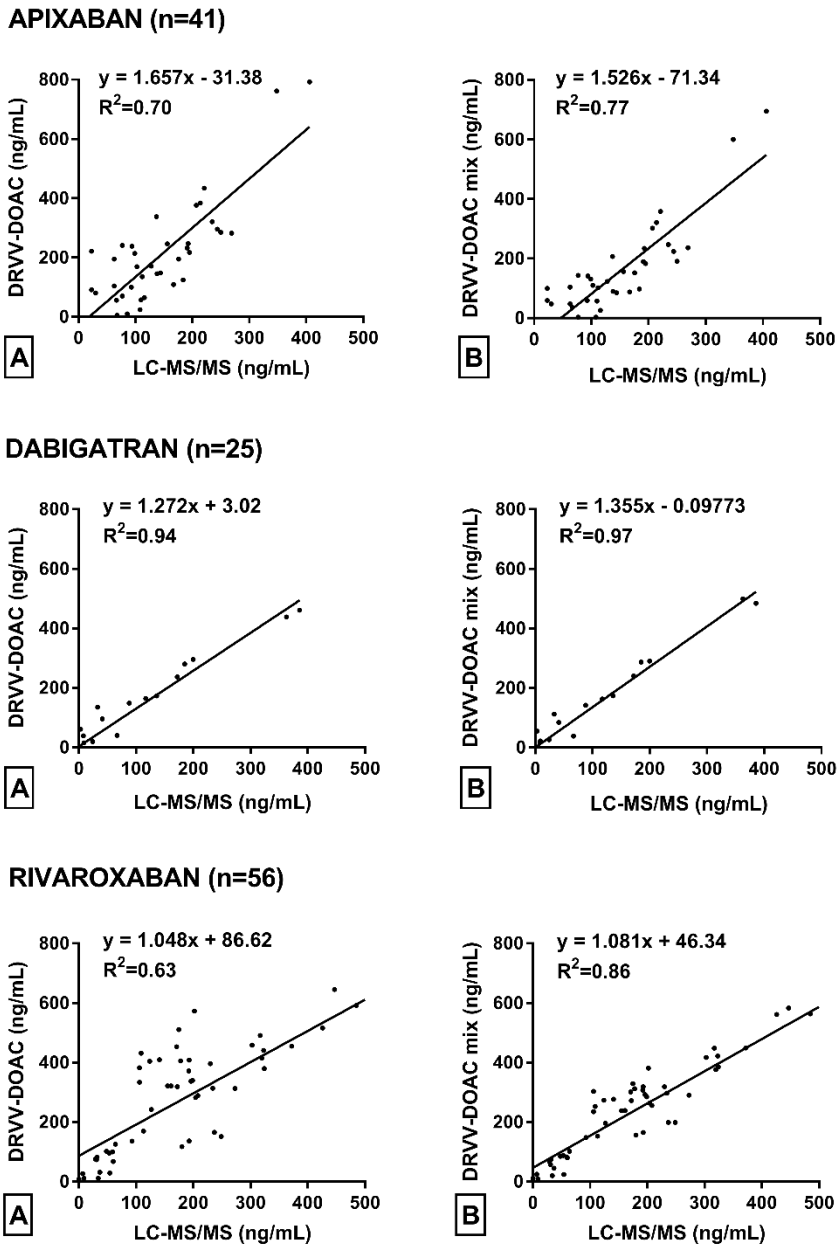
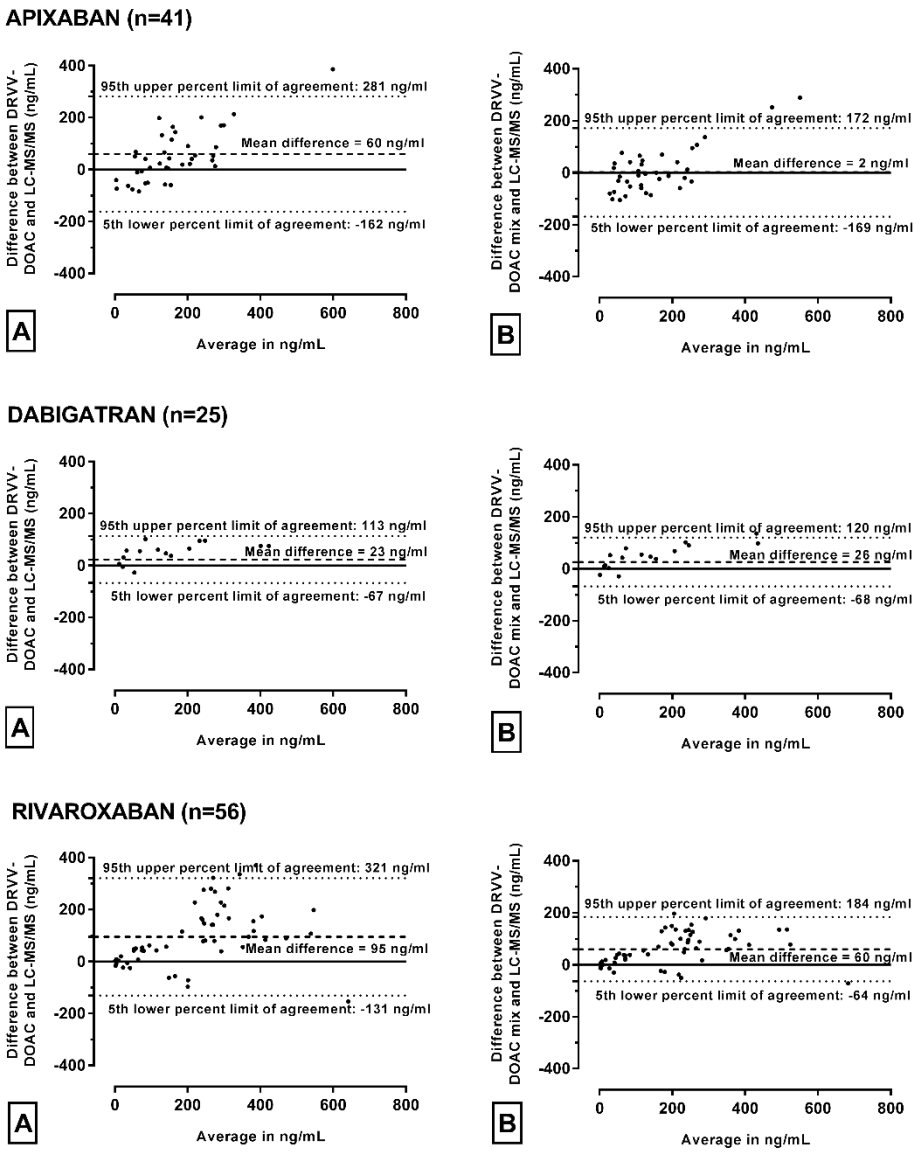


Figure 3: Results of the Bland-Altman analysis. The difference was calculated as follow: [difference (A-B) vs average] where A is the result of DRVV-DOAC assay and B the result of LC-MS/MS. Fig. 3A shows results for the non-mixed procedure, while Fig. 3B shows results for the mixed procedure. Thick and tiny dotted lines represent mean differences and 5th-95th percentiles of the limit of agreement, respectively.



Rivaroxaban: The DRVV-DOAC assay yielded a curvilinear concentration-dependent prolongation of clotting time (Fig 1). The first-order equation gave r^2-values of 0.75 and 0.76 when results were expressed in seconds or as ratios respectively. Both parameters increased to 0.90 when plasma samples were mixed with NPP. The Spearman correlation between DRVV-DOAC results expressed in ng/ml and LC-MS/MS had coefficients of 0.81 and 0.90 for the non-mixed and the mixed tests respectively. The linear regression parameters and Bland-Altman analysis are provided in Table 2 and represented in Fig 2 and 3. The Bland-Altman analysis showed a mean difference of 95 ng/ml (95% CI: -131 to 321 ng/ml) for the non-mixed test and 60 ng/ml (95% CI: -64 to 184 ng/ml) for the mixed test.

Vitamin K antagonists: The DRVV-DOAC assay was sensitive to VKA samples but poorly reflected INR values (Supplementary Material – Figure 1). The Spearman correlation coefficient between DRVV-DOAC results (in seconds or as ratios) and INR values was 0.65 (95% CI: 0.45 - 0.79; $p < 0.0001$; r^2 for the linear regression 0.39) for the non-mixed test. When patient plasma samples were mixed with NPP, DRVV-DOAC measurements returned to values close to baseline clotting time.

Heparins: The DRVV-DOAC assay was insensitive to UFH and enoxaparin up to 1 UI/ml (Supplementary Material – Table 2).

Table 2: Correlation between calibrated DRVV-DOAC assay and plasma concentrations of apixaban, dabigatran and rivaroxaban: summary of linear regression parameters and Bland-Altman analysis.

	R-square	Slope	95% CI	Intercept	95% CI	Bland-Altman bias (in ng/ml)	95% CI
APIXABAN							
DRVV-DOAC	0.70	1.66	1.30, 2.01	-31.38	-89.21, 26.46	60	-162, 281
DRVV-DOAC mix	0.77	1.53	1.26, 1.79	-71.34	-115.10, -27.54	2	-169, 172
DABIGATRAN							
DRVV-DOAC	0.94	1.27	1.14, 1.41	3.02	-14.73, 20.77	23	-67, 113
DRVV-DOAC mix	0.97	1.36	1.25, 1.46	-0.10	-14.19, 13.99	26	-68, 120
RIVAROXABAN							
DRVV-DOAC	0.63	1.05	0.83, 1.27	86.62	37.99, 135.30	95	-131, 321
DRVV-DOAC mix	0.86	1.08	0.96, 1.20	46.34	20.08, 72.59	60	-64, 184

Table 3: Summary of ROC curve analysis. Cut-offs of above on-therapy concentrations at trough (i.e. exceeding the 90th (dabigatran) or 95th (apixaban, rivaroxaban) percentile of trough levels) were defined for non-mixed and mixed procedures. Sensitivity (95% confidence interval (CI)), specificity (95% CI), positive predictive value and negative predictive value were calculated.

	Cut-off (ratio)	Sensitivity	95% CI	Specificity	95% CI	+ predictive value	- predictive value
APIXABAN							
DRVV-DOAC	1.7	100.0	100.0-100.0	88.6	73.2-96.7	60.0	100.0
DRVV-DOAC mix	1.2	100.0	100.0-100.0	80.0	63.1-91.5	46.2	100.0
DABIGATRAN							
DRVV-DOAC	3.3	100.0	100.0-100.0	100.0	100.0-100.0	100.0	100.0
DRVV-DOAC mix	2.1	100.0	100.0-100.0	100.0	100.0-100.0	100.0	100.0
RIVAROXABAN							
DRVV-DOAC	2.3	96.8	83.2-99.5	76.0	54.9-90.6	83.3	95.0
DRVV-DOAC mix	1.7	93.5	78.5-99.0	80.0	59.3-93.1	85.3	90.9

Screening performance

ROC curve analysis gave different cut-offs to exclude above on-therapy concentrations at trough. Cut-offs expressed as ratios ranged from 1.7 to 3.3 for the non-mixed test, and 1.2 to 2.1 for the mixed test. Sensitivities, specificities, positive and negative predictive values are summarised in Table 3.

Considering a cut-off ratio of 1.1 (upper limit of the normal range), DRVV-DOAC had a sensitivity and a negative predictive value (NPV) of 100% to exclude dabigatran and rivaroxaban concentrations above 30 ng/ml and 50 ng/ml. The performance was lower regarding apixaban (sensitivity of 89 and 91% and NPV of 33 and 50%, for 30 and 50 ng/ml, respectively). The performance of the mixed procedure was not assessed given the reduced sensitivity of the test and the need for urgent assessment which is not compatible with the use of freshly thawed NPP.

Discussion

In this study, DRVV-DOAC measurements (expressed in seconds or as ratios) correlated well with plasma concentrations of apixaban, dabigatran and rivaroxaban. R^2-values were better than the ones previously obtained with PT for rivaroxaban and aPTT for dabigatran[8-10, 14]. This suggests that DRVV-DOAC is more suitable than routine test for the assessment of dabigatran and rivaroxaban. No conventional test is recommended for apixaban due to a lack of sensitivity[23, 24]. The DRVV-DOAC is therefore a relevant alternative for the screening of patients treated with this drug.

In terms of sensitivity, concentrations of anticoagulant required to double the clotting time of NPP ranged from 99 ng/ml (dabigatran) to 334 ng/ml (apixaban). DRVV-DOAC showed an increased sensitivity compared to other phospholipid-rich dRVVT reagents already investigated[13]. Sensitivity of DRVV-DOAC was similar or higher than the ones of the Hemoclot Thrombin Inhibitor® for dabigatran (±340

ng/ml) and calibrated chromogenic anti-Xa assays for rivaroxaban and apixaban (±350-400 ng/ml)[24-26].

For dabigatran and rivaroxaban, LOD and LOQ of DRVV-DOAC (14-15 ng/ml and 50-53 ng/ml respectively) were similar to values obtained with the recommended specific assays, using calibrators for normal range of concentrations[8, 9]. LOD of DRVV-DOAC was higher for apixaban (55 ng/ml), limiting its use for the detection of low plasma concentrations. Bland-Altman analysis showed inconsistent mean deviations from the reference measurement, depending on the anticoagulant. The large confidence interval is likely to preclude the use of DRVV-DOAC to estimate DOACs plasma concentrations accurately. However, DRVV-DOAC measurements in the normal range ruled out dabigatran and rivaroxaban concentrations above 30 ng/ml and 50 ng/ml.

It was considered possible to improve DRVV-DOAC assay by mixing patient plasma samples with NPP before testing. DOACs do not “correct” on mixing with NPP to the same degree as plasmas which are reduced in clotting factors. The relative difference between DOAC plasmas and other plasmas in DRVV-DOAC assay was therefore expected to be increased in 1:1 mixes with NPP. Mixing improved correlation parameters for apixaban and rivaroxaban, and the Bland-Altman analysis showed closer limits of agreement. We can reasonably hypothesize that the addition of NPP reduced the inter-individual variability by the partial compensation of possible defects of plasma components. However, the mixed procedure also reduced the sensitivity of DRVV-DOAC.

Calibration of DRVV-DOAC did not improve correlation parameters, as we previously found with other dRVVT reagents[14]. These results confirm that dRVVT-based assays can be performed rapidly without the need for specific calibrators. We used home-made calibrators as none of the commercially available calibrators are designed for the DRVV-DOAC assay.

This study has limitations. First, even if DRVV-DOAC was performed with a phospholipid rich reagent, it could be affected by occasional strong lupus anticoagulants[27-29]. Our plasma samples were not tested for lupus anticoagulants. However, none of the patients had a history of this condition. Second, concentrations of calibrators used for DRVV-DOAC were not validated with the LC-MS/MS method. Then, DRVV-DOAC performance was not compared directly to other dRVVT assays or conventional tests. Nevertheless, previous studies have already investigated such tests for DOAC measurement[14, 15, 30]. We used their results for comparison, even if the instrument used could also influence the sensitivity of the test[31]. Finally, our results were generated on STA-R Evolution® coagulometer (Diagnostica Stago) and are not generalizable to other instruments. Further studies are required to investigate the potential inter-coagulometer variability of DRVV-DOAC.

Can we use the DRVV-DOAC assay to screen the intensity of anticoagulation of all DOACs?

DRVVT-based assays are currently being proposed for the screening of DOACs[32]. Their main attraction is their ability to provide a qualitative assessment of the anticoagulant effect of all DOACs, rather than using a different test for each drug. This study revealed the ex-vivo influence of apixaban, dabigatran and rivaroxaban on dRVVT-based assays. Edoxaban was not on the market at the time of the study, but in vitro data demonstrated similar results[33].

For a routine lab, dRVVT-based assays seem more suitable than aPTT or PT to screen the relative intensity of DOAC anticoagulation (better performance, lower inter-reagent variability expected)[14]. The DRVV-DOAC assay may be used to exclude the presence of dabigatran, rivaroxaban and partially apixaban. The high content in phospholipids of this optimized dRVVT-based assay permits to avoid interference from at least minor and moderate lupus anticoagulants.

The DRVV-DOAC assay appears interesting in situations requiring a rapid turnaround time, given the lack of reconstitution of its reagent and the non-requirement for specific calibrators. It takes less than 10 minutes to perform, but delays for sending blood sample to the laboratory and for centrifugation still need be considered*. In an emergency setting, physicians may suspect high DOACs levels, for instance in patients presenting with bleeding. In the absence of a defined therapeutic range, on-therapy peak and trough concentrations derived from clinical trials have been proposed for each DOAC, according to the indication[11]. The DRVV-DOAC assay could be proposed as a rapid screening test to exclude above on-therapy levels at trough. Nevertheless, thresholds were different for each DOAC.

The exclusion of clinically relevant DOAC levels is also of particular interest in patients requiring urgent surgery or thrombolysis. Moreover, the arrival of specific antidotes (i.e. idarucizumab for dabigatran or andexanet alpha for direct factor Xa inhibitors) has raised the question of when their administration is indicated. The Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH) recommends antidote administration if DOAC concentration is above 30 ng/ml (urgent surgery with a high bleeding risk) or 50 ng/ml (serious bleeding)[34]. Although accurate assays (e.g. HTI LOW, ECA-II) have been developed for the measurement of low plasma concentrations, their turnaround time may be too long (i.e. 30 minutes of reconstitution time, calibration and quality controls if not implemented in routine)[18]. The DRVV-DOAC assay could be performed as a qualitative test to rapidly exclude dabigatran and rivaroxaban concentrations above 30 ng/ml and 50 ng/ml. However, for apixaban, sensitivity was insufficient to allow assessment of low concentrations.

The mixed procedure of the DRVV-DOAC assay should not be supported in an emergency context given the longer turnaround time and the decreased sensitivity

* Adapted from the published version

compared to the non-mixed procedure. Nevertheless, it could help identify patients taking VKA when medication history is missing as mixing normalized clotting times of DRVV-DOAC in VKA samples.

Our results confirm that dRVVT-based assays cannot discriminate between apixaban, dabigatran etexilate and rivaroxaban treatments. However, when used together with conventional coagulation assays, they may be helpful in differentiating between the different drugs when clinical data are missing (e.g. unconscious patient with serious bleeding) and has already been included in previous algorithms[32].

In light of these results, the development of point-of-care (POC) devices would be helpful in the screening of the anticoagulant status of patients treated with DOACs, especially in an emergency setting. Although the CoaguCheck® (Roche diagnostics) has been proposed to detect low rivaroxaban concentrations, there is currently no specific POC test applicable for all DOACs[35]. The DRVV-DOAC represents a significant step forward in developing dRVVT-based POC devices. In this respect, the feasibility of dry chemistry with DRVV-DOAC material needs further investigations.

Conclusions

DRVV-DOAC assay might be a useful screening test to exclude above on-therapy or clinically relevant DOACs concentrations. Its short turnaround time is particularly relevant in an emergency setting. However, DRVV-DOAC is not sensitive enough to detect low apixaban concentrations. Finally, the use of DRVV-DOAC for development of POC devices is obviously promising but requires improvements.

Author contributions

ALS, TE, BC, JMD, AS, FM and JD designed the research study. ALS wrote the first draft of the manuscript and the final version. All the co-authors were responsible for the review of the manuscript. SL and ASL contributed to data collection. DRVV-DOAC assay was performed by ALS and JD. CV and LP were responsible for LC-MS/MS measurements.

Conflicts of interest

T. Exner is the owner of Haematex Research Laboratories which developed and sells the DRVV-DOAC reagent. Other authors have no relevant conflicts of interest to disclose.

Acknowledgements

The authors would like to thank Mrs. J. Baudar and Mrs M. Guldenpfennig for their contributions to this work. AL. Sennesael is a Research Fellow of the Fonds de la Recherche Scientifique – FNRS.

REFERENCES

1. Baglin T, Hillarp A, Tripodi A, Elalamy I, Buller H, Ageno W. Measuring Oral Direct Inhibitors (ODIs) of thrombin and factor Xa: A recommendation from the Subcommittee on Control of Anticoagulation of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. J Thromb Haemost 2013; 11: 756-60.
2. Tamigniau A, Douxfils J, Nicolas JB, et al. [Why, when and how to monitor new oral anticoagulants]. Rev Med Suisse 2014; 10: 326-33.
3. Cuker A, Siegal DM, Crowther MA, Garcia DA. Laboratory measurement of the anticoagulant activity of the non-vitamin K oral anticoagulants. J Am Coll Cardiol 2014; 64: 1128-39.
4. 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). J Am Coll Cardiol 2014; 63: 321-8.
5. Douxfils J, Mullier F, Dogne JM. Dose tailoring of dabigatran etexilate: obvious or excessive? Expert Opin Drug Saf 2015; 14: 1283-9.
6. Baglin T, Hillarp A, Tripodi A, Elalamy I, Buller H, Ageno W. Measuring Oral Direct Inhibitors (ODIs) of thrombin and factor Xa: A recommendation from the Subcommittee on Control of Anticoagulation of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. J Thromb Haemost 2013.
7. Douxfils J, Mani H, Minet V, et al. Non-VKA Oral Anticoagulants: Accurate Measurement of Plasma Drug Concentrations. Biomed Res Int 2015; 2015: 345138.
8. 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. Thromb Haemost 2013; 110: 543-9.
9. 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. Thromb Haemost 2013; 110: 723-31.
10. Testa S, Legnani C, Tripodi A, et al. Poor comparability of coagulation screening test with specific measurement in patients receiving direct oral anticoagulants: results from a multicenter/multiplatform study. J Thromb Haemost 2016; 14: 2194-201.
11. Douxfils J, Gosselin RC. Laboratory Assessment of Direct Oral Anticoagulants. Semin Thromb Hemost 2017; 43: 277-90.
12. Weitz JI, Eikelboom JW. Urgent Need to Measure Effects of Direct Oral Anticoagulants. Circulation 2016; 134: 186-8.
13. Exner T, Ellwood L, Rubie J, Barancewicz A. Testing for new oral anticoagulants with LA-resistant Russells viper venom reagents. An in vitro study. Thromb Haemost 2013; 109: 762-5.
14. Douxfils J, Chatelain B, Hjemdahl P, et al. Does the Russell Viper Venom time test provide a rapid estimation of the intensity of oral anticoagulation? A cohort study. Thromb Res 2015; 135: 852-60.
15. Gosselin RC, Funk DM, Taylor JM, et al. Comparison of Anti-Xa and Dilute Russell Viper Venom Time Assays in Quantifying Drug Levels in Patients on Therapeutic Doses of Rivaroxaban. Archives of Pathology & Laboratory Medicine 2014; 138: 1680-4.
16. Altman R, Gonzalez CD. Simple and rapid assay for effect of the new oral anticoagulant (NOAC) rivaroxaban: preliminary results support further tests with all NOACs. Thromb J 2014; 12: 7.

17. 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; 38: 505-13.
18. 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. Thromb Haemost 2015; 113: 862-9.
19. Delavenne X, Mismetti P, Basset T. Rapid determination of apixaban concentration in human plasma by liquid chromatography/tandem mass spectrometry: application to pharmacokinetic study. J Pharm Biomed Anal 2013; 78-79: 150-3.
20. Mueck W, Stampfuss J, Kubitza D, Becka M. Clinical pharmacokinetic and pharmacodynamic profile of rivaroxaban. Clin Pharmacokinet 2014; 53: 1-16.
21. Eliquis - Summary of Product Characteristics. European Medicines Agency; [23 March 2018]. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002148/WC500107728.pdf.
22. Pradaxa - Summary of Product Characteristics. European Medicines Agency; [23 March 2018]. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000829/WC500041059.pdf.
23. Bonar R, Favaloro EJ, Mohammed S, et al. The effect of the direct factor Xa inhibitors apixaban and rivaroxaban on haemostasis tests: a comprehensive assessment using in vitro and ex vivo samples. Pathology 2016; 48: 60-71.
24. 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: 283-94.
25. 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. Thromb Haemost 2012; 107: 985-97.
26. 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. Thromb Res 2012; 130: 956-66.
27. Devreese KM, de Laat B. Mixing studies in lupus anticoagulant testing are required at least in some type of samples. J Thromb Haemost 2015; 13: 1475-8.
28. Martinuzzo ME, Barrera LH, MA Da, Otaso JC, Gimenez MI, Oyhamburu J. Frequent false-positive results of lupus anticoagulant tests in plasmas of patients receiving the new oral anticoagulants and enoxaparin. Int J Lab Hematol 2014; 36: 144-50.
29. Depreter B, Devreese KM. Dilute Russell's viper venom time reagents in lupus anticoagulant testing: a well-considered choice. Clin Chem Lab Med 2017; 55: 91-101.
30. McGlasson DL, Fritsma GA. Measuring dabigatran with the dilute Russell viper venom confirm assay in an anticoagulation clinic population. Blood Coagul Fibrinolysis 2016; 27: 53-7.
31. Van Blerk M, Bailleul E, Chatelain B, et al. Influence of dabigatran and rivaroxaban on routine coagulation assays. A nationwide Belgian survey. Thromb Haemost 2015; 113: 154-64.
32. Lippi G, Favaloro EJ. Recent guidelines and recommendations for laboratory assessment of the direct oral anticoagulants (DOACs): is there consensus? Clin Chem Lab Med 2015; 53: 185-97.
33. Douxfils J, Chatelain B, Chatelain C, Dogne JM, Mullier F. Edoxaban: Impact on routine and specific coagulation assays. A practical laboratory guide. Thromb Haemost 2016; 115: 368-81.
34. 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. J Thromb Haemost 2016; 14: 623-7.
35. Ebner M, Peter A, Spencer C, et al. Point-of-Care Testing of Coagulation in Patients Treated With Non-Vitamin K Antagonist Oral Anticoagulants. Stroke 2015; 46: 2741-7.

Supplementary material

Table 1: Mean plasma drug concentrations according to the delay since the last pill intake. Two hours and 3 hours correspond to the expected C_{max} of the drugs while 12 hours (apixaban and dabigatran etexilate) and 24 hours (rivaroxaban) correspond to the C_{trough}. DOACs plasma concentrations were determined by LC-MS/MS.

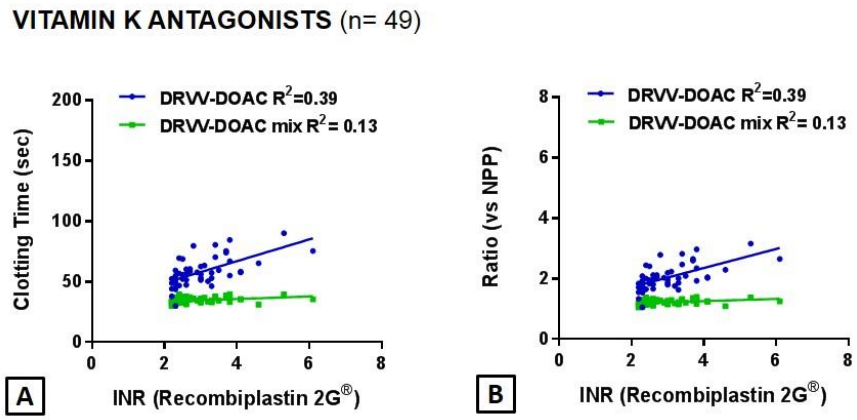
			Mean (ng/ml)	Range (min-max)
Apixaban	2h	n=6	225	99-406
	3h	n=6	225	108-348
	12h	n=16	110	24-221
Dabigatran	2h	n=4	172	0-386
	3h	n=3	233	136-363
	12h	n=2	130	88-172
Rivaroxaban	2h	n=13	267	161-447
	3h	n=15	235	106-485
	24h	n=10	43	0-93

Table 2: DRVV-DOAC measurements in heparin plasma samples. The table shows mean clotting time (CT) expressed in seconds, standard deviation (SD) and coefficient of variation (CV) for the non-mixed and the mixed procedures (N=2). Spiked samples of unfractionated heparin (UFH) and low molecular weight heparin (LMWH) were tested at the following concentrations: 0.5 UI/ml, 1.0 UI/ml and 2.0 UI/ml.

	DRVV-DOAC			DRVV-DOAC mix		
	mean CT (sec)	SD	CV (%)	mean CT (sec)	SD	CV (%)
UFH 0.5 UI/ml	29.9	0.6	1.9	29.5	0.4	1.2
UFH 1.0 UI/ml	30.7	0.2	0.7	28.4	0.6	2.2
UFH 2.0 UI/ml	103.2	5.4	5.2	28.3	0.4	1.5
LMWH 0.5 UI/ml	27.1	0.3	1.0	25.8	0.5	1.9
LMWH 1.0 UI/ml	29.4	0.2	0.7	25.9	0.1	0.3
LMWH 2.0 UI/ml	36.0	0.5	1.4	27.3	0.1	0.3

Supplementary material (continued)

Figure 1: Correlation between DRVV-DOAC measurements and INR values, measured by RecombiPlasTin 2G®, in VKA plasma samples. Fig. 1A and 1B show clotting time of DRVV-DOAC expressed in seconds and as ratios respectively. The blue circles represent the non-mixed assay, while the green squares represent the mixed procedure.



Rivaroxaban plasma levels in patients admitted for bleeding events: insights from a prospective study

Anne-Laure Sennesael, Anne-Sophie Larock, Jonathan Douxfils, Laure Elens, Gabriel Stillemans, Martin Wiesen, Max Taubert, Jean-Michel Dogné, Anne Spinewine, François Mullier

Thrombosis Journal 2018 - Accepted

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Serious bleeding events are frequently experienced by patients taking direct oral anticoagulants (DOAC).
- DOAC plasma concentrations were shown to correlate with bleeding and thromboembolic outcomes.
- Several determinants of bleeding were previously reported in DOAC patients, including age, renal function, drug interactions or genetic polymorphisms.

WHAT THIS STUDY ADDS

- Highly variable plasma levels were observed in 10 rivaroxaban patients admitted to the emergency department for bleeding.
- When using a population pharmacokinetic model, four patients had higher-than-expected plasma concentrations at trough.
- Drug interactions and inappropriate use of rivaroxaban were frequently observed. However, no clear association between patient genotype and plasma levels was shown.

Abstract

BACKGROUND: Serious bleeding events have been frequently described in patients taking direct oral anticoagulants (DOAC). In secondary analyses of phase 3 trials, DOAC plasma concentrations were shown to correlate with bleeding outcomes. This study aimed to describe rivaroxaban plasma levels in patients admitted to the emergency department (ED) for bleeding events. For each patient, risk factors for experiencing bleeding events were also investigated.

METHODS: This analysis was part of an observational study conducted in the ED of two teaching hospitals. Plasma samples from 10 rivaroxaban-treated patients admitted for bleeding events were collected. Rivaroxaban plasma concentrations were determined by calibrated chromogenic anti-Xa assay. The measured rivaroxaban levels were then extrapolated at trough using a published population pharmacokinetic (PopPK) model, and compared to on-therapy ranges observed in large clinical trials. For each patient, clinical, medication and *ABCB1* genotype data were collected.

RESULTS: Rivaroxaban measurements varied from 5 to 358 ng/ml, with a post-intake delay ranging from 9 to 38 hours. At trough, estimated plasma concentrations were between 12 and 251 ng/ml (median value 94 ng/ml). Four patients had higher-than-expected rivaroxaban levels. Inadequate dose regimen, excessive alcohol consumption and lack of treatment reassessment were observed in several patients. Half of patients were taking ≥ 1 drug with potential pharmacokinetics interactions (e.g. amiodarone, diltiazem), while half of patients were taking ≥ 1 drug increasing the risk of bleeding. All 3 patients with available genotyping data and higher-than-expected rivaroxaban levels were heterozygous or homozygous mutated for the *ABCB1* 1236C>T, 2677G>T, 3435 C>T and rs4148738 single nucleotide polymorphisms (SNP).

