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Comparison of Biophen® Heparin LRT and HemosIL® Liquid Anti-Xa assays for the measurement of direct Xa inhibitors in patients on treatment with apixaban, edoxaban, or rivaroxaban

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Abstract

Introduction: Over the last decade, the number of patients anticoagulated with direct oral anticoagulants is markedly increasing. Even though direct oral anticoagulants do not require routine monitoring, there are certain clinical situations, such as bleeding, thromboembolic complications, emergency surgery, or overdose, when current guidelines recommend the measurement of these direct oral anticoagulants' plasma concentrations. For the quantitative analysis of direct factor Xa inhibitors (i.e. apixaban, edoxaban, and rivaroxaban) chromogenic anti-Xa activity assays with substance-specific calibrations for apixaban, edoxaban, and rivaroxaban are used in clinical routine. More recently a hybrid calibrator was developed for the Biophen® Heparin LRT assay, which only requires one calibrator set for the measurement of apixaban, edoxaban, and rivaroxaban. Therefore, the aim of this study was to perform an analytical evaluation of the Biophen® Heparin LRT assay using this novel hybrid calibrator set for the measurement of apixaban, edoxaban, and rivaroxaban plasma concentrations, as well as a method comparison study between the Biophen® Heparin LRT assay and the HemosIL® Liquid Anti-Xa assay, which is a well-established assay in the clinical routine.

Materials and methods: All measurements of apixaban, edoxaban, rivaroxaban using the HemosIL® Liquid Anti-Xa assay as well as the Biophen® Heparin LRT were performed on the same ACL Top 750® analyser. The detection limit and the precision of the Biophen® Heparin LRT apixaban, edoxaban, and rivaroxaban assays were determined. For the method comparison study citrated plasma samples of 159 patients on anticoagulation treatment (n=80 for apixaban, n=23 for edoxaban, and n=55 for rivaroxaban) were measured in parallel with the respective the Biophen® Heparin LRT and the HemosIL® Liquid Anti-Xa assays.

Results: The detection limit for the Biophen® Heparin LRT apixaban, edoxaban, and rivaroxaban assays were 6.6 ng/ml, 7.5 ng/ml, and 9.7 ng/ml. Within-run and total coefficients of variation were ≤3.8% and ≤5.6% for the Biophen® Heparin LRT apixaban assay, ≤5.2% and ≤5.6% for the Biophen® Heparin LRT edoxaban assay, and ≤5.4% and ≤6.1% for the Biophen® Heparin LRT rivaroxaban assay. The method comparison study showed a very strong correlation between the Biophen® Heparin LRT and the HemosIL® Liquid Anti-Xa assays for apixaban (r_s=0.996; p<0.001), edoxaban (r_s=0.994; p<0.001), and rivaroxaban (r_s=0.986; p<0.001). Passing and Bablok regression analyses revealed only a rather small constant bias as well as a proportional difference between the two methods.

Conclusion: The Biophen® Heparin LRT assay using the novel hybrid calibrator set for measurement of apixaban, edoxaban, and rivaroxaban meets the needs of quality specifications of laboratory medicine. Since the test results of the Biophen® Heparin LRT and the HemosIL® Liquid Anti-Xa assays were comparable for apixaban, edoxaban, and rivaroxaban, the tests can be considered as equivalent.

Zusammenfassung

Einleitung: In den letzten zehn Jahren hat die Anzahl an Patientinnen und Patienten, welche mit direkten oralen Antikoagulanzien behandelt werden, deutlich zugenommen. Obwohl deren Plasmakonzentration nicht regelmäßig überwacht werden muss, gibt es Indikationen, wie Blutungen, thromboembolische Komplikationen, Notfalloperationen oder Überdosierungen, für welche nach den aktuellen Guidelines die Messung der Plasmakonzentrationen von direkten oralen Antikoagulanzien empfohlen wird. Zur quantitativen Bestimmung von direkten Faktor Xa-Inhibitoren (Apixaban, Edoxaban und Rivaroxaban) werden chromogene Anti-Xa-Aktivitätsassays mit spezifischen Kalibratoren für die einzelnen Substanzen in der klinischen Routine eingesetzt. Unlängst wurde ein Hybridkalibrator für die Biophen® Heparin LRT Assays entwickelt, wodurch nur mehr ein Satz Kalibratoren für die Messung von Apixaban, Edoxaban und Rivaroxaban benötigt wird. Das Ziel dieser Studie war es, eine analytische Evaluierung des Biophen® Heparin LRT Assays mit diesem neuartigen Hybridkalibrator zur Messung von Apixaban-, Edoxaban- und Rivaroxaban-plasmakonzentrationen sowie eine Methodenvergleich zwischen den Biophen® Heparin LRT und den HemosIL® Liquid Anti-Xa-Assays, welcher bereits in der klinischen Routine etabliert ist.

Material und Methoden: Alle Messungen von Apixaban, Edoxaban, Rivaroxaban wurden mittels HemosIL® Liquid Anti-Xa und Biophen® Heparin LRT Assays auf dem gleichen ACL Top 750®-Messgerät durchgeführt. Im Rahmen dieser Studie wurden die Nachweisgrenze und die Präzision der Biophen® Heparin LRT Apixaban-, Edoxaban- und Rivaroxaban-Assays bestimmt. Für den Methodenvergleich wurden Citratplasmaproben von 159 Patientinnen und Patienten unter laufender Antikoagulation (n=80 für Apixaban, n=23 für Edoxaban und n=55 für Rivaroxaban) sowohl mit dem entsprechenden Biophen® Heparin LRT als auch HemosIL® Liquid anti-Xa-Assay vermessen.

Resultate: Die Nachweisgrenzen für die Biophen® Heparin LRT Apixaban-, Edoxaban- und Rivaroxaban-Assays lagen bei 6,6 ng/ml, 7,5 ng/ml und 9,7 ng/ml. Die Serien- und

Gesamtvariationskoeffizienten waren ≤3,8% und ≤5,6% für den Biophen® Heparin LRT Apixaban Assay, ≤5,2% und ≤5,6% für den Biophen® Heparin LRT Edoxaban Assay und ≤5,4% und ≤6,1% für den Biophen® Heparin LRT Rivaroxaban Assay. Der Methodenvergleich zeigte eine sehr starke Korrelation zwischen den Biophen® Heparin LRT und den HemosIL® Liquid Anti-Xa-Assays für Apixaban (r_s=0,996; p<0,001), Edoxaban (r_s=0,994; p<0,001) und Rivaroxaban (r_s=0,986; p<0,001). Passing-Bablok-Regressionsanalysen zeigten nur eine geringe, aber gleichbleibende Abweichung sowie einen proportionalen Unterschied zwischen den beiden Methoden.

Schlussfolgerung: Der Biophen® Heparin LRT Assay mit dem neuartigen Hybridkalibratorsystem zur Messung von Apixaban, Edoxaban und Rivaroxaban erfüllt die Anforderungen der Qualitätsspezifikationen für Labormedizin. Da die Testergebnisse der Biophen® Heparin LRT und der HemosIL® Liquid Anti-Xa Assays für Apixaban, Edoxaban und Rivroxaban vergleichbar Resultate lieferten, können die Tests als gleichwertig angesehen werden.

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List of Abbreviations

ADP	Adenosine Diphosphate
APC	Activated Protein C
aPCC	Activated Prothrombin Complex Concentrate
aPTT	Partial Thromboplastin Clotting Time
AT	Antithrombin
CLSI	Clinical and Laboratory Standards Institute
CPB2	Carboxypeptidase B2
COX	Cyclooxygenase
CV	Coefficient of Variation
DOAC	Direct Oral Anticoagulant
DTI	Direct Thrombin Inhibitor
EPCR	Endothelial Protein C Receptor
HIT	Heparin-Induced Thrombocytopenia
HMWK	High Molecular Weight Kininogen
IL	Interleukin
INR	International Normalized Ratio
LMWH	Low-Molecular-Weight Heparins
NO	Nitric Oxide
NOAC	Novel Oral Anticoagulants
PAI	Plasminogen Activator Inhibitor
PAR	Protease-activated Receptor
PCC	Unactivated Prothrombin Complex Concentrate
PCI	Percutaneous Coronary Intervention
PDGF	Platelet-derived Growth Factor
PDI	Protein Disulfide Isomerase
pNA	Paranitroaniline
PT	Prothrombin Clotting Time
SD	Standard Deviation
SERPIN	Serine Protease Inhibitor.
TAFI	Thrombin-activatable Fibrinolysis Inhibitor
TF(PI)	Tissue Factor (Pathway Inhibitor)
TM	Thrombomodulin
tPA	Tissue-type Plasminogen Activator

TSOAC	Target-specific Oral Anticoagulant
TT	Thrombin Time
VKA	Vitamin K antagonist
VTE	Venous Thromboembolism
vWF	von Willebrand Factor

1 Introduction

This chapter presents an abbreviated version of the background on hemostasis and anticoagulation, whereas chapters 2 to 4 provide a more detailed insight on these topics.

1.1 *Hemostasis*

Hemostasis is the general term for the processes involved in the formation of blood clots at the site of injured vessels. In case of a disruption of a blood vessel wall, the hemostatic response is responsible for a fast and localised regulation to prevent bleeding or thrombosis. Therefore, the dysregulation of the hemostatic reaction often leads to these pathophysiological conditions.

As shown in Figure 1, the formation of a clot is activated via two different mechanisms which both lead to a common pathway:

- The intrinsic pathway is mediated by blood exposure to a negatively charged surface, for instance, celite, kaolin, or silica in the in vitro activated partial thromboplastin clotting time (aPTT).
- The extrinsic pathway is mediated by the exposure of tissue factor (TF). The TF generation is triggered by the injury of endothelial tissue or TF-like material, such as thromboplastin, which is used for the measurement of the prothrombin clotting time (PT).

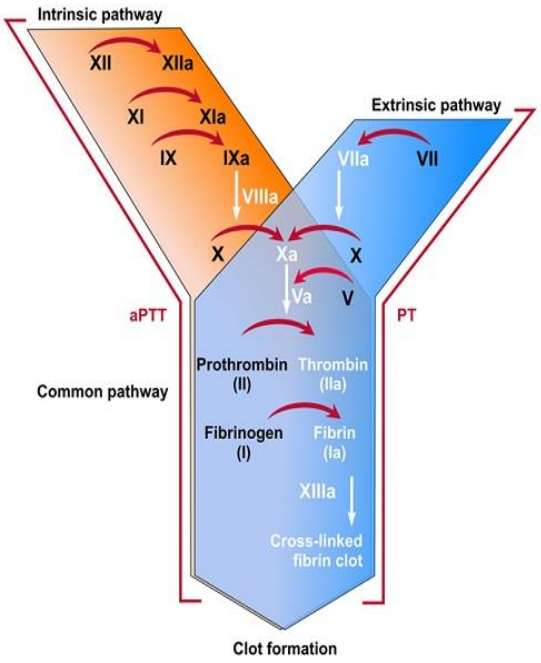

Figure 1 Overview of the coagulation pathways and involved factors (Diapharma)

- The convergence of both pathways happens through the activation of factor X, which subsequently contributes to the conversion of prothrombin to thrombin, and consequently the conversion of fibrinogen to the insoluble fibrin, which forms the cross-linked clot. This process is measured by the thrombin time (TT).

Both pathways lead to the formation of thrombin-stimulated fibrin clots and subsequently to the plasmin-induced clot lysis (Lane et al., 2005). Both processes are interlinked, and in case of correct regulation, they result in tissue remodelling, whereas flawed hemostatic processes, such as diminished thrombin generation (factor VIII deficiency), or enhanced clot lysis

(resulting from alpha-2-antiplasmin deficiency) may result in abnormal bleeding. On the other hand, excessive thrombin production, which might result from inherited thrombophilia, is associated with the formation of clots and, subsequently, thrombosis.

1.2 *Anticoagulation*

Over the last few decades, the number of agents for the prevention and management of the thromboembolic disease has significantly increased. In addition to the already well established and well-proven anticoagulants such as antiplatelet agents, heparins, and vitamin K antagonists (VKAs), direct parenteral and oral anticoagulants (DOACs) which forthright target the enzymatic activity of thrombin and factor Xa have been developed.
An overview of the coagulation cascade as well as the respective anticoagulant's point of action is shown in Figure 2.

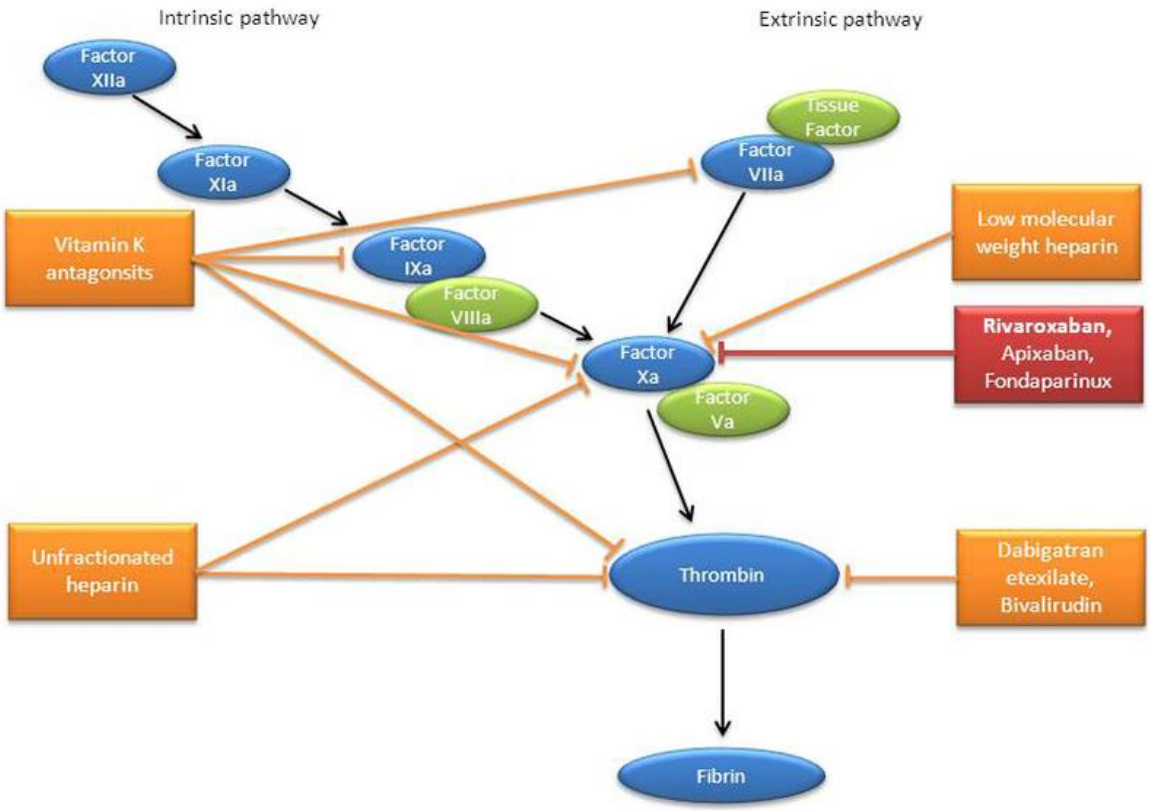

Figure 2 Schematic representation of the coagulation cascade with the sites of action of traditional and new anticoagulants (Lakatos et al., 2014)

The main hemostatic processes, which are addressed by anticoagulants include platelet activation, fibrin generation by activated coagulation factors, inhibition of procoagulant

factors to prevent excessive clot propagation, and fibrinolysis to dissolve the fibrin clot as the endothelial surface is repaired. These processes share numerous and substantial interlinkages, such as crosstalk between platelets, procoagulant factors, endogenous anticoagulant, and fibrinolytic factors, as well as the endothelium. Anticoagulants inhibit either one or multiple steps in the coagulation cascade and have a vast amount of mechanisms, such as direct enzymatic inhibition, indirect inhibition by antithrombin (AT) binding, and, by preventing their synthesis in the liver and/or modifying of their calcium-binding properties, antagonisation of vitamin K-dependent factors. Examples for anticoagulant agents are unfractionated and low molecular weight heparins (LMWHs), VKAs, direct thrombin inhibitors, as well as direct factor Xa inhibitors. The agents involved in anticoagulation are differentiated according to their respective target sites.

- Antithrombotic agents mainly target platelets, for instance, acetylsalicylic acid or clopidogrel.
- The thrombin inhibitors prevent fibrin production, which depends on thrombin generated from the proteolytic cleavage of prothrombin by factor Xa. Besides this function, thrombin activates numerous elements of the coagulation cascade, including factors V, VIII, XI, and XIII and platelets (Di Nisio et al., 2005). Thrombin's active site is in a deep groove, which allows the enhancement of the enzyme's specificity by the surrounding amino acids (Grütter et al., 1990, Rydel et al., 1990). This active site is either the sole or one out of two targets for the direct thrombin inhibitors (DTIs), with the other target site being the exosite I, which is a positively charged domain of the thrombin molecule, physically separated from the active site (Hirsh and Weitz, 1999, Di Nisio et al., 2005). Furthermore, this exosite I is the interaction site of thrombin substrates, such as fibrinogen, factor V, protein C, thrombomodulin, and PAR1 and PAR4 on platelets (Grütter et al., 1990, Rydel et al., 1990, Sheehan and Sadler, 1994, Hall et al., 1999, Hirsh and Weitz, 1999). Unlike heparins, which can only inhibit thrombin in the fluid phase via AT, the DTIs can block thrombin in both circulating and clot-bound forms (Weitz et al., 1990, Berry et al., 1994, Lefkovits and Topol, 1994, Turpie, 2008). The only licensed DTI with an oral administration is dabigatran exilate (Pradaxa®), ximelagatran (Exanta®) was withdrawn in 2006 because of its hepatotoxicity and cardiovascular events (Laux et al., 2009); an example for a parenteral DTI is bivalirudin (Angiomax®), which is licensed for the anticoagulation of patients undergoing percutaneous coronary intervention (PCI) and heparin-induced thrombocytopenia (HIT) (Lehman and Chew, 2006, Serruys et al., 2006).

- The factor Xa inhibitors target the convergence point of the intrinsic and extrinsic coagulation pathways by binding the active site of factor Xa, which is generated by the proteolytic cleavage of factor X by one of two X-ases, resulting in the inhibition of the cleavage of prothrombin to thrombin. Unlike heparins, which can only inhibit factor Xa in the fluid phase, the direct factor Xa inhibitors can block factor Xa in both, circulating and clot-bound, forms (Roehrig et al., 2005, Laux et al., 2009, Samama, 2011). Parenteral direct factor Xa inhibitors are not in use, but several oral direct factor Xa inhibitors are available, such as rivaroxaban (Xarelto®), apixaban (Eliquis®) and edoxaban (Lixiana®) as well as their respective generic products.

Besides DOACs there are other acronyms, which are commonly used for oral DTI and direct factor Xa inhibitors, such as NOACs (novel oral anticoagulants) and TSOACs (target-specific oral anticoagulants) (Ansell et al., 2014a, Husted and Lip, 2014, Husted et al., 2014, Barnes et al., 2015).

Since prolonged PT and aPTT are not considered reliable indicators of anticoagulant effect in case of direct factor Xa inhibitors, their activity is measured with chromogenic anti-Xa activity tests, which are calibrated for the respective substance. Although anti-Xa activity assays calibrated for a different factor Xa inhibitor may deliver results, these results provide only qualitative information and do not reflect real drug activities. Therefore, the measurement of direct factor X inhibitor plasma concentrations requires a specialised test for each substance, including the respective calibrators and controls. Because plasma concentration measurements of direct factor Xa inhibitors, as well as direct thrombin inhibitors, is recommended only under certain conditions, the test volume is rather small and thus, especially for smaller laboratories outside the clinical setting, the establishment of the currently established tests is laborious and unprofitable. Therefore, assays, such as the Biophen® Heparin LRT assay (CoaChrom Diagnostica), were further developed to allow direct factor Xa inhibitor measurements for apixaban, edoxaban, and rivaroxaban using only a single hybrid calibrator set for the measurement of all three Xa inhibitors.

Therefore, the aim of this study was to perform an analytical evaluation of the Biophen® Heparin LRT assay calibrated with this novel hybrid calibrator as well as an method comparison study with the clinically established and routinely used HemosIL® Liquid Anti-Xa assay (Werfen) calibrated with three different substance-specific calibrator sets for apixaban, edoxaban, and rivaroxaban.

2 *Hemostasis*

Despite its dynamic and interwoven processing arrays, the hemostatic process can be divided into phases, which shall be discussed in this chapter (Furie and Furie, 2008).

1. Injured endothelial tissue and formation of the platelet plug
2. Enhancement of the clotting process by the coagulation cascade
3. Antithrombotic control mechanisms leading to a stop of the clotting process
4. Fibrinolysis and the subsequent clot removal

2.1 *Platelet plug formation*

The initial and local hemostatic response is triggered by an injury of the vascular endothelial membrane and results in the formation of a platelet plug, which leads to diminished bleeding. Because of the injured endothelial membrane, the circulating blood gets exposed to subendothelial elements, which leads to endothelial cell activation, which subsequently promotes the recruitment of platelets along with other cell types, and procoagulant factors.
The activation of the platelets is triggered by numerous physiological stimuli, such as adenosine diphosphate (ADP), epinephrine, thrombin, and collagen, and can be grouped in four different process phases:

- Adhesion of the platelets on the subendothelial matrix
- Aggregation which results in the cohesion of platelets
- Secretion is the release of platelet granule proteins
- Procoagulant activity which enhances the generation of thrombin

While ADP and epinephrine have only a low impact on the platelet activation, collagen and thrombin pose the most potent platelet activators. Under physiological blood flow conditions, the start of the platelet activation is triggered by thrombin, which results in a core of platelets in the hemostatic plug. The subsequent steps are controlled by ADP, which activates loosely packed platelets overlying the core, and thromboxane, which activates further platelets in the newly formed shell region (Shen et al., 2017).
As mentioned above, another factor involved in the platelet plug formation is collagen, which is present in the endothelial membrane, and prevents the platelet adherence in uninjured tissue by the production of nitric oxide (NO) and prostacyclin. In case of an intimal injury, these factors are extenuated, and microfibrils, laminin, and collagen (elements of the subendothelial membrane) are exposed and lead to platelet adherence as well as platelet activation and secretion. Significant influence has the group of platelet collagen receptors, namely the

integrins, a superfamily of adhesive protein receptors, which are expressed by a vast number of cell types. The most critical platelet adhesion and activation integrins are the glycoproteins GPIa/IIa and GPVI, respectively. Therefore, the deficiency of GPIa/IIa is associated with mild bleeding diathesis, whereas GPVI deficiency leads to severe bleeding (Watson, 1999).

Next in line for the platelet plug regulation process is thrombin. The activation of thrombin is controlled and mediated by G-protein coupled protease-activated receptors (PARs) (Coughlin, 2000, Brass, 2003, Coughlin, 2005, Leger et al., 2006). The platelets have a dual receptor system for thrombin consisting of two distinct receptors, PAR-1, which is a high-affinity receptor mediating the effect of low concentrated thrombin, and PAR-4, which is a low-affinity receptor, whose activation requires high thrombin concentrations (Coughlin, 2005). The receptor activation works through cleavage of the PAR's amino-terminal exodomain, exposing a new amino-terminus, that serves as a tethered ligand binding intramolecularly to the receptor for the initiation of transmembrane signalling (Kahn et al., 1998). Since the PARs play an essential role in the platelet plug formation, they are also potential targets for antiplatelet agents, such as vorapaxar (Zontivity®) (Morrow et al., 2012, Tricoci et al., 2012). In animal models, the main route for platelet activation found was the TF-mediated thrombin generation pathway and not the collagen-induced GPVI-mediated pathway (Dubois et al., 2007).

After discussing the more potent platelet activators, the lower impact substances, ADP and epinephrine shall be discussed. ADP works by the binding of P2Y1 and P2Y12, two G-protein coupled purinergic receptors. While the activation of P2Y1 is involved in the mobilisation of calcium, platelet shape change, and rapidly reversible aggregation, P2Y12 activation leads to platelet secretion and stable aggregation (Hollopeter et al., 2001). The platelet activation process leads to the secretion of ADP, which results in the recruitment of platelets and amplification of platelet aggregation; this pathway is targeted by clopidogrel (Plavix®).

2.1.1 *Platelet adhesion*

After the initial activation, the platelets experience shape changes, which results in an extremely adhesive nature of the platelets. The adhesion process is mediated by the interaction of the platelet surface receptor GPIb/IX/V complex with the von Willebrand factor (vWF) within the subendothelial matrix domain (Clemetson and Clemetson, 1995). Because this interaction is the main trigger for the adhesion process, a deficiency of either factor is associated with a congenital bleeding disorder, the Bernard-Soulier, and von Willebrand

disease, respectively (López et al., 1998). Another factor contributing to platelet adhesion is the interaction of the platelet collagen receptor GPIa/IIa to collagen fibrils in the subendothelial matrix (Sixma et al., 1997).

2.1.2 *Platelet aggregation*

This step of the platelet activation is mediated by the exposure as well as conformational changes in the GPIIb/IIIa (integrin superfamily, like GPIa/IIa and GPVI) receptor on the platelet surface, which results in the binding of immobilised vWF and fibrinogen, which is a divalent symmetrical molecule bridging activated platelets (Bennett and Vilaire, 1979, Savage et al., 1992, Savage et al., 1996). The GPIIb/IIIa complex is the most frequent receptor present on the platelet surface (~80,000 complexes per platelet) and is involved in fibrinogen binding after platelet stimulation (for instance by thrombin, collagen, or ADP) because this stimulation triggers a conformational change of the GPIIb/IIIa and subsequently a conversion to a high-affinity fibrinogen receptor.

Furthermore, the interaction of GPIIb/IIIa and vWF is accompanied by the GPIIb/IIIa complex binding of the platelet cytoskeleton, which leads to platelet spreading and clot retraction – this process is called `inside-out´ integrin signalling. This receptor-ligand interaction is typical for all platelet stimulation modes and marks the final common pathway for platelet aggregation (Shattil et al., 1998, Coller and Shattil, 2008).

A deficiency in the GPIIb/IIIa receptor system leads to bleeding disorders in Glanzmann thrombasthenia. This disorder is usually associated with a gene mutation in the alpha IIb or the beta-3 subunit (Hodivala-Dilke et al., 1999).

2.1.3 *Platelet secretion*

The secretion of platelets derives from two different granules types, namely the alpha and dense granules. Whereas the alpha granules contain an abundant number of proteins such as fibrinogen, vWF, thrombospondin, platelet-derived growth factor (PDGF), platelet factor 4, and P-selectin, the latter contain ADP, ATP, ionised calcium, histamine, and serotonin. Therefore, platelets can release a vast number of substances from their granules upon cell stimulation with many different effects.

- ADP and serotonin

  ADP causes the stimulation and recruitment of additional platelets (Kroll and Schafer, 1989), whereas released serotonin usually causes vasodilation, but in the presence of injured or dysfunctional endothelial membrane tissue, it leads to vasoconstriction.

  ADP-activated platelets promote the number of expressed intercellular adhesion molecule 1 on the surface of endothelial cells (Gawaz et al., 1998).

- Fibronectin and thrombospondin

  Adhesive proteins are leading to the reinforcement and stabilisation of platelet aggregates.

- Fibrinogen

  Fibrinogen is secreted by alpha granules in the case of injured endothelial tissue (Harrison et al., 1990).

- Thromboxane A2

  Thromboxane A2 is a prostaglandin metabolite promoting the vasoconstriction and enhances platelet aggregation.

- PDGF

  The release of growth factors from platelets is caused by injured endothelial tissue and provides a mitogenic effect on smooth muscle cells. This effect might be involved in the mediation of physiological tissue repair and, in case of repeated injury/dysfunction, the development of atherosclerosis and coronary occlusion following angioplasty.

- Thiol and protein disulfide isomerase

  Secreted at sites of endothelial tissue injury and are most likely involved in the activation of TF and enhancement of fibrin generation and platelet thrombus formation (Cho et al., 2008, Reinhardt et al., 2008).

2.1.4 *Pro- and anticoagulant activity*

Platelet procoagulation involves the exposure of procoagulant phospholipids, primarily phosphatidylserine, as well as the formation of enzyme complexes involved in the clotting cascade on the platelet surface (Kojima et al., 1994). The close interaction between these enzyme complexes shows the close interlinkage of platelet and clotting cascade activation.

Compared to the processes mentioned above, the anticoagulant activities associated with platelets are still less well studied and understood. One possible mechanism is the activity of

thrombospondin 5, an endogenous thrombin inhibitor involved in vascular regulation, which shall be further discussed in the chapter `Control mechanisms and termination of clotting.´

2.2 *Clotting cascade and propagation of the clot*

The clotting cascade is mediated by the activation of a sequence of proenzymes of inactive precursor proteins, such as zymogens, to active enzymes, which lead to a sequentially amplified response. For instance, low numbers of factor VIIa molecules trigger the activation of a large amount factor X, which subsequently activates a vast number of thrombin molecules, leading to the conversion of fibrinogen to fibrin, which results in an inforced platelet plug. The primary enzymes involved in this section of the clotting cascade are the X-ases (tenases), which activate factor X and the prothrombinase, which facilitates the conversion of prothrombin to thrombin.

All procoagulant factors are synthesised in the liver; the exceptions are vWF and factor VIII, which is stabilised by vWF. The vWF is produced in megakaryocytes and endothelial cells, whereas the latter is synthesised in the lymphatics and renal glomeruli as well as in endothelial liver cells. Furthermore, both factors are co-expressed in postcapillary high endothelial venules (Everett et al., 2014, Fahs et al., 2014, Pan et al., 2016).

