BioMed Research International

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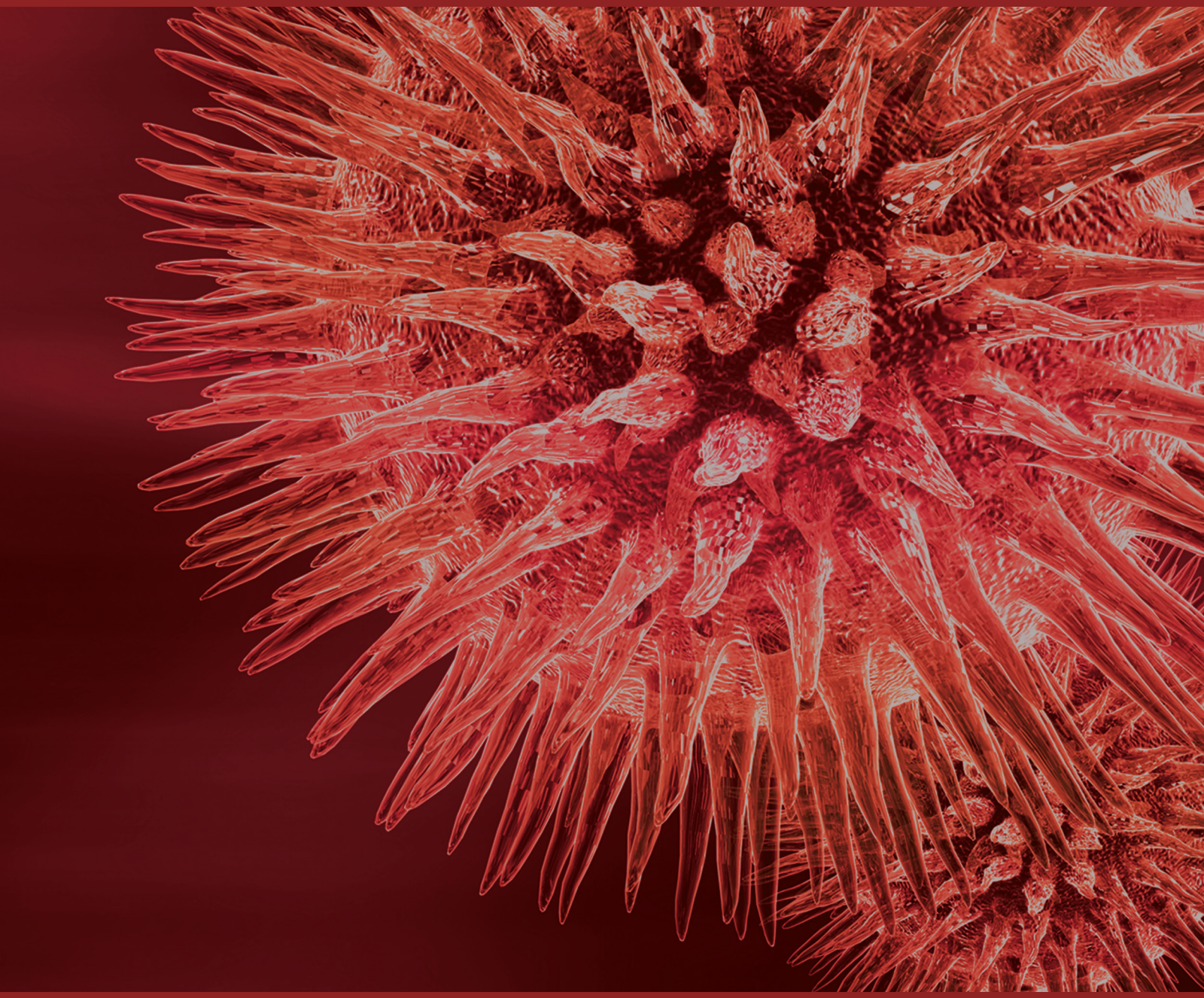
Guest Editors: Helen Mani, Jonathan Douxfils, and Jack Ansell



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Contents

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Editorial

Target-Specific Oral Anticoagulants—New Approaches in the Field of Oral Anticoagulation

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Direct oral anticoagulants targeting specific coagulation factors have been introduced as alternatives to conventional anticoagulants for both prophylactic and therapeutic indications. They have succeeded in overcoming the limitations of vitamin K antagonists.

Although the favourable efficacy and side-effect profiles compared to vitamin K antagonists is proven, there is significant concern about management of hemorrhage under direct oral anticoagulants. There is no specific antidote or method for reversing the antithrombotic effect of these drugs available, at present. Most of the management of hemorrhage is based on expert opinion and case reviews. Specific treatment regimens are needed to avoid consequences of acute hemorrhage while patients are on anticoagulation. Therefore, the focus of this special issue is to define a management system for oral anticoagulants in the peri- and postoperative setting.

Moreover, the long-term safety in clinical routine life of direct oral anticoagulants is not well-known. One study performed in clinical practice included a small cohort of patients with acute cerebral hemorrhage starting dabigatran etexilate for secondary stroke prevention to investigate efficacy and safety of this drug for one year. Another point which has to be considered for treatment with direct oral anticoagulants depends on the cost-effectiveness. In this issue a comparison of the cost-utility analysis between edoxaban, dabigatran, rivaroxaban, and apixaban in German population is described.

Due to the heterogeneous mode of action and pharmacokinetic profile, each direct oral anticoagulant will vary in its effects on laboratory assays, and to avoid mismanagement clinicians are placed in a difficult position. One review published in this issue provides an overview of current knowledge regarding appropriate coagulation assays to measure the pharmacodynamics of the direct oral anticoagulants.

For this special issue we have also invited investigators to contribute original research articles that continue the development of strategies performed by the direct oral anticoagulants. The synthesis of a novel compound, trehalose octasulfate, is presented that employs a multitarget strategy to modulate the activity of various components of thrombus formation.

Good quality observational studies and data analyses are often sufficient to provide a guide for better treatment with drugs as the new anticoagulants. Different models of anticoagulation treatment might have different impacts on patients' satisfaction. However, more investigation is needed on the management of the target-specific oral anticoagulants.

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Helen Mani
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Review Article

Non-VKA Oral Anticoagulants: Accurate Measurement of Plasma Drug Concentrations

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Non-VKA oral anticoagulants (NOACs) have now widely reached the lucrative market of anticoagulation. While the marketing authorization holders claimed that no routine monitoring is required and that these compounds can be given at fixed doses, several evidences arisen from the literature tend to demonstrate the opposite. New data suggests that an assessment of the response at the individual level could improve the benefit-risk ratio of at least dabigatran. Information regarding the association of rivaroxaban and apixaban exposure and the bleeding risk is available in the drug approval package on the FDA website. These reviews suggest that accumulation of these compounds increases the risk of experiencing a bleeding complication. Therefore, in certain patient populations such as patients with acute or chronic renal impairment or with multiple drug interactions, measurement of drug exposure may be useful to ensure an optimal treatment response. More specific circumstances such as patients experiencing a haemorrhagic or thromboembolic event during the treatment duration, patients who require urgent surgery or an invasive procedure, or patient with a suspected overdose could benefit from such a measurement. This paper aims at providing guidance on how to best estimate the intensity of anticoagulation using laboratory assays in daily practice.

1. Introduction

The fact that non-VKA oral anticoagulants (NOACs) do not require frequent biological monitoring led the products' manufacturers to put forward this advantage as one of their main selling points. However, the "one dose fits to all" policy suffers from several criticisms both on the scientific literature side [1, 2] and on the regulatory side. Effectively, dose adaptations are proposed by the different regulatory agencies for dabigatran etexilate, rivaroxaban, and apixaban regarding the degree of renal insufficiency [3–8]. Moreover, even if they are less frequent compared with vitamin K antagonists (VKA), several drug-drug interactions have been listed [9], such as concomitant treatment with drug affecting the glycoprotein-P (P-gp) and/or CYP3A4/5. These interactions also led to dose adaptation or to a nonrecommendation of concomitant administration [3–8]. In addition, several conditions such as renal and hepatic function, age, gender, and body weight impact on the exposure [10–12]. It is acknowledged that some of these factors do not or minimally alter pharmacokinetics of these agents if taken independently. However, taken concomitantly, they may have clinical implications if drugs are accumulating and/or are less easily eliminated or metabolized.

Different authors have already pointed out the fact that one should not abolish the opportunity to further improve

the benefit-risk balance of NOACs. This could require the use of occasional monitoring to assess the response at the individual level to ensure that the optimal dose is delivered, taking into account the patient characteristics [1, 2, 13, 14]. Accumulative data of case reports from patients with major bleeding and regulatory documents associated supratherapeutic level of anticoagulants and bleeding outcomes [15–18]. This suggests that certain populations, especially those with impaired renal function and the elderly, may accumulate the drug to such extent that they put them at an increasing risk of bleeding event [19–23].

Thus, while the absence of monitoring was one of the main selling arguments in favour of NOACs, it appears that occasional monitoring could be implemented in routine practice to ensure a safe and efficient treatment. However, although the gold standard to measure plasma drug concentrations is mass-spectrometry (LC-MS/MS), several limitations, that are, the availability, the laboratory experience, the local validation, and the turn-around time in emergency situations, restrict its implementation in the clinical setting. Hence, less restrictive coagulation tests have been tested to assess the pharmacodynamics of NOACs.

This paper aims at providing an overview of our current knowledge regarding how to accurately estimate plasma drug concentrations or the intensity of anticoagulation using conventional or more specific coagulation assays.

2. Rationale for Dose Tailoring NOACs in Specific Situations

This question is subject to a debate in the literature. Some authors argue that the anticoagulant effect of a fixed dose of all of these new agents is highly predictable and claim that there is no evidence that (re-)thrombosis or bleeding correlates with any measured biologic activity or drug concentrations in plasma [24]. In addition, there is not yet evidence that an individualized regimen with occasional monitoring and dose tailoring is safer or more effective than a standard dose regimen. Others argue that, in "real life," opposing to the clinical trial setting, there are abundant factors, such as body weight, age, renal and liver function, concomitant interacting drug and genetic variants, and probably many other unknown causes that influence the plasma levels and the anticoagulant effect of all of these agents. The most conservative approach would suggest that fine tunings of the dosage to the individual needs might be preferable [13].

Since these claims, information has been released in the literature regarding dabigatran etexilate, the first NOAC that reached the market and therefore the most studied to date. Thus, recent analyses of the RE-LY trial, evaluating dabigatran etexilate versus warfarin in patients with nonvalvular atrial fibrillation, revealed a high interindividual variability. Genetic variants were investigated to explain a part of the large interindividual variability and it was demonstrated that the rs2244613 intronic SNP to the esterase gene *CES1* was associated with a decreased trough concentrations and a decreased risk of bleeding [15]. In addition, a reanalysis of the RE-LY trial mentions that compared with the median C_{trough} concentration of 88 ng/mL, the rate of major bleeding doubled at a concentration of 210 ng/mL [16]. A higher residual C_{trough} plasma concentration also reduced the risk of stroke but to a lesser extent compared with the increase in major bleeding. The authors concluded that, in patients with the highest risk for events, such as the very elderly and/or those with poor renal function, an adjustment of dabigatran dose to optimize exposure might improve the benefit-risk balance.

For rivaroxaban and apixaban, data on drug levels associated with therapeutic or harmful ranges are currently lacking in the literature, but information on the risk of bleeding and recurrence of thrombosis associated with plasma drug levels can be found in the FDA-Clinical Pharmacology and Biopharmaceutics Review(s).

For patients treated with rivaroxaban 20 mg *od* for the treatment of acute DVT, the 90th percentile of rivaroxaban plasma concentrations measured at trough (24 hours after the previous dose) was about 249 ng/mL [12]. Similar concentrations were found in the setting of NVAF. The FDA-Clinical Pharmacology Biopharmaceutics Review(s) of Xarelto also mentions that a two-fold increase in exposure due to intrinsic and extrinsic factors will increase the risk of major bleeding by 50% [17]. For apixaban, no data regarding the plasma trough or max level versus bleeding or recurrence of thrombosis are available. However, it is mentioned that the risk of major bleeding increased with the exposure to apixaban, measured as AUC_{SS} [10, 18].

Of course, the overall performance of NOACs in the large phase-III clinical trials is noninferior or better, compared to INR-adjusted warfarin. However, we should keep in mind that these were carefully selected patients to begin with while bleeding and other side effects were still encountered at significant percentages [25].

3. In Which Patients and When Should We Measure Plasma Concentrations or Estimate the Intensity of Anticoagulation?

There are several situations in which it may be of assistance to assess the anticoagulant effects of NOAC [26]. In addition to the above-mentioned reasons, biological monitoring would also be valuable in acute situations such as the following [2, 14, 16, 27–29]: recurrence of thrombosis or bleeding, before surgery or invasive procedure, before fibrinolytic therapy of acute ischaemic stroke, in case of bridging therapy, or also in case of cardioversion.

Moreover, it could be useful in patients with risk factors that could lead to an accumulation or to insufficient levels of NOACs such as in patients with renal or hepatic impairment, in polymedicated patients with multiple drug-drug interactions, in patients with genetic variants interfering on the metabolic pathways (e.g., rs2244613 intronic SNP to the esterase gene *CES1* for dabigatran: no mutations linked to changes in the pharmacokinetics are known for the other NOACs at that time), or in patients with extreme body weight. These situations are summarized in Table 1.

Table 1: Summary of patients/situations that could benefit from point measurement of plasma concentrations.

(i) Bleeding or recurrence of thrombosis
(ii) Before invasive procedure or surgery
(iii) In patient with potential drug-drug interactions
(iv) In patients with genetic mutations (e.g., rs2244613 minor allele carriers for dabigatran etexilate—no mutations are currently known for the other NOACs)
(v) In patients with extreme body weight (<50 kg or >110 kg)
(vi) In elderly patients (>75 years of age)
(vii) In a case of accumulating interfering factors

The collection of information is mandatory when prescribing a biological test for patients treated with NOACs. Therefore, information on the age, the weight and the renal function of the patient, the concomitant therapies, the dosage of the NOAC, the number of administration(s) per day, the delay since the last administration, and the indication should be collected in order to obtain sufficient materials to appropriately interpret the results of such tests.

4. How to Accurately Estimate Plasma Drug Concentrations?

4.1. Sample Acquisition. Sample acquisition is of a great importance since it was proven that each component of the specimen collection system (needle gauge, composition of the collecting tube, and concentration of sodium citrate) might potentially impact the results for coagulation testing. Blood should be taken with 21-gauge needles at the antecubital veins to avoid activation of the coagulation due to a slower rate of blood flow (with >25-gauge needles) or haemolysis of the sample due to turbulence of flow through the needle (with <16-gauge needle).

Samples for plasma-based haemostasis testing should be anticoagulated with sodium citrate. The World Health Organization (WHO) and Clinical Laboratory Standards Institute (CLSI) recommend 105 to 109 mmol/L (3.13% to 3.2%) of the dehydrate form of trisodium citrate, buffered or nonbuffered as the anticoagulant of choice for haemostasis testing. The blood anticoagulant ratio is understandably a source of variability and collection containers that are underfilled contain proportionally more sodium citrate per volume of plasma leading to longer clotting times. As for routine coagulation testing, cold storage of citrate whole blood should be avoided since it may lead to platelet activation, activation of FVII, and significant time-dependent loss of both FVII and VWF [30].

4.2. Specimen Processing. The centrifugation should take place as soon as possible after the blood sampling during no less than 15 minutes at 1500 ×g. Double centrifugation can be performed to ensure the plasma is platelet-poor. The use of relative centrifugal forces (RCFs) greater than 1500 ×g is not recommended as this may induce platelet activation and red blood cell lysis.

4.3. Stability and Storage of Plasma Samples. There is currently no recommendation based on samples stability and storage of plasma samples from patients treated with NOACs. Thus, laboratories may choose to perform their own studies and validate sample stability. However, assays such as the aPTT should be performed within 4 hours if the sample is maintained at room temperature. Therefore, as a conservative approach we propose that each coagulation testing on NOACs plasma samples should be performed within 4 hours if maintained at room temperature. For long-term storage, samples should remain at −20°C for no more than 2 weeks. If longer storage periods are required, samples can be maintained at −70°C or colder.

4.4. Assessment of NOACs in Plasma. Different routine coagulation tests could be used to estimate the intensity of anticoagulation in patients treated with NOACs. More specific assays are used to accurately estimate plasma drug concentrations using specific calibrators and controls. All of these tests present advantages and drawbacks that should be discussed in order to implement, in a clinical setting, the rational of using one or more coagulation tests to help clinicians in their daily practise. Table 2 summarizes the coagulation assays that could be used to estimate either the plasmatic concentration of NOACs or the relative intensity of anticoagulation in specific situations.

4.4.1. Global Coagulation Tests

(1) Dabigatran: Activated Partial Thromboplastin Time (aPTT). The recent recommendations of the *Subcommittee of Control of Anticoagulation of the Scientific and Standardisation Committee* of the *International Society on Thrombosis and Haemostasis* (ISTH) mention that aPTT using most available reagents can be used to determine the relative intensity of anticoagulation due to dabigatran. However, this Subcommittee states that aPTT should not be used to quantify the plasma drug concentration and that further studies are required to determine the relative sensitivity of aPTT reagents to dabigatran. They add that each laboratory should be aware of the sensitivity of their aPTT assays to dabigatran and this can be achieved using commercially available plasma calibrators [31]. However, it is unknown if these specific dabigatran calibrators, used out of their specific platform context, are reliable calibrators that could reflect accurately the impact of

Table 2: Advantages and drawbacks of coagulation tests that could be used to estimate plasma concentrations of NOACs or to estimate the relative intensity of anticoagulation.

	Molecule(s)	Utility	Laboratory experience	Availability	Sensitivity[†]/ specificity	Dependence of the reagent	Cut-off for a risk of bleeding (unit(s) of expression)
aPTT	Dabigatran	Limited: poorly reflects the intensity of anticoagulation	Not required	24/7—all laboratories	±100 ng/mL/no	Yes	Yes: depends on the indication and the reagent (ratio and seconds)
TT	Dabigatran	Limited: only to exclude the presence of dabigatran. Useful in the perioperative setting	Not required	24/7—all laboratories	Too sensitive[‡]/no	Yes	Not established
dTT	Dabigatran	Proven: accurately estimates the plasma concentrations—results expressed in ng/mL	Required: requirement of calibrators and controls	Requirement of trained personnel—only in specialized laboratories	±10 ng/mL/no	No	Yes: depends on the indication (ng/mL)
ECT	Dabigatran	Limited: standardisation and validation required	Required: interlot variability probably requiring calibrators and controls	Requirement of trained personnel—only in specialized laboratories	±15 ng/mL/no	Probably not but an interlot variability has been reported	Yes: depends on the indication (ratio and seconds)
ECA	Dabigatran	Proven: accurately estimates the plasma concentrations—results expressed in ng/mL	Required: requirement of calibrators and controls	Requirement of trained personnel—only in specialized laboratories	±10 ng/mL/no	No	Yes: depends on the indication (ng/mL)
PT	Rivaroxaban	Limited: poorly reflects the intensity of anticoagulation	Not required	24/7—all laboratories	from ±100 to >500 ng/mL (depending on the reagent)/no	Yes	Not established

Table 2: Continued.

	Molecule(s)	Utility	Laboratory experience	Availability	Sensitivity[†]/ specificity	Dependence of the reagent	Cut-off for a risk of bleeding (unit(s) of expression)
Chromogenic anti-Xa assays	Rivaroxaban/ Apixaban	Proven: accurately estimates the plasma concentrations—results expressed in ng/mL	Required: requirement of calibrators and controls	Requirement of trained personnel—only in specialized laboratories	±10 ng/mL/yes-no (depending on the anti-Xa assay)	No	Not established
DRVV-T	Dabigatran/ Rivaroxaban/ Apixaban	Partially proven: confirmation should be done in plasma samples from patients treated with dabigatran and apixaban	Not required	Only in specialized laboratories	±100 to 200 ng/mL (depending on the reagent and the molecule)/no	Yes, but less importantly than for PT or aPTT	Not established

aPTT: activated partial thromboplastin time; DRVV-T: dilute Russell's viper venom time; dTT: dilute thrombin time; ECA: ecarin chromogenic assay; ECT: ecarin clotting time; PT: prothrombin time; TT: thrombin time.

[†]Sensitivity is defined as the concentration required to double or to halve the clotting time (for chronometric assays) or the OD/min (for chromogenic assays), respectively.

[‡]Plasma concentrations of dabigatran >30 ng/mL frequently excess the maximal time measured on most coagulometers.

dabigatran in plasma from patient's sample. Effectively, the aPTT is affected by numerous preanalytical and biological variables that could restrict the use of these calibrators since we ignore how these artificially spiked plasmas are prepared.

Thus, aPTT could provide guidance for the assessment of bleeding risk. For example, it is stated in the EU-Summary of Product Characteristics (EU-SmPC) that when dabigatran was used for the prevention of stroke in NVAF with a *bid* dosing regimen, an aPTT ratio greater than 2 × ULN (or an aPTT prolongation of about 80 seconds) at trough (10–16 h after the previous dose) reflected the 90th percentile of observations (i.e., 200 ng/mL at C_{trough}) and is considered to be associated with a higher risk of bleeding [4]. While the EU-SmPC attempts to provide guidance on coagulation tests value associated with a bleeding risk, studies revealed that the interreagent variability prevents using an aPTT of about 80 seconds as reflecting plasma dabigatran concentration of 200 ng/mL [32] (Figure 1). For example, in one of previous *in vitro* studies, we found aPTT from 48.6 to 62.5 seconds for the concentration of 200 ng/mL, depending on the reagent. Similar observations have been demonstrated for the threshold proposed in prevention of venous thromboembolism regarding the bleeding risk [32]. Moreover, the reagent/instruments combination can also influence the sensitivity, increasing further the possibility to standardize the results among different laboratories [33]. Therefore, laboratories should be aware about the sensitivity of their aPTT reagents (respective to the instrument on which the test is performed) towards dabigatran. More recently, aPTT values from 50–90 seconds with dabigatran concentrations >200 ng/mL were found in patient's plasmas, and aPTT results could be as high as 75 seconds with only 50 ng/mL dabigatran in plasma [34–37].

Thus, aPTT is a global assay that does not accurately reflect plasma dabigatran concentrations, especially at higher values. It has limited sensitivity depending on the reagent and is not suitable for precise quantification of the anticoagulant effect for several reasons. First, the aPTT is affected by preanalytical (inappropriate collection, handling, and/or storage) and biological variables (lupus anticoagulant, hereditary or acquired factor deficiencies, hepatic insufficiency, vitamin K deficiency, disseminated intravascular coagulation, increased risk of thromboembolic events, hyperthyroidism, patients with diabetes, cancer, or myocardial infarction, and in pregnant women) [38, 39]. Secondly, a prolonged aPTT is not strongly predictive of haemorrhage and patients may experience bleeding while displaying a normal aPTT [39–41]. Finally, the dose-response is not linear, precluding the possibility to differentiate minor versus major overdoses (Figure 1). In definitive, we should rely on aPTT only when other more specific coagulation assays are not available. On the other hand, the main advantage of aPTT is that it is widely available and does not require specific laboratory experience. Importantly, this test could be the only one available to assess dabigatran therapy in nonexperienced hospital but each laboratory should know the sensitivity of its own reagent and deal with the several variables if aPTT is used in this context.

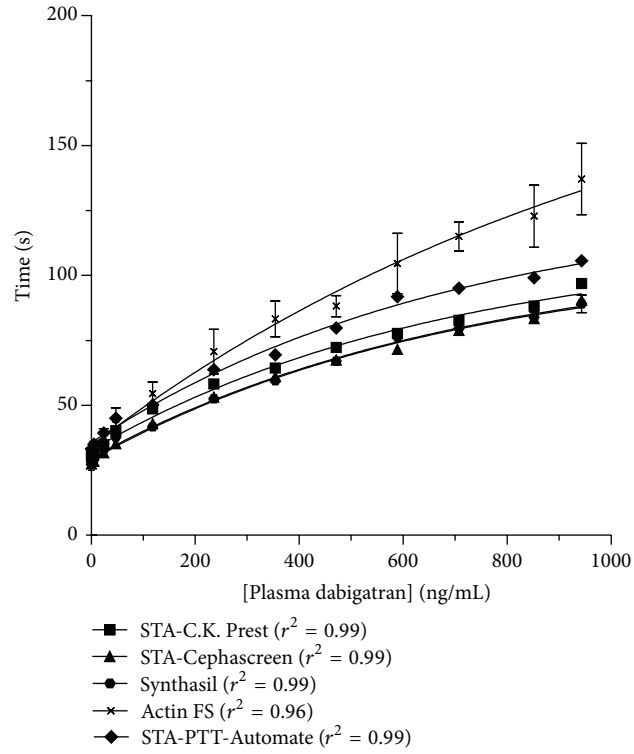

Figure 1: Impact of dabigatran on activated partial thromboplastin time (aPTT). There is a concentration-dependent prolongation of aPTT with a plateau at higher concentrations. The sensitivity depends on the reagent (*adapted from* Douxfils et al. [32]).

(2) Rivaroxaban: Prothrombin Time (PT). The *Subcommittee of Control of Anticoagulation of the Scientific and Standardization Committee* of the ISTH mentions that PT, with a sensitive reagent, can be used to determine the relative intensity of anticoagulation in emergency situations when required but should not be used to quantify drug plasma concentrations [31]. Effectively, initial pharmacokinetic studies sponsored by the drug manufacturer revealed that PT could be a useful assay to assess the pharmacodynamics of rivaroxaban due to a close linear correlation between PT and plasma drug concentrations [42, 43]. However, the investigators of these PK studies already pointed out that rivaroxaban measurement should not be translated to INR values since INR was developed to normalize PT in patients treated by VKA using the International Sensitivity Index (ISI) [42, 43]. Recently, a proposal for a specific $\text{ISI}_{\text{rivaroxaban}}$ has been investigated [44]. This method requires further investigations but could probably provide reliable standardization of the results obtained with the different thromboplastins [45].

In phase-II studies assessing the PK and PD of rivaroxaban, only two thromboplastins were assessed, that is, STA-Neoplastin and Innovin, a rabbit-brain and human recombinant thromboplastin, respectively [42]. These results already showed considerable interreagent variability in terms of sensitivity [42]. Therefore, *in vitro* studies aiming at investigating the between reagent variability have been proposed [46–49]. A large variability was reported suggesting that

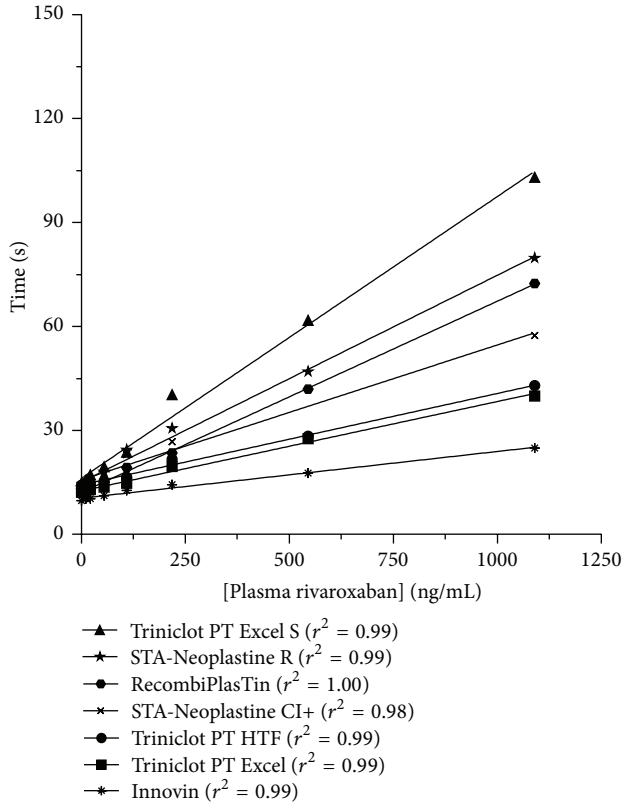

Figure 2: Impact of rivaroxaban on prothrombin time (PT). There is a linear concentration-dependent prolongation of PT. The sensitivity highly depends on the reagent (*adapted from* Douxfils et al. [49]).

the laboratories should be aware about the sensitivity of their own reagent towards rivaroxaban (Figure 2). Importantly, as mentioned for dabigatran, the reagent/instrument combination can also influence the sensitivity and therefore it is recommended that each laboratory assess the sensitivity of their reagent on the instrument on which the test is performed [33]. The *Subcommittee of Control of Anticoagulation of the Scientific and Standardization Committee* of the ISTH supports this statement [31]. Nevertheless, one weakness of this approach is that commercially available calibrators are labelled to be used with its corresponding chromogenic anti-Xa assay. Therefore, as for dabigatran and the aPTT, the quality and the accuracy of these calibrators for the calibration of PT reagents are not warranted.

The use of a calibrated-PT was assessed in an *ex vivo* study that revealed a poor correlation between calibrated-PT and measured rivaroxaban plasma concentration [50]. Moreover, in the literature, one can find data showing that depending on the reagent, normal PT may be obtained with therapeutic concentrations of rivaroxaban [51] while some authors stated that it may be possible to exclude therapeutic level of rivaroxaban by using sensitive reagent (i.e., RecombiPlasTin 2G) [52]. We also observed that RecombiPlasTin 2G was more performing than other rivaroxaban-sensitive PT reagents (personal own data), showing that this reagent is probably less influenced by interindividual variability.

Therefore, depending on the reagent, PT must not be used to estimate rivaroxaban concentrations in plasma and reflects poorly the intensity of anticoagulation due to rivaroxaban. The poor sensitivity (especially for reagents like Innovin), the important variability (less pronounced with RecombiPlasTin 2G), and the poor linear correlation with the LC-MS/MS preclude the use of PT to estimate rivaroxaban concentration in plasma. In addition, drugs or hematologic abnormalities affecting at least one factor assessed by PT could bias the conclusions. However, as for the aPTT with dabigatran, PT is the most widely available test to assess the intensity of anticoagulation of rivaroxaban. It is available 24/7 and does not require specific laboratory experience. Importantly, results should be given as ratio or as seconds and should never be expressed as INR. In addition, the knowledge of the sensitivity of the reagent is mandatory.

(3) Apixaban: Prothrombin Time (PT). The *Subcommittee of Control of Anticoagulation of the Scientific and Standardization Committee* of the ISTH elected to perform a collaborative study to analyse the anticoagulant effects of apixaban on several coagulation tests. The method includes the measurement of PT using RecombiPlasTin 2G and Technoclot reagents, the prothrombinase-induced clotting time (PiCT), and 5 chromogenic anti-Xa assays. They mention that all methods adequately determine the concentration of apixaban if added to normal human pool plasma *in vitro*. They also revealed that using assays procedures validated by manufacturers for individual coagulation platforms could further reduce error variances [53]. However, initial PK/PD studies in patients treated with apixaban assessed the effect of apixaban on coagulation with PT/INR, aPTT, and chromogenic anti-Xa assays [54]. INR values were prolonged modestly, on average increasing by 20–40% with RecombiPlasTin as reagent. aPTT increased minimally and did not differ significantly between the different apixaban dosing groups in secondary prevention of acute coronary syndrome (2.5 mg *bid*; 10 mg *od*; 10 mg *bid* and 20 mg *od*) [54]. As stated for rivaroxaban, INR must not be used for the assessment of apixaban while PT, expressed either in seconds or as ratio, is inappropriate to ensure an accurate quantitative measurement of apixaban [55]. Prothrombin time is not sensitive enough to ensure a quantitative measurement of apixaban at the plasma level obtained in initial PK/PD studies with the doses given in approved indications [54–56]. Moreover, depending on the reagent, PT may be normal with therapeutic concentration of the drug (±100 ng/mL at C_{max} and 30 ng/mL at C_{trough} in healthy subject) [56, 57]. For the most sensitive reagents it may only inform the clinician if the patient is taking the drug. This interreagent variability (Figure 3) prevents valid recommendations of cut-offs in seconds associated with a bleeding risk applicable to all reagents [55]. In addition, drugs or haematologic abnormalities affecting at least one factor assessed by PT could bias the conclusions. We definitively do not recommend PT to estimate plasma concentration of apixaban.