CONCLUSIONS: Rivaroxaban patients admitted to the ED for bleeding events showed highly variable plasma concentrations. This analysis underlines the usefulness of rapid DOAC measurement and the value of PopPK models to estimate concentrations at trough in a context where the post-intake delay is unmanageable. Close patient follow-up, including renal function assessment and drug interactions review, is essential for bleeding risk minimization.

Introduction

Direct oral anticoagulants (DOAC) are increasingly used in clinical practice, to prevent venous thrombosis or thrombus formation in non-valvular atrial fibrillation[1, 2]. However, serious bleeding events have been frequently described for patients taking DOACs. In 2013-2014, rivaroxaban and dabigatran were among the drugs most commonly implicated in emergency department (ED) admissions in the United States[3]. During phase 3 trials, risk of major bleeding of DOACs was similar to or lower than warfarin, whereas the risk of intracranial hemorrhage was substantially reduced[4, 5]. Recent observational studies have shown similar results in a real-life setting[6].

Although DOACs do not require close therapeutic monitoring, their measurement remains useful in specific clinical situations such as major bleeding and emergency surgery[7]. Assessment of the individual response may also benefit patients with suspected drug accumulation or therapeutic failure. In secondary analyses of phase 3 trials, DOAC plasma concentrations were shown to correlate with bleeding outcomes[8-10]. Conversely, a recent observational study has highlighted the relationship between low DOAC levels, measured in the first month of treatment, and the occurrence of thromboembolic events[11]. However, a therapeutic range has not been defined yet for each DOAC.

To our knowledge, few studies have investigated the intensity of DOAC anticoagulation in patients presenting to the ED for bleeding events. In the RE-VERSE AD study, the median (unbound) dabigatran plasma concentration was 110 ng/ml in patients with uncontrollable or life-threatening bleeding, before the administration of idarucizumab[12]. Bouget and Oger described DOAC levels in 5 patients admitted for major bleeding[13]. However, measurements were not interpreted by taking into account the post-intake delay and other influencing individual factors.

In this study, we aimed to describe rivaroxaban levels in patients admitted to the ED for bleeding events. Measured plasma concentrations were extrapolated at trough using population pharmacokinetic (popPK) modeling, and compared to the expected on-therapy range. Several determinants of bleeding were previously reported in rivaroxaban-treated patients, including older age, renal impairment or drug interactions[14-17]. The inappropriate use of DOAC has also been highlighted in this respect[18, 19]. In addition, *ABCB1* genetic polymorphisms have recently been suggested to contribute to high rivaroxaban levels in a patient admitted for gastrointestinal bleeding[20]. Therefore, a second objective was to investigate and discuss the presence of such risk factors for bleeding events in our rivaroxaban patients.

Materials and Methods

Setting and population

This analysis was part of a prospective observational cohort study, conducted in two teaching hospitals in Belgium to assess the preventability of serious adverse drug reactions related to the use of oral anticoagulants[21]. We collected plasma samples from rivaroxaban patients admitted to the ED for a bleeding event, between July 2015 and January 2016. The study was approved by the Ethics Committees of the Cliniques Universitaires Saint-Luc (Brussels, Belgium) and the CHU UCL Namur (Yvoir, Belgium), and registered with clinicaltrials.gov (NCT02720328). Written informed consent was obtained from each patient.

Rivaroxaban measurement

Blood sampling was performed closely after management and inclusion of the patient in the study. Blood was taken by antecubital venipuncture and collected into 0.109 M sodium citrate (9:1 v/v) tubes. Platelet-poor plasma (PPP) was obtained from the supernatant fraction after double centrifugation for 15 min at

1,500 g at room temperature. PPP was next aliquoted and frozen at -80°C without delay. Frozen plasma samples aliquots were thawed by heating to 37°C for at least 5 min just before experiments.

The following routine clotting assay was performed: prothrombin time (PT) using RecombiPlasTin2G® (Werfen, Lexington, USA; normal range 12.4-14.5 sec). The dilute Russel's Viper Venom Time (dRVVT) was measured with STA®-Staclot®DRVV-Screen (Diagnostica Stago) and STA®-Staclot®DRVV-Confirm (Diagnostica Stago), containing low and high phospholipid concentrations respectively*.

Rivaroxaban plasma concentrations were estimated with the Biophen® Direct Factor Xa Inhibitors (Biophen®DiXaI, Hyphen BioMed, Neuville-Sur-Oise, France), a calibrated chromogenic anti-Xa assay. The procedure was performed on a STA-R® Evolution analyzer according to the manufacturer recommendations, using calibrators from Hyphen BioMed. Commercial rivaroxaban anti-Xa assays have previously demonstrated good accuracy (bias below 8 %) and acceptable precision (inter-laboratory coefficients of variation (CV) 6-25 %)[22]. A procedure dedicated to low concentrations was applied to rivaroxaban plasma concentrations < 50 ng/ml, where plasma samples were diluted 1:8 in buffer and low concentrations standards were used[23].

We extrapolated rivaroxaban plasma concentrations at trough (i.e. 12 h and 24 h after the last drug intake for twice daily and once daily regimen, respectively). To this end, we used a Pop PK model that was previously developed to predict adequate discontinuation intervals in a cohort of rivaroxaban patients scheduled for cardiac catheterization[24]. Residual rivaroxaban concentrations were described by a 2-compartment model with first-order absorption and elimination. Creatinine was the only covariate included in the final model*. Results were then compared to the expected on-therapy ranges at trough (5^{th}-95^{th} percentiles): 12-137 ng/ml

* Adapted from the accepted version

(rivaroxaban 20 mg) and 18-136 ng/ml (rivaroxaban 15 mg) for stroke prevention in non-valvular atrial fibrillation (NVAF), and 6-87 ng/ml for treatment and secondary prevention of venous thromboembolism (VTE)[25].

Data collection

A comprehensive medication history was performed with patients and/or relatives to collect sociodemographic, clinical and medication data (including dose regimen, indication and timing of the last rivaroxaban intake). Patient renal function was estimated based on serum creatinine on admission using Cockroft-Gault equation. We reviewed electronic medical records and contacted GPs, community pharmacists or relatives when necessary. Bleeding severity was assessed using the International Society on Thrombosis and Haemostasis definition. Patient follow-up was undertaken to assess 3-month mortality.

Appropriateness of rivaroxaban prescribing was analyzed according to the Medication Appropriateness Index (MAI)[26]. We applied a DOAC-specific form, previously developed in a pilot study[27]. We documented the concomitant use of P-glycoprotein (P-gp) and CYP3A4 inhibitors or inducers. Regarding potential pharmacodynamics interactions, we considered other anticoagulants, antiplatelet therapy, non-steroidal anti-inflammatory drugs (NSAID), selective serotonin reuptake inhibitors (SSRI) and serotonin-norepinephrine reuptake inhibitors (SNRI). Patient adherence to anticoagulant therapy was evaluated during medication history using an adapted version of the Morisky Medication Adherence Scale[28].

ABCB1 genetic polymorphisms

For all patients except one (because of misunderstanding), an additional blood sample was drawn in EDTA tube and frozen at -20°C without delay until experiments. Genomic DNA was extracted from whole blood, using QIAamp DNA Blood Mini kit (Qiagen, CA, USA). The following single nucleotide polymorphisms

(SNP) of *ABCB1* were detected: rs1128503 (1236C>T, Gly412Gly), rs2032582 (2677G>T, Ala893Ser), rs1045642 (3435 C>T, Ile1145Ile) and rs4148738. Allelic discrimination was performed by TaqMan® (Applied Biosystems, CA, USA) Drug Metabolism Genotyping Assays (C___7586662_10, C_11711720C_30, C__15951365_20, C___1253813_10). Statistical analyses were performed using JMP Pro 13.2.0 (SAS Institute, Cary, NC, USA). Kruskal-Wallis tests were carried out to compare estimated trough concentrations between different genotypes. A p-value of less than 0.05 was considered statistically significant.

Results

Study population

Plasma samples from 10 rivaroxaban patients were included in this analysis. Median age of patients was 75 years, and 80% were female. Five patients had moderate renal impairment on admission (creatinine clearance < 60 ml/min). Indications of rivaroxaban therapy were stroke prevention in NVAF (6/10) and treatment and secondary prevention of VTE (4/10). Clinical characteristics are detailed in Table 1 for each patient. The most frequent adverse events were gastrointestinal bleeding (4/10). Half of bleeding events were considered major and half occurred spontaneously. Red blood cells transfusion and prothrombin complex concentrates administration were operated in 4 and 1 patients, respectively. All patients were alive at 3-month follow-up. Details of bleeding events are presented in Table 2.

Table 1: Characteristics of rivaroxaban patients

No	Sex, age (years)	Weight (kg)	BMI (kg/m^2)	CLcr (ml/min)	Hb (g/dl)	Indication	Dosage	Duration	CHA_2DS_2-VASC	HAS-BLED
1	M, 83	79	23.1	58	13.7	SPAF	20 mg OD	≤ 1 year	5	3
2	F, 70	N/K	N/K	N/K	3.1	SPAF	15 mg OD	> 1 year	3	1
3	F, 87	65	25.7	48	6.4	VTE	20 mg OD	< 30 days	N/A	2
4	F, 67	80	24.7	53	7.2	SPAF	20 mg OD	> 1 year	6	2
5	F, 77	63	25.2	61	14.3	SPAF	15 mg OD	> 1 year	7	3
6	M, 66	100	35	86	15.6	SPAF	20 mg OD	≤ 1 year	2	2
7	F, 72	66	21.6	46	12.8	VTE	20 mg OD	> 1 year	N/A	3
8	F, 90	70	N/K	60	13.8	SPAF	15 mg OD	N/K	6	1
9	F, 86	89	31.2	57	14.9	VTE	15 mg BID	< 30 days	N/A	1
10	F, 69	73	26.8	76	11.3	VTE	20 mg OD	> 1 year	N/A	2

BID: twice-daily, BMI: body mass index, CLcr: creatinine clearance (Cockroft-Gault equation), Hb: haemoglobin, OD: once-daily, N/A: not applicable, N/K: not known, SPAF: stroke prevention in atrial fibrillation, VTE: venous thromboembolism

Table 2: characteristics, management and clinical outcomes of bleeding events

No	Site of bleeding	Bleeding severity	Bleeding occurrence	Management	Length of stay (days)	90-day outcome
1	Hematuria	NMCR	Trauma	-	0	Alive
2	GI	Major	Spontaneous	RBC (3 units)*	N/K	N/K
3	GI	Major	Spontaneous	RBC (2 units)*	3	Alive
4	GI	Major	Spontaneous	RBC (3 units)*	1	Alive
5	Intracranial	Major	Trauma	PCC (2500 IU)	43	Alive
6	GI	NMCR	Post-intervention	-	0	Alive
7	Epistaxis	NMCR	Spontaneous	-	2	Alive
8	Intracranial	Major	Trauma	-	1	Alive
9	Hematuria	NMCR	Spontaneous	-	0	Alive
10	Hematoma	NMCR	Trauma	-	0	N/K

GI: gastro-intestinal, N/K: not known, NMCR: non-major clinically relevant, PCC: prothrombin complex concentrates, RBC: red blood cells. * Transfusion before blood sampling

Rivaroxaban measurement

Measured rivaroxaban levels varied from 5 to 358 ng/ml, with a delay since the last drug intake ranging from 9 to 38 hours. PT was above the normal range in all patients with rivaroxaban concentrations above 30 ng/ml (Table 3). DRVV-Screen was prolonged in all patients while the clotting time of DRVV-Confirm was shorter[†]. When using PopPK model, estimated plasma concentrations at trough were between 12 and 251 ng/ml (median value 94 ng/ml). Elimination clearance (CL/F) was between 2.0 and 7.9 L/h (median value 3.3 L/h). Measured and extrapolated rivaroxaban concentrations are described in Table 3 for each patient, as well as the elimination clearance. Four patients had rivaroxaban levels at trough higher than the expected on-therapy range (No 2, 3, 5, 9).

[†] Adapted from the accepted version

Table 3: Measurement of rivaroxaban plasma concentrations

No	Delay (h)	PT (sec)	DRVV Screen (sec)	DRVV Confirm (sec)	Biophen® DiXaI (ng/ml)	Estimated cc at trough (ng/ml)	Clearance (L/h)	Potential PK drug interactions	Potential PD drug interactions
1	28	18.7	76.7	54.9	84.8	98	3.3	-	Ibuprofen
2	22	19.6	76.3	57.7	237.5	**181***	2.0	Diltiazem (↑) Clarithromycin (↑)	Escitalopram
3	38	15.4	52.6	46.9	70.8	**125***	2.9	Simvastatin (↑)	/
4	27	17.7	70.8	54.4	58.3	69	3.9	-	Diclofenac Escitalopram
5	27	N/K	N/K	N/K	139.4	**134***	2.4	Amiodarone (↑)	Aspirin
6	26	12.9	47.7	38.9	4.9	12	7.9	Amiodarone (↑)	/
7	37	14.4	50.0	42.0	18.3	44	4.8	Diltiazem (↑)	/
8	27	15.3	50.0	45.8	86.5	89	3.0	/	/
9	9	26.0	123.1	75.4	357.8	**251***	3.2	/	/
10	13	N/K	93.5	69.0	184.7	72	3.9	/	Duloxetine Paroxetine

Biophen®DiXaI: Biophen® Direct Factor Xa Inhibitors, cc: concentration, DRVV: dilute Russell's Viper Venom, N/K: not known, PD: pharmacodynamics, PK: pharmacokinetics, PT: prothrombin time (normal range: 12.4-14.5 s), ↑: increased plasma concentrations, ↓: decreased plasma concentrations.

* **Estimated trough concentration above the on-therapy range.** We considered higher-than-expected rivaroxaban levels for patient 5, as measured concentration was higher than 136 ng/ml. The estimated trough concentration presented by patient 9 corresponds to a 12h-delay since the last drug intake, as this patient was taking rivaroxaban BID.

Analysis of risk factors for bleeding events

The analysis of rivaroxaban prescriptions revealed that at least one criterion of the MAI was inappropriate in 8 of 10 patients. Three patients received an inadequate dosing regimen: 20 mg OD instead of 15 mg OD (No 3, 7), and 15 mg BID instead of 20 mg OD (No 9). Two of them had estimated trough concentration above the on-therapy range. Three patients had an excessive alcohol consumption (≥ 2 units/day; No 1, 4, 7). Moreover, in two patients treated for VTE prevention, treatment indication had not been reassessed for years (No 3, 10).

Half of patients were taking at least 1 drug with potential pharmacokinetic interactions (Table 3). Three of them had higher-than-expected rivaroxaban plasma concentrations at trough. The most frequent interacting drugs were amiodarone and diltiazem. Moreover, half of patients were taking at least 1 drug increasing the risk of bleeding (Table 3). We mainly observed the concomitant use of SSRI (n=3) and NSAID (n=2). Regarding adherence to anticoagulant therapy, three patients reported forgetting to take their medicine in rare circumstances (No 1, 4, 7). One patient mentioned a variable time of intake (No 7).

ABCB1 genetic polymorphisms were analyzed in 9 of 10 patients. Genotyping data are presented in Table 4 for the *ABCB1* 1236C>T, 2677G>T, 3435 C>T and rs4148738 SNP. Of the four patients with higher-than-expected rivaroxaban trough levels, three carried at least one variant allele for each of these SNP (No 2, 3, 5 – blood sample not available for patient No 9). However, no clear association between ABCB1 genotype and estimated trough concentrations was observed ($p>0.05$).

Table 4: *ABCB1* genotyping in 10 rivaroxaban patients with bleeding events

No	1236C>T	2677G>T	3435C>T	rs4148738
1	CC	GG	CC	AA
2	**CT**	**GT**	**CT**	**GA**
3	**CT**	**GT**	**CT**	**GA**
4	TT	TT	CT	GG
5	**CT**	**GT**	**TT**	**GA**
6	TT	TT	TT	GG
7	CT	GT	TT	GA
8	TT	TT	TT	GG
9	**N/K**	**N/K**	**N/K**	**N/K**
10	CC	GG	CT	AA

N/K: not known. Patients with higher-than-expected rivaroxaban levels are shown in bold.

Discussion

In this analysis of 10 patients admitted for bleeding events, we observed a large range of rivaroxaban levels. The application of a previously published PopPK model revealed that four patients had higher-than-expected plasma concentrations at trough. Several risk factors for bleeding were found at the individual level, including older age, inappropriate use of rivaroxaban and drug interactions.

To our knowledge, this is the first study to analyze in depth rivaroxaban measurements in the context of bleeding events. Inter-individual variability in DOAC exposure has been previously investigated in routine care, showing plasma levels outside the on-therapy range in 40 % of rivaroxaban patients[29]. In four Italian anticoagulation clinics, rivaroxaban measurements varied nearly 15-fold among NVAF patients, with a mean trough level around 40 ng/ml[30]. This variability is in agreement with our results, although we estimated a higher median trough concentration (94 ng/ml). This reflects a delayed rivaroxaban clearance in our

bleeding patients (median CL/F 3.3 L/h, compared to 4.9 L/h in patients scheduled for cardiac catheterization or 6.1 L/h in AF patients as previously reported)[24, 31]. Two recent cohort studies have shown median rivaroxaban levels of 124 ng/ml in patients with severe bleeding events, and 102 ng/ml in patients admitted for intracranial hemorrhage[32, 33]. Delay since the last drug intake was 12 hours (median value) in the former case, and less than 24 hours for most patients in the latter case.

Rivaroxaban measurement for bleeding management

The management of DOAC-related bleeding includes the temporary discontinuation of the oral anticoagulant, supportive measures and the administration of reversal agents, depending on the severity of the event [34, 35]. Andexanet alpha has recently been approved by the FDA to reverse the anticoagulant effect of rivaroxaban, while prothrombin complex concentrates were also suggested as an alternative [36-38]. In this context, rapid laboratory measurement may provide valuable assistance to warrant and monitor antidote administration, or manage urgent interventions [7].

In the present study, we observed that PT and DRVVT assays were prolonged in all patients with clinically relevant concentrations (>30 ng/ml) of rivaroxaban. This is in line with practical guidance stating that normal PT or DRVVT can exclude above on-therapy levels of rivaroxaban[7, 39].‡ However, we employed a sensitive thromboplastin reagent (RecombiPlasTin 2G®) for the PT assay [40]. A nationwide Belgian survey has previously highlighted a wide variation in response to rivaroxaban according to the reagent used [41]. Commercial specific assays are currently available for all DOACs [42]. They are more accurate but require calibrators and controls. Turnaround times around 30 minutes have nevertheless been reported in a daily practice context [43, 44].

‡ Adapted from the accepted version

Factors associated with an increased risk of bleeding

Several risk factors for bleeding events were highlighted in our 10 rivaroxaban patients. First, half of them were older than 75 years, and half of them had moderate renal impairment. Older age and renal insufficiency were previously demonstrated as contributing factors to major bleeding in rivaroxaban patients[14]. Recently, glomerular filtration rates below 60 ml/min have been independently associated with higher-than-expected residual rivaroxaban levels in a perioperative setting[45]. This is not surprising since one third of the rivaroxaban dose is eliminated unchanged by the kidneys[25]. However, in our analysis, only two patients with moderate renal impairment had trough concentrations above the on-therapy range. Second, despite the highest convenience and fixed-dose regimen of DOAC, some of our patients did not receive the appropriate dose of rivaroxaban. In particular, one patient (No 9) was still taking rivaroxaban 15 mg BID while the 21-day treatment phase of VTE had been completed. Yao and colleagues highlighted that patients with indication for dose reduction were often potentially overdosed, leading to an increased risk of major bleeding[19].

Drugs interactions with P-gp or CYP3A4 inhibitors were frequent in our patients, as previously reported[32, 46]. One patient (No 2) was taking diltiazem and clarithromycin, two inhibitors of both P-gp and CYP3A4. He had an estimated rivaroxaban level of 181 ng/ml at trough, exceeding the 95th percentile of the on-therapy range (136 ng/ml). In healthy volunteers, clarithromycin has been shown to promote a 2-fold increase in rivaroxaban exposure[47]. Similarly, we extrapolated a rivaroxaban trough concentration of 125 ng/ml in a patient taking simvastatin (No 3). This lipid-lowering agent, described as a P-gp inhibitor based on *in vitro* findings, has been associated with a higher risk of major bleeding in patients taking dabigatran[48]. Pharmacodynamic interactions were also widely observed. Aspirin has often no valid indication in anticoagulated patients, while combination therapy has been shown to increase the risk of major bleeding[16, 49]. A similar increase was associated with the addition of SSRIs, the most prescribed class of

antidepressants[50]. It should be noted that rivaroxaban could also influence the pharmacokinetics of other drugs sharing the same metabolic or transport pathways. However, no significant effect of rivaroxaban on the pharmacokinetics of digoxin, atorvastatin or midazolam was observed in healthy volunteers[25]. In a clinical context, a limited increase in trough concentrations of calcineurin inhibitors (<20%) was shown in transplant patients[51].[§]

An original aspect of this work was the investigation of several *ABCB1* polymorphisms. All 3 patients with higher-than-expected rivaroxaban levels and available genotyping data were at least heterozygous mutated for the 1236C>T, 2677G>T, 3435C>T and rs4148738 SNP. Previous experiments have shown that the 1236T-2677T-3435T variant haplotype decreased ABCB1 expression or transport towards several drugs such as anticancer agents[52-54]. These results support our observations, as a decreased efflux of rivaroxaban may have led to drug accumulation. However, in a recent study conducted in 60 healthy volunteers, the 1236T-2677T-3435T haplotype was not a significant determinant of rivaroxaban pharmacokinetics[47]. This strengthens the need for additional studies to clarify the impact of *ABCB1* polymorphisms on rivaroxaban transport. Indeed, our findings might also be due to the high frequency of the variant genotype 1236T-2677T-3435T (up to 50% of the Caucasian population is expected to be heterozygous mutated). Furthermore, these three patients were also receiving concomitant interacting medications, as previously discussed.

As only 3 patients had a HAS-BLED score ≥ 3, the suitability of this score to predict bleeding risk in DOAC patients may be questioned. No bleeding risk score has been developed specifically in DOAC population[55]. The HAS-BLED score was validated in VKA-treated patients and showed modest predictive ability, as other bleeding risk scores in AF patients[56]. In 2016, the European Society of Cardiology provided a combined list of risk factors for bleeding, instead of recommending a specific

[§] Adapted from the accepted version

scale[57]. Based on this analysis, we could suggest adding significant pharmacokinetics drug-drug interactions or the inadequacy of dosing to modifiable risk factors for bleeding in DOAC patients. Moreover, a creatinine clearance < 60 ml/min should be considered as it was for the recent ORBIT bleeding score[58].**

The study presents several limitations. First, statistical analysis was limited by the small number of plasma samples collected. However, this allowed an in-depth analysis of rivaroxaban measurements, including clinical, medication and genetic characteristics at the individual level. Second, three patients (No 2-4) were transfused with red blood cells (RBC) before sampling. For these patients, we cannot exclude that rivaroxaban measurements were not influenced by fluid volume or factor Xa content of packed RBC. However, Biophen®DiXaI was designed for minimizing the interference of plasma factors. Third, estimation of renal function was only based on serum creatinine on admission, as previous laboratory results performed in primary care were not available. Finally, we used a Pop PK model developed in patients scheduled for cardiac catheterization[24]. On average, our 10 patients were older (76.7 vs 66.8 years) and had lower creatinine clearance (60.6 vs 78.7 ml/min), while BMI was similar (26.7 vs 27.0 kg/m^2). We cannot exclude the influence of demographics and overall health status on PK parameters, even if similar estimates were reported between AF, VTE and ACS populations[31].** The Pop PK model assumed no variability in the volume of distribution, while inter-individual variability in V/F was 18% in another Pop PK model from AF patients[31]. However, simulations showed the limited influence of this variability on predicted exposure estimates[24]. Another limitation of the model is that it did not take into account drug-drug interactions.**

** Adapted from the accepted version

Conclusion

In conclusion, rivaroxaban patients admitted to the ED for bleeding events showed highly variable plasma concentrations and a delayed elimination clearance. This underlines the usefulness of rapid DOAC measurement to assess contribution to the adverse event, manage urgent procedures or warrant antidote administration. Close patient follow-up, including renal function assessment and drug interactions review, is essential for bleeding risk minimization. Further studies are needed to investigate the impact of *ABCB1* polymorphisms on DOAC transport.

Acknowledgements

A.L. Sennesael is a Research Fellow of the Fonds National de la Recherche Scientifique (FNRS). The authors would like to thank the emergency departments of the Cliniques Universitaires Saint-Luc and CHU UCL Namur, Pr F. Verschuren, Dr X. Muschart, Mrs J. Baudar, Mrs M. Guldenpfennig for their contributions to this work.

Conflicts of interest

The authors have no relevant conflicts of interest to disclose.

REFERENCES

1. Turpie AG, Esmon C. Venous and arterial thrombosis--pathogenesis and the rationale for anticoagulation. Thromb Haemost 2011; 105: 586-96.
2. Santos-Gallego CG, Bayon J, Badimon JJ. Thrombi of different pathologies: implications for diagnosis and treatment. Curr Treat Options Cardiovasc Med 2010; 12: 274-91.
3. Shehab N, Lovegrove MC, Geller AI, Rose KO, Weidle NJ, Budnitz DS. US emergency department visits for outpatient adverse drug events, 2013-2014. JAMA 2016; 316: 2115-25.
4. Ruff CT, Giugliano RP, Braunwald E, et al. Comparison of the efficacy and safety of new oral anticoagulants with warfarin in patients with atrial fibrillation: a meta-analysis of randomised trials. Lancet 2014; 383: 955-62.
5. van Es N, Coppens M, Schulman S, Middeldorp S, Buller HR. Direct oral anticoagulants compared with vitamin K antagonists for acute venous thromboembolism: evidence from phase 3 trials. Blood 2014; 124: 1968-75.
6. Ntaios G, Papavasileiou V, Makaritsis K, Vemmos K, Michel P, Lip GYH. Real-World Setting Comparison of Nonvitamin-K Antagonist Oral Anticoagulants Versus Vitamin-K Antagonists for Stroke Prevention in Atrial Fibrillation: A Systematic Review and Meta-Analysis. Stroke 2017; 48: 2494-503.
7. Douxfils J, Ageno W, Samama CM, et al. Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians. J Thromb Haemost 2018; 16: 209-19.
8. Eikelboom JW, Quinlan DJ, Hirsh J, Connolly SJ, Weitz JI. Laboratory Monitoring of Non-Vitamin K Antagonist Oral Anticoagulant Use in Patients With Atrial Fibrillation: A Review. JAMA Cardiol 2017; 2: 566-74.
9. 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). J Am Coll Cardiol 2014; 63: 321-8.
10. 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: 2288-95.
11. Testa S, Paoletti O, Legnani C, et al. Low drug levels and thrombotic complications in high-risk atrial fibrillation patients treated with direct oral anticoagulants. J Thromb Haemost 2018; 16: 842-8.
12. Pollack CVJ, Reilly PA, van Ryn J, et al. Idarucizumab for Dabigatran Reversal — Full Cohort Analysis. New England Journal of Medicine 2017: doi: 10.1056/NEJMoa1707278.
13. Bouget J, Oger E. Emergency admissions for major haemorrhage associated with direct oral anticoagulants. Thrombosis Research 2015; 136: 1190-4.
14. 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: 955-62.
15. 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. Eur Heart J 2016; 37: 1145-53.
16. Shah R, Hellkamp A, Lokhnygina Y, et al. Use of concomitant aspirin in patients with atrial fibrillation: Findings from the ROCKET AF trial. Am Heart J 2016; 179: 77-86.

17. Washam JB, Hellkamp AS, Lokhnygina Y, et al. Efficacy and Safety of Rivaroxaban Versus Warfarin in Patients Taking Nondihydropyridine Calcium Channel Blockers for Atrial Fibrillation (from the ROCKET AF Trial). Am J Cardiol 2017; 120: 588-94.
18. Whitworth MM, Haase KK, Fike DS, Bharadwaj RM, Young RB, MacLaughlin EJ. Utilization and prescribing patterns of direct oral anticoagulants. Int J Gen Med 2017; 10: 87-94.
19. Yao X, Shah ND, Sangaralingham LR, Gersh BJ, Noseworthy PA. Non-Vitamin K Antagonist Oral Anticoagulant Dosing in Patients With Atrial Fibrillation and Renal Dysfunction. J Am Coll Cardiol 2017; 69: 2779-90.
20. Ing Lorenzini K, Daali Y, Fontana P, Desmeules J, Samer C. Rivaroxaban-Induced Hemorrhage Associated with ABCB1 Genetic Defect. Front Pharmacol 2016; 7: 494.
21. Sennesael AL, Larock AS, Devalet B, et al. Preventability of serious thromboembolic and bleeding events related to the use of oral anticoagulants: a prospective study. Br J Clin Pharmacol 2018.
22. Gouin-Thibault I, Freyburger G, de Maistre E, et al. Evaluation of dabigatran, rivaroxaban and apixaban target-specific assays in a multicenter French study. Thromb Res 2017; 158: 126-33.
23. Lessire S, Douxfils J, Pochet L, et al. Estimation of Rivaroxaban Plasma Concentrations in the Perioperative Setting in Patients With or Without Heparin Bridging. Clin Appl Thromb Hemost 2018; 24: 129-38.
24. Wiesen MHJ, Blaich C, Taubert M, et al. Residual rivaroxaban exposure after discontinuation of anticoagulant therapy in patients undergoing cardiac catheterization. Eur J Clin Pharmacol 2018; 74: 611-8.
25. Mueck W, Stampfuss J, Kubitza D, Becka M. Clinical pharmacokinetic and pharmacodynamic profile of rivaroxaban. Clin Pharmacokinet 2014; 53: 1-16.
26. Hanlon JT, Schmader KE, Samsa GP, et al. A method for assessing drug therapy appropriateness. J Clin Epidemiol 1992; 45: 1045-51.
27. Larock AS, Mullier F, Sennesael AL, et al. Appropriateness of prescribing dabigatran etexilate and rivaroxaban in patients with nonvalvular atrial fibrillation: a prospective study. Ann Pharmacother 2014; 48: 1258-68.
28. Wang Y, Kong MC, Ko Y. Psychometric properties of the 8-item Morisky Medication Adherence Scale in patients taking warfarin. Thromb Haemost 2012; 108: 789-95.
29. Gulilat M, Tang A, Gryn SE, et al. Interpatient Variation in Rivaroxaban and Apixaban Plasma Concentrations in Routine Care. Can J Cardiol 2017; 33: 1036-43.
30. 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. Thromb Res 2016; 137: 178-83.
31. Girgis IG, Patel MR, Peters GR, et al. Population pharmacokinetics and pharmacodynamics of rivaroxaban in patients with non-valvular atrial fibrillation: results from ROCKET AF. J Clin Pharmacol 2014; 54: 917-27.
32. Albaladejo P, Samama CM, Sie P, et al. Management of Severe Bleeding in Patients Treated with Direct Oral Anticoagulants: An Observational Registry Analysis. Anesthesiology 2017; 127: 111-20.
33. Seiffge DJ, Kagi G, Michel P, et al. Rivaroxaban plasma levels in acute ischemic stroke and intracerebral hemorrhage. Ann Neurol 2018; 83: 451-9.
34. Steffel J, Verhamme P, Potpara TS, et al. The 2018 European Heart Rhythm Association Practical Guide on the use of non-vitamin K antagonist oral anticoagulants in patients with atrial fibrillation. Eur Heart J 2018.