A multitude of involved factors is subject to post-translational modification (Hansson and Stenflo, 2005). Best characterised are the modifications addressing the vitamin K-dependent procoagulants, such as prothrombin, factors VII, IX, and X, as well as anticoagulants, such as protein C and protein S. These factors are mediated by calcium-binding sites of the vitamin K-dependent carboxylated glutamic acid, which supports the formation of membrane-bound macromolecular procoagulant complexes (Furie et al., 1999).

2.2.1 *Thrombin generation*

Although the differentiation in intrinsic and extrinsic pathways allows a reasonable interpretation of in vitro tests of the coagulation, for instance, PT and aPTT, it has been established that the physiological starting point of the clotting cascade is rather triggered by generated or exposed TF at the endothelial injury site and its interaction with activated factor VII(a) (Rapaport and Rao, 1995). As shown by Mann and colleagues, this initial process leads to the generation of small thrombin amounts, which, through a feedback loop with activated factor XI, results in an amplified number of newly generated thrombin molecules (Mann et al., 2006).

Most established clinical laboratory clotting tests focus on the initial fibrin clot formation, which is mainly mediated by factor X activation by TF or factor VIIa, which leads to low thrombin concentrations, which subsequently trigger the activation of factor V, factor VIII, factor XI, and platelets. Following their activation, the platelets support the formation of multi-component enzyme complexes, such as intrinsic X-ase and prothrombinase, by the exposure of the anionic phospholipid head group on the platelet surface (Camire and Bos, 2009). Hence, the following amplification and propagation phases of the clotting cascade are triggered by these initially low thrombin concentrations. An overview of the steps of the clotting cascade as well as the involved factors are provided in Figure 3.

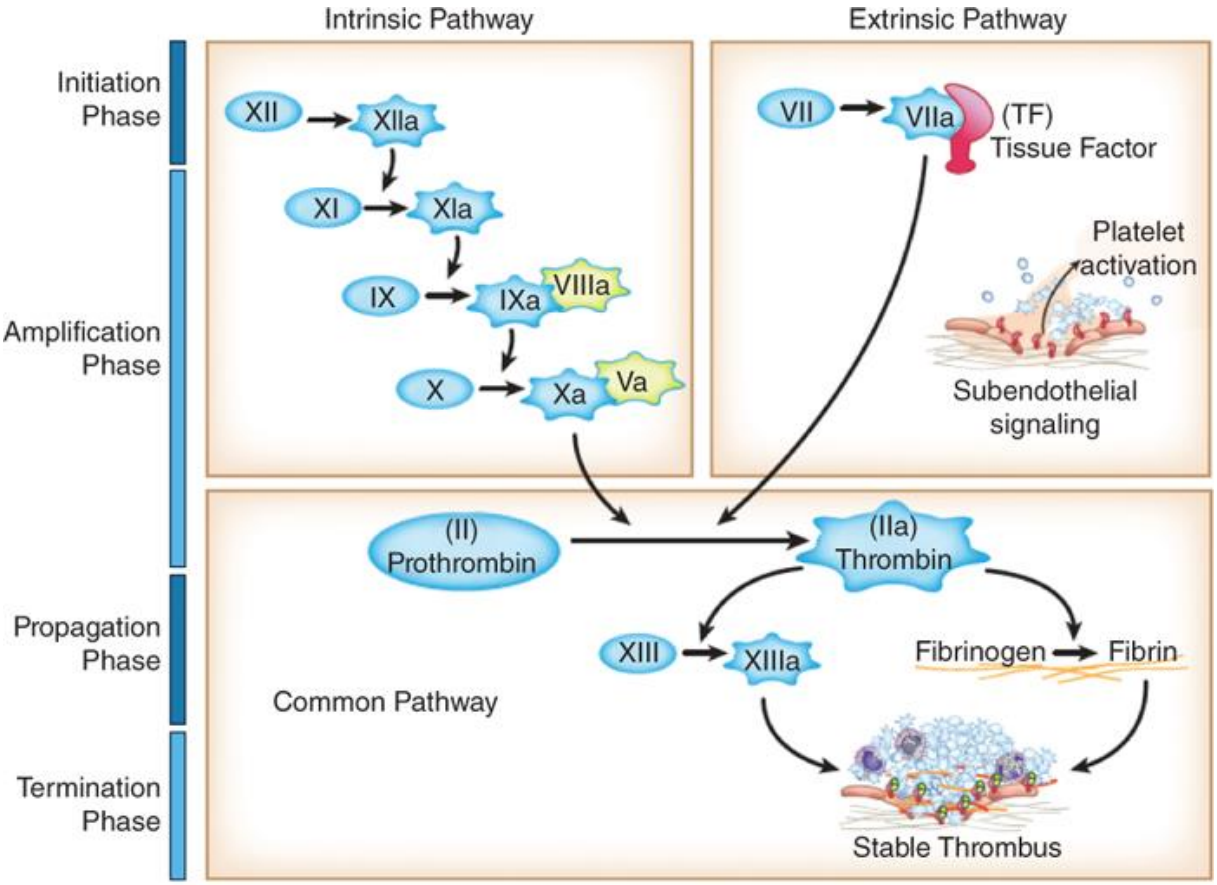

Figure 3 Individual phases of the clotting cascade (Halter et al., 2017)

For the initial thrombin generation phase, the activated platelets are crucial since they promote the formation of multi-component complexes, as well as the endothelial cells at the injury site, providing the coagulation factors' and other procoagulant effects' binding sites.

In a mouse model investigating an arteriolar injury, it was shown that the endothelial cells play an even more prominent role than platelets concerning the thrombin generation process. The study used factors Xa and Va with a fluorescence label, which, near the initially formed

platelet plug (and injury site), bound to the endothelial membrane, but did not interact with the platelets. This finding was supported by the application of a platelet aggregation inhibitor (or knock-out mice lacking platelet thrombin receptor PAR4), which showed unaffected accumulation concentrations of factors Xa, Va, and fibrin proximate the endothelial injury, which might also explain the lack of tissue hematomas and joint bleeding in patients with severe thrombocytopenia or inherited platelet defects (Ivanciu et al., 2014).

Multi-component complexes

The mediation of the coagulation cascade is mediated by multi-component macromolecular complexes, three procoagulants (extrinsic and intrinsic X-ase and prothrombinase), and the protein C anticoagulant complex. Each of these complexes consists of the enzyme, the substrate, a cofactor, and requires calcium for correct functioning.

- The extrinsic X-ase consists of factor VIIa (protease), factor X (substrate), and TF (cofactor) (Jesty et al., 1974, Baugh and Krishnaswamy, 1996, Butenas et al., 1997), and triggers the activation of factors IX and X (Osterud and Rapaport, 1977, Morrison and Jesty, 1984).
- The intrinsic X-ase consists of factor IXa (protease), factor X (substrate), and factor VIIIa (cofactor). Factor IXa either derives from the extrinsic X-ase or the intrinsic pathway, triggered by thrombin-induced activation of factor XI (Rosing et al., 1985).
- The prothrombinase consists of factor Xa (protease), prothrombin (factor II, substrate), and factor Va (cofactor).
- The protein C anticoagulant complex consists of thrombin (factor IIa, protease), protein C (substrate), and thrombomodulin (TM) (cofactor). Furthermore, the endothelial protein C receptor (ECPR), which is mainly expressed on large vessel endothelial cell membranes, supports and enhances the activation of protein C by the thrombin/TM complex (Esmon, 2006).

All factors whose activation is part of the clotting cascade are members of the trypsin-like serine protease family. Although all factors contain serine as a critical component in their catalytic site, they have a high substrate-specificity to their respective substrate due to the mediation of the enzyme-substrate complex formation by an exosite on the enzyme's surface outside the catalytic domain (Krishnaswamy, 2013).

As an example of this process, the prothrombinase complex, whose 3D crystal structure is shown in Figure 4, shall be described. The activation of platelets leads to the exposure of phosphatidylserine as well as other anionic lipids on the platelet surface, simultaneously

factor V is released from its storage in the platelet granules and binds to these anionic lipids. After the initial binding, factor V is activated (factor Va) by thrombin, which is generated by the TF-factor VIIa-interaction at the endothelial injury site in low concentrations. For the formation of the prothrombinase complex, which performs the cleavage of prothrombin to thrombin (factor II to factor IIa), the presence of a sufficient concentration of anionic membrane phospholipids and calcium is required. Compared to merely factor Xa- and prothrombin-mediated thrombin generation, the prothrombinase complex is ~300,000 times more efficient. A side effect of the prothrombinase complex reaction is the decay of factor Xa inhibition, which is facilitated by the factor Xa's binding by factor Va (resulting in shielding from factor Xa inhibitors, such as antithrombin (AT)).

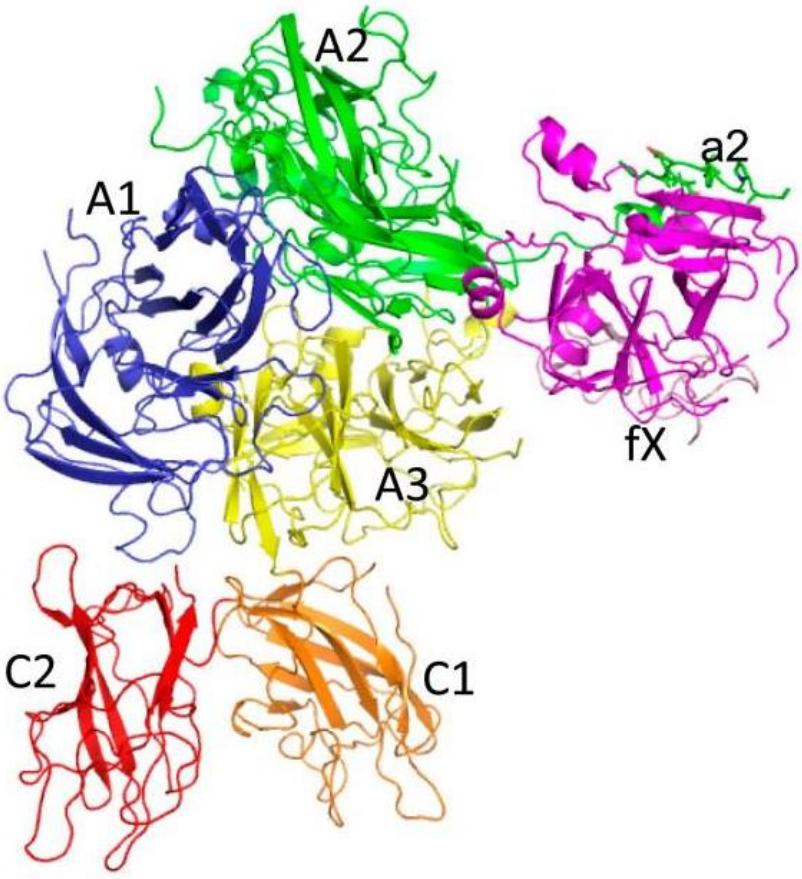

Figure 4 3D Crystal structure and domains of the prothrombinase complex (pro-pseutarin C, an intrinsically stable prothrombinase complex) (Lechtenberg et al., 2013)

Furthermore, thrombin generation is not only enhanced but regionally limited to endothelial injury sites. The importance of multi-component complexes for the platelet procoagulation process is delineated by the severity of congenital platelet function disorders, such as the Scott syndrome, an autosomal recessive disorder triggered by a mutation of TMEM16F, which is a protein involved in the lipid scramblase activity. This process results in the formation of a calcium-activated channel, promoting the disruption of the membrane gradient by transporting phosphatidylserine from the inner to the outer platelet plasma membrane layer. The presence of phosphatidylserine on the platelet surface then triggers the binding of factor Va, leading to the prothrombinase complex formation. Symptoms in patients with Scott syndrome include

deficient thrombin generation, significantly prolonged bleeding times as well as a reduced incidence rates for thrombotic events (Kojima et al., 1994, Sims, 1989 #51, Toti et al., 1996, Zhou et al., 1998, Suzuki et al., 2010, Yang et al., 2012).

2.2.2 *Extrinsic pathway*

This subchapter will discuss the substances involved in the mediation of the extrinsic pathway.

Tissue factor (TF)

Injury of blood vessel endothelial membrane results in TF expression. This integral membrane glycoprotein is usually expressed on skin, organ surfaces, vascular adventitia as well as their corresponding neoplastic tissue formations, but not by vascular endothelial cells (Bächli, 2000). Hence, exposure of TF to the blood flow only happens in case of endothelial injury (Mandal et al., 2006) and is either via direct exposure of the subendothelial matrix or cytokine-induced TF expression, by cytokine, such as interleukin 6 (IL-6), secretion by activated monocytes and endothelial cells (Levi et al., 2012).
Under physiological conditions, the TF is inactive, but cell lysis or in vitro calcium ionophore stimulation leads to its activation (decryption) and, subsequently, the FVIIa binding and factor X activation. This decryption process might be associated with the linkage of free cysteine residues (Cys186 and Cys209) into a disulfide bond and an allosteric conformational change of the TF (Chen et al., 2006, Wong and Hogg, 2010). Most likely the mediation of this process is controlled by the protein disulfide isomerase (PDI), glutathione, and NO (Wong and Hogg, 2010), although the involvement of Cys186 and/or Cys209 in the TF's activation is still researched (Bach and Monroe, 2009, Butenas et al., 2009, Kothari et al., 2010).
Another theory suggests that the TF circulates through the blood vessels in association with microvesicles, deriving from the surface of stimulated monocytes/macrophages as well as influencing the coagulation initiation of activated platelets as well as in a soluble, but differently spliced form (Giesen et al., 1999, Bogdanov et al., 2003, Del Conde et al., 2005).

Activation of factors VII, IX, and X

Another critical aspect of the extrinsic pathway is the activation of factors VII, IX, and X. The activation of factor VII is directly interlinked with the TF, which serves as a cofactor for the generation of VIIa (Konigsberg et al., 2001, Morrissey, 2001). The resultant TF-factor VIIa

complex then triggers the activation of factors X and IX. The first is activated by the generation of an Arg52-Ile53 peptide bond in the factor X heavy chain, which leads to the formation of the Xa, a serine protease (Jesty et al., 1974, Baugh and Krishnaswamy, 1996, Butenas et al., 1997), whereas the latter's active version forms a complex with its cofactor factor VIIIa (Osterud and Rapaport, 1977, Morrison and Jesty, 1984).

Factor X activation is triggered by the intrinsic and the extrinsic pathway because of of the limited amount of TF as well as the activity of tissue factor pathway inhibitor (TFPI), which binds factor Xa and thereby inhibits the TF-factor VIIa complex' activity, diminishing the intrinsic pathway's stimulus (Broze, 1995). The extrinsic pathway mediates the activation of factor X with factor IX and its cofactor factor VIII and is amplified by the presence of thrombin as well as factor Xa (Pittman and Kaufman, 1988, Butenas et al., 1997), whereas the generation of factor IXa is enhanced by thrombin-induced activation of factor XI (Di Scipio et al., 1978, Gailani and Broze, 1991, Naito and Fujikawa, 1991, von dem Borne et al., 1995, Sun and Gailani, 1996). These both feedback-loops result in an increased amount of activated factor VIII and factor IX, which subsequently facilitates Xa and thrombin generation. Recent in vitro studies proved that the extrinsic TF-factor VIIa complex utilizes a feed-forward loop for the activation of factor VIII, an element of the intrinsic pathway, and thereby enhances the thrombin generation process as well (Kamikubo et al., 2017).

2.2.3 *Intrinsic (contact activation) pathway*

The activation of the initial intrinsic pathway is mediated by plasma proteins, such as factor XII (Hageman factor), plasma prekallikrein (Fletcher factor), and high molecular weight kininogen (HMWK, Fitzgerald factor), which are activated by exposure to negatively charged surfaces. This exposure triggers the enhancement of factor XII's auto-activation, which, in combination with HMWK, subsequently triggers the generation of factors XIa and successional IXa. The latter then forms a complex with factor VIIIa, the intrinsic X-ase, resulting in the activation of factor X, and after that, jointly with thrombin, triggers the earlier mentioned feedback loop of factor VIII (Pittman and Kaufman, 1988, Butenas et al., 1997), as well as an increased generation of factors XIa and IXa (Di Scipio et al., 1978, Naito and Fujikawa, 1991, von dem Borne et al., 1995, Sun and Gailani, 1996). Furthermore, factor XIIa triggers the conversion of plasma prekallikrein to kallikrein, resulting in the release of bradykinin, an inflammatory-mediating peptide, from HMWK as well as a feedback loop of activation, because kallikrein activates factor XII (Weidmann et al., 2017).

The further intrinsic pathway cascade already is part of the common pathway, mediated by factor V, prothrombin, and fibrinogen.

Polyphosphate, a strongly anionic polymer of phosphoanhydride-linked orthophosphates, is a molecule with a varying number of orthophosphates utilised in a vast amount of organisms as well as cellular pathways. For instance, in bacteria polyphosphate is synthesised from ATP and functions as energy storage for the ATP synthesis during starvation periods (Kornberg et al., 1999), whereas in human platelets, the polyphosphate is smaller and consists of only sixty to hundred phosphate units, stored in dense granules and released during the platelet activation process (Ruiz et al., 2004). Because of its anionic nature, polyphosphate is a potent procoagulant and most likely functions as the required negatively charged surface triggering the intrinsic pathway. The polyphosphate in the intrinsic coagulation process might derive from either the injured endothelial tissue or microbial cells in the event of infections. Furthermore, polyphosphate is involved in the acceleration of factors V and XI activation, whereas the latter requires the additional presence of thrombin, the abrogation of TFPI activity, the enhancement of fibrin polymerisation, and fibrillary thickening. The efficacy of polyphosphate-mediated blood clotting is proportionally dependent on the number of phosphoanhydride-linked orthophosphates. Hence the presence of microbial polyphosphate triggers all of the process mentioned above, whereas platelet polyphosphate is less efficient in activating the intrinsic pathway and rather accelerates the blood clotting after its initiation (Morrissey et al., 2012).

2.2.4 *Deficiencies of intrinsic pathway proteins*

The physiological importance of the initial complex of the intrinsic pathway's components is still investigated, but the fact, that deficiencies in plasma prekallikrein, HMWK, and factor XII generation have no association with higher bleeding tendencies suggest that the initiation segment of the intrinsic pathway only plays a subordinate role for the in vivo coagulation cascade activation. Though particular factor XII mutations show an association with hereditary angioedema and normal C1 inhibitor concentrations, an autosomal dominant disease with recurrent attacks of upper respiratory tract oedema (Colman et al., 1975, Lämmle et al., 1991).

On the other hand, factor XI deficiencies are associated with injury-related bleeding phenomena, which hints that factor XI fulfils an essential function for the hemostasis, unaffected by contact activation and factor XII (Asakai et al., 1991). These traits most likely derive from the fact that thrombin feedback in association with polyphosphate, released from

activated platelets or derived from injured endothelial tissue, serving as a cofactor activates factor XI, which subsequently leads to enhanced thrombin generation after the clot's formation (Gailani and Broze, 1991, Naito and Fujikawa, 1991, von dem Borne et al., 1995, Choi et al., 2011). Study results in knockout mice also support the hypothesis that factor XII or factor XI deficiency are not associated with higher bleeding tendencies under physiological conditions. Furthermore, these mice showed a significantly lower number of arterial thrombosis in experimental thrombosis models (Rosen et al., 2002, Renné et al., 2005), while patients with severe deficiencies in factor XI (with less than 15% residual activity), exhibited a protection from ischemic stroke but not from myocardial infarction (Salomon et al., 2008). In another recent study, Büller and colleagues proved that an antisense therapy, which lowered factor XI activity to approximately 20% residual activity, reduced the incidence of venous thromboembolism (VTE) without increased bleeding tendency (Büller et al., 2015). Similar results were published for factor XII polymorphisms and thrombotic events (Tirado et al., 2004, Cochery-Nouvellon et al., 2007, Reuner et al., 2008), which suggests that the thrombin generation via the tertiary amplification pathway, involving factors XII or XI, might be more closely associated with the development of pathological thrombosis rather than physiological hemostasis. Hence, the association of more frequent bleeding in patients with factor XI deficiency might derive from increased fibrinolysis due to reduced activation of thrombin-activatable fibrinolysis inhibitor (TAFI) (von dem Borne et al., 1995, Von dem Borne et al., 1997, Minnema et al., 1998). Since the TAFI activation requires a high thrombin concentration within the clot, which seems to derive from thrombin-mediated factor XI activation after the initial clot formation, the absence of factor XI only allows for an insufficient TAFI generation resulting in the clot's lysis.

2.2.5 *Continuation of the coagulation cascade*

After the factor X activation and the convergence of both pathways, the coagulation cascade proceeds with the following factors:

- Factor V is secreted from platelet alpha granules during platelet activation and activated by thrombin via a cleavage process subsequently binds factor Xa (Mann et al., 1981, Suzuki et al., 1982, Tracy et al., 1982, Mann et al., 1992). For the formation of the prothrombinase complex, platelet factor V seems more significant than circulating factor V (Tracy et al., 1984). The inactivation of factor Va is triggered by protein C and protein S and prevents excessive coagulation.

- Factor Xa, interlinked with factor Va, leads to the formation of the prothrombinase complex on the platelet surface. This complex is responsible for the conversion of prothrombin (factor II) to thrombin (factor IIa) as well as the simultaneous release of the prothrombin activation fragment F1 and F2 (Kane et al., 1980, Tracy et al., 1985).
- Thrombin mediates the conversion of fibrinogen to fibrin (Mosesson, 1992)
- Factor XIIIa is responsible for the stabilisation and crosslinking of overlapping fibrin stands (Pisano et al., 1968). In association with fibrinogen, it regulates the volume of red blood cells within the thrombus and thus the clot's size. Knockout mice with either factor XIII deficiency or fibrinogen mutations associated with factor XIIIa binding abrogation show a much smaller clot size compared to wildtype mice, because of red blood cell extrusion during the clot retraction; these results are coherent with findings in patients with factor XIII-deficiency (Aleman et al., 2014). The generation of factor XIIIa is enhanced by a complex formation of thrombin, fibrin, and factor XIII (Greenberg et al., 1987).

2.3 *Control mechanisms and termination of clotting*

The activated platelet and clotting cascade interaction lead to a fast hemostatic response, restricted to the endothelial injury site. Since an excessive clotting response might result in thrombosis, vascular inflammation, as well as tissue damage, the coagulation process is tightly modulated by numerous protection mechanisms, such as the procoagulants' dilution in the blood flow, the limitation of activated factors through the reticuloendothelial system and of activated procoagulants and platelets by natural antithrombotic pathways, which are anchored on endothelial cells of the vascular system and therefore regulate and maintain the blood's fluidity (Lane et al., 2005).

2.3.1 *Clotting cascade inhibition*

Two major endogenous inhibitors, TFPI, which mediates the TF pathway and the C1 esterase inhibitor, which mediates the intrinsic pathway, achieve the inhibition of the endogenous coagulation pathway:

Tissue factor pathway inhibitor (TFPI)

TFPI is a Kunitz-type protease inhibitor and circulates in the plasma at very low concentrations, unlike AT (Bajaj et al., 2001). Its primary function is the inhibition of the factor X activation process by directly inhibiting factor Xa as well as the formation of the TFPI-factor Xa complex, which subsequently inhibits TF and FVIIa and thereby the triggering of the extrinsic pathway (Broze et al., 1988, Jesty et al., 1994, Baugh et al., 1998).
TFPI is mainly synthesised in the microvascular endothelium; afterwards, free TFPI (TFPI-α) and TFPI associated with low-density lipoproteins circulate in plasma, with the first accounting for 20% of the total plasma TFPI, whereas the latter accounts for 80% of total plasma TFPI and is generally associated with glycosaminoglycans on the surface of endothelial cells (TFPI-β) (Caplice et al., 2001). The administration of intravenous heparin leads to a vast increase of the TFPI plasma concentration, which might be responsible for the antithrombotic effects of heparin and low-molecular-weight heparin (LMWH) administration.
Deficiencies in the TFPI gene were shown to result in intrauterine lethality in mice; in humans, no cases of TFPI deficiency have been reported (Huang et al., 1997). On the other hand, increased TFPI concentrations and activity are associated with inherited bleeding disorders involving factor V mutations, namely factor V Amsterdam and factor V East Texas. These mutations utilise alternative splicing for factor V, which results in the expression of a short form of factor V, which forms a complex with TFPI-α, and in a 10- fold increased concentration of circulating TFPI-α. Therefore, patients with factor V Amsterdam or factor V East Texas manifest an autosomal dominant bleeding disorder (Kuang et al., 2001, Vincent et al., 2013, Cunha et al., 2015).
The potential of TFPI as an anticoagulant is still controversially discussed (Bajaj and Bajaj, 1997).

C1 esterase inhibitor

The C1 esterase inhibitor is synthesised in the liver and belongs to the family of serine protease inhibitors (SERPINs). Its primary function is the inhibition of FXIIa and plasma prekallikrein as well as the complement proteases C1r, C1s, and FXIa. Deficiencies in the C1 esterase inhibitor gene are associated with an angioedema syndrome known as C1INH-AAE (Abdulkareem et al., 2018).

2.3.2 *Regulation of the coagulation process' termination phase*

This phase is mediated by another SERPIN, AT, and the protein C pathway, an inhibitory process initiated by the clotting. Furthermore, prostacyclin, thromboxane, and NO modulate vascular and platelet reactivity.

The regulation of termination phase is crucial, since it controls the clot formation's extent, with dysregulations resulting in either thrombotic or bleeding disorders, depending on whether the mutation leads to a decreased, such as AT, protein C, and protein S deficiencies, or increased activity, such as factor V and TM mutations.

2.3.3 *Additional factors involved in the coagulation regulation process*

White blood cells

Since activated macrophages and monocytes possess the ability to express TF either on the cell's surface or embedded in microparticles, they are involved in maintaining physiologic hemostasis as well as pathologic thrombosis (Rak, 2014). A study by Perry and colleagues proposed that the TF messenger RNA concentration's regulation is controlled by PARP-14, a poly-ADP-ribose-polymerase protein, that might prevent excessive thrombosis during inflammatory periods (Iqbal et al., 2014).

Antithrombin (AT), heparin, heparan

AT belongs to the SERPIN family and circulates in plasma, where it neutralises numerous enzymes regulating the clotting cascade, such as thrombin, factors Xa and IXa. Furthermore, by the formation of equimolar, irreversible complexes with factors XIIa and/or XIa, they are rendered inoperable as well. AT contains two active sites, the reactive centre, Arg393-Ser394, as well as the heparin-binding site at the protein's amino terminus (Perry, 1994). The activity of AT is enhanced by endogenous heparins, presented in Figure 5, and heparan sulfate, a glycosaminoglycan present on the surface of endothelial cells, which contain a pentasaccharide sequence mediating the binding to AT. This binding interaction leads to a conformational change of the AT, whereby it gets activated and amplifies the inactivation of clotting factors by 1000 - 4000 times (Marcum et

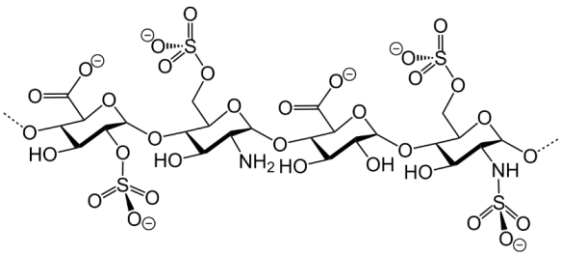

Figure 5 Structure of heparin (Chemical Book, 2019)

al., 1984, Mammen, 1998, Weitz, 2003). The effect of this pentasaccharide sequence is pharmacologically mimicked by Fondaparinux®, a LMWH.

An overview of the inactivation mechanisms of heparin, LMWH, and Fondaparinux® is provided in Figure 6.

For the swift and rapid inactivation of circulating excessive thrombin, the luminal surface of the vascular system is covered by AT-coated endothelial cells, whose inactivation capabilities is even further enhanced by the microcirculation's surface-to-volume geometry (1 ml blood is exposed to as much as 5000 cm^2 endothelial surface area). An overview of the factors involved in clotting termination and their interactions are shown in Figure 7.