(4) Dilute Russell's Viper Venom Time (DRVV-T): A Useful Assay for All NOACs. Russell's viper venom contains a potent

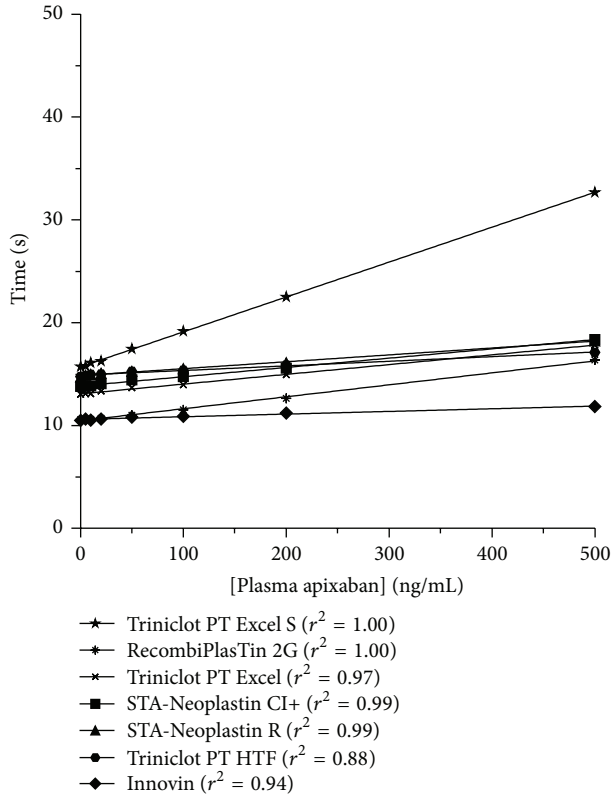

Figure 3: Impact of apixaban on prothrombin time (PT). There is a linear concentration-dependent prolongation of PT. The sensitivity highly depends on the reagent. PT is not sensitive enough to estimate plasma drug concentrations of apixaban or to assess the relative intensity of anticoagulation at therapeutic doses (±100 ng/mL at C_{max} in healthy subject taking apixaban 5 mg bid) [56] (*adapted from* Douxfils et al. [55]).

activator of factor X and is usually used for the assessment of antiphospholipid syndrome (APS) [58]. It was demonstrated previously that rivaroxaban influences APS screening tests leading to false positive results, especially on the DRVV-T [59, 60]. A study suggested that DRVV-T could be used for the evaluation of all NOACs but this needs to be validated in patient samples [61]. Recent findings confirmed this hypothesis in plasma samples from patients treated with rivaroxaban [62]. It was demonstrated that the confirmed reagent, which contains the higher amount of phospholipids, should be preferred since it is not sensitive to the cases of APS. Moreover, it is more accurate than the screening reagent despite a small loss of sensitivity. However, validation with drug level measurements in plasma using LC-MS/MS and direct comparison of the effects of dabigatran and apixaban is lacking and needs to be investigated.

4.4.2. Specific Coagulation Tests

(1) Dabigatran: Diluted Thrombin Time (dTT), Ecarin Clotting Time (ECT) and Ecarin Chromogenic Assay (ECA). Assays used to evaluate thrombin functions are more sensitive than those that are not specific toward this coagulation factor [32, 63]. Thrombin time (TT) was demonstrated to be too sensitive for dabigatran [32, 63]. However, this may guide the clinician in the perioperative setting since a normal TT indicates no clinically relevant anticoagulant effect of dabigatran.

The strong sensitivity of TT towards dabigatran led to the development of a calibrated diluted thrombin time (dTT) using dabigatran standards to calculate the dabigatran plasma concentration. Thus, the Hemoclot thrombin inhibitor (HTI) was developed and has been proposed as a rapid, standardised, and calibrated assay to determine plasma concentrations of dabigatran [32, 35, 63, 64]. Theoretically, the use of diluted plasma (1/8 initially) allows the HTI assay to measure dabigatran effects at a wide range of concentrations. It is also normalized with normal pooled plasma, thus reducing the intraindividuals variation due to abnormalities in patient's plasma characteristics, especially the fibrinogen rate. In addition, there is a linear relationship between dabigatran concentrations and the dTT, which is suitable for the precise quantitative assessment of dabigatran [32, 35, 63, 64]. It is also fully automatable and has been adapted to different coagulometers in order to be easily implemented in laboratories. Several studies showed that the HTI highly correlates with dabigatran plasma concentrations measured by LC-MS/MS in patient's plasma [34–36, 63]. However, this test is limited by the fact that, in case of switching therapy (i.e., from heparins/heparinoids to dabigatran etexilate; or from hirudin and derivatives to dabigatran etexilate), it will be slightly influenced by the presence of such inhibitors in the plasma. This implies the necessity of an accurate anamnesis of the drugs taken by the patient to avoid overestimation of drug concentrations in plasma.

Nevertheless, for the accurate determination of dabigatran plasma concentrations below 50 ng/mL, the more sensitive LC-MS/MS method is still required [34, 35].

The ECT assay provides a direct measure of the activity of direct thrombin inhibitors. When dabigatran was used for the prevention of stroke in NVAF with twice daily dosing, an ECT of greater than 3 × ULN (or an ECT prolongation of >103 seconds) at trough reflected the 90th percentile of observations and was considered to be associated with a higher risk of bleeding [4]. While development of commercial kits might improve the practicality of this test, these kits have not been standardised or validated with dabigatran [63]. For these reasons, ECT cannot be recommended for emergency monitoring of anticoagulant effects. Moreover, ECT is not widely available and is known to have interlot variability indicating that calibration is also required with this test [32].

Recently, the ECA, the chromogenic variant of the ECT, calibrated with standard calibrators, has been proposed for the accurate measurement of dabigatran in patient's plasma with a lower limit of quantitation similar to HTI [34, 36, 65]. The ECT and the ECA both use the ecarin, a highly purified metalloprotease isolated from the venom of *Echis carinatus*, to activate prothrombin into meizothrombin [66, 67]. Meizothrombin can then cleave the fibrinogen to form fibrin (ECT) or can cleave a chromogenic substrate that release *paranitroaniline* (ECA). They present the advantage to be insensitive to heparins, as the heparin-antithrombin

complex cannot inhibit meizothrombin due to steric hindrance. Therefore, only direct thrombin inhibitors could interfere with the test in case of switching therapy. Moreover, the artificial addition of prothrombin and the use of a chromogenic substrate in the ECA allow this test to be insensitive to abnormalities in patient's plasma [68]. Currently, the ECA and the HTI showed similar performances in different studies [34, 36, 65]. Head-to-head evaluations of these tests in the "real life" context, using the LC-MS/MS as reference measurement, are still required to compare the accuracy and reproducibility of these specific methodologies developed to measure dabigatran plasma concentrations.

(2) Rivaroxaban: Chromogenic Anti-Xa Assays. The initial study assessing the safety, PK, and PD of rivaroxaban suggested that rivaroxaban plasma concentrations and inhibition of FXa activity correlated closely [69]. Thus, investigations have been undertaken to optimise current chromogenic anti-Xa assays primarily used for the monitoring of LMWH [47–49, 70–75]. Thanks to specific calibrators and controls containing a defined amount of rivaroxaban, a dedicated chromogenic anti-Xa assay has been proven to accurately estimate the plasma rivaroxaban concentrations >30 ng/mL [50].

Another advantage of chromogenic anti-Xa assays is that they are less influenced than PT by the sample collection conditions and the variations in the amounts of clotting factors in patients. Several chromogenic anti-Xa assays are available on the market; however, only some of them are labelled to ensure the quantitation of rivaroxaban plasma concentrations. It is therefore important to work on specific coagulation platforms to reduce the CV in the interlaboratory setting [73]. The results from a multicentre trial involving 24 laboratories indicate that chromogenic anti-Xa methods, using rivaroxaban calibrators and controls, are suitable for the measurement of a wide range (20–660 ng/mL) of rivaroxaban plasma concentration [50, 76]. They also present a good repeatability and reproducibility and the estimated concentrations have been comparable with the concentrations measured by LC-MS/MS in a previous study [50, 75]. A limitation of chromogenic anti-Xa assays is that the standardisation across reagents and methods is not easily achieved but interesting results showed that interassay variability could be easily reduced [73]. Importantly, chromogenic anti-Xa assays, specific calibrators and controls, are not yet widely available, and their use may be difficult in emergency situations. However, the availability of liquid-stable reagents could improve the practicability of these tests.

Nevertheless, taking into account the lower sensitivity of chromogenic assays compared to LC-MS/MS and the variability of coagulation analysers that may further increase the imprecision at the lowest concentrations, detection and quantitation of lower levels (<30 ng/mL) in rivaroxaban treated patients still require LC-MS/MS analyses [50, 77].

The LC-MS/MS method is more accurate and is useful in the entire range of rivaroxaban concentrations. Consequently, the LC-MS/MS is required for quantification of very low to moderate rivaroxaban concentrations (3 to 30 ng/mL) in clinical samples.

(3) Apixaban: Chromogenic Anti-Xa Assays. Due to their good sensitivity towards the inhibition of FXa by apixaban, chromogenic anti-Xa assays calibrated with specific apixaban calibrators should be performed to estimate plasma drug concentrations [55, 57]. Another advantage of chromogenic anti-Xa assays is that they are less influenced than PT by the sample collection conditions and the variations in the amounts of clotting factors in patients [46]. Patients of the APPRAISE-1 study had participated in a PK/PD study suggesting that apixaban-mediated anticoagulant effect can be detected, even at very low plasma concentrations, using a standard laboratory chromogenic anti-Xa assay with either LMWH or apixaban calibrators [78]. However, what the authors failed to discuss in their paper regarding their data is that when comparing plasma apixaban concentrations estimated by the calibrated STA-Rotachrom and the true plasma concentration measured by LC-MS/MS, the chromogenic anti-Xa assay tended to underestimate the plasma drug concentration [78]. The French GEHT study, that assesses the reliability of different chromogenic anti-Xa assays for measurements of apixaban using specific calibrators and controls, revealed that the 3 chromogenic anti-Xa assays tested using lyophilised apixaban calibrators and controls correctly quantify a wide range of apixaban concentration. However, a limitation of this study was that it was performed in spiked samples and not from patients treated with apixaban [57]. Thus, further studies are required with validated calibrators that compare dedicated calibrated chromogenic anti-Xa assays with LC-MS/MS. As for rivaroxaban, it seems to be preferable to work on specific coagulation platforms in order to reduce the interlaboratory CV [57]. Nevertheless, confirmation of the accuracy of the quantitative measurement of apixaban by chromogenic anti-Xa assays using specific calibrators and controls should be confronted to the reference LC-MS/MS measurement in "real life" patients treated by Eliquis. In addition, chromogenic anti-Xa assays are not widely available, and their use may be difficult in emergency situations. However, as for rivaroxaban, the availability of liquid-stable reagents could improve the practicability of these tests.

5. Measurements in Other Media

NOACs can also be measured in other media such as urine or serum samples. Regarding the measurement in urine samples, it is important to mention that about 80% of dabigatran is excreted into urine as active compound and about 60% of rivaroxaban is cleared from circulation by glomerular filtration, 30% of which is excreted as active drug and the other part is metabolized in an inactive form before the renal excretion. To obtain results within minutes and possibly to develop point-of-care (POC) techniques, some authors have proposed measurement of dabigatran and rivaroxaban in urine samples [79, 80]. This testing may be useful for special patient populations such as those with acute deterioration of renal function due to any disease, before surgical interventions, during unexpected bleeding or thrombotic episodes while on therapy with NOACs, the oldest and

youngest populations, pregnancy, suspicion of overdose, and intoxication. The test is based on the development of different colours in presence and absence of oral direct FXa and thrombin inhibitors. The strength of the methods is that they are not invasive and can be performed repetitively, that the results are available within 15 min, that the patients can perform the test themselves, and that the tests do not require standards and controls. The methods are described as sensitive, specific, accurate and possess a very high interrater agreement. However, the methods are not interesting in patients with creatinine clearance below 10 mL/min, confounders may also alter the identification of the colour generated, and the tests do not provide information on compliance. Importantly, these tests are qualitative and not quantitative and therefore cannot guide the physician on bleeding or thrombotic risks [79, 80].

The measurement of rivaroxaban and apixaban in serum samples has also been proposed [81]. The determination of rivaroxaban and apixaban from serum samples of patients may be beneficial in specific clinical situations when additional blood sampling for plasma and thus the determination of FXa activity are not feasible or results are not plausible. These tests are performed with chromogenic anti-Xa assays calibrated with homemade serum samples spiked with rivaroxaban or apixaban. Results revealed that the determination of rivaroxaban and apixaban from serum samples of patients can be performed with all chromogenic assays but some differences of the results between them exist. An adaptation of the chromogenic assays to current coagulation platforms seems feasible and may even improve the results but still remains to be tested [81].

6. Perspectives

The present review summarizes the information on how to handle clinical samples from patients treated with dabigatran etexilate, rivaroxaban, or apixaban in order to accurately measure plasma concentrations/effects of these compounds. Clearly, dedicated tests should be preferred when assessing these samples in order to avoid misinterpretation that could have clinical implications (e.g., with aPTT or PT the laboratory could refer the absence of NOACs in the perioperative setting while they are still present at clinically relevant concentrations). However, these specific assays are not widely available, are frequently time-consuming, and require the use of calibrators and controls that further increase the cost of these tests. In addition, while they are interesting for the measurement of normal to high levels of inhibitors, they seem unreliable for the measurement of low plasma concentrations that could be encountered in the perioperative setting. While the majority of the elective procedure can be performed with low residual plasma concentrations (<30 ng/mL for dabigatran and rivaroxaban [82]), high-risk surgeries may require a more accurate measurement of residual plasma level of these lowest concentrations. Therefore, there is a need to develop specific coagulation tests accurate in these low concentrations.

In addition, the development of a global coagulation test, which is sensitive to all NOACs and for which a cut-off associated with bleeding or thrombotic risk can be provided, is still needed. Finally, to date, the clinical benefit of such monitoring has not been proven yet. A large, sufficiently powered, clinical trial comparing standard treatments with dose-adjusted regimens in certain categories of patients is highly requested.

7. Conclusion

Up to now, no biological assay can be recommended for the assessment of all NOACs. Specific coagulation assays, depending on the NOACs, should be used in order to provide the more reliable information on plasma concentrations. The use of calibrated chromogenic anti-Xa assays and a dilute thrombin time should be recommended for the assessment of direct FXa inhibitors and direct thrombin inhibitors, respectively. Global coagulation tests, such PT and aPTT, are not useful at all and can lead to misinterpretation that could have clinical implications if the result is not fully understood.

The use of dedicated assays, using validated platforms, may probably improve the benefit-risk profile of NOACs by identifying poor- or high-responders. Monitoring such therapies that were claimed to be independent of any biological testing may be useful to provide guidance in case of bleeding, thrombosis recurrence, before urgent surgery or procedure, for populations excluded from clinical trials, and for those with several comorbidities. However and importantly, the clinical benefit of such monitoring still needs to be proven in a large, sufficiently powered, clinical trial designed to compare standard treatments with dose-adjusted regimen of these NOACs.

Conflict of Interests

Jonathan Douxfils, Helen Mani, Valentine Minet, Bérangère Devalet, Bernard Chatelain, and Jean-Michel Dogné have no conflict of interests regarding the publication of this paper. François Mullier is orator for Bristol-Myers Squibb and Sanofi.

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Research Article

Polysulfated Trehalose as a Novel Anticoagulant Agent with Dual Mode of Action

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Physiological hemostatic balance is a coordinated outcome of counteracting coagulation and fibrinolytic systems. An imbalance of procoagulant and anticoagulant factors may result in life threatening thromboembolism. Presently, anticoagulant administration is the first line of therapy for the treatment of these conditions and several anticoagulants have been approved, including various forms of heparin. However, the polyanionic nature and multispecificity of heparin pose several complications. Generally, the polysulfated compounds with antithrombotic potential are thought to have feasible synthetic procedures with much less bleeding, thus having favourable safety profiles. Here we report the synthesis of a novel compound, trehalose octasulfate and the assessment of its anticoagulation potential. Molecular docking of trehalose and trehalose octasulfate with antithrombin showed a specificity switch in binding affinity on sulfation, where trehalose octasulfate interacts with critical residues of AT that are either directly involved in heparin binding or in the conformational rearrangement of AT on heparin binding. An *in vitro* analysis of trehalose octasulfate demonstrated prolonged clotting time. Lead compound when intravenously injected in occlusion induced thrombotic rats showed remarkable reduction in the size and weight of the clot at a low dose. Delay in coagulation time was observed by analysing blood plasma isolated from rats preinjected with trehalose octasulfate. A decrease in Adenosine 5′-Diphosphate (ADP) induced platelet aggregation indicated a probable dual anticoagulant and antiplatelet mechanism of action. To summarize, this study presents trehalose octasulfate as a novel, effective, dual acting antithrombotic agent.

1. Introduction

Thromboembolic disorders that include deep vein thrombosis (DVT) and pulmonary embolism (PE) have an annual incidence of approximately 1 in 1000 in western populations [1]. Thrombosis underlies one of the most frequent causes of cardiovascular diseases like myocardial infarction and stroke [2]. Further, venous thrombosis has been reported to be the second leading factor of death in cancer patients and is a major cause of morbidity and mortality during pregnancy and child birth. Besides the acute morbidity, thrombosis is generally followed by post-thrombotic complications with patients being prone to recurrent episodes of crippling pain and skin ulceration. In addition to the mortality risk, hospitalization is prolonged in patients with DVT and PE as a ramification of any surgical or medical disease, leading to increased healthcare expenditure [3, 4]. The first line in the management of venous thromboembolism (VTE) is to restrict the extension of thrombus so as to reduce the risk of PE and minimize the post-thrombotic complications [5]. This is mainly achieved by the use of anticoagulants, with heparins and coumarins being the most widely used. Even with the remarkable success in managing thrombotic events, the long-term use of heparins and coumarins is associated with a number of drawbacks which chiefly include unavoidable risk of bleeding, narrow therapeutic window, variable patient response and various other complications owing to their non-specific binding, food and drug interactions and

immunologic reactions [6–8]. The introduction of new anticoagulants based on low molecular weight heparins (LMWH) and a minimal antithrombin (AT) binding pentasaccharide sequence, for example, fondaparinux, dabigatran, and rivaroxaban, has successfully overcome certain limitations posed by heparin therapy. However, they still are associated with bleeding risk and lack an effective antidote to reverse excessive anticoagulation. Further, their synthesis is a complex, multistep and low-yield procedure [9–12]. Thus, the drawbacks and limitations of current antithrombotic agents have prompted a search for new antithrombotic drugs with reliable safety profiles and feasible synthetic procedures. Towards the design of new oral or intravenous molecules with anticoagulant properties as an alternative to heparin therapy, scaffolds with less anionic and more hydrophobic nature are anticipated to have reduced non-specific interactions compared to heparin. Several efforts in this direction have yielded the synthesis of heparin and heparan sulfate mimetics that mimic the binding of heparin to anticoagulant proteins like AT and heparin cofactor II (HCII) and/or to procoagulant factors, thrombin (fIIa) and factor Xa (fXa) [13]. These molecules include sulfated flavonoids [14–18], benzofurans [19], sulfated dehydrogenation polymers (DHPs) of lignin type [20], isoquinolines [21], and xanthones [22]. In addition, oligosaccharides, namely, dermatan sulfate hexasaccharides, and sulfated bis-lactobionic and bis-maltobionic acid amides have been reported to inhibit fIIa via the activation of its endogenous inhibitor, HCII [23–25]. Lately, another sulfated disaccharide, sucrose octasulfate was shown to exhibit anticoagulant action through a HCII-dependent thrombin inhibition and has been used as an alternative to heparin, yet it is unclear whether it is an effective heparin mimic in its interaction with thrombin [26]. Owing to their structural diversity and hydrophobic nature, better modulators are expected from these scaffolds and compared to heparins, these small sulfated compounds are increasingly gaining importance as inhibitors of coagulation [14–18, 27, 28].

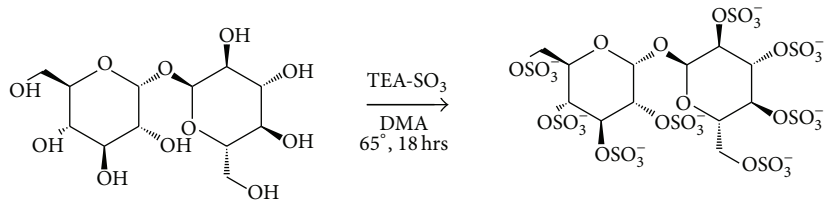
Here, we aimed to synthesize a new small sulfated molecule based on a saccharide skeleton and test its antithrombotic potential. On the basis of molecular docking based screening of small sugars, we selected disaccharide trehalose for sulfation. We report the synthesis and characterization of novel trehalose octasulfate and the assessment of its *in vitro* and *in vivo* anticoagulant properties. The anticoagulant activity of trehalose octasulfate showed a 2-3-fold prolongation of activated partial thromboplastin time (APTT) and prothrombin time (PT) at micromolar range, indicating its promising role in delaying coagulation. The effect of the test compound on thrombus formation was determined in an occlusion induced thrombosis model and by monitoring the clotting times in the blood plasma isolated from rats preinjected with trehalose octasulfate. We observed a remarkable reduction in the thrombus size and weight in the rats preinjected with trehalose octasulfate. Further, the clotting tests of the plasma isolated from the trehalose octasulfate preinjected animals showed a delay in coagulation time. A decrease in ADP induced platelet aggregation in the presence of trehalose octasulfate indicated its dual mechanism of action.

2. Materials and Methods

2.1. Docking. Autodock Vina (that employs the iterated local search global optimizer for global optimization for local minima search) [29] was used to find the relative affinity of trehalose and trehalose octasulfate with antithrombin. AT (PDB ID:1E05) and HCII (PDB ID:1JMJ) were processed in Autodock tools (ADT) [30], all water molecules were removed, polar hydrogens were added, Kollman charges were assigned to all atoms, and Gasteiger charges were calculated. The ligand PDB files were also processed in ADT. Polar hydrogens were added and Gasteiger charges were calculated and the rigid root and rotable bonds were defined by the Autotors tool of ADT. Blind docking was performed with affinity grid maps of $62 \times 58 \times 60$ points (for IJMJ) and $62 \times 62 \times 56$ points (for IE05) and 1.00 Å grid point spacing centered on whole protein encompassing the heparin binding domain (HBD) using the autogrid tool of ADT. We have considered the minimum energy conformation state of both ligands, showing binding affinity in kcal/mol. Images of ligand and receptor bound complexes were prepared in Ligplot visualizing program [31] and polar contacts between them were noted down.

2.2. Chemistry. Triethylamine sulfur trioxide adduct was purchased from Sigma-Aldrich and trehalose from MP-Biomedicals. The solvents used were of HPLC grade procured from Sigma-Aldrich.Precoated aluminium sheets (Silica gel 60 F_{254}, Merck Germany) were used for thin-layer chromatography (TLC) and spots were visualized using 5% H_2SO_4 in methanol as the developing reagent. Purity of the compound was checked by UHPLC on UHPLC-ELSD-MS Agilent 3100 MS using Acquity UPLC BEH C18 Column (1.7 μm, 2.1 mm $\times$ 50 mm I.D). The mobile phases were degassed for 15 min before use. 5 mM ammonium acetate in water and acetonitrile was used as mobile phase at a flow rate of 0.6 mL/min with detection at 214 nm. The sample was prepared in methanol + acetonitrile + water mixture (1 : 1 : 1). The IR spectra of compounds were taken on Agilent Cary 630 FT-IR spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were obtained at ambient temperature using a Bruker Spectrospin DPX-400 MHz NMR instrument in D_2O using tetramethylsilane (TMS) as an internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Chemical shift values are given in parts per million (ppm). Mass spectra were recorded on a Q Star XL hybrid electron spray ionization high resolution mass spectrometer (Applied biosystems) in a scan range of 100 to 1000 atomic mass units (amu). Melting point was recorded on a digital Buchi melting point apparatus (M-560) and was reported uncorrected.

2.2.1. Synthesis of Trehalose Octasulfate. Modification was done using previously reported method [27]. In a solution of trehalose (378 mg, 1 mmol) in DMA (15 mL), triethylamine-sulfur trioxide adduct (4 equiv/OH, 5.8 gm, 32 mmol) was added and the reaction mixture was stirred overnight at 65°C (Scheme 1). After completion of the reaction as checked



Trehalose Trehalose octasulfate

Scheme 1

by TLC, the reaction mixture was poured into 150 mL cold acetone under basic conditions (a few drops of triethylamine were added) and left at 4°C for 24 hours. On the next day, acetone was removed under vacuum and the crude oil was washed with acetone and diethyl ether (3 times). The oil was then dissolved in 30% sodium acetate aqueous solution and the suspension obtained was precipitated in ethanol.

(1) Trehalose 3, 3′, 4, 4′, 5, 5′, 6, 6′-O-Octasulfate. Creamy white solid; melting point: 210–215°C; yield: 60% IR (neat): 1637, 1229, 951, 808, 894, 1032 cm^{-1}; ^{1}H-NMR (D_2O, 400 MHz) (δ, ppm): 5.49 (d, 2H), 4.43-4.20 (m, 12 H); ^{13}C-NMR (D_2O, 100 MHz) (δ, ppm): 92.18, 75.84, 74.42, 73.69, 68.61, 65.92; ESI-MS (m/z): 713.5, 487.2, 283.2, 255.2.

2.3. Coagulation Assays. Human blood was collected from healthy individuals without any history of bleeding, thrombosis, or consumption of medication known to alter blood coagulation for 2-3 weeks prior to collection. Venous blood collected in citrated vials containing 3.8% sodium citrate solution was centrifuged for 20 min at 2400 g to separate the plasma. The supernatant plasma separated from the cell debris was pooled and used immediately for coagulation time measurements. The clotting assays were performed using commercial kits according to the manufacturer's instructions. Clotting time kits used for APTT (00597) and TT (00611) were from C.K. Prest, France, and PT kit (00667) was from Neoplastine C1 Plus. Trehalose and trehalose octasulfate were dissolved in phosphate sodium EDTA (PNE) buffer. The working concentration of trehalose in the clotting assays ranged from 0 to 10^{-3} M and 0 to 1^{-3} M for trehalose octasulfate. For APTT assay, 100 μL citrated plasma was mixed with 100 μL of test compound or buffer solution for test and control reactions respectively and incubated at 37°C for 1 minute; 200 μL of APTT reagent was then added and the mixture was again incubated at 37°C for 4 minutes. 200 μL of 20 mMol/L $CaCl_2$ was then added and tubes were monitored for clotting time. For PT assay, 100 μL citrated plasma was mixed with 100 μL of buffer or test solution for control and test reactions respectively and incubated for 1 minute at 37°C; 200 μL of PT reagent was then added and the time taken for clot formation was noted. TT test was carried out by mixing 100 μL citrated plasma with 100 μL of buffer or test solution for control and test reactions respectively and incubating at 37°C for 1 minute, followed by the addition of 200 μL of TT reagent. Time taken for clot formation was noted. All the tests were performed in triplicate and repeated at least three times. PNE was taken in control sets to correct the buffer contributions and was compared with the values of coagulation times of plasma alone. Coagulation time prolonging ratio was calculated by comparing the clotting time in the presence of test compound with that when buffer was used in place of test.

Table 1: Experimental groups considered for *in vivo* antithrombotic studies.

Experimental group	Treatment
A	Vehicle control
B	Thrombotic control (occluded)
C	Trehalose injected
D	Trehalose octasulfate injected

2.4. In Vivo Studies. Sprague Dawley rats (200–250 g) were used for the study. Animals were maintained in polypropylene cages under an ambient temperature of 25°C ± 1°C and a relative humidity of 45% to 55% in a hygienic environment under 12:12 hours light-dark cycle. The animals had free access to food pellets and purified water. All the experimental procedures were approved by the animal ethics committee of Jamia Millia Islamia, New Delhi, India.

Sprague Dawley rats were divided into four groups (Table 1). The test compounds were preinjected to the experimental animals by tail vein injection method [32, 33]. Following the injection, all the animals in the G-A to G-D underwent inferior vena caval (IVC) ligation for the thrombus to be inducted by flow restriction approach. This approach provides a total stasis environment and results in a very severe vein wall reaction to thrombosis [34]. Animals were anesthetized and IVC exposed through a midline laparotomy by dissecting at the level of renal veins. The infrarenal IVC was identified and ligated below the renal veins until the iliac trunk (1–1.2 cm in length) with nonreactive 7-0 prolene suture. After 24 hours, animals were euthanized and thrombosed inferior vena cava was harvested. Since thrombus size is a direct measure of clot formation and dissolution, the following parameters were recorded in all the animals: (i) total length of the ligated IVC, (ii) length and (iii) weight of the thrombus formed inside the ligated IVC. Using these directly measurable quantitative parameters, the ratio

of the weight of the thrombus (mg) to the length of the IVC ligation (mm) were calculated as

$$\text{Ratio (Weight to Length)} = \frac{\text{Weight of Thrombus formed (mg)}}{\text{Length of IVC ligation (mm)}}. \quad (1)$$

The quantitative parameters obtained in test compound injected groups (G-C and G-D) (Table 1) were compared with corresponding positive controls (G-A and G-B) (Table 1). In the initial phase of experiments, dosage regimen was determined by injecting the compounds at varying dosages of 10 mg/kg, 5.0 mg/kg, 2.5 mg/kg and 0.5 mg/kg body weight of rat. Among these doses, 2.5 mg/kg of body weight of trehalose octasulfate yielded quantifiable amounts of vein wall tissue and thrombus and was thus set as an optimum dosage to study its effect on thrombus formation. At the time of euthanasia blood was collected from retro-orbital plexus of animals. Plasma was isolated and APTT and PT assays were performed.

2.5. Platelet Aggregation Assay. The antiplatelet activity of test compounds was determined by whole blood aggregation. Platelet aggregation was monitored in whole blood by using a Lumi-Aggregometer (Chrono-log model 700, USA) as per manufacturer's instructions. Platelet aggregation was measured in freshly collected whole blood with constant stirring at 1200 rpm. ADP was used as the agonist. The maximum impedance (ohms) was recorded as a measure of platelet aggregation in whole blood. The experiment was repeated with blood collected from the compound treated rats (Table 1) and the impedance of aggregation of these experimental animal groups was compared to that of vehicle control ones to study the effect of trehalose octasulfate on agonist induced platelet aggregation response.

3. Results and Discussion

3.1. Docking Studies. Molecular docking study of trehalose and trehalose octasulfate was carried out to check their relative affinity towards the target proteins AT and HCII. AT and HCII are the most critical endogenous anticoagulant molecules that regulate coagulation by inhibiting procoagulant proteases, namely, thrombin, fXa, fIXa, and fXIa (AT) and thrombin (HCII). Both AT and HCII require heparin as a cofactor to inhibit these proteases at physiologically relevant rates, underlying the principal use of heparin as anticoagulant [35–37]. Heparin binding domain of AT and HCII comprises of positively charged residues of the helix D of both AT and HCII and helix A of AT [38]. In consistency with our earlier study [39], we observed a switch in the specificity of binding affinity of trehalose on sulfation. We observed that trehalose octasulfate, apart from interacting with AT residues, Glu163, Tyr166, Trp189, and Lys193, interacts with some critical residues (Ile7, Tyr131, Val141, Ser142, Arg145, and Gly167) (Figure 1) that are involved either in heparin binding or in the conformational rearrangement of AT on heparin binding. There are several reports that support the involvement of AT residues 41–49 and 124–145 in heparin binding [40], other residues have been implicated as well, for example, natural variant Ile7Asn is associated with decreased heparin binding indicating its involvement in heparin based modulation of AT [41]. Arg145 is placed within the heparin binding site of human AT [42]. Further, in native AT, Tyr131-Asn127-Leu130-Leu140-Ser142 forms a tight cluster at the helix D-strand 2A interface and tight interactions between Tyr131 and neighbouring hD, and s2A stabilizes the native conformation of AT; it has also been hypothesized and tested that disrupting this cluster would activate AT independently of heparin [43]. Helix D residues 120–124 make multiple van der Waals contacts with residues 161–166 of helix E, where the rotation of helix D on binding to heparin pivots on the side chain of Phe123 and clashes into helix E. Here, the movement of Tyr166 is considered one of the important events in the propagation of conformational change from the heparin binding site to the distant hinge region of the RCL, required for the allosteric activation of AT [44]. Thus on the basis of the interaction profile of trehalose octasulfate with AT residues, Ile7, Tyr131, Val141, Ser142, Arg145 and Gly167, we speculated that it may modulate AT-based coagulation and hence set forth for its synthesis.

3.2. Synthesis of Trehalose Octasulfate. Trehalose octasulfate was synthesised from its precursor in moderate yield in one step reaction as shown in Scheme 1. Purity of the compound was checked by UHPLC and the structure was confirmed by FT-IR, ^{1}H-NMR, ^{13}C-NMR, and mass analyses. In IR analysis, absence of characteristic bands at 3400–3150 cm^{-1} corresponding to OH group indicates that all the hydroxyl groups have undergone sulfation. In addition to this, two strong bands at 1229 cm^{-1} corresponding to S=O stretch indicated the presence of sulfate group. The modification was further confirmed by ^{1}H-NMR spectral analysis, in which all the protons of sugar ring were found deshielded in the range 5.49–4.20 ppm in comparison to unmodified trehalose (5.17–3.40). All the OH protons of trehalose disappeared in modified trehalose establishing the formation of octasulfated trehalose. In ^{13}C-NMR spectra, carbons to which sulfate group is attached in the modified product were found to be deshielded as compared to trehalose providing confirmatory evidence for the substitution of –OH group by sulfate group. It has been reported that electron ionization (EI) of certain scaffolds involves complex fragmentation due to the broad spectra of internal energy of the molecular ion peak ($M^{+\bullet}$) which suppresses the $M^{+\bullet}$ and other primary fragments containing the structural information [45]. In our case as well, no distinct molecular ion peak was obtained in modified trehalose that may be due to fragmentation of sulfated trehalose into various fragment ions.

