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DEBATE

New oral antithrombotics: a need for laboratory monitoring. Against

H. BOUNAMEAUX and G. REBER

Division of Angiology and Hemostasis, Department of Internal Medicine, University Hospital of Geneva and Faculty of Medicine, Geneva, Switzerland

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Monitoring anticoagulant therapy is a 'must' according to many laboratory specialists with the advocated aim of improving treatment efficacy or safety or both. Needless to say, monitoring also increases the need for such specialists (results must be interpreted, nuanced and sometimes disqualified), led to the creation of structures such as anticoagulant clinics and may be associated with financial advantage.

The weight of tradition and the lightness of evidence

The recent registration by the European Medicines Agency (EMEA) of new oral anticoagulant drugs such as rivaroxaban, a direct factor (F)Xa inhibitor and dabigatran etexilate, a direct thrombin inhibitor, may change this paradigm in the next years [6].

Why monitoring drugs?

Drug monitoring aims to optimize dosage regimens in order to increase efficacy and/or safety. However, few drugs exhibit

'Monitoring' vs. 'measuring' the anticoagulant effects of the new anticoagulant drugs

It is not just semantic: monitoring does not equal measuring. As a matter of fact, monitoring implies not only measuring but also possible dosage adaptation according to the test result. We suggest that while monitoring may be needless with the new oral anticoagulants, measuring the drug or its activity might be useful in a few special situations, including overdosage, urgent surgery, pregnancy, extreme body weights, children and renal insufficiency.

Management of overdosage mainly depends upon the clinical situation (does the patient bleed or not? What is the extent of the overdose?) and usually consists of watchful waiting or administration of antidotes if available. Protamine

Conclusion

The evidence available does not support laboratory *monitoring* of the new oral anticoagulant drugs: (i) the anticoagulant activity of fixed-dosed, orally administered doses of rivaroxaban and dabigatran etexilate are highly predictable; (ii) there is no evidence that the antithrombotic effect and/or risk of bleeding correlate with any related biologic activity or drug concentration measured in plasma; (iii) the safety of administering fixed doses of these new compounds has been demonstrated in thousands of patients enrolled in clinical trials on thromboprophylaxis after major orthopedic surgery, and very recently (at least for dabigatran etexilate) also with therapeutic doses for prevention of stroke and other thromboembolic events in atrial fibrillation.

Admittedly, iterative plasma *measurement* of the anticoagulant activity after a therapeutic dose of rivaroxaban or dabigatran etexilate may be occasionally informative, such as in cases of overdosage or urgent surgery to reassure the clinician if the measured activity happens to be low. However, in the absence of specific antidotes, a measured high activity merely allows us to approximate after which time the drug activity will vanish, according to its pharmacokinetic properties, which could have been calculated if the timing of administration and the exact dose are known.

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DEBATE

New oral antithrombotics: a need for laboratory monitoring. For

P. MISMETTI*†‡ and S. LAPORTE*‡

*University Jean Monnet of Saint-Etienne, EA3065, F-42023, Saint-Etienne; †Department of Therapeutic Medicine, Teaching Hospital of Saint-Etienne, F-42055, Saint-Etienne; and ‡Department of Clinical Pharmacology, Teaching Hospital of Saint-Etienne, F-42055, Saint-Etienne, France

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Introduction

In general, the need for laboratory monitoring depends on the properties of the drug considered, monitoring being warranted if the following five criteria are met:

Criterion #1 (inter-individual variability). The inexplicable inter-individual variability (i.e. variability between subjects) is high, justifying identification of the optimal dose for each patient at the start of treatment. This criterion may be waived if Criterion #2 is met (or vice versa).

The need for laboratory monitoring: the story with anticoagulant drugs

To illustrate the need for laboratory monitoring, vitamin K antagonists (VKA), the first oral anticoagulants, are perfect candidates.

The best candidates – VKA (Table 1)

With respect to Criterion #1 (inter-individual variability), the optimal dose required to reach the target INR value, even in

Criterion #2 (intra-individual instability). The unpredictable intra-individual instability in drug exposure (i.e. variability in the same patient) over time is high and could adversely affect the benefit-to-risk ratio of the drug. This variability over time could reflect a pathophysiological change, a drug-drug or food-drug interaction, or an unknown factor.

Criterion #3 (assay method). The variability of the assay method used to assess the drug concentration or effect is low and reproducible, and the optimal timing between drug administration and assessment of its concentration, or effect, is well established.

Criterion #4 (correlation). The correlation between the drug concentration, or effect, and clinical events (efficacy and safety endpoints) is well established, allowing identification of an optimal therapeutic range between the minimum effective dose and the maximum tolerated dose, and this therapeutic range is sufficiently narrow to necessitate dose adjustment in view of the variability defined in Criteria #1 and #2.

Criterion #5 (validation). Therapeutic drug monitoring as a basis for dose increase or decrease has been shown to prevent thromboembolic events and/or hemorrhagic events.

All these criteria should be met to justify the need for laboratory monitoring of a given drug.

stable patients, must be determined for each individual. In fact, age, sex, genetic polymorphism (2C9, VKORC) and drug-drug interactions explain only 60% of the inter-individual variability, hence the need to monitor the effect of VKA to determine the optimal dose for each patient [1].

As regards Criterion #2 (intra-individual variability), several drug-drug and food-drug interactions with VKA, as well as several pathological conditions, have been reported to influence VKA response in the same patient. Apart from these potential sources of variability, the highly unstable response to vitamin K antagonists has been extensively described. Even in the more recent clinical trials, including highly selected populations, patients remained within the therapeutic range for approximately 60% of the time [2].

The inter-laboratory variability of assay methods (Criterion #3) has been greatly reduced by the use of INR values instead of percentage activity, significantly improving the uniformity of anticoagulation level measurements. Several studies have demonstrated the validity of the INR/ISI system [3–5]. A further advantage of this laboratory test is that it does not depend on sampling time, due to the very long pharmacodynamic half-life of VKA.

Over the past years, several randomized studies and reviews have examined the risk of bleeding and thrombo-

Table 1 Respect of the criteria supporting laboratory monitoring by current and new anticoagulants

	Current anticoagulants			New oral anticoagulants
	VKA	UFH	LMWH fonda parinux	Dabigatran, edoxaban, apixaban, rivaroxaban
Criterion #1. Intra-individual instability in drug level	X	X		
Criterion #2. Inter-individual variability in drug level → optimal dose finding at the start of treatment	X	X		X
Criterion #3. Low variability and reproducibility of the assay method	X		X	X
Criterion #4. Correlation between drug level and clinical events → optimal therapeutic range	X			X
Criterion #5. Value of therapeutic drug monitoring demonstrated	X			

VKA, vitamin K antagonist; UFH, unfractionated heparin; LMWH, low-molecular-weight heparin.

New oral anticoagulants: a need for laboratory monitoring

The new oral anticoagulants have several advantages compared with VKA in that their pharmacokinetics (shorter half life) and pharmacodynamics (direct inhibition) are simpler and less variable [28,29]. In view of these advantages, with probably more than 80% of the population presenting a low variability, is it really worth discussing the need for laboratory monitoring? The clinical trials performed in current drug development programmes enrolled large, even if frequently selected, populations, including enough patients to enable identification of those at risk of variability (e.g. the elderly and patients with renal insufficiency, even in the case of drugs not excreted solely via the kidneys, such as rivaroxaban), who could benefit from drug monitoring (Criteria #1 and #2, Table 1). This could be particularly important in that we have already identified some drug-drug interactions with these new compounds (interaction with the P-glycoprotein system and/or cytochrome P450 isoenzymes) [30,31]. Some of these drug-drug interactions are sufficiently clinically relevant to result in contraindications or precautions for use (protease and some antifungal drugs for rivaroxaban, quinidine for dabigatran, etc.). Other interactions or polymorphism of the P-glycoprotein and/or cytochrome P450 system could also prove to be clinically relevant in clinical practise in non-selected populations and could necessitate dose adjustments, possibly based on drug monitoring. This drug monitoring, if necessary, should be performed simply to determine the initial optimal dose for a particular patient and repeated only in the event of introduction or withdrawal of a drug significantly interacting with the oral anticoagulant administered.

Conclusion

The first results observed during the clinical development of new oral anticoagulants have shown that the inexplicable variability of drug response is quite low in highly selected populations, so there is no sense in recommending drug monitoring for such patients. However, we have already identified some sources of inter- and intra-individual variability, such as renal and/or hepatic function, advanced age, and certain clinically relevant drug-drug interactions. These criteria concern a restricted population, but one at very high risk of clinical events. Laboratory monitoring should be assessed for these patients, to avoid denying them treatment with these very promising compounds.

Impact of Dabigatran Dose Adjustment on Clinical Outcome in AF Patients

A Clinical Trial Simulation Analysis

May 08, 2012

Boehringer Ingelheim

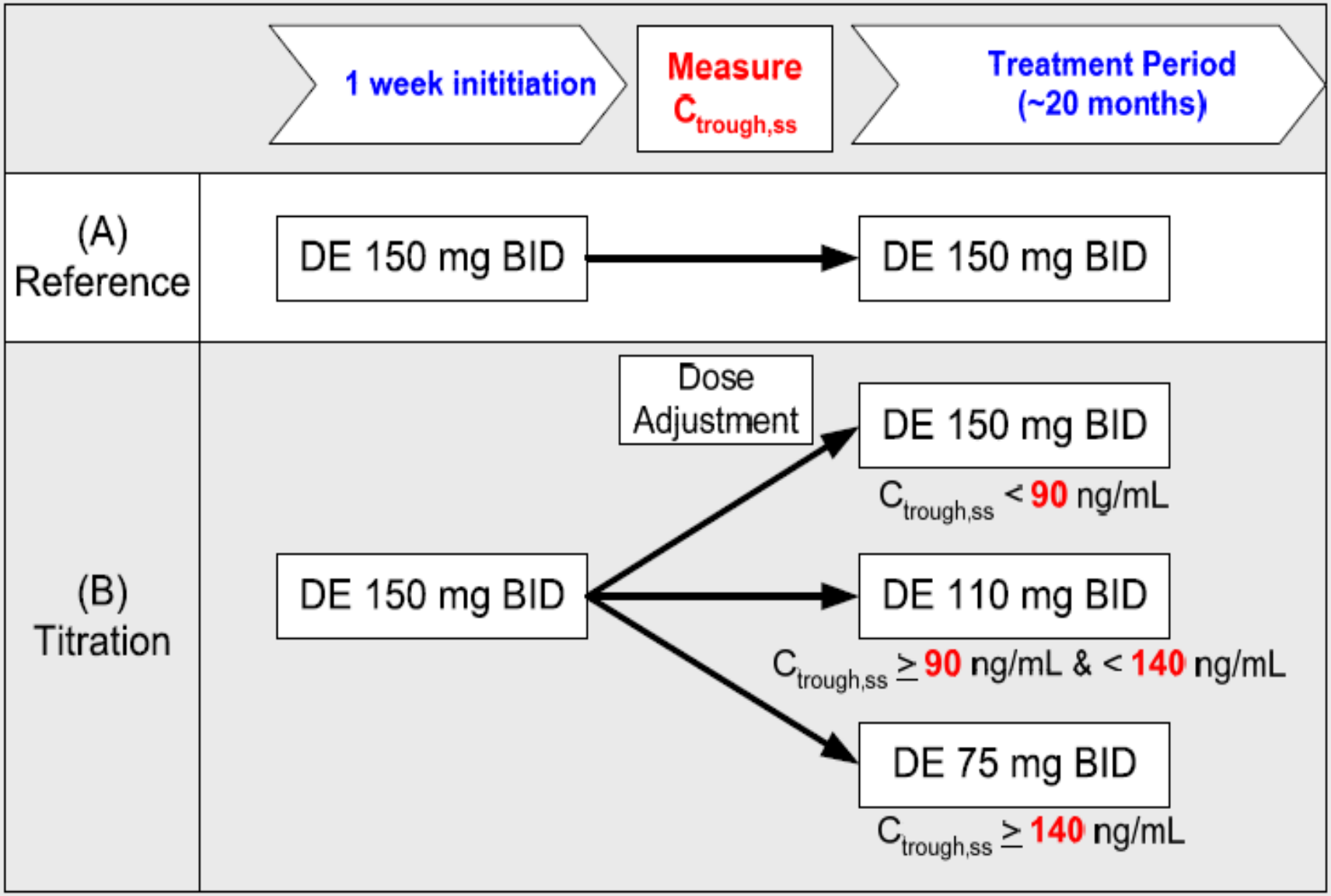
Analytic Approach To Determine Target Plasma Levels