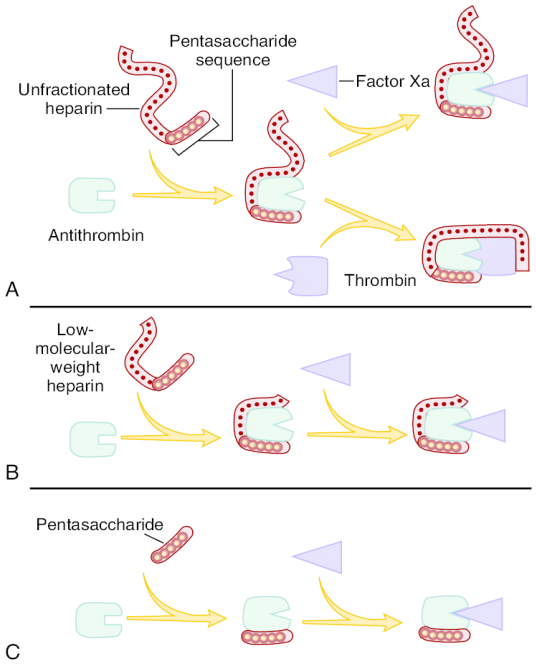

Figure 6 Mechanisms of factor Xa inactivation by (A) heparin, (B) LMWH, and (C) fondaparinux (Jaffer, 2014)

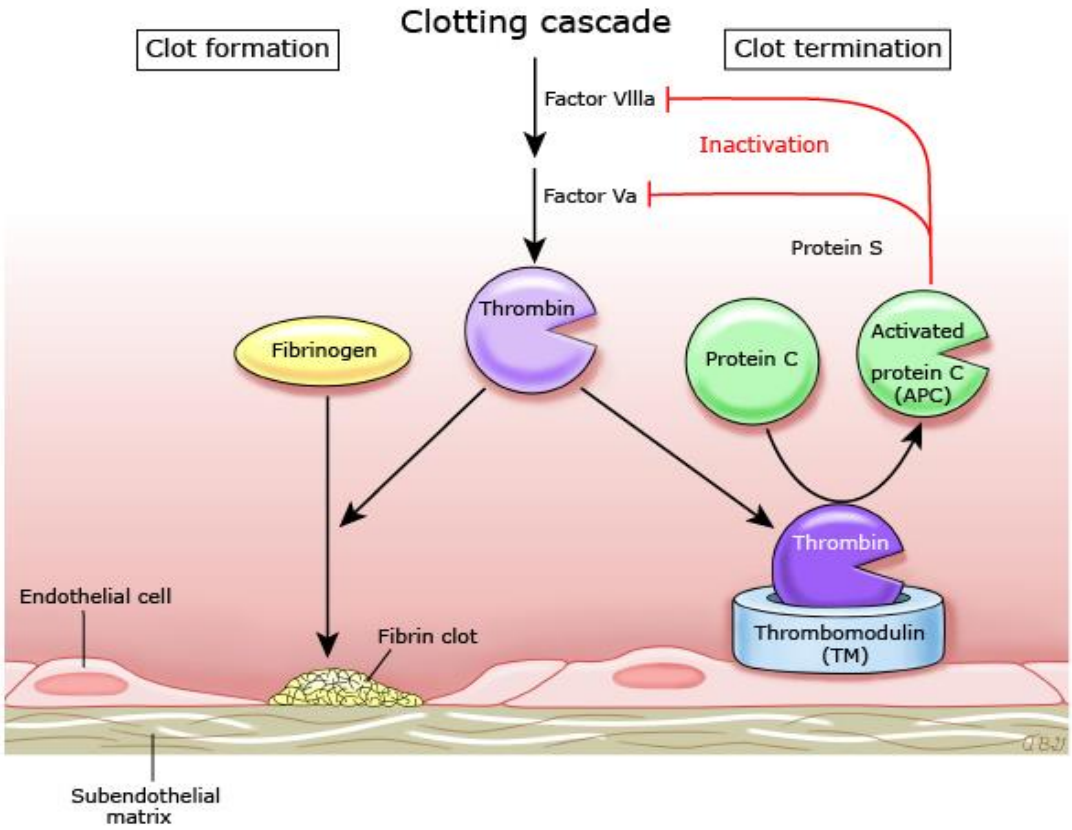

Figure 7 Hemostatic control mechanisms and termination of clotting (Leung, 2019)

Activated protein C (APC) and protein S

With progressing clot formation, thrombin binds to thrombomodulin (TM), an integral membrane protein located on the surface of endothelial cells (Esmon et al., 1982), which results in a change in thrombin substrate specificity and the loss of its procoagulant functions, including platelet activation and fibrin clot formation. Instead, it acquires the ability to activate protein C, and thus gains an anticoagulant effect (Esmon, 1989, Esmon, 2003, Dutt and Toh, 2008). This transformation is assumed to depend on the interaction of TM with thrombin's exosite I, which is the binding site for thrombin's procoagulant substrates, resulting in competitive inhibition of the thrombin (Fuentes-Prior et al., 2000). Protein C binds this complex and is subsequently cleaved and activated; in the presence of EPCR, this activation step is further enhanced (Esmon et al., 1999, Taylor et al., 2001, Saposnik et al., 2004). In TM knockout mice an association with uncontrolled activation of the coagulation system and widespread thrombosis is found, whereas a naturally-occurring TM point mutation C1611A, which leads to the shedding of TM from the endothelial surface and subsequently to its circulation at very high plasma concentrations, shows an association with bleeding events (Isermann et al., 2001).

In combination with protein S the TM triggers the proteolytic inactivation of factors Va and VIIIa on phospholipid surfaces, thus inactivating the prothrombinase and the intrinsic X-ase, respectively (Walker, 1981, Fulcher et al., 1984, Kalafatis et al., 1995, O'Brien et al., 2000). An overview of the termination of the clotting cascade is provided in Figure 8.

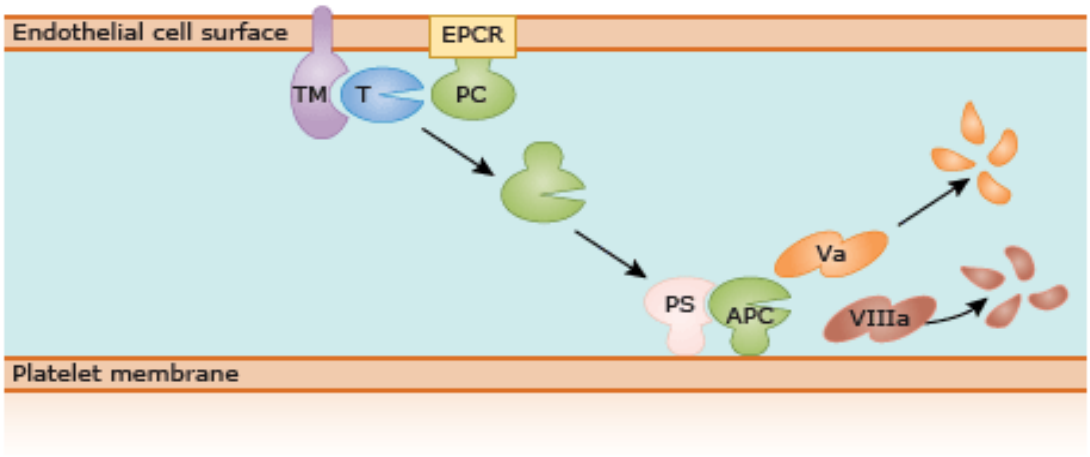

Figure 8 Protein C and S function in the termination of coagulation (Bunn and Bauer, 2011)

The initial process is the cleavage of factor Va at Arg506 by TM, which subsequently triggers the exposure of Arg306 and Arg679, and afterwards, these two amino acids are cleaved by TM. Hence, factor V Leiden, caused by the factor V point mutation R506Q, is insusceptible to

the respective position's cleavage by TM, resulting in its slower inactivation as well as a hypercoagulable state (Kalafatis et al., 1995).

Furthermore, position 506 cleaved factor V might be a cofactor, which, in association with protein S, is involved in the degradation of factors Va and VIIIa by TM (Thorelli et al., 1999). Therefore, the shortage of this cleavage product leads to a decreased anticoagulant activity of TM, which results in further amplification of the hypercoagulable state in patients with factor V Leiden (Castoldi et al., 2004).

The inactivation of factor VIIIa is mediated by TM (enhanced by protein S, decreased by factor IXa) cleaving at Arg336 and then Arg562. Furthermore, the process is enhanced by protein S, whereas the effect decreases in the presence of factor IXa (O'Brien et al., 2000).

The circulating protein S has two different forms. While the free form exerts the anticoagulant activity, the bound form is complexed to C4b binding protein of the complement system and exerts no hemostatic effect. Since C4b binding protein is an acute-phase protein, inflammatory conditions lead to its amplification, and thus, the number of free protein S is reduced, which results in an enhanced likelihood of thrombotic events (Amiral and Seghatchian, 2019).

Prostacyclin and thromboxane

Triggered by phospholipase A2, uninjured endothelium, adjacent to the disrupted endothelial membrane releases arachidonic acid from its cell membrane phospholipids. Subsequently, the arachidonic acid is converted into thromboxane A2, shown in Figure 9, which stimulates platelet aggregation and vasoconstriction, by cyclooxygenase-1 (COX-1) in platelets, whereas prostacyclin, delineated in Figure 10, via activation of adenylate cyclase, inhibits platelet aggregation and antagonises thromboxane A2-mediated vasoconstriction, derives from COX-2 and its production is stimulated by physiological and laminar blood flow (Smith, 1989, Topper et al., 1996, Grosser et al., 2006).

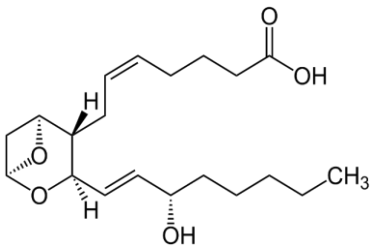

Figure 9 Structure of thrombo-xane A2 (Chemical Book, 2016)

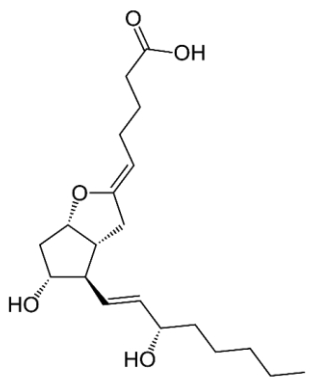

Figure 10 Structure of prostacyclin (Chemical Book, 2017d)

Therefore, the application of low-dose acetylsalicylic acid leads to the irreversible acetylation and inhibition of COX-1, whereas COX-2 is only mildly extenuated, which results in the inhibition

of thromboxane A2 for the entire platelet's life (Phillips et al., 2005). On the other hand, the inhibition of the prostacyclin, as well as COX-1 and COX-2 production by endothelial cells, requires the administration of higher acetylsalicylic acid doses (Clarke et al., 1991b).

Nitric oxide (NO)

The production of NO, an endothelium-derived relaxing factor, which is generated by the catalytic reaction of L-arginine with the enzyme NO synthase and functions as vasodilator via soluble guanylate cyclase in smooth muscle cells of the vascular system, resulting in the production of cyclic guanosine monophosphate, thus triggering the vasodilatation effect. Furthermore, NO acts as a platelet adhesion and aggregation inhibitor (Moncada and Higgs, 1993). Another production site of NO are platelets, whose synthesising activity is enhanced by platelet adhesion to fibrillar collagen under shear flow, which might provide negative feedback to prevent excessive platelet adhesion and vasoconstriction at sites of endothelial injury (Cozzi et al., 2015).

Due to its ability to freely cross cell membranes, NO functions as a paracrine hormone with a short half-life, which is caused by its rapid degradation by haemoglobin present in adjacent erythrocytes. Therefore, the circulation of free haemoglobin, like during intravascular hemolysis, results in a significant decline of NO plasma concentrations.

Intravenous administration of an arginine analogue inhibiting the NO production leads to a significantly increased blood pressure, which hints at a continuous release of NO for the regulation of the vascular tone (Qiu et al., 1998).

Besides lifestyle factors, such as regular exercising, smoking, and high low-density lipoprotein concentrations, ageing, as well as vascular diseases, influence the NO plasma concentrations. Except for exercising, which is associated with upregulation, the mentioned factors are associated with endothelial dysfunction, thus resulting in lower NO concentrations (Vanhoutte et al., 2017).

Thrombospondin 5

Another regulator of the vascular tone is thrombospondin 5, also known as cartilage oligomeric matrix protein, which is expressed in cartilage and smooth muscle tissue cells. In a mouse model, it was found that platelets are an additional production site of thrombospondin 5, which is released upon platelet activation, and subsequently inhibits thrombin-dependent platelet aggregation and serves as an anticoagulant, leading to a dose-dependent prolonged TT (Liang et al., 2015).

2.4 *Clot dissolution and fibrinolysis*

Following hemostasis, the reorganisation and removal of the clot by plasmin, a proteolytic enzyme, as well as wound healing and tissue remodelling are essential to restore the vessel's patency.

Plasmin

Plsmin's precursor molecule, plasminogen, binds tissue plasminogen activator, and fibrin. This complex then triggers the plasminogen's conversion to the active, proteolytic plasmin (Collen, 1980, Hoylaerts et al., 1982).

Plasmin has a broad substrate specificity and is capable of cleaving fibrin, fibrinogen, and a vast number of plasma proteins and clotting factors (Samis et al., 2000). The cleavage of polymerised fibrin takes place at multiples sites and results in fibrin degradation products, such as D-dimer, which consists of two D domains from adjacent fibrin monomers crosslinked by factor XIIIa. The latter is another cleavage target for plasmin, unlike factor XIII, which leads to a downregulation of fibrin crosslinking (Hur et al., 2015).

The plasminogen/plasminogen-activator system is complex, paralleling the coagulation cascade. Serine protease plasminogen activators mediate the regulation of plasmin activity (tissue-type plasminogen activator and urokinase-type plasminogen activator) and plasminogen activator inhibitors (PAI-1 and PAI-2), which are secretory products of vascular endothelial cells (Kolev and Machovich, 2003).

Tissue-type plasminogen activator (tPA)

tPA is an enzyme deriving from endothelial cells, which gets released upon stimulation by various substances, such as thrombin, serotonin, bradykinin, cytokines, and epinephrine. In plasma, tPA is circulating complexed with its natural inhibitor PAI-1, and it is cleared swiftly by the liver (Yamamoto et al., 1994, Stein et al., 1998).

The site for the plasmin generation by tPA, analogous to the prothrombin complex, is the fibrin clot's surface, where tPA, as well as fibrinogen, form a complex with fibrin by binding lysine residues in the fibrin clot. Triggered by interacting with the fibrin as well as the resulting binding interaction, tPA and plasminogen are aligned on the fibrin surface (van Zonneveld et al., 1986), which leads to an increase of tPA's catalytic efficiency by several hundred times (Hoylaerts et al., 1982).

Urokinase

Urokinase is mainly found in high concentrations in urine, and like tPA, it is a physiologic plasminogen activator. But unlike tPA, which is mostly responsible for the initiation of intravascular fibrinolysis, urokinase is the primary fibrinolysis activator in the extravascular compartment. It is secreted as prourokinase, also known as a single-chain urokinase-type plasminogen activator, which is converted to urokinase by plasmin, though its amount of proteolytic activity depends on the urokinase's exposure to fibrin (Bugge et al., 1996).

Plasminogen activator inhibitors (PAIs) and alpha-2-antiplasmin

First, the activity and synthesis of PAIs, which inhibit tPA, shall be described in this subchapter (Sprengers and Kluft, 1987, van Meijer and Pannekoek, 1995). PAI-1 is synthesised by endothelial cells and platelets, which also control the PAI-1 release during fibrinolysis. A possible theory for PAI-1 deficiencies' association with surgery or trauma-related bleeding diathesis, might be the activated platelet-mediated PAI-1 release's influence on the relative resistance of platelet-rich arterial thrombi and the corresponding thrombolysis (Fay et al., 1992, Fay et al., 1997). PAI-2 is mainly synthesised in white blood cells as well as the placenta, which results in a significantly increased PAI concentration during pregnancy. Since PAI-2 is a less effective plasminogen inhibitor than those mentioned above, its importance for maintaining physiological conditions is still unknown (Schwartz, 1994), although was found to interact with alpha-2-antiplasmin, which is involved in the plasmin inhibition (Edelberg and Pizzo, 1992). Alpha-2-antiplasmin is synthesised and secreted by the liver and is found in platelets as well. Furthermore, it is crosslinked into the fibrin clot by factor XIIIa, leading to the thrombus' resistance against plasmin by complexing with it. Hence circulating plasmin is swiftly inactivated by alpha-2-antiplasmin, although the fact that the alpha-2-antiplasmin's concentration is lower than plasminogen plasma concentrations can result in the alpha-2-antiplasmin's depletion, whereas plasmin is still generated (Favier et al., 2001).

Carboxypeptidase B2 (CPB2)

CPB2 is a thrombin-activatable fibrinolysis inhibitor, which removes carboxy-terminal lysines in the partially disintegrated fibrin clot. These carboxy-terminal lysines are usually exposed due to the fibrin's degradation to plasmin, which provides additional interaction sites in the clot for plasminogen and tPA incorporation and results in a positive feedback loop

enhancing the clot lysis. By the removal of the carboxy-terminal lysines, CPB2 weakens this clot lysis effect (Redlitz et al., 1995, Nesheim, 1998).

The precursor molecule of the CPB2 is the plasma-circulating TAFI (pro-CPB2), which, similar to protein C, is a substrate for the thrombin-TM complex (Booth, 2001, Binette et al., 2007). This complex increases the activation speed of pro-CPB2 about 1000 times compared to thrombin alone. After its activation, the CPB2 inhibits fibrinolysis by cleaving C-terminal lysines from partially digested fibrin, which leads to diminished plasminogen incorporation and activation, resulting in the clot lysis' delay (Bajzar et al., 1996, Mosnier et al., 2001b).

Premature plasmin degradation of the freshly formed fibrin clot is achieved by the crosslinking of CPB2 to fibrin by factor XIIIa, which might also enhance the activation of pro-CPB2 stabilise CPB2's enzymatic activity and prevents enzyme degradation (Valnickova and Enghild, 1998).

The activation of protein C, as well as pro-CPB2 by the thrombin-TM complex, is reasonable because it results in the depletion of the clotting cascade as well as the fibrin clot's protection from premature degradation. Since the thrombin concentrations required for pro-CPB2 are significantly higher than those for fibrinogen clotting, the activation of CPB2 requires an enhanced and accelerated thrombin generation, which is achieved by a feedback loop of factors V, VIII, and XI as well as the small amount of initially generated thrombin.

The reason for several acquired and congenital coagulation disorders is this necessity of high thrombin concentrations for pro-CPB2 activation:

- Factor VIII deficiency, more commonly known as haemophilia A, results in bleeding diathesis, whereas factor XI deficiency is associated with extenuated pro-CPB2 activation, which leads to enhanced and premature clot lysis (Broze and Higuchi, 1996, Von dem Borne et al., 1997, Minnema et al., 1998, Mosnier et al., 2001a, Lisman et al., 2002, Foley and Nesheim, 2009).
- The insufficient fibrinolysis in patients with chronic liver disease might be associated with the low concentrations of pro-CPB2 (Van Thiel et al., 2001).
- The prothrombin mutation G20210A is associated with excessive thrombin generation, which might lead to higher pro-CPB2 activation concentrations and thus result in inhibited fibrinolysis and increased thrombotic risk (Colucci et al., 2004).

2.5 *Blood coagulation and the immune system*

The hemostatic process shows a multi-stage interaction between coagulation factors and the immune system, with the hemostatic factors supporting the defence as well as the immune mediators influencing the hemostasis (van der Poll and Levi, 2012, Engelmann and Massberg, 2013, Vazquez-Garza et al., 2017).

- For instance, platelets tested in patient samples from malaria-endemic regions showed interactions with several plasmodial species and the capability of killing the parasites; in vitro assays verified that the parasite killing effects were dependent on platelet factor 4 (Kho et al., 2018).
- Besides its anticoagulant activity, TM was shown to exert the initiation of anti-inflammatory and anti-apoptotic activities as well as the stabilisation of endothelial membranes. This effect is triggered by TM binding to EPCR and the subsequent cleavage of the endothelial PAR-1 and most likely coupling sequences with different G protein-coupled receptors. This signalling cascade and its effects are quite intriguing since the cleaving of endothelial PAR-1 by thrombin results in a prothrombotic effect and disruption of the endothelial barrier, which is the APC/EPCR-PAR-1 signalling effect's opposite (Esmon, 2012). But unlike Thrombin, APC/EPCR cleaves PAR-1, preferably at Arg46 (rather than Arg41, which is cleaved by thrombin), which might be the reason for the different signalling programs compared to thrombin (Griffin et al., 2016).
- The CPB2 is capable of the inactivation of activated complement components C3a and C5a, which are the complement activation's main anaphylatoxins, as well as bradykinin. All three molecules are potent inflammatory mediators, which are inactivated by the removal of the carboxy-terminal arginine residue (Leung et al., 2008). Therefore, the activation of protein C and pro-CPB2 might result in a negative feedback mechanism that downregulates thrombin's inflammatory activity (Leung and Morser, 2012).
- Another trigger for the coagulation factor's involvement in immunoactivity is the presence of pathogens like bacteria. While pathogen- or damage-associated molecular patterns are recognised by monocytes, which deliver tissue factor, and neutrophils, which activate factor XII and bind von Willebrand factor via neutrophil extracellular traps, the identification of bacterial virulence factors works as bacterial plasminogen activator for plasmin-mediated fibrinolysis, which leads to microbial dissemination (Degen et al., 2007, Engelmann and Massberg, 2013).

- Infectious and inflammatory conditions lead to a reduction of free protein S via complement protein C4b, an acute phase reactant, which leads to an enhanced clotting process.
- Furthermore, factor V seems to have inflammatory capabilities besides its proteolytic activity against factors Va and VIIIa (Liang et al., 2018).

3 *Classic anticoagulants*

The decision, which anticoagulant is suitable for a patient, depends on the clinical setting, such as required dosing, monitoring, cost, and risks. Therefore, advantages, as well as disadvantages of each drug, should be considered and adjusted to fit the patient's needs. The guidelines for each agent's indication are mainly based on its efficacy and safety in the respective patient population. In the case of equivalency of two or more drugs, additional factors, like the physician's familiarity with the respective agent, should be considered (Schulman and Crowther, 2012).

3.1 *Antiplatelet agents*

The targets for antiplatelet agents are numerous platelet functions, such as aggregation, the release of granule contents, and platelet-mediated vascular constriction. The most common classification of these substances is based on their respective mechanisms of action.

- Acetylsalicylic acid and non-steroidal anti-inflammatory drugs

  These compounds target the cyclooxygenase, which is involved in the prostaglandin G/H synthesis and mediates the biosynthesis of prostaglandins and thromboxanes from arachidonic acid.

- Platelet P2Y12 inhibitors

  This group comprises compounds like clopidogrel, ticagrelor, and cangrelor, which block the adenosine diphosphate's binding to the specific platelet receptor P2Y12, leading to the inhibition of platelet activation. Furthermore, they target the irreversible degranulation, shape change, and aggregation of the platelets (Foster et al., 2001). These substances are usually administered as oral medications.

- Vorapaxar

  Vorapaxar, depicted in Figure 11, targets the PAR1 receptor on the platelet surface and leads to the inhibition of thrombin-induced and thrombin receptor agonist peptide-induced platelet aggregation.

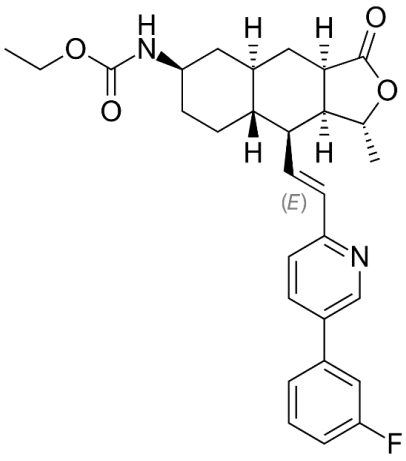

Figure 11 Structure of vorapaxar (Chemical Book, 2017e)

- Glycoprotein (GP) IIb/IIIa antibody and small molecule inhibitors

  These substances target the cross-bridging of platelets, which is mediated by fibrinogen binding to the GP IIb/IIIa receptor, thus inhibiting the final common pathway of platelet aggregation. These substances are only available as intravenous injections.

Acetylsalicylic acid

The indications for acetylsalicylic acid, presented in Figure 12, include cardiovascular disorders, for instance, primary and secondary prevention of coronary heart disease, transient ischemic attack, stroke, and in the acute therapy of patients with acute coronary syndrome. The first evidence for the benefit of acetylsalicylic acid administration was established by the Antithrombotic Trialists' Collaboration, which reviewed about 200,00 patients with antiplatelet therapy (Antithrombotic Trialists' Collaboration, 2002). Newer studies suggest the start of an acetylsalicylic acid therapy in non-ST elevation acute coronary syndrome with a loading dose of up to 325 mg, followed by a maintenance (daily) dose of 100 mg for secondary prevention, while higher doses showed no benefit, but increased bleeding risk (Yusuf et al., 2001, Mehta et al., 2010, Wright et al., 2011, Xian et al., 2015).

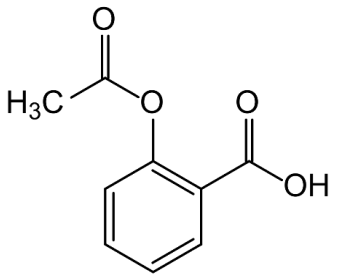

Figure 12 Structure of acetylsalicylic acid (Chemical Book, 2017a)

P2Y12 inhibitors

For the treatment of an acute ST-elevation myocardial infarction, acetylsalicylic acid should be administered with the dose above, with concomitant early application of a P2Y12 inhibitor, such as ticagrelor (Wallentin et al., 2009, Mahaffey et al., 2011). Depending on the dosage acetylsalicylic acid therapy might be associated with gastrointestinal bleeding or enhanced preexistent bleeding outside the gastrointestinal tract, in higher doses with dyspepsia and nausea, which is usually addressed by the administration of a proton pump inhibitor (Harrington et al., 2008).

As mentioned above, in case of a non-ST elevated acute coronary syndrome, especially when undergoing percutaneous coronary intervention, a dual antiplatelet therapy, concomitant administration of P2Y12 inhibitors and acetylsalicylic acid, is indicated. These combination targets and inhibits the platelet adhesion and aggregation at the beginning of an occlusive coronary artery thrombotic event, which is essential in the long-term treatment of patients

with coronary stents. The benefits of a dual antiplatelet therapy with P2Y12 inhibitors and acetylsalicylic acid were established in the CURE trial, which found that a treatment with acetylsalicylic acid and clopidogrel, depicted in Figure 13, resulted in significantly lower incidence rates of cardiovascular death, nonfatal myocardial infarct, or stroke, although the administration was associated with more frequent major bleeding, but not life-threatening bleeding or hemorrhagic stroke. (Yusuf et al., 2001).

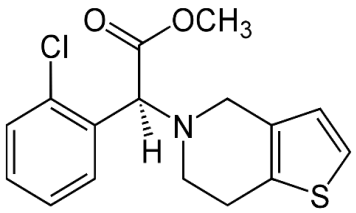

Figure 13 Structure of clopidogrel (Chemical Book, 2017c)

The reduced risk for thromboembolic events was found in low-, intermediate-, and high-risk patients (Mehta et al., 2001, Budaj et al., 2002, Steinhubl et al., 2002). More recent studies suggest the interchange of clopidogrel with ticagrelor, since it provided significantly lower incidence rates for cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke, although it had higher rates of bleeding events compared to clopidogrel (Wiviott et al., 2007, Wiviott et al., 2008, Wallentin et al., 2009). In case of prasugrel, the incidence rates did not significantly differ compared to clopidogrel, but it was associated with a higher major bleeding risk, especially in patients with a history of stroke/transient ischemic attack, over 75 years, and body weight below 60 kg (Antman et al., 2008).

Therefore, ticagrelor is the recommended interchange option for clopidogrel, although patients with multiple comorbidities might benefit more from clopidogrel administration, due to its reduced bleeding risk.

Glycoprotein IIb/IIIa inhibitors

Usually, patients receiving dual antiplatelet therapy do not require glycoprotein IIb/IIIa inhibitor therapy, except for patients who might require urgent cardiac surgery following their coronary angiography. Other indications are the occurrence of ischemia during dual antiplatelet therapy, and patients with a risk for the formation of large thrombus, especially when prasugrel or ticagrelor have not been administered before.

However, the role of GP IIb/IIIa inhibitors in treatment regimes of non-ST elevated acute coronary syndrome is not well studied, therefore it is not recommended as first-line therapy for this condition (Boersma et al., 2002).

3.2 *Heparins*

Heparins are endogenously produced, linear polysaccharides consisting of pyranosyl-uronic acid and glucosamine residue units (Munoz and Linhardt, 2004, Masuko and Linhardt, 2012), with numerous indications:

- VTE prophylaxis
- Deep vein thrombosis treatment
- Pulmonary embolism prophylaxis
- Myocardial infarction treatment
- Acute coronary syndrome treatment
- Stroke/transient ischemic attack treatment
- Neuraxial anaesthesia
- Perioperative setting

They are involved in numerous anticoagulant, anti-inflammatory, and possibly anti-angiogenic effects (Grigas et al., 1978, Ceri et al., 1990, Fatma et al., 2000, Wijelath et al., 2010, Masuko and Linhardt, 2012). While the commonly used heparin is isolated directly from animals and consists of about 45 saccharide units, the LMWHs derive from enzymatic or chemical depolymerisation of unfractionated heparin, resulting in a product of about 15 saccharide units, whereas Fondaparinux® consists of only the smallest possible AT-binding region of heparin, containing only five saccharide units (Hirsh and Levine, 1992, Weitz, 1997, Prandoni, 2001, White and Ginsberg, 2003, Hirsh et al., 2008). Their mechanism of action is the binding of AT, which is mediated by a unique pentasaccharide sequence randomly distributed within the heparin (Choay et al., 1983, Hirsh et al., 2008). The AT's heparin-binding site is located at the amino terminus, and the binding triggers a conformational change of AT, which results in an enhanced and sped-up inactivation of coagulation factors (Marcum et al., 1984, Perry, 1994).

Among the heparins, unfractionated heparin is the most potent thrombin inactivator, because the inactivation depends on the formation of a ternary complex between heparin, AT, and thrombin, which requires a heparin chain of at least 18 saccharide units (Danielsson et al., 1986). Therefore, every heparin inhibits factor Xa, but only unfractionated heparin can inactivate thrombin (Jordan et al., 1980, Weitz, 1997).