35. Tomaselli GF, Mahaffey KW, Cuker A, et al. 2017 ACC Expert Consensus Decision Pathway on Management of Bleeding in Patients on Oral Anticoagulants: A Report of the American College of Cardiology Task Force on Expert Consensus Decision Pathways. J Am Coll Cardiol 2017; 70: 3042-67.
36. Connolly SJ, Milling TJ, Jr., Eikelboom JW, et al. Andexanet Alfa for Acute Major Bleeding Associated with Factor Xa Inhibitors. N Engl J Med 2016; 375: 1131-41.
37. Andexxa-An Antidote for Apixaban and Rivaroxaban. JAMA 2018; 320: 399-400.
38. Eerenberg ES, Kamphuisen PW, Sijpkens MK, Meijers JC, Buller HR, Levi M. Reversal of rivaroxaban and dabigatran by prothrombin complex concentrate: a randomized, placebo-controlled, crossover study in healthy subjects. Circulation 2011; 124: 1573-9.
39. Cuker A. Laboratory measurement of the non-vitamin K antagonist oral anticoagulants: selecting the optimal assay based on drug, assay availability, and clinical indication. J Thromb Thrombolysis 2016; 41: 241-7.
40. 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. Thromb Res 2012; 130: 956-66.
41. Van Blerk M, Bailleul E, Chatelain B, et al. Influence of dabigatran and rivaroxaban on routine coagulation assays. A nationwide Belgian survey. Thromb Haemost 2015; 113: 154-64.
42. Douxfils J, Gosselin RC. Laboratory Assessment of Direct Oral Anticoagulants. Semin Thromb Hemost 2017; 43: 277-90.
43. Seiffge DJ, Traenka C, Polymeris A, et al. Feasibility of rapid measurement of Rivaroxaban plasma levels in patients with acute stroke. J Thromb Thrombolysis 2017; 43: 112-6.
44. Dincq AS, Lessire S, Pirard G, et al. Reduction of the turn-around time for the measurement of rivaroxaban and apixaban: Assessment of the performance of a rapid centrifugation method. Int J Lab Hematol 2018.
45. Kaserer A, Schedler A, Jetter A, et al. Risk Factors for Higher-than-Expected Residual Rivaroxaban Plasma Concentrations in Real-Life Patients. Thromb Haemost 2018; 118: 808-17.
46. Jungbauer L, Dobias C, Stollberger C, Weidinger F. The frequency of prescription of P-glycoprotein-affecting drugs in atrial fibrillation. J Thromb Haemost 2010; 8: 2069-70.
47. Gouin-Thibault I, Delavenne X, Blanchard A, et al. Interindividual variability in dabigatran and rivaroxaban exposure: contribution of ABCB1 genetic polymorphisms and interaction with clarithromycin. J Thromb Haemost 2017; 15: 273-83.
48. Antoniou T, Macdonald EM, Yao Z, et al. Association between statin use and ischemic stroke or major hemorrhage in patients taking dabigatran for atrial fibrillation. Cmaj 2017; 189: E4-e10.
49. Hernandez I, Baik SH, Pinera A, Zhang Y. Risk of bleeding with dabigatran in atrial fibrillation. JAMA Intern Med 2015; 175: 18-24.
50. Quinn GR, Singer DE, Chang Y, et al. Effect of selective serotonin reuptake inhibitors on bleeding risk in patients with atrial fibrillation taking warfarin. Am J Cardiol 2014; 114: 583-6.
51. Vanhove T, Spriet I, Annaert P, et al. Effect of the Direct Oral Anticoagulants Rivaroxaban and Apixaban on the Disposition of Calcineurin Inhibitors in Transplant Recipients. Ther Drug Monit 2017; 39: 77-82.
52. Dessilly G, Panin N, Elens L, Haufroid V, Demoulin JB. Impact of ABCB1 1236C > T-2677G > T-3435C > T polymorphisms on the anti-proliferative activity of imatinib, nilotinib, dasatinib and ponatinib. Sci Rep 2016; 6: 29559.

53. Salama NN, Yang Z, Bui T, Ho RJ. MDR1 haplotypes significantly minimize intracellular uptake and transcellular P-gp substrate transport in recombinant LLC-PK1 cells. J Pharm Sci 2006; 95: 2293-308.

54. Hoffmeyer S, Burk O, von Richter O, et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proc Natl Acad Sci U S A 2000; 97: 3473-8.

55. Hellenbart EL, Faulkenberg KD, Finks SW. Evaluation of bleeding in patients receiving direct oral anticoagulants. Vasc Health Risk Manag 2017; 13: 325-42.

56. Proietti M, Hijazi Z, Andersson U, et al. Comparison of bleeding risk scores in patients with atrial fibrillation: insights from the RE-LY trial. J Intern Med 2018; 283: 282-92.

57. Kirchhof P, Benussi S, Kotecha D, et al. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. Europace 2016; 18: 1609-78.

58. O'Brien EC, Simon DN, Thomas LE, et al. The ORBIT bleeding score: a simple bedside score to assess bleeding risk in atrial fibrillation. Eur Heart J 2015.

Chapter III

Impact of ABCB1 polymorphisms on the intra-individual variability in DOAC levels

Effect of ABCB1 genetic polymorphisms on the transport of rivaroxaban in HEK293 recombinant cell lines

Anne-Laure Sennesael, Nadtha Panin, Christelle Vancraeynest, Lionel Pochet, Anne Spinewine, Vincent Haufroid, Laure Elens

Scientific Reports 2018; 8:10514

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- ABCB1 (also called P-glycoprotein) is involved in the transport of all DOAC.
- *ABCB1* polymorphisms have been shown to influence the transport activity towards several drugs such as immunosuppressants.
- Significant inter-individual variability in DOAC plasma concentrations has been described in clinical practice.

WHAT THIS STUDY ADDS

- The intracellular accumulation of rivaroxaban was decreased in recombinant cell lines overexpressing ABCB1.
- However, the *ABCB1* 1236C>T-2677G>T-3435C>T and 1199G>A SNP had no significant effect on the transport of rivaroxaban.
- These genetic polymorphisms are unlikely to contribute to the inter-individual variability in rivaroxaban plasma levels.

Abstract

Direct oral anticoagulants (DOAC) are substrates for the ABCB1 transporter (also called P-glycoprotein), an active efflux pump. *ABCB1* polymorphisms have been previously reported to influence the pharmacokinetics of several drugs such as immunosuppressants and tyrosine kinase inhibitors. Recently, *in vivo* studies have suggested that genetic variants might contribute to the inter-individual variability in DOAC plasma concentrations. Therefore, we evaluated the *in vitro* effect of the most common coding *ABCB1* single nucleotide polymorphisms (SNP), 1236C>T-2677G>T-3435C>T, and the coding *ABCB1* 1199G>A SNP on the transport activity towards rivaroxaban.

HEK293 cells were transfected to overexpress the ABCB1 wild-type (1236C-2677G-3435C, 1199G) or variant proteins (1236C-2677G-3435T, 1236T-2677T-3435T or 1199A). ABCB1 expression decreased the intracellular accumulation of rivaroxaban, when compared to control cells. This confirms the involvement of ABCB1 in the active transport of rivaroxaban. However, the *ABCB1* 1236C>T-2677G>T-3435C>T and 1199G>A SNPs had no significant influence on the intracellular accumulation of rivaroxaban when compared to the wild-type protein.

These results suggest that the *ABCB1* coding SNPs investigated in the present study are unlikely to contribute to the inter-individual variability in rivaroxaban plasma concentrations.

Introduction

The landscape of oral anticoagulant therapy has significantly changed in the last years. Direct oral anticoagulants (DOAC) have been approved for stroke prevention in non-valvular atrial fibrillation and the treatment as well as the secondary prevention of venous thromboembolism. They are increasingly used in clinical practice, partly due to their higher convenience for clinicians and patients (fixed-dose regimen, fewer interactions with drugs and food) compared with vitamin K antagonists (VKA)[1]. Four DOACs are currently available: three direct factor Xa inhibitors (apixaban, edoxaban, rivaroxaban) and one direct thrombin inhibitor (dabigatran etexilate). In 2016, guidelines issued by the European Society of Cardiology or the American College of Chest Physicians have recommended DOACs over VKAs in eligible patients[2, 3].

All DOACs are substrates for the ABCB1 transporter, also called P-glycoprotein (P-gp) or formerly designated as the multidrug resistance 1 (MDR1) protein[4]. This active efflux pump belongs to the ATP-binding cassette transporter superfamily and is involved in the disposition of multiple drugs from diverse classes such as anticancer agents, immunosuppressants or antibiotics[5]. ABCB1 expression at the apical membrane of enterocytes limits absorption, while its localization on the luminal membrane of hepatocytes and renal tubular cells enhances biliary and renal excretion respectively. In patients taking rivaroxaban, the ABCB1 protein expression in renal tubular cells is particularly important given that more than one third of the dose is eliminated unchanged in the urine[6]. Many medications that are commonly prescribed in patients with atrial fibrillation (AF) can inhibit or induce ABCB1 activity and thereby influence DOAC pharmacokinetics[7]. For instance, it has been demonstrated that the concurrent use of moderate inhibitors of ABCB1 such as amiodarone or verapamil led to a nearly 40% increase in rivaroxaban exposure[8, 9]. Combining this with renal impairment or older age, two common characteristics in AF patients, produced even stronger effect on rivaroxaban pharmacokinetics.

Genetic polymorphisms have also been reported to influence ABCB1 activity and/or expression[5]. The *ABCB1* gene, located on chromosome 7, is composed of 29 exons in a 251.3-kb genomic region. More than 60 coding single nucleotide polymorphisms (SNP) have been described for *ABCB1*[10]. The three most common SNPs in the coding region are rs1128503 (1236C>T, Gly412Gly), rs2032582 (2677G>T, Ala893Ser) and rs1045642 (3435 C>T, Ile1145Ile). They are in strong linkage disequilibrium and present a minor allelic frequency around 50% in the Caucasian population. They have been widely investigated, with inconsistent effects on drug disposition, drug response and toxicity[5]. Another SNP of interest is the 1199G>A non-synonymous coding SNP (rs2229109), with an allelic frequency around 6% in Caucasians. The amino acid change (Ser400Asn) caused by this SNP is located in a cytoplasmic loop involved in substrate recognition. In previous *in vitro* studies, it has been shown that 1199G>A affects the transport of tacrolimus, vinblastine or tyrosine kinase inhibitors but that cyclosporine A, Rh123 and doxorubicin are transported in a similar extent by the wild-type and variant ABCB1 proteins[11, 12].

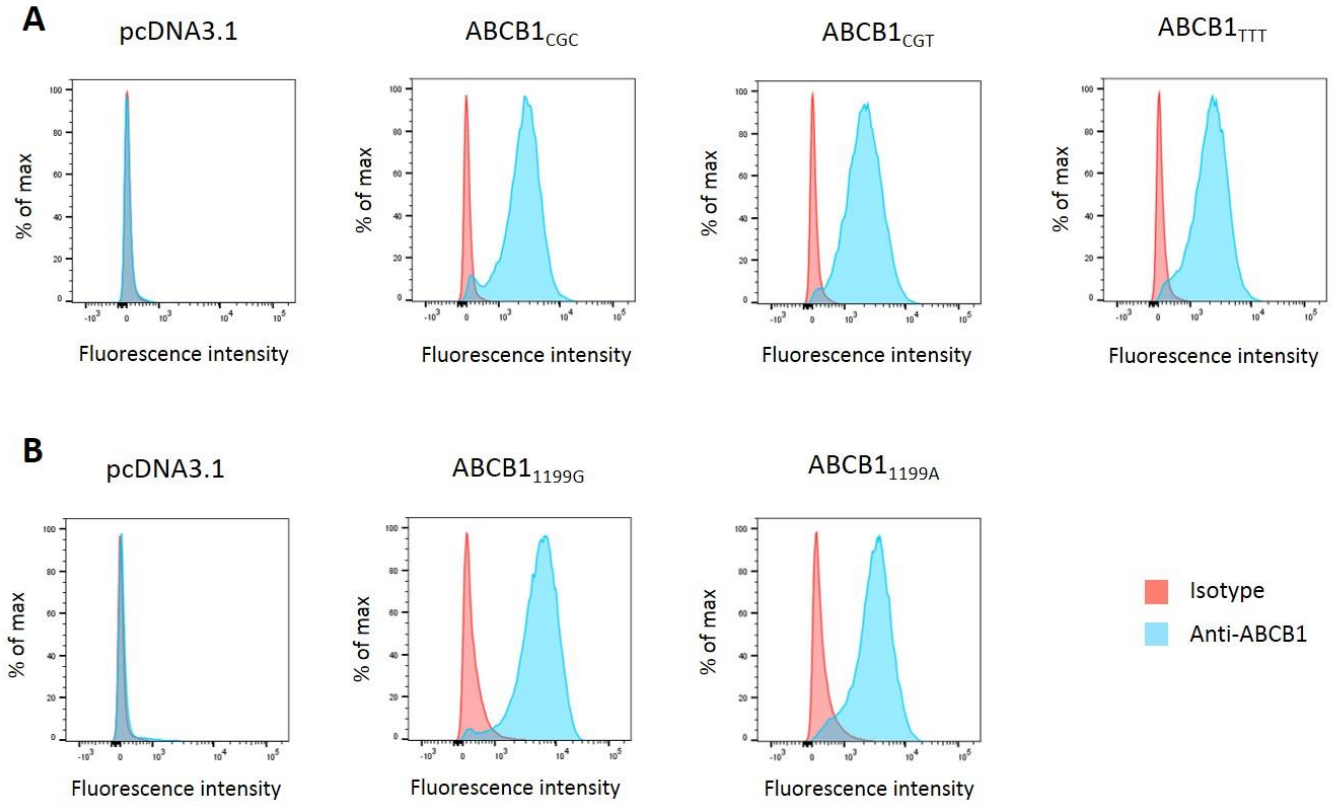
Despite the fixed-dose regimen of DOACs, significant inter-individual variability in peak and trough plasma concentrations has been described[13, 14]. In a prospective cohort study, 40% of patients with atrial fibrillation had rivaroxaban plasma measurements outside the 5^{th}-95^{th} percentile interval observed in phase 3 trials[15]. Moreover, in an analysis of the ROCKET-AF trial, rivaroxaban exposure predicted the risk of major bleeding[16]. Inversely, it was recently highlighted that DOAC patients experiencing thromboembolic events had lower plasma levels in the first month of treatment[17]. Genetic polymorphisms have been proposed to explain why patients taking the same DOAC dose present highly variable plasma concentrations[18]. Therefore, this study aimed to evaluate the in *vitro* effect of the *ABCB1* 1236C>T-2677G>T-3435C>T and 1199G>A SNPs on the transport activity towards rivaroxaban.

Results

Generation of ABCB1 recombinant cell lines

HEK293 cells overexpressing ABCB1 wild-type and variant proteins have been previously generated and characterized[11, 19]. For *ABCB1* 1236C>T-2677G>T-3435C>T, the recombinant models used consisted of stably transfected cell lines with the pcDNA3.1 empty vector, $HEK_{pcDNA3.1,}$ the wild-type vector, $HEK_{1236C\text{-}2677G\text{-}3435C}$ or two different combinations of the variant cDNA, $HEK_{1236C\text{-}2677G\text{-}3435T}$ and $HEK_{1236T\text{-}2677T\text{-}3435T}$, hereafter referred to as $HEK_{control,}$ $HEK_{CGC,}$ HEK_{CGT} and HEK_{TTT} respectively. For *ABCB1* 1199G>A, three recombinant cell lines were used: $HEK_{control,}$ HEK_{1199G} (wild-type protein) and HEK_{1199A} (variant protein). To ensure similar ABCB1 surface expression among the different cell lines, cells were sorted using fluorescence activated cell sorting (FACS) with fluorescence parameters gated on the same level of intensity. As shown in Figure 1A, similar ABCB1 surface expression was observed by analytical flow cytometry among recombinant cell lines, both for the *ABCB1* 1236C>T-2677G>T-3435C>T and 1199G>A SNPs. In contrast, there was no fluorescence detected in $HEK_{control}$ ensuring no or at least very low basal expression in our negative controls.

Figure 1. ABCB1 cell surface expression. Flow cytometry histograms of HEK293 cells transfected with **(A)** the empty pcDNA3.1 vector, *ABCB1*$_{CGC}$, *ABCB1*$_{CGT}$ or *ABCB1*$_{TTT}$ and (B) the empty pcDNA3.1 vector, *ABCB1*$_{1199G}$, *ABCB1*$_{1199A}$. Cells were incubated with a FITC anti-ABCB1 antibody (blue) or a matched isotypic control (red)



Impact of *ABCB1* 1236C>T-2677G>T-3435C>T polymorphisms on the intracellular accumulation of rivaroxaban

First, we assessed the impact of *ABCB1* 1236C>T-2677G>T-3435C>T on the ABCB1 transport activity towards rivaroxaban. $HEK_{control,}$ $HEK_{CGC,}$ HEK_{CGT} and HEK_{TTT} were incubated with different concentrations of rivaroxaban ranging from 50 to 1000 ng/ml. These concentrations were chosen to cover rivaroxaban plasma levels encountered in clinical practice (concentrations up to 600 ng/ml were described in real-life patients with AF)[14, 15]. As shown in figure 2, the intracellular accumulation of rivaroxaban was significantly decreased in recombinant cell lines overexpressing ABCB1 when compared to control cells (figure 2, [50, 250 and 500 ng/ml], $p<0.01$; [100 and 1000 ng/ml], $p<0.001$). However, we observed that the intracellular accumulation of rivaroxaban was similar between HEK_{CGC}, HEK_{CGT} and HEK_{TTT} (figure 2, $p>0.05$ at all concentrations).

Impact of *ABCB1* 1199G>A polymorphism on the intracellular accumulation of rivaroxaban

Same experiments were conducted to investigate the impact of *ABCB1* 1199G>A on the ABCB1 activity towards rivaroxaban. Again, the intracellular accumulation of rivaroxaban was lower in cell lines overexpressing ABCB1, when compared with control cells whatever their genotype (figure 3, [from 50 to 1000 ng/ml], $p<0.001$). As for the three other variants, we did not find any statistical difference in the intracellular accumulation of rivaroxaban between cells overexpressing the ABCB1 1199G and 1199A proteins (figure 3, $p>0.05$ at all concentrations).

Figure 2. Impact of *ABCB1* 1236C>T-2677G>T-3435C>T on the intracellular accumulation of rivaroxaban. Intracellular accumulation of rivaroxaban after 120 min of incubation (N=3) at different concentrations in $HEK_{control}$, HEK_{CGC}, HEK_{CGT} and HEK_{TTT}.

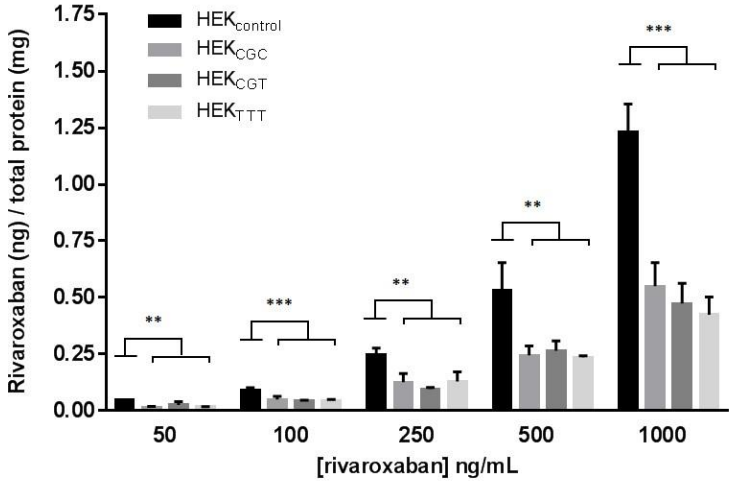
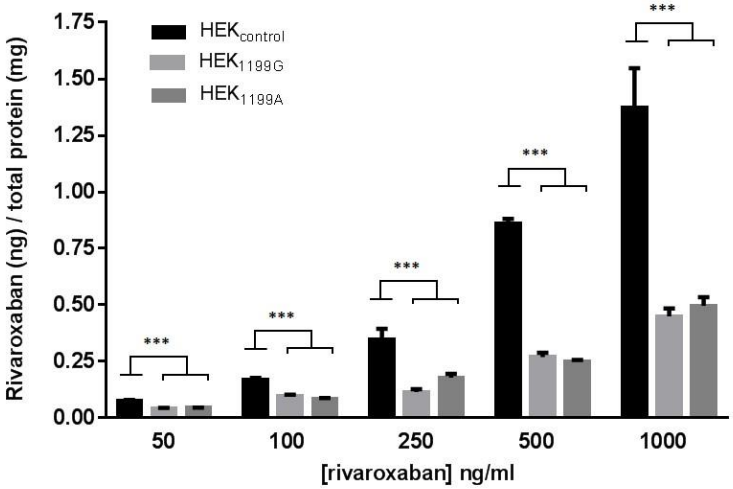


Figure 3. Impact of *ABCB1* 1199G>A on the intracellular accumulation of rivaroxaban. Intracellular accumulation of rivaroxaban after 120 min of incubation (N=3) at different concentrations in $HEK_{control}$, HEK_{1199G} and HEK_{1199A}.



The absolute amount of rivaroxaban (in ng) was divided by the total amount of proteins in cell extracts (in mg). * Compared to control cells: *p<0.05 **p<0.01 ***p<0.001

Discussion

In this study, we investigated the *in vitro* impact of *ABCB1* genetic polymorphisms on the transport activity towards rivaroxaban. Previously validated HEK293 recombinant cell models were used[11, 19]. We confirmed the involvement of ABCB1 in the active transport of rivaroxaban[6]. Indeed, we found that ABCB1 expression decreases the intracellular accumulation of the drug. However, we failed to show a significant effect of the different SNPs investigated. The *ABCB1* 1236C>T-2677G>T-3435C>T and 1199G>A SNPs had no significant influence on the intracellular accumulation of rivaroxaban when compared to the wild-type protein.

To our knowledge, this is the first *in vitro* study to explore the role of *ABCB1* genetic determinants in the disposition of DOACs. Few *in vivo* studies have been published so far. Recently, a case has been described reporting a rivaroxaban-treated patient admitted at the hospital for severe anemia related to gastrointestinal bleeding[20]. This patient presented abnormally high levels of anti-Xa activity and rivaroxaban plasma concentrations as well as an unexpected delayed clearance. The authors suggested that the homozygous presence of *ABCB1* variant alleles 2677-3435TT might have contributed to the altered drug clearance. This observation seems in contradiction with our results showing no influence of those SNPs using *in vitro* models. However, it must be stressed that this observation relates to an isolated clinical case and, given the relatively high frequency of the homozygous genotype (up to 25% of the population of Caucasian origin is expected to be 2677-3435TT), it might simply reflect a spurious association that is only due to chance. Furthermore, this patient received concomitant simvastatin (40 mg q.d) which was suggested to inhibit CYP3A4 enzyme but also the ABCB1 and ABCG2 transporters, based on *in vitro* findings[21, 22].* Finally, the patient presented a moderate renal impairment that could have worsened the renal elimination of the drug.

* Adapted from the published version

More recently, a randomized, two-center, crossover study was performed in healthy volunteers to study the influence of *ABCB1* polymorphisms on the pharmacokinetics of dabigatran and rivaroxaban. Peak plasma concentration and area under the curve (AUC) were compared across three different *ABCB1* genotypes i.e. 2677GG-3435CC, 2677GT-3435CT and 2677TT-3435TT and the effect of clarithromycin co-administration on the pharmacokinetics was also assessed. In agreement with the fact that rivaroxaban and dabigatran etexilate are both substrates for ABCB1, clarithromycin co-administration led to a two-fold increase in both drugs' AUC, irrespective of *ABCB1* genotype. Their conclusion meets the observations made in the present investigation as they inferred that *ABCB1* genotype is not a significant determinant of inter-individual variability in dabigatran and rivaroxaban pharmacokinetics but that co-administration of a P-gp/CYP3A4 inhibitor with dabigatran etexilate or rivaroxaban may warrant caution.

ABCB1 is involved in the transport of many endogenous, dietary and drug compounds that do not share obvious common structural features. The *ABCB1* 1236C>T-2677G>T-3435C>T SNPs have been inconstantly described to influence the clinical pharmacokinetics of immunosuppressants, anticancer agents, antiepileptic drugs or antidepressants[5]. A trend towards higher concentrations in patients carrying the variant haplotype seems to emerge, but therapy adjustments are currently not required or even recommended. The *ABCB1* 1199G>A SNP has previously shown a substrate-dependent impact on transport activity. For instance, the variant allele (1199G) decreased ABCB1 activity towards tacrolimus in HEK293 and K562 recombinant cell lines, but did not affect the transport of cyclosporine[11]. Similarly, the *in vitro* effect of 1199G>A was lower for nilotinib compared to imatinib, two tyrosine kinase inhibitors[12]. Structural flexibility and multiple binding sites were suggested as explanations for the polyspecificity of ABCB1[23]. In that way the 1199G>A SNP, which is located in a cytoplasmic loop involved in substrate recognition, may affect the binding of some ABCB1 substrates while the transport of the others remains unchanged as this seems to be the case for rivaroxaban[11].

Another explanation for our negative findings may rely in the fact that rivaroxaban is described as a weak to moderate substrate for ABCB1[4, 24]. In Caco-2 cells for instance, ABCB1 inhibition reduced rivaroxaban efflux by only 23% whereas the efflux of dabigatran was inhibited by 87%[4]. Moreover, rivaroxaban is also substrate for the ABCG2 transporter, known as breast cancer resistance protein (BCRP), and the cytochrome P450 (CYP) 3A4/3A5 enzyme. The active renal secretion by ABCB1 and ABCG2 and hepatic transformation by CYP3A4/3A5 account for 30% and 18% of total drug elimination respectively[6]. Although combined ABCB1, ABCG2 and CYP3A4 inhibitors were shown to increase rivaroxaban plasma concentrations by up to 150%, the impact of ABCB1 inhibition alone seems marginal[25, 26]. Gong and colleagues demonstrated that rivaroxaban clearance was significantly reduced in mice lacking both ABCB1 and ABCG2, but did not differ for individual transporter knockout[27]. This suggests a compensatory role of one transporter when the other is inhibited. It is important to note that, in HEK293 cells, the endogenous expression of ABCB1 and ABCG2 is reported to be very low[28].

Other genetic determinants could contribute to the inter-individual variability in DOAC plasma concentrations. Future studies are needed to assess the impact of *ABCG2* polymorphisms on the transport of rivaroxaban. The *ABCG2* rs2231142 (421C>A, Gln141Lys) SNP, with an allelic frequency around 5-10% in Caucasians and 30-60% in East Asians, is of particular interest as it significantly affected plasma trough concentrations in patients treated with apixaban[29, 30]. In the same study the *CYP3A5*3* allele, which is highly prevalent in Caucasians, also contributed to higher apixaban levels. Finally, it could be relevant to investigate the *CYP3A4*22* allele, which was associated with a lower CYP3A4 expression and/or activity[31].

In summary, the *ABCB1* 1236C>T-2677G>T-3435C>T and 1199G>A SNPs did not affect the efflux of rivaroxaban in HEK293 recombinant cell lines. Therefore, we believe that these SNPs are unlikely to contribute to the inter-individual variability in rivaroxaban plasma concentrations. Future studies should focus on the role of *ABCG2*, *CYP3A4* and *CYP3A5* genetic polymorphisms in the transport of rivaroxaban.

Materials and methods

Materials

Rivaroxaban and [^{13}C6]-rivaroxaban-d5 were purchased from Alsachim (Strasbourg, France). G418 was purchased from Roche Applied Science (Vilvoorde, Belgium).

Cell culture

Human Embryonic Kidney (HEK293) cells were grown in Dulbecco's Modified Eagle medium (DMEM) Glutamax 4.5 g/l glucose (Gibco, Invitrogen) supplemented with 10% (v/v) of Fetal Bovin Serum (Gibco, Invitrogen) and 1% (v/v) of Penicillin-Streptomycin solution (Gibco, Invitrogen) at a temperature of 37°C in the presence of 5% of CO_2.

Generation of stable recombinant cell lines

The generation of stable recombinant cell lines has been described in previous studies[11, 19]. Briefly, the expression vectors pcDNA3.1 with cDNA encoding ABCB1 (*ABCB1*$_{1199G}$ and *ABCB1*$_{1236T\text{-}2677T\text{-}3435T}$) were used. Mutated plasmids *ABCB1*$_{1199A,}$ *ABCB1*$_{1236C\text{-}2677G\text{-}3435C}$ and *ABCB1*$_{1236C\text{-}2677G\text{-}3435T}$ were obtained by site-directed mutagenesis. Plasmids were sequenced to confirm the presence of mutations and HEK293 cells were then transfected with pcDNA3.1 vectors. G418 (concentration 1

mg/ml) was added to select stable recombinant cell lines, which were characterized by flow cytometry, western-blot and immunofluorescence.

Characterization of ABCB1 cell surface expression

After one week of culture in the presence of G418, ABCB1 cell surface protein expression was characterized by flow cytometry as previously described with minor changes[11]. For each recombinant cell line, 10^6 cells were harvested by centrifugation and washed twice with ice-cold buffer solution (phosphate buffer saline (PBS), FBS 1%, EDTA 1mM). Cells were then incubated 45min on ice in the dark in buffer solution containing FITC Mouse anti-P-glycoprotein antibody diluted 1:10 (clone17F9 557002, BD Pharmingen) or its isotypic control diluted 1:10 (FITC Mouse IgG 2bk, clone27-35 555742, BD Pharmingen). Cells were finally washed with ice-cold buffer solution, centrifuged and resuspended in buffer solution. Samples were analyzed using Fluorescence-activated cell sorting (FACS) Canto II (BD).

Rivaroxaban accumulation

Rivaroxaban accumulation experiments were performed as previously described for tacrolimus or cyclosporin A[11]. One day before the experiment, 350.000 cells were seeded in poly-L-lysine-coated 24-well plates in 500 µl of complete medium. Rivaroxaban was added at 5 different concentrations: 10, 50, 100, 500 and 1000 ng/ml. Recombinant cells were incubated for 120 min at 37°C, 5% of CO_2[24]. Cells were then washed twice with ice-cold PBS. Cold conditions were applied to block enzymatic activity and to avoid active transport out of the cells, thereby preventing any drug loss during washing steps. After centrifugation and removal of the supernatant, cells were detached with ice-cold PBS containing 0.2% EDTA. Cell pellets were conserved at -80°C until quantification by LC-MS/MS analysis.

Rivaroxaban quantification

The amount of rivaroxaban extracted from the cell pellet was quantified by LC-MS/MS analysis according to a previously described method, with minor adaptive changes in the sample preparation[32]. The chromatography system was made up of an UPLC Acquity H-Class system (Waters) coupled with a tandem-quadrupole mass spectrometer Xevo TQ-S (Waters). Separation of the analytes was achieved on a Waters Cortecs UPLC C18 column (2.1 x 100 mm, 1.6 µm). Rivaroxaban was extracted with 100 µl of methanol and water (volume ratio 7:3) containing the internal standard ([^{13}C6]-rivaroxaban-d5) at 2 ng/ml. Blank cell pellets were prepared similarly for the calibration curve, with extraction solutions containing rivaroxaban at concentrations ranging from 0.125 ng/ml to 50 ng/ml. Samples were vortexed and then sonicated for 10 minutes in an ultrasound bath. After centrifugation at 20,000 g for 10 minutes, the supernatant was transferred to a HPLC vial (Macheray-Nagel, Düren, Germany). An aliquot (2 µL) of the final extract was injected into the LC-MS/MS system. For each experiment, the absolute amount of rivaroxaban recovered from the cell extracts was normalized to the amount of proteins measured with the BCA kit (Thermoscientific, Ghent, Belgium).