Boehringer Ingelheim

- Cut-off values (Ctrough,ss) between 0 ng/mL and 250 ng/mL (step size 10 ng/mL) were assessed for both major bleeding and ischemic stroke/SEE prevention (352 different combinations)
- 500 clinical trials with 5000 patients each were simulated to evaluate each of the 352 cut-off combinations for both the safety and efficacy endpoints
- Patient characteristics were bootstrapped from RE-LY database

The goal was to identify the optimal trough dabigatran plasma cut-off values for a dose adjustment scheme which produces simulated results that are better (balancing ischemic stroke/SEE and major bleeding event rates) than a fixed dose Pradaxa regimen and a well-controlled warfarin regimen

Boehringer Ingelheim



Results

Model Predicted Outcome

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Absolute Event Rates, not annualized

	Ischemic Stroke/SEE		Major Bleeding	
	Mean*	90% CI[$]	**Mean***	90% CI[$]
(A) Reference	**1.26**	1.01 – 1.55	**4.38**	3.91 – 4.89
(B) Titration	**1.34**	1.08 – 1.63	**3.49**	3.08 – 3.96

*risk of event within median RE-LY duration [~20 months], not annualized;

[$] Clopper-Pearson (Exact); [$]Range 10^{th} percentile – 90^{th} percentile

Relative Risk

(B) Titration vs. (A) Reference	**Rel. Risk**	90% CI
Ischemic Stroke/SEE	**1.06**	(0.76 – 1.50)
Major Bleeding	**0.80**	(0.66 – 0.97)

Titration vs. Reference

- Risk of ischemic stroke/SEE events comparable (Relative Risk 1.06)
- Risk of major bleeding events significantly reduced (Relative Risk 0.8)

Summary

Clinical Trial Simulation Analysis

Boehringer Ingelheim

- Extensive and comprehensive clinical trial simulation analyses were performed to investigate the impact of dabigatran dose titration on outcomes in AF patients.
- Dabigatran Ctrough,ss values of 90 ng/mL and 140 ng/mL were identified as promising cut-off values to assign dabigatran doses of 150 mg bid, 110 mg bid and 75 mg bid.
- Compared to a reference treatment (=DE 150 mg bid), dose adjustment showed a significant reduction of major bleeding events (RR 0.8), while the ischemic stroke protection was maintained (RR 1.06).
- Compared to warfarin treatment, dose adjustment showed a significant reduction of ischemic stroke/SEE (RR 0.8) and major bleeding events (RR 0.6).

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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)

Paul A. Reilly, PhD,* Thorsten Lehr, PhD,†‡ Sebastian Haertter, PhD,† Stuart J. Connolly, MD,§ Salim Yusuf, MD, DPhil,§ John W. Eikelboom, MB BS,§ Michael D. Ezekowitz, MD, PhD,‖ Gerhard Nehmiz, PhD,† Susan Wang, PhD,* Lars Wallentin, MD, PhD,¶ on behalf of the RE-LY Investigators

Ridgefield, Connecticut; Biberach and Saarbrücken, Germany; Hamilton, Ontario, Canada; Wynnewood, Pennsylvania; and Uppsala, Sweden

Objectives The goal of this study was to analyze the impact of dabigatran plasma concentrations, patient demographics, and aspirin (ASA) use on frequencies of ischemic strokes/systemic emboli and major bleeds in atrial fibrillation patients.

Background The efficacy and safety of dabigatran etexilate were demonstrated in the RE-LY (Randomized Evaluation of Long-Term Anticoagulation Therapy) trial, but a therapeutic concentration range has not been defined.

Methods In a pre-specified analysis of RE-LY, plasma concentrations of dabigatran were determined in patients treated with dabigatran etexilate 110 mg twice daily (bid) or 150 mg bid and correlated with the clinical outcomes of ischemic stroke/systemic embolism and major bleeding using univariate and multivariate logistic regression and Cox regression models. Patient demographics and ASA use were assessed descriptively and as covariates.

Results Plasma concentrations were obtained from 9,183 patients, with 112 ischemic strokes/systemic emboli (1.3%) and 323 major bleeds (3.8%) recorded. Dabigatran levels were dependent on renal function, age, weight, and female sex, but not ethnicity, geographic region, ASA use, or clopidogrel use. A multiple logistic regression model (c-statistic 0.657, 95% confidence interval [CI]: 0.61 to 0.71) showed that the risk of ischemic events was inversely related to trough dabigatran concentrations ($p = 0.045$), with age and previous stroke (both $p < 0.0001$) as significant covariates. Multiple logistic regression (c-statistic 0.715, 95% CI: 0.69 to 0.74) showed major bleeding risk increased with dabigatran exposure ($p < 0.0001$), age ($p < 0.0001$), ASA use ($p < 0.0003$), and diabetes ($p = 0.018$) as significant covariates.

Conclusions Ischemic stroke and bleeding outcomes were correlated with dabigatran plasma concentrations. Age was the most important covariate. Individual benefit-risk might be improved by tailoring dabigatran dose after considering selected patient characteristics. (Randomized Evaluation of Long Term Anticoagulant Therapy [RE-LY] With Dabigatran Etexilate; NCT00262600) (J Am Coll Cardiol 2014;63:321–8) © 2014 by the American College of Cardiology Foundation

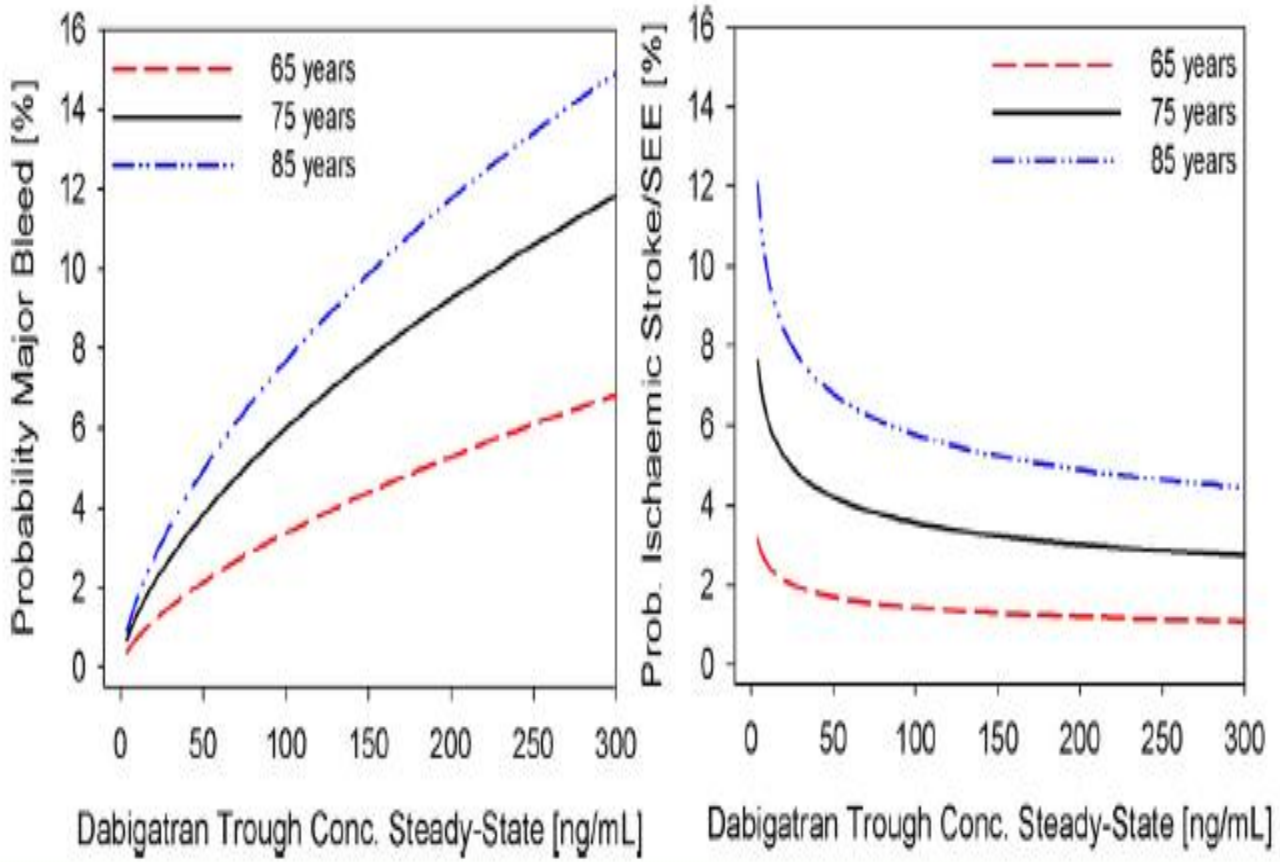


Figure 1 **Probability of Clinical Outcomes Versus Dabigatran Plasma Concentrations**

Major bleeding event **(left)** and ischemic stroke/systemic embolic event **(right)** versus trough dabigatran plasma concentration in atrial fibrillation patients by age (65, 75, and 85 years). Covariates include sex, prior stroke, and diabetes. Conc. = concentration.

The concentration range achieved for either dose in RE-LY ranged over 5-fold for the 10th to 90th percentiles, with a large overlap of concentrations, approximately 70%, between the 2 doses. Given that the primary analyses of the whole population, without consideration of plasma levels, showed that the 2 doses of DE in RE-LY were effective and safe, this suggests that there is a wide therapeutic range.

Application of findings to clinical practice. In this RE-LY substudy, demographic characteristics played the strongest role in determining risk of clinical events. In patients at highest risk for events, such as the very elderly and/or those with poor renal function, an adjustment of DE dose to optimize exposure might improve the benefit–risk if they are at either extreme of the concentration range. However, an assay of dabigatran concentrations is not yet widely available, and at least in the United States, only the 150-mg bid dose is available except for a 75-mg bid dose in patients with severe renal failure. This substudy, therefore, can only serve as a basis for future endeavors in this area.