Unfractionated heparin

Unfractionated heparin's metabolisation takes place in the reticuloendothelial system and the liver, and it is excreted via urine. Effects of the renal function for the elimination of therapeutic doses are not described; thus, no dose adjustments are required in patients with renal insufficiency or renal failure, although the renal function might play a role in high-dose regimes (Cruickshank et al., 1991, Hull et al., 1992, Hirsh et al., 2008, Kearon et al., 2008,

Garcia et al., 2012). One advantage of heparin is its fast onset of action, which is instantaneous for intravenous administration and with plasma peak concentrations two to four hours after subcutaneous application. Its metabolism is dose-dependent with a half-life of about one hour (Baglin et al., 2006), while heparin's bioavailability depends on the competitive inhibitory effect of AT, and thus the concentrations of plasma proteins, proteins secreted by platelets and endothelial cells (Turpie, 1998, Hirsh et al., 2008).

Low-Molecular-Weight Heparin (LMWH)

In comparison to unfractionated heparin, the renal clearance of LMWH contributes 10 – 40% to the heparin's elimination. Therefore, a renal impairment leads to reduced LMWH excretion, resulting in slightly higher plasma concentrations, whereas renal insufficiency with a creatinine clearance rate below 30 millilitres per minute show significantly increased plasma concentrations, which makes dose adjustment in patients with renal failure necessary. Peak concentrations for LMWH following subcutaneous administration are three to five hours, whereas intravenous administration results in peak plasma concentrations after two hours, with a half-life ranging from three to seven hours (Garcia et al., 2012).
For unfractionated as well as LMWH no placenta crossing or accumulation in breast milk are described.

3.3 *Vitamin K antagonists (VKAs)*

VKAs, which are also termed coumarins, for instance, phenprocoumon (Marcoumar®), have a wide range of indications, such as:

- Atrial fibrillation therapy
- Acute coronary syndrome therapy
- Heart failure therapy
- Prosthetic heart valve therapy
- Stroke prophylaxis
- Deep vein thrombosis treatment
- Pulmonary embolism prophylaxis
- Antiphospholipid syndrome treatment

Although their management requires a close monitoring of coagulation parameters, such as the PT and/or international normalised ratio (INR), due the respective therapeutic range of coumarins is narrow and is affected by various factors such as genetic variation, hepatic, renal, and cardiac function, drug interactions, and dietary habits, coumarins are widely used because of the clinicians' long experience with the agent and their effectiveness in the reduction of venous and arterial thromboembolic events. Increased PT, as well as INR levels, are associated with higher bleeding risks, whereas too low values only have an insufficient

effect and thus increase the risk of thromboembolic complications (Lefrere et al., 1986, Hallak et al., 1993, Hulse, 1996, Limdi et al., 2010).

Advantages and disadvantages

The main benefits of VKAs are the already mentioned large body of clinical experience and possibility of measuring and adjusting the dose (effect), the efficiency in patients with prosthetic heart valves, their low prices and good availability, as well as the possibility of antagonisation in case of bleeding events with vitamin K (Konakion®), fresh frozen plasma, or prothrombin complex concentrates.

On the other hand, coumarins have disadvantages like higher incidence rates of thromboembolic and bleeding complications in patients with atrial fibrillation, the requirement of regular monitoring and the affection of the drug concentrations by illness, drug interactions, and dietary habits (Lefrere et al., 1986, Hallak et al., 1993, Hulse, 1996, Limdi et al., 2010). Despite the feasibility of influenced plasma concentrations by genetic variations, for instance, *CYP2C9* and *VKORC1* mutations, numerous multicentered studies showed no significant outcome difference for major bleeding events as well as overall bleeding incidence rates between a genotype-guided therapy regime group and a control group (Kimmel et al., 2013, Pirmohamed et al., 2013, Verhoef et al., 2013, Gage et al., 2017).

Monitoring

While the first INR increases are detectable within two to three days, the deployment of the full anticoagulation effect takes about a week. The INR increase within the first few days reflects the diminishment of factor VII, with a half-life of only four to six hours, whereas the vitamin K-dependent factors, such as prothrombin, factors IX and X, have longer half-lives and their depletion takes about five days. Therefore, the initial time frame of coumarin therapy should be bridged with additional heparin administration. The usual therapeutic ranges for VKAs are between an INR of 2.0 - 3.0 for patients with VTE or 2.5 - 3.5 in patients with mechanical heart valves (Jones et al., 2005, Van Spall et al., 2012, Wallentin et al., 2010).

The monitoring frequency should be between four and six weeks for patients in the therapeutic range and once or twice weekly for patients outside the therapeutic range or if changes occur that might influence the coumarin absorption/metabolism such as intercurrent illness, new medication or a change of dietary habits. Only small deviations from the

therapeutic range should be re-monitored within a week without any dose adjustments (Rose et al., 2009, Van Spall et al., 2012)

Another option besides the outpatient monitoring is the patient self-monitoring with a point-of-care device and self-adjusted dosing in patients with suitable compliance and competence in self-testing (Siebenhofer et al., 2004, Fitzmaurice et al., 2005, Gardiner et al., 2005, Siebenhofer et al., 2008, Ryan et al., 2009, Verret et al., 2012, Nagler et al., 2013, Ward et al., 2015, Witt et al., 2018). Studies suggested that appropriately trained patients using self-monitoring and self-adjustment of VKA therapy had at least comparable results to patients managed in anticoagulation clinics (Heneghan et al., 2006, Connock et al., 2007, Garcia-Alamino et al., 2010, Bloomfield et al., 2011, Heneghan et al., 2016). Regardless of the used management approach, studies have demonstrated that patient education and review of medication changes by clinicians resulted in less bleeding incidences as well as higher patient satisfaction (Chiquette et al., 1998, Anderson, 2004, Jowett et al., 2006, Connock et al., 2007, Gray et al., 2007, Metlay et al., 2008, Saokaew et al., 2010, Aziz et al., 2011, Jennings et al., 2014).

As already mentioned, VKAs are subject to numerous drug interactions with a variety of mechanisms (Delaney et al., 2007, Juurlink, 2007, Schelleman et al., 2010, Lopes et al., 2011, Vazquez, 2018), such as:

❖ Alteration of the intestinal flora and subsequent intestinal vitamin K synthesis ❖ Inhibition of hepatic *CYP2C9*, with a reduction of coumarin metabolism ❖ Interruption of vitamin K recycling	Prolonged PT/Increased INR
❖ Injured gastrointestinal mucosa plus acetylsalicylic acid and nonsteroidal anti-inflammatory drugs ❖ Interference with platelet function	Increased bleeding risk independent of PT/INR
❖ Induction of hepatic CYP2C9 and thus increased coumarin metabolism ❖ High vitamin K intake	Reduced anti-coagulation

Therefore, the determination of clinically relevant drug interactions is challenging, since often the evidence for a respective drug's interaction is limited, or the effect's magnitude is small, and patients are advised to disclose any new medication, herbal products or dietary supplement to the clinician in charge of the anticoagulation management (Holbrook et al., 2005).

Another issue in coumarin management are non-responders, also termed patients with coumarin resistance. These patients require very high coumarin doses to attain therapeutic INR levels, and in some cases, there are patients that, despite having therapeutic INR levels, develop thromboembolic events. These cases require the administration of a different anticoagulant agent, which are mainly DOACs nowadays.

4 *Direct anticoagulants*

The direct anticoagulants consist of the direct thrombin and direct factor Xa inhibitors. Their administration is either via the parenteral or oral route. The first clinically available direct oral anticoagulant (DOAC) was dabigatran, an oral direct thrombin inhibitor approved in 2010 in the United States. The oral direct factor Xa inhibitors, such as apixaban (Eliquis®), edoxaban (Lixiana®) and rivaroxaban (Xarelto®), became available in subsequent years. While there are numerous approved parenteral direct thrombin inhibitors, such as bivalirudin, argatroban, and desirudin, no approved parenteral direct factor Xa inhibitor exists. A depiction of the DOACs' point of action is presented in Figure 14.

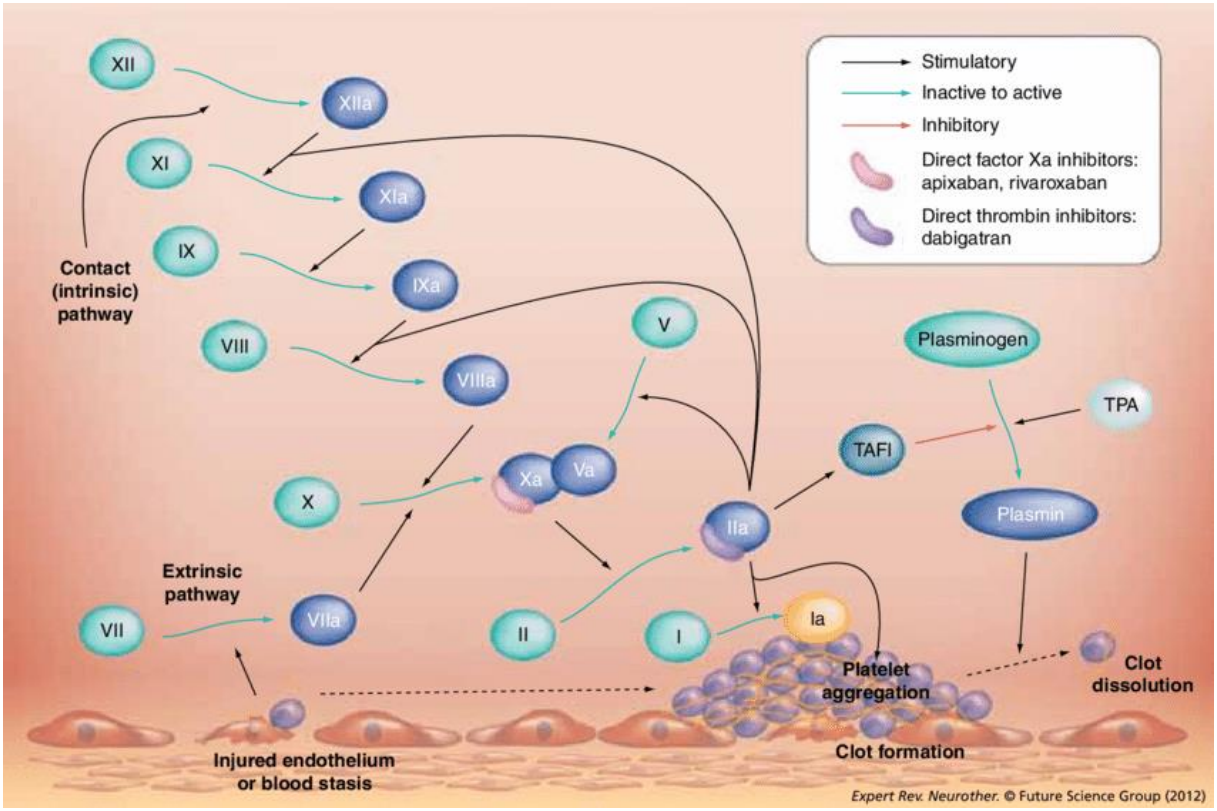

Figure 14 Coagulation cascade and action sites of direct thrombin and factor Xa inhibitors (Morales-Vidal et al., 2012)

Since their market launch, several years have passed, yet reports showed that many clinicians are still unfamiliar with the appropriate dosing of these substances. In 2017, Trujillo-Santos and colleagues found that in over 1500 patients with VTE under DOAC treatment 14 – 46% of all patients had dose derivations from the recommended dose (mainly underdose regimes), the highest concentrations were found in dabigatran, the lowest in rivaroxaban. Although bleeding incidence and death rates for this group was similar to the patient group with the

recommended doses, the patient group with divergent doses had higher rates of VTE recurrence (Trujillo-Santos et al., 2017).

The renal system plays an important role in the metabolisation of DOACs, with the following renal metabolism rates:

Dabigatran: 80 – 85%

Edoxaban: ~35%

Rivaroxaban: ~ 35%

Apixaban: ~ 25%

Therefore, some reservations about the application and dose management in renally impaired patients have arisen. Despite these concerns, a meta-analysis reported that the application of DOACs in mild-to-moderate chronic kidney disease proves significant benefits compared to VKA treatment regimes, without increased bleeding risk but improved survival rates, which is a result similar to patients' groups without renal insufficiency (Ha et al., 2019). Yet the adjustment of DOAC doses in respect to the renal function seems like a viable option, in patients with severe renal impairment, with creatinine clearance rates below 30 ml/min, and require long-term anticoagulation, the application of dose-adjusted LMWH or VKAs are the preferred choices. For patients with creatinine clearance rates below 15 ml/min, the application of direct factor Xa inhibitors, as well as dabigatran, is not recommended by the manufacturer (FDA, 2015a, FDA, 2016a, FDA, 2016b, FDA, 2016c)

For drug adherence maximation accompanied by minimal bleeding incidence numbers, Gladstone and colleagues have published a set of strategies, such as the evaluation of compliance, proper medication storage, conduct in case of missed intake, planning for surgical procedures, prevention of interactions with additional medication, especially in case of platelet inhibitors, renal function monitoring (Gladstone et al., 2015). In a meta-analysis investigating drug discontinuation rates in patients with VTE or atrial fibrillation and an anticoagulation of at least 12 weeks with either DOAC or a VKA, it was reported that DOAC patients missing the medication intake have a longer period during which they are not therapeutically anticoagulated compared to patients with VKAs (Chatterjee et al., 2014).

On the other hand, DOACs seem to have a lower bleeding risk, especially for lethal intracranial bleedings, compared to VKAs (Chai-Adisaksopha et al., 2015), although the impact of meta-analytical studies suffer from differences in patient populations and clinical settings, as well as the combination of different doses of the same anticoagulant; thus a direct comparison of bleeding risk with different agents seems hardly feasible (Mannucci, 2013).

Unlike heparin and VKAs DOACs do not require frequent monitoring, whereas heparin and VKA require a regular check of the clotting times due to their narrow therapeutic window, as well as their variability of the dose-response relationship resulting from a variety of factors, such as bioavailability, diet, or acute illnesses (Eitzman et al., 1994, Hirsh et al., 2008). Therefore, patients burdened by frequent monitoring or experience difficulties in maintaining a therapeutic INR may benefit from DOACs. The latter is especially convenient for patients with the persistent need for antibiotics or numerous concomitant and variable medications, thus experiencing unavoidable drug-drug interactions.

Furthermore, the possibility of heparin-induced thrombocytopenia, which induces an aggressive hypercoagulation, in heparin treatment regimen is absent in DOAC application, leaving it the more advantageous option for percutaneous cardiac interventions. Additionally, the incidence rates for osteoporotic fractures under anticoagulation seem lower for DOACs compared to VKAs (Lau et al., 2017a, Lau et al., 2017b, Sugiyama, 2017).

On the other hand, for some conditions like a prosthetic heart valve or pregnancy heparins and/or VKAs are the preferred option. The reason direct thrombin inhibitors and direct factor Xa inhibitors are not applied in patients with prosthetic heart valves are due to their increased risk for valve thrombosis, as well as the increased bleeding risk in patients with gastrointestinal diseases and direct factor Xa inhibitor therapy. The administration of DOACs during pregnancy is usually avoided, since sufficient information is not available, thus making heparin the preferred option for anticoagulation in this setting.

The anticoagulation of patients with renal insufficiency is usually conducted with heparin or VKAs since the latter's' metabolism and excretion are not as dependent on the creatinine clearance as DOACs. Similar reasonings of physicians apply for severe liver diseases and the resulting reduced hepatic activity and metabolisation of DOACs.

Anticoagulation in patients with the antiphospholipid syndrome is usually done with heparin because presently only a few data about the treatment of antiphospholipid syndrome with DOACs are published, but the setting is currently researched in an ongoing randomised trial comparing rivaroxaban with VKA treatment (ClinicalTrials, 2016).

Moreover, patients with stable INR control and minimal bleeding risk most likely have no gain from a switch to a DOAC and the administration of DOACs requires sufficient compliance, since there is no routine monitoring and the short half-lives of the DOACs require a steady and sometimes more frequent intake to prevent insufficient anticoagulation.

The indications, as well as the contraindications of DOACs, are presented in Table 1.

Table 1 Indications and contraindications of DOACs

Indications	VTE prophylaxis
	VTE therapy
	Atrial fibrillation therapy
	Acute coronary syndrome therapy
	Heparin-induced thrombocytopenia (HIT) therapy
Contraindications	Prosthetic heart valves
	Severe renal insufficiency
	Pregnancy
	Antiphospholipid syndrome

4.1 *Direct thrombin inhibitors*

The parenteral, as well as the oral direct thrombin inhibitors, target and inactivate circulating as well as clot-bound thrombin (factor IIa), which seems to play a very significant role in patients with coronary thrombosis. Unlike heparin, which binds to platelet factor 4, leaving it unable to induce or react with the anti-heparin/platelet factor 4 antibodies causing heparin-induced thrombocytopenia, parenteral direct thrombin inhibitors, such as bivalirudin, argatroban, and desirudin, can target these antibodies and thus preventing heparin-induced thrombocytopenia, making them feasible anticoagulants in patients with this condition. Lepirudin, another parenteral direct thrombin inhibitor, was discontinued in 2012 by the manufacturer, unrelated to safety concerns (Schiele et al., 1995, Greinacher et al., 1999).

4.1.1 *Parenteral direct thrombin inhibitors*

Bivalirudin

Bivalirudin (Angiomax®), depicted in Figure 15, consists of a synthetic 20 amino acid peptide and has a molecular weight of 2180.29 g/mol (Chemical Book, 2000). It is an analogue of hirudin and binds to the thrombin catalytic site and exosite I, which leads to reversible inhibition of thrombin's enzymatic activity (Angiomax, 2013). The major metabolisation sites for bivalirudin are the kidney and the liver; thus, it can be treated with hemodialysis (Di Nisio et al., 2005). The most important indications for its application are heparin-induced thrombocytopenia (HIT) and percutaneous coronary interventions in high-risk patients for the development of HIT. For patients with HIT, the drug is administered as a bolus followed by continuous intravenous infusions. For renal impaired patients with creatine clearance rates

below 30 ml/minute, only the infusion rate has to be adjusted, whereas the bolus dose remains the same (Angiomax, 2013). The intravenous administration of bivalirudin procures an immediate, but short-termed (half-life of about 25 minutes) anticoagulant effect, which results in normal coagulation times within one hour after discontinuation (Warkentin et al., 2008).

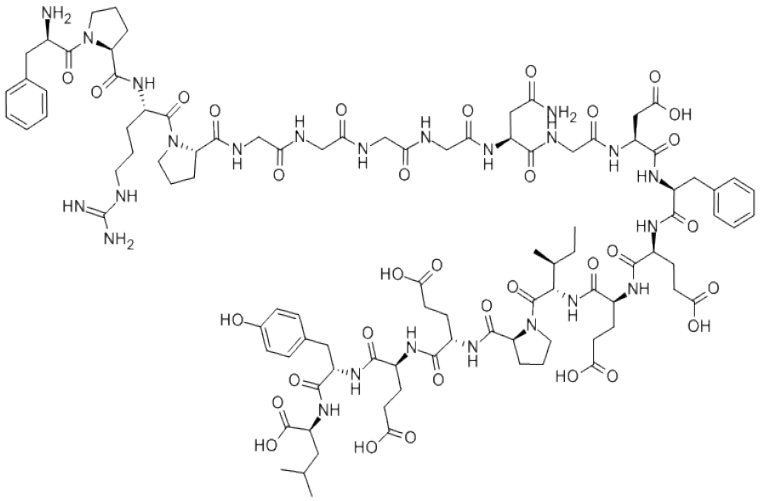

Figure 15 Structure of bivalirudin (Chemical Book, 2000)

Two coagulation times are used for the monitoring of bivalirudin activity: the activated clotting time as well as the aPTT, whose target range is about two times the normal range, but depends on the measurement device.

Argatroban

Argatroban (Arganova®, Novastan®), shown in Figure 16, is a synthetic peptide, has a molecular weight of 508.63 g/mol and interacts with the active site of thrombin (Clarke et al., 1991a, Chemical Book, 2005). Compared to bivalirudin, argatroban's plasma half-life is about twice as long, with about 40 to 50 minutes, and its metabolisation is controlled by the hepatic system (Di Nisio et al., 2005). Therefore, dose adjustments are recommended in, while renal impairment is not an issue for argatroban application (Swan and Hursting, 2000). While the indications for argatroban are similar to bivalirudin, its administration differs, because argatroban is only applied as a continuous infusion without an initial bolus in HIT, whereas for PCI in high-risk patients for HIT the administration combines an initial bolus with continuous infusions, like with bivalirudin. The monitoring of argatroban activity is done by aPTT; the target range is about 2 to 3 times the normal range, but should not exceed 100 seconds (GSKsource, 2014).

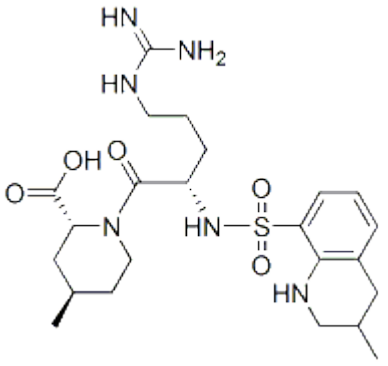

Figure 16 Structure of argatroban (Chemical Book, 2005)

Since argatroban prolongs the PT and subsequently the INR, patients transitioning to VKAs require the usage of an adjusted INR during the overlap and after the argatroban's discontinuation.

Desirudin

Desirudin (Iprivask®, Revasc®) (structure not shown due to its large dimension), has a molecular weight of 6963.4245 g/mol and is a recombinant derivative of hirudin, which targets and inhibits free and clot-bound thrombin (Chemical Book, 1998, Graetz et al., 2011). Compared to the parenteral direct thrombin inhibitors mentioned above, desirudin's half-life is longer, with approximately two hours, and depends on the renal function. Desirudin is commonly used as prophylaxis for hip arthroplasty administered as a subcutaneous injection every 12 hours (Eriksson et al., 1997, Jove et al., 2014).
For other properties of desirudin see the already described direct thrombin inhibitor substances.

4.1.2 *Oral direct thrombin inhibitor(s)*

The sole licensed oral direct thrombin inhibitor for clinical use is dabigatran exilate (Pradaxa®), although other agents are currently under development (ClinicalTrials, 2019). Its application form is a prodrug, that is converted in the liver to dabigatran, presented in Figure 17, which is a direct thrombin inhibitor inhibiting clot-bound as well as circulating thrombin, has a molecular weight of 471.5 g/mol and a half-life of about 12 to 15 hours, depending on the renal and hepatic function (Hauel et al., 2002, Chemical Book, 2008).

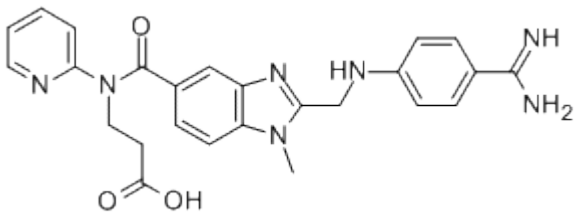

Figure 17 Structure of dabigatran (Chemical Book, 2008)

The indications, as well as the contraindications of dabigatran, are presented in the respective list for DOACs in Table 1.
The dosing of dabigatran usually does not require regular monitoring but is prescribed at a fixed, appropriate dose to prevent underdosing. The peak value of the anticoagulant effect is reached 2 to 3 hours after ingestion (van Ryn et al., 2010), with the predominant elimination pathway via renal excretion of the mainly unchanged (about 80%) active substance (Blech et al., 2008, Stangier et al., 2010). Therefore, dose adjustments considering the patient's renal function, the indication as well as drug interactions are recommended.

Indications for oral direct thrombin inhibitors include VTE prophylaxis in surgical patients, VTE treatment as well as secondary prevention and stroke prevention in AF.

Interactions

Since the main metabolisation of dabigatran takes place in the kidney, a renal impairment results in an extended half-life of the substance of up to 34 hours in patients with severe chronic kidney disease (Stangier et al., 2010). Therefore, in patients with a low creatinine clearance below 30 ml/minute dose reductions are recommended to prevent drug accumulation and bleeding events (Gulseth et al., 2011). For patients with creatinine clearance rates below 30 ml/minute either the dose should be reduced to 75 mg (United States) twice-daily or avoided completely (Canadian and European Medicines Agency) (CMA, 2019, EMA, 2019, EMC, 2019).

Possible drug interactions of dabigatran, which is a substrate for P-glycoprotein, and P-glycoprotein inhibitors or inducers should also be considered in dose adjustments of dabigatran. While P-glycoprotein inducers, such as rifampin, lead to a reduced anticoagulant effect of dabigatran, thus a combination of these two substances should be avoided. On the other hand, a combination of dabigatran with P-glycoprotein inhibitors, such as ketoconazole, verapamil, results in an increased anticoagulant effect of dabigatran, which may result in a significantly increased bleeding risk, especially in patients with renal failure. For dabigatran, no interactions with the cytochrome p450 system are known; thus, no dose changes are required with concomitant administration of CYP inducers or inhibitors (Boehringer-Ingelheim, 2014).

Monitoring

Before administration of dabigatran, the patient's PT and aPTT should be measured to allow an assessment of the coagulation status before anticoagulation as well as the serum creatinine concentration to establish a baseline of the renal function. Aside from these initial tests, no regular coagulation monitoring is required for patients taking dabigatran, although the regular monitoring of dabigatran plasma concentrations might result in improved efficacy and safety. Especially cases of suspected dabigatran overdoses (and bleeding) or dabigatran patients requiring emergency surgery as well as absorption and drug adherence concerns seem to profit from the determination of the drug's plasma concentration (Moore et al., 2014, Rao, 2014, Reilly et al., 2014b, Reilly et al., 2014a, Powell, 2015, Chan et al., 2018). The expected peak concentrations, published by the International Council for Standardization in

Haematology, for dabigatran 150 mg twice-daily dose are approximately 157 ng/ml, with 25th to 75th percentile of 117 to 275 ng/ml and trough concentrations of approximately 60 to 91 ng/ml, with 25th to 75th percentile 39 to 143 ng/ml (Gosselin et al., 2018).
The bleeding risk can be estimated with the ecarin clotting time, whereas the dabigatran activity is commonly measured with other coagulation tests, such as the dilute TT, aPTT and activated clotting time, which show prolonged coagulation times. PT is not a reliable measurement tool for dabigatran activity. In studies, TT was found to have the highest sensitivity, although some clinicians seem to prefer the aPTT for dabigatran assessment (Dager et al., 2012, van Ryn et al., 2012).

Bleeding and antagonisation

For the antagonisation of dabigatran and to manage possible bleeding events, the antidote idarucizumab (Praxbind®) has been developed. Idarucizumab is a monoclonal antibody fragment, which binds dabigatran and reverses its anticoagulant effect within minutes after intravenous injection. The injection dose is commonly 5 grams, and the reversion of the anticoagulant effect was achieved within 15 minutes and lasted for approximately 24 hours. (FDA, 2015b). The main indications are patients with dabigatran therapy, who require urgent surgery or have major bleeding, while only patients with relevant dabigatran concentrations and a prolonged TT should receive idarucizumab. The first positive results for idarucizumab treatment were found in the RE-VERSE AD study, which subsequently led to the drug's approval (Pollack et al., 2015, Pollack et al., 2017). Idarucizumab antagonises dabigatran activity at nanomolar concentrations and no mimicry of thrombin function, such as binding to thrombin substrates, altering of clotting parameters, or independent platelet aggregation, was found in animal models, thus no anticoagulant or prothrombotic effect for idarucizumab is to be expected (Schiele et al., 1995).

Further examples for anticoagulation antidotes

- Clotting factor products

  These agents can be administered either in their activated or unactivated state.
- Unactivated prothrombin complex concentrates (PCCs)

  PCCs contain purified human clotting factors: either factors II, IX, and X (3-factor PCCs) or factors II, IX, X, and VII (4-factor PCCs), both along protein C and S in their inactive/zymogen form. The most experience of PCCs is with VKA antagonisation, data regarding DOAC antagonisation efficacy is mainly limited to

observational studies, studies in healthy individuals, as well as animal models (Eerenberg et al., 2011, Dumkow et al., 2012, Godier et al., 2012, Lambourne et al., 2012, Marlu et al., 2012, Pragst et al., 2012, Dager et al., 2013, Perzborn et al., 2013, Dickneite and Hoffman, 2014, Htun et al., 2014, Ross et al., 2014, Wong and Keeling, 2014, Zahir et al., 2015, Majeed et al., 2017), but recently a systemic review on PCC administration in direct factor Xa inhibitor-associated major bleeding was published. Since no significant difference between bleeding management with PCC administration and only anticoagulant discontinuation was found, the authors recommended PCC administration only in case of life-threatening bleeding. Dose recommendations vary from 25 to 50 units of factor IX activity per kilogram. Unless the PCC therapy is followed by a hemostatic response, a readministration is not recommended; otherwise, the application should only be repeated in patients with an impaired renal function after 12 to 24 hours (Piran et al., 2019).

- Activated prothrombin complex concentrates (aPCCs)

  aPCCs contain purified human clotting factors: either factors II, IX, and X (3-factor PCCs) or factors II, IX, X, and VII (4-factor PCCs) with at least one factor in its activated form, most commonly factor VII. Similar to PCCs, the data for aPCCs in DOAC associated major bleeding is scarce, although studies indicated correction of rivaroxaban-induced thrombin generation impairment with no increased risk of DOAC-associated intracerebral haemorrhage or thrombotic/hemorrhagic complications at the recommended dose (Marlu et al., 2012, Dibu et al., 2016, Honickel et al., 2016). The recommended initial dose is 50 units of factor IX activity per kilogram. Unless the aPCC therapy is followed by a hemostatic response, a readministration is not recommended; otherwise, the application should be only repeated in patients with an impaired renal function after 12 hours, but the daily dose should not exceed 200 units/kg(Gruber et al., 2009).