3.3. In Vitro Clotting Assays. To assess the anticoagulant property of trehalose octasulfate, the *in vitro* anticoagulant activity was analyzed for both sulfated and nonsulfated trehalose in human plasma by the three conventional coagulation assays, APTT, PT, and TT and the results are summarized in Figures 2 and 3. These results were expressed as ratio of

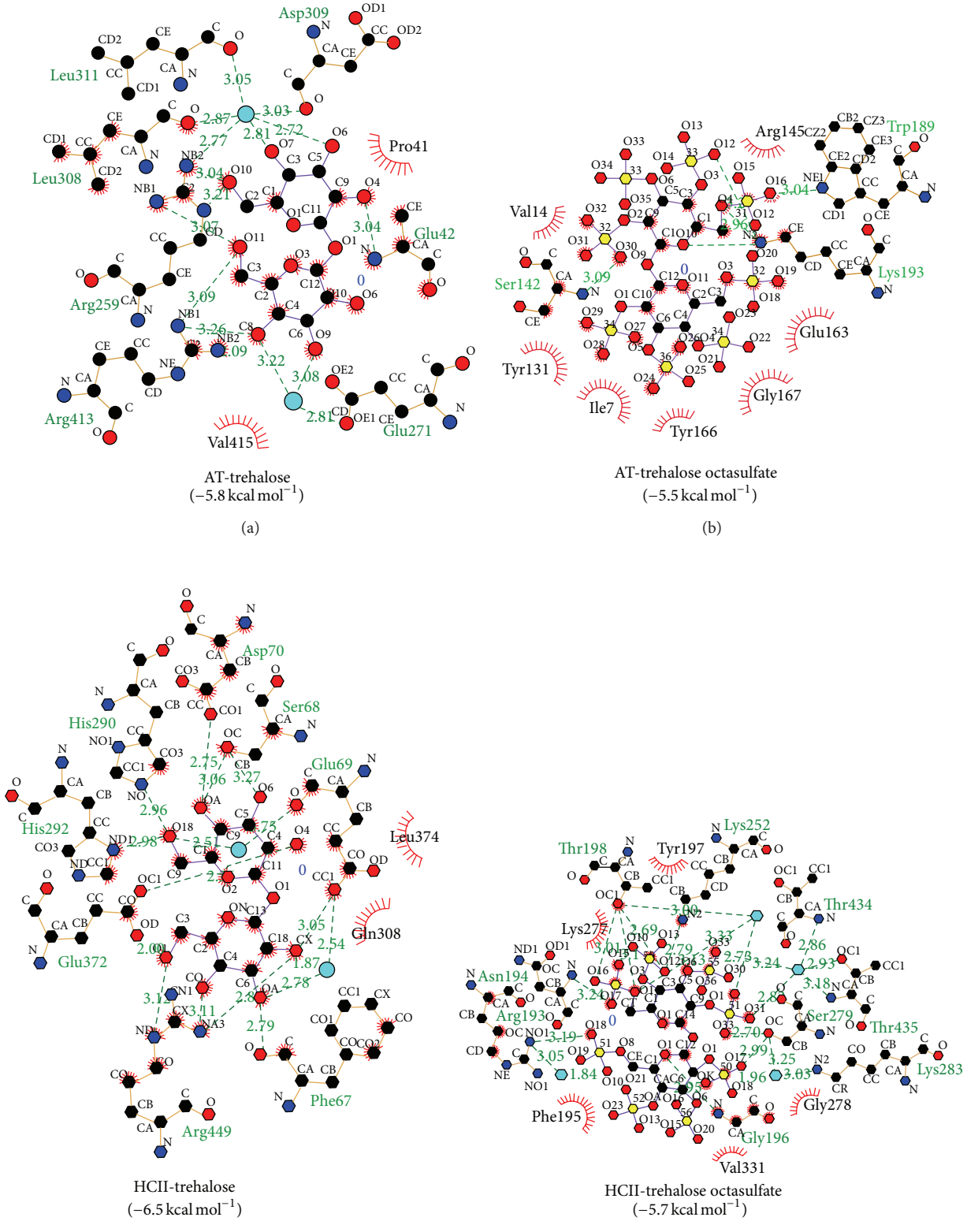


Figure 1: Ligplot analysis of binding of trehalose and trehalose octasulfate to antithrombin (1E05) heparin cofactor II (1JMJ).

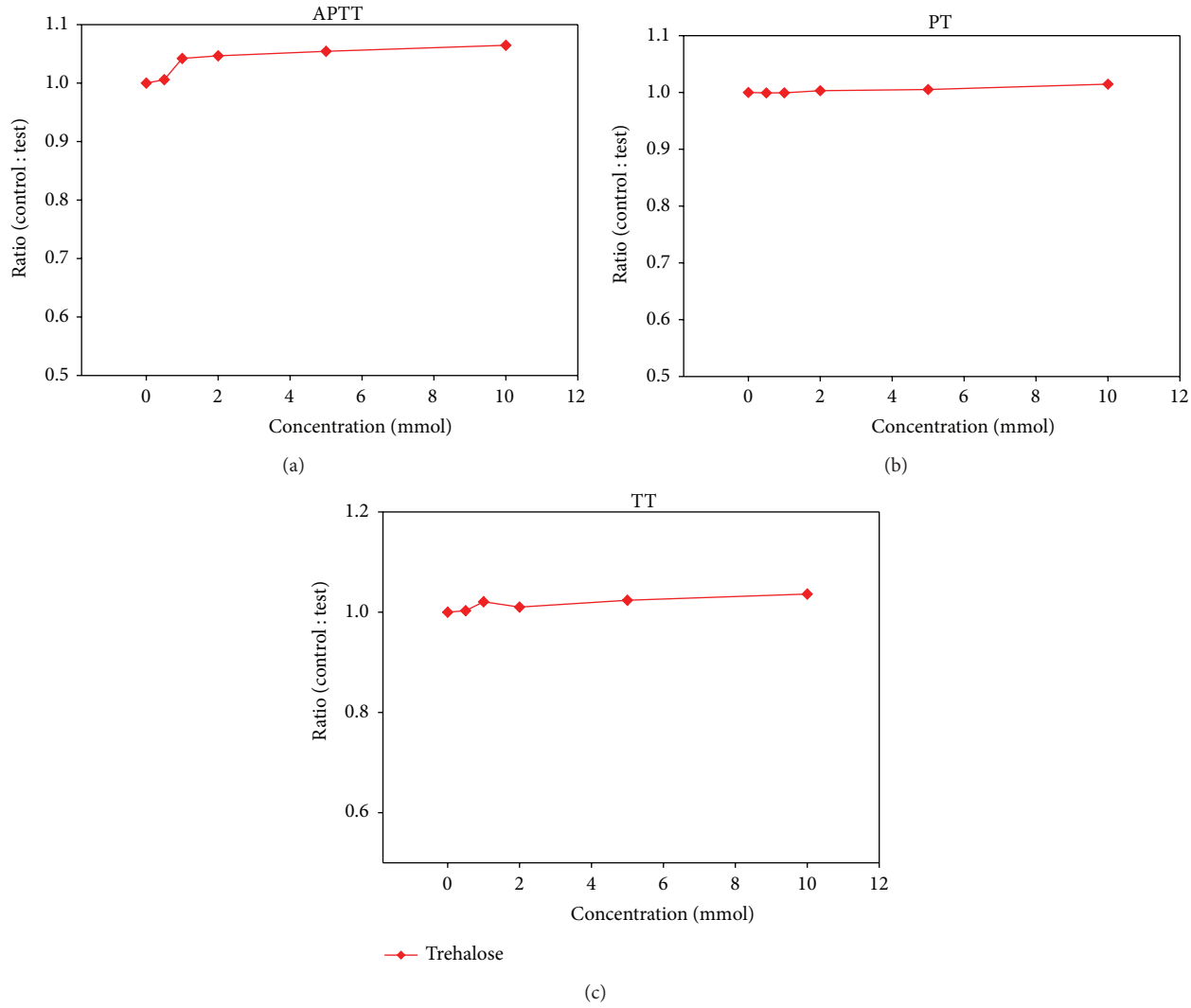


Figure 2: Effect of trehalose on clotting assays (a) APTT, (b) PT and (c) TT using human pooled plasma, expressed as ratio of clotting time in the presence and absence of trehalose octasulfate. Values represent an average of three independent experiments.

clotting time in the presence or absence of test compound to find out the extent of modulation with respect to the control. We observed that the ratio of the coagulation times in the presence of the nonsulfated compound with that of control plasma was close to 1 in all the three clotting tests, indicating that trehalose does not alter the coagulation pathways (at 10 mM) (Figure 2). However, the sulfated derivative exhibited promising anticoagulant properties in a dose-dependent manner. Trehalose octasulfate prolonged the clotting times APTT and PT significantly (Figure 3). At 1 mM concentration of trehalose octasulfate, there was an approximately 8-fold increase in APTT and 3-fold increase in PT. Preliminary information about the mode of action of these lead compounds could be inferred based on the varying effect on clotting assays, since each assay gives interaction of different stages of the coagulation pathway [46]. In view of the fact that trehalose octasulfate prolonged APTT and PT (APTT more than PT), its probable mode of action may be linked to the common pathway and can also be implicated in the intrinsic pathway. Further, in order to set an optimum anticoagulating dosage of the lead compound, we analysed the concentration required to double the coagulation times ($APTT_2$ and PT_2) (Table 2). The rationale was to find an effective concentration that only prolongs/delays clotting to a limited and controlled extent so as not to shift the equilibrium towards bleeding.

Table 2: Concentration of trehalose octasulfate required to double the clotting times.

Compound	$APTT_2$	PT_2
Trehalose octasulfate	400 μM	900 μM

3.4. In Vivo Antithrombotic Effect. In our study, inferior vena caval ligation caused prominent thrombus formation in the occluded region of the vena cava in Sprague Dawley rats. Control (thrombotic) animals developed a prominent

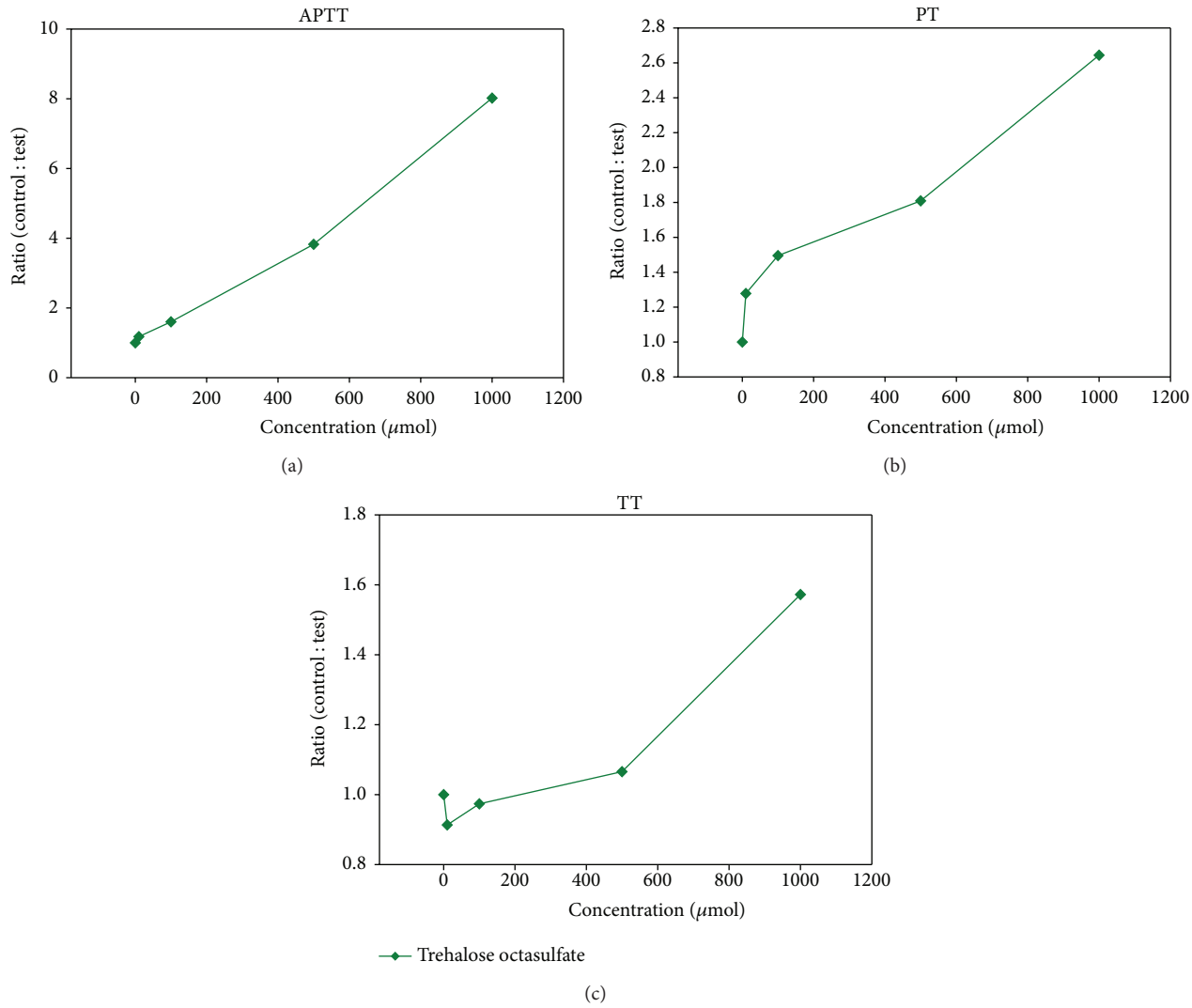


Figure 3: Effect of trehalose octasulfate on clotting assays (a) APTT, (b) PT and (c) TT using human pooled plasma, expressed as ratio of clotting time in the presence and absence of trehalose. Values represent an average of three independent experiments.

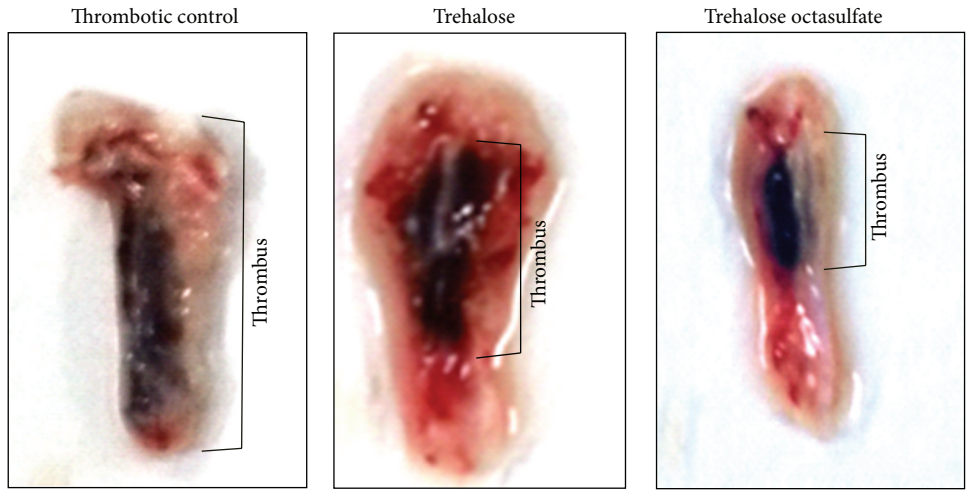


Figure 4: Comparison of thrombi harvested from control, trehalose and trehalose octasulfate treated thrombosis model animals.

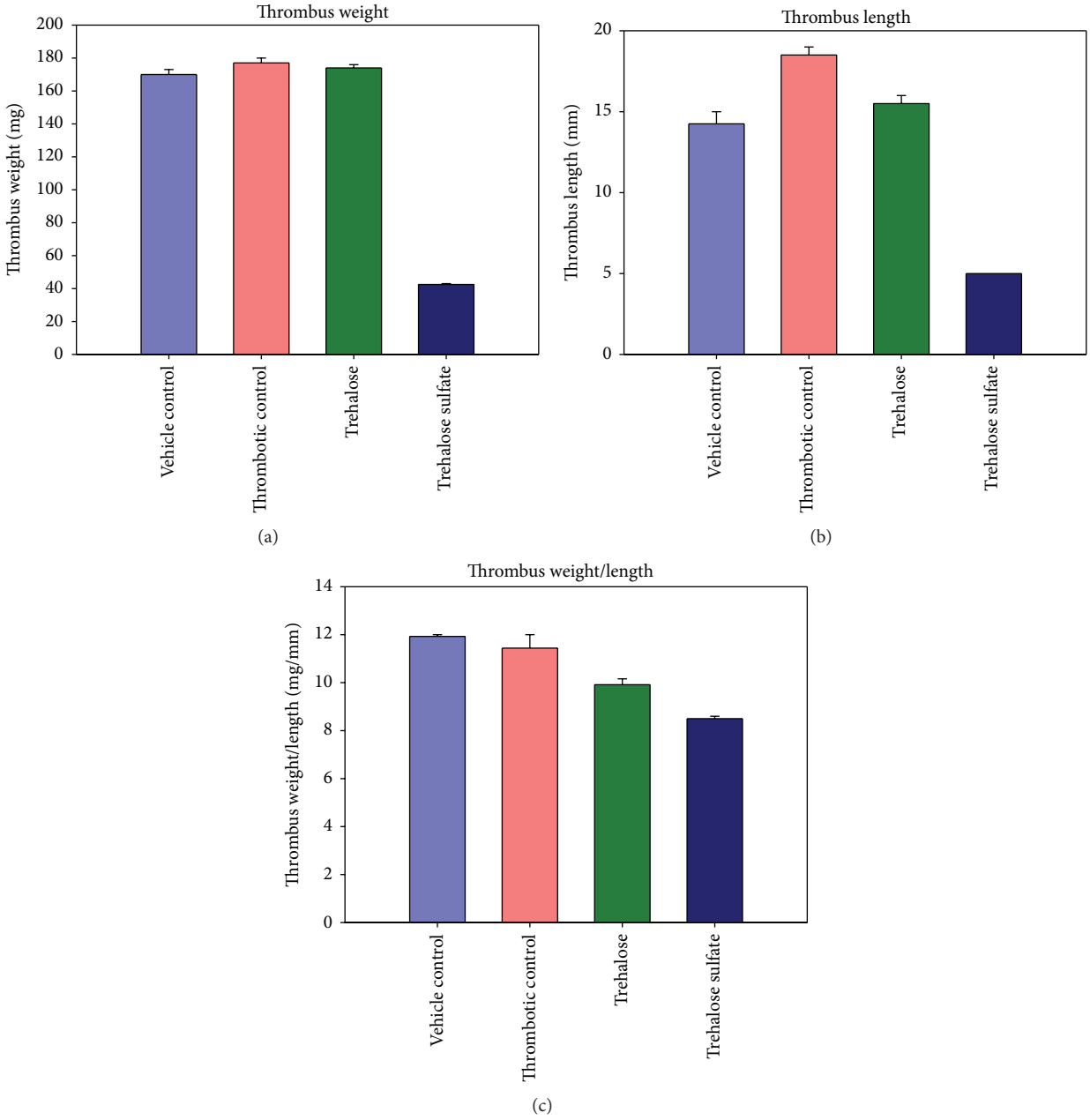


Figure 5: Comparison of thrombi from control, trehalose and trehalose octasulfate treated thrombosis model animals; (a) weight, (b) length, and (c) weight/length ratio of the thrombus formed inside the ligated IVC.

thrombus exhibited with an increased length and weight of the thrombus and were associated with a significant increase in the derived parameter, ratio of thrombus weight to IVC length. The thrombus harvested from the vehicle control group of rats (vehicle or buffer injected) was also considerably prominent, with dimensions close to the thrombotic control (no treatment). The preinjection with trehalose octasulfate (2.5 mg/kg body weight) caused a marked decrease in thrombus size, as evident by the prominent reductions in the thrombus dimensions as compared to the corresponding control group (Figures 4 and 5). Figures 4 and 5 show the antithrombotic effect of trehalose octasulfate, an appreciable reduction in thrombus weight and length in the trehalose octasulfate treated rat as compared to the thrombotic control rat was observed. A consequent decrease in thrombus weight/length ratio as compared to the thrombotic rat could be observed (Figure 5(c)). These results clearly indicate the *in vivo* antithrombotic potential of trehalose octasulfate. Thus, in addition to *ex vivo* anticlotting potency, trehalose octasulfate was found to possess *in vivo* antithrombotic activity, as evident by the significant inhibition of occlusion-induced venous thrombosis in rats injected with this compound. Further, while establishing the optimum dosage regimen, trehalose octasulfate was able to completely inhibit thrombus

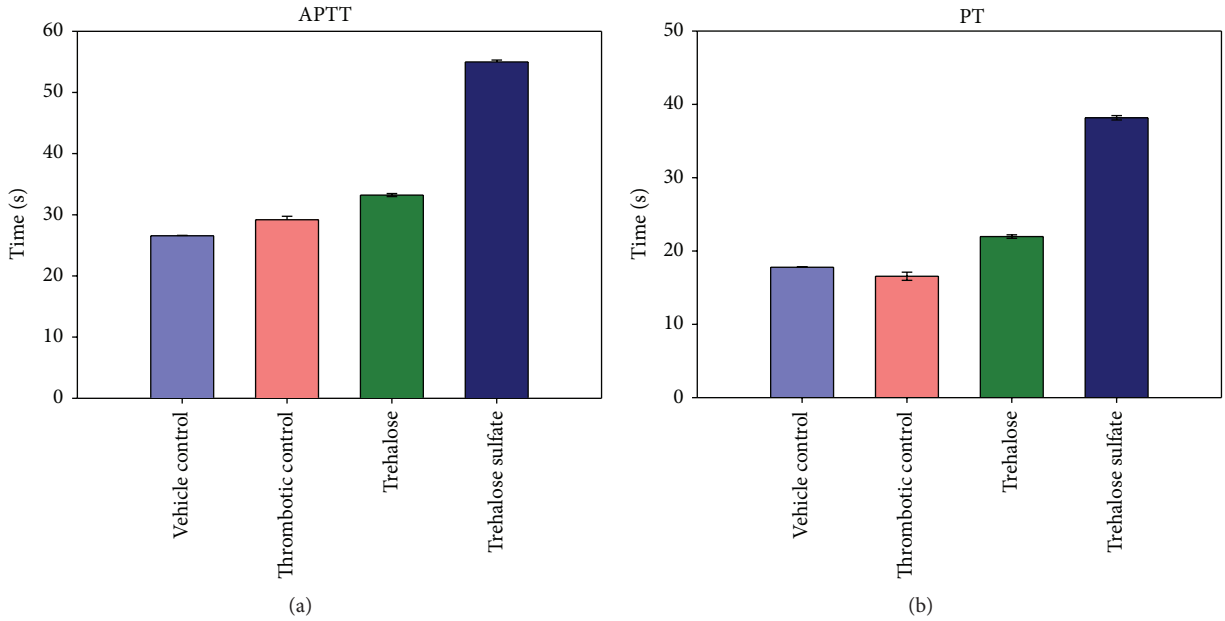


Figure 6: Effect of trehalose and trehalose octasulfate on (a) APTT and (b) PT in rat plasma after IV infusion of the compounds.

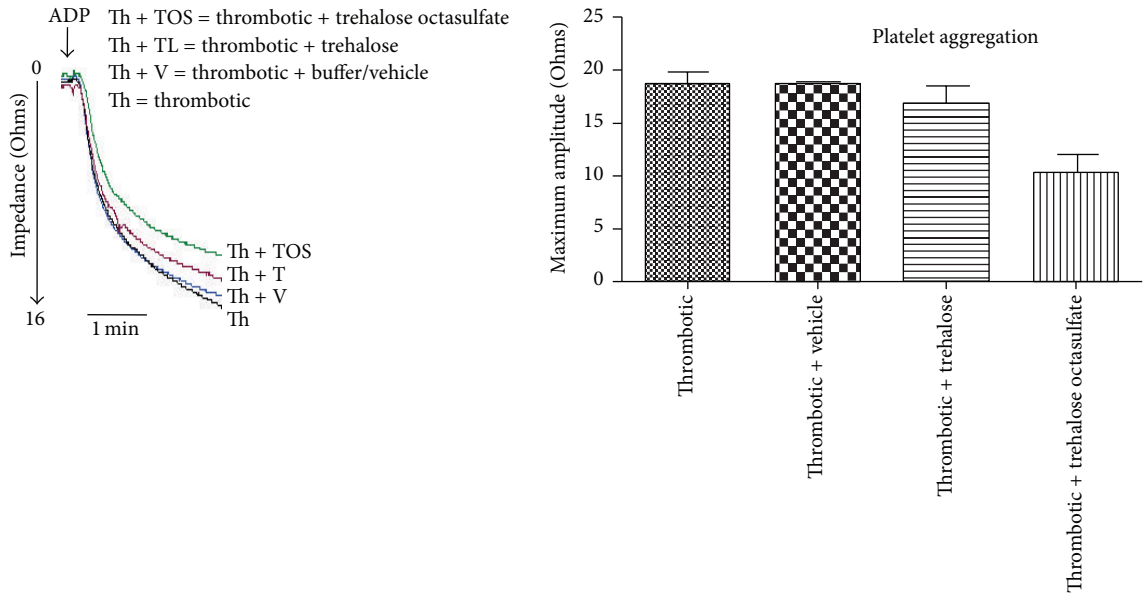


Figure 7: Effect of trehalose octasulfate on platelet aggregation, in whole blood of rat injected with the compounds, $P < 0.05$.

formation at the highest dose tested (10 mg/Kg body weight). This further ascertained a dose-dependent effect of trehalose octasulfate, which may enable to set its optimum dosage.

3.5. In Vivo Clotting Assays. In consistency with the *in vitro* anticoagulating effect of trehalose octasulfate, coagulation parameters APTT and PT were prolonged *in vivo* as well. Plasma isolated from the trehalose octasulfate injected rats showed a delayed coagulation in both the intrinsic and extrinsic pathways at the optimum antithrombotic dosage. Figure 6 shows the APTT and PT profile of trehalose octasulfate treated rat. Approximately 2-fold rise in both the clotting times was observed as compared to both the control plasma and the plasma isolated from ligated (thrombotic) animal.

3.6. Antiplatelet Effect. Whole blood aggregation study was conducted to evaluate the efficacy of trehalose octasulfate on platelet aggregation. Trehalose octasulfate appreciably decreased the impedance of ADP induced platelet aggregation. Figure 7 shows a representative platelet aggregation curve of trehalose octasulfate treated animals, measured in

freshly collected whole blood. A decrease in platelet aggregation in blood collected from trehalose octasulfate injected animals was observed. Therefore, platelet aggregation studies revealed the antiplatelet activity of trehalose octasulfate, suggesting a dual mechanism of action in modulating coagulation.

4. Conclusion

Trehalose octasulfate in the present study showed a significant and dose-dependent retardation of intrinsic and extrinsic coagulation pathways both *in vivo* and *ex vivo*. Unmodified/nonsulfated trehalose did not affect coagulation, indicative of sulfation being an ideal strategy to impart anticoagulant properties in natural scaffolds. Trehalose octasulfate also showed promising antithrombotic property against IVC statis-induced venous thrombosis model in rats, which is one of the most efficient models for screening antithrombotic activity of the drugs. Further, the antiplatelet activity of trehalose octasulfate in terms of ADP induced platelet aggregation indicated their antiplatelet activity. Thus, from the mechanistic point, trehalose octasulfate employs a multitarget strategy to modulate the activity of multiple components of thrombus formation, suggesting a dual mechanism of action. However, the molecular details of its role in reducing the coagulation need to be completely understood.

Abbreviations

AT: Antithrombin
HCII: Heparin cofactor II
ADP: Adenosine 5′-Diphosphate
HBD: Heparin binding domain
DVT: Deep vein thrombosis
PE: Pulmonary embolism
VTE: Venous thromboembolism
LMWH: Low molecular weight heparin
fIIa: Thrombin
fXa: Factor Xa
DHPs: Dehydrogenation polymers
APTT: Activated partial thromboplastin time
PT: Prothrombin time
TLC: Thin-layer chromatography
DMA: Dimethylacetamide.

Conflict of Interests

The authors have no conflict of interests to declare.

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Research Article

Cost-Effectiveness of Anticoagulation in Patients with Nonvalvular Atrial Fibrillation with Edoxaban Compared to Warfarin in Germany

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We compared the cost-utility analysis for edoxaban at both doses with that of dabigatran at both doses, rivaroxaban, and apixaban (non vitamin K antagonist oral anticoagulants, NOAC) in a German population. Data of clinical outcome events were taken from edoxaban's ENGAGE-AF, dabigatran's RE-LY, rivaroxaban's ROCKET, and apixaban's ARISTOTLE trials. The base-case analyses of a 65-year-old person with a CHADS2 score >1 gained 0.17 and 0.21 quality-adjusted life years over warfarin for 30 mg od and 60 mg od edoxaban, respectively. The incremental cost-effectiveness ratio was 50.000 and 68.000 euro per quality-adjusted life years for the higher and lower dose of edoxaban (Monte Carlo simulation). These findings were also similar to those for apixaban and more cost-effective than the other NOAC regimens. The current market costs for direct oral anticoagulants are high in relation to the quality of life gained from a German public health care insurance perspective. The willingness-to-pay threshold was lowest for 60 mg edoxaban compared to all direct oral anticoagulants and for 30 mg edoxaban compared to dabigatran and rivaroxaban.

1. Introduction

In a large prospective randomized double blind and double dummy study (ENGAGE-AF), once-daily dosing of the oral direct factor Xa inhibitor edoxaban 60 mg (dose adjusted to 30 mg) was noninferior warfarin for the prevention of the primary endpoint of stroke and systemic embolic events in patients with nonvalvular atrial fibrillation (NVAF), when compared to warfarin with INR (international normalized ratio) adjusted to 2 to 3; edoxaban 60 mg was also associated with a significantly lower rate of major bleeding and cardiovascular mortality [1]. Other nonvitamin K oral antagonist oral anticoagulants (NOAC, dabigatran, rivaroxaban, and apixaban) have proven to be superior or at least equivalent for stroke prevention and occurrence of severe bleeding complications in patients with NVAF compared to dose-adjusted warfarin. In the RE-LY trial 110 mg bid dabigatran was noninferior and 150 mg bid dabigatran was superior to dose-adjusted warfarin for prevention of stroke and systemic embolism and both doses resulted in less intracranial bleeding [2]. In the double blind ROCKET-AF trial patients on rivaroxaban 20 mg od had reduced rates of stroke and systemic embolism and comparable major bleeding incidences compared to warfarin [3]. In the double blind ARISTOTLE trial, apixaban was associated with lower rates of strokes and major bleeding and reduced incidence of cardiovascular deaths compared to warfarin [4].

One of the main limitations for prescribing NOAC in real life is their higher daily price compared to warfarin. The pharmacoeconomic aspects of dabigatran, rivaroxaban, and

apixaban were analysed for many countries. All have been demonstrated to be cost-effective in many countries for the health care system mainly based on the reduced incidence of major bleeding complications but also for some of the NOAC, due to a lower incidence of ischemic stroke and systemic embolism [5]. Related analyses include a willingness to pay, an incremental cost-effectiveness ratio (ICER) of a currency (euro, USD, or any other currency) per quality-adjusted life years (QALY) for dabigatran, rivaroxaban, or apixaban compared with warfarin. As the results of all these investigations were similar regarding the socioeconomic benefit of NOAC compared to warfarin, it was argued if these analyses have to be performed separately for every country. National guidelines for economic evaluation agree that a given country's unit costs should be applied to calculations of costs when adapting an analysis for local decision making [6]. As an example, lower prices for dabigatran in Europe compared with those in the USA were resulted in more favourable cost-effectiveness ratios despite smaller estimated gains in quality-adjusted survival [7]. Here we determined the cost-effectiveness of 60 mg od and 30 mg od edoxaban from a German payers perspective and compared the results with those obtained for the approved NOAC dabigatran (both doses 110 mg bid and 150 mg bid), rivaroxaban, and apixaban [5].