Canadian Journal of Cardiology 29 (2013) S24–S33

Review

Importance of Pharmacokinetic Profile and Variability as Determinants of Dose and Response to Dabigatran, Rivaroxaban, and Apixaban

Inna Y. Gong, BMSc,[a,b] and Richard B. Kim, MD[a,b]

[a] Division of Clinical Pharmacology, Department of Medicine, University of Western Ontario, London, Ontario, Canada

[b] Department of Physiology and Pharmacology, University of Western Ontario, London, Ontario, Canada

ABSTRACT

Warfarin has been the mainstay oral anticoagulant (OAC) medication prescribed for stroke prevention in atrial fibrillation (AF) patients. However, warfarin therapy is challenging because of marked interindividual variability in dose and response, requiring frequent monitoring and dose titration. These limitations have prompted the clinical development of new OACs (NOACs) that directly target the coagulation cascade with rapid onset/offset of action, lower risk for drug-drug interactions, and more predictable response. Recently, NOACs dabigatran (direct thrombin inhibitor), and rivaroxaban and apixaban (factor Xa [FXa] inhibitors) have gained regulatory approval as alternative therapies to warfarin. Though the anticoagulation efficacy of these NOACs has been characterized, differences in their pharmacokinetic and pharmacodynamic profiles have become a significant consideration in terms of drug selection and dosing. In this review, we outline key pharmacokinetic and pharmacodynamic features of each compound and provide guidance on selection and dosing of the 3 NOACs relative to warfarin when considering OAC therapy for AF patients. Importantly, we show that by better understanding the effect

RÉSUMÉ

La warfarine a été le pilier des anticoagulants oraux (ACO) prescrit pour la prévention de l'accident vasculaire cérébral chez les patients ayant une fibrillation auriculaire (FA). Cependant, le traitement par la warfarine est difficile en raison de la variabilité interindividuelle marquée de la dose et de la réponse, ce qui rend nécessaire une surveillance fréquente et un réglage posologique. Ces limites ont suscité le développement clinique de nouveaux ACO (NACO) qui ciblent directement la cascade de coagulation par un délai d'action rapide et une durée d'action, un plus faible risque d'interactions médicamenteuses et une réponse plus prévisible. Récemment, les NACO dont le dabigatran (inhibiteur direct de la thrombine), le rivaroxaban et l'apixaban (inhibiteurs du facteur Xa) ont obtenu l'homologation à titre de solution de rechange au traitement par la warfarine. Tandis que l'efficacité de l'anticoagulation de ces NACO a été établie, les différences dans leurs profils pharmacocinétiques et pharmacodynamiques sont devenues une préoccupation importante en ce qui a trait à la sélection du médicament et du dosage. Dans cette revue, nous exposons les grandes lignes des principales caractéristiques pharmacocinétiques et

When dabigatran was coadministered with amiodarone or ketoconazole (a P-gp and strong CYP3A4 inhibitor), AUC was increased by approximately 50%.[30] Similarly, the potent P-gp inhibitor verapamil increased AUC by 2.4-fold when administered 1 hour before dabigatran and AUC increased by 71% when coadministered.[31] Interestingly, patients of the RE-LY trial coadministered with amiodarone and verapamil only had a 13% and 23% increased bioavailability, respectively.[3] Coadministration of ketoconazole with rivaroxaban increased the AUC by 160% along with similar increases in coagulation measurements.[26] The strong P-gp/CYP3A4 inhibitor ritonavir elevated rivaroxaban AUC by 150%. Coadministration of ketoconazole increased apixaban AUC by 2-fold.[32] Overall, coadministration of rivaroxaban and apixaban with P-gp/CYP3A4 inhibitors (namely azoles and protease inhibitors) should be avoided and coadministration with moderate inhibitors (ie, erythromycin) should be exercised with caution (Table 2).

Inducers of CYP3A4/P-gp such as rifampicin have been shown to result in 65% reduction in dabigatran bioavailbility and 50% decrease in AUC and clotting parameters for rivaroxabin and apixaban.[26,32,33] Thus, concomitant use of rivaroxaban/apxiaban with strong CYP3A4/P-gp inducers is contraindicated because of concern of reduced anticoagulation efficacy.

Table 2. List of dual substrates, inhibitors, and inducers of CYP3A4 and P-glycoprotein

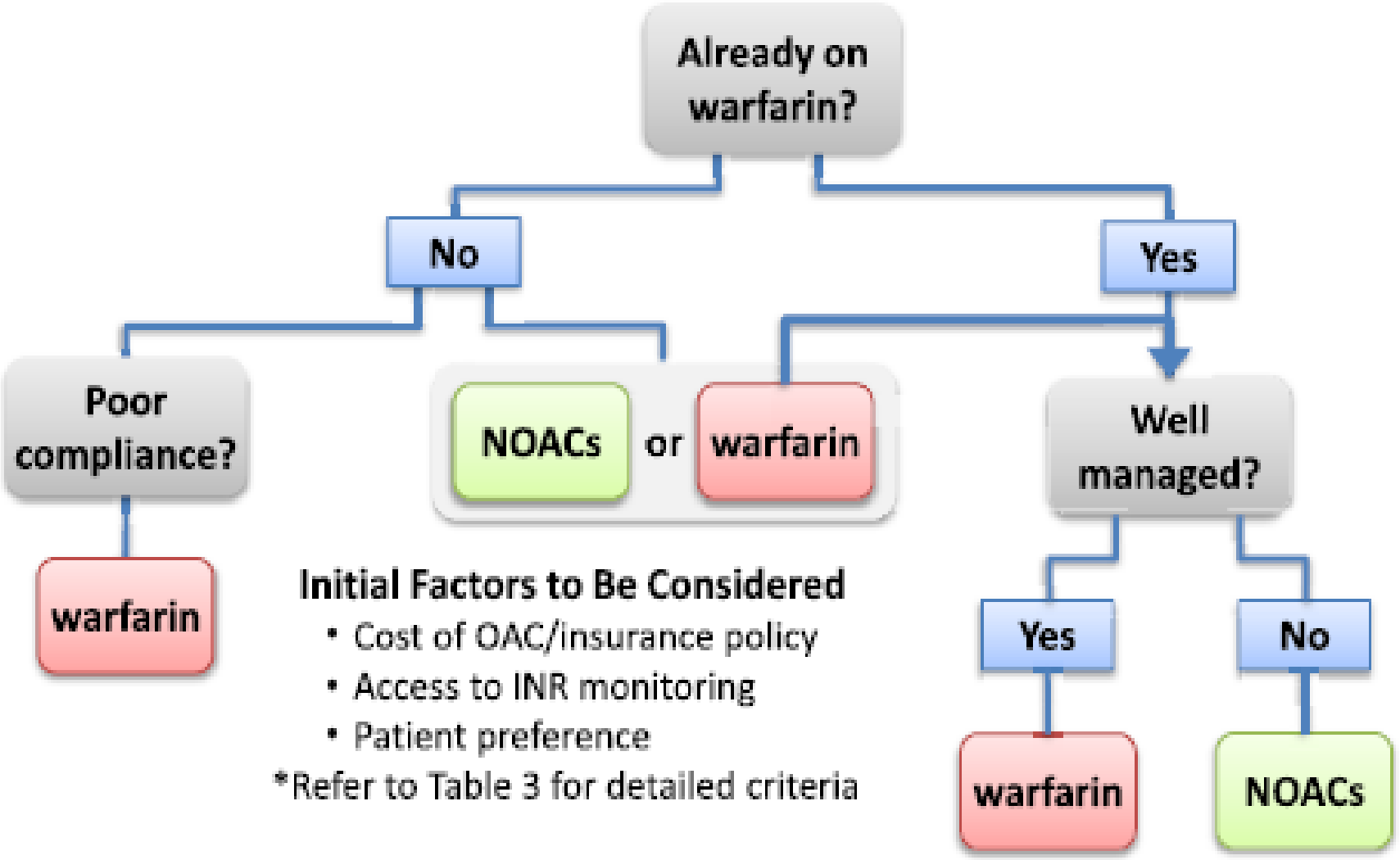
Substrates	Inhibitors	Inducers
Apixaban	Amiodarone	Carbamazepine
Atorvastatin	Cimetidine	Phenobarbital
Celiprolol	Clarithromycin	Rifampicin
Cyclosporine	Erythromycin	St John's wort
Docetaxel	Fluconazole	
Paclitaxel	Ketoconazole	
Rivaroxaban	Itraconazole	
Tacrolimus	Nifedipine	
	Nelfinavir	
	Ritonavir	
	Saquinavir	
	Verapamil	
	Voriconazole	

Selecting the Right OAC

Although the NOACs have shown efficacy similar or greater than warfarin, it is unlikely that they will fully replace warfarin. The interindividual variability in exposure/response of NOACs and bleeding risk associated with anticoagulation therapy remains a pertinent issue. Indeed, even in a clinical trial setting with stringent enrollment criteria, the 1-dose-fits-all dosing regimen strategy did not appear successful for NOACs, likely due to the various clinical covariates that significantly affected extent of drug exposure and response (Fig. 2).[41,44-47] Moreover, dabigatran and rivaroxaban use outside of the clinical trial setting has recently been noted to exhibit large interindividual variability in concentration and response.[47,48] The same trend is likely to be observed with apixaban as its clinical use increases.