  For PCCs and aPCCs alike, the maximum dose in VKA antagonisation approved by the FDA is 5000 units, due to PCCs and aPCCs potentially prothrombotic nature. This dose cap is recommended, because the substances' effect can be determined by measuring the PT and INR, allowing additional case-sensitive dosing, when the effect of VKAs has been incompletely reversed (FDA, 2018). On the other hand, the application of unactivated PCCs for the treatment of factor Xa inhibitor-associated bleeding is off-label, and there is a measurement to determine the PCCs' effect on hemostasis and thus the need for additional administration.

- Recombinant activated factor VII

  The administration of recombinant activated factor VII is not recommended in DOAC-associated bleeding treatment.
- Pro-hemostatic therapies
- This antidote group consists of antifibrinolytic agents, such as tranexamic acid and epsilon-aminocaproic acid, and desmopressin. Both substances are usually administered intravenously as a bolus and followed by a continuous infusion. Whereas the antifibrinolytic agents interfere with plasmin formation (from its precursor plasminogen) by plasminogen activators, thus inhibiting fibrinolysis, desmopressin targets platelets with impaired activity and restores their functionality. Studies found that pro-hemostatic therapeutics, specifically tranexamic acid, are not associated with an increased thrombosis risk, making these agents a possible antidote for DOAC-associated bleeding (Chornenki et al., 2019).
- Transfusions

  Available transfusion products include red blood cells, platelets or plasma, such as fresh frozen plasma.

Risks and side effects

Concerning the risks of dabigatran administration, it should be noted that it is accompanied by the risk of spinal or epidural hematoma in patients undergoing neuraxial anaesthesia or spinal puncture (Boehringer-Ingelheim, 2014).

Numerous studies found that the bleeding risk for dabigatran is comparable to warfarin, although it seems like dabigatran is associated with slightly less intracranial haemorrhage and death, but a somewhat higher risk of gastrointestinal bleeding in high-dose regimes (Desai et al., 2013, Mannucci, 2013, Southworth et al., 2013).

Another common side effect of dabigatran is the occurrence of non-bleeding gastrointestinal events, such as dyspepsia, dysmotility, gastrointestinal reflux, which was found to be twice as common for dabigatran compared to warfarin (Bytzer et al., 2013). On the other hand, dabigatran administration is not associated with an increased risk of severe liver injury, unlike its predecessor ximelagatran. It might be possible that the application of DOACs might even reduce the risk of severe liver injury, although this finding did not reach statistical significance in a study published by Douros and colleagues (Douros et al., 2018).

4.2 *Direct factor Xa inhibitors*

Direct factor Xa inhibitors target and inactivate circulating and clot-bound factor Xa. As mentioned above, there are no parenteral direct factor Xa inhibitors approved for clinical use - otamixaban was developed as an intravenous factor Xa inhibitor, but its development was discontinued, because of its increased risk of bleeding while there are numerous oral direct factor Xa inhibitors., but its development was discontinued because of an increased bleeding risk in patients with acute coronary syndromes (Guertin and Choi, 2007, Steg et al., 2013) – while there are numerous approved oral direct factor Xa inhibitors, such as apixaban (Eliquis®), edoxaban (Lixiana®) and rivaroxaban (Xarelto®).

Interactions

As mentioned before, the metabolisation of direct factor Xa inhibitors takes mainly place in the kidney and the liver, the allocation depends on the particular drug. Hence, a severe renal and/or hepatic impairment results in an accumulation of the substances, although it seems, that direct factor Xa inhibitors, similar to dabigatran, are not associated with hepatotoxicity, but rather have a liver protective effect, although not statistically significant (Douros et al., 2018). For patients with a high body mass index, direct oral factor Xa inhibitors are commonly not recommended (Martin et al., 2016), although a recent study concluded that they could be administered to these patients without dose adjustments (Moore and Kröll, 2017). Furthermore, dose adjustments considering the patient's ethnicity were recently found to be inappropriate, since these dose adjustments would result in inferior efficacy (Cho et al., 2018).

Monitoring

Like dabigatran, the oral direct factor Xa inhibitors are administered at a fixed dose without monitoring. Therefore, the correct dosing plays a critical role to prevent deviations from the recommended plasma concentrations. The anti-Xa activity, as well as the respective substance's plasma concentration, can be measured in patients with potential interactions, either by other drugs or conditions which influence the drug absorption rate, for instance, altered gastrointestinal anatomy. Therefore, these measurements are applied for the detection of proper drug absorption and drug concentrations are not deviating. Since no established therapeutic ranges exist for these drugs, the recommended concentrations are mostly derived from data from clinical trials, and these measurements should not be used for the

determination of a target range (Garcia et al., 2013, Gosselin et al., 2018). When the anti-Xa activity is determined it should be considered that the assay is specifically calibrated for the respective agent since heparin-calibrated assays are not clinically validated for these measurements and might reflect significantly different values (Beyer et al., 2016).

Bleeding and antagonisation

For the management of major bleeding in patients with direct oral factor Xa inhibitor treatment, andexanet alfa was developed and approved in 2018 for life-threatening or uncontrolled bleeding associated with rivaroxaban and apixaban (FDA, 2018).
Andexanet alfa is a recombinant, catalytically inactive form of factor Xa, which competitively binds to and inhibits these anticoagulants, which was proven in the ANNEXA-4 study for patients with factor Xa inhibitor therapy due to atrial fibrillation or VTE. Studies showed a significant reduction of the anti-Xa activity for apixaban, rivaroxaban, and enoxaparin, whereas thromboses and other adverse effects remained below the 10% threshold. The number of thrombotic events associated with the antagonisation rather than the patient's individual risk might be even lower since only 3% of the patients had a thrombosis within the first 5 days following antagonisation (Connolly et al., 2016, Connolly et al., 2019). Control studies conducted in healthy individuals showed no thromboses or other significant adverse effects for andexanet alfa (Siegal et al., 2015, Siegal et al., 2017). Furthermore, it seems like andexanet alfa might reverse the anticoagulant effects of all direct factor Xa inhibitors, including LMWHs and fondaparinux, but this application is still researched (Lu et al., 2013).
Andexanet alfa can be either administered in low dose, with an initial bolus of 400 mg given at 30 mg/minute, and subsequently followed by a continuous infusion of 4 mg/minute for up to 120 minutes, or as high dose, with an initial bolus of 800 mg given at 30 mg/minute, and subsequently followed by a continuous infusion of 8 mg/minute for up to 120 minutes. The low dose is administered for low concentrations of factor Xa inhibitor intake, for rivaroxaban below 10 mg or apixaban below 5 mg or if the period since the last intake is longer than 8 hours.

Further examples for anticoagulation antidotes

Since the list of antidotes for antagonisation of direct factor Xa inhibitors and direct thrombin inhibitors comprises the same substances, the list will not be presented again. It is found in chapter 4.1.2, subchapter `Further examples for anticoagulation antidotes´.

4.2.1 *Rivaroxaban (Xarelto®)*

Rivaroxaban, shown in Figure 18, has a molecular weight of 435.88 g/mol, a plasma half-life of 5 to 9 hours and is usually administered as 15 and 20 mg tablets, which should be taken with food (Chemical Book, 2011, FDA, 2013, Beyer-Westendorf and Siegert, 2015). The dose is adjusted according to the clinical indication (a complete list of DOAC indications and contraindications is presented in Table 1) and the patient's renal and hepatic function.

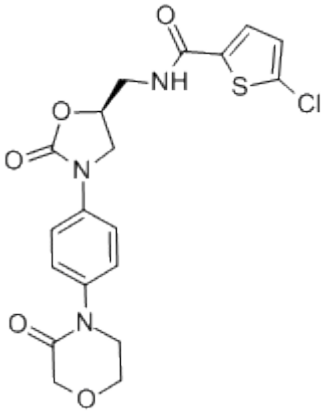

Figure 18 Structure of rivaroxaban (Chemical Book, 2011)

- VTE prophylaxis in surgical patients
  10 mg daily for a duration of 12 to 35 days, depending on the type of surgery
- Treatment of VTE/secondary prevention of VTE
  15 mg twice daily for 3 weeks, followed by 20 mg once-daily, after six months another reduction to a daily dose of 10 mg can be considered, depending on the patient's risk profile for VTE development (FDA, 2017a)
- Stroke prophylaxis in atrial fibrillation
  15 to 20 mg in a once-daily dose in the evening, depending on the patient's creatinine clearance rate (below or above 50 ml/minute)

Since the metabolisation and elimination of rivaroxaban largely depend on the patient's renal and hepatic functions, it is not recommended for VTE prophylaxis, treatment, or secondary prevention in patients with a creatinine clearance below 30 ml/minute or severe hepatic impairment (Poulsen et al., 2012).

Interactions

Since rivaroxaban shows a potent inhibitory interaction with of CYP-3A4 and P-glycoprotein, such as ketoconazole, itraconazole, voriconazole, posaconazole, its concurrent use is contraindicated (CMA, 2019). It seems like CYP-3A4 or P-glycoprotein inhibitors have no effect on rivaroxaban, whereas CYP-3A4 inducers, such as rifamycins, and carbamazepine, reduce rivaroxaban activity (Eikelboom and Weitz, 2011, Nutescu et al., 2011, Altena et al., 2014).

The parameters measured before the start of rivaroxaban administration should include PT, and aPTT to assess the coagulation status before anticoagulation as well as creatinine clearance rate and liver function tests to judge if dose adjustment is necessary.

Monitoring

Before administration of rivaroxaban, the patient's PT and aPTT should be measured to allow an assessment of the coagulation status before anticoagulation as well as the serum creatinine concentration to establish a baseline of the renal function as well as liver function tests for a baseline of the hepatic function.

Similar to other DOACs, no routine monitoring of coagulation times is done for rivaroxaban therapy, although it seems that a future establishment of therapeutic ranges, as well as regular measurements, might improve efficacy and safety (Powell, 2015, Chan et al., 2018). Instances for recommended measurement of anti-Xa activity in rivaroxaban patients include major bleeding events, suspected deviating plasma concentrations, emergency surgery, as well as suspected drug absorption or adherence issues. The expected peak concentrations for rivaroxaban, published by the International Council for Standardization in Haematology, are approximately 250 – 270 ng/ml for a 20 mg once-daily dose, and a trough concentration of approximately 26 – 44 ng/ml (Gosselin et al., 2018). As mentioned above, these concentrations are not intended for therapeutic target screening, but to prove sufficient drug absorption, and the corresponding anti-Xa activity should be measured with an assay specifically calibrated for rivaroxaban. It was found that in cases this specifically calibrated anti-Xa assay is not available, another anti-Xa assay calibrated to another anticoagulant, such as LMWH, are applicable, PT and aPTT however are not reliable (Siegal and Konkle, 2014).

Risks and side effects

Since hepatotoxic effects and liver injury associated with rivaroxaban administration were only reported for individual cases but were not seen in larger trials, the incidence rate of these complications is still unknown (Caldeira et al., 2014, Liakoni et al., 2014).

Due to its anticoagulant nature, rivaroxaban, like all other anticoagulants, increases the bleeding risk, the product labelling warns explicitly about the possible occurrence of spinal/epidural hematoma caused by neuraxial anaesthesia or spinal puncture (Bayer AG, 2011).

4.2.2 *Apixaban (Eliquis®)*

Apixaban, depicted in Figure 19, has a molecular weight of 459.504 g/mol, a plasma half-life of approximately 12 hours and is usually administered as 2.5 and 5 mg tablets. The dose is adjusted according to the clinical indication (a complete list of DOAC indications and contraindications is provided in Table 1) and the patient's renal and hepatic function (Chemical Book, 2012, Agnelli et al., 2013).

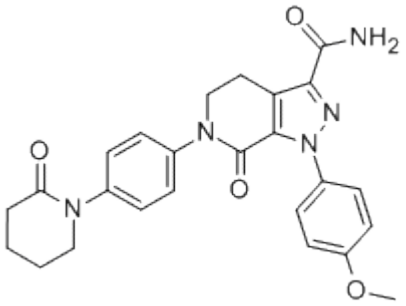

Figure 19 Structure of apixaban (Chemical Book, 2012)

- VTE prophylaxis in surgical patients
  2.5 mg twice daily for a duration of 12 to 35 days, depending on the type of surgery
- Treatment of VTE/secondary prevention of VTE
  10 mg twice daily for seven days, followed by 5 mg twice daily, after six months another reduction to a daily dose of 5 mg can be considered, depending on the patient's risk profile for VTE development
- Stroke prophylaxis in atrial fibrillation
  2.5 to 5 mg twice-daily dose in the evening, depending on the patient's creatinine clearance rate (below or above 50 ml/minute), high age, low body weight and serum creatinine above 1.5 mg/dL

Interactions

Dose adjustments for apixaban are recommended for patients with concomitant intake of CYP-3A4 and P-glycoprotein inhibitors (Pfizer, 2014). Among the direct factor, Xa inhibitors apixaban's metabolisation is the least dependent on renal clearance, although apixaban administration is not recommended in patients with a creatinine clearance rate below 15 ml/minute. Dose adjustments should be made based on creatinine clearance, body weight, and age. Apixaban administration is not recommended in patients with prosthetic heart valves or during pregnancy (FDA, 2016b, Pfizer, 2016).

Monitoring

Before administration of apixaban, the patient's PT and aPTT should be measured to allow an assessment of the coagulation status before anticoagulation as well as the serum creatinine

concentration to establish a baseline of the renal function as well as liver function tests for a baseline of the hepatic function.

Similar to other DOACs, no routine monitoring of coagulation times is done for apixaban therapy, although it seems that a future establishment of therapeutic ranges, as well as regular measurements, might improve efficacy and safety (Powell, 2015, Chan et al., 2018). Instances for recommended measurement of anti-Xa activity in apixaban patients include major bleeding events, suspected deviating plasma concentrations, emergency surgery as well as suspected drug absorption or adherence issues (Wong et al., 2008). The expected peak concentrations for apixaban, published by the International Council for Standardization in Haematology, are approximately 132 to 171 ng/ml for a 5 mg twice-daily dose, and a trough concentration of approximately 63 to 103 ng/ml (Gosselin et al., 2018). As mentioned above, these concentrations are not intended for therapeutic target screening, but to prove sufficient drug absorption, and the corresponding anti-Xa activity should be measured with an assay specifically calibrated for apixaban. It was found that in cases this specifically calibrated anti-Xa assay is not available, another anti-Xa assay calibrated to another anticoagulant, such as LMWH, are applicable, PT and aPTT however are not reliable.

Risks and side effects

Due to its anticoagulant nature, apixaban, like all other anticoagulants, increases the bleeding risk, the product labelling warns explicitly about the possible occurrence of spinal/epidural hematoma caused by neuraxial anaesthesia or spinal puncture (Pfizer, 2014).

4.2.3 *Edoxaban (Lixiana®)*

Edoxaban, presented in Figure 20, has a molecular weight of 548.065 g/mol, a plasma half-life of 10 to 14 hours and is usually administered as 30 and 60 mg tablets (Chemical Book, 2015). The dose is adjusted according to the clinical indication (a complete list of DOAC indications and contraindications is presented in Table 1) and the patient's renal and hepatic function.

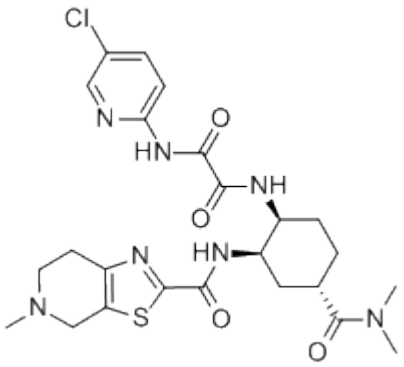

Figure 20 Structure of edoxaban (Chemical Book, 2015)

Recommended administration for edoxaban in VTE prophylaxis and stroke prophylaxis in atrial fibrillation is 30 or 60 mg once-daily following parenteral anticoagulation for a duration of 5 to 10 days (Camm and Bounameaux, 2011,

Daiichi-Sankyo, 2012, Dentali et al., 2012, Büller et al., 2013).

Interactions

Edoxaban is a substrate for P-glycoprotein, hence dose adjustments for edoxaban are recommended for patients with concomitant intake of P-glycoprotein inhibitors (FDA, 2016c). Dose adjustments should be made based on creatinine clearance, body weight, and age. Edoxaban administration is not recommended in patients with creatinine clearance rates above 95 or below 15 ml/minute (FDA, 2016c).

Monitoring

Before administration of edoxaban, the patient's PT and aPTT should be measured to allow an assessment of the coagulation status before anticoagulation as well as the serum creatinine concentration to establish a baseline of the renal function as well as liver function tests for a baseline of the hepatic function.

Similar to other DOACs, no routine monitoring of coagulation times is done for apixaban therapy, although it seems that a future establishment of therapeutic ranges, as well as regular measurements, might improve efficacy and safety (Powell, 2015). Instances for recommended measurement of anti-Xa activity in edoxaban patients include major bleeding events, suspected deviating plasma concentrations, emergency surgery as well as suspected drug absorption or adherence issues (Wong et al., 2008). The expected peak concentrations for edoxaban, published by the International Council for Standardization in Haematology, are approximately 170 to 234 ng/ml for a 60 mg once-daily dose, and a trough concentration of approximately 19 to 36 ng/ml (Gosselin et al., 2018). As mentioned before, these concentrations are not intended for therapeutic target screening, but to prove sufficient drug absorption, and the corresponding anti-Xa activity should be measured with an assay specifically calibrated for edoxaban. It was found that in cases this specifically calibrated anti-Xa assay is not available, another anti-Xa assay calibrated to another anticoagulant, such as LMWH, are applicable, PT and aPTT however are not reliable (Siegal and Konkle, 2014).

Risks and side effects

Due to its anticoagulant nature, edoxaban, like all other anticoagulants, increases the bleeding risk, the product labelling warns explicitly about the possible occurrence of spinal/epidural hematoma caused by neuraxial anaesthesia or spinal puncture. Furthermore, it warns about a

reduced efficacy in nonvalvular atrial fibrillation in patients with a high clearance rate (FDA, 2015c).

4.2.4 *Betrixaban (Bevyxxa®)*

Betrixaban, shown in Figure 21, is currently only approved in the United States, not in Europe. Betrixaban has a molecular weight of 451.905 g/mol, a plasma half-life of 19 to 27 hours and is usually administered as 40, 80 and 160 mg tablets, which should be taken with food (Cohen et al., 2016, FDA, 2017b, Gibson et al., 2017, Chemical Book, 2017b). The dose is adjusted according to the clinical indication (a complete list of DOAC indications and contraindications is presented in Table 1) and the patient's renal and hepatic function. In the case of VTE prophylaxis, a dose of 160 mg for the first day, followed by 80 mg once daily for a duration of 35 to 42 days is recommended (Cohen et al., 2016).

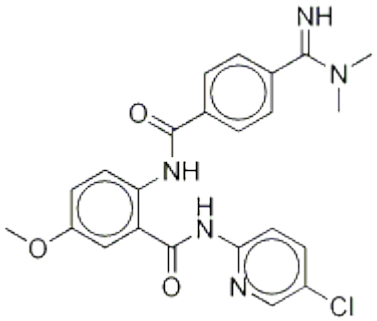

Figure 21 Strucure of betrixaban (Chemical Book, 2017b)

Interactions

Betrixaban is a substrate for P-glycoprotein, hence dose adjustments for betrixaban are recommended for patients with concomitant intake of P-glycoprotein inhibitors, for instance, patients with a creatinine clearance below 15 ml/minute and concomitant P-glycoprotein inhibitor taking, a dose of 80 mg on the first day followed by 40 mg daily is recommended. Dose adjustments should be made based on creatinine clearance, body weight, and age. Betrixaban administration is not recommended in patients with prosthetic heart valves or during pregnancy (FDA, 2017b).

Monitoring

Since monitoring of betrixaban has the same essential recommendations and further advice is not published yet, see the respective factor Xa inhibitor's monitoring subchapters.

Risks and side effects

Due to its anticoagulant nature, betrixaban, like all other anticoagulants, increases the bleeding risk, the product labelling warns explicitly about the possible occurrence of spinal/epidural hematoma caused by neuraxial anaesthesia or spinal puncture (FDA, 2017b).

4.3 *Transitioning between anticoagulants*

During the transitioning period between anticoagulants, stable anticoagulation should be maintained. Therefore, when transitioning from DOAC to VKA, it is essential to maintain the anticoagulant effect from DOACs, because the onset of VKA takes a few days, even when the prolongation of PT/INR is detectable earlier (Cairns and Weitz, 2015, Granger et al., 2015). Similar, in a transition from VKA to DOAC, the VKA effect requires a few days to wear off. The transition recommendations by the American Society of Hematology are presented in Table 2 for DOAC to VKA, and Table 3 for VKA to DOAC. For a transition between two DOACs, it is recommended to start the first intake of the new DOAC when the next dose of the first DOAC would have been due, without overlapping (Witt et al., 2018).

Table 2 DOAC to VKA transition recommendations by the American Society of Hematology (Witt et al., 2018)

Switching from DOAC to VKA	
Dabigatran	Overlap VKA and dabigatran for 3 or 1 to 2 days for reduced renal activity. Overlap VKA and dabigatran until the INR is therapeutic on VKA.
Apixaban	Discontinuation of apixaban, start of parenteral VKA until the INR is therapeutic on VKA. Overlap VKA and apixaban until the INR is therapeutic on VKA, measurement right before the next apixaban.
Edoxaban	Halving of edoxaban dose and start of VKA until the INR is therapeutic on VKA, discontinuation of edoxaban. Discontinuation of edoxaban, start of parenteral VKA until the INR is therapeutic on VKA. Overlap VKA and edoxaban until the INR is therapeutic on VKA, measurement right before the next edoxaban.
Rivaroxaban	Discontinuation of rivaroxaban, start of parenteral VKA until the INR is therapeutic on VKA. Overlap VKA and rivaroxaban until the INR is therapeutic on VKA, measurement right before the next rivaroxaban.

Table 3 VKA to DOAC transition recommendations by the American Society of Hematology (Witt et al., 2018)

Switching from VKA to DOAC	
Dabigatran	Stop of VKA and subsequent monitoring of the PT/INR, start of dabigatran when INR is below 2.
Apixaban	Stop of VKA and subsequent monitoring of the PT/INR, start of apixaban when INR is below 2.
Edoxaban	Stop of VKA and subsequent monitoring of the PT/INR, start of edoxaban when INR is below 2.5.
Rivaroxaban	Stop of VKA and subsequent monitoring of the PT/INR, start of rivaroxaban when INR is below 3.

5 Methods

All coagulation tests used for data generation in this study were conducted on the ACL Top 750®, utilising a chromogenic measurement of coagulation times and drug plasma concentrations. The quantification of coagulation times and drug concentrations is based on the optical attenuation based on the Beer-Lambert law, which is shown in [1] and explained in Table 4 (Mills et al., 1993).

$$A_\lambda = \log\frac{I_0}{I} = \log\frac{1}{\tau} = \varepsilon_\lambda * c * d \qquad [1]$$

Table 4 Beer-Lambert law

Symbol	Unit	
A_λ	[1]	Optical attenuation
I_0	[W/m^2]	Incident intensity
I	[W/m^2]	Transmitted intensity
τ	[1]	Transmission factor
ε_λ	[m^2/mol]	Molar attenuation coefficient/ absorptivity of the attenuating species
c	[mol/L]	Concentration of the attenuating species
d	[m]	Optical path length

5.1 *HemosIL® Liquid Anti-Xa assays*

The HemosIL® Liquid Anti-Xa kit is a one stage chromogenic assay based on a synthetic chromogenic substrate and factor Xa inactivation. This assay requires calibration with the respective HemosIL calibrator for the specific measurement of apixaban, edoxaban, and rivaroxaban on the ACL TOP® series. This assay measures the factor Xa neutralisation by apixaban, edoxaban or rivaroxaban. The residual Factor Xa hydrolyses a specific chromogenic substrate releasing paranitroaniline (pNA). The amount ofpNA released (measured by absorbance at 405nm) is inversely proportional to the concentration of heparin (or other anti-Xa products) present in plasma.

Measurement range and detection limit of the HemosIL® Liquid Anti-Xa assays

As claimed by the manufacturer and according to the package inserts the HemosiL® Liquid Anti-Xa apixaban assay has a measurement range of 15 – 1000 ng/ml, with a supposed limit of detection of 6 ng/ml, the HemosiL® Liquid Anti-Xa edoxaban assay has a measurement range of 20 – 400 ng/ml, with a supposed limit of detection of 20 ng/ml, while the HemosiL®

Liquid Anti-Xa rivaroxaban assay has a measurement range of 20 – 1000 ng/ml, with a supposed limit of detection of 10 ng/ml (Werfen GmbH, 2017).

5.2 *Biophen® Heparin LRT assays*

Biophen® Heparin LRT is a chromogenic kinetics assay, which is based on the inhibition of a constant and an excess amount of Factor Xa. The residual Factor Xa hydrolyses a specific chromogenic substrate releasing pNA. The amount of pNA released (measured by absorbance at 405nm) is inversely proportional to the concentration of heparin (or other anti-Xa products) present in plasma. For this assay, plasma is diluted with physiological saline (reference buffer). To evaluate heparin inhibition in one stage chromogenic assay, specific direct factor Xa inhibitor calibrators and controls are used on the ACLTOP® family for measuring Anti-Factor Xa activity of DiXaIs, such as apixaban, edoxaban, and rivaroxaban.

Measurement Range and detection limit of the Biophen® Heparin LRT assays

As claimed by the manufacturer the Biophen® Heparin LRT assays have a limit of detection of 10.0 ng/ml for rivaroxaban, 6.0 ng/ml for apixaban and 0.04 IU/ml for heparin, with a measurement range of 20.0 – 1000.0 ng/ml for rivaroxaban, and 15.0 – 1000.0 ng/ml for apixaban (CoaChrom Diagnostica GmbH, 2015).

5.3 *Analytical evaluation of the Biophen® Heparin LRT apixaban, edoxaban, and rivaroxaban assays*

Calibration curves of the Biophen® Heparin LRT assays

This subchapter will discuss the respective calibration curves of the Biophen® Heparin LRT assays. For the calibration of the 3 assays, only the measurement of a single hybrid calibrator set with 5 calibrators is required, since the apixaban and edoxaban assays are calibrated based on the data of the rivaroxaban calibration curve. For the Biophen® Heparin LRT assays, the interpolation applies a linear regression without an axis transformation within the range of 0 – 100 ng/ml and a polynomial of second order with a logarithmic transformation above 100 ng/ml based on the absorption rates. This necessity is based on the relatively low dilution range's linear relation between concentrations and colour development (and therefore the absorption), which does not apply for higher concentration ranges. Therefore, the plasma

samples are diluted either 1:4 for edoxaban or 1:5 for apixaban and rivaroxaban assays for the interpolation.

The Biophen® Heparin LRT calibration curve for apixaban is presented in Figure 22 and exerts a linear and a polynomial of second-order behaviour between the absorption rate and the concentration depending on the concentration range, similar to the HemosIL® Liquid Anti-Xa apixaban assay.

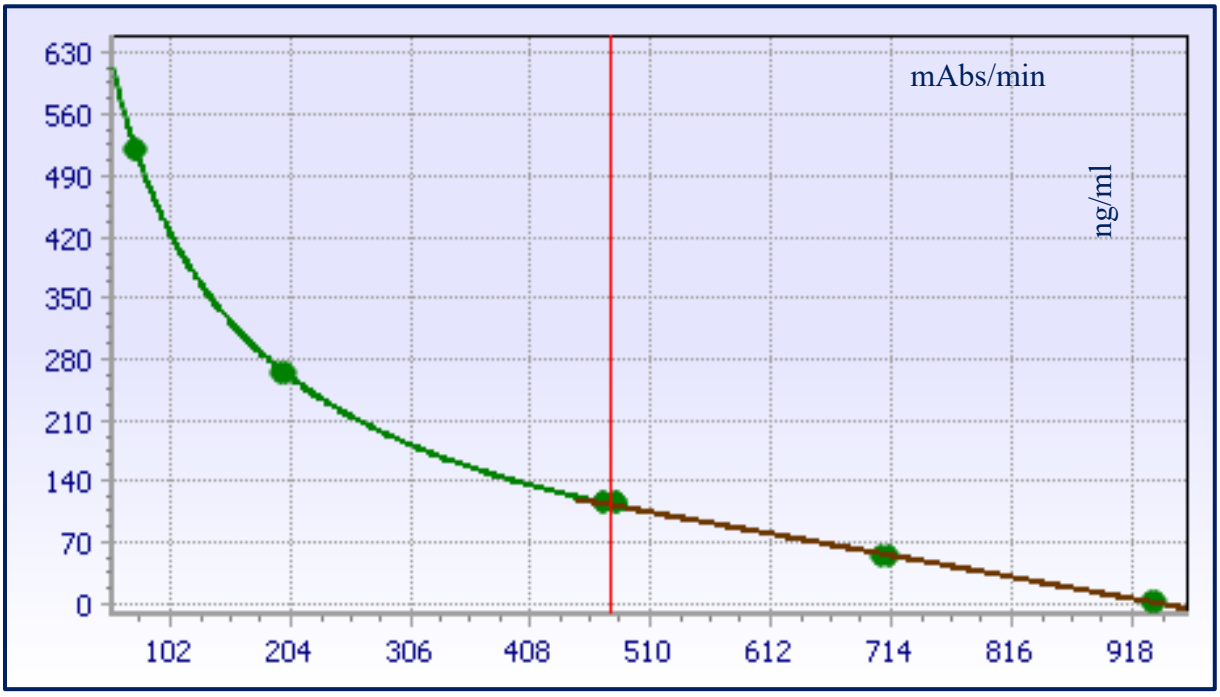

Figure 22 Biophen® Heparin LRT apixaban assay calibration curve

The Biophen® Heparin LRT calibration curve for edoxaban is presented in Figure 23 and exerts a linear and a polynomial of second-order behaviour between the absorption rate and the concentration depending on the concentration range, which significantly differs from the HemosIL® Liquid Anti-Xa edoxaban assay. Therefore, we expected a difference between the HemosIL® Liquid Anti-Xa and Biophen® Heparin LRT assays in lower concentration ranges.