2. Methods

2.1. Markov Decision Model and Data Sources. We used the Markov decision model to analyse the QALYs, total costs (one-time costs for events, rehabilitation costs for inpatient and ambulatory care, inpatient medical treatment costs, and daily costs for drugs), and ICER based on the data of the ENGAGE-AF [1] study. The results were compared with our data previously derived from the RE-LY, ROCKET-AF, and ARISTOTLE trials [5] under a German health care insurance perspective. The following health states and outcome events were included: healthy with NVAF, transient ischemic attack, ischemic stroke (fatal, moderate to severe, and mild), haemorrhage (fatal, moderate to severe intracranial, mild intracranial, major noncerebral, and minor noncerebral), myocardial infarction (MI), recurrent and combined events, and cardiovascular mortality using the results from the ENGAGE-AF trial and costs for the German population [8] (Figure 1). Definitions of these events were taken from the ENGAGE-AF study [1]. Event probabilities were not included if they were not reported consistently across the studies (systemic embolism, pulmonary embolism, hemorrhagic stroke, and bleeding in other locations) (Table 1).

For the base-case analysis we used a hypothetical population cohort of patients with the starting age of 65 years with NVAF who were at increased risk for stroke (CHADS2-score >1) with no contraindications to anticoagulation as reported in the ENGAGE study [1]. Our results were expressed in quality-adjusted life years (QALY), 2012 euro, and incremental cost-effectiveness ratios (ICER: total costs (€, edoxaban) - total costs (€, warfarin)/QALY (edoxban) - QALY (warfarin)). We applied utilities and costs to each outcome yearly or event driven and discounted costs and benefits at 5% annually [9, 10]. A half cycle correction was done for each model, using a cycle length of 1 year. We quantified QALYs, risk for adverse events, and net cost for a time horizon of 20 years for the German population [11]. This time frame is used for CEA investigations taking well in account the much shorter treatment period of the studies from which the data are used (12).

2.2. Probability of Adverse Outcome Events and of Endpoints. The adverse outcome events and endpoints with the 95% confidence intervals (CIs) were taken from the ENGAGE-AF study [1] and were found to be similar with those reported by Freeman et al. [12]. Intention to treat (ITT) values were taken for ischemic stroke, myocardial infarction, death from cardiovascular cause, and death from nonvascular cause and on-treatment values (OT) for bleeding events (minor bleeding, major bleeding, and ICH) for calculations in the sensitivity analyses (Table 1).

2.3. Severity of Stroke and Haemorrhage. Ischemic stroke was classified into fatal, moderate to severe, mild, and no neurologic deficit (4 categories) as reported [12]. A second mild ischemic stroke was defined to result in a moderate to severe ischemic stroke or death and a second moderate stroke in a severe ischemic stroke, reduced life quality, or death [13].

Haemorrhage was categorized into fatal, ICH with moderate to severe neurologic sequelae, ICH with no neurological deficit, major extracerebral haemorrhage, and minor extracerebral haemorrhage [12, 13]. A moderate to severe ischemic stroke followed by an ICH was categorized into a moderate to severe neurologic outcome. Decrease of quality of life depended on the severity of outcome and resulted in different costs according to the German health care system [14].

2.4. Mortality Rates. The mortality rates (death from vascular cause and death from any other cause) were taken from the ENGAGE-AF study [1]. The annual rates for death from any other cause were taken from published German mortality tables [8].

2.5. Quality of Life Utilities. The quality-adjusted life years (QALY = survived life years adjusted for quality of life) [15] were calculated by multiplying the time spent within a health state with the corresponding utility value. The utility values for warfarin were taken from data on patients with NVAF who underwent time trade-off and standard gamble methods to estimate their quality of life [12, 16]. All utility values were discounted in our model [17]. A utility of "1" represents a completely healthy status and a utility of "0" represents death. The mean utility for warfarin was 0.987 [12, 16, 18]. The utility for edoxaban was estimated as published for ximelagatran [12, 18], dabigatran, rivaroxaban, and apixaban [5, 12, 13, 16, 18–22].

2.6. Costs for Drugs and Outcome Events. One-time costs for most events were taken from the institute for payment regulations in German hospitals (Institut für Entgeltsystem

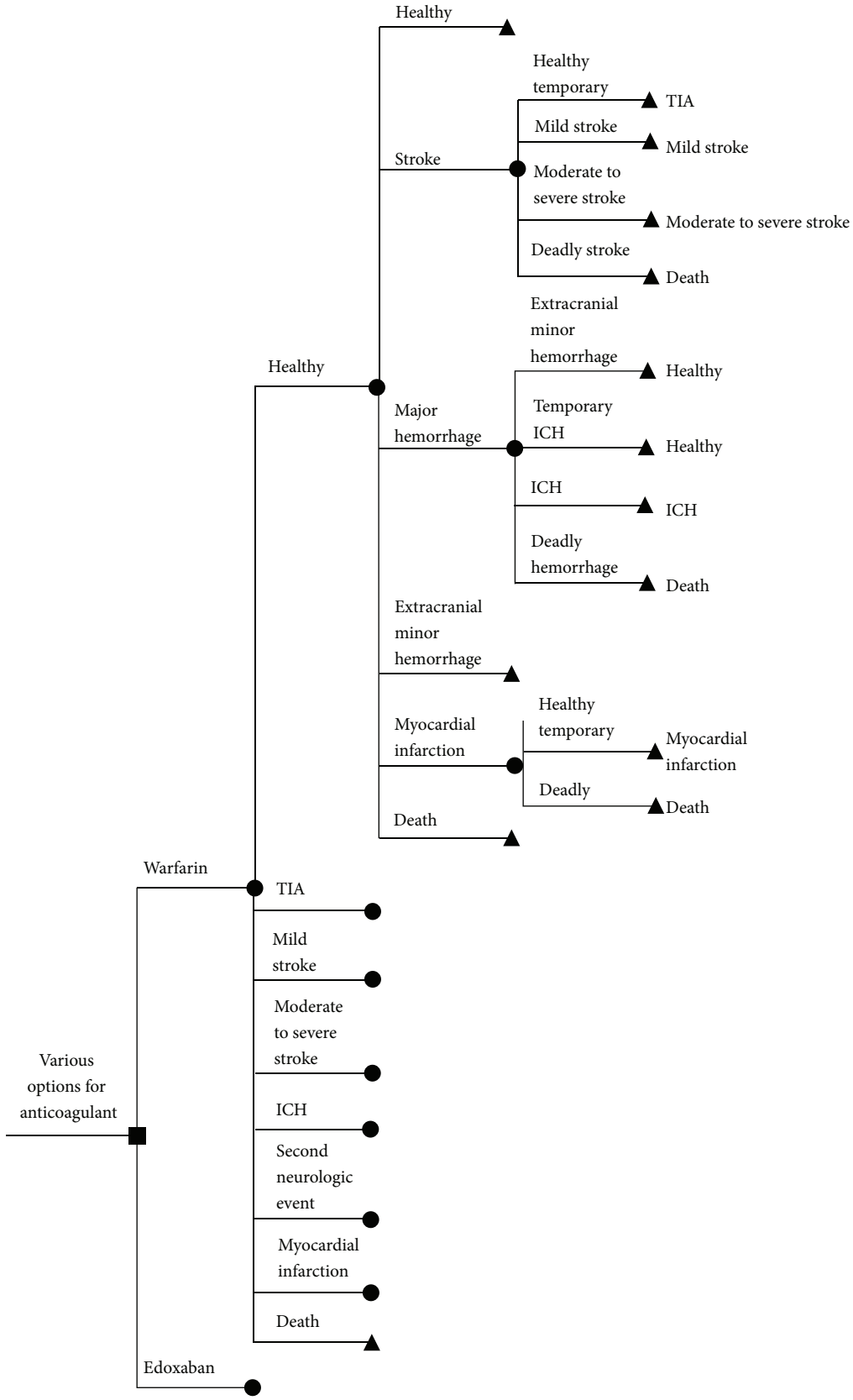

Figure 1: Outline of the Markov model for data of the ENGAGE-AF study. Here one dose of edoxaban is given as an example used in the Markov model. ICH intracerebral haemorrhage; TIA transient ischemic attack (modified from [5]).

Table 1: Base-case values and ranges for probabilities of stroke, haemorrhage, myocardial infarction, and death used for sensitivity analyses of NOACs.

Variable	Edoxaban 30 mg	Edoxaban 60 mg	Reference	Dabigatran 110 mg	Dabigatran 150 mg	Reference [5]	Rivaroxaban	Reference [5]	Apixaban	Reference [5]
Stroke										
Annual rate of ischemic stroke (%)										
NOAC	1.77 (1.58–1.96)	1.25 (1.09–1.41)	[1]	1.34 (1.13–1.55)	0.92 (0.75–1.09)	[2]	1.34 (1.12–1.55)	[3]	0.97 (0.82–1.12)	[4]
Warfarin	1.25 (1.09–1.41)	1.25 (1.09–1.41)	[1]	1.2 (1.00–1.40)	1.2 (1.00–1.40)	[2]	1.42 (1.20–1.63)	[3]	1.05 (0.89–1.21)	[4]
Ischemic strokes with warfarin or NOAC (%)										
Fatal (within 30 d)	8.20 (5.50–10.90)	8.20 (5.50–10.90)	[12, 18]	8.20 (5.50–10.90)	8.20 (5.50–10.90)	[12, 18]	8.20 (5.50–10.90)	[12, 18]	8.20 (5.50–10.90)	[12, 18]
Moderate to severe neurologic sequelae	40.20 (35.30–45.10)	40.20 (35.30–45.10)	[12, 18]	40.20 (35.30–45.10)	40.20 (35.30–45.10)	[12, 18]	40.20 (35.30–45.10)	[12, 18]	40.20 (35.30–45.10)	[12, 18]
Mild neurologic sequelae	42.50 (37.60–47.40)	42.50 (37.60–47.40)	[12, 18]	42.50 (37.60–47.40)	42.50 (37.60–47.40)	[12, 18]	42.50 (37.60–47.40)	[12, 18]	42.50 (37.60–47.40)	[12, 18]
No residual neurologic sequelae	9.10 (6.20–12.00)	9.10 (6.20–12.00)	[12, 18]	9.10 (6.20–12.00)	9.10 (6.20–12.00)	[12, 18]	9.10 (6.20–12.00)	[12, 18]	9.10 (6.20–12.00)	[12, 18]
Hemorrhage										
Annual rate of ICH (%)										
NOAC	0.26 (0.18–0.34)	0.39 (0.29–0.49)	[1]	0.23 (0.14–0.32)	0.30 (0.20–0.40)	[2]	0.5 (0.37–0.63)	[3]	0.33 (0.24–0.42)	[4]
Warfarin	0.85 (0.70–0.99)	0.85 (0.70–0.99)	[1]	0.74 (0.58–0.90)	0.74 (0.58–0.90)	[2]	0.7 (0.55–0.85)	[3]	0.80 (0.66–0.94)	[4]
Annual rate of extracranial hemorrhage (%)										
NOAC	1.37 (1.19–1.55)	2.36 (2.12–2.60)	[1]	2.51 (2.23–2.79)	2.84 (2.54–3.14)	[2]	3.11 (2.78–3.44)	[3]	1.79 (1.58–2.00)	[4]
Warfarin	2.6 (2.35–2.85)	2.6 (2.35–2.85)	[1]	2.67 (2.38–2.96)	2.67 (2.38–2.96)	[2]	2.71 (2.40–3.02)	[3]	2.27 (2.03–2.51)	[4]
Annual rate of major hemorrhage (%)										
NOAC	1.61 (1.41–1.81)	2.75 (2.49–3.01)	[1]	2.71 (2.41–3.01)	3.11 (2.80–3.24)	[2]	3.6 (3.24–3.96)	[3]	2.13 (1.90–2.36)	[4]
Warfarin	3.34 (3.14–3.72)	3.34 (3.14–3.72)	[1]	3.36 (3.03–3.69)	3.36 (3.03–3.69)	[2]	3.4 (3.06–3.74)	[3]	3.09 (2.81–3.37)	[4]
Annual rate of minor hemorrhage (%)										
NOAC	6.6 (6.18–7.02)	8.67 (8.18–9.16)	[1]	13.20 (12.51–13.81)	14.80 (14.15–15.53)	[2]	11.8 (11.13–12.47)	[3]	14.03 (13.37–14.69)	[4]

Table 1: Continued.

Variable	Edoxaban 30 mg	Edoxaban 60 mg	Reference	Dabigatran 110 mg	Dabigatran 150 mg	Reference [5]	Rivaroxaban	Reference [5]	Apixaban	Reference [5]
Warfarin	10.15 (9.62–10.68)	10.15 (9.62–10.68)	[1]	16.40 (15.64–17.10)	16.40 (15.64–17.10)	[2]	11.4 (10.74–12.06)	[3]	19.79 (18.96–20.62)	[4]
Myocardial infarction										
Annual rate of myocardial infarction (%)										
NOAC	0.89 (0.76–1.02)	0.7 (0.58–0.82)	[1]	0.72 (0.57–0.87)	0.74 (0.59–0.89)	[2]	0.91 (0.73–1.09)	[3]	0.53 (0.42–0.64)	[4]
Warfarin	0.75 (0.63–0.87)	0.75 (0.63–0.87)	[1]	0.53 (0.40–0.66)	0.53 (0.40–0.66)	[2]	1.12 (0.92–1.32)	[3]	0.61 (0.49–0.73)	[4]
Death										
Age at start (years)	65	65	Assumption	65	65	Assumption	65	Assumption	65	Assumption
Death of cardiovascular cause (%/yr)										
NOAC	2.71 (2.48–2.94)	2.74 (2.51–2.97)	[1]	2.43 (2.15–2.71)	2.28 (2.01–2.55)	[2]	1.53 (1.30–1.76)	[3]	1.80 (1.60–2.00)	[4]
Warfarin	3.17 (2.92–3.42)	3.17 (2.92–3.42)	[1]	2.69 (2.39–2.99)	2.69 (2.39–2.99)	[2]	1.71 (1.47–1.95)	[3]	2.02 (1.81–2.23)	[4]
Death of causes other than cardiovascular or of unknown cause (%/yr)	Age adjusted from mortality tables (see reference)	Age adjusted from mortality tables (see reference)	[8]	Age adjusted from mortality tables (see reference)	Age adjusted from mortality tables (see reference)	[8]	Age adjusted from mortality tables (see reference)	[8]	Age adjusted from mortality tables (see reference)	[8]

im Krankenhaus, *InEK*) which included German-diagnosis related groups (G-DRGs) [14] and were expressed in euro and reflected from the health care insurance perspective in Germany in 2012. Costs for bleeding events are not included in the G-DRGs and were taken from the literature [23]. Of note, our analysis did not include indirect costs and they were calculated over a time horizon of 20 years for the German population [11] with a discount of 5% (0% and 10% in the sensitivity analyses) per year [9, 10, 24, 25]. Rehabilitation costs were included following ischemic strokes, ICH, and myocardial infraction, but not major bleedings without need for rehabilitation.

Cost for warfarin therapy, a mean of three-week interval for INR measurements with patient office visits, was set at 153€ annually [26, 27]. The retail costs and daily costs for edoxaban according to costs of other NOACs (3.37 Euro per day) and phenprocoumon (0.20€ per day) were taken from pharmacies and the "red list" (German equivalent of "The Physicians' Desk Reference Manual" in USA, e.g.) [26]. Total costs for the drugs warfarin respected the event costs according to the *InEK*. These entries were used to examine the cost-effectiveness based on the event probabilities (Tables 1 and 3).

2.7. Sensitivity Analyses. One-way sensitivity analyses of all variables were included in the decision models over their plausible ranges. Ranges for clinical events were derived from confidence intervals (CI) for event probabilities [1]. Medication costs for edoxaban and phenprocoumon were included as reported above. Two-way sensitivity analyses were performed for combinations of stroke and ICH using the values of warfarin [1].

2.8. Probabilistic Sensitivity Analysis. The Monte Carlo simulations (MCS) were made using random sampling and random distribution of variables for 10 000. Beta-distribution of the event probabilities was assumed for the calculation except for subcategories of stroke using Dirichlet distribution [12]. The Dirichlet distribution was chosen to show the probability of our subclassifications. Maximum and minimum ranges of costs for each adverse event were calculated using the German *InEK* and a gamma- and log normal distribution.

2.9. Statistical Methods. The models and analyses were created with TreeAge Pro 2013 and Microsoft Excel 2003.

3. Results

3.1. Base-Case Analysis, One-Way Sensitivity Analyses, and Two-Way Sensitivity Analysis. The calculated QALY, the total costs, and the ICER with edoxaban and warfarin are shown in Tables 2 and 3. For comparison the data derived from the outcome events of the RE-LY, ROCKET-AF, and ARISTOTLE trials are included as published [5]. The ICER for edoxaban 60 mg was lower compared to edoxaban 30 mg daily. Compared to the other NOAC regimens, edoxaban 60 mg had the lowest ICER (Table 4). This difference is mainly due to the lower QALY of the warfarin group in the ENGAGE-AF study compared to the other studies.

The results of the one-way sensitivity analysis showed that the costs for edoxaban (both doses), the quality of life utilities, the treatment of ischemic stroke, and the treatment of major and intracerebral bleeding complications were important values in our model. Edoxaban and apixaban thus were most cost-effective compared to the other NOAC [5].

3.2. Two-Way Sensitivity Analyses. The two-way sensitivity analyses of key variables for varying risk rates for ischemic stroke and intracerebral haemorrhage showed that both doses of edoxaban were preferable as a therapy for combinations of moderate to high risks for ischemic stroke and high risk of intracerebral haemorrhage at a set willingness to pay of 50.000€ per QALY against INR-dose-adjusted warfarin. These findings were also similar to those for apixaban and more cost-effective than the other NOAC regimens [5]. Using the probabilistic sensitivity analysis (PSA) in the Monte Carlo simulation by varying all variables simultaneously resulted in a willingness-to-pay threshold (PSA ICER) of 52.000€ per QALY for edoxaban 60 mg od and 69.600€ per QALY for edoxaban 30 mg od (Table 4). A similar market price was assumed for edoxaban at both doses as for the other NOAC. These data were similar to the base-case results and similar to apixaban and lower than for rivaroxaban and dabigatran at both doses as reported earlier [5]. The analysis demonstrates the cost-effectiveness of both doses of edoxaban for prevention of cerebral and noncerebral embolic events in patients with nonvalvular AF. The ICERs are comparable to apixaban and lower compared to dabigatran at both doses and rivaroxaban, despite an almost identical daily price for all NOACs.

3.3. Probabilistic Sensitivity Analyses: Monte Carlo Simulation. The various willingness-to-pay thresholds were analysed by using the probabilistic sensitivity analysis (PSA) in the Monte Carlo simulation (MCS) and varying all variables simultaneously. As a result edoxaban 60 mg od and edoxaban 30 mg od were cost-effective at willingness-to-pay threshold of 52.000€ per QALY and 67.000€ per QALY higher (Figure 2). The results of the cost-effectiveness at willingness-to-pay thresholds for the other NOAC treatment regimens are shown for comparison at Krejczy et al. [5]. The PSA results were similar to the base-case results.

3.4. Subgroup Analyses. Total costs increased and the ICER decreased in a base-case analysis for a 65- to 85-year-old cohort from the German public health care insurance perspective excluding a discount for costs and utility values.

The absolute numbers of QALY and total costs decreased when costs and utility values were discounted by 10%. In the same time the ICER increased in a base-case data for a 65- to 85-year-old cohort from the German public health care insurance perspective.

Table 2: Base-case values and ranges for quality of life estimates used in sensitivity analyses for NOACs.

Variable	Edoxaban 30 mg	Edoxaban 60 mg	Reference	Dabigatran 110 mg bid	Dabigatran 150 mg bid	Reference [5]	Rivaroxaban	Reference [5]	Apixaban	Reference [5]
Quality of life estimates (utility)										
NOAC	0.994 (0.975–1.00)	0.994 (0.975–1.00)	[12, 13, 16, 18]	0.994 (0.975–1.00)	0.994 (0.975–1.00)	[12, 13, 16, 18]	0.994 (0.975–1.00)	[12, 13, 16, 18]	0.994 (0.975–1.00)	[12, 13, 16, 18]
Warfarin	0.987 (0.953–1.00)	0.987 (0.953–1.00)	[12, 16, 18]	0.987 (0.953–1.00)	0.987 (0.953–1.00)	[12, 16, 18]	0.987 (0.953–1.00)	[12, 16, 18]	0.987 (0.953–1.00)	[12, 16, 18]
Neurological sequelae										
Mild	0.87 (0.00–1.00)	0.87 (0.00–1.00)	[21]	0.87 (0.00–1.00)	0.87 (0.00–1.00)	[21]	0.87 (0.00–1.00)	[21]	0.87 (0.00–1.00)	[21]
Moderate	0.68 (0.00–1.00)	0.68 (0.00–1.00)	[21]	0.68 (0.00–1.00)	0.68 (0.00–1.00)	[21]	0.68 (0.00–1.00)	[21]	0.68 (0.00–1.00)	[21]
Serious	0.52 (0.00–1.00)	0.52 (0.00–1.00)	[21]	0.52 (0.00–1.00)	0.52 (0.00–1.00)	[21]	0.52 (0.00–1.00)	[21]	0.52 (0.00–1.00)	[21]
Recurrent event	0.12 (0.00–1.00)	0.12 (0.00–1.00)	[13]	0.12 (0.00–1.00)	0.12 (0.00–1.00)	[13]	0.12 (0.00–1.00)	[13]	0.12 (0.00–1.00)	[13]
Myocardial infarction	0.5 (0.00–1.00)	0.5 (0.00–1.00)	Assumption [12, 20]	0.5 (0.00–1.00)	0.5 (0.00–1.00)	Assumption [12, 20]	0.5 (0.00–1.00)	Assumption [12, 20]	0.5 (0.00–1.00)	Assumption [12, 20]
Hemorrhage										
Major hemorrhage	0.85 (0.00–1.00)	0.85 (0.00–1.00)	Assumption [12, 19, 22]	0.85 (0.00–1.00)	0.85 (0.00–1.00)	Assumption [12, 19, 22]	0.85 (0.00–1.00)	Assumption [12, 19, 22]	0.85 (0.00–1.00)	Assumption [12, 19, 22]
Minor hemorrhage	0.95 (0.00–1.00)	0.95 (0.00–1.00)	[12, 18]	0.95 (0.00–1.00)	0.95 (0.00–1.00)	[12, 18]	0.95 (0.00–1.00)	[12, 18]	0.95 (0.00–1.00)	[12, 18]

Table 3: Base-case values and ranges for costs used in sensitivity analyses for NOACs.

Variable	Edoxaban 30 mg	Edoxaban 60 mg	Ref	Dabigatran 110 mg bid	Dabigatran bid 150 mg	Ref [5]	Rivaroxaban	Ref [5]	Apixaban	Ref [5]
Costs										
Daily cost of medicine (euro)										
NOAC	3.37 (0.00–5.00)	3.37 (0.00–5.00)	Ass	3.38 (0.00–5.00)	3.38 (0.00–5.00)	[26]	3.20 (0.00–5.00)	[26]	3.54 (0.00–5.00)	[26]
Warfarin	0.20 (0.00–1.00)	0.20 (0.00–1.00)	[26]	0.20 (0.00–1.00)	0.20 (0.00–1.00)	[26]	0.20 (0.00–1.00)	[26]	0.20 (0.00–1.00)	[26]
Costs per INR determination	0.64 (0.46–0.79)	0.64 (0.46–0.79)	Ass	0.64 (0.46–0.79)	0.64 (0.46–0.79)	Ass	0.64 (0.46–0.79)	Ass	0.64 (0.46–0.79)	Ass
One-time costs of neurologic event (stroke or intracranial hemorrhage) (euro)										
Serious	7 000 (901–46 558)	7 000 (901–46 558)	[14]	7 000 (901–46 558)	7 000 (901–46 558)	[14]	7 000 (901–46 558)	[14]	7 000 (901–46 558)	[14]
Moderate	4 233 (901–46 558)	4 233 (901–46 558)	[14]	4 233 (901–46 558)	4 233 (901–46 558)	[14]	4 233 (901–46 558)	[14]	4 233 (901–46 558)	[14]
Mild	3 942 (2 014–4 233)	3 942 (2 014–4 233)	[14]	3 942 (2 014–4 233)	3 942 (2 014–4 233)	[14]	3 942 (2 014–4 233)	[14]	3 942 (2 014–4 233)	[14]
One-time costs for myocardial infarction (euro)	10 000 (2 743–48 023)	10 000 (2 743–48 023)	[14]	10 000 (2 743–48 023)	10 000 (2 743–48 023)	[14]	10 000 (2 743–48 023)	[14]	10 000 (2 743–48 023)	[14]
One-time costs for hemorrhage (euro)										
Major hemorrhage	2 500 (891–5 415)	2 500 (891–5 415)	[23]	2 500 (891–5 415)	2 500 (891–5 415)	[23]	2 500 (891–5 415)	[23]	2 500 (891–5 415)	[23]
Minor hemorrhage	50 (0.00–100)	50 (0.00–100)	Ass	50 (0.00–100)	50 (0.00–100)	Ass	50 (0.00–100)	Ass	50 (0.00–100)	Ass
Rehabilitation costs (euro)										
Annual ambulant rehabilitation costs	2 300 (1 800–2 800)	2 300 (1 800–2 800)	[43]	2 300 (1 800–2 800)	2 300 (1 800–2 800)	[43]	2 300 (1 800–2 800)	[43]	2 300 (1 800–2 800)	[43]
Inpatient rehabilitation costs per patient	8 000 (2 000–14 000)	8 000 (2 000–14 000)	[43]	8 000 (2 000–14 000)	8 000 (2 000–14 000)	[43]	8 000 (2 000–14 000)	[43]	8 000 (2 000–14 000)	[43]
Annual costs for further medical treatment	2 900 (2 300–4 000)	2 900 (2 300–4 000)	[43]	2 900 (2 300–4 000)	2 900 (2 300–4 000)	[43]	2 900 (2 300–4 000)	[43]	2 900 (2 300–4 000)	[43]
Costs in case of death (euro)	2 500	2 500	[14]	2 500	2 500	[14]	2 500	[14]	2 500	[14]
Discounting (%)	5 (0–10)	5 (0–10)	[9, 10, 24]	5 (0–10)	5 (0–10)	[9, 10, 24]	5 (0–10)	[9, 10, 24]	5 (0–10)	[9, 10, 24]

Ref = reference; Ass = assumption.

Table 4: Results of the base-case analysis for a 65-year-old population over a time horizon of 20 years from a German healthcare insurance perspective.

Trial	Anticoagulant	QALY	Total costs €	ICER €/QALY	Daily price €/d	PSA ICER €/QALY
ENGAGE-AF	Edoxaban 30 mg od	7.65	21 052	68 275	3.37	69 600
	Warfarin	7.48	9 747		0.20	
	Edoxaban 60 mg od	7.69	20 157	50 411	3.37	52 000
	Warfarin	7.48	9 747		0.20	
RE-LY [5]	Dabigatran 110 mg bid	7.68	20 048	294 349	3.38	278 000
	Warfarin	7.64	7 622		0.20	
	Dabigatran 150 mg bid	7.71	19 537	163 184	3.38	174 000
	Warfarin	7.64	7 622		0.20	
ROCKET-AF [5]	Rivaroxaban 20 mg od	7.67	19 874	133 926	3.20	130 500
	Warfarin	7.59	9 069		0.20	
ARISTOTLE [5]	Apixaban 5 mg bid	7.75	19 885	57 245	3.54	55 500
	Warfarin	7.56	8 915		0.20	

4. Discussion

The present study shows that the two dosage regimens of edoxaban 60 mg od and edoxaban 30 mg od are nearly cost-effective bid for prevention of ischemic stroke and systemic embolic events in patients with NVAF based on the data of the ENGAGE-AF study based on a societal willingness to pay comparable to data from other countries. Comparing these data obtained with the other four treatment regimens with dabigatran 110 mg bid, dabigatran 150 mg bid (RE-LY study [2]), rivaroxaban 20 mg od (ROCKET-AF study [3]), and apixaban 5 mg (ARISTOTLE trial [4]) which are available in Germany and many other countries edoxaban 60 mg was the most cost-effective followed by apixaban 5 mg bid, edoxaban 30 mg od, and the 3 remaining treatment regimens (Table 4). Of note these data were obtained using the same inputs into the Markov model based on the German insurance system. From the public health care insurance view, only edoxaban 60 mg od treatment was nearly cost-effective at a hypothetical willingness-to-pay threshold of 50.000 EUR for patients at a moderate or higher risk of stroke (CHADS2-score >1) compared to INR-adjusted warfarin with current German market costs.

Similar analyses were reported for the two doses of dabigatran based on the health care costs and willingness to pay in USA [12, 13], Canada [28], United Kingdom [29, 30], Denmark [31], Sweden [32], and Portugal [33], for rivaroxaban in USA [34], for all three NOACs in USA [35], Canada [36], Germany [5], and Italy [37], and as a comparative analysis for dabigatran and rivaroxaban in Canada [38]. All analyses for dabigatran used the Markov model for calculation of the QALYs and ICERs and a one-way and two-way sensitivity analysis. In addition, we calculated these data for rivaroxaban and apixaban as well as for a certain range of daily costs for warfarin and daily costs of the NOACs for Germany. The cost data we used for the Markov model were comparable to those used in other countries [12, 28–31, 34].

Despite differences in model designs and structures of the cost-effectiveness analyses, it was mostly possible to replicate the results published by different authors in different countries like USA, UK, and Canada and identify variables responsible for differences between ICERs using a reference model approach [39]. All analyses for dabigatran used the Markov model for calculation of the QALYs and ICERs and a one-way and two-way sensitivity analysis. In addition, we calculated these data for rivaroxaban and apixaban as well as for a certain range of daily costs for warfarin and daily costs of the NOACs for Germany. The cost data we used for the Markov model were comparable to those used in other countries [5, 12, 28–31, 34]. This enables a better interpretation of published findings by focusing attention on the assumptions underlying the key model features accounting for differences [39]. A real patient data analysis favoured dabigatran for stroke prophylaxis in patients with nonvalvular AF under the current hospital's perspective in a Hong Kong teaching hospital and provided a reference for further comparisons under patient and subsidization perspectives [40].