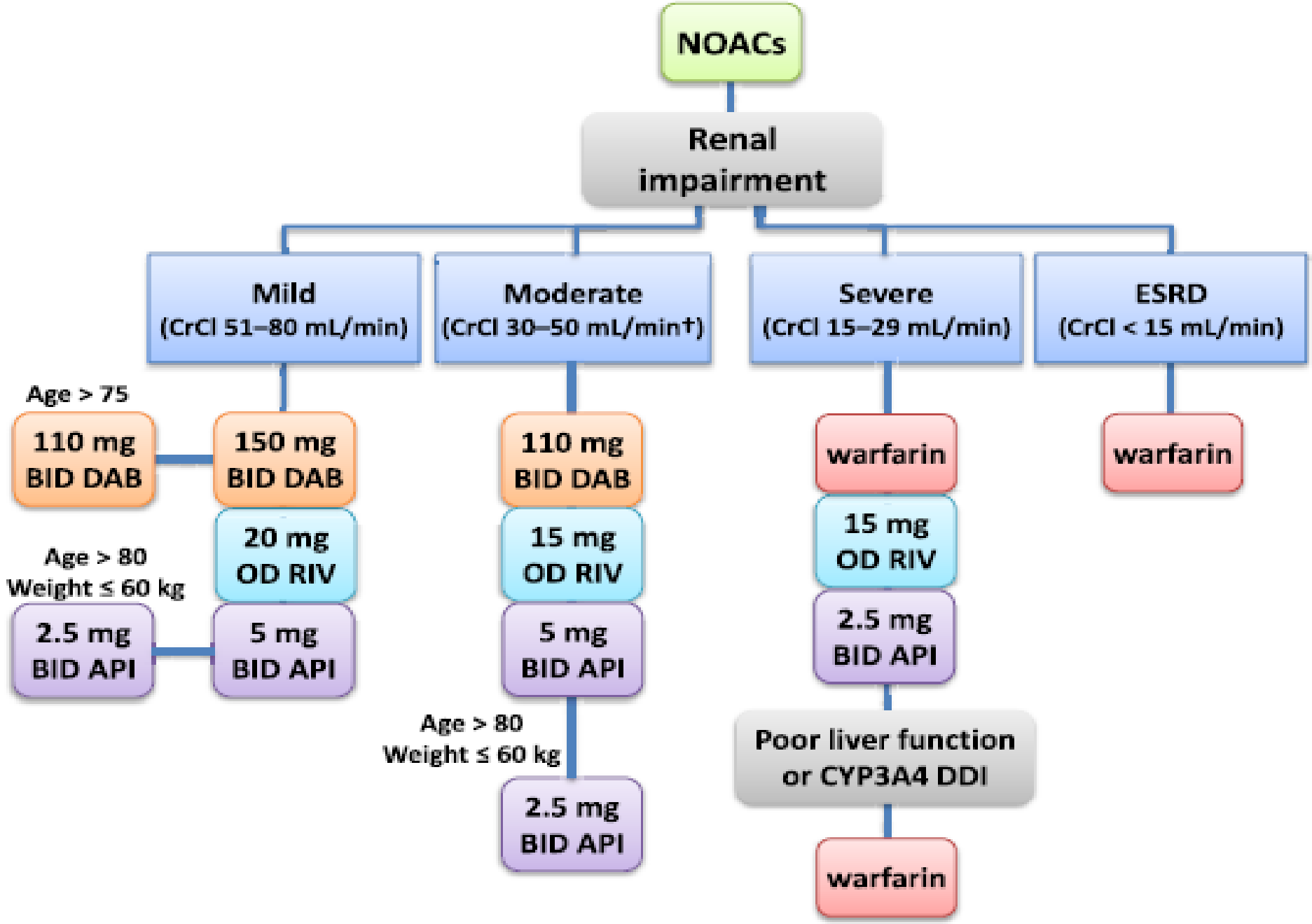
A

Selection of warfarin vs NOAC for AF patients



B

Selection of appropriate NOAC for AF patients



C

Selection of appropriate NOAC continued

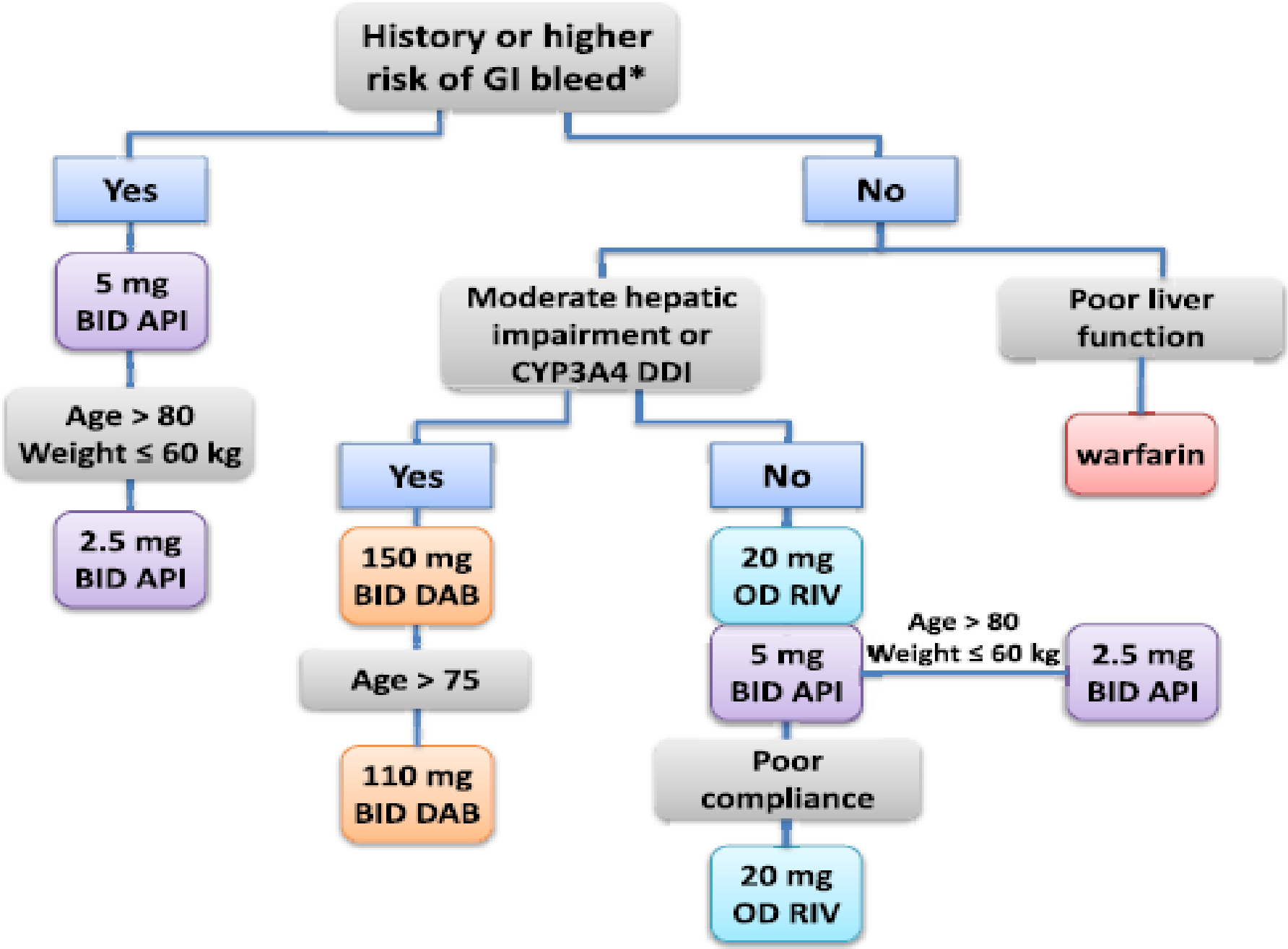


Table 3. Summary of patient criteria for selecting warfarin vs NOACs

Select warfarin	Select NOACs
Stable and well controlled INR	Previously taking warfarin with poor INR control
CrCl < 30 mL/min	Normal renal function or mild renal dysfunction
Low cost to patient/lack of insurance coverage	High cost of drug affordable
Good compliance	Inadequate access to routine INR monitoring
History of gastrointestinal bleeding	Require rapid onset of action
Require rapid reversal (antidote)	Harbouring multiple variant alleles in CYP2C9 and VKORC1 known to confer warfarin sensitivity*
Concomitant use of P-gp/CYP3A4 inhibitor or inducer	Patient preference

CrCl, creatinine clearance; CYP, cytochrome P450; INR, international normalized ratio; NOAC, new oral anticoagulant; P-gp, P-glycoprotein; VKORC1, vitamin K epoxide reductase enzyme subunit 1.

*Particularly CYP2C9 poor metabolizer genotype combined with VKORC1 sensitive genotype.

Notably, we are starting to realize the role of genetic variation to interindividual variability of NOAC exposure and clinical outcomes. The *CES1* rs2244613 SNP was not only associated with lower dabigatran exposure but also with reduced bleeding risk, indicating the potential for pharmacogenetic-based dosing adjustments using this intronic SNP.[16] Because clinically relevant polymorphisms also exist in CYP3A4/5, P-gp, and BCRP, these genetic variations might also play an important role in determining NOAC exposure. Overall, the full spectrum of NOAC efficacy and safety within the context of pharmacogenetics, demographic characteristics, concomitant medications, and comorbidities should be characterized as the use of these new agents increase in AF patients outside of the clinical trial setting.

More importantly, meaningful interpretation of the results is difficult because of the lack of guidance on extrapolating coagulation results to bleeding and thrombosis risk. Accordingly, directly measuring plasma NOAC drug concentrations might be more desirable and more accurate for monitoring anticoagulation. An important consideration for measuring either coagulation or NOAC drug exposure is the standardization of sampling time from the last dose. The trough concentration (C_{trough}) is preferred over the peak concentration (C_{max}), avoiding misinterpretation of results because of variability in the absorption phase.

Conclusions

NOACs are promising alternatives to warfarin, demonstrating at least similar antithrombotic efficacy and decreased rate of intracranial hemorrhage. However, the availability of multiple NOACs has introduced difficulty in deciding the best agent because head-to-head trials are unavailable and unlikely to be performed. Rather, clinicians are required to make informed decisions in selecting the appropriate agent based on characteristics of the patient and OAC pharmacology. As the clinical use of NOACs increases, surveillance using therapeutic monitoring (measurement of plasma drug concentration or anticoagulation response) throughout the treatment period might be valuable in minimizing the risk of bleeding and lack of efficacy. Finally, because of the extent of interindividual variation in the metabolism and clearance of NOACs, it is likely that a greater range of NOAC doses will be needed to more precisely treat our patients.

Combined Administration of Antibiotics and Direct Oral Anticoagulants: A Renewed Indication for Laboratory Monitoring?

Giuseppe Lippi, MD[1] Emmanuel J. Favaloro, PhD, FFSc (RCPA)[2] Camilla Mattiuzzi, MD[3]

[1] Laboratory of Clinical Chemistry and Hematology, Academic Hospital of Parma, Parma, Italy
[2] Department of Haematology, Institute of Clinical Pathology and Medical Research (ICPMR), Pathology West, Westmead Hospital, Westmead, New South Wales, Australia
[3] Service of Service of Clinical Governance, General Hospital of Trento, Trento, Italy

Address for correspondence Giuseppe Lippi, MD, U.O. Diagnostica Ematochimica, Azienda Ospedaliero-Universitaria di Parma, Via Gramsci, 14, 43126 Parma, Italy (e-mail: glippi@ao.pr.it; ulippi@tin.it).

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The recent development and marketing of novel direct oral anticoagulants (DOACs) represents a paradigm shift in the management of patients requiring long-term anticoagulation. The advantages of these compounds over traditional therapy with vitamin K antagonists include a reportedly lower risk of severe hemorrhages and the limited need for laboratory measurements. However, there are several scenarios in which testing should be applied. The potential for drug-to-drug interaction is one plausible but currently underrecognized indication for laboratory assessment of the anticoagulant effect of DOACs. In particular, substantial concern has been raised during Phase I studies regarding the potential interaction of these drugs with some antibiotics, especially those that interplay with permeability glycoprotein (P-gp) and cytochrome 3A4 (CYP3A4). A specific electronic search on clinical trials published so far confirms that clarithromycin and rifampicin significantly impair the bioavailability of dabigatran, whereas clarithromycin, erythromycin, fluconazole, and ketoconazole alter the metabolism of rivaroxaban in vivo. Because of their more recent development, no published data were found for apixaban and edoxaban, or for potential interactions of DOACs with other and widely used antibiotics. It is noteworthy, however, that an online resource based on Food and Drug Administration and social media information, reports several hemorrhagic and thrombotic events in patients simultaneously taking dabigatran and some commonly used antibiotics such as amoxicillin, cephalosporin, and metronidazole. According to these reports, the administration of antibiotics in patients undergoing therapy with DOACs would seem to require accurate evaluation as to whether dose adjustments (personalized or antibiotic class driven) of the anticoagulant drug may be advisable. This might be facilitated by direct laboratory assessments of their anticoagulant effect ex vivo.