Statistical analysis

All experiments were repeated twice. Statistical analyses were performed using GraphPad Prism 7.0 for Windows (San Diego California, USA). Analyses of variance were used under the null hypothesis that the means of the different groups were equal. When differences among means were significant, post-hoc Student-Newman-Keuls tests were carried out. In all cases, a p-value of less than 0.05 was considered statistically significant.

Data availability

All data generated or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgements

The authors would like to thank S. Ibouraadaten and F. Uwambayinema for their assistance in analytical flow cytometry, Dr N. Dauguet for his help in cell sorting and R. Siriez for his contribution to LC-MS/MS analysis. AL Sennesael is a Research Fellow of the Fonds National de la Recherche Scientifique (FNRS).

Author Contributions

ALS, LE, VH, AS designed the research study. ALS and NP performed the experiments and analysed the results. CV and LP supervised the LC-MS/MS analysis. ALS wrote the first draft of the manuscript and the final version. All the co-authors were responsible for the review of the manuscript.

Competing financial interests

The authors have no conflicts of interest to disclose.

REFERENCES

1. Czuprynska J, Patel JP, Arya R. Current challenges and future prospects in oral anticoagulant therapy. Br J Haematol 2017: 10.1111/bjh.14714.
2. Kirchhof P, Benussi S, Kotecha D, et al. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. Europace 2016; 18: 1609-78.
3. Jain A, Cifu AS. Antithrombotic therapy for venous thromboembolic disease. JAMA 2017; 317: 2008-9.
4. Hodin S, Basset T, Jacqueroux E, et al. In Vitro Comparison of the Role of P-Glycoprotein and Breast Cancer Resistance Protein on Direct Oral Anticoagulants Disposition. Eur J Drug Metab Pharmacokinet 2018; 43: 183-91.
5. Wolking S, Schaeffeler E, Lerche H, Schwab M, Nies AT. Impact of Genetic Polymorphisms of ABCB1 (MDR1, P-Glycoprotein) on Drug Disposition and Potential Clinical Implications: Update of the Literature. Clin Pharmacokinet 2015; 54: 709-35.
6. Mueck W, Stampfuss J, Kubitza D, Becka M. Clinical pharmacokinetic and pharmacodynamic profile of rivaroxaban. Clin Pharmacokinet 2014; 53: 1-16.
7. Jungbauer L, Dobias C, Stollberger C, Weidinger F. The frequency of prescription of P-glycoprotein-affecting drugs in atrial fibrillation. J Thromb Haemost 2010; 8: 2069-70.
8. Greenblatt DJ, Patel M, Harmatz JS, Nicholson WT, Rubino CM, Chow CR. Impaired Rivaroxaban Clearance in Mild Renal Insufficiency With Verapamil Coadministration: Potential Implications for Bleeding Risk and Dose Selection. J Clin Pharmacol 2018; 58: 533-40.
9. Cheong EJY, Goh JJN, Hong Y, Kojodjojo P, Chan ECY. Rivaroxaban With and Without Amiodarone in Renal Impairment. J Am Coll Cardiol 2018; 71: 1395-7.
10. Hodges LM, Markova SM, Chinn LW, et al. Very important pharmacogene summary: ABCB1 (MDR1, P-glycoprotein). Pharmacogenet Genomics 2011; 21: 152-61.
11. Dessilly G, Elens L, Panin N, et al. ABCB1 1199G>A genetic polymorphism (Rs2229109) influences the intracellular accumulation of tacrolimus in HEK293 and K562 recombinant cell lines. PLoS One 2014; 9: e91555.
12. Dessilly G, Elens L, Panin N, Karmani L, Demoulin JB, Haufroid V. ABCB1 1199G>A polymorphism (rs2229109) affects the transport of imatinib, nilotinib and dasatinib. Pharmacogenomics 2016; 17: 883-90.
13. Chaussade E, Hanon O, Boully C, et al. Real-Life Peak and Trough Dabigatran Plasma Measurements over Time in Hospitalized Geriatric Patients with Atrial Fibrillation. J Nutr Health Aging 2018; 22: 165-73.
14. 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. Thromb Res 2016; 137: 178-83.
15. Gulilat M, Tang A, Gryn SE, et al. Interpatient Variation in Rivaroxaban and Apixaban Plasma Concentrations in Routine Care. Can J Cardiol 2017; 33: 1036-43.
16. Eikelboom JW, Quinlan DJ, Hirsh J, Connolly SJ, Weitz JI. Laboratory Monitoring of Non-Vitamin K Antagonist Oral Anticoagulant Use in Patients With Atrial Fibrillation: A Review. JAMA Cardiol 2017; 2: 566-74.

17. Testa S, Paoletti O, Legnani C, et al. Low drug levels and thrombotic complications in high-risk atrial fibrillation patients treated with direct oral anticoagulants. J Thromb Haemost 2018; 16: 842-8.

18. Pare G, Eriksson N, Lehr T, et al. Genetic determinants of dabigatran plasma levels and their relation to bleeding. Circulation 2013; 127: 1404-12.

19. Dessilly G, Panin N, Elens L, Haufroid V, Demoulin JB. Impact of ABCB1 1236C > T-2677G > T-3435C > T polymorphisms on the anti-proliferative activity of imatinib, nilotinib, dasatinib and ponatinib. Sci Rep 2016; 6: 29559.

20. Ing Lorenzini K, Daali Y, Fontana P, Desmeules J, Samer C. Rivaroxaban-Induced Hemorrhage Associated with ABCB1 Genetic Defect. Front Pharmacol 2016; 7: 494.

21. Sakaeda T, Fujino H, Komoto C, et al. Effects of acid and lactone forms of eight HMG-CoA reductase inhibitors on CYP-mediated metabolism and MDR1-mediated transport. Pharm Res 2006; 23: 506-12.

22. Glodkowska-Mrowka E, Mrowka P, Basak GW, et al. Statins inhibit ABCB1 and ABCG2 drug transporter activity in chronic myeloid leukemia cells and potentiate antileukemic effects of imatinib. Exp Hematol 2014; 42: 439-47.

23. Chufan EE, Sim HM, Ambudkar SV. Molecular basis of the polyspecificity of P-glycoprotein (ABCB1): recent biochemical and structural studies. Adv Cancer Res 2015; 125: 71-96.

24. Gnoth MJ, Buetehorn U, Muenster U, Schwarz T, Sandmann S. In vitro and in vivo P-glycoprotein transport characteristics of rivaroxaban. J Pharmacol Exp Ther 2011; 338: 372-80.

25. Mueck W, Kubitza D, Becka M. Co-administration of rivaroxaban with drugs that share its elimination pathways: pharmacokinetic effects in healthy subjects. Br J Clin Pharmacol 2013; 76: 455-66.

26. Hellwig T, Gulseth M. Pharmacokinetic and pharmacodynamic drug interactions with new oral anticoagulants: what do they mean for patients with atrial fibrillation? Ann Pharmacother 2013; 47: 1478-87.

27. Gong IY, Mansell SE, Kim RB. Absence of both MDR1 (ABCB1) and breast cancer resistance protein (ABCG2) transporters significantly alters rivaroxaban disposition and central nervous system entry. Basic Clin Pharmacol Toxicol 2013; 112: 164-70.

28. Ahlin G, Hilgendorf C, Karlsson J, Szigyarto CA, Uhlen M, Artursson P. Endogenous gene and protein expression of drug-transporting proteins in cell lines routinely used in drug discovery programs. Drug Metab Dispos 2009; 37: 2275-83.

29. Ueshima S, Hira D, Fujii R, et al. Impact of ABCB1, ABCG2, and CYP3A5 polymorphisms on plasma trough concentrations of apixaban in Japanese patients with atrial fibrillation. Pharmacogenet Genomics 2017; 27: 329-36.

30. Hira D, Terada T. BCRP/ABCG2 and high-alert medications: Biochemical, pharmacokinetic, pharmacogenetic, and clinical implications. Biochem Pharmacol 2018; 147: 201-10.

31. Elens L, van Gelder T, Hesselink DA, Haufroid V, van Schaik RH. CYP3A4*22: promising newly identified CYP3A4 variant allele for personalizing pharmacotherapy. Pharmacogenomics 2013; 14: 47-62.

32. Sennesael AL, Exner T, Chatelain B, et al. An optimized dRVVT-based assay to estimate the intensity of anticoagulation in patients treated with direct oral anticoagulants. Thromb Res 2017; 157: 29-37.

Chapter IV

Computerized clinical decision support systems to improve DOAC prescribing

CHAPTER IV

Do computerized clinical decision support systems improve oral anticoagulants prescribing? A systematic review

Anne-Laure Sennesael, Bruno Krug, Barbara Sneyers, Anne Spinewine

Article in preparation

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Computerized clinical decision support systems (CDSS) were shown to reduce medication errors and improve practitioner performance.
- Prescribing issues were frequently involved in serious adverse drug reactions (ADR) related to the use of oral anticoagulants.

WHAT THIS STUDY ADDS

- CDSS for the prescribing of oral anticoagulants improved practitioner performance in 9 of 15 studies, while clinical outcomes were poorly reported.
- CDSS mainly tackled the underuse of oral anticoagulants and warfarin-drug interactions.
- Most systems provided specific recommendations automatically, at the time and location of decision making.

Abstract

BACKGROUND: Despite the highest convenience of direct oral anticoagulants (DOAC), evidence suggest that ensuring appropriate and safe use of anticoagulant therapy remains challenging in practice. Computerized clinical decision support systems (CDSS) were previously shown to improve practitioner performance in different areas. Therefore, we systematically reviewed the impact of CDSS for the prescribing of oral anticoagulants and described CDSS features associated with success versus failure.

METHODS: We searched Medline, Embase, CENTRAL, CINHAL and PsycINFO for full-text reports published since 1998. We included studies comparing CDSS for the initiation or monitoring of oral anticoagulants to routine care, in a real clinical setting. Two reviewers performed study selection, data collection and risk of bias assessment according to pilot-tested tools. Disagreements were discussed with the help of a third reviewer. Potentially important CDSS features, identified from previous literature, were evaluated.

RESULTS: Fifteen studies were included in qualitative synthesis. Most studies were performed in primary care (n=6) or hospital (n=6) setting, and included patients with atrial fibrillation (AF) (n=8). CDSS recommendations were mainly related to the indication of oral anticoagulation (n=10) and drug interactions (n=5). Most CDSS were integrated in electronic health records or electronic prescribing, and provided recommendations automatically at the time and location of decision making. Overall, significant improvements in practitioner performance were found in 9 of 15 studies.

CONCLUSIONS: CDSS mainly focused on the underuse of oral anticoagulation in AF patients or on warfarin drug interactions, with variable impact. None CDSS delivered individualized assessment for drug selection among DOAC and VKA.

Introduction

Oral anticoagulants are effective drugs for stroke prevention in atrial fibrillation and the treatment and secondary prevention of venous thromboembolism (VTE) [1, 2]. Nevertheless, they have been frequently described as a cause of serious adverse drug reactions (ADR) in both inpatient and outpatient settings, requiring higher level of care or hospital admission[3-6]. In 2013-2014, vitamin K antagonists (VKA) and direct oral anticoagulants (DOAC) were among the most commonly implicated drugs in emergency department visits in older patients[7]. Most importantly, data suggest that a significant part of these ADR results from medication errors and could therefore have been avoided[8, 9]. In a recent prospective study performed in emergency departments, we have shown that more than half of serious ADRs related to the use of oral anticoagulants were potentially preventable, both for DOAC and VKA[10]. Therefore, despite the availability and highest convenience of DOAC, ensuring appropriate and safe use of oral anticoagulants remains challenging in practice.

Computerized clinical decision support systems (CDSS) provide assistance to clinicians in the process of decision-making by comparing individual patient characteristics against a computerized knowledge base[11]. Specifically, CDSS for drug prescribing may help initiating appropriate therapy, identifying drug-drug interactions or determining the optimal dose regimen[12, 13]. In previous systematic reviews, CDSS were shown to reduce medication errors and improve practitioner performance in different areas[13-15]. Their impact on patient outcomes remains nevertheless understudied and inconsistent, although Varghese et al. have recently reported positive effect[16]. Several features have been suggested to explain why some CDSS were successful and others not. For instance, systems providing decision support automatically, at the time and location of decision making were more likely to improve clinical practice[17, 18].

In the pre-DOAC era, many studies investigated the computer-assisted management of anticoagulant therapy. However, these CDSS mostly dealt with INR-based dose adjustments of warfarin in in- and outpatients[19, 20]. Practitioner performance (e.g. proportion of time with therapeutic INR) was inconsistently improved across studies, while most trials were not powered to evaluate clinical outcomes [19]. More recently, evidence has accumulated on CDSS for VTE prophylaxis in hospitalized patients. CDSS implementation proved to be effective in enhancing the appropriate use of thromboprophylaxis and reducing VTE occurrence[21, 22]. Results may nevertheless not be generalizable to the management of an existing long-term treatment.

Now that DOAC have broadened the landscape of anticoagulant therapy, clinicians are facing new challenges such as choosing the appropriate individualized drug treatment or juggling different pharmacokinetic properties and dose regimens [23, 24]. CDSS might be helpful in this context to ensure appropriate use of oral anticoagulants. Therefore, we systematically reviewed studies evaluating the impact of CDSS for the prescribing of oral anticoagulant therapy, compared to routine care. We also performed a descriptive analysis of CDSS features associated with success versus failure.

Methods

This systematic review was conducted in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) statement[25]. Review protocol was registered with PROSPERO under CRD42018093507.

Studies eligible for review

We included studies comparing the effect of a CDSS for the prescribing of oral anticoagulants (i.e. DOAC or VKA) to routine care, in a real clinical setting. We defined CDSS as a system that matches individual patient characteristics to a

computerized clinical knowledge base, and provides patient-specific assessments or recommendations in an electronic format[11]. The decision support had to be designed to influence directly the prescriber, at the time of prescribing or at a later time. Recommendations could be relayed to the prescriber by another health care professional (HCP). According to Pearson et al., we considered CDSS for the initiation (i.e. support when DOAC or VKA is started, before or after the prescribing choice) or the monitoring (i.e. support when patients are already taking DOAC or VKA) of drug therapy[26]. We excluded CDSS exclusively dedicated to the therapeutic drug monitoring (TDM) and dosing of VKA, CDSS aiming at improving thromboprophylaxis in hospitalized patients and patient-oriented CDSS such as shared-decision making tools. Multifaceted interventions were included in this systematic review if the CDSS effect was reported separately. In the same way, CDSS involving many drugs were included if specific results were available for oral anticoagulants. Eligibility criteria were pilot tested on a sample of 50 reports by two reviewers (ALS and BK). Cases of disagreement were discussed with a third reviewer (AS) and eligibility criteria were refined accordingly.

Search strategy

We searched Medline (PubMed, 1998 – March 2018), Embase (Ovid, 1998 – March 2018), CENTRAL (Wiley, 1998 – April 2018), CINHAL (EBSCOhost, 1998 – April 2018) and PsycINFO (ProQuest, 1998 – April 2018). We limited our search to full-text reports published in English since 1998, as 2 previous reviews on oral anticoagulation management were published that year [19, 20]. Free text terms and subject headings related to the two following search concepts were combined: 1) anticoagulation and 2) CDSS. The Medline search strategy was developed first, and improved after careful consideration of relevant articles. The final version of search strategy, available in Appendix 1, was then applied to the other databases. In order to identify additional potential studies, we searched reference lists of all included studies. We also reviewed: 1) references of previous systematic reviews in the field,

2) citing articles of included studies using Scopus, 3) the trial register clinicaltrials.gov, and 4) Google Scholar.

After duplicate removal, titles and abstracts of identified articles were screened independently by two reviewers (ALS and BK) for compliance with inclusion criteria. Full texts of reports thought to be potentially eligible were then assessed by the two same reviewers. At any stage, disagreements were resolved by consensus and the help of a third reviewer (AS) where necessary. Reviewers were not blinded to journal's name, author's details or outcomes when applying eligibility criteria. A list of excluded full-text reports, with primary reason for exclusion, is available on request.

Data extraction

Our data collection form was designed based on the Cochrane Handbook for Systematic Reviews of Interventions[27] and the Effective Practice and Organization of Care (EPOC) resources for review authors[28]. We extracted information on study design and setting, inclusion and exclusion criteria, number and characteristics of participants, outcome measures and corresponding results. Regarding the intervention, we recorded details about scope and application of CDSS recommendations (initiation vs. monitoring of oral anticoagulation; support related to indication, choice, dosage or interactions). Potentially important CDSS features, identified from previous literature, were evaluated[17, 18, 29]. They are listed in Table 1.

Table 1: CDSS features that were evaluated in the present systematic review

General system features
Integration with EMR and/or CPOE
Clinician-system interaction features
Automatic provision as part of physician workflow No need for additional clinician data entry Recommendations viewed by the prescriber directly on the computer screen Request documentation of the reason for not following CDSS recommendations Provision at time and location of decision making Recommendations executed by noting agreement
Communication content features
Provision of a recommendation, not just an assessment Promotion of action rather than inaction Justification of decision support via provision of reasoning/research evidence
Auxiliary features
Local user involvement in development process Provision of decision support results to patients as well as providers Periodic performance feedback CDSS accompanied by conventional education Support for CDSS use (e.g. active training, passive instructions, helpdesk) Monitoring of unintended effects of CDSS Clear incentives for use

The data collection form was pilot-tested on 3 studies (2 randomized controlled trials (RCT) and 1 interruptive time series analysis (ITS)) by all authors involved in this systematic review (ALS, BK, BS and AS). Imprecisions were highlighted and the tool was improved accordingly (full version available on request). The final data collection process was performed initially by one reviewer (ALS), and then checked for accuracy by a second reviewer (BK). Disagreements were discussed to reach consensus. We contacted study authors to provide further information where necessary.

Quality assessment

Methodological quality of RCT was evaluated using the Cochrane Collaboration's tool for assessing risk of bias[30]. Domains of assessment included selection bias (sequence generation and allocation concealment), performance bias (blinding of patients and HCP), detection bias (blinding of assessors), attrition bias (incomplete outcome data) and reporting bias (selective outcome reporting). For non-randomized studies, evaluation was based on the Risk Of Bias In Non-randomised Studies – of Interventions (ROBINS-I) tool[31]. We drew up a table containing the different domains, with examples of low and high risk of bias judgments (Appendix 2). This quality assessment tool was first pilot-tested on three studies by three reviewers (ALS, BK and AS), and improved accordingly. Then, for each included study, two reviewers (ALS and BK) independently assessed the risk of bias. Disagreements were resolved by discussion. All studies were considered for data synthesis, irrespective of their risk of bias.

Data synthesis and analysis

As high variability in interventions and outcome measurements were expected, we decided not to perform a meta-analysis. The following outcomes were reported: practitioner performance (e.g. appropriateness of oral anticoagulant prescribing), patient outcomes (e.g. adverse events, mortality, or morbidity) and implementation outcomes (e.g. adoption, acceptability, appropriateness or adverse effects of the system). Main results of included studies were summarized in the form of table. The presence or absence of CDSS features was reported for each study. Feature prevalence among studies and median number of CDSS features per study were described separately for trials demonstrating or not improvement in primary outcome measure.

Results

Study selection

The search strategy identified 8513 records (Figure 1). After duplicate removal, titles and abstracts of 7271 records were screened. Among these, 43 full-text articles were reviewed as they were thought to be potentially eligible. Fifteen studies were finally included in qualitative synthesis. One additional study was recently identified through searching the trial register clinicaltrials.gov and will be integrated in the near future. For 10 studies, authors were contacted to provide further information. After sending one reminder, answers were received for 7 studies. We are planning to send one last reminder.

Study characteristics

Characteristics of the 15 included trials are detailed in Table 2. Most studies were conducted in Europe (n=7)[32-38] or in North America (n=6)[39-44], in primary care setting (n=6)[32, 33, 37, 39, 43, 45] or in hospitals (n=6)[34, 36, 38, 40-42]. Half of them included AF patients (n=8)[32-34, 37, 39, 40, 45, 46]. Ten studies were randomized controlled trials (RCT)[32-34, 36, 37, 39, 41, 42, 44, 45]. Randomization was performed at cluster level (primary care practices, hospitals or long-stay units, n=6)[32, 33, 36, 37, 39, 44], provider level (n=3)[41, 42, 45] or patient level (n=1)[34]. Other non-randomized designs included 2 interrupted time series (ITS)[43, 46], 2 historically controlled studies (HCT)[38, 40] and 1 controlled before-after study (CBA)[35].

All trials assessed practitioner performance as a primary outcome measure. Implementation outcomes were further investigated in 10 studies[32-37, 39-41, 45], and 2 studies described patient outcomes[32, 33]. Only one study reported potential financial benefits if the CDSS tested was commercialized[35]. Seven studies were previously registered in clinical trials databases[32-34, 37, 39, 41, 45] and, for 4 of these, protocols were published[32, 33, 37, 45].

Figure 1: Study selection (PRISMA flow diagram)[25]

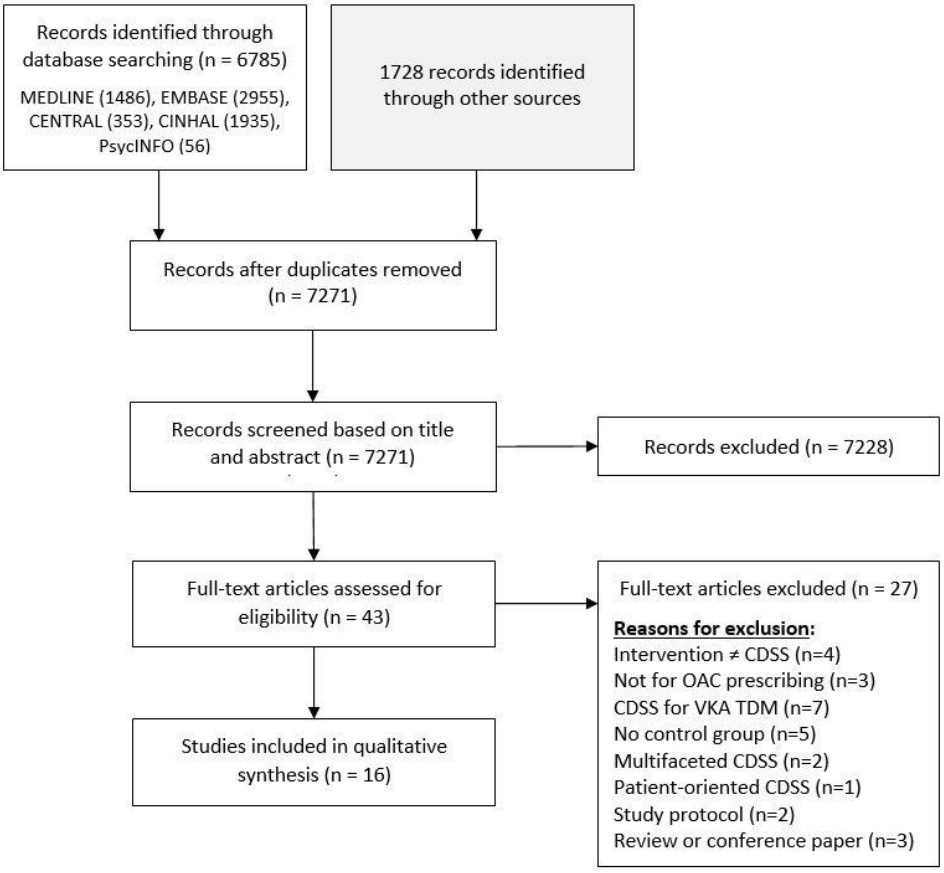


Table 2: characteristics and main results of included studies

Study	Design	Setting and patients	No. patient/ HCP/centre	CDSS description	Control	1ary outcome	CDSS effect
Arts, 2017[37]	RCT	1ary care practices, The Netherlands, AF patients	781/39/18*	Tx recommendations in AF patients (OAC or AP)	No CDSS	P: proportion of patients treated according to the guideline	NS
Bajorek, 2016[45]	RCT	1ary care practices, Australia, AF patients	393/48*/–	Tx recommendations in AF patients (warfarin, ASA or none)	No CDSS	P: proportion of patients receiving OAC	↑
Cook, 2015[40]	HCT	Hospital, USA, new AF inpatients	494/–/1	Alert for OAC-naïve new AF patients	No CDSS	P: proportion of eligible patients who were prescribed warfarin I: accuracy of notifications	NS 44%
Eckman, 2016[39]	RCT	1ary care practices, USA, AF patients	1493/70/15*	Tx recommendations in AF patients (OAC, ASA or none)	No CDSS	P: discordance between CDSS recommendation and actual Tx	NS
Feldstein, 2006[43]	ITS	1ary care clinics, USA,VKA-treated patients	9910/–/15	Alert for co-prescribing of warfarin and 5 interacting drugs	No CDSS	P: interacting prescription rate	↓
Holt, 2017[33]	RCT	1ary care practices, UK, AF patients	5339/–/46*	Alert for eligible OAC-naïve AF patients	No CDSS	P: proportion of eligible patients who were prescribed OAC	NS
Judge, 2006[44]	RCT	Academic long-stay units, USA, all residents	445/–/7*	Alert for co-prescribing of warfarin and interacting drugs + INR >3	No CDSS	P: proportion of alerts followed by an appropriate action	↑
Karlsson, 2018[32]	RCT	1ary care clinics, Sweden, AF patients	14800/–/42*	Alert for eligible OAC-naïve AF patients	No CDSS	P: proportion of eligible patients who were prescribed OAC	↑

Table 2: characteristics and main results of included studies (continued)

Study	Design	Setting and patients	No. patient/ HCP/centre	CDSS description	Control	1ary outcome	CDSS effect
Kruger, 2011[35]	CBA	Nursing homes, Norway, all residents	388/504/2	Alert for OAC-naïve AF patients	No CDSS	P: use of warfarin	↑
Sheibani, 2017[46]	ITS	Offices of cardiologists, Iran, new AF patients	373/10/10	Mobile application to assess stroke and bleeding risks in AF patients	No CDSS	P: proportion of patients treated according to the guideline	↑
Silbernagel, 2016[34]	RCT	Academic hospital, Switzerland, OAC-naïve AF inpatients	889*/–/1	Alert for OAC-naïve AF patients	No CDSS	P: rate of adequate OAC prescription at discharge	↑
Strom, 2010 (A)[42]	RCT	Academic hospitals, USA, all inpatients	–/1963*/2	Alert for co-prescribing of warfarin and NSAID	Passive alert	P: not reordering the alert-triggering drug after firing	NS
Strom, 2010 (B)[41]	RCT	Academic hospitals, USA, all inpatients	96/1971*/2	Hard-stop alert for co-prescribing of warfarin and TMP-SMX	No CDSS	P: not reordering the alert-triggering drug after firing	↑
Velez, 2014[38]	HCT	Academic hospital, Spain, VKA-treated inpatients	–/–/1	Alert for co-prescribing of OAC and interacting drugs	No CDSS	P: medication errors caused by drug interactions	↓
Weir, 2003[36]	RCT	Hospitals, UK, stroke or TIA patients	637/–/16*	Ischemic and bleeding risks associated with Tx (warfarin, ASA, DPY, ASA+ DPY, warfarin + ASA or none)	No CDSS	P: risk reduction in ischemic and bleeding events achieved by Tx	NS

1ary: primary, AF: atrial fibrillation, AP: antiplatelet therapy, ASA: aspirin, CBA: controlled before-after study, DPY: dipyridamole, HCP: health care professional, HCT: historically controlled trial, I: implementation outcome, ITS: interrupted time series, NS: not significant, NSAID: non-steroidal anti-inflammatory drugs, OAC: oral anticoagulant, P: practitioner performance, RCT: randomized controlled trial, TIA: transient ischemic attack, TMP-SMX: co-trimoxazole, Tx: treatment, VKA: vitamin K antagonist. * Unit of allocation

When looking at CDSS that were evaluated, 8 were exclusively dedicated to the prescribing of oral anticoagulants[32-34, 38, 39, 43, 45, 46]. CDSS were developed in-house (n=6)[34-36, 39, 45, 46] or resulted from close collaboration with commercial vendors (n=9)[32, 33, 37, 38, 40-44]. CDSS developers were also evaluators in 11 trials[32-38, 43-46]. Thirteen systems were pilot-tested before study initiation[32-35, 37, 39-46]. CDSS provided support for the initiation of therapy (n=13)[32-37, 39-42, 44-46] or for treatment monitoring (n=7)[38, 39, 41-45]. Recommendations were mainly related to the indication of oral anticoagulation (n=10)[32-37, 39, 40, 45, 46] and to drug-drug interactions (n=5)[38, 41-44]. Half of CDSS focused specifically on the use of warfarin (n=8)[35, 36, 40-45]. None individualized assessment regarding the choice between DOAC and VKA or among DOAC was identified. CDSS targeted physicians in all studies, except for one that was unclear[36]. In 5 studies performed in the US and the UK, nurse practitioners were also CDSS users[33, 41-44]. Clinical practice guidelines (n=7)[32-34, 37, 39, 45, 46] and expert opinion (n=5)[36, 38, 43-45] were common sources of knowledge.

Methodological quality

Methodological quality of included studies is presented in Table 3. Overall 5 studies were assessed at low risk of bias[32, 33, 37, 40, 43], while one or more bias domains were considered at high risk for the 10 other trials. Performance bias was frequently involved with high risk assessment, especially in RCT. This was partly due to the potential for contamination when physicians allocated to different groups were used to work together[41, 42, 44] or when the unit of allocation was the patient[34]. Three non-randomized studies were deemed at high risk of detection bias, because methods of outcome assessment differed between groups[35, 46] or subjective outcomes were not blindly assessed[38].

Table 3: assessment of risk of bias for the 15 included studies

Study	Design	Sequence generation	Allocation concealment	Bias due to confounding	Selection bias	Performance bias	Misclassifi-cation bias	Attrition bias	Detection bias	Reporting bias	Other risk of bias
Arts, 2017[37]	RCT	Low	Low			Low		Low	Low	Low	
Bajorek, 2016[45]	RCT	Low	Low			Low		Low	Low	High	High
Eckman, 2016[39]	RCT	Unclear	Unclear			Low		Low	Low	Low	High
Holt, 2017[33]	RCT	Low	Low			Low		Low	Low	Low	
Judge, 2006[44]	RCT	Unclear	Unclear			High		Low	Low	Low	
Karlsson, 2018[32]	RCT	Low	Low			Low		Low	Low	Low	
Silbernagel, 2016[34]	RCT	Low	Low			High		Low	Low	Low	
Strom, 2010 (A)[42]	RCT	Unclear	Unclear			High		Low	Low	Low	
Strom, 2010 (B)[41]	RCT	Low	Low			High		Low	Low	Low	
Weir, 2003[36]	RCT	Low	Low			High		Low	Low	Low	
Cook, 2015[40]	HCT			Low	Low	Low	Low	Low	Low	Low	
Feldstein, 2006[43]	ITS			Low	Low	Low	Low	Low	Low	Low	
Kruger, 2011[35]	CBA			High	High	Low	Low	Low	High	Low	
Sheibani, 2017[46]	ITS			Low	High	High	Low	Low	High	Low	
Velez, 2014[38]	HCT			Low	Low	Low	Low	Low	High	Low	

CBA: controlled before-after study, HCT: historically controlled trial, ITS: interrupted time series, RCT: randomized controlled trial, Low: low risk of bias, High: high risk of bias. Grey zone = not applicable.

Impact of CDSS on measured outcomes

Practitioner performance

Overall, significant improvements in practitioner performance were found in 9 studies[32, 34, 35, 38, 41, 43-46]. First, CDSS interventions had a positive effect in 3 of the 8 studies assessing adherence to guidelines or recommendations (2 RCT and 1 ITS) [32, 34, 46]. Absolute differences between intervention and control groups were nevertheless modest, ranging from 2 to 18% in terms of adequate prescription rates. Second, the prevalence of use of oral anticoagulation was significantly increased in 2 of the 3 studies evaluating this process measure[35, 36, 45]. Third, practitioner performance was improved in 4 of the 5 studies providing support for the management of drug-drug interactions[38, 41, 43, 44]. Results on practitioner performance are detailed in Appendix 3.