Limitations of pharmacoeconomic analyses include that they are not prespecified. Therefore the trials did not include patient-level documentation of medical resource use for estimates of total medical costs and administration of the EQ-5D for evaluation of health preferences (i.e., quality of life). The economic evaluation is more dependent on assumptions to calculate costs and the use of literature-based estimates of quality of life to generate QALYs [7]. Other limitations include differences between the studies: open [2] versus double blind study design [1, 3, 4], age, gender, creatinine clearance, CHADS2 score, history of stroke, previous therapy with warfarin, time in therapeutic range (TTR) of the INR, other biographic data of patients, and reporting minor and nonmajor bleeding complications. It has to be considered that the TTR of the INR may be higher in the studies if anticoagulation clinics such as in The Netherlands or in Italy or self-monitoring systems are used. Therefore, the individual warfarin-control groups of every study have to

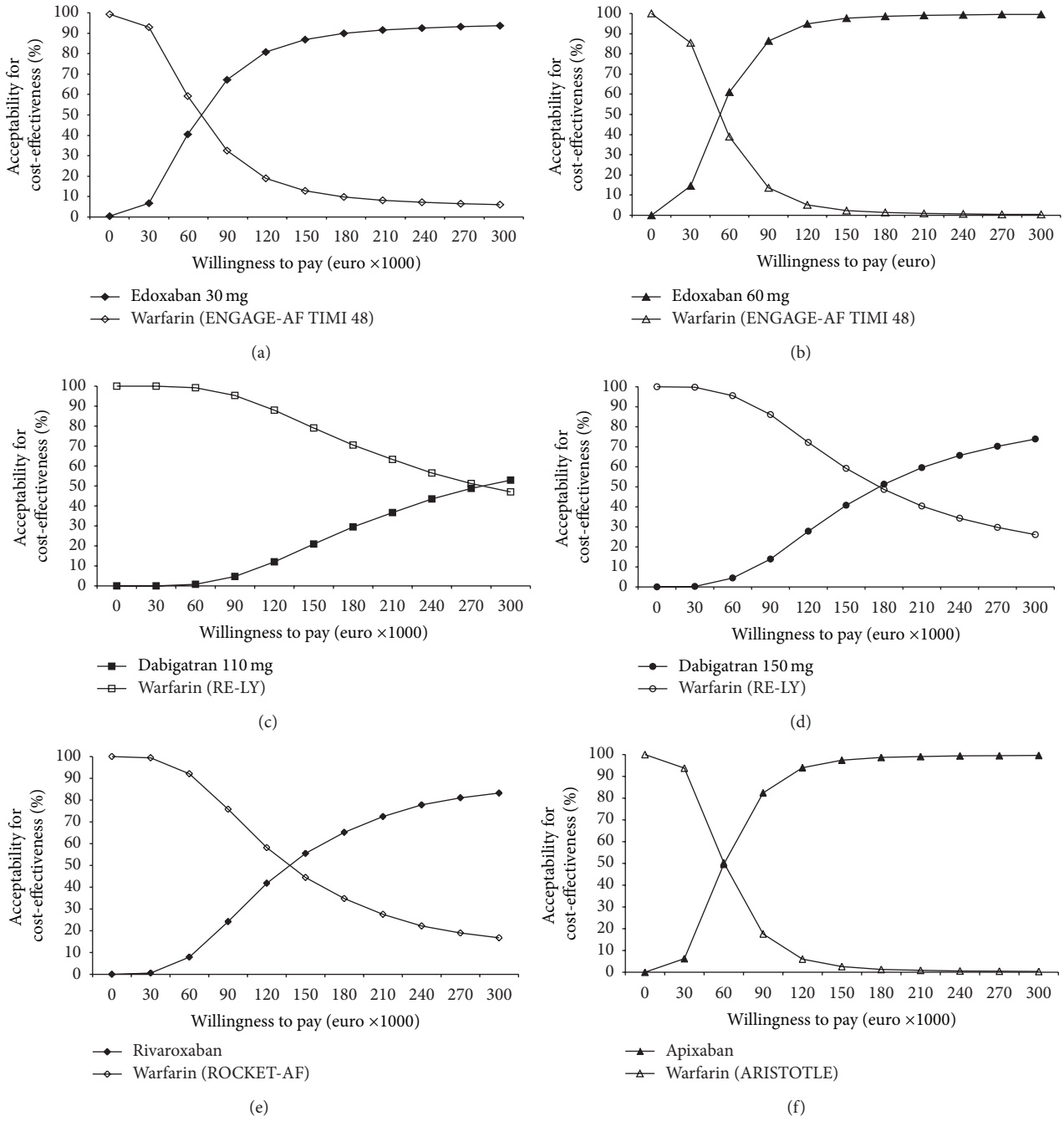

Figure 2: Monte Carlo simulation: acceptability curve for edoxaban 30 mg od (a), edoxaban 60 mg od (b), dabigatran 110 mg bid (c), dabigatran 150 mg bid (d), rivaroxaban 20 mg od (e), and apixaban 5 mg bid (f) compared to warfarin (results obtained from data of every NOAC study) with current market prices for a population starting with 65 years from a German health insurance perspective (reproduction of (c) to (f) with permission of the publisher of [5]).

be used for such investigations [41]. Other limitations of the study are the extrapolation from the shorter treatment period of the studies to a 20-year-time horizon for the cost-effectiveness analysis, the fact that the willingness to pay may be set to a lower range of 20.000 to 30.000€, and the fact that in Germany no willingness-to-pay threshold exists for the health insurance system. This has to be respected for a comparison of the data across countries. The amount of willingness to pay depends substantially on the market price of the NOAC. It may be assumed that they will be reduced over time by the several economic fine-tunings. The lack of head-to-head trials makes it difficult to determine the most cost-effective agent [42]. Therefore we performed our cost-effectiveness analysis strictly only using the results of the

individual studies without indirect treatment comparisons and German mortality tables to decrease the variance of the results [5].

In conclusion, edoxaban in addition to apixaban may be regarded as the most cost-effective NOAC from a German public health care insurance perspective. The larger reduction in medical cost was mainly driven by reductions in the risks major bleeding events. Additional real life use of NOAC has to substantiate the present results for specific countries, which should be collected with a support of scientific and other independent organizations.

Conflict of Interests

Job Harenberg is an advisor or consultant for AstraZeneca, Bayer HealthCare, Boehringer Ingelheim, Bristol-Myers Squibb, Novartis, Pfizer, Polymedix, Roche Diagnostics, Sanofi-Aventis; speaker or a member of speakers bureau for AstraZeneca, Bayer HealthCare, Boehringer Ingelheim, Novartis, Sanofi-Aventis; Harenberg obtained grants for research from Bayer HealthCare; Boehringer Ingelheim, Bristol-Myers Squibb, Novartis, Pfizer, Sanofi-Aventis, Roche Diagnostics. Gregory Y. H. Lip is an advisor or consultant for Boehringer Ingelheim; Sanofi-Aventis; Bayer HealthCare; Merck & Co.; Astellas; Portola; BIOTRONIK, Inc.; AstraZeneca; Bristol-Myers Squibb; Pfizer; Boehringer Ingelheim; Sanofi-Aventis; Bayer HealthCare; Merck & Co.; Astellas; Bristol-Myers Squibb; Pfizer; Lip obtained grants for clinical research from Bayer HealthCare; Sanofi-Aventis. Martin Wehling was employed by AstraZeneca R&D, Mölndal, as director of discovery medicine (translational medicine) from 2004 to 2006, while being on sabbatical leave from his professorship at the University of Heidelberg. After returning to this position in January 2007, lecturing and consulting for Novartis, Takeda, Roche, Pfizer, Bristol-Myers, Daichii-Sankyo, Lilly, Leo-Pharma, Shire, and Novo-Nordisk. Martin Krejczy and Konrad Obermann do not have to declare conflict of interests.

Authors' Contribution

Conception and design of the work, acquisition of data, or analysis and interpretation of data were performed by Martin Krejczy and Job Harenberg. Drafting the paper or revising it critically for important intellectual content was done by Martin Krejczy, Job Harenberg, Martin Wehling, Konrad Obermann, and Gregory Y. H. Lip. Final approval of the version to be published was done by Martin Krejczy, Job Harenberg, Martin Wehling, Konrad Obermann, and Gregory Y. H. Lip.

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Review Article

Management of Non-Vitamin K Antagonist Oral Anticoagulants in the Perioperative Setting

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The field of oral anticoagulation has evolved with the arrival of non-vitamin K antagonist oral anticoagulants (NOACs) including an anti-IIa agent (dabigatran etexilate) and anti-Xa agents (rivaroxaban and apixaban). The main specificities of these drugs are predictable pharmacokinetics and pharmacodynamics but special attention should be paid in the elderly, in case of renal dysfunction and in case of emergency. In addition, their perioperative management is challenging, especially with the absence of specific antidotes. Effectively, periods of interruption before surgery or invasive procedures depend on half-life and keeping a permanent balance between bleeding and thromboembolic risks. In addition, few data regarding the link between plasma concentrations and their effects are provided. Routine laboratory tests are altered by NOACs and quantitative measurements are not widely performed. This paper provides a review on the management of NOACs in the perioperative setting, including the estimation of the bleeding and thrombotic risk, the periods of interruption, the indication of heparin bridging, the usefulness of laboratory tests before surgery or invasive procedure, and the time of resuming. Most data are based on expert's opinions.

1. Introduction

Three non-vitamin K antagonist oral anticoagulants (NOACs) [1] are already widely used in the clinical setting: rivaroxaban and apixaban, two direct factor Xa (FXa) inhibitors, and dabigatran etexilate (DE)—the prodrug of dabigatran, a direct thrombin inhibitor. Both of these drugs will progressively tend to replace vitamin K antagonists (VKAs) in most of their indications. NOACs indications vary among countries. They are licensed for long-term prevention of thromboembolic events in nonvalvular atrial fibrillation (NVAF), for thromboprophylaxis of venous thromboembolism (VTE) including deep venous thromboembolism (DVT) and pulmonary embolism (PE) after hip and knee arthroplasty, and for the treatment and secondary prophylaxis of VTE. Rivaroxaban is also approved in Europe for secondary prevention of atherothrombotic events after acute coronary syndrome (ACS) with elevated cardiac biomarkers [2–7].

Advantages of NOACs include rapid onset and offset of action and relatively predictable anticoagulation effects [8]. In most patients, routine laboratory monitoring of the anticoagulant effect is not required but the assessment of the estimated renal clearance is necessary [9]. In some cases (e.g., emergencies, bleeding, overdose, and trauma), the anticoagulation status and the alteration of standard laboratory data must be known [10, 11]. An increasing number of patients on long-term treatment with NOACs are encountered in the perioperative setting and it is essential for physicians to be aware of the pharmacological properties of these drugs. The management of those patients requires an involvement of

all participating teams (general practitioners, surgeons, anesthesiologists, and other healthcare professionals involved in invasive procedures). Their cessation is indisputable in most elective procedure, but the risk between thrombosis and bleeding should be balanced [12]. In some settings, the therapeutic window is bridged by low molecular weight heparin (LMWH) or unfractionated heparin (UFH) to prevent thromboembolic risk [13, 14]. No specific antidote is currently available in case of bleeding so clinicians have to deal with rescue treatments [15]. The optimal time for NOAC's resumption depends mainly on the postoperative risk of bleeding [16].

This paper aims at providing a review on the management of NOACs in the perioperative setting in accordance with the current literature. This includes the estimation of the bleeding and thrombotic risk of each patient, the period of NOAC's interruption before an invasive procedure, the conditions for heparin bridging during this interruption, the usefulness of common and specific laboratory tests to assess the remaining anticoagulant effect preoperatively, and the time of NOAC's resumption prerequisites for the perioperative management of NOACs. The literature search was performed in PubMed using the following keywords: perioperative, anticoagulant, dabigatran, rivaroxaban, and apixaban. Overall inclusion of papers was limited to studies published until May 30, 2014.

2. Indications and Posology of NOACs

Three molecules are currently available in the clinical setting: dabigatran etexilate (Pradaxa, Boehringer-Ingelheim Pharma GmBH, Ingelheim am Rhein, Germany): 75 mg, 110 mg, and 220 mg capsules, rivaroxaban (Xarelto, Johnson and Johnson/Bayer HealthCare AG, Leverkusen, Germany): 2.5 mg, 10 mg, 15 mg, and 20 mg tablets, and apixaban (Eliquis, Bristol Myers Squibb/Pfizer, Bristol Myers Squibb House, Uxbridge, United Kingdom): 2.5 mg and 5 mg tablets.

Table 1 summarizes the approved indications by the Food and Drug Administration and the European Commission, the posology, and the dose adaptation of the different NOACs.

Table 2 summarizes the main studies leading to the approved indications of NOACs [17–27].

3. Clinician Oriented Overview of Pharmacokinetic Properties of NOACs

3.1. Dabigatran Etexilate (Pradaxa, Boehringer-Ingelheim Pharma GmBH, Ingelheim am Rhein, Germany): 75 mg, 110 mg, and 220 mg Capsules. Dabigatran etexilate is the prodrug of dabigatran, a selective and reversible oral direct thrombin inhibitor. The plasma peak after ingestion is at 1.5–3.0 hours and the plasma trough level is 11–14 hours after ingestion in healthy volunteers [28]. Bioavailability varies from 3 to 7% depending on the pH encountered in the microenvironment of the gastrointestinal tract. Dabigatran is 35% bound to plasma proteins, allowing theoretically its elimination by hemodialysis. Eighty percent of the drug is directly eliminated in the urine, explaining that, in the setting of renal insufficiency, the anticoagulant effect accumulates. Its elimination half-life rises to 18–24 hours in patients with significantly impaired renal function compared to healthy elderly subjects [29]. Creatinine clearance (CrCl) estimation based on the Cockroft and Gault formula [30, 31] is recommended in elderly patients to calculate doses and avoid overmedication [9]. Twenty percent is conjugated as glucuronides by hepatic metabolism. Dabigatran etexilate is contraindicated in case of severe renal impairment (CrCl < 30 mL/min) in Europe while a lower dose is proposed in North America for CrCl between 30 and 15 mL/min [32]. Hepatic impairment or liver disease with expected impact on survival is also a contraindication [33, 34].

3.2. Rivaroxaban. Rivaroxaban is a selective and reversible oral direct FXa inhibitor. The plasma peak after ingestion is at 2–4 hours and the half-life elimination is 5–9 hours in healthy volunteers and 11–13 hours in the elderly [28]. Bioavailability is between 80 and 100% for 10 mg and around 66% for 15 or 20 mg under fasting conditions. Rivaroxaban is bound to plasma proteins in more than 90%, making hemodialysis ineffective to eliminate this drug. About one-third (36%) of the dose is eliminated by renal pathway as unchanged active drug, and the approximately remaining two-thirds of the dose is subject to metabolic degradation. Metabolites are eliminated equally via renal pathway and via hepatobiliary route in the feces [35]. Clearance is mildly influenced by renal function [36]. Rivaroxaban is not recommended in severe renal impairment (ClCr < 15 mL/min) [32].

3.3. Apixaban. Apixaban is a selective and reversible oral direct FXa inhibitor. The plasma peak after ingestion is at 2-3 hours and the half-life elimination is 8–15 hours in healthy volunteers [28]. Apixaban is 87% bound to plasma proteins. Bioavailability is around 50%. Apixaban is eliminated via multiple pathways: predominantly via the fecal route (56%) and 25–29% via renal excretion [37]. Apixaban is not recommended in severe renal impairment (ClCr < 15 mL/min) [32].

Table 3 proposes an overview of the main pharmacokinetic properties of direct oral anticoagulants.

4. Drugs Interactions

Two mechanisms are mainly involved in NOACs' metabolism and elimination: the efflux operated by P-glycoprotein (P-gp) and the CYP450 isoform CYP3A4. Dabigatran etexilate is metabolized in dabigatran in the plasma and liver via CYP450-independent mechanisms [38], but DE acts as a substrate for P-gp. Therefore, strong inhibitors or inducers of P-gp may alter the absorption of DE [39]. Rivaroxaban and apixaban are metabolized by CYP3A4/5 and are both substrates for P-gp. Thus, drugs that strongly inhibit or induce CYP3A4 or P-gp or both influence the pharmacokinetic (PK) profile of these NOACs [39, 40].

5. Perioperative Management of NOACs

As illustrated in the RE-LY (randomized evaluation of long-term anticoagulation therapy) study [41], 25% of the patients

Table 1: Summary of approved indications, posology and dose adaptation of the different NOACs.

	Dabigatran etexilate (Pradaxa)	Rivaroxaban (Xarelto)	Apixaban (Eliquis)
VTE Prophylaxis	(i) 220 mg/day (2 capsules of 110 mg OD) or (ii) 150 mg/day (2 capsules of 75 mg OD) if CrCl 30–50 mL/min, if >75 years, if verapamil, amiodarone and quinidine THR: 28–35 days TKR: 10 days	10 mg/day (1 tablet of 10 mg OD) THR: 5 weeks TKR: 2 weeks	5 mg/day (1 tablet of 2.5 mg BID) THR: 32–38 days TKR: 10 days
Non-valvular atrial fibrillation	(i) 300 mg/day (1 capsule of 150 mg BID) (ii) 220 mg/day (EU) (1 capsule 110 mg BID) (a) if >80 y or verapamil 150 mg/day (US) (1 capsule of 75 mg BID) (b) if CrCl between 15–30 mL/min (c) if dronedarone/ketoconazole (US)	(i) 20 mg/day (1 tablet of 20 mg OD) (ii) 15 mg/day (1 tablet of 15 mg OD) if CrCl between 15–49 mL/min	(i) 10 mg/day (1 tablet of 5 mg BID) (ii) 5 mg/day (1 tablet of 2.5 mg BID) if at least 2 of the following conditions: ≥80 years, ≤60 kg or serum creatinine ≥1.5 mg/dL; or if CrCl 15–29 mL/min
VTE treatment and secondary prophylaxis	(i) 150 mg BID after 5–10 days parenteral anticoagulation (ii) 1 capsule 75 mg BID if CrCl <30 mL/min (US) (iii) Adopted indication CHMP° (april 2014) (EU)	(i) Treatment phase: 30 mg/day (1 tablet of 15 mg BID) for 21 days (ii) Secondary prevention: 20 mg/day (1 tablet of 20 mg OD) 15 mg/day (1 tablet of 15 mg OD) if CrCl between 15–49 mL/min and the risk of bleeding outweighs the risk of recurrent DVT or PE	✗
Prevention of atherothrombotic events after ACS with elevated cardiac biomarkers	✗	5 mg/day (1 tablet of 2.5 mg BID) in association with ASA (75–100 mg) alone or ASA + clopidogrel (75 mg)	✗

✗: Off-label; BID: twice daily; CrCl: creatinine clearance; VTE: venous thromboembolism; OD: once daily; PE: pulmonary embolism; THR: total hip replacement TKR: total knee replacement; °Committee for Medicinal Products for Human Use (CHMP), ASA: acetylsalicylic acid.

Table 2: Summary of the main studies leading to approved indications of NOACs.

Clinical context	NOAC	Other anticoagulant	Conclusion (NOACs versus other drugs)
VTE[0] prophylaxis after orthopedic surgery			
RE-MODEL	Dabigatran etexilate 150 or 220 mg OD	Enoxaparin 40 mg OD SC[5]	Same efficacy and safety profile after TKR[1]
RE-NOVATE II	Dabigatran etexilate 220 mg OD	Enoxaparin 40 mg OD SC	Same profile in term of safety and bleeding after THR[2]
RECORD	Rivaroxaban 10 mg OD	Enoxaparin 40 mg OD SC	More effective, without increasing major bleeding after THR/TKR
ADVANCE II	Apixaban 2.5 mg BID	Enoxaparin 40 mg OD SC	More effective without increased bleeding after TKR
ADVANCE III	Apixaban 2.5 mg BID	Enoxaparin 40 mg OD SC	Lower rate of VTE without increased bleeding after THR
Non-valvular atrial fibrillation			
RE-LY[3]	Dabigatran etexilate 110 mg or 150 mg BID	Adjusted dose warfarin (INR 2-3)	Efficacy superior for the prevention of stoke with a similar rate of major bleeding
ROCKET-AF[4]	Rivaroxaban daily dose 20 mg	Adjusted dose warfarin	Non-inferiority, no significant difference in term of bleeding
ARISTOTLE	Apixaban 5 mg BID	Adjusted dose warfarin (INR 2-3)	Superior in preventing stroke or systemic embolism, less bleeding and lower mortality
VTE Treatment			
RE-COVER II	Dabigatran etexilate 150 mg BID after 5–11 days of LMWH[6] or UFH[7]	Warfarin	Similar effect on VTE recurrence, lower risk of bleeding for the treatment of acute VTE
EINSTEIN	Rivaroxaban 15 mg BID for 3 weeks following by 20 mg OD	Enoxaparin SC following by vitamin K antagonist	Simple, single drug approach. Improve benefit-to-risk of anticoagulation
Acute coronary syndrome			
ATLAS ACS	Rivaroxaban 2.5 BID	Placebo	Reduced the composite endpoint of death from cardiovascular causes, myocardial infarction or stroke. No increase of fatal bleeding.

[0]VTE: venous thromboembolism, [1]TKR: total knee replacement, [2]THR: total hip replacement, [3]RE-LY: Randomized Evaluation of Long-Term Anticoagulation therapy, [4]ROCKET-AF: Rivaroxaban Once Daily Oral, Direct factor Xa Inhibition Compared with Vitamin K antagonism for Prevention of Stroke and Embolism in Atrial Fibrillation, [5]SC: Subcutaneously, [6]LMWH: low molecular weight heparin, [7]UFH: unfractionated heparin.

Table 3: Overview of main pharmacokinetic properties of NOACs.

	Dabigatran etexilate	Rivaroxaban	Apixaban
Plasma peak (hours)	1.5–3.0	2.0–4.0	3.0-4.0
Elimination half-life (hours)	11–14: healthy volunteers 18–24: significantly impaired renal function	5–9: healthy volunteers 11–13: elderly	8–15: healthy volunteers
Protein binding (%)	35%	>90%	87%
Elimination (%)	80% active renal 20% faecal	33% non-active renal 66% metabolized: (metabolism: 50% renal and other half by hepatobiliary route)	Multiples pathways: 25%–29% renal 56% by faecal route
Bioavailability	3–7% PH sensitive	80–100% 10 mg 66%: 15–20 mg under fasting conditions	±50%

Table 4: CHA_2DS_2-VASc Score.

Acronym	CHA_2DS_2-Vasc Score	Points
C	Congestive heart failure	1
H	Hypertension	1
A_2	Age ≥ 75 years	2
D	Diabetes mellitus	1
S_2	Stroke, transient attack, or thromboembolism	2
V	Vascular disease (prior myocardial infarction, peripheral arterial disease, aortic plaque)	1
A	Age 65–74 years	1
Sc	Sex category: female	1

on anticoagulant therapy required a transitory cessation during the two years of follow-up while, in the ROCKET-AF (rivaroxaban once daily oral, direct factor Xa inhibition compared with vitamin K antagonism for prevention of stroke and embolism in atrial fibrillation) study [42], 33% of the patients experienced a temporary interruption of anticoagulant therapy. Forty percent of these interruptions were before surgical or invasive procedure.

In the perioperative context, the balance between thrombosis (in case of anticoagulation interruption) and bleeding (in case of anticoagulation continuation) should be assessed for each patient [43].

5.1. The Thromboembolic Risk

5.1.1. For the Arterial Side. In developed countries, atrial fibrillation (AF) affects about 1.5 to 2% of the population. This arrhythmia is associated with a 5-fold increased risk of stroke (AF is associated with 15–20% of all strokes [44]), a 3-fold increased incidence in congestive heart failure, and a higher mortality [45]. Scores that assess thrombotic risk in the perioperative setting are not well established, whereas in certain chronic conditions risks like AF, stratification scores help in decision making when the risk of thrombosis and bleeding must be weighted [46, 47]. The most widely used score is the $CHADS_2$ score (congestive heart failure, hypertension, age > 75 years, diabetes, and prior stroke/transient ischemic attack). It is validated for predicting AF-related thromboembolic risk events and helps for the optimal selection of VKAs and NOACs therapies [46, 47]. Since 2010, the CHA_2DS_2-VASc score (including cardiovascular disease, atherosclerotic disease, and female sex as additional risk factors) (Table 4) improves the predictive value for thromboembolism. Only a small proportion of patients belong to the low risk and intermediate risk categories [46, 48]. Patients with CHA_2DS_2-VASc score ≥ 2 are considered at high risk of thrombosis [46].

The ARISTOTLE (apixaban for reduction in stroke and other thromboembolic events in atrial fibrillation) trial shows a superiority of apixaban over warfarin in terms of stroke or systemic embolism prevention [20, 49] whatever the assessment of stroke risks by $CHADS_2$ and CHA_2DS_2-VASc [50]. Actually, the assessment of periprocedural thrombotic risk is extrapolated from the risk outside the periprocedural period [12].

5.1.2. For the Venous Side. Patients with venous thromboembolism are at high risks of recurrent thrombosis, thrombus propagation, and embolization until 3 months after diagnosis and initiation of anticoagulation therapy [51]. The risk of recurrence is conditioned by the underlying cause. It decreases if the etiology is provoked (e.g., trauma, fractures, and pregnancy), but if the underlying cause is idiopathic, the risk of recurrence is higher [52].

5.2. The Bleeding Risk.

The bleeding risk is multifactorial and its assessment needs to consider patient-specific and procedure-specific variables [53].

Table 5: HAS-BLED score.

HAS-Bled Score	Risk Factor	Points
H	Hypertension (uncontrolled, systolic blood pressure >160 mmHg)	1
A	Abnormal renal function or liver function	1 or 2 (each 1)
S	Stroke	1
B	Bleeding history or predisposition to bleeding (e.g., bleeding diathesis, anemia)	1
L	Labile INR	1
E	Elderly (age > 65 years)	1
D	Drug (antiplatelet, nonsteroïdal anti-inflammatory drugs) or alcohol abuse	1 or 2 (each 1)

5.2.1. Patient-Specific Variables

For the Arterial Side. HAS-BLED score is used to assess 1-year risk of major bleeding in patients with AF under VKAs [54] (Table 5). Other scores such as ATRIA [55] and $HEMORR_2HAGES$ [56] scores are also used [57]. All these scores offer a modest predictive performance of the estimation of the bleeding risk in NOACs treated patients with AF. However, HAS-BLED and $HEMORR_2HAGES$ scores are superior in terms of clinically relevant bleeding for patients on NOACs [58]. Nevertheless, a second analysis of the ARISTOTLE trial shows less bleeding under apixaban compared with warfarin, independently of the HAS-BLED score [50].

For the Venous Side. The RIETE score (Registro Informatizado de Enfermedad Tromboembólica) assesses the risk of fatal bleeding in VTE patients and seems better in predicting gastrointestinal than intracranial fatal bleeding [59].

5.2.2. Procedure-Specific Variables. There is little data available to identify the risk of blood loss related to the invasive procedure or surgery [60], except for cardiac surgery [61]. The estimation of bleeding risk for surgery/invasive procedure remains controversial for certain procedures and has low level of evidence [13]. Furthermore, there is a high intercenter variability in red cell blood loss for the main procedure, mainly reflecting differences in surgical techniques. Similarly, classifications into different surgery bleeding risks according to the severity of trauma and the risk of periprocedural bleeding [12, 60, 62–65] are not always easy to use in daily practice. Therefore, it is recommended to develop an institutional guideline and a hospital-wide policy concerning perioperative anticoagulation management in different procedures [63].

5.3. Interval between Last Dose and Invasive Procedure or Surgery. The interval between the last dose and the invasive procedure or surgery depends on the bleeding risk of the procedure and the drug half-life (Table 3).

5.3.1. The Invasive Procedure or Surgery. Some procedures defined as minimal procedures with little tissue trauma are at low risk for bleeding [14, 63, 66] and can be achieved without interruption of NOACs (e.g., superficial skin and oral mucosal surgery, wound revisions, nonextraction dental treatment, or cataract surgery). Gastrointestinal endoscopic biopsy without cessation of DE seems to be safe according to current Japanese guidelines [67]. It is recommended to perform the procedure at the trough drug concentration (12 or 24 hours after the last intake) [13, 63, 68].

5.3.2. Drug Metabolism and Elimination. The percentage of drug eliminated after 2, 3, 4, and 5 half-lives is 75%, 87.5%, 93.8%, and 96.9%, respectively [69]. Dabigatran etexilate, rivaroxaban, and apixaban have different drug metabolic and elimination pathways (Table 3). Creatinine clearance (CrCl) must be assessed by the Cockroft and Gault method. Estimation of renal function by Modification of Diet in Renal Disease Equation 4 (MDRD 4) leads to an overestimation of the renal function at lower levels. Thus, many elderly patients with AF would either become incorrectly eligible for NOACs or would receive a too high dose, which may explain the serious incidences of bleeding reported [9, 30].

Some specific populations have an increased half-life of NOACs' elimination and need therefore special attention. This concerns patients with renal or hepatic impairment, particularly the elderly with fluctuating renal function, diuretic use, hypovolemia, liver chronic infections, liver cirrhosis, alcohol abuse, obstructed bile flow, hepatorenal syndrome, and associated coagulopathy. Assessing both renal and liver function must be done preoperatively for every patient on NOACs. The three agents can be used in mild hepatic impairment (Child-Pugh A), while DE and apixaban are allowed in moderate hepatic impairment (Child-Pugh B), but not in severe hepatic impairment (Child-Pugh C) [70].

It is also important to check preoperatively any off-label use or misuse of these NOACs, including the concomitant medications (verapamil, dronedarone, ketoconazole, amiodarone, tacrolimus, etc.), older ages, and extreme body weights. All of these groups should probably require a longer interval of NOACs' arrest, and a measurement of the anticoagulant residual activity should be considered before an invasive procedure.

Several interval schemes based on expert's opinions are proposed taking into account NOACs' pharmacokinetics, patient, and/or type of invasive procedure or surgery (Table 6).

The "*Groupe d'Intérêt en Hémostase Périopératoire*" (GIHP) proposes a unique scheme: 1 day of interruption in case of low risk bleeding surgery or procedure and 5 for other procedures, whatever the molecule, renal function, and concomitant medications [13]. The duration of stopping is

proposed empirically to ensure no residual anticoagulant effect in the absence of a validated antidote.

Except for minimal procedures which can be achieved without stopping NOACs, other experts or expert groups propose a window without any NOAC, according to the CrCl and/or the type of surgery. An interval of at least 48 hours (about 3 half-lives) should be maintained for a patient with a CrCl above 50 mL/min to perform surgery or invasive procedure, whatever the molecule. The free interval is increased to at least 4 days for patients on DE with CrCl between 30 and 50 mL/min or for patients on rivaroxaban/apixaban with a CrCl between 15 and 30 mL/min (Table 6). Further large prospective studies are needed to confirm the safety of these perioperative procedures.

5.4. To Bridge or Not to Bridge during the Perioperative Interruption. For patients on VKA, the procedure is well established [12, 60]. Guidelines recommend to bridge patients at high risk for thromboembolism (mechanical heart valve, AF, or VTE) with LMWH. For patients at low risk for thromboembolism, they suggest no bridging in case of stopping [60, 71]. Patients classified as being at high risk have more than 10% annual risk for thromboembolism while this risk is reduced to less than 5% for those classified as being at low risk [60].

For VKAs, Siegal et al. [71] as well as Feng et al. [72] showed an increased risk of bleeding with similar thrombotic risk among patients who underwent periprocedural bridging therapy with heparin bridging. Recently, Omran et al. [73] have validated a HAS-BLED score ≥ 3 as the most predictive variable for hemorrhage for patients who had heparin bridging during a perioperative interruption of VKAs before an elective invasive procedure.

Expert's opinions recommend no heparin bridging for NOACs [62, 63, 74], except the GIHP [13]. The last group proposes to stop NOACs 5 days before a surgery with medium or high risk of bleeding, to ensure a complete elimination of the drug in all patients. And if the patient is at high thromboembolic risk (e.g., AF with a history of stroke), they suggest bridging with LMWH or UFH.

Beyer-Westendorf et al. [14] had recently presented the first prospective data from a national registry that supported the concept of short-term interruption without heparin bridging.

They concluded that if a surgery or an invasive procedure requires a NOAC's arrest, most patients can safely interrupt NOACs for a short period without heparin bridging. In case of heparin bridging therapy, the rate of cardiovascular events is not reduced. There is a significantly higher rate of bleeding complications due to heparin bridging or major procedures. However, patients at cardiovascular risk undergoing major procedures may benefit from heparin bridging because their outcome in terms of cardiovascular risk is increased and because, in this particular setting, heparin bridging is not an independent factor for bleeding risk.

Those data do not support a systematic bridging therapy but highlight its probable benefit in patients at cardiovascular risk undergoing high risk surgery. Table 7 shows categories of procedures defined by the severity of tissue trauma and the risk for periprocedural bleeding [14].

During the ROCKET-AF trial, patients who required temporary interruption of anticoagulant therapy for surgery and invasive procedure were bridged only in 6% of the cases, predominantly by LMWH. The rate of major bleeding was similar in bridged and nonbridged patients. The incidence of bleeding (major and nonmajor clinically relevant bleeding according to International Society on Thrombosis and Hemostasis definitions [75]) was higher in case of bridging therapy, while stroke and systemic embolism were similar in both groups [42]. The study suffers several biases such as the use of bridging therapy in a nonrandomized way and the too low number of events.

Devices implants in most of Canadian centers are performed with NOAC interruption without LMWH bridging [76]. Again, this study demonstrates the necessity of an individual assessment of each patient, on a case-by-case basis [77].

Thus, there is no universal strategy for periprocedural management of NOACs, but a stepwise approach can be considered. Some prerequisites are essential to allow periprocedural decision (Table 8).

The decision about perioperative NOACs management should be written in the medical record.

5.5. A Particular Case: Anesthesia Procedures

5.5.1. Neuraxial Anesthesia. For patients without thrombotic risk (assessed by CHA_2D_2-VASc score), Benzon et al. [78] recommend 5 half-life intervals between NOAC's discontinuation and a neuraxial puncture (with or without epidural catheter placement). For patients with high risk of stroke or VTE, this interval can be shortened to 2 or 3 half-lives, or it can stay at 5 half-lives if LMWH bridging is associated. Llau and Ferrandis [79] provided recommendations based on NOAC's pharmacokinetics in the setting of thromboprophylaxis. For spinal anesthesia, if the puncture is atraumatic, the first dose of DE can be administrated 1–4 hours after the end of surgery and after 6–8 hours for rivaroxaban. If the puncture is traumatic or hemorrhagic, the first dose of DE or rivaroxaban is delayed by 24 hours. For epidural anesthesia, DE cannot be administrated if a permanent catheter is inserted. For rivaroxaban, the first dose after epidural anesthesia is administered 6–10 hours after the end of surgery. An interval of 18 hours is recommended before the removal of the epidural catheter (22–26 hours for elderly patients) and at least 4 hours after removal. The European Society of Anaesthesiology (ESA) [80] recommends extreme caution when using neuraxial blockade in the presence of rivaroxaban/apixaban. For dabigatran, the manufacturer advises against its use in the presence of neuraxial blockade. Because of a potential risk of retroperitoneal hematoma in lumbar plexus and paravertebral blocks, ESA recommends the same guidelines for these two peripheral nerve blocks as for neuraxial blockades [78, 80].

Table 6: Schemes of discontinuation of NOACs.