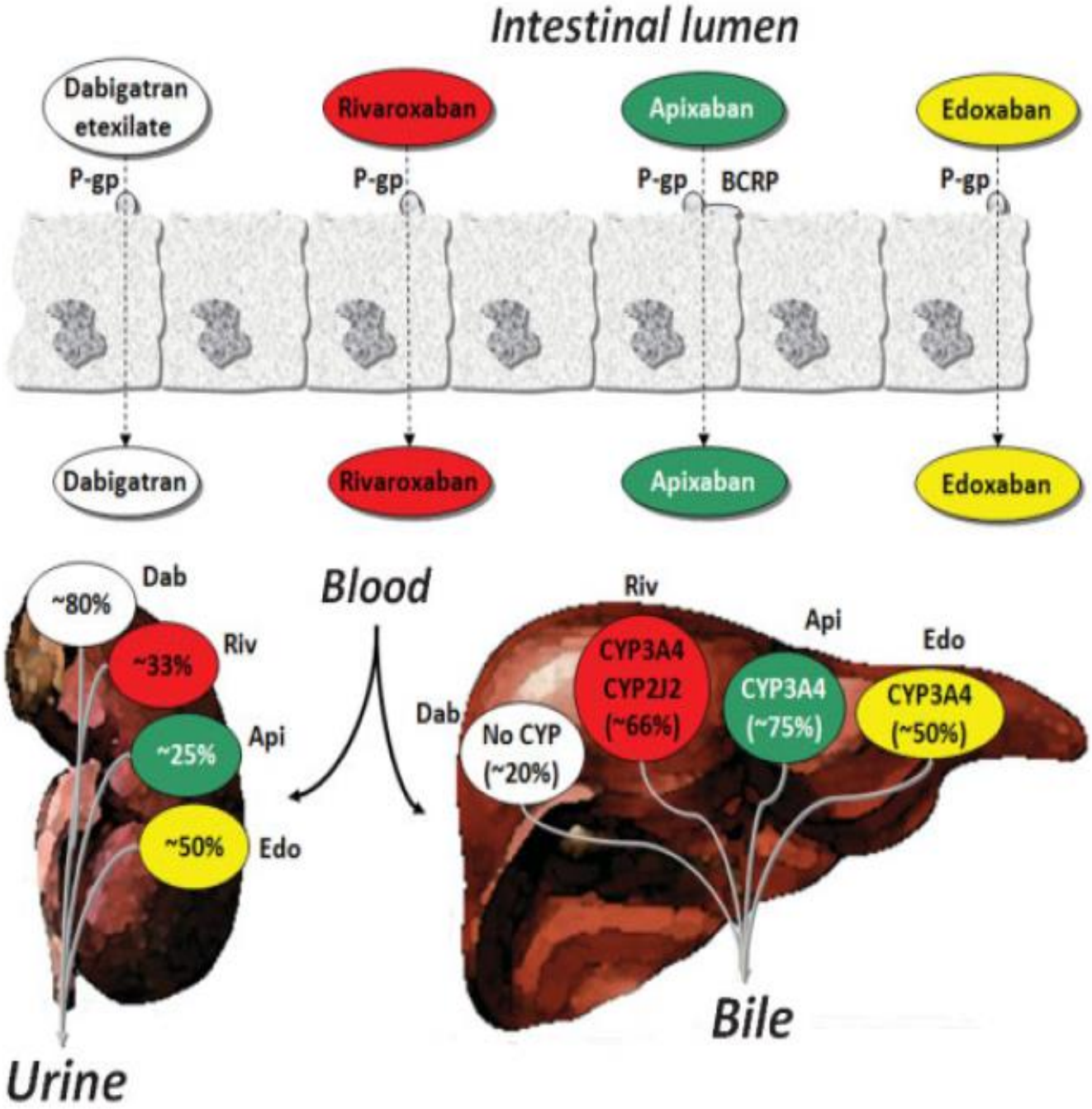

Fig. 2 Metabolism of novel oral anticoagulants. BCRP, breast cancer resistance protein; CYP, cytochrome; P-gp; permeability glycoprotein.

Monitoring new oral anticoagulants, managing thrombosis, or both?

Hugo ten Cate[1,2]

[1]Department of Internal Medicine and Cardiovascular Research Institute Maastricht, Maastricht University Medical Center, Maastricht, the Netherlands; [2]Maastricht Anticoagulation Clinic, Maastricht, the Netherlands

of) thrombosis. While NOACs have been developed under the assumption that monitoring and dose adjustment would be abolished (illustrated by provocative paper headlines like "Funeral of the Anticoagulation Clinic"), the implication may be that patients are left with a prescription and a single advice, but without any kind of formalised support (including laboratory control). I consider this development potentially harmful to the patient, requiring critical consideration of causes and consequences. Pharmaceutical companies have invested heavily in the development of novel, targeted synthetic drugs that directly interfere with a specific coagulation protease, blocking its clotting activity. After many years of struggle (including the failure of ximelagatran to reach the market due to liver function problems), several NOACs have now successfully reached the clinical arena. Competition among several large companies is strong and rapid penetration of the most important segments of the market (including the AF population) is economically important.

The assumption is that NOACs, in contrast with VKA, neither require monitoring with laboratory tests, nor frequent dose adjustment, thanks to reduced food and drug interactions, contributing to increased pharmacokinetic stability. However, several issues should be considered. First, the NOACs are all prescribed on a fixed-dose basis. One size fits all, comparable to the poly-pill concept. In practice, this assumption has weaknesses; pharmacokinetic studies show that drugs like dabigatran show considerable variation in plasma drug concentrations (11), such that while the majority of patients will obtain an adequate plasma drug level, a measurable proportion will either achieve an insufficient, or a supra-therapeutic drug level, when given a fixed dose. Advocates of a fixed-dose policy will argue that the overall performance of NOACs in the large clinical trials is non-inferior or better, as compared to INR-adjusted warfarin. This may be so, but one should keep in mind that these were carefully selected patients to begin with (excluding those with assumed poor compliance, renal insufficiency, bleeding

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THROMBOSIS JOURNAL

EDITORIAL Open Access

New oral anticoagulants: discussion on monitoring and adherence should start now!

Hugo ten Cate[1,2]

Abstract

New oral anticoagulants (NOACs) have been introduced to improve anticoagulant therapy worldwide, but safe implementation may require additional measures. First, optimization of dose adjustment based on therapeutic levels of the drug may be more appropriate than fixed dose therapy. The development and implementation in quantitative laboratory assays will enable further dose optimization. Second, non-adherence to medication is a potential threat to the safe use of NOACs. Since cardiovascular medication may not be optimally used in about 50% of patients, procedures to improve adherence are imperative, also for NOAC therapy and in particular in elderly patients.

Concluding remarks

NOACs have been introduced to improve anticoagulant therapy worldwide. In particular in countries where current VKA control is difficult to organize, NOACs may provide a promising alternative. Two issues need to be taken into account in order to obtain safe anticoagulation with NOACs (or VKA). First, for NOACs therapeutic ranges of each agent should become available based on concentrations and/or dose response effects in laboratory tests. This will ultimately provide a means of optimizing dose adjustment in individual patients, more so than by current algorithms.

Secondly, stimulation of adherence is of utmost importance. Given the body of data showing poor adherence in patients on long term medication, similar problems may be expected in those using NOACs. This requires measures to prevent non-adherence, preferably in a patient centered manner. Further discussion and studies are needed to raise awareness for this adherence to medication problem, amongst patients, authorities and prescribers.

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Regular Article

On the monitoring of dabigatran treatment in "real life" patients with atrial fibrillation

Mika Skeppholm [a,b,*], Paul Hjemdahl [a], Jovan P. Antovic [c], Josephine Muhrbeck [b], Jaak Eintrei [c], Yuko Rönquist-Nii [d], Anton Pohanka [d], Olof Beck [d], Rickard E. Malmström [a]

[a] Department of Medicine Solna, Clinical Pharmacology Unit, Karolinska Institutet & Clinical Pharmacology, Karolinska University Hospital, Stockholm, Sweden
[b] Department of Cardiology, Danderyd Hospital & Department of clinical sciences, Karolinska Institutet, Stockholm, Sweden
[c] Department of Coagulation Research, Institute for Molecular Medicine and Surgery, Karolinska Institutet, Department of Clinical Chemistry, Karolinska University Hospital, Stockholm, Sweden
[d] Department of Laboratory Medicine Huddinge, Clinical Pharmacology Unit, Karolinska Institutet & Clinical Pharmacology, Karolinska University Hospital, Stockholm, Sweden

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ABSTRACT

Introduction: The oral direct thrombin inhibitor dabigatran is increasingly used to prevent thromboembolic stroke in patients with atrial fibrillation (AF). Routine laboratory monitoring is currently not recommended, but measurements of dabigatran and/or its effect are desirable in certain situations. We studied dabigatran exposure and compared different tests for monitoring of dabigatran in a real-life cohort of AF patients.
Material and methods: Ninety AF patients (68 ± 9 years, 67% men, mean $CHADS_2$ score 1.5) were treated with dabigatran 150 (n = 73) or 110 mg BID (n = 17). Trough plasma concentrations of total and free dabigatran by liquid chromatography-tandem mass-spectrometry (LC-MS/MS) were compared to indirect measurements by Hemoclot thrombin inhibitors (HTI) and Ecarin clotting assay (ECA), as well as PT-INR and aPTT.

Results: Total plasma dabigatran varied 20-fold (12–237 ng/mL with 150 mg BID) and correlated well with free dabigatran ($r^2 = 0.93$). There were strong correlations between LC-MS/MS and HTI or ECA ($p < 0.001$) but these assays were less accurate with dabigatran below 50 ng/mL. The aPTT assay was not dependable and PT-INR not useful at all. There were weak correlations between creatinine clearance (Cockcroft-Gault) and LC-MS/MS, HTI and ECA ($p < 0.001$ for all). A high body weight with normal kidney function was associated with low dabigatran levels.
Conclusions: HTI and ECA reflect the intensity of dabigatran anticoagulation, but LC-MS/MS is required to quantify low levels or infer absence of dabigatran. Most real life patients with a normal creatinine clearance had low dabigatran levels suggesting a low risk of bleeding but possibly limited protection against stroke.

Ninety patients treated with dabigatran due to non-valvular atrial fibrillation were recruited from Danderyd's Hospital in Stockholm County where they were followed as outpatients. The study was performed in

Blood sampling was performed at steady state, i.e. after a minimum of three days of treatment. All patients reported full compliance during the last 3 days. All samples were obtained in the morning before intake of the next capsule (i.e. at trough, 10–16 hours after the last dose). Blood

Exposure levels to dabigatran observed in our real-life cohort were somewhat lower for the 150 mg BID and higher for the 110 mg BID dose, respectively, when compared to data from the RE-LY trial. Thus, we found 75 (10th to 90th percentile = 31-127) ng/mL total dabigatran in our individually dosed patients compared to 93 (40–215) ng/mL in the fixed dosed patients on 150 mg BID in the RE-LY study [12]. This is at least partially explained by clinical decisions to prescribe the 110 mg BID dose to patients with risk factors for higher exposure (higher age, lower body weight, worse renal function). Our present and previ-

Analytical results	All patients (n = 90)	Dabigatran 110 mg BID* (n = 17)	Dabigatran 150 mg BID* (n = 73)
LC-MS/MS total, ng/mL, Median (range)	70 (12 - 237)	75 (29 - 217)	62 (12 - 237)
LC-MS/MS free, ng/mL, Median (range)	54 (8–188)	60 (23 - 168)	52 (8 - 188)
LC-MS/MS total, ng/mL, 10th-90th percentile	33 - 145	38 - 199	31 - 127
LC-MS/MS free, ng/mL, 10th-90th percentile	24 - 106	26 - 158	23 - 96
HTI, ng/mL, Median (range)	48 (0–173)	53 (8–173)	46 (0–172)
ECA, ng/mL, Median (range)	56 (9–239)	61 (17–236)	54 (9–239)
aPTT, seconds, Median (range)	44 (27–75)	45 (32–75)	44 (27–65)
PT-INR, Median (range)	1.1 (0.9 -1.6)	1.1 (1.0 -1.3)	1.1 (0.9 -1.6)

*For comparison; the concentrations of total dabigatran in the fixed dosage groups in the pivotal RE-LY trial [9] were:

66 (median), 28–155 (10th to 90th percentile) ng/mL in patients dosed 110 mg BID and

93 (median), 40–215 (10th to 90th percentile) ng/mL in patients dosed 150 mg BID.