Patient outcomes

In the 2 studies assessing patient outcomes[32, 33], CDSS intervention did not influence the incidence of stroke, transient ischemic attack (TIA) or systemic embolism. Karlsson et al. found a lower incidence of significant bleeding in CDSS group (12 vs. 16 events per 1000 patients, p=0.04) [32]. However, Holt et al. reported comparable incidences between intervention and control groups (35 vs 50 events per 1000 patients, p=0.054) [33]. In both studies, clinical outcomes were measured 12 months after study initiation.

Implementation outcomes

Cook et al. defined alert accuracy as a primary outcome[40]. They found that, among 604 notifications of newly-diagnosed AF received by providers, 44% were confirmed as newly-diagnosed. CDSS utilization was described in 3 studies and ranged between 5 and 30%[34, 37, 39]. Barriers to the use of CDSS were not reported,

although one study team was planning to publish separate data regarding the usefulness, ease of use and acceptance of their CDSS[32]. Reasons for deviating from CDSS recommendations were explored in 5 studies[32, 37, 39, 40, 45]. Disagreements with recommendations[39, 45], drug management by the specialist[39, 45] and patient preferences[32, 39] were each mentioned by providers in 2 studies. Bleeding risk and falls were also reported reasons in primary care[32]. Planning of a surgical procedure was another reason for the non-prescribing of warfarin in eligible inpatients[40]. Four cases of delay in prescribing or administration of adequate drugs were monitored in the study of Strom et al., which investigated a hard-stop alert[41]. All adverse events were probably or definitely related to the intervention, leading to the early termination of the study. Implementation outcomes are described in Appendix 4.

Description of CDSS features

CDSS features are detailed for each study in Table 4. Most CDSS were integrated in electronic medical records (EMR) or computerized provider order systems (CPOE), and were delivered automatically as part of physician workflow (n=11)[32-35, 37, 38, 40-44]. Among these, 5 CDSS were flexible (i.e. the user could choose to postpone the decision)[32-34, 37, 40] while 1 was a hard-stop alert (i.e. drug orders were blocked) [41]. Non-integrated CDSS provided support through web-based systems (n=2)[39, 45], smartphone application (n=1)[46] or software (n=1)[36]. In all but one study, recommendations were viewed directly on the computer screen by the prescriber[36], and were provided at the time and location of decision making[39]. Thirteen CDSS gave specific recommendations instead of just an assessment[32-34, 37-46]. Providers were for instance prompted to order acetaminophen when warfarin and NSAID were prescribed simultaneously[42].

Table 4: CDSS features evaluated

	Arts, 2017[37]	Cook, 2015[40]	Eckman, 2016[39]	Holt, 2017[33]	Strom, 2010[42] (A)	Weir, 2003[36]	**Prevalence, n (%) NS effect (N=6)**	Bajorek, 2016[45]	Feldstein, 2006[43]	Judge, 2006[44]	Karlsson, 2018[32]	Kruger, 2011[35]	Sheibani, 2017[46]	Silbernagel, 2016[34]	Strom, 2010[41](B)	Velez, 2014[38]	**Prevalence, n (%) SS effect* (N=9)**
GENERAL SYSTEM FEATURES																	
Integration with EMR and/or CPOE	+	+	−	+	+	−	**4 (67)**	−	+	+	+	+	−	+	+	+	**7 (78)**
CLINICIAN-SYSTEM INTERACTION FEATURES																	
Automatic provision as part of physician workflow	+	+	−	+	+	−	**4 (67)**	−	+	+	+	+	−	+	+	+	**7 (78)**
No need for additional clinician data entry	+	+	−	−	+	−	**3 (50)**	?	+	+	+	+	−	−	+	+	**6 (67)**
Rec. viewed directly on the computer screen	+	+	+	+	+	−	**5 (83)**	+	+	+	+	+	+	+	+	+	**9 (100)**
Justification for not following CDSS rec.	+[a]	−	−	+	−	−	**2 (33)**	+	−	−	+	−	−	−	−	−	**2 (22)**
Provision at time and location of decision making	+	+	−	+	+	+	**5 (83)**	+	+	+	+	+	+	+	+	+	**9 (100)**
Recommendations executed by noting agreement	−	−	−	−	+	−	**1 (17)**	−	+	−	+	?	−	−	+	−	**3 (33)**
COMMUNICATION CONTENT FEATURES																	
Recommendation, not just an assessment	+	+	+	+	+	−	**5 (83)**	+	+	+	+	−	+	+	+	+	**8(89)**
Action rather than inaction	+	+	+	+	+	+	**6 (100)**	+	+	+	+	+	+	+	+	+	**9 (100)**
Provision of reasoning or research evidence	+	+	+	−	−	−	**3 (50)**	?	+	+	+	−	+	+	−	+	**6 (67)**

Table 4: CDSS features evaluated (continued)

	Arts, 2017[37]	Cook, 2015[40]	Eckman, 2016[39]	Holt, 2017[33]	Strom, 2010[42] (A)	Weir, 2003[36]	**Prevalence, n (%) NS effect (N=6)**	Bajorek, 2016[45]	Feldstein, 2006[43]	Judge, 2006[44]	Karlsson, 2018[32]	Kruger, 2011[35]	Sheibani, 2017[46]	Silbernagel, 2016[34]	Strom, 2010[41](B)	Velez, 2014[38]	**Prevalence, n (%) SS effect* (N=9)**
AUXILIARY FEATURES																	
Local user involvement in development process	?	+	+	+	−	−	**3 (50)**	−	+	+	+	+	+	−	−	+	**6 (67)**
Provision of decision support results to patients	−	−	−	−	−	−	**0 (0)**	−	−	−	−	−	−	−	−	−	**0 (0)**
Periodic performance feedback	−	−	+	−	−	−	**1 (17)**	−	−	−	−	−	−	−	−	−	**0 (0)**
CDSS accompanied by conventional education	−	−	+	−	−	−	**1 (17)**	−	−	?	+	+	−	−	−	−	**2 (22)**
Support for CDSS use	+	−	−	+	−	−	**2 (33)**	+[b]	+	−	+	+	+	+	−	−	**6 (67)**
Monitoring of unintended effects of CDSS	−	−	−	+	−	−	**1 (17)**	−	−	−	+	−	−	−	+	−	**2 (22)**
Clear incentives for use	−	−	−	−	−	−	**0 (0)**	−	−	−	−	−	−	−	−	−	**0 (0)**
TOTAL NUMBER OF FEATURES PER STUDY	**10**	**9**	**7**	**10**	**8**	**2**		**6**	**11**	**9**	**14**	**9**	**7**	**8**	**9**	**9**	

EMR: electronic medical record, CPOE: computerized provider order entry, NS: no significant, rec: recommendation, SS: statistically significant, − : absence of feature, + : presence of feature, ? : unclear. * Significant improvement in 1ary outcome (practitioner performance). [a] 4 of 13 intervention practices justified reasons for deviating from recommendations (but pooled analysis); [b] 7 of 15 clinics received academic detailing (no improved effect)

Several features were observed in around half of CDSS, such as the provision of reasoning or research evidence (n=9)[32, 34, 37-40, 43, 44, 46] or the requirement for response with indication of intention to comply (n=8)[32-34, 37, 41-43, 45]. Among the latter, 4 studies requested justification for not following recommendations[32, 33, 37, 45]. In one third of studies, CDSS users were required to enter or confirm clinical data, before (n=2)[36, 46] or after (n=3)[33, 34, 39] provision of first decision support. Recommended drugs could be ordered by a single click in only 4 studies[32, 41-43].

Among auxiliary features, the involvement of local users in the development process was inconsistently reported (n=9)[32, 33, 35, 38-40, 43, 44, 46]. In 8 studies[32-35, 37, 43, 45, 46], CDSS implementation was accompanied by support in the form of active training (n=7)[32, 33, 35, 37, 43, 45, 46], passive instructions (n=4)[32, 34, 37, 45] or helpdesk (n=1)[37]. Feedback on the compliance with CDSS recommendations was observed in only 1 study[39]. Finally, 3 studies were closely monitored for safety outcomes or inappropriate drug use triggered by the CDSS[32, 33, 41].

The prevalence of CDSS features among studies is presented in Table 4. Although the number of trials included in the descriptive analysis was small, a trend towards higher prevalence of CDSS features in studies demonstrating improvement in primary outcome seemed to emerge. Particularly, support for CDSS use was more frequently provided in this subgroup. The median number of CDSS features per study was 9, both in trials showing no significant effect (range 2-10) and in trials showing statistically significant effect (range 6-14). Two studies specifically assessed the contribution of a single characteristic to the impact of CDSS. As previously mentioned, Strom et al. found that the requirement for indication of intention did not improve practitioner performance[42]. In the study of Feldstein et al., academic detailing addressing barriers to the use of alerts did not influence interacting prescription rates [43].

Discussion

We systematically reviewed 15 studies evaluating the impact of CDSS for the prescribing of oral anticoagulant therapy. All studies assessed practitioner performance, with significant improvements found in 9 trials. Patient-related outcomes were poorly reported. CDSS were mainly designed to tackle the issues of anticoagulation underuse and drug-drug interactions. Most systems provided specific recommendations automatically, at the time and location of decision making.

Previous authors have conducted more general reviews of CDSS effects, including systems for diagnosis, for prevention, or for the prescription of any drug. In 2005, Garg and colleagues demonstrated that CDSS improved practitioner performance in 64% of 97 controlled trials[13]. Similarly, a systematic review investigating CDSS for drug prescribing revealed improvement in process of care outcomes in 63% of 59 studies[12]. This is consistent with current results, specific to the prescribing of oral anticoagulants and showing positive impact on practitioner performance in 60% of trials. Although an important quality of care outcome[47], adherence to guidelines may not translate into patient benefit. In this review, only 2 studies explored the impact of CDSS on clinical outcomes[32, 33], with no statistically significant influence on the rate of thromboembolic events. However, these studies also showed no or minimal changes in guidelines adherence. In addition, they were not powered for the detection of adverse events.

A difficulty encountered when conducting this systematic review was the heterogeneity of outcomes, limiting the comparability of studies[48]. Future work should focus on developing a core outcome set (COS) for interventions to improve anticoagulant prescribing, as has been done in older patients[49, 50]. The appropriateness of oral anticoagulant therapy appears a relevant process-related outcome and should be measured using comprehensive instruments such as the

Medication Appropriateness Index[24, 51]. To assess CDSS effect on clinical outcomes like ADR or drug-related hospital admissions, studies with a larger sample size and a longer follow-up period are needed. Cluster RCT, recommended to avoid contamination bias, are nevertheless unlikely to be performed on that scale given the considerable time and resources they would require[13].

This systematic review highlighted a shift over time from CDSS developed for the management of warfarin-drug interactions (4 studies published in 2006-2010[41-44]) to notifications regarding the indication of anticoagulation (8 studies published in 2015-2018[32-34, 37, 39, 40, 45, 46]). Although oral anticoagulants are strongly recommended in AF patients at high risk of stroke, their underuse remains substantial and represents a key safety issue[1, 52, 53]. Several national and international organizations focused on raising the awareness of the importance of anticoagulation[54, 55]. It is therefore not surprising that CDSS moved in the same direction. However, none CDSS provided recommendations regarding the choice between VKA and DOAC or among DOAC. In a previous analysis, we have shown that prescribing issues were frequently involved in serious ADRs related to the use of oral anticoagulants[10]. It included inadequate drug choice or dose for DOAC, or pharmacodynamics interactions for both VKA and DOAC. Therefore, CDSS for selecting the most appropriate oral anticoagulant, tailored to patient characteristics, should be developed. It is also definitely worth integrating alerts in CPOE systems to notify prescribers when DOAC dose is not adapted to renal function or for the management of DOAC-drug interactions. In Canada, the IMPACT-AF randomized trial is currently investigating a CDSS for primary care management of atrial fibrillation[56]. The tool includes individualized medication and dosage recommendations regarding anticoagulation with VKA and DOAC as therapeutic options, based on patient clinical characteristics and Canadian guidelines[57]. It also pre-fills DOAC authorization form. Results are expected in fall 2018.

As CDSS interventions improved process outcomes inconsistently, we may ask which characteristics are essential to ensure success. A few meta-regression analyses were carried out for that purpose[17, 58, 59]. They were nevertheless constrained by the limited number of good quality studies and the lack of consistent and systematic report of CDSS characteristics[60]. Four independent predictors of improved clinical practice were identified by Kawamoto and Lobach in 2005 and 2012: automatic provision as part of physician workflow, recommendations viewed by the prescriber directly on the computer screen, provision of a recommendation (not just an assessment), and provision at time and location of decision making[17, 58]. These characteristics were highly prevalent for the CDSS we investigated (73 to 93 %), similarly to previous reports[17, 18]. Two thirds of our included studies combined all 4 features, but proportions were identical whether or not a significant effect of CDSS intervention was proved. This suggests that other content or implementation characteristics may play a pivotal role in CDSS effectiveness. For instance, having to click many times to access information could negatively influence CDSS effects in a context of click burden and physician burnout[61]. In 11 studies, decision support was integrated with electronic records or electronic prescribing. CPOE + CDSS systems have been increasingly implemented as a patient safety strategy, especially in the United States where financial incentives were offered. They were reported to reduce prescribing errors, while their effect on adverse drug events remains unclear[62]. However, in two previous systematic reviews, integrated CDSS were less likely to be effective compared to stand-alone systems[12, 59]. Reasons advanced for this apparent paradox include the phenomenon of alert fatigue, which is more likely to occur when many alerts are generated. This is illustrated in 2 of our studies[37, 40], showing no significant effect of multifaceted CDSS on the prescribing of oral anticoagulants.

One striking finding was the low usage of CDSS – between 5 and 30% for the 3 studies reporting this outcome[34, 37, 39]. Arts and colleague have recently carried out a mixed method evaluation to gain a better understanding of this issue[63]. They found that 60% of physicians stopped using their primary care CDSS during the study period. Shortage of time was a main barrier, especially as notifications were often not related to the patient's reason for consultation. To overcome such situations, CDSS used in primary care should have the ability to suggest and remind follow-up visits dedicated to the management of oral anticoagulation. This is especially important for DOAC patients for whom clinical follow-up seems overlooked due to the lack of regular therapeutic monitoring[64]. A too high intensity of alerts and irrelevant recommendations were other significant discouraging factors, as previously highlighted[65, 66]. Therefore, efforts should be made to reduce the risk for alert fatigue when designing a CDSS for the prescribing of oral anticoagulants[67]. Future work is needed to determine the clinical relevance of drug-drug interactions (DDI) with DOAC (e.g. statins, bisoprolol) and thereby restrict notifications[68, 69]. In recent years the concept of context-aware DDI has emerged to make decision support more relevant[70, 71]. It integrates patient-specific contextual data (e.g. laboratory results, demographic characteristic) for risk assessment. Regarding oral anticoagulants, it would be interesting to develop alerts for pharmacodynamics interactions that are triggered only if hemoglobin or platelet count are out of range, or if the patient has other risk factors for bleeding[67].

There is growing acceptance that an effective CDSS implementation is as important as a relevant and accurate content[72]. The GUIDES checklist, headed by the Norwegian Institute of Public Health, has recently been developed to help professionals implement CDSS successfully[73]. According to this tool, key factors affecting CDSS integration include a clear communication and training sessions, an assessment of barriers and facilitating factors, and feedback and monitoring to detect CDSS malfunctions. In this review, we observed a trend towards a higher prevalence of support for CDSS use in successful studies. This hypothesis remains

nevertheless to be confirmed, given the small number of trials. Only 3 studies mentioned monitoring procedures for unintended consequences. The alarming frequency of CDSS malfunctions and the risk of e-iatrogenesis have been clearly highlighted in previous reports, but often remain undetected[74-76]. Finally, targeting the right user is essential when designing effective CDSS interventions[77]. In our review, CDSS for the prescribing of oral anticoagulants were equally assessed in primary care and hospital settings. Interestingly, Eckman and colleagues considered targeting cardiologists or clinical pharmacists in addition to primary care physicians, as the latter indicated they were not taking decisions about anticoagulation[39]. On the contrary, Weir and colleagues hypothesized that their CDSS may have greater utility in primary care as physicians have less experience of anticoagulation[36].

This systematic review has several limitations. First we limited our search strategy to full-text papers published in English. We cannot exclude publications in other languages, even though 93% of Medline records were in English. Publication bias may also have influenced results. However, we searched the trial register clinicaltrials.gov and contacted authors of registered studies without subsequent publication. Second, in order to limit variability in interventions and outcome measurements, we focused on CDSS designed to influence the prescriber only. The incorporation of patient preferences into antithrombotic therapy decisions is increasingly emphasized[54, 78]. As an example, the "Excellence in anticoagulation care" guidance from the NHS recommends that all decisions should be made in partnership with patients[79]. In 2017, O'Neill and colleagues systematically reviewed patient decision aids for stroke prevention in AF[80]. Most interventions were educational booklets that were viewed outside the clinical visit.

Conclusion

Fifteen studies investigating CDSS for the prescribing of oral anticoagulants were reviewed. A positive impact on practitioner performance was found in 60% of trials, while the effect on clinical outcomes remains unclear. The scope of CDSS, currently limited to the underuse of oral anticoagulants and warfarin-drug interactions, should evolve to help prescribers choose the most appropriate medication. Systems were overall well designed, including several potentially successful features. However, efforts should be made to improve the relevance and accuracy of notifications. Closer attention should also be paid to implementation outcomes, such as identifying barriers to adoption or monitoring CDSS adverse effects. Future research is needed to evaluate the impact of CDSS tackling the misuse of DOAC on prescribing appropriateness.

REFERENCES

1. Freedman B, Potpara TS, Lip GY. Stroke prevention in atrial fibrillation. Lancet 2016; 388: 806-17.
2. Castellucci LA, Cameron C, Le Gal G, et al. Clinical and safety outcomes associated with treatment of acute venous thromboembolism: a systematic review and meta-analysis. JAMA 2014; 312: 1122-35.
3. Damen NL, Baines R, Wagner C, Langelaan M. Medication-related adverse events during hospitalization: a retrospective patient record review study in The Netherlands. Pharmacoepidemiol Drug Saf 2017; 26: 32-9.
4. Hafner JW, Jr., Belknap SM, Squillante MD, Bucheit KA. Adverse drug events in emergency department patients. Ann Emerg Med 2002; 39: 258-67.
5. Pirmohamed M, James S, Meakin S, et al. Adverse drug reactions as cause of admission to hospital: prospective analysis of 18 820 patients. Bmj 2004; 329: 15-9.
6. Marquet K, Claes N, De Troy E, Kox G, Droogmans M, Vleugels A. A multicenter record review of in-hospital adverse drug events requiring a higher level of care. Acta Clin Belg 2017; 72: 156-62.
7. Shehab N, Lovegrove MC, Geller AI, Rose KO, Weidle NJ, Budnitz DS. US emergency department visits for outpatient adverse drug events, 2013-2014. JAMA 2016; 316: 2115-25.
8. Harper P, Young L, Merriman E. Bleeding risk with dabigatran in the frail elderly. N Engl J Med 2012; 366: 864-6.
9. Perrone V, Conti V, Venegoni M, et al. Seriousness, preventability, and burden impact of reported adverse drug reactions in Lombardy emergency departments: a retrospective 2-year characterization. Clinicoecon Outcomes Res 2014; 6: 505-14.
10. Sennesael AL, Larock AS, Devalet B, et al. Preventability of serious thromboembolic and bleeding events related to the use of oral anticoagulants: a prospective study. Br J Clin Pharmacol 2018.
11. Sim I, Gorman P, Greenes RA, et al. Clinical decision support systems for the practice of evidence-based medicine. J Am Med Inform Assoc 2001; 8: 527-34.
12. Hemens BJ, Holbrook A, Tonkin M, et al. Computerized clinical decision support systems for drug prescribing and management: a decision-maker-researcher partnership systematic review. Implement Sci 2011; 6: 89.
13. Garg AX, Adhikari NK, McDonald H, et al. Effects of computerized clinical decision support systems on practitioner performance and patient outcomes: a systematic review. Jama 2005; 293: 1223-38.
14. Bright TJ, Wong A, Dhurjati R, et al. Effect of clinical decision-support systems: a systematic review. Ann Intern Med 2012; 157: 29-43.
15. Jia P, Zhang L, Chen J, Zhao P, Zhang M. The Effects of Clinical Decision Support Systems on Medication Safety: An Overview. PLoS One 2016; 11: e0167683.
16. Varghese J, Kleine M, Gessner SI, Sandmann S, Dugas M. Effects of computerized decision support system implementations on patient outcomes in inpatient care: a systematic review. J Am Med Inform Assoc 2018; 25: 593-602.
17. Kawamoto K, Houlihan CA, Balas EA, Lobach DF. Improving clinical practice using clinical decision support systems: a systematic review of trials to identify features critical to success. Bmj 2005; 330: 765.

18. Mollon B, Chong J, Jr., Holbrook AM, Sung M, Thabane L, Foster G. Features predicting the success of computerized decision support for prescribing: a systematic review of randomized controlled trials. BMC Med Inform Decis Mak 2009; 9: 11.
19. Chatellier G, Colombet I, Degoulet P. An overview of the effect of computer-assisted management of anticoagulant therapy on the quality of anticoagulation. Int J Med Inform 1998; 49: 311-20.
20. Fitzmaurice DA, Hobbs FD, Delaney BC, Wilson S, McManus R. Review of computerized decision support systems for oral anticoagulation management. Br J Haematol 1998; 102: 907-9.
21. Borab ZM, Lanni MA, Tecce MG, Pannucci CJ, Fischer JP. Use of Computerized Clinical Decision Support Systems to Prevent Venous Thromboembolism in Surgical Patients: A Systematic Review and Meta-analysis. JAMA Surg 2017; 152: 638-45.
22. Kahn SR, Morrison DR, Diendere G, et al. Interventions for implementation of thromboprophylaxis in hospitalized patients at risk for venous thromboembolism. Cochrane Database Syst Rev 2018; 4: Cd008201.
23. Sennesael AL, Dogne JM, Spinewine A. Optimizing the Safe Use of Direct Oral Anticoagulants in Older Patients: A Teachable Moment. JAMA Intern Med 2015; 175: 1608-9.
24. Larock AS, Mullier F, Sennesael AL, et al. Appropriateness of prescribing dabigatran etexilate and rivaroxaban in patients with nonvalvular atrial fibrillation: a prospective study. Ann Pharmacother 2014; 48: 1258-68.
25. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009; 6: e1000097.
26. Pearson SA, Moxey A, Robertson J, et al. Do computerised clinical decision support systems for prescribing change practice? A systematic review of the literature (1990-2007). BMC Health Serv Res 2009; 9: 154.
27. Higgins J, Green S. Cochrane Handbook for Systematic Reviews of Interventions - Version 5.1.0. The Cochrane Collaboration; 2011. Available at: www.handbook.cochrane.org.
28. Cochrane Effective Practice and Organisation of Care (EPOC). Data collection form. EPOC Resources for review authors; 2017. Available at: http://epoc.cochrane.org/epoc-specific-resources-review-authors.
29. Berlin A, Sorani M, Sim I. A taxonomic description of computer-based clinical decision support systems. J Biomed Inform 2006; 39: 656-67.
30. Higgins JP, Altman DG, Gotzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. Bmj 2011; 343: d5928.
31. Sterne JA, Hernan MA, Reeves BC, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. Bmj 2016; 355: i4919.
32. Karlsson LO, Nilsson S, Bang M. A clinical decision support tool for improving adherence to guidelines on anticoagulant therapy in patients with atrial fibrillation at risk of stroke: A cluster-randomized trial in a Swedish primary care setting (the CDS-AF study). 2018; 15: e1002528.
33. Holt TA, Dalton A, Marshall T, et al. Automated Software System to Promote Anticoagulation and Reduce Stroke Risk: Cluster-Randomized Controlled Trial. Stroke 2017; 48: 787-90.
34. Silbernagel G, Spirk D, Hager A, Baumgartner I, Kucher N. Electronic Alert System for Improving Stroke Prevention Among Hospitalized Oral-Anticoagulation-Naive Patients With Atrial Fibrillation: A Randomized Trial. J Am Heart Assoc 2016; 5.
35. Kruger K, Strand L, Geitung JT, Eide GE, Grimsmo A. Can electronic tools help improve nursing home quality? ISRN Nurs 2011; 2011: 208142.

36. Weir CJ, Lees KR, MacWalter RS, et al. Cluster-randomized, controlled trial of computer-based decision support for selecting long-term anti-thrombotic therapy after acute ischaemic stroke. Qjm 2003; 96: 143-53.

37. Arts DL, Abu-Hanna A, Medlock SK, van Weert HC. Effectiveness and usage of a decision support system to improve stroke prevention in general practice: A cluster randomized controlled trial. PLoS One 2017; 12: e0170974.

38. Velez-Diaz-Pallares M, Ruano M, Villamañán E, Larrubia Y, Wagner E, Herrero A. Impact of electronic prescription alerts on medication errors related to Vitamin K antagonists in hospitalised patients. European Journal of Hospital Pharmacy 2014; 21: 29-33.

39. Eckman MH, Lip GY, Wise RE, et al. Impact of an Atrial Fibrillation Decision Support Tool on thromboprophylaxis for atrial fibrillation. Am Heart J 2016; 176: 17-27.

40. Cook DA, Enders F, Caraballo PJ, Nishimura RA, Lloyd FJ. An automated clinical alert system for newly-diagnosed atrial fibrillation. PLoS One 2015; 10: e0122153.

41. Strom BL, Schinnar R, Aberra F, et al. Unintended effects of a computerized physician order entry nearly hard-stop alert to prevent a drug interaction: a randomized controlled trial. Arch Intern Med 2010; 170: 1578-83.

42. Strom BL, Schinnar R, Bilker W, Hennessy S, Leonard CE, Pifer E. Randomized clinical trial of a customized electronic alert requiring an affirmative response compared to a control group receiving a commercial passive CPOE alert: NSAID--warfarin co-prescribing as a test case. J Am Med Inform Assoc 2010; 17: 411-5.

43. Feldstein AC, Smith DH, Perrin N, et al. Reducing warfarin medication interactions: an interrupted time series evaluation. Arch Intern Med 2006; 166: 1009-15.

44. Judge J, Field TS, DeFlorio M, et al. Prescribers' responses to alerts during medication ordering in the long term care setting. J Am Med Inform Assoc 2006; 13: 385-90.

45. Bajorek BV, Magin PJ, Hilmer SN, Krass I. Optimizing Stroke Prevention in Patients With Atrial Fibrillation: A Cluster-Randomized Controlled Trial of a Computerized Antithrombotic Risk Assessment Tool in Australian General Practice, 2012-2013. Prev Chronic Dis 2016; 13: E90.

46. Sheibani R, Sheibani M, Heidari-Bakavoli A, Abu-Hanna A, Eslami S. The Effect of a Clinical Decision Support System on Improving Adherence to Guideline in the Treatment of Atrial Fibrillation: An Interrupted Time Series Study. J Med Syst 2017; 42: 26.

47. What outcomes should be reported in EPOC reviews? . Cochrane Effective Practice and Organisation of Care (EPOC). ; 2017. Available at: https://epoc.cochrane.org/resources/epoc-resources-review-authors.

48. Seidling HM, Bates DW. Evaluating the Impact of Health IT on Medication Safety. Stud Health Technol Inform 2016; 222: 195-205.

49. Millar AN, Daffu-O'Reilly A, Hughes CM, et al. Development of a core outcome set for effectiveness trials aimed at optimising prescribing in older adults in care homes. Trials 2017; 18: 175.

50. Beuscart JB, Knol W, Cullinan S, et al. International core outcome set for clinical trials of medication review in multi-morbid older patients with polypharmacy. BMC Med 2018; 16: 21.

51. Spinewine A, Schmader KE, Barber N, et al. Appropriate prescribing in elderly people: how well can it be measured and optimised? Lancet 2007; 370: 173-84.

52. Jones NR, Hobbs FDR, Taylor CJ. Underuse of anticoagulation therapy for atrial fibrillation: Are we failing our patients? Can Fam Physician 2017; 63: 943-4.

53. Xian Y, O'Brien EC, Liang L, et al. Association of Preceding Antithrombotic Treatment With Acute Ischemic Stroke Severity and In-Hospital Outcomes Among Patients With Atrial Fibrillation. Jama 2017; 317: 1057-67.
54. Kirchhof P, Benussi S, Kotecha D, et al. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. Europace 2016; 18: 1609-78.
55. Jones C, Pollit V, Fitzmaurice D, Cowan C, Guideline Development G. The management of atrial fibrillation: summary of updated NICE guidance. BMJ 2014; 348: g3655.
56. Cox JL, Parkash R, Abidi SS, et al. Optimizing primary care management of atrial fibrillation: The rationale and methods of the Integrated Management Program Advancing Community Treatment of Atrial Fibrillation (IMPACT-AF) study. Am Heart J 2018; 201: 149-57.
57. Abidi SR, Cox J, Abusharekh A, Hashemian N, Abidi SS. A Digital Health System to Assist Family Physicians to Safely Prescribe NOAC Medications. Stud Health Technol Inform 2016; 228: 519-23.
58. Lobach D, Sanders GD, Bright TJ, et al. Enabling health care decisionmaking through clinical decision support and knowledge management. Evid Rep Technol Assess (Full Rep) 2012: 1-784.
59. Roshanov PS, Fernandes N, Wilczynski JM, et al. Features of effective computerised clinical decision support systems: meta-regression of 162 randomised trials. Bmj 2013; 346: f657.
60. Lobach DF. The road to effective clinical decision support: are we there yet? Bmj 2013; 346: f1616.
61. Collier R. Rethinking EHR interfaces to reduce click fatigue and physician burnout. Cmaj 2018; 190: E994-e5.
62. Ranji SR, Rennke S, Wachter RM. Computerised provider order entry combined with clinical decision support systems to improve medication safety: a narrative review. BMJ Qual Saf 2014; 23: 773-80.
63. Arts DL, Medlock SK, van Weert H, Wyatt JC, Abu-Hanna A. Acceptance and barriers pertaining to a general practice decision support system for multiple clinical conditions: A mixed methods evaluation. PLoS One 2018; 13: e0193187.
64. Sennesael AL, Larock AS, Devalet B, et al. Preventability of serious thromboembolic and bleeding events related to the use of oral anticoagulants: a prospective study. Br J Clin Pharmacol 2018; 84: 1544-56.
65. Lugtenberg M, Weenink JW, van der Weijden T, Westert GP, Kool RB. Implementation of multiple-domain covering computerized decision support systems in primary care: a focus group study on perceived barriers. BMC Med Inform Decis Mak 2015; 15: 82.
66. Cornu P, Steurbaut S, De Beukeleer M, Putman K, Van De Velde R, Dupont AG. Physician's expectations regarding prescribing clinical decision support systems in a Belgian hospital. Acta Clinica Belgica 2014; 69: 157-64.
67. Tolley CL, Slight SP, Husband AK, Watson N, Bates DW. Improving medication-related clinical decision support. Am J Health Syst Pharm 2018; 75: 239-46.
68. Antoniou T, Macdonald EM, Yao Z, et al. Association between statin use and ischemic stroke or major hemorrhage in patients taking dabigatran for atrial fibrillation. Cmaj 2017; 189: E4-e10.
69. Nehaj F, Sokol J, Ivankova J, Mokan M, Mokan M. Effect of Bisoprolol on the Level of Dabigatran. Am J Ther 2018.
70. Duke JD, Bolchini D. A successful model and visual design for creating context-aware drug-drug interaction alerts. AMIA Annu Symp Proc 2011; 2011: 339-48.