	Dabigatran	Rivaroxaban	Apixaban
Baron et al. [12]	CrCl ≥ 50 mL/min: 1 or 2 days CrCl < 50 mL/min: 3–5 days	≥1 day if CrCl normal CrCl 60–90 mL/min: 2 days CrCl 30–59 mL/min: 3 days CrCl 15–29 mL/min: 4 days	CrCl > 60 mL/min: 1 or 2 days CrCl 50–59 mL/min: 3 days CrCl 30–49 mL/min: 5 days
Liew et al. [64]	No/minimal residual effect at surgery (4-5 drug half-lives) CrCl > 50 mL/min: 3 days CrCl 30–50 mL/min: 4 to 5 days Mild-moderate effect at surgery (2-3 drug half-lives) CrCl > 50 mL/min: 2 days CrCl 30–50 mL/min: 3 days	No/minimal residual effect at surgery (4-5 drug half-lives) CrCl > 50 mL/min: 3 days CrCl 30–50 mL/min: 4 to 5 days Mild-moderate effect at surgery (2-3 drug half-lives) CrCl > 50 mL/min: 2 days CrCl 30–50 mL/min: 3 days	No/minimal residual effect at surgery (4-5 drug half-lives) CrCl > 50 mL/min: 3 days CrCl 30–50mL/min: 4 to 5 days Mild-moderate effect at surgery (2-3 drug half-lives) CrCl >50 mL/min: 2 days CrCl 30–50 mL/min: 3 days
Kozek-Langenecker [93]	2 days-interval may be sufficient for low-risk intervention 4 days at least for high risk intervention and/or CrCl < 50 mL/min	1 day for low-bleeding interventions 2 days in high bleeding risk interventions	1 day for low-bleeding interventions 2 days in high bleeding risk interventions
Spyropoulos and Douketis [62]	CrCl > 50 mL/min: Low risk bleeding surgery°: last dose 2 days before surgery High risk bleeding surgery*: Last dose 3 days before surgery CrCl 30–50 mL/min: Low risk bleeding surgery°: Last dose 3 days before surgery High risk bleeding surgery*: Last dose 4-5 days before surgery	CrCl > 30 mL/min: Low risk bleeding surgery°: last dose 2 days before surgery High risk bleeding surgery*: Last dose 3 days before surgery CrCl 15–29.9 mL/min: Low risk bleeding surgery°: Last dose 3 days before surgery High risk bleeding surgery*: Last dose 4 days before surgery	CrCl > 50 mL/min: Low risk bleeding surgery°: last dose 2 days before surgery High risk bleeding surgery*: Last dose 3 days before surgery CrCl 30–50 mL/min: Low risk bleeding surgery°: Last dose 3 days before surgery High risk bleeding surgery*: Last dose 4 days before surgery
Sié et al. [13]	Low risk procedure[1]: 1 day Other procedures than low risk bleeding procedures[2]: stop 5 days before surgery/invasive procedures	Low risk procedure[1]: 1 day Other procedures than low risk bleeding procedures[2]: stop 5 days before surgery/invasive procedures	Low risk procedure[1]: 1 day Other procedures than low risk bleeding procedures[2]: stop 5 days before surgery/invasive procedures

°Low-risk bleeding surgery: 2-day risk of major bleed 0%–2%; *High-risk bleeding surgery: 2%–4%.
[1]Low risk procedure: in case of bleeding, if it occurs, will be low abundance, non-critical in its location and/or easily controlled by simple mechanical hemostasis; [2]High risk procedure: the probability of significant bleeding cannot be excluded or, any surgery that is usually hemorrhagic or for which the risk of bleeding would be unacceptable.

Table 7: Categories of procedures according to the severity of tissue trauma and the risk for peri-procedural bleeding.

Minor procedures with little tissue trauma
Superficial skin and oral mucosal surgery, skin biopsies Wound revisions Non-extraction dental treatment
Minor procedures with little tissue trauma, but relevant bleeding risk
Transluminal cardiac, arterial, and venous interventions Pacemaker-related surgery Pleura and ascites punctures Cataract surgery Arthroscopy, endoscopy, laparoscopy Organ biopsies Dental extraction Hernia repair Intramuscular and paravertebral injections
Major procedures with relevant tissue trauma and high bleeding risk
Open pelvic, abdominal and thoracic surgery Brain surgery Major orthopedic and trauma surgery Vascular surgery

5.5.2. Other Peripheral Nerve Blocks. ESA does not routinely apply the same guidelines as for neuraxial blockades [81], but the American Society of Regional Anesthesia (ASRA) does [78].

5.6. A Particular Case: Atrial Fibrillation Ablation. Uninterrupted warfarin therapy with therapeutic anticoagulation has been shown to be associated with lower risk of periprocedural thromboembolic events after AF ablation and is increasingly being accepted as the preferred anticoagulation strategy [82]. However, increasingly more patients are being treated with NOACs, thereby complicating the periprocedural anticoagulation management.

For dabigatran, there is no consensus regarding the management of patients taking dabigatran who are referred for AF ablation. The results depend on the period of dabigatran interruption. Nearly uninterrupted anticoagulation (holding the morning dose), which theoretically has a better protection from periprocedural thromboembolism, was associated with both increased bleeding and thromboembolic events, especially in the nonparoxysmal AF [83]. Other observational studies with a more interrupted approach showed equivalent bleeding and embolic events compared to therapeutic warfarin [84–87].

For rivaroxaban, uninterrupted rivaroxaban appears to be a feasible and safe alternative to uninterrupted warfarin therapy in patients undergoing AF ablation. Future larger and randomized trials are needed to confirm those preliminary data [83].

6. Management in an Emergency Situation

In case of a surgery or invasive procedure, the degree of emergency should be assessed in order to decrease the bleeding risk: NOACs must be discontinued, timing of the last dose intake should be known, and if possible, invasive procedure should be delayed until the NOACs reach trough concentration. Waiting and postponing semiurgent procedures may be a reasonable strategy to prevent bleeding. In case of bleeding emergencies under NOACs, several difficulties are encountered: there is currently no antidote and no rapid quantitative measurement of the anticoagulant effect (see laboratory testing for NOACs in a perioperative setting), strategies to reverse the anticoagulant effect are poor, and, depending on the residual concentration of NOACs, administration of factors is rendered ineffective [15, 63].

Table 8: Essential prerequisites allowing perioperative decisions.

Molecule	Type
	Dose
	Indication
Patient	Thromboembolic risk—bleeding risk
	Renal function (CrCl—Cockroft and Gault formula)
	Hepatic function
	Concomitant drugs
	Approved NOAC's indication
Procedure	Type and technique
	Bleeding risk
	Date of its achievement (Day 0)
Anesthesia	General and/or regional (neuroaxial or peripheral nerve blocks)

It is very important to establish a hospital-wide policy for bleeding management in patients taking NOACs, based on the available blood products and laboratory assays in each institution. The procedure must be easily accessible (e.g., intranet site, personal digital assistant (PDA)). Some workgroups propose algorithms based on NOAC's plasma concentration, but their quantitative measurements are not currently routinely performed [88].

In case of bleeding that does not respond to supportive measures (surgical hemostasis, embolization, fluid replacement, etc.) in patients taking DE, ensure adequate diuresis and suggest hemodialysis. Dabigatran is 35% bound to plasma proteins, theoretically allowing its elimination by hemodialysis, but clinical experience is limited [89–91]. However, hemodialysis might be more effective than unspecific reversal treatment with factor concentrates. A single-center study including patients with end-stage renal disease measured DE elimination with hemodialysis. Four hours of hemodialysis with 200 mL/min and 400 mL/min targeted blood flow removed 48.8% and 59.3% of total dabigatran from the central compartment, respectively. There was a linear relation between anticoagulant activity of dabigatran and its plasma levels. Minor redistribution of dabigatran (<16%) after the end of the hemodialysis session occurred. These results support hemodialysis as a suitable approach to eliminate dabigatran in emergency situations [92]. An extracorporeal renal replacement therapy filter can also be easily added during emergent cardiac surgery [93]. However, a

Table 9: Coagulation factor and pro-hemostatic agents.

Concentrate of factors (II, (VII), IX et X): prothrombin complex concentrate, PCC, PPSB (Cofact, Confidex, Octaplex, Beriplex)	25 U/kg, once or two times*
Concentrate of activated factors: idem PCC + VIIa, FEIBA, (FEIBA)	50 IE/kg, max 200 IE/kg/day*
Factor VIIa: (Novoseven)	Needs further evaluation: 90 μg/kg
Antifibrinolytics (Tranexamic acid (Exacyl), Aminocaproïc acid), Desmopressin (Minirin)	No clinical data in this context
Fresh Frozen Plasma (FFP)	Not useful to reverse anticoagulation, expand plasma volume in case of massive transfusion
Protamin, vitamin K	No effect in case of NOACs bleeding

*PCC or aPCC utilization is based of few experimental data, can be considered if immediate hemostatic support is essential in case of life-threating bleeding (need for ≥4 red cells transfusions and exogenous cathecolamins for hemodynamic stabilization).

special care to bleeding at the punctures sites is necessary and therefore ultrasound guided techniques are very useful. Hemoperfusion over a charcoal filter is under evaluation [89]. Oral activated charcoal may be effective only in case of a recent ingestion within 2 hours [89].

An analysis of major bleeding patients in the RE-LY study concludes that the overall resources required to manage bleeding were not greater than those after warfarin-related bleeding. For patients treated with DE, red cells transfusions were higher, plasma transfusions were lower, the stay in intensive care unit was shorter, and there was a lower trend in mortality compared with patients treated with warfarin. Based on these results, they concluded on a safety profile of DE [94].

There is currently no specific hemostatic agent for the reversal of NOACs in case of bleeding or in emergency situations but different antidotes are under development. Andexanet alfa (PRT064445) is a truncated form of enzymatically inactive FXa: it reverses dose-dependently the inhibitory activity and corrects *ex vivo* clotting times [95]. For dabigatran, a humanized selective and specific monoclonal antibody fragment (idarucizumab) is under development [96]. Aripazine (PER977), another small synthetic molecule, reverses anticoagulant activity of all clinically used NOACs in rat bleeding models [97].

Hemostatic agents used for life-threatening bleeding are shown in Table 9.

7. Laboratory Testing for NOACs in a Perioperative Setting

NOACs do not require routine coagulation monitoring. This point is considered as an advantage for the physicians and an improvement of quality of life for their patients. However, point measurement of NOACs may be required in several clinical situations including the perioperative setting [8, 10, 11, 78, 98, 99].

For VKAs, it is well established that, at time of surgery (INR on the day before surgery), an elevated INR (i.e., ≥2) will increase the risk of bleeding and a near normal or normal INR (i.e., ≤1.5) will not [60]. But, for NOACs, the residual drug level that can be considered as safe is not known, except for dabigatran. The residual dabigatran concentration that is recommended before special intervention (such as surgery) is provided in the Committee for Medicinal Products for Human Use (CHMP) assessment report from the European Medicine Agency that states "(…) *dabigatran concentration under 48 ng/mL is equivalent to at least 75% of dabigatran's elimination and should be recommended*" [100]. A French group of experts called GIHP proposed the threshold of 30 ng/mL (for dabigatran and rivaroxaban) [88].

Details of these recommendations are presented in Table 10 [88].

7.1. Which Laboratory Tests Should We Use in the Perioperative Setting and How to Interpret Them? Activated partial thromboplastin time (aPTT) and prothrombin time (PT) are global assays not reflecting plasma NOACs concentrations. They are not suitable for precise quantification of the anticoagulant effect. Thrombin time (TT) was demonstrated to be too sensitive towards dabigatran [89, 101]. However, this may guide the clinician in the perioperative setting since a normal TT indicates no clinically relevant anticoagulant effect of dabigatran. The strong sensitivity of TT towards dabigatran leads to the development of a calibrated diluted thrombin time (dTT) with dabigatran standards to calculate the dabigatran plasma concentration. Several studies showed that the dTT or the ecarin chromogenic assay (ECA) highly correlates with dabigatran plasma concentrations measured by LC-MS/MS in patient's plasma [102–104]. However, one limitation of dTT is that, in case of switching therapy (i.e., from heparins/heparinoids to dabigatran etexilate or from hirudin and derivatives to dabigatran etexilate), they will be slightly influenced by the presence of such inhibitors in the plasma. This implies the necessity of an accurate anamnesis of the drugs taken by the patient to avoid overestimation of drug concentrations in plasma. In addition, for the accurate determination of dabigatran plasma concentrations below 50 ng/mL, the more sensitive liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) method is still required [102, 104].

For rivaroxaban, chromogenic anti-Xa assay has been proven to accurately estimate the plasma rivaroxaban concentrations > 30 ng/mL [105]. Due to their good sensitivity

Table 10: Perioperative management of NOACs (dabigatran and rivaroxaban)—Proposal for recommendations from the GIHP ("Groupe d'Intérêt en Hémostase Périopératoire").

Measured concentration	Recommendations
<30 ng/mL	Operate
30–200 ng/mL	Wait up to 12 h and obtain new dosage or (if time is not compatible with emergency) Operate, if abnormal bleeding: antagonise the anticoagulant effect
200–400 ng/mL	Wait up to 12 h and obtain new dosage or (if time is not compatible with emergency) Maximise delay surgery Discuss hemodialysis, especially if CrCl < 50 mL/min (with dabigatran only) Operate, if abnormal bleeding: antagonise
>400 ng/mL	Overdose-Major haemorrhagic risk Discuss haemodialysis before surgery (with dabigatran only)

Table 11: Influence of dabigatran, rivaroxaban and apixaban on coagulation tests used in the perioperative setting.

	Dabigatran	Rivaroxaban	Apixaban
Prothrombin Time (PT)	Time prolonged + (relative to reagent sensitivity)	Time prolonged + to +++ (relative to reagent sensitivity)	Time non-prolonged or prolonged + (relative to reagent sensitivity)
Activated Partial Thromboplastin Time (aPTT)	Time prolonged + to +++ (relative to reagent sensitivity)	Time prolonged + (relative to reagent sensitivity)	Time prolonged + (relative to reagent sensitivity)
PT-based coagulation factors measurement (II, VII, IX, X)	Slightly decreased (depending on the reagent)	Slightly decreased (depending on the reagent)	Slightly decreased (depending on the reagent)
APTT-based coagulation factors measurement (VIII, IX, XI)	Slightly decreased (depending on the reagent)	Slightly decreased (depending on the reagent)	Slightly decreased (depending on the reagent)
Fibrinogen	No influence or slightly decrease (depending on the reagent)	No influence	No influence
Thrombin Time	Time prolonged +++++	No influence	No influence
Anti-Xa based antithrombin measurement	No influence	Increased: 10% per 100 ng/mL	Increased: 10% per 100 ng/mL
Anti-IIa based antithrombin measurement	Increased: 5–10% per 100 ng/mL	No influence	No influence

towards the inhibition of FXa by apixaban, chromogenic anti-Xa assays calibrated with specific apixaban calibrators should be performed to estimate plasma drug concentration [106–108]. However, taking into account the lower sensitivity of chromogenic assays compared to LC-MS/MS and the variability of coagulation analysers that may further increase the imprecision at the lowest concentrations, detection and quantitation of lower levels (<30 ng/mL for rivaroxaban and <15 ng/mL for apixaban) still require LC-MS/MS analyses [108, 109].

Even if these specific coagulation tests to assess NOACs are promising, they suffer from difficulties to be implemented easily in the clinical setting. Moreover, assessment of lower levels of NOACs encountered in the perioperative setting is challenging due to the limit of quantitation of these tests but improvements in the low range are under development by several companies.

In addition, to correctly interpret the results of laboratory assay, some information needs to be collected: drug, indication, and the timing of the last dose administration (due to the short half-life of NOACs) [110]. The interpretation of the results requires education of the front staff in many specialties [98]. Finally, it has been clearly shown that NOACs influence the results of different coagulation assays used in the perioperative setting leading to misinterpretations [41] (Table 11).

7.2. A Particular Case: Atrial Fibrillation Ablation. The ideal management of oral anticoagulation during catheter ablation for AF 112–114 is still controversial with a wide range of procedures available. During AF ablation, it is now recommended to achieve and maintain an ACT of 300 to 400 seconds in order to reduce the risk of systemic thromboembolism [111]. However, the ACT is affected by a lot of preanalytical [112] and analytical variables [113, 114]. Finally, target ACT should be redetermined for the periprocedural use of NOACs for AF ablation [111–117].

8. Resumption of the NOACs after Invasive Procedure or Surgery

Once again, in the postoperative period, the bleeding risk must be weighted with the thromboembolic risk; however the

risk for major bleeding exceeds the risk for thromboembolic complications after surgery [118]. Regarding the thromboembolic risk, Kaatz et al. [119] concluded that patients with chronic AF had twice as much risks of postoperative stroke, especially in neurosurgery and vascular surgery. The bleeding risk in the postoperative period is mainly due to patient's characteristics (bridging therapy, mitral mechanic heart valve, active cancer, prior bleeding history, and reinitiation of heparin therapy within 24 hours after surgery), even if a premature heparin resumption is an avoidable risk factor [120]. But first of all surgical bleeding risk must be under control [121]. For NOACs, given the fact that full anticoagulation occurs between 2 and 4 hours (Table 3) and that no antidote is available, resumption of DE, rivaroxaban, and apixaban should be performed at least 48 hours after the high risk procedures [12]. This delay can expose patients at risk for thromboembolism. Twenty-four hours are recommended before resuming oral anticoagulation after a procedure at low risk of bleeding [60, 62]. Other schemes exist in order to minimize bleeding risks: consider a stepwise resumption of NOACs [16, 77] or administer prophylactic doses of LMWH early after the surgery, and restart full doses of NOACs only after 3 or 4 days [122]. Heparin bridging can be useful if the patient cannot tolerate oral medication (e.g., in ileus or postoperative nausea and vomiting) [66]. In the postoperative setting, it is essential to reassess patient's renal function, especially for elderly who are subject to dehydration and for patients taking DE. For LMWH, doses must be adapted in case of extreme body weight (body mass index above 30 kg/m^2) and poor renal function. If CrCl is less than 30 mL/min, the use of unfractionated heparin is more indicated [12, 123].

9. Conclusions

In the field of oral anticoagulation, clinicians will be more frequently confronted about their management of NOACs in the pre- and postoperative setting. Prerequisites are essential for NOAC's pharmacokinetics, indications, drug interactions, and alterations of standard laboratory assays. Actual data are based mainly on expert's opinions, except one national registry study. In some situation, interruption periods of NOACs are necessary and should be based on their respective half-life, on the bleeding risk of procedures, and on the thromboembolic risk of patients. Some scores such as CHA_2DS_2-VASc and HAS-BLED should help clinicians in their decisions. Given their shorter half-life, no heparin bridging during the interruption interval seems necessary, except for patients at high cardiovascular risk undergoing major surgery.

Further clinical trials over perioperative management of patients under NOACs are still required. Emergency surgeries, invasive procedures, or bleeding patients remain a real challenge given the absence of antidote. Possibilities of reversal are poor and based on few experimental data and case reports. Furthermore, rapid quantitative measurements of the anticoagulant effect are not available in most institutions. Awaiting future clinical trial data, it seems to be important to establish a hospital-wide policy for bleeding management in patients taking NOACs, in accordance to the available blood products and laboratory assays of each institution.

Conflict of Interests

The authors have no conflict of interests to disclose.

Authors' Contribution

Anne-Sophie Dincq and Sarah Lessire contributed equally to this work.

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Review Article

Management of the Bleeding Patient Receiving New Oral Anticoagulants: A Role for Prothrombin Complex Concentrates

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Ease of dosing and simplicity of monitoring make new oral anticoagulants an attractive therapy in a growing range of clinical conditions. However, newer oral anticoagulants interact with the coagulation cascade in different ways than traditional warfarin therapy. Replacement of clotting factors will not reverse the effects of dabigatran, rivaroxaban, or apixaban. Currently, antidotes for these drugs are not widely available. Fortunately, withholding the anticoagulant and dialysis are freqnently effective treatments, particularly with rivaroxaban and dabigatran. Emergent bleeding, however, requires utilization of Prothrombin Complex Concentrates (PCCs). PCCs, in addition to recombinant factor VIIa, are used to activate the clotting system to reverse the effects of the new oral anticoagulants. In cases of refractory or emergent bleeding, the recommended factor concentrate in our protocols differs between the new oral anticoagulants. In patients taking dabigatran, we administer an activated PCC (aPCC) [FELBA] due to reported benefit in human in vitro studies. Based on human clinical trial evidence, the 4-factor PCC (Kcentra) is suggested for patients with refractory rivaroxaban- or apixaban-associated hemorrhage. If bleeding continues, recombinant factor VIIa may be employed. With all of these new procoagulant agents, the risk of thrombosis associated with administration of factor concentrates must be weighed against the relative risk of hemorrhage.

1. Introduction

Hemorrhage is the major cause of early mortality after injury and a leading risk in any operative procedure. Recently developed target-specific oral anticoagulants defy traditional reversal protocols previously used with warfarin. In the face of new oral anticoagulants, we have developed additional approaches to management of bleeding. Patients with life threatening hemorrhage may benefit from use of a new 4-factor prothrombin complex concentrate (PCC) (Kcentra, CSL Behring Gmbh, Marburg, Germany). Recent data suggest that there may be a role for factor concentrates including PCCs, activated PCCs (aPCC), and recombinant factor VIIa (rfVIIa, NovoSeven, Novo Nordisk, Bagsvaerd, Denmark) in dabigatran, rivaroxaban, and apixaban-associated bleeding. This report will briefly review the mechanism of action of the oral anticoagulants, present our bleeding management protocols, and discuss the rationale for our use of prothrombin complex concentrates and rfVIIa in refractory hemorrhage.

2. Mechanism of Action

Understanding the mechanism of action of oral anticoagulants provides a biologic foundation for use of PCCs, aPCCs, and rfVIIa in treatment of uncontrolled bleeding. Warfarin creates a deficiency of factors II, VII, IX, and X through inhibition of vitamin K-dependent carboxylation of the clotting proteins [1]. Replacement of functional coagulation factors can reverse the anticoagulant effect of warfarin. The newer oral anticoagulants interact with the coagulation cascade in different ways. Dabigatran directly inhibits thrombin

Table 1: Prothrombin complex concentrates (PCC) available in the United States.

Product	Coagulation factors in product	FDA approved indications to manage hemorrhage	Heparin in product?
Nonactivated			
3-factor PCC			
Profilnine	Nonactivated II, IX, X, and small amounts of VII	Hemophilia B	No
Bebulin	Nonactivated II, IX, X, and small amounts of VII	Hemophilia B	Yes
4-factor PCC			
Kcentra	Nonactivated II, VII, IX, X, protein C, and protein S	Warfarin	Yes
Activated PCC			
Feiba	Activated VII, nonactivated II, IX, and X	Hemophilia A & B with inhibitors	No

(activated factor II), which limits the formation of fibrin [2]. Rivaroxaban and apixaban directly inhibit activated factor X [3, 4]. Replacement of clotting factors will not reverse the effects of dabigatran, rivaroxaban, and apixaban and we currently do not have antidotes for these drugs.

Rates of bleeding seen with the new oral anticoagulants in real world patients treated with dabigatran are consistent with those seen in clinical trials and less than those seen with warfarin. Most of the data available at this time comes from clinical trials. Gastrointestinal and intracranial hemorrhage are the two most important bleeding complications reported with the new oral anticoagulants. In postmarketing surveillance, there did not appear to be higher bleeding rates associated with dabigatran as opposed to warfarin. Some of the largest trials are in patients with atrial fibrillation. In these studies, reduced rates of intracranial hemorrhage are seen with dabigatran in comparison to warfarin as well as a reduced death rate. The rate of gastrointestinal hemorrhage is similar between dabigatran and warfarin-treated patients. Factor Xa inhibitors are also associated with reduced risk of mortality and intracranial hemorrhage compared to warfarin in trials conducted in patients with atrial fibrillation and may be used in ablation procedures [5–8].

PCCs are plasma-derived products containing factors II, VII, IX, and X. The 3-factor PCCs contain a minimal amount of factor VII. Table 1 shows the PCCs available in the United States. FEIBA (Baxter AG, Vienna, Austria) is unique because it contains factor VII primarily in the activated form (activated PCC (aPCC)) [9]. Kcentra contains all of the vitamin K-dependent proteins (factors II, VII, IX, and X and proteins C and S) [10]. Kcentra and Bebulin (Baxter AG, Vienna, Austria) contain small amounts of heparin which is insufficient to cause anticoagulation but contraindicates use of these products in patients with a history of heparin-induced thrombocytopenia. Recombinant factor VIIa initiates coagulation independent of tissue factor, factor VIII and factor IX, and is approved for use in patients with factor VII deficiency and hemophilia with factor inhibitors [11]. PCCs provide replacement of functional vitamin K-dependent proteins to more rapidly reverse the anticoagulant effect of warfarin [12]. In contrast, for patients with uncontrolled bleeding while taking dabigatran, rivaroxaban, or apixaban, PCCs, aPCCs, and rVIIa can be used to activate the coagulation system.

3. Animal and Human Data for Management of Dabigatran, Rivaroxaban, and Apixaban-Associated Hemorrhage

The PCC selection in our protocols for dabigatran, rivaroxaban, and apixaban-associated hemorrhage is based upon the available human and animal data presented in detail in Table 2 and summarized in Table 3. Human in vitro and animal studies have shown improved thrombin generation after administration of aPCCs in dabigatran treated animals [13, 14]. In a prospective crossover study, 4-factor PCCs failed to correct coagulation times or thrombin generation in humans taking dabigatran [15] suggesting that 4-factor PCCs are not useful for dabigatran-associated hemorrhage. rfVIIa could be considered in dabigatran-associated bleeding based on corrected time to thrombin generation from in vitro studies and decreased bleeding time in rats [13, 14, 16]. However, one case report suggested decreased bleeding after administration of rfVIIa in a patient taking dabigatran [17] and two reports noted continued bleeding [18–20]. Due to the mixed efficacy reported in case reports of rfVIIa, we use the aPCC (Feiba) prior to rfVIIa in refractory bleeding associated with dabigatran [21, 22]. In contrast to dabigatran, 4-factor PCCs improved rivaroxaban-induced coagulation testing abnormalities in a prospective crossover study in healthy volunteers [15], informing our decision to use a 4-factor PCC (Kcentra) in the setting of rivaroxaban-associated refractory bleeding. Given the mechanistic similarity and positive in vitro data, we also use a 4-factor PCC (Kcentra) in patients with refractory apixaban-associated bleeding [23]. Animal studies suggest that correction of coagulation testing does not always correlate to improvement in bleeding outcomes [14]. Outcomes of patients receiving factor concentrates via these protocols must be closely monitored.

Table 2: Animal and human studies using factor concentrates to reverse anticoagulant effect of dabigatran, rivaroxaban, and apixaban.

Author	Reversal agent	Test system	Coagulation test effect	Bleeding outcome effect
Dabigatran				
Van Ryn et al. [16]	aPCC	Rat	aPTT unchanged	Decreased bleeding time
	rfVIIa	Rat	aPTT decreased	Decreased bleeding time
Van Ryn et al. [14]	4-Factor PCC	Rat	PT decreased, aPTT unchanged	Decreased bleeding time
	aPCC	Rat	PT decreased, aPTT unchanged, increased thrombin generation	Decreased bleeding time
	rfVIIa		PT and aPTT decreased	Decreased bleeding time
Lambourne et al. [38]	4-Factor PCC	Mouse	No effect	No effect
	rfVIIa	Mouse	aPTT decreased	No effect
	4-Factor PCC + rfVIIa	Mouse	TT and aPTT decreased	No effect
	aPCC	Mouse	No effect	No effect
Pragst et al. [39]	4-Factor PCC	Rabbit	PT decreased, aPTT unchanged	Normalized blood loss
Zhou et al. [40]	4-Factor PCC	Mouse ICH		Prevented hematoma expansion; control level mortality
	rfVIIa	Mouse ICH		Ineffective control of hematoma expansion
Marlu et al. [13]	4-Factor PCC	Human in vitro	Increased amount of thrombin generation	
	rfVIIa	Human in vitro	Corrected time to thrombin generation	
	aPCC	Human in vitro	Corrected time to thrombin generation	
Eerenberg et al. [15]	4-Factor PCC	Human	No effect on aPTT, TT, or ECT	
Khoo et al. [41]	aPCC	Human in vitro	Increased thrombin generation and corrected time to thrombin generation	
Warkentin et al. [17]	rfVIIa	Human case report	Decreased aPTT and PT	Decreased blood loss
Garber et al. [18]	rfVIIa	Human case report		Worsening ICH
Truumees et al. [19]	rfVIIa	Human case report		Continued blood loss
Lillo-Le Louët et al. [20]	4-Factor PCC + rfVIIa	Human case report	PT and aPTT unchanged	Continued bleeding
	4-Factor PCC + rfVIIa	Human case report	PT and aPTT unchanged	Bleeding stopped with dialysis
Wychowski and Kouides [42]	3-Factor PCC	Human case report	TT and aPTT unchanged, PT decreased	No further bleeding
Dumkow et al. [43]	3-Factor PCC	Human case report		Clinical bleeding and hemoglobin stabilized
Rivaroxaban				
Perzborn and Tinel [44]	4-factor PCC	Rat	PT decreased	Bleeding time decreased
Godier et al. [45]	4-factor PCC	Rabbit	aPTT normalized, PT decreased	No effect on blood loss
	rfVIIa	Rabbit	aPTT normalized, PT decreased	Decreased bleeding time, no effect on blood loss

Table 2: Continued.

Author	Reversal agent	Test system	Coagulation test effect	Bleeding outcome effect
Perzborn et al. [46]	4-Factor PCC	Rat	Decreased PT, normalized TAT concentration	Reduced bleeding time
	aPCC	Rat	Decreased PT	Reduced bleeding time
		Primate	Reduced PT	Normalized bleeding time
	rVIIa	Rat	Decreased PT	Reduced bleeding time
		Primate	Decreased PT	Bleeding time unchanged
Marlu et al. [13]	4-Factor PCC	Human in vitro	Increased amount of thrombin generation	
	rfVIIa	Human in vitro	Corrected time to thrombin generation	
	aPCC	Human in vitro	Corrected all thrombin generation parameters	
Eerenberg et al. [15]	4-Factor PCC	Human in vivo	Normalized PT and thrombin generation	
Dinkelaar et al. [47]	4-Factor PCC	Human in vitro	No effect on PT or time to thrombin generation Normalized amount of thrombin generation	
Körber et al. [48]	4-Factor PCC	Human in vitro	No effect on aPTT, PT	
	rfVIIa	Human in vitro	Decreased clotting time, no effect on aPTT, PT	
Apixaban				
Escolar et al. [23]	PCC	Human in vitro	Increased thrombin generation	
	rfVIIa	Human in vitro	Increased thrombin generation	
	aPCC	Human in vitro	Increased thrombin generation	

aPCC: activated prothrombin complex concentrate; rfVIIa: recombinant factor VIIa.

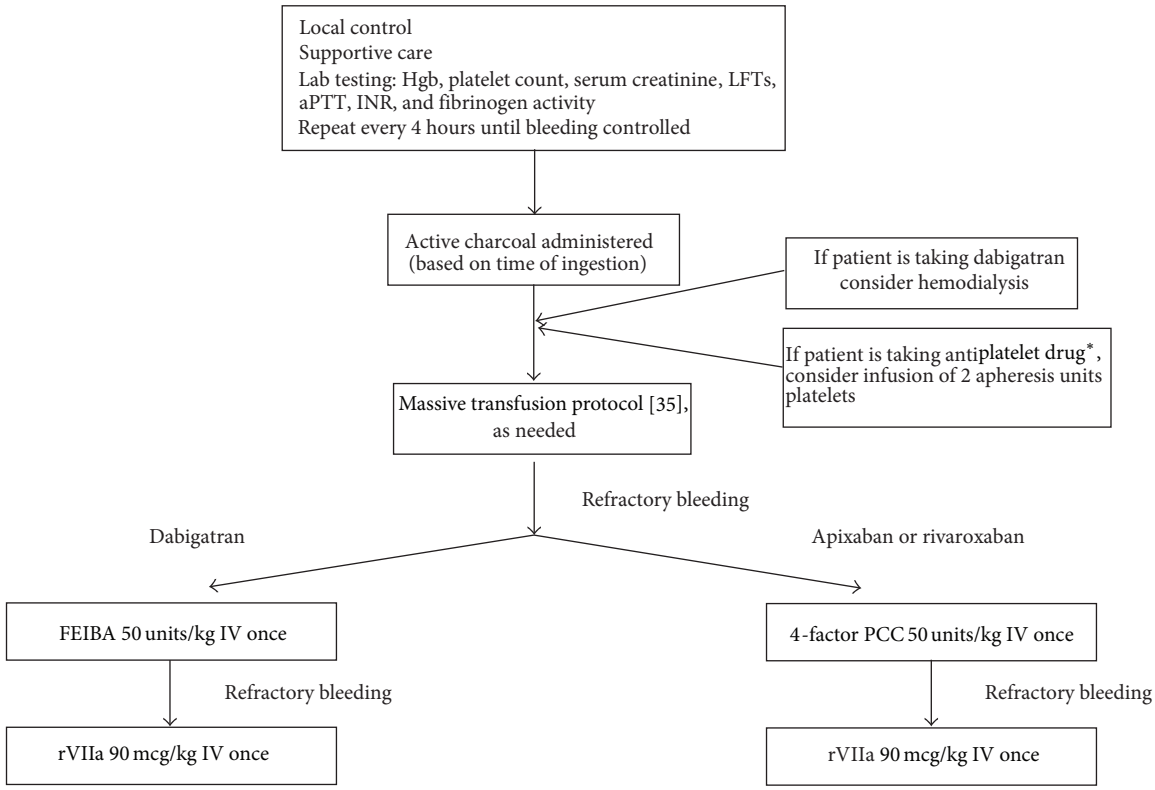
Table 3: Summary of animal and human data for reversal of dabigatran, rivaroxaban, and apixaban using factor concentrates.