The Biophen® Heparin LRT calibration curve for rivaroxaban is presented in Figure 24 and exerts a linear and a polynomial of second-order behaviour between the absorption rate and the concentration depending on the concentration range, similar to the HemosIL® Liquid Anti-Xa rivaroxaban assay.

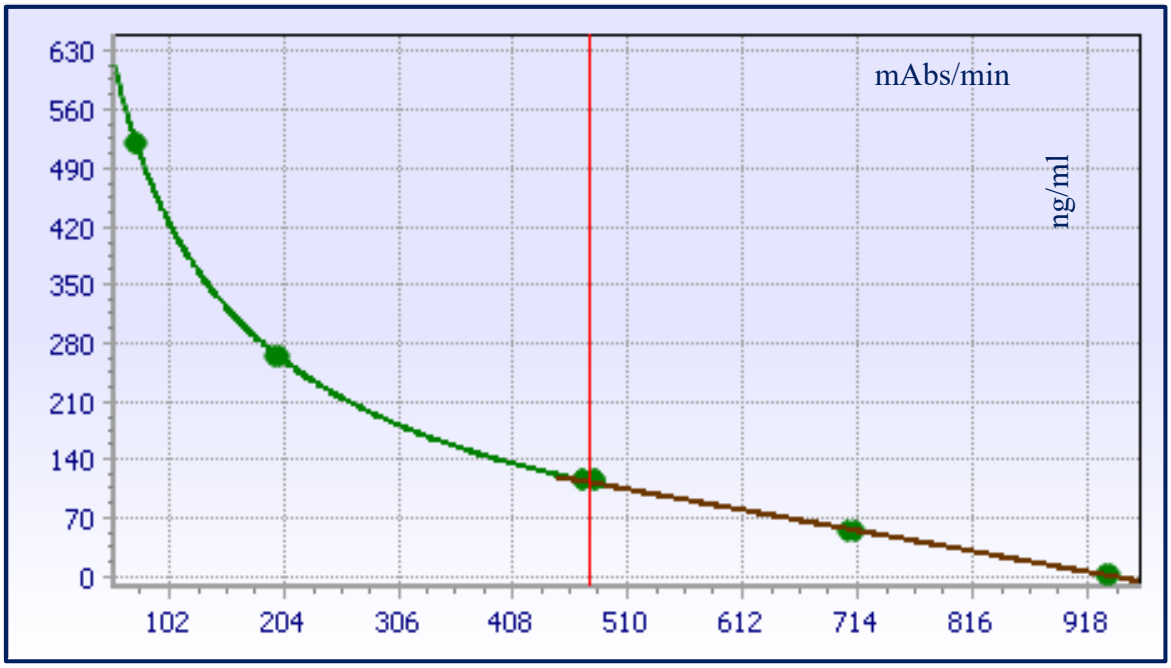

Figure 23 Biophen® Heparin LRT edoxaban assay calibration curve

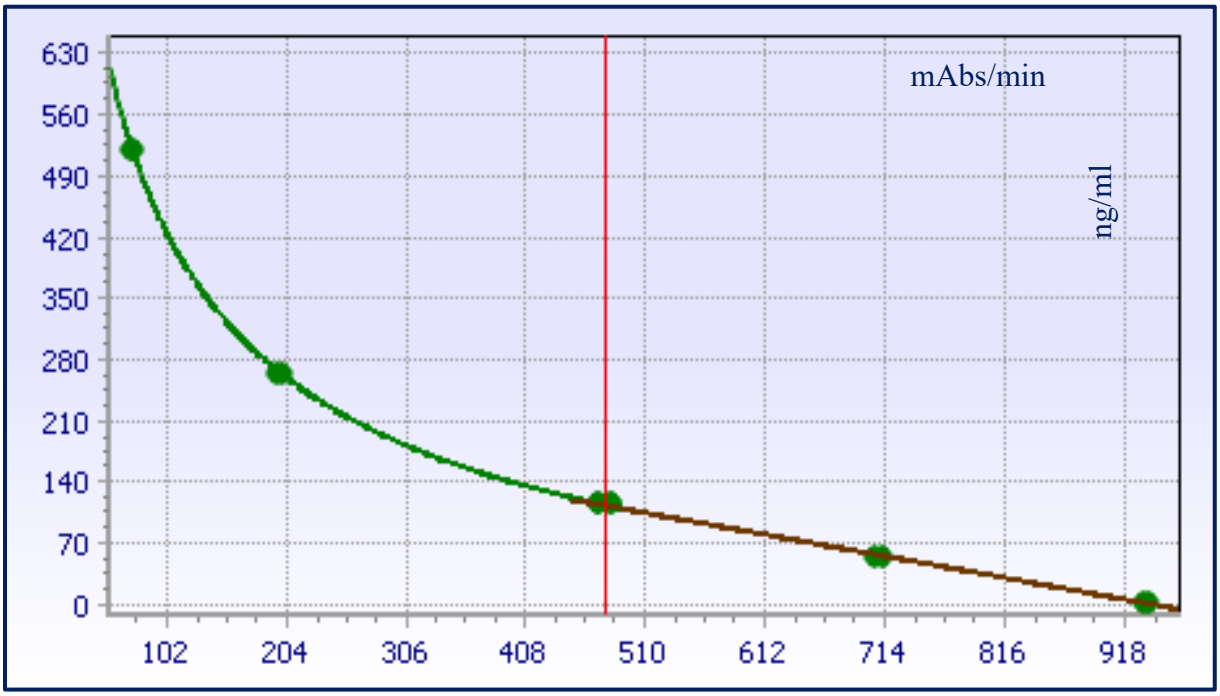

Figure 24 Biophen® Heparin LRT rivaroxaban assay calibration curve

As aforementioned, the Biophen® Heparin LRT apixaban and edoxaban assays do not require an individual calibrator; rather their calibration curves are based on the interpolated data of the Biophen® Heparin LRT rivaroxaban calibration curve.

Detection limit of the Biophen® Heparin LRT assays

The detection limits for the Biophen® Heparin LRT assays for apixaban, edoxaban, and rivaroxaban measurements in our laboratory were determined by assaying a citrate plasma pool sample of healthy individuals without any anticoagulation therapy in replicates of 20 samples and was calculated as 3 standard deviations (SD) added to the mean response of the citrate plasma pool sample.

Precision study of the Biophen® Heparin LRT assays

The evaluation of the precision of the Biophen® Heparin LRT assays for apixaban, edoxaban, and rivaroxaban tests in our laboratory was performed with a replication study according to the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) guideline EP05-A3. Four different daily reconstituted controls (low, medium, high, and very high) were analysed in duplicate in one run per day for 20 days on a single ACL Top 750® analyser. Within-run and total analytical imprecision (CV) was calculated with the CLSI single-run precision evaluation test (Clinical and Laboratory Standards Institute, 2014).

5.4 *Analytical method comparison of the Biophen® Heparin LRT assays with the HemosIL® Liquid Anti-Xa assays*

For the method comparison of the Biophen® Heparin LRT assays with the HemosIL® Liquid Anti-Xa assays blood samples of 159 patients (52.2% male, 47.8% female) with an age of 78.65 ± 10.63 years were analysed in this study. The distributions for the respective treatment groups were:

- 80 patients (52.5% male, 47.5% female) with an age of 77.13 ± 11.59 years for apixaban
- 23 patients (41.7% male, 58.3% female) with an age of 81.08 ± 8.48 years for edoxaban
- 55 patients (56.4% male, 43.6% female) with an age of 77.13 ± 11.59 years for rivaroxaban

After the measurement of the coagulation parameters, the routine samples were preserved and stored at -80°c. The samples were collected within the period from October 2017 until May 2019 in the B&S Zentrallabor. The preservation and scientific utilisation of leftover blood samples were approved by the ethical review committee of the St. John of God Hospital in Linz, Austria.

The preparation of the citrate plasma samples for the measurements comprised thawing in a water quench for 10 minutes and centrifugation at 4500 rpm for 10 minutes at 4°c. Afterwards, the plasma was transferred into a test tube and loaded on the ACL Top 750® for the measurement of anti-Xa activity with the respective assays.

Statistical analysis was conducted with IBM SPSS Statistics, versions 23 and 24 (IBM Deutschland, Ehningen, Germany), GraphPad (GraphPad Software, Inc., California, United States) and MedCalc, version 17.2 (MedCalc Software, Ostend, Belgium).

Passing and Bablok regression, as well as Bland-Altman plots and Spearman rank correlation, were used for the method comparison of the Biophen® Heparin LRT assays with the HemosIL® Liquid Anti-Xa assay as a comparative method (Spearman, 1904, Passing and Bablok, 1983, Bland and Altman, 1999). The Cusum test was performed to evaluate the linearity of the paired data (Brown et al., 1975).

6 Results

For a better outline of the evaluation of the assays, this chapter is subdivided into subchapters for each respective substance.

6.1 Apixaban

6.1.1 Detection limit and precision study for the Biophen® Heparin LRT apixaban assay

The detection limit for the Biophen® Heparin LRT apixaban assay was 6.6 ng/ml. The Biophen® Heparin LRT apixaban assay had a within-run coefficient of variation (CV) of 3.8% and a total CV of 5.6% at a mean concentration of 24 ng/ml (low control), a within-run CV of 2.3% and a total CV of 3.8% at a mean concentration of 72 ng/ml (medium control), a within-run CVA of 1.8% and a total CV of 2.2% at a mean concentration of 194 ng/ml (high control) and a within-run CV of 1.7% and a total CV of 2.3% at a mean concentration of 412 ng/ml (very high control). An overview of the results is presented in Table 5.

Table 5 Results of the within-run and total CVs for apixaban

Apixaban	*Within-run CV*	*Total CV*
Low [24 ng/ml]	*3.8%*	*5.6%*
Medium [77 ng/ml]	*2.3%*	*3.8%*
High [194 ng/ml]	*1.8%*	*2.2%*
Very high [412 ng/ml]	*1.7%*	*2.3%*

6.1.2 Analytical method comparison of the apixaban assays

The data on the 80 patient plasma samples with apixaban plasma concentrations measured with the HemosIL® Liquid Anti-Xa and the Biophen® Heparin LRT apixaban assays is shown in Table 6 and Figure 25.

In the 80 patients on apixaban treatment, median apixaban plasma concentrations were 91 ng/ml (range: 10 – 431 ng/ml; 25th – 75th percentiles: 56 – 143 ng/ml) as measured by the HemosIL® Liquid Anti-Xa apixaban assay and 77 ng/ml (range: 7 – 420 ng/ml, 25th – 75th percentiles: 35 – 121 ng/ml) by the Biophen® Heparin LRT apixaban assay.

*Table 6 Data on the analytical assay comparison of the HemosIL® Liquid Anti-Xa and Biophen® Heparin LRT apixaban assay**

Assay	*Lowest value* *Median value* *Highest value*	*25th percentile value* *75th percentile value*
HemosIL® Liquid Anti-Xa	*10 ng/ml* *91 ng/ml* *431 ng/ml*	*56 ng/ml* *143 ng/ml*
Biophen® Heparin LRT	*7 ng/ml* *77 ng/ml* *420 ng/ml*	*35 ng/ml* *121 ng/ml*

* Plasma samples of 80 patients on apixaban treatment

The method comparison graph for HemosIL® Liquid Anti-Xa apixaban and Biophen® Heparin LRT apixaban assays in the 80 patients, shown in Figure 25, displays the scatter diagrams with the regression line (solid line) and the confidence intervals for the regression line (dashed lines) according to Passing and Bablok. Non-parametric correlation analyses of the 80 patients revealed a Spearman´s coefficient of rank correlation of 0.993 (95% CI, 0.989-0.995; p<0.001) between the HemosIL® Liquid Anti-Xa apixaban and the Biophen® Heparin LRT apixaban assays.

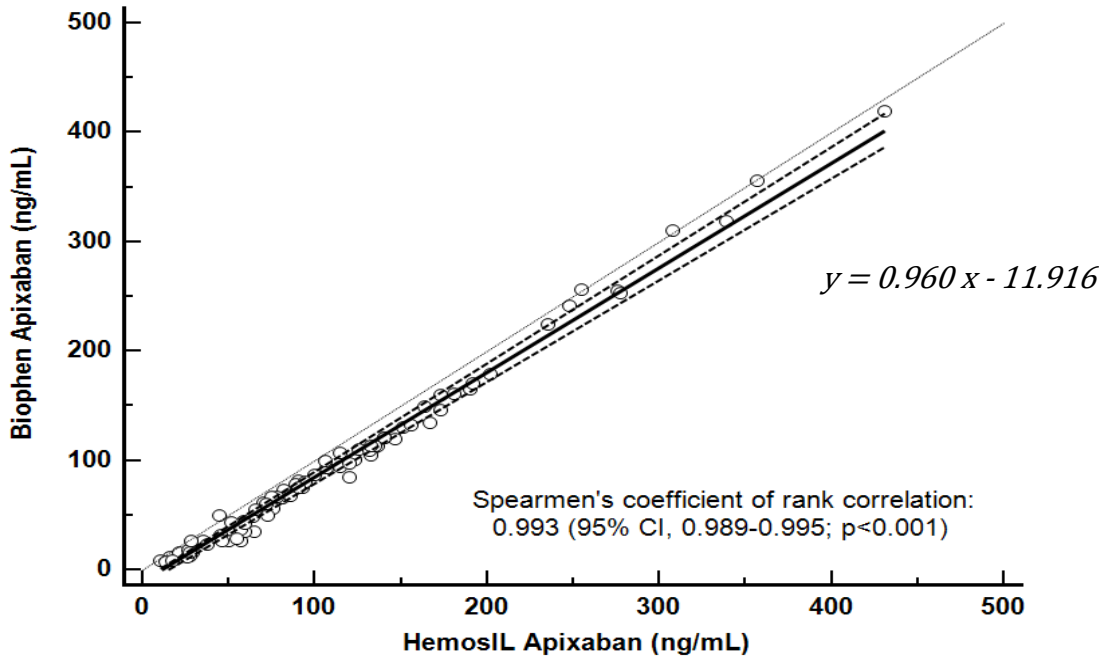

Figure 25 Passing and Bablok regression line and equations for HemosIL® Liquid Anti-Xa and Biophen® Heparin LRT apixaban assays

The respective regression equation presented in *[2]* suggests a rather small constant bias as well as a proportional difference between the two methods.

$$y = -11.92\ (95\%\ CI, -14.21\ to\ -9.19) + 0.96\ (95\%\ CI, 0.93\ to\ 0.99)\ x \qquad [2]$$

The Cusum test showed no significant deviation from linearity (p=0.25). Bland-Altman difference plots are shown in Figure 26.

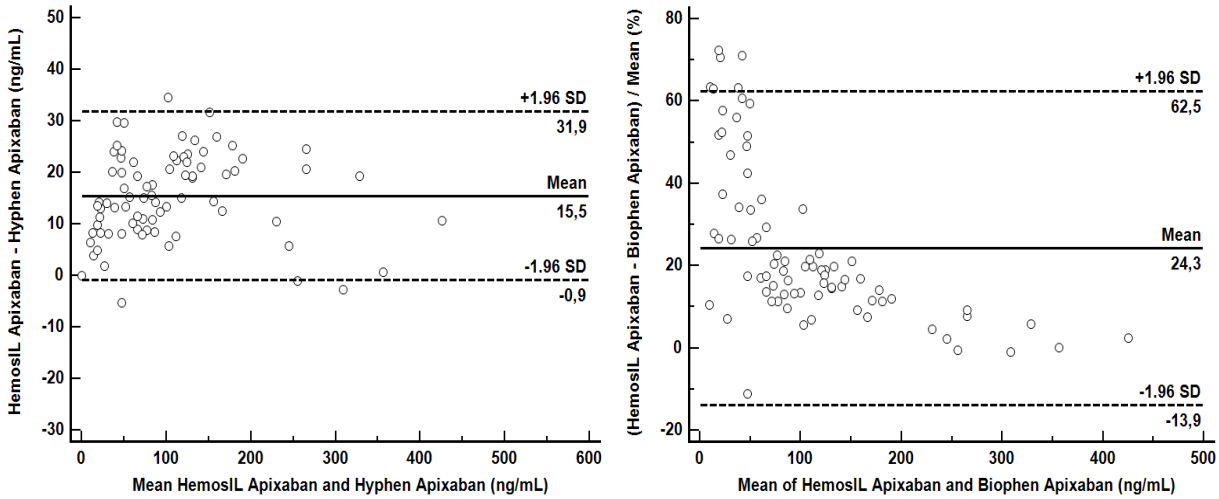

Figure 26 Bland–Altman plots for HemosIL® Liquid Anti-Xa and Biophen® Heparin LRT apixaban assays. Left: absolute deviations. Right: relative deviations.

6.2 *Edoxaban*

6.2.1 *Detection limit and precision study for the Biophen® Heparin LRT edoxaban assay*

The detection limit for the Biophen® Heparin LRT edoxaban assay was 7.5 ng/ml. The Biophen® Heparin LRT edoxaban assay had a CV of 5.2% and a total CV of 5.6% at a mean concentration of 25 ng/ml (low control), a within-run CV of 3.8% and a total CV of 6.0% at a mean concentration of 81 ng/ml (medium control), a within-run CV of 1.7% and a total CV of 2.8% at a mean concentration of 150 ng/ml (high control) and a within-run CV of 1.3% and a total CV of 2.3% at a mean concentration of 290 ng/ml (very high control). An overview of the results is presented in Table 7.

Table 7 Results of the within-run and total CVs for edoxaban

Apixaban	*Within-run CV*	*Total CV*
Low [25 ng/ml]	*5.2%*	*5.6%*
Medium [81 ng/ml]	*3.8%*	*6.0%*
High [150 ng/ml]	*1.7%*	*2.8%*
Very high [290 ng/ml]	*1.3%*	*2.3%*

6.2.2 *Analytical method comparison of the edoxaban assays*

The data on the 23 patient plasma samples with edoxaban plasma concentrations measured with the HemosIL® Liquid Anti-Xa and the Biophen® Heparin LRT apixaban assays is shown in Table 8 and Figure 27.

*Table 8 Data on the analytical assay comparison of the HemosIL® Liquid Anti-Xa edoxaban assay and the Biophen® Heparin LRT edoxaban assay**

Assay	*Lowest value* *Median value* *Highest value*	*25th percentile value* *75th percentile value*
HemosIL® Liquid Anti-Xa	*9 ng/ml* *57 ng/ml* *347 ng/ml*	*22 ng/ml* *142 ng/ml*
Biophen® Heparin LRT	*8 ng/ml* *44 ng/ml* *354 ng/ml*	*18 ng/ml* *140 ng/ml*

* Plasma samples of 23 patients on edoxaban treatment

In the 23 patients on edoxaban treatment, median edoxaban plasma concentrations were 57 ng/ml (range: 9 – 347 ng/ml; 25th – 75th percentiles: 22 – 142 ng/ml) as measured by the HemosIL® Liquid Anti-Xa edoxaban assay and 44 ng/ml (range: 8 – 354 ng/ml, 25th – 75th percentiles: 18 – 140 ng/ml) by the Biophen® Heparin LRT edoxaban assay.

The method comparison graph for HemosIL® Liquid Anti-Xa edoxaban and Biophen® Heparin LRT edoxaban assays in the 23 patients, shown in Figure 27, displays the scatter diagrams with the regression line (solid line) and the confidence intervals for the regression line (dashed lines) according to Passing and Bablok. Non-parametric correlation analyses of the 23 patients revealed a Spearman´s coefficient of rank correlation of 0.994 (95% CI: 0.985

– 0.995; p<0.001) between the HemosIL® Liquid Anti-Xa edoxaban and the Biophen® Heparin LRT edoxaban assay.

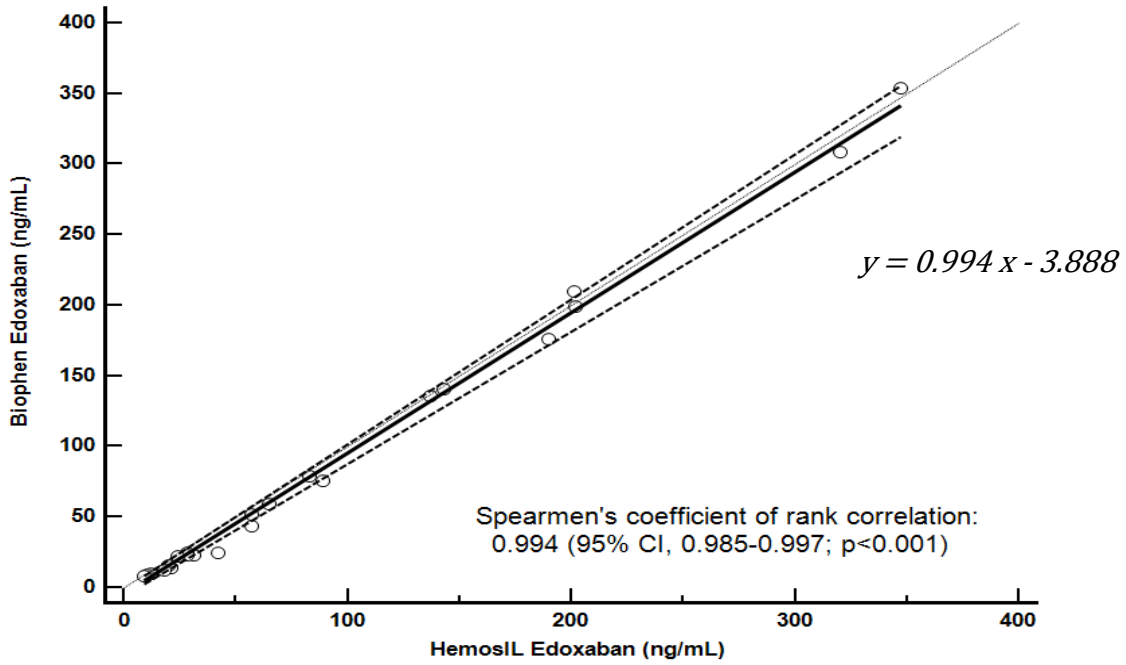

Figure 27 Passing and Bablok regression line and equations for HemosIL® Liquid Anti-Xa and Biophen® Heparin LRT edoxaban assays

The respective regression equation presented in *[3]* suggests a rather small constant bias as well as a proportional difference between the two methods. Thus, Passing and Bablok regression suggests, in addition to a rather small but significant constant bias, a high proportional difference between the two methods.

$$y = -3.89\ (95\%\ CI, -6.31\ to\ -1.25) + 0.99\ (95\%\ CI, 0.94\ to\ 1.03)\ x \qquad [3]$$

The Cusum test showed no significant deviation from linearity (p=0.78). Bland-Altman difference plots are shown in Figure 28.

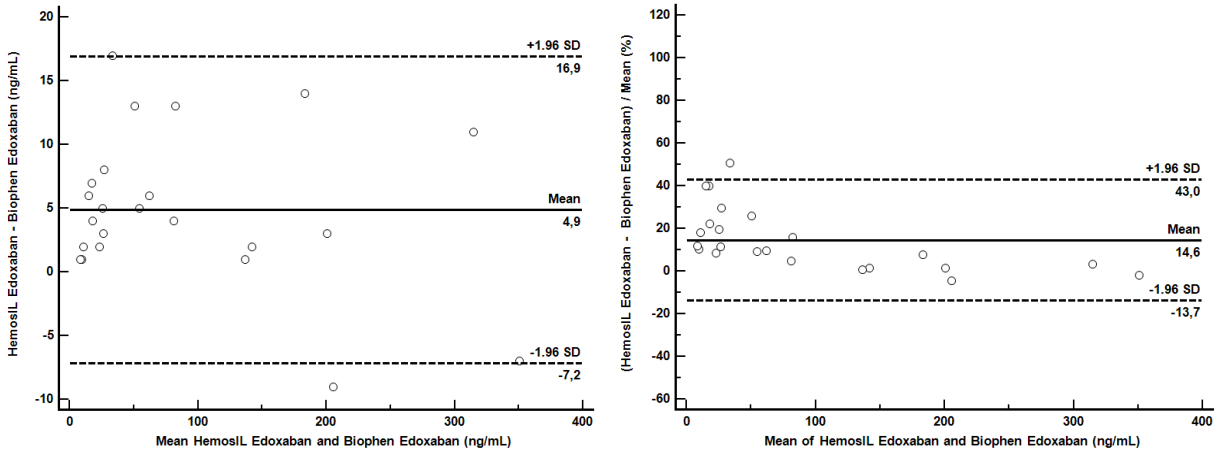

Figure 28 Bland–Altman plot for HemosIL® Liquid Anti-Xa and Biophen® Heparin LRT edoxaban assays. Left: absolute deviations. Right: relative deviations.

6.3 *Rivaroxaban*

6.3.1 *Detection limit and precision study for the Biophen® Heparin LRT rivaroxaban assay*

The detection limit for the Biophen® Heparin LRT rivaroxaban assay was 9.7 ng/ml. The Biophen® Heparin LRT rivaroxaban assay had a CV of 5.4% and a total CV of 6.1% at a mean concentration of 24 ng/ml (low control), a within-run CV of 3.8% and a total CV of 4.9% at a mean concentration of 80 ng/ml (medium control), a within-run CV of 3.5% and a total CV of 3.3% at a mean concentration of 104 ng/ml (high control) and a within-run CV of 1.8 % and a total CV of 2.9% at a mean concentration of 308 ng/ml (very high control). An overview of the results is presented in Table 9.

Table 9 Results of the within-run and total CVs for rivaroxaban

Apixaban	*Within-run CV*	*Total CV*
Low [24 ng/ml]	*5.4%*	*6.1%*
Medium [80 ng/ml]	*3.8%*	*4.9%*
High [104 ng/ml]	*3.5%*	*3.3%*
Very high [308 ng/ml]	*1.8%*	*2.9%*

6.3.2 *Analytical method comparison of the rivaroxaban assays*

The data on the 55 patient plasma samples with rivaroxaban plasma concentrations measured with the HemosIL® Liquid Anti-Xa and the Biophen® Heparin LRT apixaban assays is shown in Table 10 and Figure 29.

In the 55 patients on rivaroxaban treatment, median rivaroxaban plasma concentrations were 69 ng/ml (range: 13 – 515 ng/ml; 25th – 75th percentiles: 43 – 131 ng/ml) as measured by the HemosIL® Liquid Anti-Xa rivaroxaban assay and 69 ng/ml (range: 11 – 627 ng/ml, 25th – 75th percentiles: 47 – 144 ng/ml) by the Biophen® Heparin LRT rivaroxaban assay.

*Table 10 Data on the analytical assay comparison of the HemosIL® Liquid Anti-Xa rivaroxaban assay and the Biophen® Heparin LRT rivaroxaban assay**

Assay	*Lowest value* *Median value* *Highest value*	*25th percentile value* *75th percentile value*
HemosIL® Liquid Anti-Xa	*13 ng/ml* *69 ng/ml* *515 ng/ml*	*43 ng/ml* *131 ng/ml*
Biophen® Heparin LRT	*11 ng/ml* *69 ng/ml* *627 ng/ml*	*47 ng/ml* *144 ng/ml*

* Plasma samples of 55 patients on rivaroxaban treatment

The method comparison graph for HemosIL® Liquid Anti-Xa rivaroxaban and Biophen® Heparin LRT rivaroxaban assays in the 55 patients, shown in Figure 29, displays the scatter diagrams with the regression line (solid line) and the confidence intervals for the regression line (dashed lines) according to Passing and Bablok. Non-parametric correlation analyses of the 23 patients revealed a Spearman´s coefficient of rank correlation of 0.986 (95% CI, 0.977 – 0.992, p<0.001) between the HemosIL® Liquid Anti-Xa rivaroxaban and the Biophen® Heparin LRT rivaroxaban assay.