71. Cornu P, Steurbaut S, Gentens K, Van de Velde R, Dupont AG. Pilot evaluation of an optimized context-specific drug-drug interaction alerting system: A controlled pre-post study. Int J Med Inform 2015; 84: 617-29.

72. Beeler PE, Bates DW, Hug BL. Clinical decision support systems. Swiss Med Wkly 2014; 144: w14073.

73. Van de Velde S, Kunnamo I, Roshanov P, et al. The GUIDES checklist: development of a tool to improve the successful use of guideline-based computerised clinical decision support. Implement Sci 2018; 13: 86.

74. Wright A, Hickman TT, McEvoy D, et al. Analysis of clinical decision support system malfunctions: a case series and survey. J Am Med Inform Assoc 2016; 23: 1068-76.

75. Stone EG. Unintended adverse consequences of a clinical decision support system: two cases. J Am Med Inform Assoc 2018; 25: 564-7.

76. Weiner JP, Kfuri T, Chan K, Fowles JB. "e-Iatrogenesis": The Most Critical Unintended Consequence of CPOE and other HIT. Journal of the American Medical Informatics Association : JAMIA 2007; 14: 387-8.

77. Sirajuddin AM, Osheroff JA, Sittig DF, Chuo J, Velasco F, Collins DA. Implementation pearls from a new guidebook on improving medication use and outcomes with clinical decision support. Effective CDS is essential for addressing healthcare performance improvement imperatives. J Healthc Inf Manag 2009; 23: 38-45.

78. Kearon C, Akl EA, Ornelas J, et al. Antithrombotic Therapy for VTE Disease: CHEST Guideline and Expert Panel Report. Chest 2016; 149: 315-52.

79. Excellence in anticoagulant care - Defining the elements of an excellent anticoagulation service NHS, 2016.

80. O'Neill ES, Grande SW, Sherman A, Elwyn G, Coylewright M. Availability of patient decision aids for stroke prevention in atrial fibrillation: A systematic review. Am Heart J 2017; 191: 1-11.

Appendix 1: Medline search strategy – PubMed interface (15.03.2018)

1. Anticoagula*[Title/Abstract] OR anti-coagula*[Title/Abstract] OR antithrombotic[Title/Abstract] OR anti-thrombotic[Title/Abstract]
2. DOAC[Title/Abstract] OR DOACS[Title/Abstract] OR NOAC[Title/Abstract] OR NOACS[Title/Abstract] OR factor Xa inhibitor*[Title/Abstract] OR factor Xa antagonist*[Title/Abstract] OR thrombin inhibitor*[Title/Abstract] OR dabigatran[Title/Abstract] OR pradaxa[Title/Abstract] OR rivaroxaban[Title/Abstract] OR xarelto[Title/Abstract] OR apixaban[Title/Abstract] OR eliquis[Title/Abstract] OR edoxaban[Title/Abstract] OR lixiana[Title/Abstract]
3. Vitamin K antagonist*[Title/Abstract] OR vitamin K inhibitor*[Title/Abstract] OR VKA[Title/Abstract] OR VKAS[Title/Abstract] OR coumadin*[Title/Abstract] OR warfarin[Title/Abstract] OR marevan[Title/Abstract] OR acenocoumarol[Title/Abstract] OR sintrom[Title/Abstract] OR phenprocoumon[Title/Abstract] OR marcoumar[Title/Abstract] OR fluindione[Title/Abstract] OR previscan[Title/Abstract]
4. Anticoagulants[MeSH Terms]
5. Thrombin/antagonists and inhibitors[MeSH Terms]
6. Factor Xa Inhibitors[MeSH Terms]
7. Apixaban[Supplementary concept]
8. Dabigatran[MeSH Terms]
9. Edoxaban[Supplementary concept]
10. Rivaroxaban[MeSH Terms]
11. Coumarins[MeSH Terms]
12. Vitamin K/antagonists and inhibitors[MeSH Terms]
13. **OR/1-12**
14. ((Computerized[Title/Abstract] OR computerised[Title/Abstract] OR computer[Title/Abstract]) AND order entry[Title/Abstract]) OR computerized prescribing[Title/Abstract] OR computerised prescribing[Title/Abstract] OR electronic prescribing[Title/Abstract] OR electronic order entry[Title/Abstract] OR medical order entry[Title/Abstract] OR CPOE[Title/Abstract]

15. Alert[Title/Abstract] OR alerts[Title/Abstract] OR reminder*[Title/Abstract] OR ((computerized[Title/Abstract] OR computerised[Title/Abstract]) AND assessment[Title/Abstract]) OR decision support*[Title/Abstract] OR decision aid*[Title/Abstract] OR decision tool*[Title/Abstract] OR decision making aid*[Title/Abstract] OR decision making tool*[Title/Abstract] OR *CDS*[Title/Abstract]
16. Ipad[Title/Abstract] OR smartphone[Title/Abstract] OR mobile application[Title/Abstract] OR computer assist*[Title/Abstract]
17. Decision support systems, clinical[MeSH Terms]
18. Decision-making, computer-assisted[MeSH Terms]
19. Decision support techniques[MeSH Terms]
20. Expert systems[MeSH Terms]
21. Reminder systems[MeSH Terms]
22. Clinical pharmacy information systems[MeSH Terms]
23. Medical order entry systems[MeSH Terms]
24. Electronic prescribing[MeSH Terms]
25. Software[MeSH Terms]
26. **OR/14-25**
27. **13 AND 26**
28. Filter English
29. Date limitations (01/01/1998 → 15/03/2018)

Appendix 2: developed tool for risk of bias assessment

	ADEQUATE (LOW RISK OF BIAS)	INADEQUATE (HIGH RISK OF BIAS)
Selection bias (differences between baseline characteristics of the groups compared)		
Sequence generation **(RCT)**	Randomized sequence of assignments (e.g. random number table, computer generated list)	Nonrandom method of assignment (e.g. date of birth, hospital record number, patient's preference, availability of intervention)
Allocation concealment **(RCT)**	Central randomization (web-based, phone, pharmacy), sequentially numbered, opaque, sealed envelopes	Open random allocation schedule, envelopes without appropriate safeguards
Confounding **(non-RCT)**	No baseline differences between groups that were potentially linked to the study outcome OR appropriate statistical adjustments (e.g. stratification, matching, inverse probability weighting)	Baseline differences between groups that were potentially linked to the study outcome AND no statistical adjustment made
Selection bias **(non-RCT)**	Inclusion of all patients who would have been eligible Start of follow-up and intervention coincide for all subjects	Selection into the study related to intervention and outcome Start of follow-up and intervention do not coincide for all subjects
Performance bias (differences in the care that is provided, or in exposure to other factors than the intervention)		
Deviations from intended interventions	Blinding ensured and unlikely to have been broken Patients or HCP aware of assignment but outcome not likely to be influenced For CDSS-RCT: unit of allocation = practices or clinicians	Patients or HCP aware of assignment and outcome likely to be influenced Blinding attempted but likely that could have been broken For CDSS-RCT: unit of allocation = individual patients
Misclassification of intervention **(non-RCT)**	Intervention status is well defined and based solely on information collected at the time of intervention (without knowledge of subsequent outcomes)	Intervention status is not well defined, or the assignment of intervention status could have been affected by knowledge of outcomes

Appendix 2: developed tool for risk of bias assessment (continued)

	ADEQUATE (LOW RISK OF BIAS)	INADEQUATE (HIGH RISK OF BIAS)
Attrition bias (differences in withdrawals from a study)		
Missing data	Outcome data available for all patients Missing outcome data, but unlikely to bias the results (reasons reported and balanced across groups, unlikely to be connected with outcome)	Missing outcome data, and likely to bias the results (differences in proportion and reasons across groups, related to outcomes)
Detection bias (differences in how outcomes are determined)		
Blinding of assessors	Measurement errors are unrelated to the intervention received (objective outcome, subjective outcome with blinded assessment)	Subjective outcome without blinded assessment Use of different methods to assess outcomes in the different groups
Reporting bias (differences between reported and unreported outcomes)		
	No evidence that outcomes were selectively reported (all pre-specified main outcomes are reported)	Some important outcomes are subsequently omitted from the results (not all pre-specified main outcomes are reported)

Appendix 3: main results on practitioner performance

Study	Outcome	Interv.	Control	Effect
Adherence to guidelines or CDSS recommendations				
Arts, 2017[37]	Prop. of AF patients treated according to the guidelines	55%	50%	NS
Cook, 2015[40]	Prop. of new AF patients treated according to the guidelines	27%	36%	NS
Eckman, 2016[39]	Discordance between CDSS recommendation and actual Tx	41%	40%	NS
Holt, 2017[33]	Prop. of AF patients treated according to the guidelines	66%	64%	NS
Karlsson, 2018[32]	Prop. of AF patients treated according to the guidelines	73%	71%	↑
Sheibani, 2017[46]	Prop. of AF patients treated according to the guidelines	66%	48%	↑
Silbernagel, 2016[34]	Prop. of OAC-naïve AF patients treated according to the guidelines	22%	16%	↑
Weir, 2003[36]	Risk reduction in ischemic and bleeding events achieved by Tx	17%	16%	NS
Use of oral anticoagulation				
Bajorek, 2016[45]	Prop. of patients receiving OAC	-	-	↑
Kruger, 2011[35]	Use of warfarin	10%	3%	↑
Weir, 2003[36]	Prop. of patients receiving warfarin	14%	12%	-
Prescription of interacting drugs				
Judge, 2006[44]	Prop. of alerts followed by an appropriate action	25%	7%	↑
Strom, 2010 (A)[42]	Not reordering the alert-triggering drug after firing	25%	28%*	NS
Strom, 2010 (B)[41]	Not reordering the alert-triggering drug after firing	58%	14%	↑
Feldstein, 2006[43]	Interacting prescriptions per 10000 warfarin users per month	2804	3294	↓
Velez, 2014[38]	Medication errors caused by interactions per month	3	11	↓

AF: atrial fibrillation, Interv. : intervention, OAC: oral anticoagulant, NS: not significant, Prop. : proportion, Tx: treatment

* CDSS compared to a more passive alert

Appendix 4: main results on implementation outcomes

Study	Implementation outcome
Use of the CDSS	
Arts, 2017[37]	Opening of notifications: 5%
Eckman, 2016[39]	Review of CDSS recommendations: 30%
Silbernagel, 2016[34]	Use of the score calculation tool by the physician: 11%
Reasons for deviating from recommendations	
Arts, 2017[37]	*Insufficient data for analysis*
Bajorek, 2016[45]	Treatment considered appropriate, Following the cardiologist's recommendation
Cook, 2015[40]	Surgery planned (23%), choice of aspirin (19%), no reason (16%), warfarin use (11%)
Eckman, 2016[39]	Patient preference (27%), management by specialist (24%), disagreement with recommendation (9%)
Karlsson, 2018[32]	Patient preference (18%), bleeding risk (15%), risk of falls (10%), terminal illness (8%), other contraindication (6%), other reason (43%)
Monitoring of CDSS unintended consequences (other than bleeding events)	
Holt, 2017[33]	No reports of inappropriate clinical decisions triggered by CDSS use
Strom, 2010 (B)[41]	4 cases of delay in prescribing of TMP-SMX or administration of warfarin
Accuracy of notifications	
Cook, 2015[40]	Notifications of new AF judged to be truly newly-diagnosed: 44%
Acceptability of the CDSS	
Weir, 2003[36]	Feeling that the CDSS influenced the prescribing practice: 5/9 clinicians

AF: atrial fibrillation, TMP-SMX: co-trimoxazole

Discussion

I. Key findings of this work

Direct oral anticoagulants (DOAC) have transformed the landscape of oral anticoagulant therapy, with the promise of allegedly safer, one-size-fits-all and more convenient drugs. They are increasingly used in clinical practice, mainly for stroke prevention in non-valvular atrial fibrillation (NVAF). However, serious adverse drug reactions (ADR) have been frequently described in patients taking DOAC, including bleeding and thromboembolic events. Evidence has suggested that the inappropriate use of DOAC or genetic determinants might contribute to the occurrence of ADR. Moreover, the management of DOAC-related ADR is undergoing fundamental changes with the arrival of specific reversal agents, requiring validation and implementation of coagulation assays.

It is within this context that we carried out this research project. The objectives were: 1) to determine the preventability and explore underlying reasons of serious ADR associated with the use of DOAC, 2) to assess the performance of an original coagulation assay to screen rapidly the intensity of DOAC anticoagulation, 3) to evaluate the *in vitro* effect of *ABCB1* polymorphisms on rivaroxaban transport, and 4) to review the impact of clinical decision support systems (CDSS) for the prescribing of oral anticoagulants.

In *chapter I*, we conducted a prospective cohort study in the emergency departments (ED) of two teaching hospitals. Patients admitted for a thromboembolic or bleeding event while under DOAC or vitamin K antagonist (VKA) were included. More than half of serious ADR were deemed potentially preventable, both for DOAC and VKA. Prescribing and adherence issues were frequently observed in DOAC patients. Several factors contributing to ADR were identified during interviews with general practitioners. Some were common to both classes of oral anticoagulants, such as the concomitant and inadequate use of antiplatelet therapy or communication issues.

In *chapter II*, the performance of an original coagulation assay (DRVV-DOAC) was determined using clinical samples from DOAC patients. The DRVV-DOAC was a ready-to-use dilute Russell's viper venom time (dRVVT) reagent, with an enhanced sensitivity to DOAC. Results suggest that DRVV-DOAC could be rapidly performed as a qualitative test to exclude clinically significant DOAC concentrations. However, the sensitivity of the assay regarding apixaban requires improvement.

In connection with *Chapter I* and *Chapter III*, we measured rivaroxaban plasma concentrations in 10 patients admitted to the ED for bleeding events. Observations confirmed the high inter-individual variability in DOAC levels. Four patients had higher-than-expected rivaroxaban levels. Several risk factors for bleeding were found at the individual level, including inappropriate use of rivaroxaban and drug interactions. No clear association between plasma concentrations and *ABCB1* genotype data was shown.

In *chapter III*, the potential contribution of several *ABCB1* genetic polymorphisms to DOAC-related ADR was investigated *in vitro*. We found that the 1236C>T-2677G>T-3435C>T and 1199G>A single nucleotide polymorphisms (SNP) had no significant effect on the transport of rivaroxaban in HEK293 recombinant cell lines. Therefore, they are unlikely to contribute to the inter-individual variability in DOAC concentrations.

Finally, in *chapter IV*, we systematically reviewed evidence regarding the impact of CDSS for the prescribing of oral anticoagulants. Fifteen studies were identified, more than half of them including AF patients. CDSS recommendations were mainly related to the indication of oral anticoagulation and drug interactions. None CDSS delivered individualized assessment for drug selection among DOAC and VKA. Most were integrated in electronic health records or electronic prescribing, providing support automatically at the time and location of decision making. Significant improvements in practitioner performance were reported in 9 of 15 studies.

II. Critical appraisal

1) Research relevance

As several studies have stressed, anticoagulants are an important cause of serious ADR, leading to hospital admissions or requiring higher level of care[1, 2]. A significant part of these ADR was shown to result from medication errors and the subsequent inappropriate use of anticoagulants[3]. As an example, before the arrival of DOAC, 70% of anticoagulant-related ADR were found to be potentially preventable in inpatients. Warfarin was involved in one ADR out of 5, while others concerned parenteral anticoagulants[4].

During this thesis, we extended knowledge of ADR associated with the use of DOAC. Major bleeding events have been well described in real-life DOAC patients, including incidence rates, management strategies or outcomes[5, 6]. However, their preventability had not been investigated yet. Our findings therefore constitute an important step forward in addressing this patient safety issue. The identification of contributing factors to ADR should help effectively target interventions to optimize the use of DOAC in primary and secondary care[7, 8]. Moreover, we also took into consideration the common and critical issue of therapeutic failure – that is the occurrence of thromboembolic events resulting from inadequate anticoagulant therapy[9].

Literature is exhibiting a growing interest in DOAC measurement, particularly for urgent situations[10-13]. Concern is even greater since the introduction of DOAC reversal agents, as the approved dose of idarucizumab may be insufficient to reverse dabigatran in case of drug accumulation[14, 15]. Nevertheless, in emergency departments, assays that rapidly provide the intensity of DOAC anticoagulation are still lacking. Several studies had previously described dRVVT-based assays for dabigatran and rivaroxaban measurement[16, 17]. For the first time, we used an optimized and liquid-stable reagent. This feature is of paramount importance to

reduce routine turnaround time, given the 30 additional minutes required for reconstitution of commercially available reagents. Moreover, the performance of dRVVT-based assays regarding apixaban had not been investigated previously. Results were eagerly awaited as no conventional assay can be recommended for the screening of apixaban levels[18].

Pharmacogenomics is increasingly being suggested as a potential source for improving patient safety[19]. For instance, the US Food and Drug Administration has now provided recommendations with consideration of genotype in labelling of more than 150 medications, including warfarin. With regard to DOAC, the effect of some *ABCB1* genetic polymorphisms on drug exposure had been previously evaluated in healthy volunteers and NVAF patients, showing conflicting results[20]. High inter-individual variability and small sample size might have contributed to negative findings in some of these studies [21, 22].Therefore, it was important to clarify *in vitro* the impact of *ABCB1* genetic polymorphisms on DOAC transport. We provided the first *in vitro* data concerning rivaroxaban.

2) Strengths

Translational and interdisciplinary approach

Translational research is critical to improve patient care from basic science discoveries. Several research funding agencies such as the National Institutes of Health (NIH) recognized the importance of translation and made it a priority[23]. Far more than the simplistic view of "bench-to-bedside", translational research has been described as an iterative process including 5 key phases (T0 to T4)[24]. Consideration was given to moving from basic knowledge to a new health intervention (e.g. therapy, diagnosis, prevention), but also to translating research findings into practice and policy. Figure 1 illustrates the different aspects of translational research that were investigated during the present thesis to address

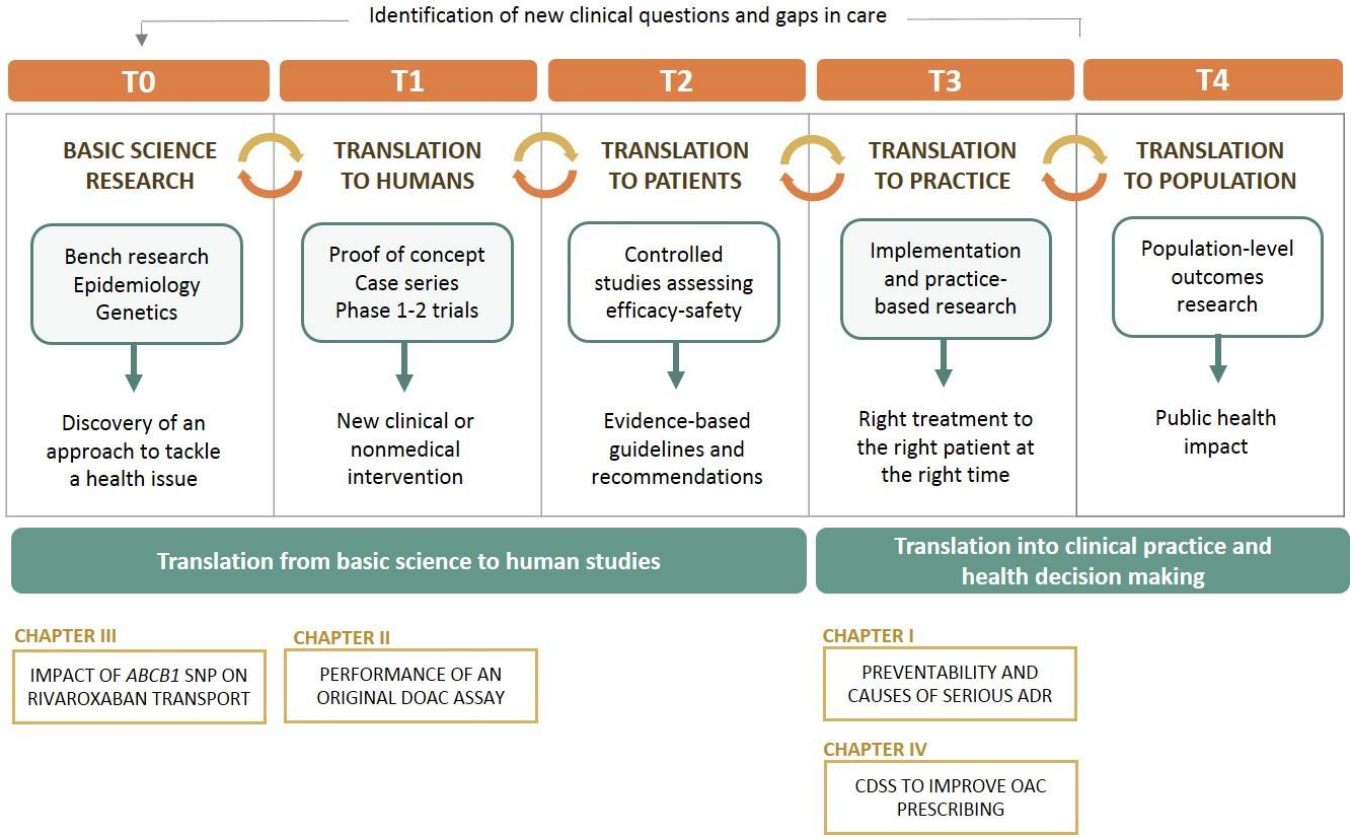
the issue of DOAC-related ADR. We explored a wide range of research disciplines including medication errors, laboratory measurement, pharmacogenomics, and health information technology. Moreover, connections were established between the different fields of research. As an example, in *chapter II.2*, plasma samples were drawn from a few rivaroxaban patients included in our prospective study. Genotyping and prescribing data were examined to interpret DOAC measurement.

For each part of the project, we benefited from a favorable working environment. The evaluation of DRVV-DOAC performance was based on the measurement of DOAC plasma concentrations by LC-MS/MS. This reference method, only available in specialized laboratories, is considered the most accurate to address drug concentrations[25]. Regarding pharmacogenomics experiments, we took advantage from recombinant cell lines overexpressing ABCB1, which were previously generated and validated[26-28]. Finally, all tools for ADR assessment and semi-structured interviews were reviewed by experts in the field of clinical pharmacy, thrombosis and hemostasis or qualitative research.

Comprehensive overview of ADR

We gave an overall and reliable view of ADR associated with the use of DOAC. ADR were characterized after prospective data collection and medication history by experienced clinical pharmacists. Their preventability was assessed by four independent reviewers from different backgrounds. Upstream, we explored potential contributing factors. Perceptions of general practitioners (GP) were highlighted using qualitative research and genetic determinants were analyzed. Downstream, we looked at the management of DOAC-related ADR, especially the contribution of DOAC measurement. To close the loop, interventions to reduce ADR were approached by investigating the impact of CDSS for the prescribing of oral anticoagulants.

Figure 1: Phases of translational research investigated in the present thesis[24, 29, 30]



Adapted from Blumberg et al, 2012[31]

3) Limitations and validity of this work

We believe that this research project presents two main limitations. First, our prospective study focused on patients who were prescribed DOAC or VKA. However, we did not consider the other side of the coin: thromboembolic events related to the underuse of oral anticoagulants[32]. As an example, it was recently shown in patients with acute ischemic stroke and a known history of AF (CHA_2DS_2-VASC≥2) that 70% were not receiving anticoagulation[33]. These potentially preventable strokes represent another key safety issue in AF patients and would have deserved further investigation. Among underlying reasons for underuse, the feeling of responsibility when serious bleeding occurs and concerns about the application of evidence to daily practice were previously reported by physicians[34, 35]. To increase the use of oral anticoagulants, several interventions were designed such as the IMPACT-AF trial where the combination of education, monitoring and feedback resulted in positive outcomes[36]. In the same way, our systematic review highlighted that most CDSS developed to improve oral anticoagulants prescribing tackled the problem of underuse.

Second, although comprehensive medication history and adherence assessment were performed with patients, their perspectives were not considered in the present thesis. Patient involvement in research is increasingly recognized as a key factor in fulfilling patients' need and achieving translational research[37, 38]. This comprises patient participation in identification of priorities and design of consistent research projects, but also the inclusion of patient-reported outcome measures (PROM) in clinical trials[39, 40]. However, from 2005 to 2008, only 16% of cardiovascular trials reported PROM in 10 leading journals[41]. Health-related quality of life and treatment satisfaction have been previously investigated in DOAC patients, using recently developed questionnaires such as the Anti-Clot Treatment Scale (ACTS) or the Perception of Anticoagulant Treatment Questionnaire (PACT-

Q)[42, 43]. It would have been worthwhile to investigate the perceived burden of anticoagulation in patients who have experienced serious ADR.

Given the time-consuming character of qualitative research, it was not feasible to include both physicians and patients' views about factors contributing to ADR. We chose to conduct interviews with GP as they play a central role in managing DOAC patients. Nevertheless, some findings may have benefited from patient insights, as illustrated in Box 1. In a previous qualitative study exploring causes of preventable drug-related hospital admissions, perspectives of GP, patients and community pharmacists were triangulated[44]. It allowed the identification of communication problems between patients and HCP, as well as patient knowledge gaps about medication. The educational needs of DOAC patients have been recently highlighted in a qualitative study, including the development of educational materials to help understanding[45].

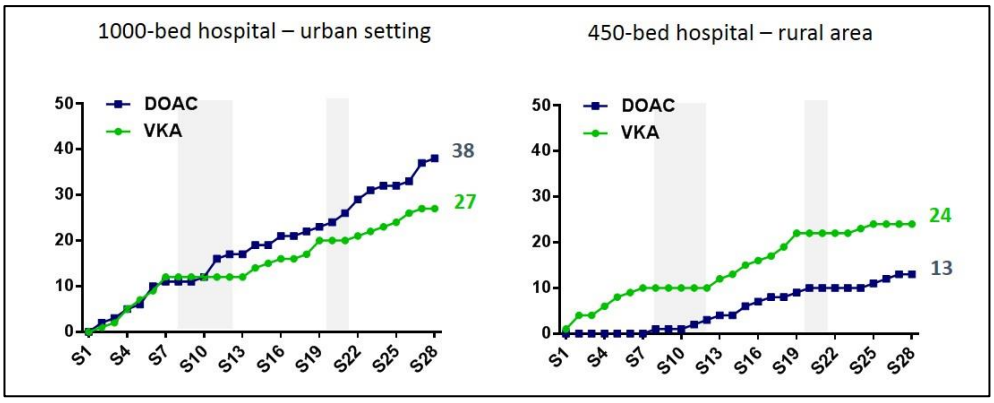
Box 1: examples of qualitative findings (GPs' interviews) that may have benefited from patient insights

- **Patient behavior towards anticoagulation**
  « Je pense qu'il y a certaines situations où les gens ne se rendent pas compte de la gravité de ce qu'ils prennent, de l'intérêt d'être vigilant avec ses médicaments, de les prendre à temps et à heure, de ne pas faire n'importe quoi avec. »
- **Patient non-adherence**
  « Sous anticoagulants oraux ça m'arrive d'avoir des gens qui font des hématuries. Alors vous avez des gens qui avec deux prises par jour ne veulent plus en prendre qu'une. Avec toutes les conséquences...»
- **Patient education**
  « Une fois qu'ils sortent du spécialiste avec le traitement, ils reçoivent tellement d'informations d'un coup, ils n'enregistrent pas forcément tout et ils ne font pas très attention. »
- **Communication between general practitioners and patients**
  « Je suis devenu juste prescripteur. Alors parfois je refuse, je leur dis vous devez venir. "Ah mais j'en parlerai avec mon spécialiste, il me fera des ordonnances pour 3 mois comme ça vous ne devrez plus en faire." Ça évolue comme ça. »
- **Banalization of anticoagulation**
  « C'est un médicament comme un autre, peut-être que justement il passe trop inaperçu par rapport à d'autres en disant… quand même se rappeler que c'est un médicament dangereux. »

Generalizability of findings

Although our prospective study was performed in an academic setting, the two hospitals sharply differed in size and location. One of them was a 1000-bed hospital in an urban setting while the other was a 450-bed hospital in a rural area. Rates of and reasons for ED admissions were not similar, as well as the proportion of DOAC- vs. VKA-related admissions (figure 1). Therefore, our results are expected to be representative of the current situation in Belgium.

Figure 2: cumulated number of inclusions per week of study



VKA-patients enrolment was temporarily stopped from S8 to S12 and from S20 to S21.

In addition, characteristics of GP who participated in interviews were well-balanced regarding gender, experience and population density of practice site. The 3 regions of the country were covered. Most issues that have emerged from GP interviews were previously reported in a more general context (e.g. non-adherence, drug-drug interactions or communication issues) [46-49]. However, some findings might be less relevant to countries with a different anticoagulation management in primary care. For example, anticoagulation clinics are the major site for INR measurement in Italy, Spain and the UK, while it is not the case in Belgium[50]. Moreover, national healthcare systems may adopt more or less stringent eligibility criteria for the use of DOAC[51].

Regarding the more fundamental aspects of this work, the performance of the DRVV-DOAC assay was determined using a STA-R Evolution coagulometer (Diagnostica Stago). We cannot exclude that thresholds to rule out clinically significant DOAC concentrations would differ depending on the coagulometer used. Instrument-related differences in sensitivity were indeed demonstrated for routine tests of hemostasis[52]. Moreover, as pharmacogenomics experiments were carried out for rivaroxaban only, we may ask whether results are generalizable to other DOAC. *In vitro* experiments have recently shown that ABCB1 was involved in the disposition of all DOAC, but at varying degrees[53]. The transport of dabigatran etexilate was the most influenced by ABCB1 inhibition, with an 87% reduction of the efflux. In addition, the very low and highly variable oral bioavailability of dabigatran etexilate makes this prodrug a suitable candidate for additional experiments[54].

III. Clinical perspectives

1) Impact of this work

Recommendations for general practitioners

The arrival of DOAC has brought profound changes in the management of oral anticoagulation in primary care, with a shift from biological to clinical monitoring. Even if DOAC prescriptions are often initiated by a specialist physician, general practitioners (GP) are key actors in minimizing the risk of ADR in DOAC patients. Our prospective study highlighted that four main issues were involved in 80% of potentially preventable DOAC-related ADR: 1) inappropriate drug choice, 2) inadequate DOAC dose, 3) pharmacodynamics drug interactions, and 4) patient non-adherence. For each of these issues, recommendations for the daily practice of GP are provided in Box 2.

Box 2: recommendations for the daily practice of general practitioners

- **Inappropriate choice of treatment**
  - To individualize anticoagulation according to patient characteristics and preferences
  - To prescribe DOAC cautiously in older patient with low body weight and moderate or severe renal impairment
  - To reassess treatment choice after serious ADR (instead of using off-label doses)
- **Inappropriate DOAC dose**
  - To monitor renal function before DOAC initiation and during follow-up
- **Pharmacodynamics drug interactions**
  - To reassess aspirin (and discontinue if primary prevention or stable arterial disease)
  - To avoid the prescription of non-steroidal anti-inflammatory drugs (NSAID)
- **Patient non-adherence**
  - To evaluate drug management at home and patient adherence
  - To understand reasons for non-adherence and reinforce patient education

Restarting anticoagulation appropriately after a bleeding event is critical, as in most cases the risk-benefit balance remains positive. Among patients we included, several were prescribed inadequately reduced dosage because they had experienced bleeding. First the indication of anticoagulation should be reassessed. If there is still a valid indication, risk factors should be identified and corrected if possible (e.g. drug interactions, dose not adapted). Switching to another oral anticoagulant should be considered, for instance VKA in case of severe renal impairment. Once this is achieved, anticoagulation can be restarted provided that the patient is not at high risk of re-bleeding and that bleeding did not occur at a critical site[55].