	Dabigatran		Rivaroxaban		Apixaban	
	Animal	Human	Animal	Human	Animal	Human
3-factor PCC		Case report +/−				
4-factor PCC	Rats +/− Rabbits + Mice − Mice ICH +	In vitro + In vivo − Case report −	Rats + Rabbits +/−	In vitro +/− In vivo +		In vitro +
aPCC	Rats +/− Mice −	In vitro +	Rat + Primate +	In vitro +		In vitro +
rfVIIa	Rats +/− Mice +/− Mice ICH −	In vitro + Case report +/−	Rat + Rabbits +/− Primate +/−	In vitro +/−		In vitro +

+: effective; −: ineffective; +/−: mixed results between studies or between coagulation testing and bleeding outcomes; PCC: prothrombin complex concentrate; aPCC: activated prothrombin complex concentrate; rfVIIa: recombinant factor VIIa.

4. Protocols

Our institution has created protocols to help direct the use of factor concentrates in the treatment of life threatening bleeding in patients taking new oral anticoagulants (Figure 1). The initial measures are the same for any bleeding patient, with local intervention and supportive care. In addition, confirmation of medication, dosing, and duration since the last dose guides further therapy. Renal and hepatic function are evaluated to determine patient metabolism of



* Aspirin, clopidogrel, prasugrel, ticagrelor, and aspirin-dipyridamole

Figure 1: Management protocol for hemorrhage in patients taking dabigatran, rivaroxaban, or apixaban.

medication. Transfusion of packed RBCs and a transfusion protocol featuring a balance of packed red blood cells, plasma, and platelets may be utilized depending on the severity of hemorrhage. Based largely on retrospective data, the optimal ratio of plasma to packed RBCs administered is thought to be 1 : 1 or 1 : 2 [24]. The effects of antiplatelet agents are reversed by transfusion of 2 apheresis units of platelet concentrates if needed (see below) [25, 26].

Our management protocols in the setting of life threatening bleeding in the patient taking dabigatran, rivaroxaban, or apixaban are summarized in Figure 1. Activated charcoal may be administered if the last dose of anticoagulant was ingested less than 2 hours (dabigatran [2] or rivaroxaban [27]) or 3 hours (apixaban [28]) before presentation. Hemodialysis removes dabigatran and should be considered based on clinical status and need for surgical intervention. In a survey of nonmalignant hematologists, withholding the anticoagulant and dialysis were the most effective treatment used in 80% of bleeding episodes associated with rivaroxaban and dabigatran, respectively [29]. Thus, the use of specialized resuscitation protocols is often not required.

After transfusion of 4 units of PRBCs, we transfuse PRBCs, plasma, and platelets in a 1 : 1 : 1 ratio with a goal of hemoglobin of 9-10 g/dL [24]. We employ this transfusion strategy to avoid additional mechanisms of coagulopathy as the new oral anticoagulants prolong the PTT and INR and, thus, interfere with laboratory monitoring of other coagulation abnormalities. For patients on antiplatelet drugs we consider administration of two apheresis units of platelets.

In cases of refractory bleeding, the recommended factor concentrate in our protocols differs between the new oral anticoagulants. In patients taking dabigatran, we administer 50 units/kg IV of aPCC (Feiba) due to suggested benefit in human in vitro studies [13]. Based on human clinical trial evidence, 50 units/kg IV of the 4-factor PCC (Kcentra) is suggested for patients with refractory rivaroxaban or apixaban-associated hemorrhage. If bleeding continues, rfVIIa may be employed. Thrombosis has been reported with administration of all of the factor concentrates and the relative risk of hemorrhage and thrombosis must be considered.

Platelets are an essential but poorly understood component of hemostasis after injury. Previous evidence identifies admission platelet counts as inversely correlated with early mortality and supports transfusion of platelets with critical injury and trauma, even for platelet counts in the normal reference range. Quantitative platelet deficits have predicted progression of intracranial hemorrhage and mortality after traumatic brain injury. Study of platelet dysfunction has been hindered by technical complexity of existing platelet assays [30, 31].

Given the controversy which continues in the trauma and acute surgery literature, a number of observations can be made [30, 32–37]. Patients receiving antiplatelet agents

have an increased number of comorbidities. While recent retrospective work suggests limited impact of antiplatelet medications on outcome with trauma, limited prospective work suggests that the elderly and patients with intracranial hemorrhage are at greater risk for poor outcomes if taking antiplatelet agents. A normal platelet count is not reassuring in this setting. Normal clotting studies also do not predict good outcome. Despite this, a specific role for empiric platelet therapy has not been identified. Finally, while a variety of assays for platelet function have been reported, consensus regarding the optimal assay and the standard for acute management of injury has not been reached. Of assays which can be performed at the bedside, thromboelastography is the most promising bedside assay [31]. Our recommendation favoring consideration of platelet administration in the setting of life threatening bleeding must be understood in the context of the quality of studies which are available.

5. Conclusion

Our institution developed management protocols in an effort to standardize treatment of severe bleeding associated with use of new oral anticoagulants and approval of new factor concentrates. These protocols are based on limited human data but serve as a tool to guide therapy as providers gain experience with these anticoagulants and PCCs. Standardizing therapy allows the collection of clinical data, which can guide further trials. We anticipate continued modification of these protocols as laboratory and clinical experience expand. In particular, thrombosis risk with powerful newer agents designed to enhance coagulation must be monitored. At this time, data related to thrombosis risk is limited to anecdotes [5].

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Lisa Baumann Kreuziger and Joseph Keenan completed a literature search, primary paper writing, and revisions. Colleen Morton assisted with literature search, content review, and revisions and developed protocols. David Dries, MSE, MD, initiated the project, developed protocols, and completed content review and revisions.

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Clinical Study

Dabigatran in Secondary Stroke Prevention: Clinical Experience with 106 Patients

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Introduction. Our aim was to analyze our clinical experience with dabigatran etexilate in secondary stroke prevention. *Methods*. We retrospectively included patients starting dabigatran etexilate for secondary stroke prevention from March 2010 to December 2012. Efficacy and safety variables were registered. *Results*. 106 patients were included, median follow-up of 12 months (range 1–31). Fifty-six females (52.8%), mean age 76.4 (range 50–95, SD 9.8), median CHADS2 4 (range 2–6), CHA2DS2-VASc 5 (range 2–9), and HAS-BLED 2 (range 1–5). Indication for dabigatran etexilate was ischemic stroke in 101 patients and acute cerebral hemorrhage (CH) due to warfarin in 5 (4.7%). Dabigatran etexilate 110 mg bid was prescribed in 71 cases (67%) and 150 mg bid was prescribed in the remaining. Seventeen patients (16%) suffered 20 complications during follow-up. Ischemic complications (10) were 6 transient ischemic attacks (TIA), 3 ischemic strokes, and 1 acute coronary syndrome. Hemorrhagic complications (10) were CH (1), gastrointestinal bleeding (6), mild hematuria (2), and mild metrorrhagia (1), leading to dabigatran etexilate discontinuation in 3 patients. Patients with previous CH remained uneventful. Three patients died (pneumonia, congestive heart failure, and acute cholecystitis) and 9 were lost during follow-up. *Conclusions*. Dabigatran etexilate was safe and effective in secondary stroke prevention in clinical practice, including a small number of patients with previous history of CH.

1. Introduction

Ischemic stroke is one of the most common complications of atrial fibrillation (AF). The mean annual rate for ischemic stroke in patients with nonvalvular AF (NVAF) is 5%, rising to 23% in patients over 80 years old [1, 2].

Vitamin K antagonists (VKA) have been shown to be effective in reducing the incidence of stroke, even in the very elderly [3], and in secondary stroke prevention [4]. However, significant limitations such as individual variability in pharmacokinetics, the need for monitoring, interactions with both drugs and foods, and the risk of bleeding have led to the development of the new oral anticoagulants (NOAC) [5].

One of these NOACs is dabigatran etexilate, a direct thrombin inhibitor approved by the European Medicines Agency in August 2011, based on the results of the clinical trial entitled "Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY)" [6], for primary and secondary prevention of cardioembolic stroke in patients with NVAF [7]. In the direct comparison with warfarin, dabigatran etexilate 150 mg bid (D150) was superior in preventing stroke and systemic embolism, with a 24% relative risk reduction (RRR) for stroke, while the 110 mg bid dose (D110) was shown to be noninferior to warfarin and safer [6]. Furthermore, both doses resulted in a lower risk of cerebral hemorrhage (CH) compared to warfarin, even in patients over 75 years old [5, 6, 8, 9]. A subgroup analysis in secondary stroke prevention with 3,623 patients showed a nonsignificant trend in favor of dabigatran etexilate having greater efficacy, with a significantly lower rate of CH [10]. Dabigatran etexilate has also been shown in postmarketing surveillance studies to be both safe [11] and cost-effective [12]. Clinical guidelines recommend the use of dabigatran etexilate over VKA in

Table 1: Baseline characteristics.

Baseline clinical variables	*N*
Number of patients (females, %)	106 (56, 52.8)
Mean age ± SD (range)	76.4 ± 9.8 (50–95)
Hypertension (%)	84 (79.2)
Diabetes (%)	35 (33.0)
Peripheral artery disease (%)	5 (4.7)
Ischemic heart disease (%)	9 (8.5)
Previous systemic bleeding (%)*	8 (7.5)
Heart failure (%)**	8 (7.5)
Mean GFR ± SD (range)***	72.6 mL/min ± 21.3 (32–122)
GFR < 60 mL/min (%)	29 (27.9)
Median NIHSS (range)	1 (0–18)
Median mRS (range)	1 (0–4)
Median CHADS (range)	4 (2–6)
Median CHA2DS2-VASc (range)	5 (2–9)
CHA2DS2VASc > 5 (%)	75 (70.8)
Median HAS-BLED (range)	2 (1–5)
Reason for starting dabigatran	
Stroke/TIA (%)	101 (95.3)
VKA-related bleeding (%)	5 (4.7)
Previous anticoagulation (%)	39 (36.8)
Ischemic stroke (*n* with INR < 2)	34 (29)
Bleeding (*n* with INR > 2)	5 (5)
Dabigatran dose	
150 mg/12 hours (%)	35 (33)
110 mg/12 hours (%)	71 (67)
Age > 75	66
HAS-BLED ≥ 3	14
GFR 30–50 mL/min	9
Concomitant antiaggregation therapy (%)	9 (8.5)

*Any bleeding with anemia considered as 2-point drop in hemoglobin measured in g/dL and/or need for packed red blood cell transfusion (RCC). **Congestive heart failure or left ventricular ejection fraction <40. ***Measured by Crockcroft-Gault formula. SD: standard deviation, DM: diabetes mellitus, GFR: glomerular filtration rate, NIHSS: National Institutes of Health Stroke Scale, and mRS: Modified Rankin Scale.

primary and secondary prevention of stroke in patients with NVAF, in case of the AHA guidelines with a level of evidence B [13–16]. The most effective dose of dabigatran etexilate is 150 mg/12 hours, although 110 mg/12 hours should be used in certain circumstances (over 75 years old, creatinine clearance 30–50 mL/min, patients at high risk of hemorrhage (HAS-BLED ≥ 3) and in patients treated with verapamil, as recommended by the European Union guidelines [8, 17, 18]. In the United States the Food and Drug Administration approved dabigatran etexilate 75 mg bid instead of 110 mg bid.

Despite the above, there is a lack of quality efficacy and safety data on the use of dabigatran etexilate in secondary prevention of stroke in routine clinical practice [19]. Moreover, ischemic and hemorrhagic risk in the patients selected for the clinical trials may be lower than in actual clinical practice. Data regarding efficacy and safety in unselected populations is lacking, although at least two observational registries are ongoing, and the FDA reported data regarding safety in clinical practice [20–23]. Our aim was to analyze our clinical experience with dabigatran etexilate in secondary stroke prevention.

2. Methods

This was a retrospective analysis of patients treated with dabigatran etexilate for secondary prevention of stroke at our large teaching hospital between March, 1, 2010 and December, 31, 2012. Independent Ethics Committee approval was obtained.

Criteria for starting treatment with dabigatran etexilate were diagnosis of stroke or TIA secondary to NVAF, no absolute contraindications for anticoagulation, normal liver function, and glomerular filtration rate (GFR) ≥ 30 mL/min.

Demographic and clinical variables, history of treatment with VKA, and analytical data (INR, GFR) were all recorded. The stroke or systemic embolism and hemorrhage risks were assessed using the CHADS2, CHA2DS2-VASc, and HAS-BLED scores. The dabigatran etexilate dose and the concomitant use of antiaggregation therapy were specified.

Patients were followed up through outpatient appointments and review of electronic records until their death or loss to follow up. Observation time (months), ischemic and hemorrhagic complications, and time to the event (months) were recorded. During follow-up, adherence to dabigatran etexilate was assessed by means of clinical questioning. CH and any bleeding with anemia, considered as 2-point drop in hemoglobin measured in g/dL, and/or need for red blood cell transfusion were considered serious adverse events, following the definition of RE-LY of major bleeding [6].

Statistical Methods. We performed a descriptive analysis of baseline clinical variables (gender, age > 75, hypertension, DM, reason for starting dabigatran etexilate, previous anticoagulation, CHA2DS2-VASc > 5, GFR < 60, dabigatran etexilate dose, concomitant antiaggregation therapy). The data were analyzed using SPSS 20 statistical software. Categorical variables are presented as absolute numbers and frequencies and quantitative variables are expressed as mean (standard deviation) and median (min-max range). Treatment continuation is represented by Kaplan-Meier survival curve.

3. Results

106 patients were included with a median follow-up of 12 months (range 1–33). 56 were female (52.8%), with a mean age 76.4 years (range 50–95, SD 9.8), median CHADS2 4 (range 2–6), CHA2DS2-VASc 5 (range 2–9), and HAS-BLED 2 (range 1–5). Indication for dabigatran etexilate was ischemic stroke in 101 patients (66.3% anticoagulation naïve and 33.7% previously on warfarin) and acute CH due to VKA in 5 (4.7%). Dabigatran etexilate 110 mg bid was prescribed in 71 cases (67%), mostly due to age over 75 years (Table 1), and 150 mg bid was prescribed in the remaining. Adherence was

Table 2: Ischemic complications.

Patient	Age	Baseline mRS	CHA2DS2-VASc	HAS-BLED	Dabigatran dose	Concomitant antiaggregation therapy	Event	TE (months)	Discontinued yes/no
#1	85	3	5	2	110	No	CI	1	Yes
#2	95	1	6	3	110	No	TIA	10	No
#3	79	1	6	3	110	No	CI	1	No
#4	83	1	7	3	110	No	TIA	1	No
#5	50	4	3	1	150	No	TIA	1	No
#6	83	2	6	2	110	No	TIA	1	No
#7	83	2	6	2	110	No	CI*	1	No
#8	78	0	6	2	110	Yes	TIA	4	No
#9	73	0	6	2	110	No	TIA	6	No
#10	85	3	9	4	110	No	NSTE-ACS	8	No

CI: cerebral infarct. *Treated with intravenous tPA. mRS: Modified Rankin Scale. TE: time to event. TIA: transient ischemic attack. NSTE-ACS: non-ST-segment elevation acute coronary syndrome.

confirmed for every patient. Sixty-six patients who suffered ischemic stroke were previously diagnosed as NVAF, only 34 (51.5%) were on VKA, and 29 (85,3%) had INR below 2. All patients with CH due to VKA had INR above 3 at the time of diagnosis. Nine patients received concomitant antiaggregation therapy due to vascular disease. Baseline characteristics, treatment indication, and concomitant medication are summarized in Table 1. Seventeen patients (16%) suffered 20 events during follow-up and 3 patients suffered 2 events. Ischemic complications (10) (Table 2) consisted of 6 transient ischemic attacks (TIAs), 3 ischemic strokes (2 of them disabling), 1 treated with intravenous thrombolysis, and 1 acute coronary syndrome. Three TIAs and all ischemic strokes occurred during the first month of treatment. Hemorrhagic complications (10) (Table 3) were CH (1, not disabling), gastrointestinal bleeding (6, of which 3 required blood transfusions), mild hematuria (2), and mild metrorrhagia (1). Four patients suffered serious adverse events. Events only led to dabigatran etexilate discontinuation in 4 patients (Figure 1).

All patients treated with dabigatran etexilate because they had a previous CH due to VKA remained uneventful. Three patients died due to pneumonia, congestive heart failure, and acute cholecystitis and 9 were lost during follow-up.

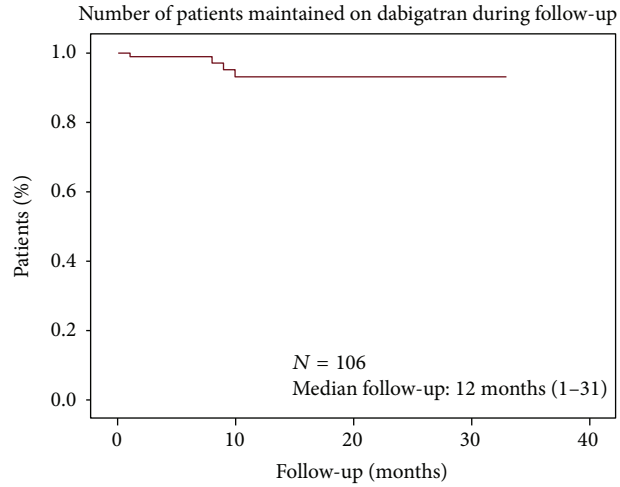

Figure 1: Number of patients maintained on dabigatran during follow-up (Kaplan-Meier survival curve).

4. Discussion

We present a series of patients treated with dabigatran etexilate for secondary prevention of stroke with reasonably favourable results in terms of efficacy and safety, taking into account the high-risk features of our patients. The prevention of stroke in patients with NVAF is a serious problem which is far from being resolved. A study of NVAF patients admitted for a first stroke found that 60% were not receiving anticoagulation and of those taking VKA, 75% had subtherapeutic INR [24]. Our data are similar in that only 34 of the 66 patients with a history of NVAF with ischemic stroke were treated with VKA, and 29 (85,3%) of them had a subtherapeutic INR. These results reflect the continued underuse of anticoagulant therapy, despite the clinical guideline recommendations and the difficulty in maintaining the INR in the therapeutic range in patients treated with VKA. This phenomenon is not considered to be explained by suboptimal adherence, but for VKA erratic pharmacokinetics [24].

Our patients treated with dabigatran etexilate had a higher ischemic and hemorrhagic risk profile than the patients included in the RE-LY study [6, 25]. The mean age was higher (76 versus 71 years) and there was a larger proportion of women (52.8% versus 36.4%). In addition, all had previously suffered a stroke or TIA, as opposed to only 20% of the RE-LY patients. The RE-LY secondary-prevention subgroup had a similar CHADS2 score to our cohort but with a lower age (mean 70.5 years) [10]. Moreover, we included 5 patients with CH, which was an exclusion criterion in RE-LY. In our series, a majority (67%) received D110, mainly, because they were aged > 75 and due to their high hemorrhagic risk.

Table 3: Bleeding complications.

Patient	Age	Baseline mRS	CHA2DS2-VASc	HAS-BLED	Dabigatran dose	Concomitant antiaggregation therapy	Event	TE (months)	Discontinued yes/no	BT
#3	80	1	6	3	110	Yes	UGIB	10	Yes	Yes
#10	85	3	9	4	110	Yes	Rectal bleeding	9	No	No
#11	90	3	6	4	110	No	Rectal bleeding	1	No	No
#12	86	2	5	2	110	No	UGIB	9	Yes	Yes
#12	86	2	5	3	110	No	CH**	9	Yes	No
#13	63	0	3	1	150	No	Metrorrhagia	1	No	No
#14	81	2	6	3	110	No	UGIB	8	Yes	Yes
#15	87	0	5	3	110	No	UGIB	6	No	No
#16	77	3	6	3	110	No	Hematuria	1	No	No
#17	78	0	3	1	110	No	Hematuria	8	No	No

** Administration of prothrombin complex concentrate. mRS: Modified Rankin Scale, TE: time to event, BT: blood transfusion, UGIB: upper gastrointestinal bleeding, and CH: cerebral hemorrhage.

During follow-up, a total of 20 adverse events were recorded in 17 patients, 10 ischemic (9.4%) (Table 2) and 10 hemorrhagic (9.4%) (Table 3). The mortality rate was low (2.8%) and unrelated to dabigatran etexilate, despite the fact that the patients were elderly and suffered from multiple medical problems. It is also worth noting that treatment only had to be permanently discontinued in 4 patients (Figure 1).

The RE-LY study provided us with an estimated annual risk of stroke in patients on treatment with dabigatran etexilate of around 1% in primary prevention and 2% in secondary prevention [6, 10]. Although our event rate may seem higher (around 10%), the higher risk basal features of our cohort may be accounted for this increase [25]. In addition, if only cerebral infarcts are considered, we observed 3 cases (2 of them disabling), a figure consistent with RE-LY study. The majority of cerebral ischemic events occurred during the first month of treatment, which might suggest that this is a high-risk period. In terms of other ischemic events, in our series, a patient with a history of revascularized chronic ischemic heart disease suffered an acute coronary syndrome which required stenting.

Recently, a Danish registry of dabigatran etexilate in clinical practice and a FDA report on dabigatran etexilate safety confirm a bleeding risk of this drug comparable to warfarine, with lower CH rate [20, 23]. Moreover, Weber effect (an increased likelihood of reporting adverse events in newly approved drugs) may account for the excess of reports of bleeding complications in patients treated with dabigatran etexilate [23]. In our cohort there were 9 systemic bleeding complications, none of which were fatal, and only 3 required blood transfusions. Only in 3 cases dabigatran etexilate had to be discontinued permanently. The treatment for the bleeding episodes consisted of temporarily discontinuing dabigatran etexilate. None of the patients required prothrombin complex concentrates or hemodialysis. Although there is considered to be a lower risk of bleeding with D110, [5, 6] in our series, the majority of patients with hemorrhagic complications were taking this dose. Therefore, despite D110 seeming to be the best option in patients at high risk of bleeding, clinical and laboratory data should be monitored for signs of such events. Several factors such as age, renal function impairment, and comedications increase the risk of hemorrhagic complications [26]. The concomitant use of antiaggregation therapy increased the risk of major bleeding in the RE-LY patients [27]. In our series, of the 9 patients treated with dual therapy, 2 had hemorrhagic complications; although neither was serious, in one case dabigatran etexilate was discontinued permanently for safety concerns. These data suggest that the concomitant use of antiaggregation therapy may be safe, but further evidence is required to confirm this and these patients need to be closely monitored for bleeding events.

The most feared complication of anticoagulant treatment is CH. The risk is however lower with either dose of dabigatran etexilate than with warfarin [10]. In our series, there was only 1 CH during the follow-up period, with the 110 mg dose. None of the patients on dual therapy or with a history of previous VKA-related CH suffered a CH. The reasons for the lower rate of intracranial bleeding with either dose of dabigatran etexilate are not fully understood. It is speculated that dabigatran etexilate's single therapeutic target could preserve certain hemostatic mechanisms in the brain that may be protective against spontaneous CH [8]. However, ongoing registries will help clarify safety of dabigatran etexilate in routine clinical practice [21, 22].

Our study had a high percentage of anticoagulation-naïve patients (63.2%), slightly higher than that in the RE-LY study (50.4%). As in RE-LY, their outcome did not differ significantly from that of the rest of the patients [28, 29]. Dabigatran etexilate therefore appears to be a safe and effective alternative for patients who have not previously received VKA.

Our study has some limitations, such as the limited number of patients and the short follow-up period in a proportion of cases. Nevertheless, these were 106 patients with high baseline risk treated with dabigatran etexilate for secondary stroke prevention with a satisfactory overall outcome, with high treatment adherence and a relatively low rate of severe or disabling events. These data confirm in routine clinical practice the published benefits of dabigatran etexilate in secondary stroke prevention found in controlled clinical trials. However, caution is needed, as this treatment is not exempt of complications, as our data show.

Disclosure

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Preventive Strategies against Bleeding due to Nonvitamin K Antagonist Oral Anticoagulants

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Dabigatran etexilate (DE), rivaroxaban, and apixaban are nonvitamin K antagonist oral anticoagulants (NOACs) that have been compared in clinical trials with existing anticoagulants (warfarin and enoxaparin) in several indications for the prevention and treatment of thrombotic events. All NOACs presented bleeding events despite a careful selection and control of patients. Compared with warfarin, NOACs had a decreased risk of intracranial hemorrhage, and apixaban and DE (110 mg BID) had a decreased risk of major bleeding from any site. Rivaroxaban and DE showed an increased risk of major gastrointestinal bleeding compared with warfarin. Developing strategies to minimize the risk of bleeding is essential, as major bleedings are reported in clinical practice and specific antidotes are currently not available. In this paper, the following preventive approaches are reviewed: improvement of appropriate prescription, identification of modifiable bleeding risk factors, tailoring NOAC's dose, dealing with a missed dose as well as adhesion to switching, bridging and anesthetic procedures.

1. Introduction

Nonvitamin K antagonist oral anticoagulants (NOACs) [1] have been approved by the European Commission, as an alternative to vitamin K antagonists (VKAs) and parenteral anticoagulants, for the following indications: prevention of venous thromboembolism (VTE) in adult patients undergoing elective hip or knee surgery (apixaban [2–4], dabigatran etexilate (DE) [5–7], and rivaroxaban [8–11]), prevention of stroke and systemic embolism in adult patients with nonvalvular atrial fibrillation (NVAF) (apixaban [12], DE [13], and rivaroxaban [14]), treatment and secondary prevention of

deep vein thrombosis (DVT) and pulmonary embolism (PE) in adults (rivaroxaban and DE [15, 16]), and prevention of atherothrombotic events after an acute coronary syndrome with elevated cardiac biomarkers, combined with a single or dual antiplatelet therapy (acetylsalicylic acid alone or associated with clopidogrel or ticlopidine) (rivaroxaban [17, 18]). In NVAF trials, NOACs proved to be either superior or noninferior to warfarin for the prevention of stroke and systemic embolus [12–14]. Several guidelines (European Society of Cardiology, American College of Chest Physicians, and Canadian Cardiovascular Society) recommend NOACs as broadly preferable to VKAs in most patients with NVAF. This will lead to a wider use of NOACs in the future.

Compared with warfarin, the NOACs showed less risk of intracranial hemorrhage, and apixaban and DE (110 mg bid) showed less risk of major bleeding from any site [12–14]. Unfortunately, rivaroxaban and DE had an increased risk of gastrointestinal (GI) bleeding compared with warfarin. Apixaban was associated with fewer GI bleeding compared with warfarin, but it was not statistically significant [19].

Bleeding events were reported despite a regular monitoring of adverse events, a strong medication adherence and a careful selection of patients in the pivotal clinical trials (exclusion of patients with assumed poor compliance, bleeding risks, renal insufficiency, etc.). Extension of adverse events into clinical practice is currently under research and postmarketing registers, like the GLORIA-AF registry, are recruiting [20, 21].

The aim of this review is to highlight the bleeding risks with NOACs in the clinical practice and to broach different prevention strategies to minimize these adverse events.

2. NOACs and Major Bleeding

Large randomized controlled trials (RCT) allowing head-to-head comparison between NOACs are not available. Only indirect comparison on bleeding can be proposed since the three pivotal NOAC trials contain a common comparator (i.e., adjusted-dose warfarin). Even so there are limits in the conclusiveness of such comparisons, like differences in the study populations (differences in reporting age, renal function, exclusion criteria, and additional risk factors), in the definition of adverse events, in study protocols (open or double-blind design) and in time in therapeutic range (TTR) of the international normalized ratio (INR) values among these RCTs. In the three pivotal trials comparing NOACs with warfarin, evidence of the validation of the stated INR was not provided. This makes cross-trial comparisons difficult [30–32].

Few data exist regarding the safety of NOACs in clinical practice, and the available information reflects the limitations of post-authorization studies, such as reporting bias. Recent evidence provides contradiction to earlier safety reports that suggested that the major bleeding rates in patients receiving NOACs in clinical practice did not exceed the rates reported in the pivotal trials [21, 33].

McConeghy et al. evaluated DE adverse event reports with a reported bleeding event and/or reported fataloutcome compared with warfarin [34]. This retrospective analysis of the FDA Adverse Event Reporting System (FAERS) database suggested increased odds of bleed-related mortality in clinical practice with dabigatran compared with the clinical trials [34].

The bleeding reports were driven by patients who were older, renally impaired, acutely injured, and had low body weight. These patients were underrepresented in the RELY trial and may have higher risks of dabigatran-induced bleeding. Furthermore, reports from FAERS showed underreporting bias [34].

For rivaroxaban, the following clinical characteristics were associated with an increased risk for major GI bleeding [32]: concurrent aspirin or nonsteroidal anti-inflammatory drugs (NSAID) use, prior vitamin K antagonist use, decreased creatinine clearance, prior stroke, transient ischemic attack or systemic embolization, sleep apnea, cigarette smoking, chronic obstructive pulmonary disease, male gender, patient treated with histamine-2 receptor antagonist or proton pump inhibitor (PPI), and prior upper and lower GI bleeding. Most of these characteristics were also associated with an increased risk of major GI bleeding in patients treated with warfarin [32].

Concerning apixaban, Hylek et al. recently analyzed the bleeding events of all patients who received at least one dose of a study drug in the ARISTOTLE trial. All major bleedings (defined by the criteria of the International Society on Thrombosis and Haemostasis (ISTH)) that appear from the time of the first dose until 2 days after the last dose was received were included [19]. Apixaban, compared with warfarin, was associated with a 31% reduction of first major bleeding and with half of the death within 30 days following a major hemorrhage. Independent factors associated with first major hemorrhage were: older age, prior hemorrhage, prior stroke or transient ischemic attack (TIA), diabetes, lower creatinine clearance, and decreased hematocrit. Female gender and liver disease were more associated with apixaban randomization, compared with warfarin. A subgroup analysis showed that patients with renal dysfunction and low body weight had a greater reduction in bleeding with apixaban versus warfarin than in patients with normal renal function and higher body weight.

The use of aspirin and nonsteroidal anti-inflammatory drugs (NSAID) with apixaban increased independently the risk of major bleeding by around 30% [19].

Based on the RE-LY trial, DE 150 mg BID, combined with a single or dual antiplatelet therapy, increased the rate of extracranial bleeding [35].

A brief summary of NOAC's pharmacology is available in Table 1 [22–25].

3. Prevention of Major Bleeding in Patients Receiving NOACs

The following preventive strategies are achievable to reduce the incidence rate of NOAC-related major bleeding:

Table 1: Summary of pharmacokinetic properties of nonvitamin K antagonist oral anticoagulants (NOACs) [22–25].

	Dabigatran	Rivaroxaban	Apixaban
Target	Factor lla	Factor Xa	Factor Xa
Prodrug	Yes	No	No
Tmax (h)	1.5–3.0	2.0–4.0	3.0–4.0
Distribution volume (L)	60–70	±50	23
Half-life (h)	11: healthy individuals 12-13: elderly	5–9: healthy individuals 11–13: elderly	8–15: healthy individuals
Bioavailability	3–7% pH sensitive	80–100%: 10 mg 66%: 15–20 mg under fasting conditions	±50%
Protein binding	35%	>90%	87%
Metabolism	Conjugation	CYP-dependent and independent mechanism	CYP-dependent mechanism
Active metabolites	Yes glucuronide conjugates	No	No
Elimination	80% renal 20% bile (glucuronide conjugation)	33% unchanged via the kidney 66% metabolized in the liver into inactive metabolites then eliminated via the kidney or the colon in an approximate 50% ratio	25% renal 75% through the liver while the residue is excreted by the hepatobiliary route in the feces
Effects of food	Tmax delayed; Cmax and AUC unchanged	Tmax delayed; Cmax and AUC increased (76% and 30–40%, respectively)	Tmax delayed; Cmax and AUC unchanged
CYP substrate	No	CYP3A4, CYP2J2	CYP3A4
P-gp substrate	DE: yes	Yes	Yes

(1) improving appropriate prescription,

(2) identifying modifiable bleeding risk factors,

(3) improving individual benefit-risk by tailoring NOACs dose,

(4) dealing with a missed dose,

(5) adhering to switching procedures,

(6) adhering to bridging procedures,

(7) adhering to anesthetic recommendations.