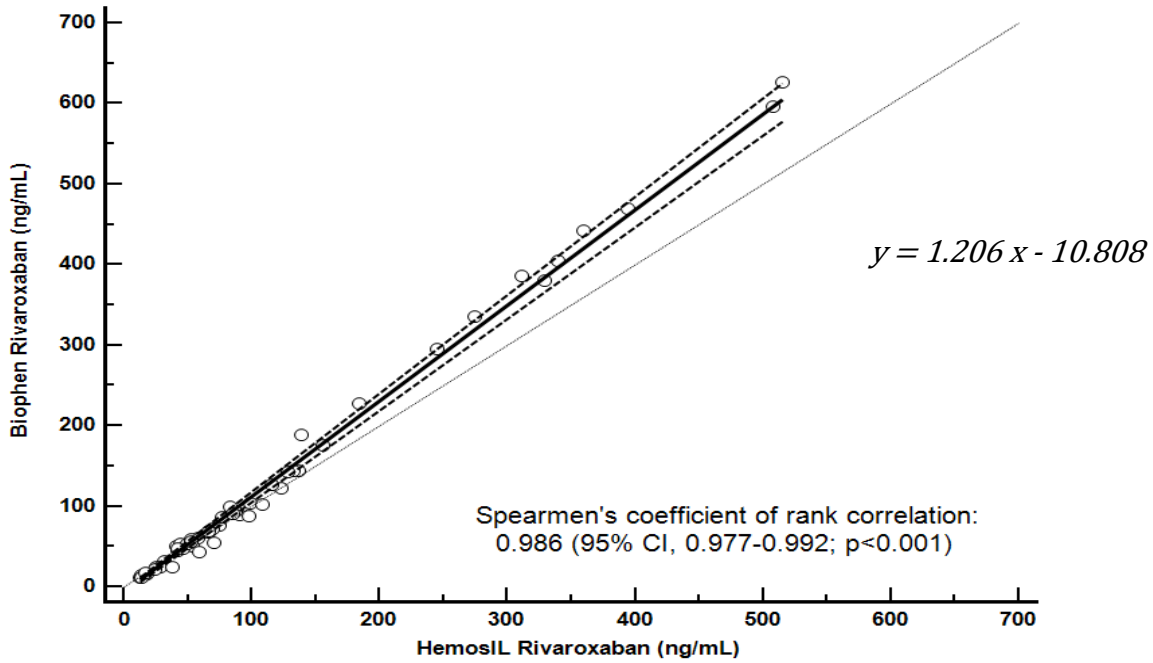

Figure 29 Passing and Bablok regression line and equations for HemosIL® Liquid Anti-Xa and Biophen® Heparin LRT rivaroxaban assays

The respective regression equation presented in *[4]* suggests a rather small constant bias as well as a proportional difference between the two methods.

$$y = -6.72\ (95\%\ CI, -9.66\ to\ -4.24) + 1.19\ (95\%\ CI, 1.14\ to\ 1.22)\ x \qquad [4]$$

The Cusum test showed no significant deviation from linearity (p=0.17). Bland-Altman difference plots are shown in Figure 30.

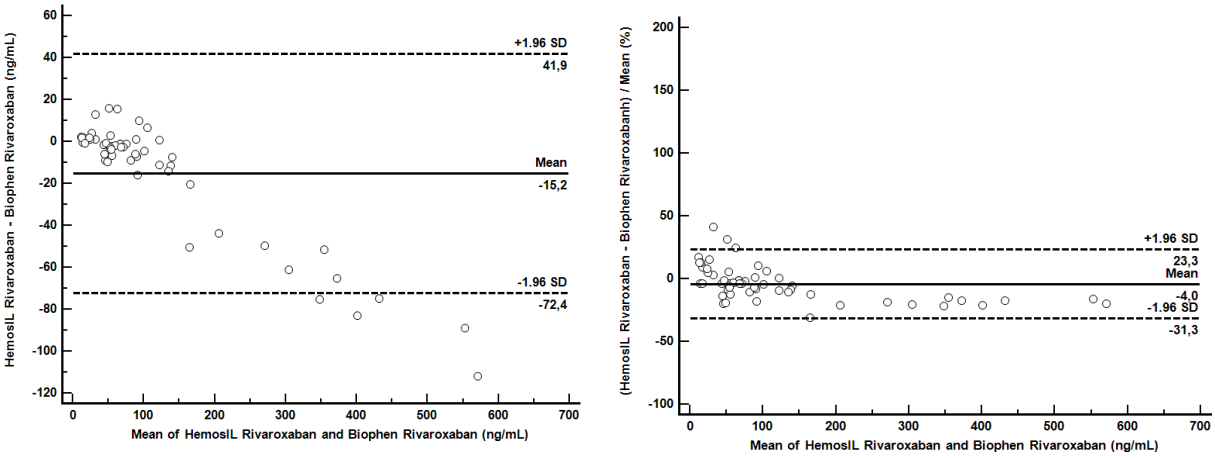

Figure 30 Bland–Altman plot for HemosIL® Liquid Anti-Xa and Biophen® Heparin LRT rivaroxaban assays. Left: absolute deviations. Right: relative deviations.

7 Discussion

This study compared the Biophen® Heparin LRT and HemosIL® Liquid Anti-Xa assays for clinical routine samples of apixaban, edoxaban and rivaroxaban controls as well as patients on the respective anticoagulation treatment. The main question was if the respective assays show comparable results and are therefore equivalent in clinical routine use. Because the Biophen® Heparin LRT assays require only a single hybrid calibrator set, in comparison to the HemosIL® Liquid Anti-Xa assays, which requires a calibrator set for each respective substance, the establishment of the Biophen® Heparin LRT assays would reduce the number of required calibrators, making the direct factor Xa inhibitor concentration measurements more attractive for laboratories with smaller sample volumes. This reduction of necessary calibrator sets is achieved by the interpolation of the calibrated curve for compiling the calibration curves of apixaban and edoxaban. Since these curves are based on the interpolated data from another direct factor Xa inhibitor substance rather than their calibrators, they are called hybrid curves. Therefore, the measurement of the direct factor Xa inhibitor plasma concentration with the Biophen® Heparin LRT assays requires only one calibrator set (CoaChrom Diagnostica GmbH, 2015).

The detection limits for all 3 Biophen® Heparin LRT assays were significantly below the clinically relevant concentration measurement threshold of 30 ng/ml (Margetic et al., 2020), allowing for an explicit determination of the absence of clinically relevant factor Xa inhibitor plasma concentrations, which supports well-founded therapeutic decision-making. The inter-run, as well as the total CVs, were significantly below the recommended clinical measurement threshold of 10%, which proved the Biophen® Heparin LRT assays' precision. Therefore, our study has shown good consistencies for the hybrid curve calibration system compared to the clinically established assays as well as that the Biophen® Heparin LRT assays measure up to the manufacturer's claims regarding detection limits and precision, although our test results suggest a rather small constant bias as well as proportional difference between the two methods. Since the measurement of direct factor Xa inhibitor plasma concentrations is only indicated for a limited number of clinical conditions, such as patients requiring emergency surgery or with absorption and drug adherence concerns, the disparities between the HemosIL® Liquid Anti-Xa and Biophen® Heparin LRT assays were found to have no relevance for clinical decision-making.

By exploiting the limited impact of the exact plasma concentration on therapeutic decision-making, another study found that it is possible to measure relevant apixaban and rivaroxaban

plasma concentrations with the more commonly used LMWH anti-Xa activity assay instead of a drug-specific anti-Xa activity assay; rather than the exact drug plasma concentration values they investigated negative predictive values, which described the absence of apixaban and rivaroxaban (plasma concentrations below the measurement threshold of 30 ng/ml). For a negative predictive value of 100% for apixaban, the corresponding cut-off was 0.05 IU/ml, whereas for rivaroxaban the corresponding cut-off was 0.1 IU/ml, which allows the exclusion of clinically relevant direct factor Xa inhibitor drug concentrations even in the absence of drug-specifically calibrated chromogenic assays, therefore allowing the assessment of the necessity of reversal agent administration (Margetic et al., 2020). The low measurement threshold of the study by Margetic and colleagues was chosen according to the recommendations for reversal agent administration by the International Society on Thrombosis and Hemostasis at direct factor Xa inhibitor plasma concentrations above 30 ng/ml in the perioperative setting as well as above 50 ng/ml in patients with bleeding (Levy et al., 2016). The fact that LMWH-calibrated anti-Xa activity assays can specifically detect the plasmatic absence of direct anti-Xa inhibitors, but not determine the inhibitor concentrations quantitatively was already confirmed by another study (Gosselin et al., 2015). Therefore, the application of LMWH-calibrated anti-Xa activity assays for the measurement of plasma concentrations - or rather the absence - of direct factor Xa inhibitor is only recommended for emergency assessments of the necessity of reversal agent administration in the absence of a drug-specifically calibrated assay. In the future this issue might be addressed by a „Da-Xa inhibition assay“, a single anti-Xa based laboratory assay for all directly and indirect factor Xa inhibitors, by reporting the inhibitory activity rather than drug concentrations, which would make drug-specific calibrations for anti-Xa substances obsolete (van Pelt et al., 2018).

Besides the drug-specific calibrations requirement of the particular calibrator sets, heparin interference is a known disturbing factor for the measurement of plasma concentrations of direct factor Xa inhibitors. Therefore, a dedicated heparin neutralisation buffer was developed (CoaChrom Diagnostica), which is supposed to work in combination with the Biophen® Heparin LRT assays. Because the reduction of heparin interference may support clinical decision-making in anticoagulated patients (with DOACs), especially for the assessment of bridging necessity, the successful establishment of this heparin neutralisation buffer system might largely influence the decision on the used assay system, we propose an analytical method comparison of the Biophen® Heparin LRT assays plus the heparin neutralisation buffer with the sole assays as a follow-up project. First data for the successful application of

the heparin neutralisation buffer was reported on the ISTH 2019 for a company-internal experimental run conducted by Hyphen Biomed (Molton et al., 2019).

Since the measurement of low direct factor Xa inhibitor concentrations requires the application of LC-MS/MS, this method is considered the gold standard for the measurement of direct factor Xa inhibitor plasma concentrations (Cuker and Siegal, 2015). Due to the long measurements times as well as labour-intensive preparation work for LC-MS/MS measurements, this method is commonly not applicable in the clinical setting. Therefore, the limitation of this study is the lack of LC-MS/MS measurements, since we compared the clinically approved Biophen® Heparin LRT and HemosIL® Liquid Anti-Xa assays. These assays were already compared to LC-MS/MS measurements by DiaChrom Diagnostica and Werfen at the respective assays' market launch.

In conclusion, our study suggests that the Biophen® Heparin LRT assays for measuring direct Xa inhibitor activity as well as anti-Xa concentrations are equivalent to the established HemosIL® Liquid Anti-Xa assays. However, it should be considered that the latter provides the advantage of only requiring a single calibrator set due to its hybrid curve technology, making it especially feasible for smaller, non-clinical laboratories. On the other hand, the application of the Biophen® Heparin LRT assays for coagulation tests on Werfen instruments, such as the ACL Top® family, which are widely used, introduce foreign reagents which require special setups and adjustments, therefore complicating the overall process of direct Xa inhibitor measurements.

8 Outlook

This chapter provides an overview of the anticoagulants as well as antidotes currently under development.

8.1 *Anticoagulants under development*

Currently, numerous anticoagulant agents are under development, such as TFPI, factor VIII inhibitor, TM, factor IXa inhibitor, Factor XI inhibitor, factor XIIa inhibitor, protein disulfide isomerase inhibitors, and polyphosphate inhibitors.

8.1.1 *Tissue factor pathway inhibitors (TFPI)*

These inhibitors are a recombinant form of the physiological TFPI, which targets the TF/factor VIIa complex, or individual TF and factor VIIa inhibitors (Stassens et al., 1996, Lee et al., 2001, Presta et al., 2001, Giugliano et al., 2007).

8.1.2 *Factor VIII inhibitor*

TB-402, a human IgG4 monoclonal antibody, which partially inhibits factor VIII, was proven to be effective in preventing postoperative VTE in a randomised phase II trial. Since it has a half-life of approximately 3 weeks a single postoperative intravenous injection of TB-402 is as effective as 10 days of continuous LMW heparin administration (Verhamme et al., 2011)

8.1.3 *Thrombomodulin (TM)*

ART-123 is a recombinant form of TM's extracellular domain, which is involved in the protein C and subsequently thrombin activation, and its efficacy was shown for VTE prophylaxis in a phase II trial (Kearon et al., 2005, Carnemolla et al., 2012). Furthermore, it is already approved in Japan for the treatment of disseminated intravascular coagulation. Since it has a half-life of 2 to 3 days, anticoagulation maintenance only requires a subcutaneous injection every six days (Moll et al., 2004).

8.1.4 *Factor IXa inhibitor*

REG1 contains an injectable RNA aptamer (pegnivacogin), which specifically inactivates factor IXa, and a complementary nucleotide (anivamersen), which neutralises anti-IXa

activity, thus functioning as an antidote as well. Randomised studies showed promising results in concomitant antiplatelet-therapy in coronary artery disease (Chan et al., 2008, Cohen et al., 2010), although a more recent study found more frequent allergic reactions and increased bleeding risk compared to bivalirudin administration, but reduced stent thrombosis risk (Lincoff et al., 2016).

8.1.5 *Factor XI inhibitor*

FXI-ASO is a factor XI antisense oligonucleotide, which reduces the risk of VTE development at higher FXI-ASO doses compared to low FXI-ASO doses as well as LMWH enoxaparin, while no higher bleeding risk was found for higher FXI-ASO doses (Zhang et al., 2010, Büller et al., 2015). This gave rise to the hypothesis that antithrombotic activity and normal hemostasis might be uncoupled, which would allow maximal anticoagulation without increased bleeding risk by targeting FXIa and inhibiting the tertiary amplification pathway. Another advantage is the long half-life of antisense therapies of up to 3 months, thus requiring fewer injections and available at lower potential costs (Flaumenhaft, 2015).

8.1.6 *Factor XIIa inhibitor*

rHA-Infestin-4, a fusion protein of albumin and the Infestin-4 (factor XIIa inhibitor) was shown to prevent pathologic thrombus formation while not affecting hemostasis in animal models, making it a possible agent for the prevention and treatment of acute ischemic cardiovascular and cerebrovascular events (Hagedorn et al., 2010).

8.1.7 *Protein disulfide isomerase (PDI) inhibitors*

PDI is an oxidoreductase enzyme catalysing redox protein folding, including coagulation factor XI and TF. Since PDI is located on the platelet surface, it is also involved in enhanced platelet aggregation via integrin activation (Wang and Essex, 2017). Therefore, the inhibition of PDI results in a diminished fibrin generation and platelet activation, as shown in preclinical studies with a PDI peptide inhibitor (Sousa et al., 2017).

8.2 *Antidotes under development*

Currently, numerous antidotes are under development, and the most progress was made on small molecule antidotes and mutant forms of factor Xa.

8.2.1 *Small molecule antidotes*

For the small molecule antidote PER 977 preliminary studies showed its ability to specifically bind to direct thrombin inhibitors, factor Xa inhibitors, and heparins and their respective anticoagulant effects (Laulicht et al., 2013). Compared to edoxaban, which showed the fastest normalisation times among already approved DOACs with 12 to 15 hours, PER977 normalised the clotting time within 10 minutes, without significant adverse effects (Ansell et al., 2014b)

8.2.2 *Mutant form of factor Xa*

Factor Xa^{I16L}, which is a mutant form of factor Xa, interchanges isoleucine for leucine at position 16, and like PER 977 it might possess the ability to antagonise multiple anticoagulant agents, such as rivaroxaban and dabigatran. Instead of binding to the anticoagulant, factor Xa^{I16L} is circulating in a zymogen-like state; thus, it is insensitive to competitive inhibitors. Its activation is triggered by factor Va, which is present on damaged cellular surfaces, thereby selectively restoring hemostasis at the bleeding site (Thalji et al., 2016).

9 List of Tables

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ABDULKAREEM, A., D'SOUZA, R. S., MUNDORFF, J., SHRESTHA, P., SHOGBESAN, O. & DONATO, A. 2018. Refractory Abdominal Pain in a Patient with Chronic Lymphocytic Leukemia: Be Wary of Acquired Angioedema due to C1 Esterase Inhibitor Deficiency. *Case Rep Hematol,* 2018**,** 7809535.

AGNELLI, G., BULLER, H. R., COHEN, A., CURTO, M., GALLUS, A. S., JOHNSON, M., PORCARI, A., RASKOB, G. E. & WEITZ, J. I. 2013. Apixaban for extended treatment of venous thromboembolism. *N Engl J Med,* 368**,** 699-708.

ALEMAN, M. M., BYRNES, J. R., WANG, J. G., TRAN, R., LAM, W. A., DI PAOLA, J., MACKMAN, N., DEGEN, J. L., FLICK, M. J. & WOLBERG, A. S. 2014. Factor XIII activity mediates red blood cell retention in venous thrombi. *J Clin Invest,* 124**,** 3590-600.

ALTENA, R., VAN ROON, E., FOLKERINGA, R., DE WIT, H. & HOOGENDOORN, M. 2014. Clinical challenges related to novel oral anticoagulants: drug-drug interactions and monitoring. *Haematologica,* 99**,** e26-7.

AMIRAL, J. & SEGHATCHIAN, J. 2019. Revisiting the activated protein C-protein S-thrombomodulin ternary pathway: Impact of new understanding on its laboratory investigation. *Transfus Apher Sci,* 58**,** 538-544.

ANDERSON, R. J. 2004. Cost analysis of a managed care decentralized outpatient pharmacy anticoagulation service. *J Manag Care Pharm,* 10**,** 159-65.

ANGIOMAX. 2013. Available: http://www.angiomax.com/downloads/Angiomax_US_PI_June_2013 [Accessed November 03, 2019].

ANSELL, J., CROWTHER, M., BURNETT, A., GARCIA, D., KAATZ, S., LOPES, R. D., NUTESCU, E., OERTEL, L., SCHNURR, T., STREIFF, M., WIRTH, D., WITT, D. & WITTKOWSKY, A. 2014a. Comment on: editorial by Husted et al. "Non-vitamin K antagonist oral anticoagulants (NOACs): no longer new or novel". (Thromb Haemost 2014; 111: 781-782). *Thromb Haemost,* 112**,** 841.

ANSELL, J. E., BAKHRU, S. H., LAULICHT, B. E., STEINER, S. S., GROSSO, M., BROWN, K., DISHY, V., NOVECK, R. J. & COSTIN, J. C. 2014b. Use of PER977 to reverse the anticoagulant effect of edoxaban. *N Engl J Med,* 371**,** 2141-2.

ANTITHROMBOTIC TRIALISTS' COLLABORATION 2002. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *Bmj,* 324**,** 71-86.

ANTMAN, E. M., WIVIOTT, S. D., MURPHY, S. A., VOITK, J., HASIN, Y., WIDIMSKY, P., CHANDNA, H., MACIAS, W., MCCABE, C. H. & BRAUNWALD, E. 2008. Early and late benefits of prasugrel in patients with acute coronary syndromes undergoing percutaneous coronary intervention: a TRITON-TIMI 38 (TRial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet InhibitioN with Prasugrel-Thrombolysis In Myocardial Infarction) analysis. *J Am Coll Cardiol,* 51**,** 2028-33.

ASAKAI, R., CHUNG, D. W., DAVIE, E. W. & SELIGSOHN, U. 1991. Factor XI deficiency in Ashkenazi Jews in Israel. *N Engl J Med,* 325**,** 153-8.

AZIZ, F., CORDER, M., WOLFFE, J. & COMEROTA, A. J. 2011. Anticoagulation monitoring by an anticoagulation service is more cost-effective than routine physician care. *J Vasc Surg,* 54**,** 1404-7.

BACH, R. R. & MONROE, D. 2009. What is wrong with the allosteric disulfide bond hypothesis? *Arterioscler Thromb Vasc Biol,* 29**,** 1997-8.

BÄCHLI, E. 2000. History of tissue factor. *Br J Haematol,* 110**,** 248-55.

BAGLIN, T., BARROWCLIFFE, T. W., COHEN, A. & GREAVES, M. 2006. Guidelines on the use and monitoring of heparin. *Br J Haematol,* 133**,** 19-34.

BAJAJ, M. S. & BAJAJ, S. P. 1997. Tissue factor pathway inhibitor: potential therapeutic applications. *Thromb Haemost,* 78**,** 471-7.

BAJAJ, M. S., BIRKTOFT, J. J., STEER, S. A. & BAJAJ, S. P. 2001. Structure and biology of tissue factor pathway inhibitor. *Thromb Haemost,* 86**,** 959-72.

BAJZAR, L., MORSER, J. & NESHEIM, M. 1996. TAFI, or plasma procarboxypeptidase B, couples the coagulation and fibrinolytic cascades through the thrombin-thrombomodulin complex. *J Biol Chem,* 271**,** 16603-8.

BARNES, G. D., AGENO, W., ANSELL, J. & KAATZ, S. 2015. Recommendation on the nomenclature for oral anticoagulants: communication from the SSC of the ISTH. *J Thromb Haemost,* 13**,** 1154-6.

BAUGH, R. J., BROZE, G. J., JR. & KRISHNASWAMY, S. 1998. Regulation of extrinsic pathway factor Xa formation by tissue factor pathway inhibitor. *J Biol Chem,* 273**,** 4378-86.

BAUGH, R. J. & KRISHNASWAMY, S. 1996. Role of the activation peptide domain in human factor X activation by the extrinsic Xase complex. *J Biol Chem,* 271**,** 16126-34.

BAYER AG. 2011. Available: http://www.xareltohcp.com/sites/default/files/pdf/xarelto_0.pdf [Accessed November 03, 2019].

BENNETT, J. S. & VILAIRE, G. 1979. Exposure of platelet fibrinogen receptors by ADP and epinephrine. *J Clin Invest,* 64**,** 1393-401.

BERRY, C. N., GIRARDOT, C., LECOFFRE, C. & LUNVEN, C. 1994. Effects of the synthetic thrombin inhibitor argatroban on fibrin- or clot-incorporated thrombin: comparison with heparin and recombinant Hirudin. *Thromb Haemost,* 72**,** 381-6.

BEYER-WESTENDORF, J. & SIEGERT, G. 2015. Of men and meals. *J Thromb Haemost,* 13**,** 943-5.

BEYER, J., TRUJILLO, T., FISHER, S., KO, A., LIND, S. E. & KISER, T. H. 2016. Evaluation of a Heparin-Calibrated Antifactor Xa Assay for Measuring the Anticoagulant Effect of Oral Direct Xa Inhibitors. *Clin Appl Thromb Hemost,* 22**,** 423-8.

BINETTE, T. M., TAYLOR, F. B., JR., PEER, G. & BAJZAR, L. 2007. Thrombin-thrombomodulin connects coagulation and fibrinolysis: more than an in vitro phenomenon. *Blood,* 110**,** 3168-75.

BLAND, J. M. & ALTMAN, D. G. 1999. Measuring agreement in method comparison studies. *Stat Methods Med Res,* 8**,** 135-60.

BLECH, S., EBNER, T., LUDWIG-SCHWELLINGER, E., STANGIER, J. & ROTH, W. 2008. The metabolism and disposition of the oral direct thrombin inhibitor, dabigatran, in humans. *Drug Metab Dispos,* 36**,** 386-99.

BLOOMFIELD, H. E., KRAUSE, A., GREER, N., TAYLOR, B. C., MACDONALD, R., RUTKS, I., REDDY, P. & WILT, T. J. 2011. Meta-analysis: effect of patient self-testing and self-management of long-term anticoagulation on major clinical outcomes. *Ann Intern Med,* 154**,** 472-82.

BOEHRINGER-INGELHEIM. 2014. Available: http://bidocs.boehringer-ingelheim.com/BIWebAccess/ViewServlet.ser?docBase=renetnt&folderPath=/Prescribing%20Information/PIs/Pradaxa/Pradaxa.pdf [Accessed November 03, 2019].

BOERSMA, E., HARRINGTON, R. A., MOLITERNO, D. J., WHITE, H., THEROUX, P., VAN DE WERF, F., DE TORBAL, A., ARMSTRONG, P. W., WALLENTIN, L. C., WILCOX, R. G., SIMES, J., CALIFF, R. M., TOPOL, E. J. & SIMOONS, M. L. 2002. Platelet glycoprotein IIb/IIIa inhibitors in acute coronary syndromes: a meta-analysis of all major randomised clinical trials. *Lancet,* 359**,** 189-98.

BOGDANOV, V. Y., BALASUBRAMANIAN, V., HATHCOCK, J., VELE, O., LIEB, M. & NEMERSON, Y. 2003. Alternatively spliced human tissue factor: a circulating, soluble, thrombogenic protein. *Nat Med,* 9**,** 458-62.

BOOTH, N. A. 2001. TAFI meets the sticky ends. *Thromb Haemost,* 85**,** 1-2.

BRASS, L. F. 2003. Thrombin and platelet activation. *Chest,* 124**,** 18s-25s.

BROWN, R. L., DURBIN, J. & EVANS, J. M. 1975. Techniques for Testing the Constancy of Regression Relationships over Time. *Journal of the Royal Statistical Society. Series B (Methodological),* 37**,** 149-192.

BROZE, G. J., JR. 1995. Tissue factor pathway inhibitor and the revised theory of coagulation. *Annu Rev Med,* 46**,** 103-12.

BROZE, G. J., JR. & HIGUCHI, D. A. 1996. Coagulation-dependent inhibition of fibrinolysis: role of carboxypeptidase-U and the premature lysis of clots from hemophilic plasma. *Blood,* 88**,** 3815-23.

BROZE, G. J., JR., WARREN, L. A., NOVOTNY, W. F., HIGUCHI, D. A., GIRARD, J. J. & MILETICH, J. P. 1988. The lipoprotein-associated coagulation inhibitor that inhibits the factor VII-tissue factor complex also inhibits factor Xa: insight into its possible mechanism of action. *Blood,* 71**,** 335-43.

BUDAJ, A., YUSUF, S., MEHTA, S. R., FOX, K. A., TOGNONI, G., ZHAO, F., CHROLAVICIUS, S., HUNT, D., KELTAI, M. & FRANZOSI, M. G. 2002. Benefit of clopidogrel in patients with acute coronary syndromes without ST-segment elevation in various risk groups. *Circulation,* 106**,** 1622-6.

BUGGE, T. H., FLICK, M. J., DANTON, M. J., DAUGHERTY, C. C., ROMER, J., DANO, K., CARMELIET, P., COLLEN, D. & DEGEN, J. L. 1996. Urokinase-type plasminogen activator is effective in fibrin clearance in the absence of its receptor or tissue-type plasminogen activator. *Proc Natl Acad Sci U S A,* 93**,** 5899-904.

BÜLLER, H. R., BETHUNE, C., BHANOT, S., GAILANI, D., MONIA, B. P., RASKOB, G. E., SEGERS, A., VERHAMME, P. & WEITZ, J. I. 2015. Factor XI antisense oligonucleotide for prevention of venous thrombosis. *N Engl J Med,* 372**,** 232-40.

BÜLLER, H. R., DECOUSUS, H., GROSSO, M. A., MERCURI, M., MIDDELDORP, S., PRINS, M. H., RASKOB, G. E., SCHELLONG, S. M., SCHWOCHO, L., SEGERS, A., SHI, M., VERHAMME, P. & WELLS, P. 2013. Edoxaban versus warfarin for the treatment of symptomatic venous thromboembolism. *N Engl J Med,* 369**,** 1406-15.

BUNN, H. & BAUER, K. 2011. Thrombotic Disorders. *Pathophysiology of Blood Disorders.* New York: McGraw-Hill Education.

BUTENAS, S., ORFEO, T. & MANN, K. G. 2009. Tissue factor in coagulation: Which? Where? When? *Arterioscler Thromb Vasc Biol,* 29**,** 1989-96.

BUTENAS, S., VAN 'T VEER, C. & MANN, K. G. 1997. Evaluation of the initiation phase of blood coagulation using ultrasensitive assays for serine proteases. *J Biol Chem,* 272**,** 21527-33.

BYTZER, P., CONNOLLY, S. J., YANG, S., EZEKOWITZ, M., FORMELLA, S., REILLY, P. A. & AISENBERG, J. 2013. Analysis of upper gastrointestinal adverse events among patients given dabigatran in the RE-LY trial. *Clin Gastroenterol Hepatol,* 11**,** 246-52.e1-5.

CAIRNS, J. A. & WEITZ, J. I. 2015. Transition from apixaban to warfarin--addressing excess stroke, systemic embolism, and major bleeding. *Am Heart J,* 169**,** 1-3.

CALDEIRA, D., BARRA, M., SANTOS, A. T., DE ABREU, D., PINTO, F. J., FERREIRA, J. J. & COSTA, J. 2014. Risk of drug-induced liver injury with the new oral anticoagulants: systematic review and meta-analysis. *Heart,* 100**,** 550-6.

CAMIRE, R. M. & BOS, M. H. 2009. The molecular basis of factor V and VIII procofactor activation. *J Thromb Haemost,* 7**,** 1951-61.

CAMM, A. J. & BOUNAMEAUX, H. 2011. Edoxaban: a new oral direct factor xa inhibitor. *Drugs,* 71**,** 1503-26.

CAPLICE, N. M., PANETTA, C., PETERSON, T. E., KLEPPE, L. S., MUESKE, C. S., KOSTNER, G. M., BROZE, G. J., JR. & SIMARI, R. D. 2001. Lipoprotein (a) binds and inactivates tissue factor pathway inhibitor: a novel link between lipoproteins and thrombosis. *Blood,* 98**,** 2980-7.

CARNEMOLLA, R., PATEL, K. R., ZAITSEV, S., CINES, D. B., ESMON, C. T. & MUZYKANTOV, V. R. 2012. Quantitative analysis of thrombomodulin-mediated conversion of protein C to APC: translation from in vitro to in vivo. *J Immunol Methods,* 384**,** 21-4.

CASTOLDI, E., BRUGGE, J. M., NICOLAES, G. A., GIRELLI, D., TANS, G. & ROSING, J. 2004. Impaired APC cofactor activity of factor V plays a major role in the APC resistance associated with the factor V Leiden (R506Q) and R2 (H1299R) mutations. *Blood,* 103**,** 4173-9.