[12]. The European Medicines Agency (EMA) conservatively stated that exceeding the 90th percentile of dabigatran trough levels is associated with an increased risk of bleeding, and this level was set at about 200 ng/mL [15]; this corresponds to 160 ng/mL free dabigatran. Apart from the upper limit stated by EMA, which increases the risk of suffering a major bleed substantially, there is no recommended target concentration to achieve for optimal dabigatran treatment of individual patients. A summary graph by Reilly et al. [12] suggests that the ideal trough levels of total dabigatran in plasma may be around 50–120 ng/mL (corresponding to 40–100 ng/mL free dabigatran) when treating patients with AF. However, the optimal range of dabigatran concentrations needs to be further defined in prospective studies.

were not reported for these subgroups [9]. It is of interest that the majority of our "real life" patients with normal CrCl had plasma dabigatran concentrations in the low range of the interval measured in the RE-LY study. Thus, the risk of bleeding may not be imminent while protection against thromboembolic complications could be less efficient among patients like those in our study who are dosed according to recommendations. Furthermore, the considerable variability of dabigatran concentrations in plasma seen presently and previously [11,12] supports the contention that patients could benefit from a possibility to monitor dabigatran treatment and individualize dosages for optimal efficacy and safety. Such monitoring would, however, not need to be as intense as for warfarin treatment.

We identified a group of patients that received a potentially too low dose, i.e., patients with both high body weight and normal renal function. These patients had a median total dabigatran concentration of 54 ng/mL (free 45 ng/mL), which is close to the low cut-off of 50 ng/mL considered to be needed for adequate thromboembolic protection in the RE-ALIGN study on patients with mechanical heart valves [17]. Furthermore, in the RE-ALIGN study 8% of the patients did not reach the pre-specified target concentration with the maximal dosage of 300 mg dabigatran BID, which indicates that not all patients can achieve satisfactory protection against thromboembolic events with standard dabigatran treatment.

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Regular Article

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

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

[a] Department of Medicine Solna, Karolinska Institutet & Department of Cardiology, Danderyd Hospital, Stockholm, Sweden
[b] Department of Medicine Solna, Karolinska Institutet & Clinical Pharmacology, Karolinska University Hospital, Stockholm, Sweden
[c] Department of Coagulation Research, Institute for Molecular Medicine and Surgery, Karolinska Institutet, & Department of Clinical Chemistry, Karolinska University Hospital, Stockholm, Sweden
[d] Department of Clinical Sciences, Cardiology Unit, Karolinska Institutet, Danderyd's Hospital, and Stockholm Heart Center, Stockholm, Sweden
[e] Department of Laboratory Medicine Huddinge, Clinical Pharmacology Unit, Karolinska Institutet & Clinical Pharmacology, Karolinska University Hospital, Stockholm, Sweden

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ABSTRACT

Introduction: The direct factor-Xa inhibitor apixaban is approved e.g. for the prevention of stroke in patients with atrial fibrillation (AF). Although routine monitoring of apixaban therapy is currently not recommended, selective monitoring could be useful to optimize efficacy and safety in certain clinical situations. We studied the exposure and effect of apixaban using different laboratory methods in a clinical setting with a well-defined cohort of AF patients.

Material and methods: Seventy AF patients (72 ± 7.4 years, 64 % men, mean $CHADS_2$ score 1.7) treated with apixaban 2.5 (n = 10) or 5 mg BID (n = 60). Trough plasma apixaban concentrations determined by liquid chromatography-tandem mass-spectrometry (LC-MS/MS) were compared to the coagulation assays Anti-factor Xa, PT-INR and aPTT.

Results: The apixaban plasma concentration determined by LC-MS/MS varied more than 10-fold overall. The range was between 15-83 and 29-186 ng/mL for the 2.5 mg BID and 5 mg BID respectively, with patients receiving 5 mg BID having significantly higher apixaban concentrations ($p < 0.001$). A strong correlation between LC-MS/MS and anti-FXa-assay was found ($p < 0.001$), while aPTT and PT-INR were not sensitive enough. There were no significant correlations between gender, creatinine clearance, body weight or age and apixaban exposure.

Conclusions: Anti-FXa-assay performed well upon apixaban concentrations in a normal exposure range. Still LC-MS/MS remains the "gold standard" method, covering also low concentrations. Compared to clinical trials, we observed relatively lower apixaban exposure and a more pronounced difference between high and low dose. Additional information regarding apixaban exposure and benefit-risk profile is needed in order to individualize treatment.

Material and Methods

Seventy patients with AF treated with apixaban were recruited from the coagulation centre at Danderyd´s Hospital in the Stockholm County during the period 2013-10-01 and 2014-06-03. The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethical Review Board in Stockholm, Sweden (Dnr 2012/1232-31/4). Oral and written informed consent was obtained from each participant. The patients were treated with apixaban according to clinical routine care. Patients were excluded from the study if they were in treatment with any possibly interacting drug according to the SPC. All study patients reported full compliance during the last three days before trough plasma samples were collected in median 12.3 hours (9.8 – 18.8; min-max) after last intake of the drug. Samples were analyzed using LC-MS/MS methodology and different coagulation assays; anti-FXa assay, aPTT and PT-INR. The apixaban concentrations were also evaluated in relation to the clinical characteristics (renal function, body weight, sex, age) of the treated patients and the prescribed apixaban dose.

Analytical results in the entire cohort and divided by apixaban dosage.

Analytical results	All patients n = 70	Apixaban 2.5 mg BID n = 10	Apixaban 5 mg BID n = 60
LC-MS/MS, ng/mL Median (range)	75 (15 – 186)	48 (15 – 83)	77 (29 – 186)
LC-MS/MS, ng/mL (10th-90th percentile)	43 – 118	17 – 80	47 – 121
LC-MS/MS, ng/mL Geometric mean	72	44	78
Anti-Xa, ng/mL Median (range)	75 (16-178)	48 (16-75)	77 (31-178)
aPTT, seconds Median (range)	34 (28-50)	34 (30-37)	34 (28-50)
PT-INR Median (range)	1.1 (0.9 -1.4)	1.1 (1.0 -1.3)	1.0 (0.9 -1.4)

The median trough concentrations measured with LC-MS/MS (or anti-FXa assay) were in our study 48 and 77 ng/mL, for 2.5 and 5 mg BID, respectively. Our data are overall in line with previous reports from healthy volunteers [18,20] and ACS patients [13]. According to the manufacturer, median trough values were 79 ng/mL (34 – 162, 5th – 95th percentile) and 103 ng/mL (41 – 230, 5th – 95th percentile) for patients treated with 2.5 and 5 mg BID, respectively [8]. These concentrations are higher than in our real life patients but still with a marked variability as seen in our cohort where apixaban exposure varied 6-fold among patients within the same dose group.

clinical use. Our clinical cohort of patients had lower exposure of apixaban than patients included in the pivotal trial, and furthermore the difference in plasma concentrations between high dosed and low dosed patients were more pronounced. Clearly additional information is vital in order to understand the correlation between apixaban exposure and the benefit-risk profile in order to attain the best health benefit and a more individualized patient treatment.

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Full Length Article

Determination of dabigatran and rivaroxaban by ultra-performance liquid chromatography-tandem mass spectrometry and coagulation assays after major orthopaedic surgery

M.W.M. Schellings [a,b,*], K. Boonen [c], E.M.H. Schmitz [b,d,e,h], F. Jonkers [f,g], D.J. van den Heuvel [h], A. Besselaar [f], J.G.E. Hendriks [g], D. van de Kerkhof [e,h]

[a] MaasstadLab, Clinical Laboratory, Maasstad Hospital, Rotterdam, The Netherlands
[b] Clinical Laboratory, Máxima Medical Center, Eindhoven, The Netherlands
[c] Clinical Laboratory, Amphia Hospital, Breda, The Netherlands
[d] Laboratory of Chemical Biology and Institute of Complex Molecular Systems, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands
[e] Expert Center Clinical Chemistry Eindhoven, Eindhoven, The Netherlands
[f] Department of Orthopaedic Surgery, Catharina Hospital, Eindhoven, The Netherlands
[g] Department of Orthopaedic Surgery, Máxima Medical Center, Eindhoven, The Netherlands
[h] Clinical Laboratory, Catharina Hospital, Eindhoven, The Netherlands

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ABSTRACT

Major orthopaedic surgery is associated with an increased risk of venous thromboembolism. Direct oral anticoagulants (DOACs) are recommended as thromboprophylactic agents after orthopaedic surgery. Although routine monitoring of DOACs in general is not required, measuring DOAC concentration may be necessary in clinical settings. The effects of DOACs on routine coagulation assays in spiked material are studied extensively, however, few data are available on DOAC concentration in patients after major orthopaedic surgery.

We measured trough and peak DOAC concentrations with UPLC-MS/MS and routine coagulation tests in a prospective study including 40 patients receiving thromboprophylactic treatment with dabigatran 220 mg od and 40 patients receiving rivaroxaban 10 mg od after major orthopaedic surgery.

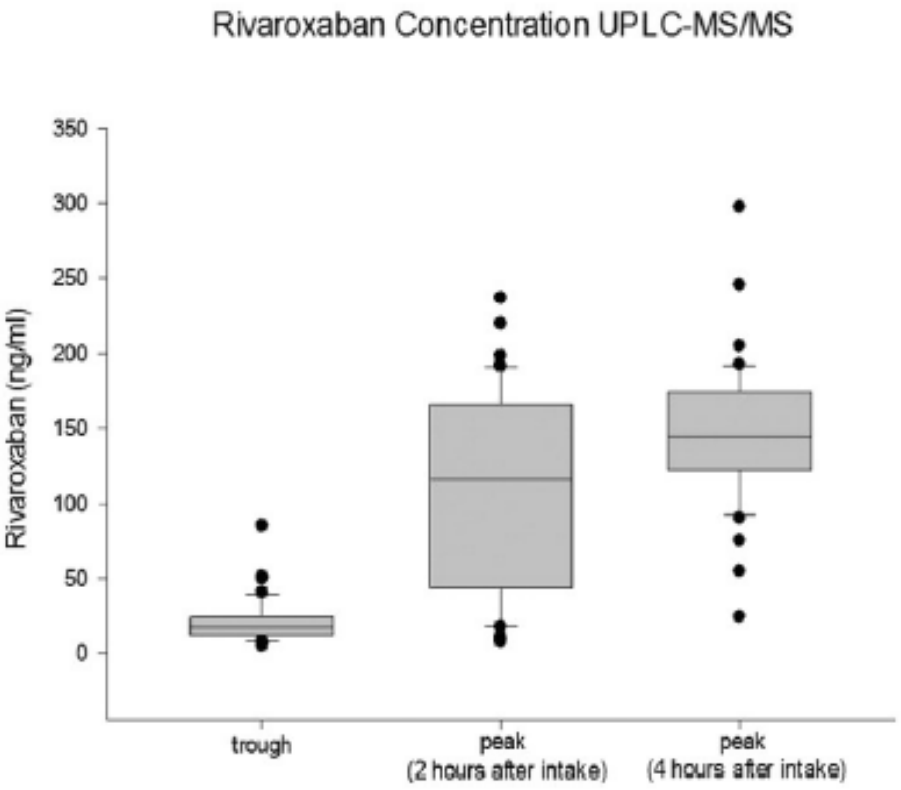
For rivaroxaban, the median trough concentration with UPLC-MS/MS was 17.1 ng/mL and median peak concentration was 149 ng/mL. The anti-Xa assay displayed a good correlation, but a positive bias in comparison to the reference method. Furthermore, trough levels were mostly below the LOD of the anti-Xa assay. For dabigatran, the median trough concentration with UPLC-MS/MS was 12.1 ng/mL, and median peak level was 80.8 ng/mL. A positive bias was found when results from coagulation assays were compared to UPLC-MS/MS data. However, the addition of glucuronidated metabolites to dabigatran concentration UPLC-MS/MS data generally resolved most of this bias. Age was found to have a significant influence on dabigatran pharmacokinetics, irrespective of kidney function, whereas no effect of age was found during rivaroxaban treatment. In both treatment groups, female subjects displayed faster pharmacokinetics in comparison to male subjects, although not reaching significance.