3.1. Improving Appropriate Prescription

3.1.1. Off-Label Use or Misuse. The off-label use or misuse of NOACs means a use outside an appropriate indication or at inappropriate doses. For example the off label uses of DE are: age >80 years, patients with liver or kidney disease, with previous bleeding, with previous ischemic heart disease or severe renal impairment, patients with a $CHADS_2$ score of 0 and patients with coadministration of systemic ketoconazole, cyclosporine, itraconazole, tacrolimus, dronedarone and aspirin [36–41].

Misuse is frequent (between 8.0 and 43.5%) and can induce supratherapeutic anticoagulation with a potential risk of severe or even fatal bleeding [36–38]. A lack of consensus in NVAF's definition and the complexity of dose regimens for different indications and populations (as illustrated in Table 2) can lead to inadequate prescriptions.

Because of an increased risk of bleeding, NOACs should be used with caution if a concomitant use of antiplatelet agents is indicated [42–44], and NSAIDs should be avoided if possible.

3.1.2. Renal Function. Several cases of severe bleedings (often leading to death) have been reported in older patients under DE [45]. Renal failure was the most recurrent risk factor associated with bleedings in these elderly patients and should therefore be reassessed during the treatment if clinically indicated (fluctuating renal function, diuretic use, and hypovolemia).

In clinical trials of DE and rivaroxaban for NVAF, drug eligibility and dosing were determined by using the Cockcroft-Gault equation to estimate creatinine clearance (Cr_{Cl}), a measure of renal function. The modification of diet in renal disease (MDRD) equation (used to estimate glomerular filtration rate (eGFR)) leads, in low GFR values, to a surestimated renal function in comparison with the Cockcroft-Gault equation [46, 47]. Thus, by using the MDRD equation, many elderly patients with AF would either become incorrectly eligible for these drugs or would receive higher doses than required. Regulatory authorities and drug companies recommend therefore the use of the Cockcroft-Gault equation instead of the MDRD-derived eGFR to calculate

Table 2: Indication and dose regimens of Dabigatran etexilate, Rivaroxaban, and Apixaban [2–18].

	Dabigatran etexilate	Rivaroxaban	Apixaban
VTE Prophylaxis	220 mg/day (2 capsules of 110 mg OD) or 150 mg/day (2 capsules of 75 mg OD) → if Cr_{Cl} 30–50 mL/min, if >75 ys, if verapamil, amiodarone and quinidine THR: 28–35 days TKR: 10 days	10 mg/day (1 tablet of 10 mg OD) THR: 5 weeks TKR: 2 weeks	5 mg/day (1 tablet of 2.5 mg BID) THR: 32–38 days TKR: 10 days
Nonvalvular atrial fibrillation	300 mg/day (1 capsule of 150 mg BID) 220 mg/day (EU) (1 capsule of 110 mg BID) → if >80 ys or verapamil 150 mg/day (US) (1 capsule of 75 mg BID) → if Cr_{Cl} between 15–30 mL/min	20 mg/day (1 tablet of 20 mg OD) 15 mg/day (1 tablet of 15 mg OD) → if Cr_{Cl} between 15–49 mL/min	10 mg/day (1 tablet of 5 mg BID) 5 mg/day (1 tablet of 2.5 mg BID) → if at least 2 of the following conditions: ≥80 ys, ≤60 kg or serum creatinine ≥1.5 mg/dL; or if Cr_{Cl} 15–29 mL/min
VTE treatment	Adopted indication by the CHMP on 25th April 2014 (EU) 300 mg/day (US) (1 capsule of 150 mg BID) after 5–10 days of parenteral anticoagulation	Treatment phase: 30 mg/day (1 tablet of 15 mg BID) for 21 days Secondary prevention: 20 mg/day (1 tablet of 20 mg OD) 15 mg/day (1 tablet of 15 mg OD) → if Cr_{Cl} between 15-49 mL/min and the risk of bleeding outweighs the risk of recurrent DVT or PE	×
Prevention of atherothrombotic events after ACS with elevated cardiac biomarkers	×	5 mg/day (1 tablet of 2.5 mg BID) in association with ASA (75–100 mg) alone or ASA + clopidogrel (75 mg)	×

×Off-label; BID: twice daily; Cr_{Cl}: creatinine clearance; DVT: Deep-vein thrombosis; OD: once daily; PE: pulmonary embolism; THR: total hip replacement; and TKR: total knee replacement; vte: venous thromboembolism; CHMP: committee for medecinal products for human use.

eligibility for NOACs and adapted dose for elderly patients with AF.

3.1.3. Bioavailability. By opening DE capsules, the bioavailability reaches 75% and increases highly the bleeding risk [43]. Therefore, gastrostomies and jejunostomies are not advised with DE.

For rivaroxaban, Moore et al. studied its relative bioavailability when administered as a whole tablet orally or as a crushed tablet mixed with applesauce or water suspension through a nasogastric tube (NGT) or a gastrostomy. There was no difference in both pharmacokinetics, so that they concluded to a safe administration for rivaroxaban through a NGT or gastrostomy [48].

To the best of our knowledge, there is currently no data available for apixaban.

3.1.4. Patient with Low Body Weight. Despite recent data suggesting that the clearance of anticoagulants increases with weight, the optimal dosing strategies for most anticoagulants remain unknown [49]. This uncertainty is mainly relevant for anticoagulants with fixed-dosing regimen such as NOACs, in contrast to anticoagulants for which efficacy monitoring is routinely required (i.e., VKAs).

In the RE-LY study, the overall mean weight of patient was 82.6 kg (ranging from 32 to 222 kg) with 17.1% of patients weighing above 100 kg. A tendency of increasing *dabigatran* concentrations with decreasing body weight was found [49]. There is very limited clinical experience in patients with a body weight <50 kg. In this population, no dose adjustment is advised but a close clinical surveillance is recommended by the different authorities [43, 50].

A study with 48 healthy participants assessed the influence of extremes of body weight (≤50 kg and >120 kg) on

the pharmacokinetics (PK) of *rivaroxaban* 10 mg OD as compared with normally weighted patients (80 kg). The results show that the C_{max} of rivaroxaban was increased by 24% in subjects weighing ≤50 kg while the area under the curve (AUC) was unaffected (difference is <25%) by body weight. The 24% increase in C_{max} in patients with low body weight resulted in a small (15%) increase in prolongation of prothrombin time (PT), which was not considered as clinically relevant [51]. However, this was performed with STA Neoplastin CI+, a reagent with a moderate sensitivity to rivaroxaban. In a population pharmacokinetic/pharmacodynamics (PK-PD) modeling study, there was a clear increase in the volume of distribution interrelated with weight and probably due to secondary increase of body volume [52]. Based on these findings, a higher exposure to rivaroxaban could be expected in patients with low body weight and, consequently, a higher risk of bleeding with a standard dose. However, these preliminary data need confirmations in larger studies. Similarly to dabigatran, no dose adjustment is currently proposed by the European and American agencies in patients with extreme body weight (<50 kg or >120 kg) [42].

Apixaban has also been evaluated in patients with extreme body weight. A 30% and 20% increase in C_{max} and AUC, respectively, has been seen in patients weighing <50 kg [53]. These modifications were considered as modest and unlikely to be clinically meaningful. However, further evaluation of clinical data is warranted. Since the body weight seems to have a modest effect on apixaban exposure, the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) recommend dose adjustment in patients weighing <60 kg (2.5 mg BID instead of 5 mg BID) in the presence of additional risk factors, namely, age ≥80 years or serum creatinine >1.5 mg/dL [54, 55].

3.1.5. Patients with Impaired Hepatic Function. Hepatic impairment may alter the pharmacokinetics of drugs that are metabolized by the liver, such as rivaroxaban and apixaban [56]. Data on the use of NOACs in hepatic impairment are scarce and mainly restricted to single-dose studies in a limited number of subjects with mild or moderate hepatic impairment. In addition, patients with elevated liver enzymes and/or bilirubin levels were excluded from clinical trials. The manufacturer's recommendations for rivaroxaban, apixaban, and DE regarding impaired hepatic function are based on both Child-Pugh classification and liver-related exclusion criteria applied in clinical trials [57].

Stangier et al. found no influence of moderate hepatic impairment on pharmacokinetic, pharmacodynamics and safety profile of DE following administration of a single 150 mg dose in 24 subjects [56]. However, patients with elevated liver enzymes above 2 times the upper limit of normal (ULN) were excluded from clinical trials [43]. The Summary of Product Characteristics of the European Commission (EU-SmPC) contraindicates the use of DE in patients with hepatic impairment or liver disease expected to have any impact on survival [43].

In contrast to dabigatran, liver metabolism is an important route of elimination for FXa inhibitors. Approximately two-thirds of the administered *rivaroxaban* dose is metabolized by the liver via CYP3A4, 2J2, and CYP-independent mechanisms to inactive metabolites [42]. The AUC following administration of a single dose of rivaroxaban 10 mg was increased by 2.27-fold in patients with moderately impaired liver function (Child-Pugh B). Moderate but not mild hepatic impairment reduced total body clearance of rivaroxaban and led to pharmacodynamics effects [58]. Therefore, EMA contraindicates its use in case of hepatic disease associated with coagulopathy and clinically relevant bleeding risk including cirrhotic patients with Child Pugh B and C.

Apixaban undergoes liver metabolism mainly via CYP3A4/5, but other isoenzymes are also involved [44]. Data on the use of apixaban in patients with moderate hepatic impairment (Child Pugh B) show that AUC increased slightly by 1.09-fold when a 5 mg single dose was administered [59]. Apixaban should be used with caution in patients with elevated liver enzymes (ALT/AST > 2 × ULN) or total bilirubin ≥ 1.5 × ULN because those were excluded from clinical trials. The EMA recommends to perform liver function test prior to initiating apixaban and to use it with caution in patients with moderate and severe hepatic impairment. Apixaban is contraindicated in patients with hepatic disease associated with coagulopathy and clinically relevant bleeding risk [44].

In conclusion, limited data indicate that the PK and PD of NOACs such as rivaroxaban or apixaban can be modified in moderate hepatic impairment, in contrast to dabigatran. Further evaluations are needed to better support clinicians in their decision process. Evaluations of liver function are recommended before prescribing NOACs and regularly during treatment in patients with possible liver impairment.

3.1.6. Drug Interactions. In addition to these specific populations, NOACs have also drug interactions with P-glycoprotein substrates or CYP3A4 inhibitors that may highly increase their plasma concentrations and hence bleeding risks. Table 3 summarizes drug-drug interactions reported in the literature as well as recommendations for dose adaptation and contraindications for the EU-SmPC and FDA prescribing guidelines [26–28].

3.2. Identifying Modifiable Bleeding Risk Factors. Identification of modifiable and nonmodifiable bleeding risk factors will help to ascertain and manage the risk of major bleeding. This can be performed before NOAC's initiation using validated bleeding scores like the International Society of Thrombosis and Hemostasis bleeding assessment tool [60]. The HAS-BLED score is a tool designed for assessing bleeding risks during anticoagulation. Despite debate in the literature on its ability to predict any clinically relevant bleeding, it may be useful for this purpose [61, 62]. For example, a HAS-BLED score ≥3 indicates high risk for hemorrhage and suggests that modifiable risk factors affecting bleeding should be reviewed and corrected (e.g., blood pressure, hepatic/renal function, INR, antiplatelet agents, NSAIDs, alcohol ingestion, and selective serotonin reuptake inhibitors (SSRI)).

Table 3: Summary of drug-drug interactions provided in the literature. When available, recommendations for dose adaptation or contraindications by the competent authorities are provided [26–28].

Molecule	Mechanism	Dabigatran	Rivaroxaban	Apixaban
		Antiarrhythmics		
Dronedarone	P-gp and CYP 3A4 inhibitor	AUC: +114% (400 mg: single dose)*	Minor effect (use with caution if CrCl 15–50 mL/min)***	No data yet
		AUC: +136% (400 mg: multiple doses)		
Quinidine	P-gp competition	AUC: +53% (1,000 mg: single dose)**	Minor effect (use with caution if CrCl 15–50 mL/min)	No data yet
Verapamil	P-gp competition and weak CYP 3A4 inhibitor	AUC: +18% (120 mg IR: single dose taken 2 h after DE intake)**	Minor effect (use with caution if CrCl 15–50 mL/min)	No data yet
		AUC: +143% (120 mg IR: single dose, 1 h before DE intake)**		
		Cmax: +12% (120 mg IR: single dose taken 2 h after DE intake)**		
		Cmax: +179% (120 mg IR: single dose, 1 h before DE intake)**		
Amiodarone	P-gp competition	AUC: +58% (600 mg: single dose)**	Minor effect (use with caution if CrCl 15–50 mL/min)	No clinically relevant effect
Diltiazem	P-gp and CYP 3A4 inhibitor	No effect	Minor effect (use with caution if CrCl 15–50 mL/min)	AUC: +40%
		Antianginal/antihypertensive drugs		
Ranolazine	P-gp and CYP 3A4 inhibitor	No data yet	Minor effect (use with caution if CrCl 15–50 mL/min)	No data yet
Felodipine	P-gp and CYP 3A4 inhibitor	No data yet	Minor effect (use with caution if CrCl 15–50 mL/min)	No data yet
		Anti-inflammatory		
Naproxen	P-gp competition	No data yet	AUC: +10% (500 mg)	AUC: +50%
		Antihypercholesterolemiant		
Atorvastatin	P-gp and CYP 3A4 substrate	AUC: +18%	No effect	No PK data yet
		Antimycotic		
Ketoconazole	P-gp and CYP 3A4 inhibitor	AUC: +138% (400 mg: single dose)*	Cmax: +72% (400 mg: single dose)	Cmax: +62% (400 mg od)
		AUC: +153% (400 mg: multiple doses)	AUC: +158% (400 mg: single dose)	AUC: +100% (400 mg od)
Itraconazole	P-gp and CYP 3A4 inhibitor	No data yet*	No data yet, but similar results than ketoconazole are expected	No data yet, but similar results than ketoconazole are expected
Voriconazole	P-gp and CYP 3A4 inhibitor	No data yet		
Posaconazole	P-gp and CYP 3A4 inhibitor	No data yet***	No data yet	
Fluconazole	CYP 3A4 inhibitor	No data yet Supposed no effect	Cmax: +28%	No data yet
			AUC: +42%	

Table 3: Continued.

Molecule	Mechanism	Dabigatran	Rivaroxaban	Apixaban
		Antibacterial		
Clarithromycin	P-gp and CYP 3A4 inhibitor	Cmax: +49% AUC: +60%	AUC: +54% (500 mg bid)	No data yet
Azithromycin	P-gp and CYP 3A4 inhibitor	No data yet	Minor effect (use with caution if CrCl 15–50 mL/min)	No data yet
Erythromycin	P-gp and CYP 3A4 inhibitor	No data yet	AUC: +34% (500 mg tid)	No data yet
		Protease inhibitors		
Ritonavir	P-gp and CYP 3A4 inhibitor	No data yet***	Cmax: +55% (600 mg bid) AUC: +153% (600 mg bid)	No PK data but strong increase
		Immunosuppressor		
Cyclosporine	P-gp competition	No data yet*	AUC: +50%	No data yet
Tacrolimus	P-gp competition	No data yet*	AUC: +50%	No data yet

*The FDA recommends reducing the dabigatran etexilate at 75 mg bid for stroke prevention in NVAF. No recommendations are given by the FDA for cyclosporine, tacrolimus, and itraconazole.
**The EMA contraindicates concomitant treatment with these drugs. EMA recommends dose reduction from 220 mg od to 150 mg od in major orthopedic surgery and from 150 mg bid to 110 mg bid in stroke prevention in patients with NVAF. No dose recommendation is provided by the FDA.
***Not recommended by the EMA.

The EMA has introduced new contraindications for all NOACs since September 2013. This statement mentions that a screening of injuries and sicknesses that may lead to major bleeding is required before starting NOAC therapies. This can be a current or recent gastrointestinal bleeding, suspected or known esophageal varicose veins, any malignancy with high bleeding risks (e.g., colon cancer), recent cerebral, spine or ophthalmic injuries, recent intracranial hemorrhage, arteriovenous malformations, vascular aneurysm, or major intraspinal and intracranial vascular anomalies [32].

Careful attention must be directed to renal protective strategies for patients under DE. These patients should know that concurrent medications (e.g., NSAIDs) or clinical comorbidities (e.g., dehydration) can deteriorate their renal function, and, as consequence, increase and prolong dabigatran anticoagulant effect [32].

3.3. Improving Individual Benefit-Risk by Tailoring NOAC's Dose

3.3.1. Why? A reanalysis of the RE-LY study has shown that bleeding outcomes were correlated with dabigatran plasma concentrations [63]. Demographic characteristics (mainly age and previous stroke) played the strongest role in determining risk of clinical events. In addition, the authors concluded that for patients at highest risk for events, such as the very elderly and/or those with poor renal function, an adjustment of dabigatran dose to optimize exposure might improve benefit-risk ratio if they are at either extreme of the concentration range.

The EU-SmPC mentions that exceeding the 90th percentile of NOAC's trough level is considered to be associated with an increased risk of bleeding. For example, patients treated with 150 mg dabigatran BID for stroke prevention in NVAF have a 90th percentile of dabigatran plasma concentrations measured at trough (10–16 hours (h) after the previous dose) about 200 ng/mL [43].

Moreover, estimation of plasma drug concentrations can also be interesting to identify high responders which are at risk of bleeding [64]. Pharmacokinetic studies showed that dabigatran has considerable variation in plasma drug concentrations [65]. Most patients will obtain an adequate plasma level when given a fixed dose. But a measurable proportion will either achieve an insufficient or a supratherapeutic drug level [66–68]. Furthermore, medication adherence is not better than 50% in unmonitored conditions [64, 69], meaning that losing track of the patients during long-term (often life-long) treatment can be worrying. For these reasons, searching for the optimal dose in patients at risk of supra- or infratherapeutic plasma level can improve the efficacy and safety of NOACs. In addition, without a structured organization, there will be no routine check on side effects, tolerance, and adherence [64, 69].

3.3.2. When? To prevent massive bleeding, biological monitoring would be valuable in the following situations [64, 68, 70, 71]:

(i) before urgent surgery or procedure (with the last administration in the last 24 h, or more, if Cr_{Cl} < 50 mL/min),

(ii) before fibrinolytic therapy of acute ischemic stroke,

(iii) for bridging therapy,

(iv) for patients with multiple risk factors for NOAC's accumulation (i.e., patients older than 75 years, drug-drug interactions as with frequently used medication like amiodarone and verapamil, extreme body weight (<50–60 kg and >110–120 kg), hepatic impairment, and renal impairment),

(v) for patients with renal impairment (progressive decrease of renal function, acute decrease due to dehydration, antibiotics administration, etc.),

(vi) in complex management of dual or triple antithrombotic therapies (e.g., patients with AF undergoing percutaneous coronary intervention or dual platelet inhibitors added to NOACs).

3.3.3. How? NOACs affect all routine coagulation assays [72]. The maximum effect of NOAC on clotting tests occurs at the same time as their maximal plasma concentrations (Table 1).

Therefore, it is essential to know the timing of NOAC's administration and the timing of blood sampling when interpreting results of a coagulation assay in a NOAC treated patient.

For example, coagulation assay results will differ if blood samples are taken 2 hours after DE intake (peak level) compared with blood sampling 12 hours after ingestion of the same dose. A French group (GIHP: Groupe d'Intérêt en Hémostase Périopératoire) proposed the following cut-off for the perioperative management of DE and rivaroxaban (Table 4): <30 ng/mL: the operation may take place; between 30 and 200 ng/mL: therapeutic zone; between 200 and 400 ng/mL: minor hemorrhagic risk; >400 ng/mL: major hemorrhagic risk [29].

For apixaban, there is no data regarding the plasma trough or max level versus bleeding or recurrence of thrombosis. Pharmacokinetic studies revealed that apixaban plasma concentrations varied modestly between peak and trough and were mainly comprised within the range of 100–300 ng/mL [73].

The recent recommendation of the International Society of Thrombosis and Hemostasis (ISTH) mentions that the activated partial thromboplastin time (aPTT) and prothrombin time (PT) can be used in emergency situations to determine the relative intensity of anticoagulation due to DE and rivaroxaban, respectively [72]. However, aPTT and PT should not be used to quantify the drug plasma concentration. Further studies are required to determine the relative sensitivity of aPTT and PT reagents in order to give more specific recommendations. In addition, aPTT and PT are global assays which are not reflecting peripheral concentrations of NOACs, especially at high plasma concentrations [72].

PT is not sensitive enough to estimate apixaban plasma concentrations. Furthermore, depending on the reagent, it may be normal with apixaban therapeutic concentration. Even for the most sensitive reagents, it may only inform the clinician if the patient is taking the drug.

Table 4: Perioperative management of NOACs (dabigatran and rivaroxaban)—proposal for recommendations from the GIHP (Groupe d'Intérêt en Hémostase Périopératoire) [29].

Measured concentration	Recommendations
<30 ng/mL	Operate
30–200 ng/mL	(i) Wait up to 12 h and obtain new dosage or (if time is not compatible with emergency) (ii) Operate, if abnormal bleeding: antagonise the anticoagulant effect
200–400 ng/mL	(i) Wait up to 12 h and obtain new dosage or (if time is not compatible with emergency) (ii) Maximise delay surgery (iii) Discuss hemodialysis, especially if Cr_{cL} <50 mL/min (with dabigatran only) (iv) Operate, if abnormal bleeding: antagonise
>400 ng/mL	Overdose major haemorrhagic risk Discuss haemodialysis before surgery (with dabigatran only)

As thrombin time (TT) is too sensitive to dabigatran [74], it is advisable to use a calibrated diluted thrombin time (dTT) with dabigatran standards to estimate dabigatran plasma concentration [74]. Therefore, Hemoclot thrombin inhibitor (HTI) is a rapid, linear, standardized, and calibrated assay which determines precisely plasma concentrations of dabigatran [74].

Chromogenic anti-Xa assays are recommended to accurately estimate rivaroxaban and apixaban plasma concentrations higher than 30 ng/mL [75, 76].

3.4. Dealing with a Missed Dose. To minimize the risk of bleeding, keep in mind that patients on DE for NVAF or on rivaroxaban for NVAF and VTE prevention should *never* take a double dose at the same time. If the missed dose is within 6 hours for dabigatran and within 12 hours for rivaroxaban, patients should take the forgotten capsule immediately. Otherwise, if the time is exceeded, they should just go on with the treatment without taking any capsule.

The only exception concerns VTE treatment with rivaroxaban, where patients may take simultaneously 2 tablets of 15 mg to ensure a total daily dose of 30 mg.

For apixaban, there is no time schedule. Patients should just take the forgotten capsule immediately and go on with the treatment [26].

3.5. Adherence to Switching Procedures. To avoid hypercoagulability during a switching procedure, it is important to consider NOAC's pharmacokinetics and patient comorbidities.

When VKAs are switched to NOACs, VKAs should be discontinued and NOACs should be started as soon as the INR is lower than 2 [77].

Inversely, if DE is switched to VKAs, physicians need to consider the renal function before starting VKAs. Creatinine

clearance (Cr_{Cl}) influences the delay of VKA's introduction, which in this case, precedes dabigatran arrest (from 3 days to 1 day, if Cr_{Cl} is over 50 mL/min, between 30 and 50 mL/min or between 15 and 30 mL/min, resp.). NOACs interfere with an elevated INR, so that a better reflect of VKA on the INR will appear only after 2 days of NOAC's arrest [77].

To switch from NOACs to parenteral anticoagulants, these last ones should be started when the next dose of NOACs is due [77]. Inversely, NOACs should be started at the same time or up to 2 hours before the next parenteral anticoagulant dose. For intravenous unfractionated heparin, NOACs should be started at the time of discontinuation of the infusion.

3.6. Adherence to Bridging Procedures. The aim of bridging procedures is to avoid thromboembolic events in patients at high risk during the perioperative period.

Unfortunately, there is a lack of consensus regarding the different bridging procedures. These differences between national recommendations make safety studies difficult [78–82].

Only one prospective study has recently evaluated the peri-interventional NOAC management in unselected patients from daily care [83]. Outcomes and bleeding risks were compared for different types of procedure (minimal, minor, or major) in patients under NOACs. In 22% of the patients, NOACs were not interrupted, and in 30%, the gap in NOAC's intake was bridged with heparin. The rest of the patients underwent an interruption of NOACs of maximal 3 perioperative days. Major procedures had the highest cardiovascular and major bleeding complications. Interestingly, the bridging therapy did not reduce cardiovascular events but led to higher rates of major bleeding complications compared with no bridging. But these bleedings were similar to VKA patients who had bridging therapy before invasive procedures.

However, a selection bias resulted from a more frequent use of bridging therapy in severe procedure (most physicians anticipated the increased cardiovascular risk in patients undergoing major procedure). This can explain why cardiovascular and bleeding events were more frequent in major procedures [83].

They concluded that a continuation or short interruption of NOACs is safe for most invasive procedures and that patients with cardiovascular risks undergoing major procedure may benefit from bridging therapy, with as a consequence a higher bleeding risk. As the bridging therapy with heparin does not reduce the risk of cardiovascular events, a benefit-risk evaluation is needed to target the appropriate candidates who could benefit from it.

NOACs are sometimes interrupted perioperatively without bridging procedure. In this case, a postoperative resumption balanced between bleeding and thromboembolic risk needs to be defined. Spyropoulos and Douketis proposed a management based on studies assessing DE as thromboprophylaxis after orthopedic surgery and on the RELY trial. These authors suggest that NOACs should be resumed 24 hours after low bleeding risk surgery and 48–72 hours after high bleeding risk surgery. In patients with high thrombotic risk, consider a reduced dose of NOACs on the evening after surgery (DE) and on the first postoperative day (DE, rivaroxaban, and apixaban). Further studies are necessary to validate the safety of this approach [82].

3.7. Adherence to Anesthetic Recommendations. Anesthesia recommendations are available to decrease bleeding risks during perioperative procedures (e.g., regional anesthesia), especially in neuraxial anesthesia, which has potential risk of spinal hematoma [71, 84, 85].

Extreme caution is recommended with neuraxial blockade for rivaroxaban and apixaban. For DE, the manufacturer advise against its use [84].

For regional nerve blockade, ultrasound is a valuable tool to optimize catheter placement and decrease accidental vascular puncture [86].

Postprocedure anticoagulation (e.g., after removing indwelling catheter) should be restarted after 8 hours minus the time to reach maximum activity (T_{max}) (8 hours equal to the time to establish a stable clot) [87].

4. Absence of Antidote

The absence of antidote for NOACs emphasizes the importance of implementing strategies to prevent massive bleeding.

NOACs have a relatively short half-life, so that stopping the drug in patients without altered renal or liver function could be already valuable to eliminate it.

In large randomized controlled trial (RCT), the number of fatal bleedings was similar between NOACs and warfarin groups, despite absence of antidote for NOACs [12–14]. Strategies against life-threatening bleedings under NOACs are needed in any cases. Currently, there is no high-quality evidence in the clinical management of severe bleedings under NOACs but rather experience-related management. Animals and *in vitro* studies are guiding treatment approaches. The discussed effectiveness of nonspecific reversal therapies must be counterbalanced with their increased thrombotic risk [88–90].

A recent paper has presented the management and outcome of major bleeding during treatment with DE or warfarin [91].

Dickneite and Hoffman [92] reviewed the currently available data of PCC's use in reversing NOAC's anticoagulant effects. Because of different study models, the results are not entirely consistent. Some studies used 3F-PCCs, which contain high concentrations of coagulation factors II, IX and X and low and/or variable amounts of FVII. Other studies used 4F-PCCs, which additionally contain high levels of FVII. Due to their ability to raise the levels of these factors and consequently enhance thrombin generation in *in vitro* models, their utility to overcome the anticoagulant effects of FIIa and FXa inhibitors is plausible. But using PCCs to attempt to overcome the effect of an inhibitor is more complicated than simply replacing factors that are deficient. For example, the still present dabigatran will continue to

inhibit thrombin activity, even if PCCs are supplied and lead to thrombin formation.

Effectiveness and appropriate doses of PCCs still need to be established, especially when there appear variations in the ability of different PCCs to reverse NOAC's anticoagulant effects [92].

Only one of the available 4F-PCCs is able to reverse anticoagulation due to dabigatran and rivaroxaban. The others are more selective for dabigatran or rivaroxaban. These differences can be due to the wide variation in the amount of factors II, VII, IX and X, of antithrombotic proteins (proteins C and S) and also of anticoagulants, such as heparin and antithrombin, among the different PCCs. For the 3F-PCCs, two of them were able to normalize increased bleeding time following dabigatran administration, but the effect was short-lasting in comparison with 4F-PCC. This can be explained by the smaller amount of FVII in 3F-PCCs and its short half-life compared with the other factors. Activated PCCs seem to enhance more the parameters of thrombin generation to supratherapeutic levels than nonactivated PCCs, but it may be consequently at greater risk of thrombosis. Thrombin generation appears to have the best predictive value in the reversal of NOAC's anticoagulant effect.

Anyway, even if some PCCs seem promising in reversing anticoagulation due to NOACs, their effectiveness needs still to be studied in bleeding human patients [92].

Piccini et al. analyzed the management and outcomes of major bleeding events in patients treated with rivaroxaban versus warfarin, using data from the ROCKET AF trial [93]. Among high-risk patients with AF who experienced major bleeding, there was a reduction in fresh frozen plasma and PCC's use in the rivaroxaban group compared with the warfarin group [93].

Other alternatives exist to treat major bleedings in patients who do not respond to supportive measures. For dabigatran, due to its important renal excretion, hemodialysis can be proposed but with limited clinical experience [94–96]. Hemodialysis can also be discussed if a patient has renal insufficiency and needs an emergent surgery that cannot be delayed [29].

If NOAC's intake is recent, oral activated charcoal may also be effective [94].

Different specific antidotes are currently under evaluation. For DE, the antidote is a humanized selective and specific monoclonal antibody fragment (aDabi-Fab), which has no effect on other molecules. A recent study compared its *ex vivo* reversal effect with PCC, aPCC, and rFVIIa in a dabigatran anticoagulated liver trauma experimental model. Coagulation was assessed by thromboelastometry (TEM), global coagulation assays and diluted thrombin time. Interestingly, rFVIIa (90 and 180 microgrammes/kg) had no significant effect on coagulation parameters, but aDabi-Fab (60 and 120 mg/kg), PCC and aPCC (30 and 60 IU/kg) were effective in reducing the anticoagulation effects of dabigatran (TEM parameters and PT). In contrast, aDabi-Fab was the only reversal agent that normalized aPTT [97].

Andexanet alpha (PRT064445), a truncated form of enzymatically inactive factor Xa, is a universal reversal agent for all anti-Xa inhibitors. It reverses the inhibition of factor Xa dose-dependently, correcting the prolonged *ex vivo* clotting times due to anti-Xa inhibitors [98, 99].

Another antidote currently under research is aripazine (PER977), a small synthetic molecule that bounds several NOACs in animal studies, without significant adverse events. It reverses the anticoagulant activity of all clinically used NOACs in the rat-tail injury model and also in a human *ex vivo* model, using aPTT and anti-Xa analysis to measure its reversal effect [98, 99].

In the absence of specific antidotes, it is important to assess the degree of emergency and the patient characteristics (which type of NOAC, timing of the last dose, drugs interactions, comorbidities, site of bleeding, etc.) [100]. If possible, delay the surgery until the NOAC reaches trough concentration.

A hospital-wide policy for the management of NOAC's related bleedings should be easily accessible for every health worker involved in the patient's care.

5. Conclusions

NOACs are indisputably an important step forward in the field of anticoagulation. However, an inappropriate use can possibly lead to a higher risk of bleeding. This highlights the importance of strengthening education of health care professionals and patients, that is, with regard to dose adjustment, modalities of administration, choice of anticoagulant, and compliance guidance. Modifiable bleeding risk factors should also be screened and reviewed before initiation of NOACs. Individual benefit-risk might be improved in some clinical settings or patient subpopulations (patients at risk of supra- or infratherapeutic plasma level) by tailoring a dose following coagulation monitoring. Adherence to switching, bridging, resuming, and anesthetic recommendations is crucial to allow an optimal management of patient.

Anticoagulation with NOACs has still risks and requires strong adherence from the patient's side and careful supervision from the physician's side. Furthermore, since February 2012, the EMA has imposed the development of an education pack for patients and prescribers with regard to the safety and effectiveness of NOACs.

A well-structured organization will help to improve the control on side effects, tolerance, and adherence.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Lessire Sarah, Dincq Anne-Sophie, Gourdin Maximilien, and Mullier François contributed equally.

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