Recommendations for hospital health care professionals

Transitions of care are high-risk periods for medication errors and ADR occurrence[49]. For instance, we observed the case of a nursing home resident who received rivaroxaban and enoxaparin at hospital discharge. He was subsequently readmitted for serious bleeding. Therefore, providing accurate discharge letters in

a timely manners is critical. Patients and/or relatives should also receive consistent treatment instructions in both oral and written form. Moreover, during patient hospitalization, communication with primary care providers should be improved in order to discuss treatment modifications and ensure continuity of care.

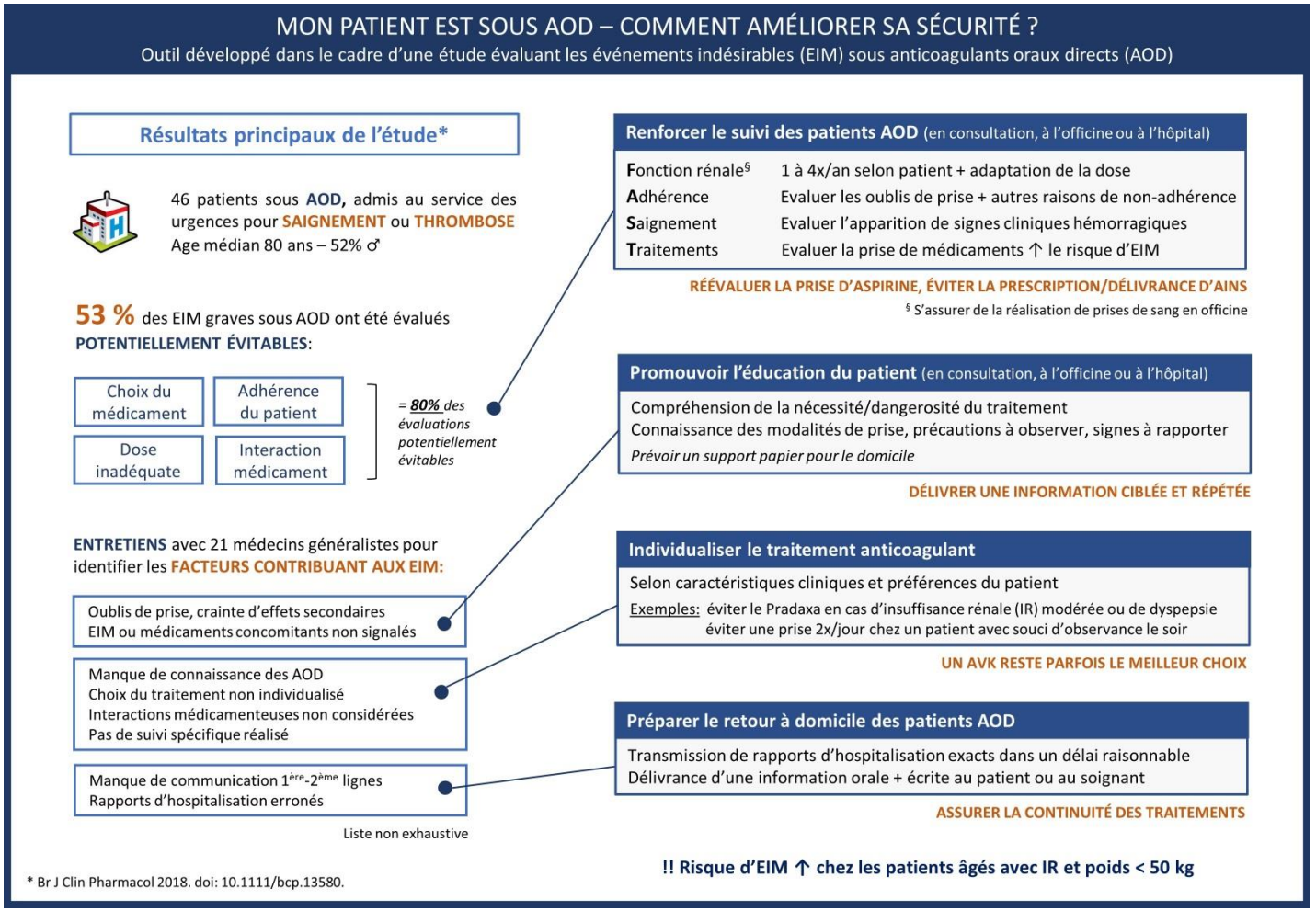
The role of clinical pharmacists in improving the use of DOAC has been previously emphasized[56-58]. They should ensure the appropriateness of DOAC prescribing in their care unit or at the hospital level. In an increasingly specialized healthcare system, clinical pharmacists are also key players in detecting clinically significant interactions with concomitant medications. Finally, they may be responsible for patient education during their hospital stay[59]. Patients should be provided with educational materials such as those developed by the High-Risk Medications working group of the Association Francophone des Pharmaciens Hospitaliers de Belgique (AFPHB).

This research work has underlined the usefulness of laboratory measurement for managing DOAC-related hospital admissions[55]. Commercial specific assays are currently available for all DOAC and can be performed using any coagulometer. In Belgium, they are reimbursed by the Institut national d'assurance maladie-invalidité (INAMI) under certain conditions. In the absence of DOAC point-of-care tests, hospital laboratories should routinely implement specific assays to provide results with a turnaround time around 30 minutes in emergency situations. For their part, clinicians involved in the acute management of DOAC-related ADR should be made aware of the added value of measuring DOAC levels, also for the subsequent reevaluation of anticoagulant therapy[21].

Recommendations for community pharmacists

Although community pharmacists lack information regarding renal function, they have the opportunity to play a pivotal role in optimizing the use of DOAC in practice. Especially, their contribution in detecting and solving drug-drug interactions has been pointed out in both previous literature and our GP interviews[60]. Moreover, community pharmacists should take advantage of their new status of referent pharmacist ("Pharmacien de Référence") to follow closely their DOAC-treated patients. They should provide them with an updated medication schedule including over-the-counter drugs, and ensure information sharing with other HCP. In this context, community pharmacists are also ideally placed to identify and discuss any DOAC-related issue such as ADR occurrence or patient non-adherence[61, 62]. Patient involvement in pharmacist-led medication reviews has recently been demonstrated to be critical in identifying drug-related problems[63].

We summarized recommendations for HCP in a practical tool, which is presented on the opposite page.


MON PATIENT EST SOUS AOD – COMMENT AMÉLIORER SA SÉCURITÉ ?
Outil développé dans le cadre d'une étude évaluant les événements indésirables (EIM) sous anticoagulants oraux directs (AOD)
Résultats principaux de l'étude*
46 patients sous AOD, admis au service des urgences pour SAIGNEMENT ou THROMBOSE
Age médian 80 ans – 52% ♂
53 % des EIM graves sous AOD ont été évalués POTENTIELLEMENT ÉVITABLES:
Choix du médicament
Adhérence du patient
Dose inadéquate
Interaction médicament
= 80% des évaluations potentiellement évitables
ENTRETIENS avec 21 médecins généralistes pour identifier les FACTEURS CONTRIBUANT AUX EIM:
Oublis de prise, crainte d'effets secondaires
EIM ou médicaments concomitants non signalés
Manque de connaissance des AOD
Choix du traitement non individualisé
Interactions médicamenteuses non considérées
Pas de suivi spécifique réalisé
Manque de communication 1ère-2ème lignes
Rapports d'hospitalisation erronés
Liste non exhaustive
* Br J Clin Pharmacol 2018. doi: 10.1111/bcp.13580.
Renforcer le suivi des patients AOD (en consultation, à l'officine ou à l'hôpital)
Fonction rénale§ 1 à 4x/an selon patient + adaptation de la dose
Adhérence Evaluer les oublis de prise + autres raisons de non-adhérence
Saignement Evaluer l'apparition de signes cliniques hémorragiques
Traitements Evaluer la prise de médicaments ↑ le risque d'EIM
RÉÉVALUER LA PRISE D'ASPIRINE, ÉVITER LA PRESCRIPTION/DÉLIVRANCE D'AINS
§ S'assurer de la réalisation de prises de sang en officine
Promouvoir l'éducation du patient (en consultation, à l'officine ou à l'hôpital)
Compréhension de la nécessité/dangerosité du traitement
Connaissance des modalités de prise, précautions à observer, signes à rapporter
Prévoir un support papier pour le domicile
DÉLIVRER UNE INFORMATION CIBLÉE ET RÉPÉTÉE
Individualiser le traitement anticoagulant
Selon caractéristiques cliniques et préférences du patient
Exemples: éviter le Pradaxa en cas d'insuffisance rénale (IR) modérée ou de dyspepsie
éviter une prise 2x/jour chez un patient avec souci d'observance le soir
UN AVK RESTE PARFOIS LE MEILLEUR CHOIX
Préparer le retour à domicile des patients AOD
Transmission de rapports d'hospitalisation exacts dans un délai raisonnable
Délivrance d'une information orale + écrite au patient ou au soignant
ASSURER LA CONTINUITÉ DES TRAITEMENTS
!! Risque d'EIM ↑ chez les patients âgés avec IR et poids < 50 kg

2) Interventions to promote DOAC rational prescribing

Now that DOAC have broaden the landscape of anticoagulant therapy, clinicians are facing new challenges such as choosing the most appropriate individualized drug treatment[64]. Below we discuss several approaches to improve DOAC prescribing. However, it is generally acknowledged that multifaceted approaches are more effective than a single intervention strategy in this context[65].

Educational approaches

During the present thesis, we highlighted a lack of DOAC knowledge among GP and a subsequent trend to prescribe the same medication for all patients. Recent evidence has indeed confirmed that many HCP felt uncomfortable prescribing oral anticoagulants[66, 67]. Although improving knowledge alone may be insufficient to predict practitioner performance, we believe these educational needs should be adequately addressed[68].

The passive distribution of printed educational materials (PEM) is inexpensive, but has previously shown modest effect if used alone[69, 70]. Channel and format of PEM are important characteristics to consider, as wells as professionals' needs[70]. From GP interviews it appeared that a schematic format was greatly appreciated, as the one provided by pharmaceutical companies for each DOAC. This is in contrast with the narrative form of some existing tools (e.g. prescribing guides as part of risk minimization plans). The Centre Belge d'Information Pharmacothérapeutique (CBIP) was a frequent independent source of information.

Box 3: Recommendations for policymakers - 1

We recommend the development of a pragmatic tool to help providers choose among the different anticoagulation options, in close collaboration with GP. This tool should be made available at the time of decision-making, in both electronic and paper form. The CBIP seems the best way for the dissemination of information.

During educational outreach visits (EOV), also referred as academic detailing, trained people visit HCP at their site of practice to discuss evidence-based approaches to a particular topic[71]. EOV have been included in many interventions to improve prescribing, with consistent positive findings[65, 71, 72]. They were acknowledged as an important source of information in primary care during our interviews. In Belgium, Farmaka is an independent organization providing GP with EOV. They covered the topic of stroke prevention in atrial fibrillation in 2014. However, the Belgian government has recently decided to stop funding EOV. This opens the way for face-to-face visits with industry delegates only.

Box 4: Recommendations for policymakers - 2

EOV should be supported to help GP individualize anticoagulant treatment. More generally, we recommend against the suppression of EOV in primary care.

Box 5: Illustrative verbatim quotes regarding educational approaches

GP 1 : « Toutes les informations que j'ai reçues c'est les informations des firmes qu'ils envoient, c'est les informations du CBIP, c'est les informations de Farmaka. »
GP 12 : « Il existe un organisme indépendant qui s'appelle Farmaka. Je les reçois régulièrement, 2-3 fois par an. Je préfère ces bons conseils-là que la publicité. »
GP 17 : « Les délégués m'apprennent beaucoup, parce que je n'ai pas le temps de suivre des formations. (…) Donc je pense que là, si j'avais des rendez-vous comme vous pour me dire « tiens on va parler de tel truc », alors je veux bien. »

Interprofessional collaboration

Interprofessional meetings or activities were suggested to improve adherence to recommended practices[73]. In recent years, local consensus processes jointly arranged by GP and community pharmacists ("Concertation médico-pharmaceutique", CMP) have received financial support from INAMI. CMP aim to discuss difficulties encountered in practice, identify solutions and adopt

recommendations. Several quality improvement initiatives have been developed and made available for this purpose, among which one targets the appropriate use of DOAC. Nevertheless, didactic material for meeting preparation is currently available in Dutch only. This interdisciplinary approach is similar to the "Physicians-Pharmacists Quality Circles" (PPQC) that emerged in Switzerland twenty years ago, showing positive impact on prescription quality and drug costs[74, 75].

Box 6: Recommendations for policymakers - 3

Collaboration between GP and community pharmacists should be encouraged. Specifically, we recommend the development and provision of didactic material to support CMP on the appropriate use of DOAC in the French-speaking part of Belgium.

In addition, interprofessional collaboration could be strengthen at the patient level. Multidisciplinary case conferences have been especially studied in the nursing home (NH) setting, suggesting prescribing improvement[76]. It was recently part of a complex intervention to optimize medication prescribing in Belgian NH[77]. Another example is the PINCER trial, which was performed in primary care in the UK. In this cluster RCT, meetings between pharmacists and members of GP practices to discuss medication errors were more effective than simple feedback to providers[78]. It is important to emphasize that, in these two studies, pharmacists had access to patient medical records. This is currently not the case for community pharmacists in Belgium, while renal function is a key element to assess the appropriateness of DOAC prescribing.

Box 7: Recommendations for policymakers - 4

For DOAC patients who have designated a referent pharmacist, face-to-face medication reviews including GP and community pharmacists should be promoted once a year to highlight and discuss DOAC-related problems. Moreover, access to patient biological data should be provided to community pharmacists.

Box 8: Illustrative verbatim quotes regarding interprofessional collaboration

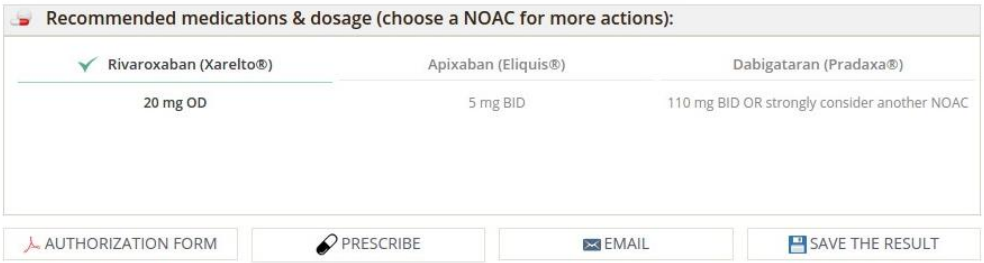
GP 7 (about medication review with pharmacists): « Si ça pouvait être réalisé pour les patients à domicile à raison d'une fois par an par exemple, où l'on rediscute de tous les traitements, je pense que ce serait vraiment très intéressant pour les médecins traitants également, parce que ce n'est pas possible de connaître toutes les indications, toutes les contre-indications de chaque médicament. »

Computer decision support interventions

In hospitalized patients, CDSS proved to be effective in enhancing the appropriate use of thromboprophylaxis and reducing the occurrence of venous thromboembolism[79, 80]. However, these interventions aimed at initiating anticoagulation for a short period of time. It would probably be more difficult to get clinicians to reconsider an existing long-term treatment – the recognized issue of clinical inertia[81]. Our systematic review has highlighted the current lack of CDSS to select the most appropriate oral anticoagulant for an individual patient. Included studies mostly focused on the indication of anticoagulation, whatever drug used.

Two CDSS recently developed are worth mentioning, as they have taken DOAC into account. First, Abidi et al. have described a CDSS to help determine patient eligibility for DOAC (according to Canadian guidelines), choose medication and dosage, and complete authorization forms (figure 3) [82]. This last feature appears valuable as, during interviews, many GP mentioned the burden of administrative procedures. Second, an Australian decision support tool to assist the selection of antithrombotic therapy (CARAT) has integrated DOAC in its most recent version [83]. CARATv2.0 considered thromboembolic and bleeding risks of patients, as well as major medication safety issues like drug interactions. According to Australian guidelines, warfarin was recommended as first choice in eligible patients. The application of the Excel prototype of CARATv2.0 increased the use of anticoagulants in hospitalized patients with AF.

Figure 3: Computerized NOAC Authorization Decision Support System (NOAC-ADSS)[82]



Several characteristics are important to consider when designing a CDSS for DOAC prescribing. In order to increase utilization rate, CDSS should be integrated in practice and should not require additional tasks from providers (e.g. clinical data entry, clicking to open another screen)[84]. Restricting notifications to severe drug-drug interactions or high-risk patients and improving their accuracy seem essential to reduce alert fatigue[85]. Moreover, involving users in CDSS development would be a proper way to enhance confidence in and agreement on recommendations[86]. Finally, user training and monitoring appear critical when implementing a CDSS. Poor knowledge of CDSS was reported as a common barrier to adoption in primary care[86, 87]. New safety issues may also arise from the introduction of health information technology, as illustrated in our systematic review.

3) <u>Interventions to improve DOAC management and adherence</u>

Patient-oriented approaches are essential to minimize the risk of ADR in DOAC patients. Indeed, one third of preventable drug-related admissions were previously associated with adherence problems[44]. Our prospective study also highlighted patient non-adherence as a common contributing factor to DOAC-related ADR. Especially, we observed thrombotic events in patients who did not follow their treatment plan properly, because of carelessness or fear of experiencing bleeding events.

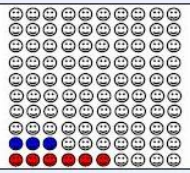
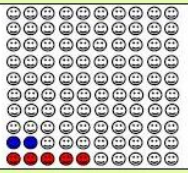
Patient decision aids

Incorporation of patient preferences, needs and values into anticoagulant treatment decisions is increasingly emphasized[88, 89]. This is of particular importance given 1-year DOAC discontinuation rates ranging from 15 to 50% in observational studies [90-92]. Patient decision aids (PtDA) are designed to help understand medical options and promote shared decision-making (SDM)[93]. They were shown to improve decision quality about treatments, but little is currently known about their effect on patient adherence and clinical outcomes[94].

A systematic review published in 2017 has found 7 studies investigating PtDA for stroke prevention in AF[95]. Most interventions were educational booklets that were viewed outside the clinical visit. We need to highlight the study of Thomson et al., performed in primary care in England[96]. A computerized PtDA, including individualized risk-benefit assessment of warfarin treatment and a SDM component, was delivered by physicians during clinical encounters. It resulted in reduced decision conflicts, although patients were less likely to start warfarin.

Recently, several PtDA have incorporated DOAC as treatment options. As an example, Holbrook et al. have developed a decision aid booklet providing three levels of decision-making: 1) anticoagulants vs. no treatment or aspirin, 2) DOAC vs. warfarin, and 3) choice among the 4 DOAC available[97]. Their PtDA was made of narrative text, visual plots and summary comparison charts (Figure 4). In a validation study, it improved patient knowledge and made patients feel more confident with their treatment decision. An electronic version of the tool should be investigated in future studies. In the same way, a multicenter randomized trial evaluating the impact of a SDM conversation tool will be performed soon[98]. The "Anticoagulation choice" tool includes individualized risks for stroke with and without anticoagulation, as well as issue cards for the consideration of practical issues (e.g. diet and medication interactions).

Figure 4: extract from a patient decision aid for choosing among anticoagulants for atrial fibrillation (2014, Dr A. Holbrook, McMaster University, Canada)

Issue	Warfarin (Coumadin)	New Oral Anticoagulants (NOACs)
Stroke (over 2 years)	3%	2.4%
Major Bleeding (over 2 years), which includes: - Intracranial bleed (MOST harmful) - Gastrointestinal bleed	5.6% 1.3% 1.3%	5% 0.6% 2.3%
No stroke or major bleeding (over 2 years)	91%	93%
Stroke Major Bleeding No Stroke or Major Bleeding (over 2 years)		
Other Adverse Effects requiring stopping drug (over 2 yrs)	8.4%	7.3%
Heart Attack (over 2 years)	1.3%	1.3%
Death (over 2 years)	6.3%	6%
Pill Taking	You MUST remember to take a pill ONCE a day	You MUST remember to take a pill ONCE or TWICE a day
Blood Tests	Yes, to monitor how thin is your blood - initially every week then every month or less often	No tests to monitor blood thinning but you will need kidney blood testing at least every year*
Activity Restrictions	Limit alcohol and injury	Limit alcohol and injury
Antidote (if you bleed, are there treatments that can stop the bleeding?)	Yes, there are specific drugs that stop the bleeding	No, you will need blood transfusions until the drug wears off
Special Diet?	No, but we would advise you not to vary your diet much	No
Taking Other Drugs	Certain drugs interact with warfarin to increase risk, especially other blood thinners	A few drugs interact with NOACs to increase risk, especially other blood thinners
Drug Cost	Approximately $9 per month (covered by ODB)**	Approximately $104 per month (restricted coverage by ODB)**

*you cannot take dabigatran, rivaroxaban, or apixaban if you have poor kidney function or have a mechanical heart valve; ** ODB = Ontario Drug Benefit (government drug plan)

These two examples raise the following question: to which extent should patient be involved in anticoagulant treatment decisions? Systematic reviews have highlighted that the frequency of admission and the availability of an antidote are important factors influencing patient decisions regarding oral anticoagulant therapy[99, 100]. Patients should therefore be involved in the choice between the 4 DOAC available, as was the case for the PtDA provided by Holbrook et al. Nevertheless, treatment selection should be mainly driven by clinical characteristics and co-medications. We could imagine a shared decision-making tool where physicians first select appropriate therapeutic options and discuss them in a second time with their patient.

Box 9: Recommendations for policymakers - 5

The development of a CDSS for the initiation of anticoagulant therapy should include the identification of patient preferences.

Patient education

Our qualitative findings highlighted the need to strengthen patient education regarding anticoagulation. In particular, GP frequently mentioned the regular delivery of repeated information as a means of improving medication use. This appeared challenging to achieve for DOAC in general practice, as patient follow-up had been only based on regular blood tests until the arrival of DOAC.

Box 10: Illustrative verbatim quotes regarding patient education

GP1 : « C'est mieux une information qui est répétée et on donne un élément intéressant de manière répétée quand on les voit régulièrement. »
GP 5: « Si on peut faire plus au niveau éducation du patient, par exemple dans le diabète, il y a des infirmières qui viennent, avec des séances d'information. Pourquoi pas avec le Sintrom aussi? Parce que oui c'est vrai, ça a des conséquences qui peuvent être dramatiques. »

GP 7: « Il faut répéter surtout dans les pathologies chroniques, qui nécessitent un suivi régulier. En fait, ne pas hésiter à répéter les mêmes choses au patient. Ça je commence tout doucement à me rendre compte que c'est utile. »

GP 9 : « Ça c'est le plus important pour moi. Donner l'information exacte et se rendre compte du fait qu'il faut parfois répéter et surtout l'avoir sur papier. »

Pharmaceutical care services have shown positive effects on adherence and knowledge[101]. In France, community pharmacists have been involved in education of VKA- and DOAC-treated patients since 2013 and 2016 respectively. Chronically anticoagulated patients (i.e. treatment > 6 months) are eligible for this program, which includes two 20-minute interviews in the first year and one interview per year for subsequent years. Pharmacists were provided with accompanying guide and patient information leaflets. Their perception of the program was broadly positive[102]. Similar interviews ("Bon Usage des Médicaments", BUM), have been introduced in Belgium. However, they currently apply to asthmatic patients only. The broadening of indications is planned in the multiannual framework for community pharmacies[103]. In a recent survey, only 63% of Belgian pharmacists were shown to feel confident or very confident when advising DOAC patients. E-learning was the preferred format to improve their skills[104]. A computerized check-list for the first DOAC dispensation has been developed by a professional pharmacy association in Antwerp (KAVA).

Box 11: Recommendations for policymakers - 6

Pharmaceutical care programs (BUM) should be extended to patients taking oral anticoagulants. Education of DOAC patients by community pharmacists is critical given the lack of regular laboratory monitoring. This implies improving skills and confidence of pharmacists when counselling DOAC patients. E-learning could be proposed as part of their ongoing training.

Two studies examined the impact of various interventions on DOAC adherence. In the first one - the AEGEAN study, apixaban patients in the intervention group received education booklet, reminder tools and virtual clinic access[105]. There was no added value of this educational program, compared to standard of care. The second one included the use of Medication Event Monitoring System (MEMS) to track DOAC intake, as well as phone calls in case of intake errors[106]. Tele monitoring and feedback improved DOAC adherence. However, in these two RCT, adherence rates were very high, even in control groups. It raises the issue of applicability of findings in everyday practice.

4) Towards an integrated care pathway for DOAC patients

Integrated care pathways (ICP) were defined in 1998 by Campbell et al. as "structured multidisciplinary care plans which detail essential steps in the care of patients with a specific clinical problem"[107]. They aim at improving quality of care, patient satisfaction and clinical outcomes, by the promotion of multidisciplinary communication and evidence translation into practice. ICP were implemented in hospitals for the management of surgical or medical inpatients, with a reduction in in-hospital complications[108]. Their use was then extended to improve hospital discharge in the elderly or routine care of patients with chronic conditions. For instance, a chronic obstructive pulmonary disease ICP has been recently shown to reduce the risk of hospitalization[109]. In Belgium, 13 pilot projects of integrated care for chronic disease management, funded by INAMI, have started this year.

Integrated care approaches are increasingly recognized as a reliable means of improving quality of healthcare in AF patients[110, 111]. Their systematic review revealed a reduction in all-cause mortality and cardiovascular hospitalizations[112]. However, the 3 included studies took place in specialized clinics in secondary care. Interestingly, a protocol has recently been published to investigate ICP in primary care in England[113]. Integrated care will be performed by practice nurses (PN), under

the supervision of GP. For DOAC patients, it will involve quarterly visits (three times a year with PN, once with GP) to check AF management. GP and PN will also receive the support of an Anticoagulation Expert Centre. Patients will no more be routinely followed by their cardiologist for their AF.

Several core components, previously described to develop effective chronic care, seem important to include in ICP for DOAC patients:

- Patient education and self-management support should be provided to help patients acquire knowledge and confidence about their anticoagulant therapy. This could be performed by community pharmacists (provided they have appropriate expertise as discussed above), but also by trained nurses during home visits in case of suspected drug management issues.

- As highlighted in the Chronic Care Model, sharp distinction between acute care and planned follow-up visits for chronic conditions should be drawn[114]. This sounds particularly relevant for DOAC patients as specific follow-up visits seem currently not to exist.

- Support to comply with guidelines should be delivered at the time and location of decision making through CDSS. In the Netherlands for instance, the Dutch College of GP developed NHGDoc, a CDSS covering multiple disease areas for general practice[115]. Electronic reminders might also facilitate scheduling of follow-up visits. The management of more complex cases could be resolved by expert assistance, provided that higher level of care is not required.

- Physicians must be given the opportunity to ask for DOAC measurement in case of suspected drug accumulation or serious ADR. In Belgium, specific assays for anti-Xa inhibitors are currently reimbursed in patients with renal

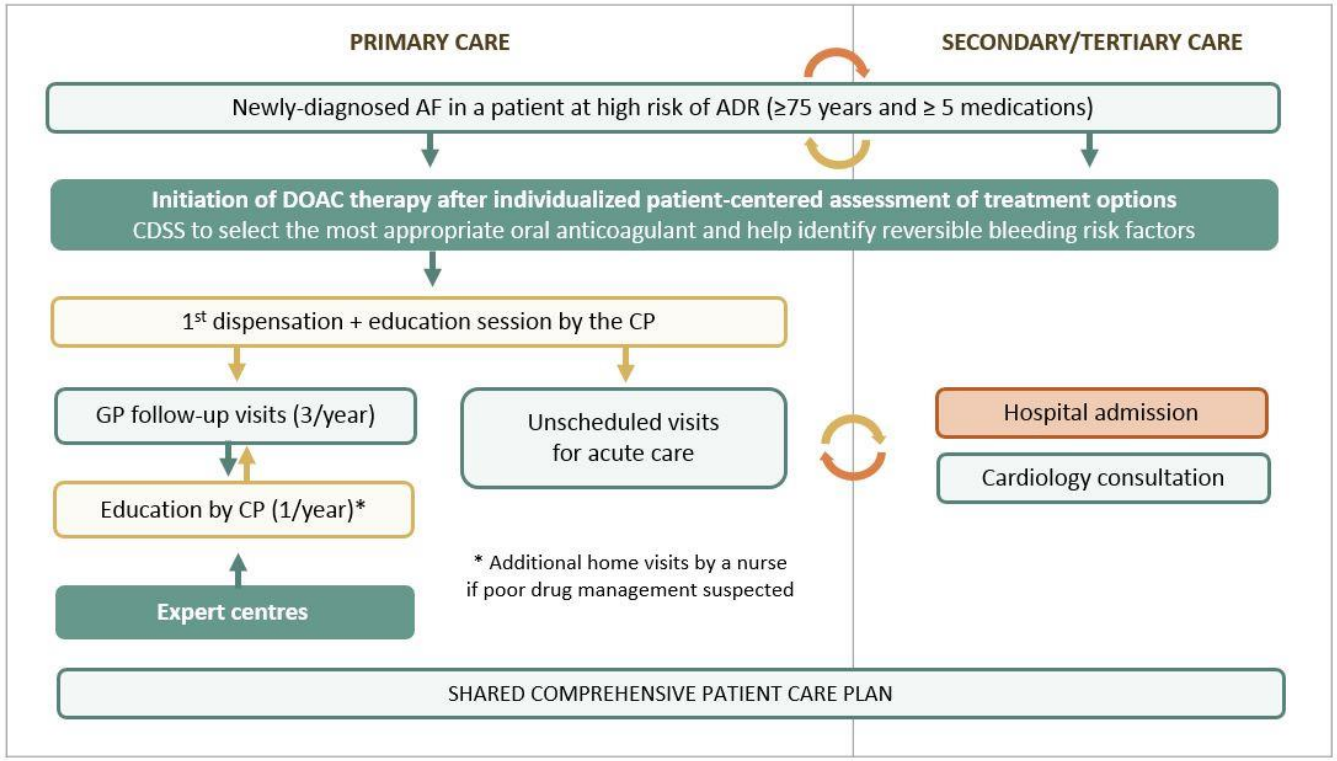
impairment, extreme body weight or bleeding. This should also be the case for dabigatran etexilate in the near future.

- All members of the multidisciplinary team must agree with and share a comprehensive patient-centered care plan[116]. This requires the implementation of computerized systems for information sharing between HCP. In 2013, the Flemish Government launched the digital platform Vitalink to facilitate communication of health data, including medication schemes. This should be achieved shortly at the national level by the current development of the VIDIS system (INAMI).

- From GP interviews, consultation cost appeared to play a role in monitoring non-adherence of VKA patients (even if later reimbursed). Therefore we could be in favor of free follow-up visits and education sessions for DOAC patients who benefit from this care plan. HCP should receive a fixed annual remuneration per patient. ICP could be restricted to patients with a higher risk of ADR (e.g. older than 75 years with polymedication) to be financially and logistically sustainable.

In figure 5 we suggest an integrated care pathway for DOAC patients. We aimed at strengthening partnerships between HCP currently involved in the management of DOAC patients, rather than creating new structures such as specialized anticoagulation clinics.