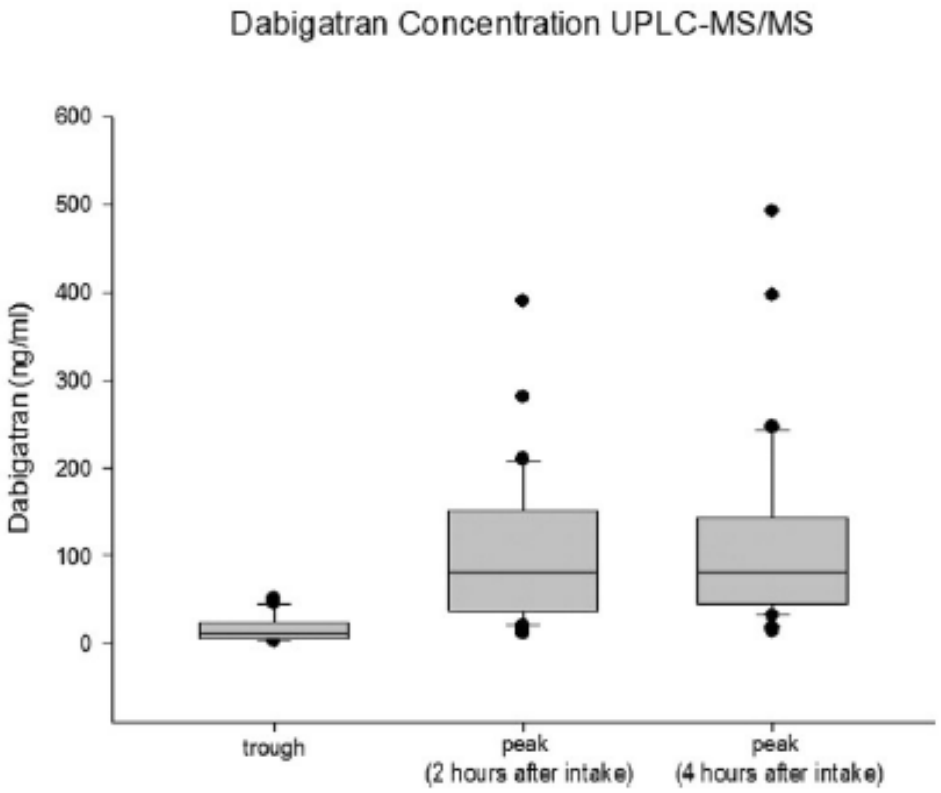
We conclude that UPLC-MS/MS is the method of choice to measure trough concentrations of DOACs in patients after orthopaedic surgery. Current coagulation assays are not suited for this purpose. We found large heterogeneity in both peak and trough concentrations of DOACs, and showed that pharmacokinetics of novel oral anticoagulants may be influenced by age and gender. Whether patients with high or low trough concentrations are at increased risk for bleeding or thromboembolic events respectively remains to be established.

Eighty patients with major orthopaedic surgery from two clinics were included. One clinic included 40 patients receiving thromboprophylactic treatment with dabigatran 220 mg od and the other clinic 40 patients receiving rivaroxaban 10 mg od. Patients were included three days after surgery and the start of thromboprophylactic treatment. Exclusion criteria were previous malignancy, concomitant use of platelet aggregation inhibitors or other anticoagulants, renal failure (eGFR <30 mL/min), age < 18 years old and BMI >30 kg/m^2. Informed consent was obtained before participation in the study. Blood was collected at day 3 of prophylaxis, just before and 2 and 4 h after taking the drugs (t0, t2 and t4, respectively). Samples were centrifuged and plasma was conserved at −80 °C until measurement.

Using UPLC-MS/MS we found a large range of rivaroxaban peak and trough values (Fig. 1a). We measured a median 2 h peak level of 124.6 ng/mL (CI 16.9–200.3 ng/mL), a median 4 h peak level of 149 ng/mL (CI 107.8–209.1 ng/mL) and a median trough level of 17.1 ng/mL (CI 7.7–49.7 ng/mL).

A wide range of peak and trough values was also found for dabigatran (Fig. 1b). Using UPLC-MS/MS, we measured a median 2 h peak level of total dabigatran (including glucuronidated metabolites) of 81 ng/mL (CI 18.6–210 ng/mL), a median 4 h peak level of 81 ng/mL (CI 18.7–247 ng/mL) and a median trough value of 12.1 ng/mL (CI 2.8–48 ng/mL) (Fig. 1b).


Rivaroxaban Concentration UPLC-MS/MS
Rivaroxaban (ng/ml)
350
300
250
200
150
100
50
0
trough
peak
(2 hours after intake)
peak
(4 hours after intake)


Dabigatran Concentration UPLC-MS/MS
Dabigatran (ng/ml)
600
500
400
300
200
100
0
trough
peak
(2 hours after intake)
peak
(4 hours after intake)

Real-world variability in dabigatran levels in patients with atrial fibrillation: comment

J. DOUXFILS,* B. CHATELAIN,† J.-M. DOGNÉ* and F. MULLIER†

Department of Pharmacy, Namur Thrombosis and Hemostasis Center (NTHC), Namur Research Institute for LIfe Sciences (NARILIS), University of Namur, Namur; and †Hematology Laboratory, Namur Thrombosis and Hemostasis Center (NTHC), Namur Research Institute for LIfe Sciences (NARILIS), CHU DinantGodinne UcL Namur, Université Catholique de Louvain, Yvoir, Belgium

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See also Chan NC, Coppens M, Hirsh J, Ginsberg JS, Weitz JI, Vanassche T, Douketis JD, Schulman S, Eikelboom JW. Real-world variability in dabigatran levels in patients with atrial fibrillation. *J Thromb Haemost* 2015; **13**: 353–9 and Chan NC, Hirsh J, Ginsberg JS, Eikelboom JW. Real-world variability in dabigatran levels in patients with atrial fibrillation: reply. This issue, pp 1168–9.

We read with interest the recent prospective observational study by Chan *et al.*[1] The authors aimed to investigate the interpatient and intrapatient variabilities in dabigatran plasma levels with the 110 and 150 mg twice-daily dose regimens in 100 patients with atrial fibrillation (AF). They also assessed the effect of physicians' dose selection on plasma levels in the two different subgroups and explored whether a single trough measurement would identify patients with extreme plasma levels on subsequent visits (i.e. at 2, 4, and 6 months) [1]. They support the practice of selecting dabigatran dose based on clinical characteristics because it results in similar levels of drug exposure in patients given the 110 or the 150 mg twice-daily dose regimen. However, they do not support the concept that a single plasma level measurement with the Hemoclot Thrombin Inhibitor® (Hyphen BioMed, Neuville-sur-Oise, France) can be used to identify patients with consistently high or low plasma levels.

In their study, Chan *et al.* revealed an impressive 17-fold variation in plasma concentrations (from ≤ 30 to 510 ng mL^{-1}) at trough (i.e. at a median of 13.3 ± 4.7 h after the last drug intake) with an interpatient geometric coefficient of variation (gCV) of 63.8%. This variation was equally

Furthermore, it would have been very interesting to obtain data on clinical outcomes as well as on the reason (s) for the six treatment cessations. However, and interestingly, Chan *et al.* found that dose adaptation based on the patients' clinical characteristics (mainly age and renal clearance) results in similar median trough and peak level. This confirms the choice of the different regulatory agencies to adopt the lower dose for patients with factors for drug accumulation [4–6].

In conclusion, these limitations clearly highlight that the conclusions of Chan *et al.* probably need to be toned down. As previously mentioned, several criteria should be taken into consideration when considering proper drug monitoring: a high (i) intraindividual and (ii) interindividual variability in drug level, both justifying identification of the optimal dose for each patient at the start of treatment; (iii) a low variability and good reproducibility in the assay method; (iv) a correlation between drug level and clinical event; and (v) the demonstration of the value of the therapeutic drug monitoring [7]. Up to now, the high intraindividual and interindividual variabilities in drug level are clearly demonstrated [8], and the results from Chan *et al.* support this observation. Techniques for

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Plasma levels of direct oral anticoagulants in real life patients with atrial fibrillation: Results observed in four anticoagulation clinics

Sophie Testa [a,*], Armando Tripodi [b], Cristina Legnani [c], Vittorio Pengo [d], Rosanna Abbate [e], Claudia Dellanoce [a], Paolo Carraro [f], Luisa Salomone [c], Rita Paniccia [e], Oriana Paoletti [a], Daniela Poli [f], Gualtiero Palareti [g], for the START-Laboratory Register

[a] Haemostasis and Thrombosis Center, Department of Laboratory Medicine, AO Istituti Ospitalieri, Cremona, Italy
[b] Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Clinical Sciences and Community Health, Università degli Studi di Milano, IRCCS Cà Granda Maggiore Hospital Foundation, Milano, Italy
[c] Angiology and Blood Coagulation, University Hospital of Bologna, Italy
[d] Department of Cardiothoracic and Vascular Sciences, University Hospital of Padua, Padua, Italy
[e] Thrombosis Centre, Department of Heart and Vessels, University Hospital of Florence, Florence, Italy
[f] Department of Laboratory Medicine, ULSS 16 and University-Hospital of Padova
[g] Cardiovascular Diseases, University of Bologna, Bologna, Italy

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ABSTRACT

Introduction: Direct oral anticoagulant (DOAC) intra- and inter-individual variability was previously reported, but its magnitude is still considered negligible for patient management.

Objective: To evaluate inter- and intra-individual variability in real-world atrial fibrillation patients on dabigatran, rivaroxaban or apixaban in four Italian anticoagulation clinics and to assess the correlation between DOAC plasma concentration and creatinine-clearance (CrCl).

Materials and Methods: A total of 330 consecutive patients were enrolled, of which 160 were on dabigatran (70 and 90 taking 150 mg or 110 mg twice-daily, respectively), 71 on rivaroxaban (37 and 34 taking 20 mg or 15 mg once-daily) and 99 on apixaban (73 and 26 taking 5 mg or 2.5 mg twice-daily). Blood was taken at trough and peak within the first month (15–25 days) of treatment. Diluted-thrombin-time (dTT) calibrated for dabigatran and anti-FXa calibrated for rivaroxaban or apixaban was performed.

Results: Mean inter-individual variability expressed as overall CV values for all drugs was lower at peak (CV = 46%) than at trough (CV = 63%). Mean CV% intra-individual variability was 36.6% at trough and 34.0% at peak. Correlation with CrCl was poor for all drugs and only dabigatran at trough showed a significant correlation.

Conclusion: This multicenter study confirms high DOAC inter-individual variability that cannot be explained by the rate of renal clearance to which the three DOAC were subjected since the correlation with CrCl was relatively poor. This poor correlation suggests caution in using CrCl as the sole laboratory parameter to indirectly evaluate residual circulating DOAC.

In this study, we report results on inter-individual variability assessed in patients with atrial fibrillation treated with dabigatran, rivaroxaban or apixaban in four Italian anticoagulation clinics. In one clinic we also evaluated intra-individual variability by measuring the DOAC anticoagulant effect over three consecutive time points. Finally, we evaluated the correlation between DOAC anticoagulant levels measured with specific coagulation tests and creatinine clearance.

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

After giving their informed consent, a total of 330 consecutive patients seen at the anticoagulation clinics from Jan. 1st 2014 to Dec. 31st 2014 were enrolled in the study providing they had been treated with DOAC for at least one week and were available to attend the clinic for blood sampling at the specified time points (see below). Before starting anticoagulation, liver function was assessed by means of liver enzymes (aspartate aminotransferase-AST and Alanine aminotransferase-ALT). A total of 160 patients were on dabigatran (70 and 90 taking 150 mg or 110 mg twice-daily, respectively), 71 on rivaroxaban (37 and 34 taking 20 mg or 15 mg once-daily, respectively), and 99 on apixaban (73 and 26 taking 5 mg or 2.5 mg twice-daily, respectively). Patients were evaluated at enrolment and the type and dosage of drug prescribed at the discretion of the attending physician based on clinical characteristics. Patients were followed in the first month (15–25 days) of treatment when trough and peak blood samples were taken. The trough sample was obtained at 12 h from the last dose intake for dabigatran and apixaban, and at 24 h for rivaroxaban. The peak sample was obtained at 2 h from ingestion for all drugs and 2 h after through sample. Concomitant food intake was ensured in patients on rivaroxaban. Plasma samples were collected in vacuum plastic tubes

Table 3

Mean (min–max) dabigatran levels (ng/mL) determined with the diluted thrombin time and CV% in patients from different clinics. The total number of observations was 320.

Dabigatran Dose (mg)	Clinic	Peak (ng/ml) Mean (min–max)	CV (%)	Trough (ng/ml) Mean (min–max)	CV (%)
110	A	211(31–595)	71	115(30–324)	64
	B	172(38–394)	56	92(36–208)	64
	C	155(50–334)	64	60(30–109)	36
	D	224(46–651)	65	107(14–386)	72
150	A	187(77–427)	45	89(30–175)	42
	B	191(104–435)	51	54(16–130)	70
	C	222(112–447)	46	95(24–232)	52
	D	240(43–538)	56	125(33–494)	92

Table 4

Mean (min–max) apixaban levels (ng/mL) determined with the anti-FXa assay and CV% in patients from different clinics. The total number of observations was 198. ND, not determined.

Apixaban dose (mg)	Clinic	Peak (ng/ml) Mean (min–max)	CV (%)	Trough(ng/ml) Mean (min–max)	CV (%)
5	A	242 (106–374)	31	110 (44–255)	47
	B	227 (102–416)	35	127 (42–283)	45
	C	ND	ND	91 (57–196)	49
	D	133 (102–164)	33	125 (92–190)	29
2.5	A	161 (109–288)	37	91 (57–196)	49
	B	166 (55–300)	42	85 (38–248)	68
	C	ND	ND	ND	ND
	D	249 (212–287)	21	61 (26–103)	44

Table 5

Mean (min–max) rivaroxaban levels (ng/mL) determined with the anti-factor Xa assay and CV% in patients from different clinics. The total number of observations was 142. ND, not determined.

Rivaroxaban Dose (mg)	Clinic	Peak (ng/ml) Mean (min–max)	CV (%)	Trough (ng/ml) Mean (min–max)	CV (%)
20	A	247 (61–449)	49	39 (16–74)	40
	B	229 (65–370)	37	41 (16–106)	67
	C	231 (138–341)	32	43 (5–119)	103
	D	ND	ND	ND	ND
15	A	190 (77–355)	50	25 (17–49)	32
	B	229 (149–365)	52	26 (19–34)	30
	C	205 (85–393)	46	32 (0–88)	79
	D	ND	ND	ND	ND

DOAC inter-individual variability has been inconsistently evaluated in published reports and guidelines, and differently commented as being either low or high [5–14]. Furthermore, few studies investigated all the DOAC currently licensed for treatment of patients with atrial fibrillation. Our multicenter study, investigating a relatively high number of patients, shows a relatively high inter-individual variability for the three DOAC in patients treated with different dosages. On average, the drug concentration levels varied more than 20-times among the patients for dabigatran, nearly 15-times for rivaroxaban and 7-times for apixaban. This variability, observed in real life patients, compares favorably with that already reported for dabigatran [11,12,18] or rivaroxaban [11] for results stemming from the clinical trials. Variability was similarly high if assessed within each clinic or evaluated as a whole, suggesting that it cannot be accounted for by the variability of the different laboratory assays. Furthermore, variability was considerably higher in patients treated with the lowest dose of DOAC. This observation may have important practical implications since on the one hand it supports the strategy of assigning lower doses to patients with specific clinical characteristics, while on the other hand it shows that the same patient may reveal a greater variability with respect to anticoagulation, regardless of the clinical criteria adopted to assign drug dosage. It is intriguing that

patients taking the same oral dose of single DOAC may present with highly variable plasma concentrations. Different DOAC metabolism patterns in individual patients may be the likely explanation for this variability. Our data show a greater variability at trough than at peak levels. Although the reason for this is not known, drug absorption is more likely predictable than elimination, since the latter is probably affected by a greater number of variables than the former [19].

A crucial role in metabolism is assigned to the kidney's ability to clear DOAC from circulation even though this is not the only mechanism for elimination. Although dabigatran, rivaroxaban and apixaban show different rates of renal excretion (nearly 80%, 35% and 27%, respectively), it can expected that their plasma concentrations at steady state do correlate with calculated creatinine clearance. No formal assessment of this correlation has however yet been reported. Our results show that while rivaroxaban or apixaban plasma concentrations are not correlated with creatinine clearance at trough, the relatively high inter-individual dabigatran variability can only in part be accounted for by a degree of inverse correlation between its plasma concentrations and creatinine clearance. In line with current recommendations, patients with creatinine clearance lower than 30 mL/min were not prescribed dabigatran in this study. Despite this, a certain degree of inverse

correlation between dabigatran plasma concentrations and creatinine clearance was observed, supporting the idea that circulating dabigatran is regulated by renal function even at near-normal creatinine clearance levels and suggesting that prescribing dabigatran to patients at the lower end of normal range for creatinine clearance may prompt excessive residual plasma concentrations. Indeed, post-hoc analysis of RE-LY trial [17] showed that trough plasma concentrations of dabigatran were associated far more with the relative risk of hemorrhage than with the relative risk of ischemic stroke, suggesting that the real concern for dabigatran might be safety rather than efficacy.

The results of our study may have practical implications for patient management.

First, there is an urgent need to establish drug-specific cut off levels to flag concern about the occurrence of clinical (hemorrhage or thrombosis) events in treated patients. It is reasonable to assume that given the relatively high inter-individual variability, it will be difficult to set

precise cut off values applicable to the general population of treated patients. Information on anticoagulant levels from real world patients are needed to increase the scanty data presently available [18].

Secondly, there is a need to establish cut off drug levels to be applied to patients receiving treatment after stopping anticoagulation in the event of surgery or invasive procedures. It is currently thought that owing to the relatively short DOAC half-life, residual circulating drug levels are reasonably low when anticoagulation is discontinued two days before surgery, provided renal function is normal. To this end, it is recommended renal function be assessed by measuring serum creatinine and calculating clearance by means of the Cockroft Gault formula. The safety of such an assessment is based on the assumption that DOAC plasma concentrations are correlated with creatinine clearance. Our study shows that this assumption does not hold true, particularly for rivaroxaban and apixaban (i.e., the anti FXa drugs that show a minor dependency on renal function), and may therefore lead to misleading interpretation of anticoagulant status in individual patients who may thus be at risk of bleeding during surgery. It is our view that, instead of proposing measurement of indirect parameters such as renal and liver function, direct measurement of the effect of drug anticoagulation is much safer and should be performed just before surgery or invasive procedure. Consequently, specific tests should be available in real time

in each hospital to guide the management of patients in the different clinical conditions. The lower limits of drug detection, in our experience through the methods used, is associated to the nearly complete absence of residual anticoagulant activity. While the cost incurred by measuring the anticoagulant effect of DOAC is probably slightly higher than that of measuring serum creatinine, the strategy would make patient management much safer.

Intra-individual variability is an interesting parameter since it provides information as to whether single DOAC time-point measurements may or may not characterize individual patients. If DOAC are metabolized at the same rate by individual patients, their concentrations at steady-state should be fairly constant over time and this might be taken as a characteristic feature for each patient. As shown by our study and elsewhere in the literature [12] this does not hold true for dabigatran where intra-individual variability is considerably higher (average CV, 55%). Rivaroxaban and apixaban showed, respectively, intermediate (average CV, 33%) and relatively small (average CV, 19%) variability. Accordingly, single DOAC measurement can hardly provide

variability. Accordingly, single DOAC measurement can hardly provide an estimation of the average anticoagulation achieved in individual patients. It should be realized that estimating the average anticoagulation achieved with DOAC is difficult as it depends on the adsorption, metabolism and clearance of the drug that may vary over time. However, it can be assumed that when DOAC measurement is performed after drug discontinuation and soon before surgery or invasive procedures even a single DOAC measurement may be useful to see whether there are still excess circulating levels that may increase the risk of bleeding.

(CV = 46%). The relatively high inter-individual variability cannot be accounted for by the rate of renal clearance the three DOAC are (although to a different extent) subjected to since the correlation of their plasma concentrations and creatinine clearance was relatively poor. The possible influence on this poor correlation brought about by the hepatic function and other confounding variables such as gender and body mass index has not been formally assessed. However, owing

to the real nature of this study, investigating relatively large numbers of patients, it is unlikely that the above effects (if any) can affect the conclusion that the poor observed correlation argues against using creatinine clearance as the sole laboratory parameter to assess whether or not residual circulating DOAC is sufficiently low to ensure low risk of bleeding during surgery and/or invasive procedures. Intra-individual variability is also relatively high for dabigatran, suggesting that single DOAC measurement cannot be used to estimate the level of anticoagulation reached at the steady-state in treated patients. All in all, the above observations may have important practical implications for the management of patients treated with DOAC.

CHARTING THE FUTURE TOGETHER

The NHLBI Strategic Vision

U.S. Department of Health and Human Services
National Institutes of Health
National Heart, Lung, and Blood Institute

Director's Message

The National Heart, Lung, and Blood Institute (NHLBI) began the Strategic Visioning process with a bold challenge: Imagine a world where we are able to prevent the burden of cardiovascular, lung, and blood diseases; a world where we are able to capture the promise of personalized precision medicine, with each person receiving the right treatment, tailored to his or her needs, at the right time.

Through a dynamic and iterative process, we engaged diverse stakeholders from across the United States and around the globe **(figure 1)** and received an unprecedented number of ideas that have informed the development of this Strategic Vision. The research priorities in this Strategic Vision will enable us to accelerate our journey toward scientific and health advances over the next decade.

This is an exciting time for behavioral and biomedical research. The convergence of innovations in areas such as computational biology, data science, bioengineering, and high-throughput "omic" technologies holds promise for

Figure 1: Global Participation in Strategic Visioning

■ Participants came from 50 states and 42 countries. Registered Users on the Strategic Visioning Forum: 4,450; Ideas Supported: 42,000

STRATEGIC VISION OBJECTIVE 5:
Develop and optimize novel diagnostic and therapeutic strategies to prevent, treat, and cure HLBS diseases

Objective 5 Compelling Questions	
5.CQ.18	Is targeted manipulation of epigenetic modifications (distinct from global suppression of histone acetylation or DNA methylation) a useful strategy for therapeutic intervention in chronic cardiopulmonary or blood diseases?
5.CQ.19	With increasing use of direct-acting oral anticoagulants for stroke prevention in atrial fibrillation and treatment of venous thromboembolism, what is the role of laboratory monitoring, and can the use of new technologies help better define those at risk of bleeding or thrombosis with use of direct-acting oral anticoagulants or warfarin?