Figure 5: proposal for an integrated care pathway for DOAC patients



AF: atrial fibrillation, ADR: adverse drug reaction, CP: community pharmacist, GP: general practitioner

IV. Research perspectives

Preventability of serious ADR associated with the use of DOAC	
What are patient's perspectives on experiencing ADR while taking oral anticoagulants?	- Semi-structured interviews to identify perceived contributing factors to ADR and evaluate the impact of ADR on beliefs and behavior regarding medication intake - Assessment of treatment satisfaction using validated scales (ACTS, PACT-Q)
How have GPs' perceptions about DOAC evolved in the last decade?	- Follow-up study with the 21 GPs who were interviewed
To what extent do physicians agree on the concomitant prescription of aspirin and oral anticoagulants?	- National survey among GP and cardiologists based on selected clinical cases, to highlight situations where disagreements arise
Does a multifaceted intervention improve hospital discharge in patients treated with oral anticoagulants?	- Prospective, controlled study to evaluate a complex intervention (patient education, communication with GP and structured discharge reports). Assessment of process measures and patient outcomes
Measurement of the intensity of anticoagulation in DOAC patients	
Can DRVV-DOAC be further improved to detect low apixaban levels?	- Modification of the reagent composition (phospholipids, ionic components), and *in vitro* assessment of sensitivity to apixaban
What is the screening performance of DRVV-DOAC in emergency situations?	- Prospective study in patients admitted to the ED with life-threatening bleeding or requiring urgent surgery/thrombolysis - Comparison of DRVV-DOAC vs specific assays on the delay to obtain results and the detection of clinically significant levels
Impact of ABCB1 polymorphisms on DOAC transport	
Can other genetic determinants contribute to the inter-individual variability in DOAC concentrations?	- *In vitro* studies examining the role of *ABCG2* rs2231142, *CYP3A5*3* and *CYP3A4*22* alleles
Do ABCB1 genetic polymorphisms impact the transport of other DOAC?	- *In vitro* studies examining the effect of *ABCB1* polymorphisms on the transport of DE
Computerized clinical decision support systems to improve DOAC prescribing	
To what extent could CDSS prevent DOAC-related medication errors?	- Retrospective study on pharmacist interventions related to the prescription of DOAC in a teaching hospital. Assessment of the proportion of interventions potentially avoided by a CDSS - Design and pilot-testing of a CDSS based on previous studies

V. Conclusion

DOAC are widely acknowledged as a cornerstone of oral anticoagulant therapy. Perceptions on their use in clinical practice have nevertheless changed over the last decade, moving from an idealized to a more nuanced view of a complex situation. Especially, the need for regular clinical follow-up and occasional laboratory testing have been highlighted. During this four-year research project, we explored serious ADR related to the use of DOAC and contributed to a better knowledge of these patient safety issues. We demonstrated that more than half of DOAC-related ADR were potentially preventable, leaving considerable room for improvement. Several contributing factors were identified, including patient non-adherence, lack of knowledge among providers or communication issues. However, the genetic polymorphisms that we investigated did not appear as a potential source for improving safety in DOAC patients. No recommendation can currently be made with regard to this point. Finally, DOAC measurement proved to be helpful for the short- and long-term management of serious ADR. The use of dRVVT-based assays sounds promising for a rapid assessment of anticoagulant intensity, but requires further improvements.

The time has now come for the prevention of DOAC-related hospital admissions. Interventions focusing on prescribing, patient follow-up and continuity of care should be designed and implemented to help improve DOAC management in clinical practice. Reviewing evidence on CDSS for DOAC prescribing was a first step in this direction.

REFERENCES

1. Budnitz DS, Lovegrove MC, Shehab N, Richards CL. Emergency hospitalizations for adverse drug events in older Americans. N Engl J Med 2011; 365: 2002-12.
2. Marquet K, Claes N, De Troy E, Kox G, Droogmans M, Vleugels A. A multicenter record review of in-hospital adverse drug events requiring a higher level of care. Acta Clin Belg 2017; 72: 156-62.
3. Perrone V, Conti V, Venegoni M, et al. Seriousness, preventability, and burden impact of reported adverse drug reactions in Lombardy emergency departments: a retrospective 2-year characterization. Clinicoecon Outcomes Res 2014; 6: 505-14.
4. Piazza G, Nguyen TN, Cios D, et al. Anticoagulation-associated adverse drug events. Am J Med 2011; 124: 1136-42.
5. Becattini C, Franco L, Beyer-Westendorf J, et al. Major bleeding with vitamin K antagonists or direct oral anticoagulants in real-life. Int J Cardiol 2017; 227: 261-6.
6. Xu Y, Schulman S, Dowlatshahi D, et al. Direct Oral Anticoagulant- or Warfarin-Related Major Bleeding: Characteristics, Reversal Strategies, and Outcomes From a Multicenter Observational Study. Chest 2017; 152: 81-91.
7. Avery AJ, Rodgers S, Franklin BD, et al. Research into practice: safe prescribing. Br J Gen Pract 2014; 64: 259-61.
8. MDS-3: Managing access to medicines and health technologies. Chapter 29: promoting rationale prescribing. Management Sciences for Health, 2012.
9. Marcum ZA, Pugh MJ, Amuan ME, et al. Prevalence of potentially preventable unplanned hospitalizations caused by therapeutic failures and adverse drug withdrawal events among older veterans. J Gerontol A Biol Sci Med Sci 2012; 67: 867-74.
10. Jabet A, Stepanian A, Golmard JL, et al. Are Screening Tests Reliable to Rule Out Direct Oral Anticoagulant Plasma Levels at Various Thresholds (30, 50, or 100 ng/mL) in Emergency Situations? Chest 2018; 153: 288-90.
11. Douxfils J, Ageno W, Samama CM, et al. Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians. J Thromb Haemost 2018; 16: 209-19.
12. Seiffge DJ, Traenka C, Polymeris A, et al. Feasibility of rapid measurement of Rivaroxaban plasma levels in patients with acute stroke. J Thromb Thrombolysis 2017; 43: 112-6.
13. Ebner M, Birschmann I, Peter A, et al. Point-of-care testing for emergency assessment of coagulation in patients treated with direct oral anticoagulants. Crit Care 2017; 21: 32.
14. Weitz JI, Eikelboom JW. Urgent Need to Measure Effects of Direct Oral Anticoagulants. Circulation 2016; 134: 186-8.
15. Simon A, Domanovits H, Ay C, Sengoelge G, Levy JH, Spiel AO. The recommended dose of idarucizumab may not always be sufficient for sustained reversal of dabigatran. J Thromb Haemost 2017; 15: 1317-21.
16. Altman R, Gonzalez CD. Simple and rapid assay for effect of the new oral anticoagulant (NOAC) rivaroxaban: preliminary results support further tests with all NOACs. Thromb J 2014; 12: 7.
17. Douxfils J, Chatelain B, Hjemdahl P, et al. Does the Russell Viper Venom time test provide a rapid estimation of the intensity of oral anticoagulation? A cohort study. Thromb Res 2015; 135: 852-60.
18. Gosselin RC, Adcock DM, Bates SM, et al. International Council for Standardization in Haematology (ICSH) Recommendations for Laboratory Measurement of Direct Oral Anticoagulants. Thromb Haemost 2018; 118: 437-50.

19. Abbasi J. Getting Pharmacogenomics Into the Clinic. JAMA 2016; 316: 1533-5.
20. Tseng AS, Patel RD, Quist HE, et al. Clinical Review of the Pharmacogenomics of Direct Oral Anticoagulants. Cardiovasc Drugs Ther 2018; 32: 121-6.
21. Gouin-Thibault I, Delavenne X, Blanchard A, et al. Interindividual variability in dabigatran and rivaroxaban exposure: contribution of ABCB1 genetic polymorphisms and interaction with clarithromycin. J Thromb Haemost 2017; 15: 273-83.
22. Ueshima S, Hira D, Fujii R, et al. Impact of ABCB1, ABCG2, and CYP3A5 polymorphisms on plasma trough concentrations of apixaban in Japanese patients with atrial fibrillation. Pharmacogenet Genomics 2017; 27: 329-36.
23. Woolf SH. The meaning of translational research and why it matters. Jama 2008; 299: 211-3.
24. Glasgow RE, Vinson C, Chambers D, Khoury MJ, Kaplan RM, Hunter C. National Institutes of Health approaches to dissemination and implementation science: current and future directions. Am J Public Health 2012; 102: 1274-81.
25. Samuelson BT, Cuker A, Siegal DM, Crowther M, Garcia DA. Laboratory Assessment of the Anticoagulant Activity of Direct Oral Anticoagulants: A Systematic Review. Chest 2017; 151: 127-38.
26. Dessilly G, Elens L, Panin N, et al. ABCB1 1199G>A genetic polymorphism (Rs2229109) influences the intracellular accumulation of tacrolimus in HEK293 and K562 recombinant cell lines. PLoS One 2014; 9: e91555.
27. Dessilly G, Elens L, Panin N, Karmani L, Demoulin JB, Haufroid V. ABCB1 1199G>A polymorphism (rs2229109) affects the transport of imatinib, nilotinib and dasatinib. Pharmacogenomics 2016; 17: 883-90.
28. Dessilly G, Panin N, Elens L, Haufroid V, Demoulin JB. Impact of ABCB1 1236C > T-2677G > T-3435C > T polymorphisms on the anti-proliferative activity of imatinib, nilotinib, dasatinib and ponatinib. Sci Rep 2016; 6: 29559.
29. Westfall JM, Mold J, Fagnan L. Practice-based research--"Blue Highways" on the NIH roadmap. Jama 2007; 297: 403-6.
30. Medicine Io. The CTSA Program at NIH: Opportunities for Advancing Clinical and Translational Research. Leshner AI, Terry SF, Schultz AM, Liverman CT, editors. Washington, DC: The National Academies Press, 2013. 178 p.
31. Blumberg RS, Dittel B, Hafler D, von Herrath M, Nestle FO. Unraveling the autoimmune translational research process layer by layer. Nat Med 2012; 18: 35-41.
32. Freedman B, Potpara TS, Lip GY. Stroke prevention in atrial fibrillation. Lancet 2016; 388: 806-17.
33. Xian Y, O'Brien EC, Liang L, et al. Association of Preceding Antithrombotic Treatment With Acute Ischemic Stroke Severity and In-Hospital Outcomes Among Patients With Atrial Fibrillation. Jama 2017; 317: 1057-67.
34. Jones NR, Hobbs FDR, Taylor CJ. Underuse of anticoagulation therapy for atrial fibrillation: Are we failing our patients? Can Fam Physician 2017; 63: 943-4.
35. Mas Dalmau G, Sant Arderiu E, Enfedaque Montes MB, Sola I, Pequeno Saco S, Alonso Coello P. Patients' and physicians' perceptions and attitudes about oral anticoagulation and atrial fibrillation: a qualitative systematic review. BMC Fam Pract 2017; 18: 3.
36. Vinereanu D, Lopes RD, Bahit MC, et al. A multifaceted intervention to improve treatment with oral anticoagulants in atrial fibrillation (IMPACT-AF): an international, cluster-randomised trial. Lancet 2017; 390: 1737-46.

37. Sacristan JA, Aguaron A, Avendano-Sola C, et al. Patient involvement in clinical research: why, when, and how. Patient Prefer Adherence 2016; 10: 631-40.

38. Patrick K. Realizing the vision of patient-relevant clinical research. Cmaj 2016; 188: 1063.

39. Calvert M, Kyte D, Mercieca-Bebber R, et al. Guidelines for Inclusion of Patient-Reported Outcomes in Clinical Trial Protocols: The SPIRIT-PRO Extension. Jama 2018; 319: 483-94.

40. Hernandez AF, Fleurence RL, Rothman RL. The ADAPTABLE Trial and PCORnet: Shining Light on a New Research Paradigm. Ann Intern Med 2015; 163: 635-6.

41. Rahimi K, Malhotra A, Banning AP, Jenkinson C. Outcome selection and role of patient reported outcomes in contemporary cardiovascular trials: systematic review. Bmj 2010; 341: c5707.

42. Prins MH, Guillemin I, Gilet H, et al. Scoring and psychometric validation of the Perception of Anticoagulant Treatment Questionnaire (PACT-Q). Health Qual Life Outcomes 2009; 7: 30.

43. Cano SJ, Lamping DL, Bamber L, Smith S. The Anti-Clot Treatment Scale (ACTS) in clinical trials: cross-cultural validation in venous thromboembolism patients. Health and Quality of Life Outcomes 2012; 10: 120.

44. Howard R, Avery A, Bissell P. Causes of preventable drug-related hospital admissions: a qualitative study. Qual Saf Health Care 2008; 17: 109-16.

45. Clarkesmith DE, Lip GYH, Lane DA. Patients' experiences of atrial fibrillation and non-vitamin K antagonist oral anticoagulants (NOACs), and their educational needs: A qualitative study. Thromb Res 2017; 153: 19-27.

46. Osterberg L, Blaschke T. Adherence to medication. N Engl J Med 2005; 353: 487-97.

47. Slight SP, Howard R, Ghaleb M, Barber N, Franklin BD, Avery AJ. The causes of prescribing errors in English general practices: a qualitative study. Br J Gen Pract 2013; 63: e713-20.

48. Dechanont S, Maphanta S, Butthum B, Kongkaew C. Hospital admissions/visits associated with drug-drug interactions: a systematic review and meta-analysis. Pharmacoepidemiol Drug Saf 2014; 23: 489-97.

49. Kripalani S, LeFevre F, Phillips CO, Williams MV, Basaviah P, Baker DW. Deficits in communication and information transfer between hospital-based and primary care physicians: implications for patient safety and continuity of care. Jama 2007; 297: 831-41.

50. Le Heuzey JY, Ammentorp B, Darius H, et al. Differences among western European countries in anticoagulation management of atrial fibrillation. Data from the PREFER IN AF registry. Thromb Haemost 2014; 111: 833-41.

51. Gómez-Outes A, Suárez-Gea ML, Lecumberri R, Terleira-Fernández AI, Vargas-Castrillón E. Direct-acting oral anticoagulants: pharmacology, indications, management, and future perspectives. European Journal of Haematology 2015; 95: 389-404.

52. Van Blerk M, Bailleul E, Chatelain B, et al. Influence of dabigatran and rivaroxaban on routine coagulation assays. A nationwide Belgian survey. Thromb Haemost 2015; 113: 154-64.

53. Hodin S, Basset T, Jacqueroux E, et al. In Vitro Comparison of the Role of P-Glycoprotein and Breast Cancer Resistance Protein on Direct Oral Anticoagulants Disposition. Eur J Drug Metab Pharmacokinet 2018; 43: 183-91.

54. Stangier J. Clinical pharmacokinetics and pharmacodynamics of the oral direct thrombin inhibitor dabigatran etexilate. Clin Pharmacokinet 2008; 47: 285-95.

55. Tomaselli GF, Mahaffey KW, Cuker A, et al. 2017 ACC Expert Consensus Decision Pathway on Management of Bleeding in Patients on Oral Anticoagulants: A Report of the American College of Cardiology Task Force on Expert Consensus Decision Pathways. J Am Coll Cardiol 2017; 70: 3042-67.

56. Larock AS, Mullier F, Sennesael AL, et al. Appropriateness of prescribing dabigatran etexilate and rivaroxaban in patients with nonvalvular atrial fibrillation: a prospective study. Ann Pharmacother 2014; 48: 1258-68.
57. Ashjian E, Kurtz B, Renner E, Yeshe R, Barnes GD. Evaluation of a pharmacist-led outpatient direct oral anticoagulant service. Am J Health Syst Pharm 2017; 74: 483-9.
58. Shore S, Ho PM, Lambert-Kerzner A, et al. Site-Level Variation in and Practices Associated With Dabigatran Adherence. Jama 2015; 313: 1443.
59. Zdyb EG, Courtney DM, Malik S, Schmidt MJ, Lyden AE. Impact of Discharge Anticoagulation Education by Emergency Department Pharmacists at a Tertiary Academic Medical Center. J Emerg Med 2017; 53: 896-903.
60. Desmaele S, De Wulf I, Dupont AG, Steurbaut S. Pharmacists' role in handling problems with prescriptions for antithrombotic medication in Belgian community pharmacies. Int J Clin Pharm 2015; 37: 656-68.
61. Elliott RA, Boyd MJ, Salema NE, et al. Supporting adherence for people starting a new medication for a long-term condition through community pharmacies: a pragmatic randomised controlled trial of the New Medicine Service. BMJ Qual Saf 2016; 25: 747-58.
62. Nkansah N, Mostovetsky O, Yu C, et al. Effect of outpatient pharmacists' non-dispensing roles on patient outcomes and prescribing patterns. Cochrane Database Syst Rev 2010: Cd000336.
63. Kari H, Kortejarvi H, Airaksinen M, Laaksonen R. Patient involvement is essential in identifying drug-related problems. Br J Clin Pharmacol 2018; 0.
64. Schaefer JK, McBane RD, Wysokinski WE. How to choose appropriate direct oral anticoagulant for patient with nonvalvular atrial fibrillation. Ann Hematol 2016; 95: 437-49.
65. Chan WV, Pearson TA, Bennett GC, et al. ACC/AHA Special Report: Clinical Practice Guideline Implementation Strategies: A Summary of Systematic Reviews by the NHLBI Implementation Science Work Group: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. J Am Coll Cardiol 2017; 69: 1076-92.
66. Piran S, Schulman S, Panju M, Pai M. Oral anticoagulant dosing, administration, and storage: a cross-sectional survey of Canadian health care providers. J Thromb Thrombolysis 2018; 45: 180-5.
67. Wang Y, Bajorek B. Decision-making around antithrombotics for stroke prevention in atrial fibrillation: the health professionals' views. International Journal of Clinical Pharmacy 2016; 38: 985-95.
68. Kamarudin G, Penm J, Chaar B, Moles R. Educational interventions to improve prescribing competency: a systematic review. BMJ Open 2013; 3: e003291.
69. Sennesael AL, Dalleur O, Henrard S, Artoisenet C, Schoevaerdts D, Spinewine A. Implementing a screening tool to improve prescribing in hospitalized older patients: a pilot study. Int J Clin Pharm 2018; 40: 15-9.
70. Giguère A, Légaré F, Grimshaw J, et al. Printed educational materials: effects on professional practice and healthcare outcomes. Cochrane Database of Systematic Reviews 2012.
71. O'Brien MA, Rogers S, Jamtvedt G, et al. Educational outreach visits: effects on professional practice and health care outcomes. Cochrane Database Syst Rev 2007: Cd000409.
72. Ostini R, Hegney D, Jackson C, et al. Systematic review of interventions to improve prescribing. Ann Pharmacother 2009; 43: 502-13.

73. Reeves S, Pelone F, Harrison R, Goldman J, Zwarenstein M. Interprofessional collaboration to improve professional practice and healthcare outcomes. Cochrane Database Syst Rev 2017; 6: Cd000072.
74. Niquille A, Ruggli M, Buchmann M, Jordan D, Bugnon O. The nine-year sustained cost-containment impact of swiss pilot physicians-pharmacists quality circles. Ann Pharmacother 2010; 44: 650-7.
75. Bugnon O, Jotterand S, Niquille Charriere A, Ruggli M, Herzig L. [Physicians-pharmacists quality circles: shared responsibility of the freedom of prescription]. Rev Med Suisse 2012; 8: 1042, 4-8.
76. Alldred DP, Kennedy MC, Hughes C, Chen TF, Miller P. Interventions to optimise prescribing for older people in care homes. Cochrane Database Syst Rev 2016; 2: Cd009095.
77. Anrys P, Strauven G, Boland B, et al. Collaborative approach to Optimise MEdication use for Older people in Nursing homes (COME-ON): study protocol of a cluster controlled trial. Implement Sci 2016; 11: 35.
78. Avery AJ, Rodgers S, Cantrill JA, et al. A pharmacist-led information technology intervention for medication errors (PINCER): a multicentre, cluster randomised, controlled trial and cost-effectiveness analysis. Lancet 2012; 379: 1310-9.
79. Borab ZM, Lanni MA, Tecce MG, Pannucci CJ, Fischer JP. Use of Computerized Clinical Decision Support Systems to Prevent Venous Thromboembolism in Surgical Patients: A Systematic Review and Meta-analysis. JAMA Surg 2017; 152: 638-45.
80. Kahn SR, Morrison DR, Diendere G, et al. Interventions for implementation of thromboprophylaxis in hospitalized patients at risk for venous thromboembolism. Cochrane Database Syst Rev 2018; 4: Cd008201.
81. Eckman MH, Lip GY, Wise RE, et al. Impact of an Atrial Fibrillation Decision Support Tool on thromboprophylaxis for atrial fibrillation. Am Heart J 2016; 176: 17-27.
82. Abidi SR, Cox J, Abusharekh A, Hashemian N, Abidi SS. A Digital Health System to Assist Family Physicians to Safely Prescribe NOAC Medications. Stud Health Technol Inform 2016; 228: 519-23.
83. Wang Y, Bajorek B. Pilot of a Computerised Antithrombotic Risk Assessment Tool Version 2 (CARATV2.0) for stroke prevention in atrial fibrillation. Cardiol J 2017; 24: 176-87.
84. Beeler PE, Bates DW, Hug BL. Clinical decision support systems. Swiss Med Wkly 2014; 144: w14073.
85. Tolley CL, Slight SP, Husband AK, Watson N, Bates DW. Improving medication-related clinical decision support. Am J Health Syst Pharm 2018; 75: 239-46.
86. Lugtenberg M, Weenink JW, van der Weijden T, Westert GP, Kool RB. Implementation of multiple-domain covering computerized decision support systems in primary care: a focus group study on perceived barriers. BMC Med Inform Decis Mak 2015; 15: 82.
87. Short D, Frischer M, Bashford J. Barriers to the adoption of computerised decision support systems in general practice consultations: a qualitative study of GPs' perspectives. International Journal of Medical Informatics 2004; 73: 357-62.
88. Kirchhof P, Benussi S, Kotecha D, et al. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. Europace 2016; 18: 1609-78.
89. Kearon C, Akl EA, Ornelas J, et al. Antithrombotic Therapy for VTE Disease: CHEST Guideline and Expert Panel Report. Chest 2016; 149: 315-52.

90. Maura G, Billionnet C, Alla F, Gagne JJ, Pariente A. Comparison of Treatment Persistence with Dabigatran or Rivaroxaban versus Vitamin K Antagonist Oral Anticoagulants in Atrial Fibrillation Patients: A Competing Risk Analysis in the French National Health Care Databases. Pharmacotherapy 2018; 38: 6-18.
91. Collings SL, Lefevre C, Johnson ME, et al. Oral anticoagulant persistence in patients with non-valvular atrial fibrillation: A cohort study using primary care data in Germany. PLoS One 2017; 12: e0185642.
92. Forslund T, Wettermark B, Hjemdahl P. Comparison of treatment persistence with different oral anticoagulants in patients with atrial fibrillation. Eur J Clin Pharmacol 2016; 72: 329-38.
93. An introduction to patient decision aids. Bmj 2013; 347: f4147.
94. Stacey D, Legare F, Lewis KB. Patient Decision Aids to Engage Adults in Treatment or Screening Decisions. Jama 2017; 318: 657-8.
95. O'Neill ES, Grande SW, Sherman A, Elwyn G, Coylewright M. Availability of patient decision aids for stroke prevention in atrial fibrillation: A systematic review. Am Heart J 2017; 191: 1-11.
96. Thomson RG, Eccles MP, Steen IN, et al. A patient decision aid to support shared decision-making on anti-thrombotic treatment of patients with atrial fibrillation: randomised controlled trial. Qual Saf Health Care 2007; 16: 216-23.
97. Fatima S, Holbrook A, Schulman S, Park S, Troyan S, Curnew G. Development and validation of a decision aid for choosing among antithrombotic agents for atrial fibrillation. Thromb Res 2016; 145: 143-8.
98. Kunneman M, Branda ME, Noseworthy PA, et al. Shared decision making for stroke prevention in atrial fibrillation: study protocol for a randomized controlled trial. Trials 2017; 18: 443.
99. Wilke T, Bauer S, Mueller S, Kohlmann T, Bauersachs R. Patient Preferences for Oral Anticoagulation Therapy in Atrial Fibrillation: A Systematic Literature Review. Patient 2016.
100. Loewen PS, Ji AT, Kapanen A, McClean A. Patient values and preferences for antithrombotic therapy in atrial fibrillation. A Narrative Systematic Review. Thromb Haemost 2017; 117: 1007-22.
101. Ryan R, Santesso N, Lowe D, et al. Interventions to improve safe and effective medicines use by consumers: an overview of systematic reviews. Cochrane Database Syst Rev 2014: Cd007768.
102. Mongaret C, Lepage C, Aubert L, Lestrille A, Slimano F. [Outcomes after a 2-year pharmaceutical care program for patients taking vitamin K antagonist therapy? Community pharmacist's perception]. Ann Pharm Fr 2018; 76: 114-21.
103. Cadre pluriannuel pour le patient avec les pharmaciens d'officine. Belgian Ministry of Health, 2017.
104. Papastergiou J, Kheir N, Ladova K, et al. Pharmacists' confidence when providing pharmaceutical care on anticoagulants, a multinational survey. Int J Clin Pharm 2017; 39: 1282-90.
105. Montalescot G. Abstract 18842: Adherence and Persistence to Apixaban Treatment in Patients With Non Valvular Atrial Fibrillation is High and Similar With Standard of Care Patient Education or With an Additional Educational Program: The Randomized AEGEAN Study. Circulation 2016; 134: A18842-A.
106. Desteghe L, Vijgen J, Koopman P, et al. Telemonitoring-based feedback improves adherence to non-vitamin K antagonist oral anticoagulants intake in patients with atrial fibrillation. European Heart Journal 2018; 39: 1394-403.
107. Campbell H, Hotchkiss R, Bradshaw N, Porteous M. Integrated care pathways. BMJ : British Medical Journal 1998; 316: 133-7.

108. Rotter T, Kinsman L, James E, et al. Clinical pathways: effects on professional practice, patient outcomes, length of stay and hospital costs. Cochrane Database Syst Rev 2010: Cd006632.

109. Wu CX, Hwang CH, Tan WS, et al. Effectiveness of a chronic obstructive pulmonary disease integrated care pathway in a regional health system: a propensity score matched cohort study. BMJ Open 2018; 8: e019425.

110. Kirchhof P. The future of atrial fibrillation management: integrated care and stratified therapy. Lancet 2017; 390: 1873-87.

111. Kotecha D, Breithardt G, Camm AJ, et al. Integrating new approaches to atrial fibrillation management: the 6th AFNET/EHRA Consensus Conference. Europace 2018; 20: 395-407.

112. Gallagher C, Elliott AD, Wong CX, et al. Integrated care in atrial fibrillation: a systematic review and meta-analysis. Heart 2017; 103: 1947-53.

113. van den Dries CJ, Oudega R, Elvan A, et al. Integrated management of atrial fibrillation including tailoring of anticoagulation in primary care: study design of the ALL-IN cluster randomised trial. BMJ Open 2017; 7: e015510.

114. Bodenheimer T, Wagner EH, Grumbach K. Improving primary care for patients with chronic illness. Jama 2002; 288: 1775-9.

115. Lugtenberg M, Westert GP, Pasveer D, van der Weijden T, Kool RB. Evaluating the uptake and effects of the computerized decision support system NHGDoc on quality of primary care: protocol for a large-scale cluster randomized controlled trial. Implement Sci 2014; 9: 145.

116. Paulus D, Van den Heede K, Mertens R. Position paper: organisation des soins pour les malades chroniques en Belgique. . Centre Fédéral d'Expertise des Soins de Santé (KCE), 2012. D/2012/10.273/83.

Anne-Laure SENNESAEL

✉ anne-laure.sennesael@uclouvain.be

EDUCATION

2014 – Present: PhD in biomedical and pharmaceutical sciences
Université catholique de Louvain (UCL), Brussels, Belgium

2012 – 2014: Master in hospital pharmacy
Université catholique de Louvain (UCL), Brussels, Belgium

2010 – 2012: Master in pharmaceutical sciences
Université catholique de Louvain (UCL), Brussels, Belgium

EXPERIENCE

2014 – Present: Research fellow in clinical pharmacy (FNRS)
Clinical Pharmacy Research Group, Louvain Drug Research Institute, Brussels, Belgium; Namur Thrombosis and Hemostasis Center, Belgium

2012 – 2014: Trainee in hospital pharmacy
CHU UCL Namur, Yvoir, Belgium

2010 – 2012: Student researcher in toxicology and cancer biology
Louvain Drug Research Institute, Brussels, Belgium

AWARDS

2012: Pharmacist and Doctor Nedeljkovic Award

PUBLICATIONS IN RELATIONSHIP WITH THIS THESIS

Sennesael AL, Dogné JM, Spinewine A. Optimizing the safe use of direct oral anticoagulants in older patients: a teachable moment. JAMA Intern Med. 2015; 175:1608-9.

Sennesael AL, Henrard S, Spinewine A. Drug interactions with non-vitamin K oral anticoagulants – Letter to the Editor. JAMA 2018; 319:829.

Sennesael AL, Larock AS, Devalet B, Mathieux V, Verschuren F, Muschart X, Dalleur O, Dogné JM, Spinewine A. Preventability of serious adverse drug reactions related to the use of direct oral anticoagulants : a prospective study. Br J Clin Pharmacol 2018; 84:1544-56.

Sennesael AL, Exner T, Chatelain B, Lessire S, Larock AS, Vancraeynest C, Pochet L, Dogné JM, Spinewine A, Mullier F, Douxfils J. An optimized dRVVT-based assay to estimate the intensity of anticoagulation in patients treated with direct oral anticoagulants. Thromb Res. 2017; 157:29-37.

Sennesael AL, Larock AS, Douxfils J, Elens L, Stillemans G, Wiesen M, Taubert M, Dogné JM, Spinewine A, Mullier F. Rivaroxaban plasma levels in patients admitted for bleeding events: insights from a prospective study. Thromb J. 2018. Accepted.

Sennesael AL, Panin N, Vancraeynest C, Pochet L, Spinewine A, Haufroid V, Elens L. Effect of ABCB1 genetic polymorphisms on the transport of rivaroxaban in HEK293 recombinant cell lines. Sci Rep. 2018; 8:10514.

POSTER PRESENTATIONS IN RELATIONSHIP WITH THIS THESIS

A DRVVT-based assay to estimate the intensity of anticoagulation in patients treated with direct oral anticoagulants. International Society on Thrombosis and Haemostasis (ISTH) 2015 Congress, 20-25 June 2015, Toronto, Canada.

Impact of apixaban on the dilute Russell's Viper Venom Time: an ex-vivo study. 62nd Annual SSC Meeting of the ISTH, 25-28 May 2016, Montpellier, France.

Preventability of serious adverse drug reactions related to the use of direct oral anticoagulants: a prospective study. Eurothrombosis 2016, 29th September-1st October 2016, London, UK.

Measurement of direct oral anticoagulants levels in patients admitted for bleeding events: a prospective study. ISTH 2017 Congress, 8-13 July 2017, Berlin, Germany.

Effect of ABCB1 genetic polymorphisms on the transport of rivaroxaban in HEK293 recombinant cell lines. 25th Annual Meeting of the Belgian Society on Thrombosis and Haemostasis, 23-24 November 2017, Malines, Belgium.

Adverse drug reactions associated with the use of oral anticoagulants in older patients. 11e congrès international francophone de gérontologie et gériatrie, 13-15 June 2018, Montreux, Suisse.

ORAL PRESENTATIONS IN RELATIONSHIP WITH THIS THESIS

Etude prospective des événements indésirables graves liés à l'utilisation des agents anticoagulants oraux directs. 6^{e} symposium annuel du Namur Thrombosis and Hemostasis Center (NTHC), 28 April 2016, Maillen, Belgium.

Emergency admissions for adverse drug reactions related to the use of direct oral anticoagulants and vitamin K antagonists: a prospective study. Annual symposium of the Belgian Society of Emergency and Disaster Medicine, 14 January 2017, Brussels, Belgium.

Can *ABCB1* genetic polymorphisms explain the inter-individual variability in DOAC plasma concentrations? 8^{e} symposium annuel du NTHC, 29 Mars 2018, Gesves, Belgium.