CERI, H., HWANG, W. S. & CHEUNG, H. 1990. Endogenous heparin-binding lectin activity in human placenta: purification and developmental expression. *Biochem Cell Biol,* 68**,** 790-5.

CHAI-ADISAKSOPHA, C., HILLIS, C., ISAYAMA, T., LIM, W., IORIO, A. & CROWTHER, M. 2015. Mortality outcomes in patients receiving direct oral anticoagulants: a systematic review and meta-analysis of randomized controlled trials. *J Thromb Haemost,* 13**,** 2012-20.

CHAN, M. Y., COHEN, M. G., DYKE, C. K., MYLES, S. K., ABERLE, L. G., LIN, M., WALDER, J., STEINHUBL, S. R., GILCHRIST, I. C., KLEIMAN, N. S., VORCHHEIMER, D. A., CHRONOS, N., MELLONI, C., ALEXANDER, J. H., HARRINGTON, R. A., TONKENS, R. M., BECKER, R. C. & RUSCONI, C. P. 2008. Phase 1b randomized study of antidote-controlled modulation of factor IXa activity in patients with stable coronary artery disease. *Circulation,* 117**,** 2865-74.

CHAN, N., SAGER, P. T., LAWRENCE, J., ORTEL, T., REILLY, P., BERKOWITZ, S., KUBITZA, D., EIKELBOOM, J., FLORIAN, J., STOCKBRIDGE, N., ROSE, M., TEMPLE, R. & SELTZER, J. H. 2018. Is there a role for pharmacokinetic/pharmacodynamic-guided dosing for novel oral anticoagulants? *Am Heart J,* 199**,** 59-67.

CHATTERJEE, S., SARDAR, P., GIRI, J. S., GHOSH, J. & MUKHERJEE, D. 2014. Treatment discontinuations with new oral agents for long-term anticoagulation: insights from a meta-analysis of 18 randomized trials including 101,801 patients. *Mayo Clin Proc,* 89**,** 896-907.

CHEMICAL BOOK. 1998. *Desirudin* [Online]. Available: https://m.chemicalbook.com/ProductChemicalPropertiesCB61116647_EN.htm [Accessed 07/12/2019].

CHEMICAL BOOK. 2000. *Bivalirudin* [Online]. Available: https://m.chemicalbook.com/ChemicalProductProperty_EN_CB9471951.htm [Accessed 07/12/2019].

CHEMICAL BOOK. 2005. *Argatroban* [Online]. Available: https://m.chemicalbook.com/ProductChemicalPropertiesCB7738830_EN.htm [Accessed 07/12/2019].

CHEMICAL BOOK. 2008. *Dabigatran* [Online]. Available: https://m.chemicalbook.com/ProductChemicalPropertiesCB61458206_EN.htm [Accessed 07/12/2019].

CHEMICAL BOOK. 2011. *Rivaroxaban* [Online]. Available: https://m.chemicalbook.com/ProductChemicalPropertiesCB91176772_EN.htm [Accessed 07/12/2019].

CHEMICAL BOOK. 2012. *Apixaban* [Online]. Available: https://m.chemicalbook.com/ProductChemicalPropertiesCB51508586_EN.htm [Accessed 07/12/2019].

CHEMICAL BOOK. 2015. *Edoxaban* [Online]. Available: https://m.chemicalbook.com/ProductChemicalPropertiesCB21518508_EN.htm [Accessed 07/12/2019].

CHEMICAL BOOK. 2016. *Thromboxane A2* [Online]. Available: https://www.chemicalbook.com/ChemicalProductProperty_EN_CB62558523.htm [Accessed 10/01/2020].

CHEMICAL BOOK. 2017a. *Acetylsalicylic acid* [Online]. Available: https://www.chemicalbook.com/chemicalproductproperty_en_cb5114818.htm [Accessed 10/01/2020].

CHEMICAL BOOK. 2017b. *Betrixaban* [Online]. Available: https://m.chemicalbook.com/ProductChemicalPropertiesCB02500171_EN.htm [Accessed 07/12/2019].

CHEMICAL BOOK. 2017c. *Clopidogrel* [Online]. Available: https://www.chemicalbook.com/ChemicalProductProperty_EN_CB3341209.htm [Accessed 10/01/2020].

CHEMICAL BOOK. 2017d. *Prostacyclin* [Online]. Available: https://www.chemicalbook.com/ChemicalProductProperty_EN_CB02131131.htm [Accessed 10/01/2020].

CHEMICAL BOOK. 2017e. *Vorapaxar* [Online]. Available: https://www.chemicalbook.com/ProductChemicalPropertiesCB32628522_EN.htm [Accessed 10/01/2020].

CHEMICAL BOOK. 2019. *Heparin* [Online]. Available: https://www.chemicalbook.com/ChemicalProductProperty_DE_cb6766085.htm [Accessed 10/01/2020].

CHEN, V. M., AHAMED, J., VERSTEEG, H. H., BERNDT, M. C., RUF, W. & HOGG, P. J. 2006. Evidence for activation of tissue factor by an allosteric disulfide bond. *Biochemistry,* 45**,** 12020-8.

CHIQUETTE, E., AMATO, M. G. & BUSSEY, H. I. 1998. Comparison of an anticoagulation clinic with usual medical care: anticoagulation control, patient outcomes, and health care costs. *Arch Intern Med,* 158**,** 1641-7.

CHO, J., FURIE, B. C., COUGHLIN, S. R. & FURIE, B. 2008. A critical role for extracellular protein disulfide isomerase during thrombus formation in mice. *J Clin Invest,* 118**,** 1123-31.

CHO, M. S., YUN, J. E., PARK, J. J., KIM, Y. J., LEE, J., KIM, H., PARK, D. W. & NAM, G. B. 2018. Outcomes After Use of Standard- and Low-Dose Non-Vitamin K Oral Anticoagulants in Asian Patients With Atrial Fibrillation. *Stroke***,** Strokeaha118023093.

CHOAY, J., PETITOU, M., LORMEAU, J. C., SINAY, P., CASU, B. & GATTI, G. 1983. Structure-activity relationship in heparin: a synthetic pentasaccharide with high affinity for antithrombin III and eliciting high anti-factor Xa activity. *Biochem Biophys Res Commun,* 116**,** 492-9.

CHOI, S. H., SMITH, S. A. & MORRISSEY, J. H. 2011. Polyphosphate is a cofactor for the activation of factor XI by thrombin. *Blood,* 118**,** 6963-70.

CHORNENKI, N. L. J., UM, K. J., MENDOZA, P. A., SAMIENEZHAD, A., SWARUP, V., CHAI-ADISAKSOPHA, C. & SIEGAL, D. M. 2019. Risk of venous and arterial thrombosis in non-surgical patients receiving systemic tranexamic acid: A systematic review and meta-analysis. *Thromb Res,* 179**,** 81-86.

CLARKE, R. J., MAYO, G., FITZGERALD, G. A. & FITZGERALD, D. J. 1991a. Combined administration of aspirin and a specific thrombin inhibitor in man. *Circulation,* 83**,** 1510-8.

CLARKE, R. J., MAYO, G., PRICE, P. & FITZGERALD, G. A. 1991b. Suppression of thromboxane A2 but not of systemic prostacyclin by controlled-release aspirin. *N Engl J Med,* 325**,** 1137-41.

CLEMETSON, K. J. & CLEMETSON, J. M. 1995. Platelet GPIb-V-IX complex. Structure, function, physiology, and pathology. *Semin Thromb Hemost,* 21**,** 130-6.

CLINICAL AND LABORATORY STANDARDS INSTITUTE, C. 2014. *EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures* [Online]. Available: https://clsi.org/standards/products/method-evaluation/documents/ep05/ [Accessed 01/01/2020].

CLINICALTRIALS. 2016. Available: https://clinicaltrials.gov/ct2/results?term=02157272 [Accessed November 03, 2019].

CLINICALTRIALS. 2019. Available: http://www.clinicaltrials.gov/ct2/results?term=AZD-0837 [Accessed November 03, 2019].

CMA. 2019. Available: http://webprod.hc-sc.gc.ca/dpd-bdpp/info.do?lang=eng&code=84384 [Accessed November 03, 2019].

COACHROM DIAGNOSTICA GMBH 2015. BIOPHEN™ Heparin LRT. CoaChrom Diagnostica GmbH.

COCHERY-NOUVELLON, E., MERCIER, E., LISSALDE-LAVIGNE, G., DAURÉS, J. P., QUÈRÈ, I., DAUZAT, M., MARÉS, P. & GRIS, J. C. 2007. Homozygosity for the C46T polymorphism of the F12 gene is a risk factor for venous thrombosis during the first pregnancy. *J Thromb Haemost,* 5**,** 700-7.

COHEN, A. T., HARRINGTON, R. A., GOLDHABER, S. Z., HULL, R. D., WIENS, B. L., GOLD, A., HERNANDEZ, A. F. & GIBSON, C. M. 2016. Extended Thromboprophylaxis with Betrixaban in Acutely Ill Medical Patients. *N Engl J Med,* 375**,** 534-44.

COHEN, M. G., PURDY, D. A., ROSSI, J. S., GRINFELD, L. R., MYLES, S. K., ABERLE, L. H., GREENBAUM, A. B., FRY, E., CHAN, M. Y., TONKENS, R. M., ZELENKOFSKE, S., ALEXANDER, J. H., HARRINGTON, R. A., RUSCONI, C. P. & BECKER, R. C. 2010. First clinical application of an actively reversible direct factor IXa inhibitor as an anticoagulation strategy in patients undergoing percutaneous coronary intervention. *Circulation,* 122**,** 614-22.

COLLEN, D. 1980. On the regulation and control of fibrinolysis. Edward Kowalski Memorial Lecture. *Thromb Haemost,* 43**,** 77-89.

COLLER, B. S. & SHATTIL, S. J. 2008. The GPIIb/IIIa (integrin alphaIIbbeta3) odyssey: a technology-driven saga of a receptor with twists, turns, and even a bend. *Blood,* 112**,** 3011-25.

COLMAN, R. W., BAGDASARIAN, A., TALAMO, R. C., SCOTT, C. F., SEAVEY, M., GUIMARAES, J. A., PIERCE, J. V. & KAPLAN, A. P. 1975. Williams trait. Human kininogen deficiency with diminished levels of plasminogen proactivator and prekallikrein associated with abnormalities of the Hageman factor-dependent pathways. *J Clin Invest,* 56**,** 1650-62.

COLUCCI, M., BINETTI, B. M., TRIPODI, A., CHANTARANGKUL, V. & SEMERARO, N. 2004. Hyperprothrombinemia associated with prothrombin G20210A mutation inhibits plasma fibrinolysis through a TAFI-mediated mechanism. *Blood,* 103**,** 2157-61.

CONNOCK, M., STEVENS, C., FRY-SMITH, A., JOWETT, S., FITZMAURICE, D., MOORE, D. & SONG, F. 2007. Clinical effectiveness and cost-effectiveness of different models of managing long-term oral anticoagulation therapy: a systematic review and economic modelling. *Health Technol Assess,* 11**,** iii-iv, ix-66.

CONNOLLY, S. J., CROWTHER, M., EIKELBOOM, J. W., GIBSON, C. M., CURNUTTE, J. T., LAWRENCE, J. H., YUE, P., BRONSON, M. D., LU, G., CONLEY, P. B., VERHAMME, P., SCHMIDT, J., MIDDELDORP, S., COHEN, A. T., BEYER-WESTENDORF, J., ALBALADEJO, P., LOPEZ-SENDON, J., DEMCHUK, A. M., PALLIN, D. J., CONCHA, M., GOODMAN, S., LEEDS, J., SOUZA, S., SIEGAL, D. M., ZOTOVA, E., MEEKS, B., AHMAD, S., NAKAMYA, J. & MILLING, T. J., JR. 2019. Full Study Report of Andexanet Alfa for Bleeding Associated with Factor Xa Inhibitors. *N Engl J Med,* 380**,** 1326-1335.

CONNOLLY, S. J., MILLING, T. J., JR., EIKELBOOM, J. W., GIBSON, C. M., CURNUTTE, J. T., GOLD, A., BRONSON, M. D., LU, G., CONLEY, P. B., VERHAMME, P., SCHMIDT, J., MIDDELDORP, S., COHEN, A. T., BEYER-WESTENDORF, J., ALBALADEJO, P., LOPEZ-SENDON, J., GOODMAN, S., LEEDS, J., WIENS, B. L., SIEGAL, D. M., ZOTOVA, E., MEEKS, B., NAKAMYA, J., LIM, W. T. & CROWTHER, M. 2016. Andexanet Alfa for Acute Major Bleeding Associated with Factor Xa Inhibitors. *N Engl J Med,* 375**,** 1131-41.

COUGHLIN, S. R. 2000. Thrombin signalling and protease-activated receptors. *Nature,* 407**,** 258-64.

COUGHLIN, S. R. 2005. Protease-activated receptors in hemostasis, thrombosis and vascular biology. *J Thromb Haemost,* 3**,** 1800-14.

COZZI, M. R., GUGLIELMINI, G., BATTISTON, M., MOMI, S., LOMBARDI, E., MILLER, E. C., DE ZANET, D., MAZZUCATO, M., GRESELE, P. & DE MARCO, L. 2015. Visualization of nitric oxide production by individual platelets during adhesion in flowing blood. *Blood,* 125**,** 697-705.

CRUICKSHANK, M. K., LEVINE, M. N., HIRSH, J., ROBERTS, R. & SIGUENZA, M. 1991. A standard heparin nomogram for the management of heparin therapy. *Arch Intern Med,* 151**,** 333-7.

CUKER, A. & SIEGAL, D. 2015. Monitoring and reversal of direct oral anticoagulants. *Hematology,* 2015**,** 117-124.

CUNHA, M. L., BAKHTIARI, K., PETER, J., MARQUART, J. A., MEIJERS, J. C. & MIDDELDORP, S. 2015. A novel mutation in the F5 gene (factor V Amsterdam) associated with bleeding independent of factor V procoagulant function. *Blood,* 125**,** 1822-5.

DAGER, W. E., GOSSELIN, R. C., KITCHEN, S. & DWYRE, D. 2012. Dabigatran effects on the international normalized ratio, activated partial thromboplastin time, thrombin time, and fibrinogen: a multicenter, in vitro study. *Ann Pharmacother,* 46**,** 1627-36.

DAGER, W. E., GOSSELIN, R. C. & ROBERTS, A. J. 2013. Reversing dabigatran in life-threatening bleeding occurring during cardiac ablation with factor eight inhibitor bypassing activity. *Crit Care Med,* 41**,** e42-6.

DAIICHI-SANKYO. 2012. Available: http://www.daiichisankyo.com/news/detail/004033.html [Accessed November 03, 2019].

DANIELSSON, A., RAUB, E., LINDAHL, U. & BJORK, I. 1986. Role of ternary complexes, in which heparin binds both antithrombin and proteinase, in the acceleration of the reactions between antithrombin and thrombin or factor Xa. *J Biol Chem,* 261**,** 15467-73.

DEGEN, J. L., BUGGE, T. H. & GOGUEN, J. D. 2007. Fibrin and fibrinolysis in infection and host defense. *J Thromb Haemost,* 5 Suppl 1**,** 24-31.

DEL CONDE, I., SHRIMPTON, C. N., THIAGARAJAN, P. & LOPEZ, J. A. 2005. Tissue-factor-bearing microvesicles arise from lipid rafts and fuse with activated platelets to initiate coagulation. *Blood,* 106**,** 1604-11.

DELANEY, J. A., OPATRNY, L., BROPHY, J. M. & SUISSA, S. 2007. Drug drug interactions between antithrombotic medications and the risk of gastrointestinal bleeding. *Cmaj,* 177**,** 347-51.

DENTALI, F., RIVA, N., CROWTHER, M., TURPIE, A. G., LIP, G. Y. & AGENO, W. 2012. Efficacy and safety of the novel oral anticoagulants in atrial fibrillation: a systematic review and meta-analysis of the literature. *Circulation,* 126**,** 2381-91.

DESAI, J., KOLB, J. M., WEITZ, J. I. & AISENBERG, J. 2013. Gastrointestinal bleeding with the new oral anticoagulants--defining the issues and the management strategies. *Thromb Haemost,* 110**,** 205-12.

DI NISIO, M., MIDDELDORP, S. & BÜLLER, H. R. 2005. Direct thrombin inhibitors. *N Engl J Med,* 353**,** 1028-40.

DI SCIPIO, R. G., KURACHI, K. & DAVIE, E. W. 1978. Activation of human factor IX (Christmas factor). *J Clin Invest,* 61**,** 1528-38.

DIAPHARMA. Available: https://diapharma.com/coagulation/ [Accessed November 10, 2019].

DIBU, J. R., WEIMER, J. M., AHRENS, C., MANNO, E. & FRONTERA, J. A. 2016. The Role of FEIBA in Reversing Novel Oral Anticoagulants in Intracerebral Hemorrhage. *Neurocrit Care,* 24**,** 413-9.

DICKNEITE, G. & HOFFMAN, M. 2014. Reversing the new oral anticoagulants with prothrombin complex concentrates (PCCs): what is the evidence? *Thromb Haemost,* 111**,** 189-98.

DOUROS, A., AZOULAY, L., YIN, H., SUISSA, S. & RENOUX, C. 2018. Non-Vitamin K Antagonist Oral Anticoagulants and Risk of Serious Liver Injury. *J Am Coll Cardiol,* 71**,** 1105-1113.

DUBOIS, C., PANICOT-DUBOIS, L., GAINOR, J. F., FURIE, B. C. & FURIE, B. 2007. Thrombin-initiated platelet activation in vivo is vWF independent during thrombus formation in a laser injury model. *J Clin Invest,* 117**,** 953-60.

DUMKOW, L. E., VOSS, J. R., PETERS, M. & JENNINGS, D. L. 2012. Reversal of dabigatran-induced bleeding with a prothrombin complex concentrate and fresh frozen plasma. *Am J Health Syst Pharm,* 69**,** 1646-50.

DUTT, T. & TOH, C. H. 2008. The Yin-Yang of thrombin and activated protein C. *Br J Haematol,* 140**,** 505-15.

EDELBERG, J. M. & PIZZO, S. V. 1992. Lipoprotein (a) promotes plasmin inhibition by alpha 2-antiplasmin. *Biochem J,* 286 (Pt 1)**,** 79-84.

EERENBERG, E. S., KAMPHUISEN, P. W., SIJPKENS, M. K., MEIJERS, J. C., BULLER, H. R. & LEVI, M. 2011. Reversal of rivaroxaban and dabigatran by prothrombin complex concentrate: a randomized, placebo-controlled, crossover study in healthy subjects. *Circulation,* 124**,** 1573-9.

EIKELBOOM, J. W. & WEITZ, J. I. 2011. New oral anticoagulants for thromboprophylaxis in patients having hip or knee arthroplasty. *Bmj,* 342**,** c7270.

EITZMAN, D. T., CHI, L., SAGGIN, L., SCHWARTZ, R. S., LUCCHESI, B. R. & FAY, W. P. 1994. Heparin neutralization by platelet-rich thrombi. Role of platelet factor 4. *Circulation,* 89**,** 1523-9.

EMA. 2019. Available: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000829/WC500041059.pdf [Accessed November 03, 2019].

EMC. 2019. Available: http://www.medicines.org.uk/EMC/medicine/20759/SPC/Pradaxa+75+mg+hard+capsules/ [Accessed November 03, 2019].

ENGELMANN, B. & MASSBERG, S. 2013. Thrombosis as an intravascular effector of innate immunity. *Nat Rev Immunol,* 13**,** 34-45.

ERIKSSON, B. I., EKMAN, S., LINDBRATT, S., BAUR, M., BACH, D., TORHOLM, C., KALEBO, P. & CLOSE, P. 1997. Prevention of thromboembolism with use of recombinant hirudin. Results of a double-blind, multicenter trial comparing the efficacy of desirudin (Revasc) with that of unfractionated heparin in patients having a total hip replacement. *J Bone Joint Surg Am,* 79**,** 326-33.

ESMON, C. T. 1989. The roles of protein C and thrombomodulin in the regulation of blood coagulation. *J Biol Chem,* 264**,** 4743-6.

ESMON, C. T. 2003. The protein C pathway. *Chest,* 124**,** 26s-32s.

ESMON, C. T. 2006. The endothelial protein C receptor. *Curr Opin Hematol,* 13**,** 382-5.

ESMON, C. T. 2012. Protein C anticoagulant system--anti-inflammatory effects. *Semin Immunopathol,* 34**,** 127-32.

ESMON, C. T., ESMON, N. L. & HARRIS, K. W. 1982. Complex formation between thrombin and thrombomodulin inhibits both thrombin-catalyzed fibrin formation and factor V activation. *J Biol Chem,* 257**,** 7944-7.

ESMON, C. T., XU, J., GU, J. M., QU, D., LASZIK, Z., FERRELL, G., STEARNS-KUROSAWA, D. J., KUROSAWA, S., TAYLOR, F. B., JR. & ESMON, N. L. 1999. Endothelial protein C receptor. *Thromb Haemost,* 82**,** 251-8.

EVERETT, L. A., CLEUREN, A. C., KHORIATY, R. N. & GINSBURG, D. 2014. Murine coagulation factor VIII is synthesized in endothelial cells. *Blood,* 123**,** 3697-705.

FAHS, S. A., HILLE, M. T., SHI, Q., WEILER, H. & MONTGOMERY, R. R. 2014. A conditional knockout mouse model reveals endothelial cells as the principal and possibly exclusive source of plasma factor VIII. *Blood,* 123**,** 3706-13.

FATMA, N., SINGH, D. P., SHINOHARA, T. & CHYLACK, L. T., JR. 2000. Heparin's roles in stabilizing, potentiating, and transporting LEDGF into the nucleus. *Invest Ophthalmol Vis Sci,* 41**,** 2648-57.

FAVIER, R., AOKI, N. & DE MOERLOOSE, P. 2001. Congenital alpha(2)-plasmin inhibitor deficiencies: a review. *Br J Haematol,* 114**,** 4-10.

FAY, W. P., PARKER, A. C., CONDREY, L. R. & SHAPIRO, A. D. 1997. Human plasminogen activator inhibitor-1 (PAI-1) deficiency: characterization of a large kindred with a null mutation in the PAI-1 gene. *Blood,* 90**,** 204-8.

FAY, W. P., SHAPIRO, A. D., SHIH, J. L., SCHLEEF, R. R. & GINSBURG, D. 1992. Brief report: complete deficiency of plasminogen-activator inhibitor type 1 due to a frame-shift mutation. *N Engl J Med,* 327**,** 1729-33.

FDA, U. S. F. A. D. A. 2013. Available: http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/022406s004lbl.pdf [Accessed November 03, 2019].

FDA, U. S. F. A. D. A. 2015a. Available: https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/022512s028lbl.pdf [Accessed November 03, 2019].

FDA, U. S. F. A. D. A. 2015b. Available: http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/761025lbl.pdf [Accessed 03/12/2019 2019].

FDA, U. S. F. A. D. A. 2015c. Available: http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/206316lbl.pdf [Accessed November 03, 2019].

FDA, U. S. F. A. D. A. 2016a. Available: http://www.accessdata.fda.gov/drugsatfda_docs/label/2016/022406s019s020lbl.pdf [Accessed November 03, 2019].

FDA, U. S. F. A. D. A. 2016b. Available: http://www.accessdata.fda.gov/drugsatfda_docs/label/2016/202155s012lbl.pdf [Accessed November 03, 2019].

FDA, U. S. F. A. D. A. 2016c. Available: http://www.accessdata.fda.gov/drugsatfda_docs/label/2016/206316s004lbl.pdf [Accessed November 03, 2019].

FDA, U. S. F. A. D. A. 2017a. Available: https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/022406s024lbl.pdf [Accessed November 03, 2019].

FDA, U. S. F. A. D. A. 2017b. Available: https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/208383s000lbl.pdf [Accessed November 03, 2019].

FDA, U. S. F. A. D. A. 2018. Available: https://www.fda.gov/downloads/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/UCM606687.pdf [Accessed December 10, 2019].

FITZMAURICE, D. A., MURRAY, E. T., MCCAHON, D., HOLDER, R., RAFTERY, J. P., HUSSAIN, S., SANDHAR, H. & HOBBS, F. D. 2005. Self management of oral anticoagulation: randomised trial. *Bmj,* 331**,** 1057.

FLAUMENHAFT, R. 2015. Making (anti)sense of factor XI in thrombosis. *N Engl J Med,* 372**,** 277-8.

FOLEY, J. H. & NESHEIM, M. E. 2009. Soluble thrombomodulin partially corrects the premature lysis defect in FVIII-deficient plasma by stimulating the activation of thrombin activatable fibrinolysis inhibitor. *J Thromb Haemost,* 7**,** 453-9.

FOSTER, C. J., PROSSER, D. M., AGANS, J. M., ZHAI, Y., SMITH, M. D., LACHOWICZ, J. E., ZHANG, F. L., GUSTAFSON, E., MONSMA, F. J., JR., WIEKOWSKI, M. T., ABBONDANZO, S. J., COOK, D. N., BAYNE, M. L., LIRA, S. A. & CHINTALA, M. S. 2001. Molecular identification and characterization of the platelet ADP receptor targeted by thienopyridine antithrombotic drugs. *J Clin Invest,* 107**,** 1591-8.

FUENTES-PRIOR, P., IWANAGA, Y., HUBER, R., PAGILA, R., RUMENNIK, G., SETO, M., MORSER, J., LIGHT, D. R. & BODE, W. 2000. Structural basis for the anticoagulant activity of the thrombin-thrombomodulin complex. *Nature,* 404**,** 518-25.

FULCHER, C. A., GARDINER, J. E., GRIFFIN, J. H. & ZIMMERMAN, T. S. 1984. Proteolytic inactivation of human factor VIII procoagulant protein by activated human protein C and its analogy with factor V. *Blood,* 63**,** 486-9.

FURIE, B., BOUCHARD, B. A. & FURIE, B. C. 1999. Vitamin K-dependent biosynthesis of gamma-carboxyglutamic acid. *Blood,* 93**,** 1798-808.

FURIE, B. & FURIE, B. C. 2008. Mechanisms of thrombus formation. *N Engl J Med,* 359**,** 938-49.

GAGE, B. F., BASS, A. R., LIN, H., WOLLER, S. C., STEVENS, S. M., AL-HAMMADI, N., LI, J., RODRIGUEZ, T., JR., MILLER, J. P., MCMILLIN, G. A., PENDLETON, R. C., JAFFER, A. K., KING, C. R., WHIPPLE, B. D., PORCHE-SORBET, R., NAPOLI, L., MERRITT, K., THOMPSON, A. M., HYUN, G., ANDERSON, J. L., HOLLOMON, W., BARRACK, R. L., NUNLEY, R. M., MOSKOWITZ, G., DAVILA-ROMAN, V. & EBY, C. S. 2017. Effect of Genotype-Guided Warfarin Dosing on Clinical Events and Anticoagulation Control Among Patients Undergoing Hip or Knee Arthroplasty: The GIFT Randomized Clinical Trial. *Jama,* 318**,** 1115-1124.

GAILANI, D. & BROZE, G. J., JR. 1991. Factor XI activation in a revised model of blood coagulation. *Science,* 253**,** 909-12.

GARCIA-ALAMINO, J. M., WARD, A. M., ALONSO-COELLO, P., PERERA, R., BANKHEAD, C., FITZMAURICE, D. & HENEGHAN, C. J. 2010. Self-monitoring and self-management of oral anticoagulation. *Cochrane Database Syst Rev***,** Cd003839.

GARCIA, D., BARRETT, Y. C., RAMACCIOTTI, E. & WEITZ, J. I. 2013. Laboratory assessment of the anticoagulant effects of the next generation of oral anticoagulants. *J Thromb Haemost,* 11**,** 245-52.

GARCIA, D. A., BAGLIN, T. P., WEITZ, J. I. & SAMAMA, M. M. 2012. Parenteral anticoagulants: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest,* 141**,** e24S-e43S.

GARDINER, C., WILLIAMS, K., MACKIE, I. J., MACHIN, S. J. & COHEN, H. 2005. Patient self-testing is a reliable and acceptable alternative to laboratory INR monitoring. *Br J Haematol,* 128**,** 242-7.

GAWAZ, M., NEUMANN, F. J., DICKFELD, T., KOCH, W., LAUGWITZ, K. L., ADELSBERGER, H., LANGENBRINK, K., PAGE, S., NEUMEIER, D., SCHOMIG, A. & BRAND, K. 1998. Activated platelets induce monocyte chemotactic protein-1 secretion and surface expression of intercellular adhesion molecule-1 on endothelial cells. *Circulation,* 98**,** 1164-71.

GIBSON, C. M., HALABY, R., KORJIAN, S., DAABOUL, Y., ARBETTER, D. F., YEE, M. K., GOLDHABER, S. Z., HULL, R., HERNANDEZ, A. F., LU, S. P., BANDMAN, O., LEEDS, J. M., GOLD, A., HARRINGTON, R. A. & COHEN, A. T. 2017. The safety and efficacy of full- versus reduced-dose betrixaban in the Acute Medically Ill VTE (Venous Thromboembolism) Prevention With Extended-Duration Betrixaban (APEX) trial. *Am Heart J,* 185**,** 93-100.

GIESEN, P. L., RAUCH, U., BOHRMANN, B., KLING, D., ROQUE, M., FALLON, J. T., BADIMON, J. J., HIMBER, J., RIEDERER, M. A. & NEMERSON, Y. 1999. Blood-borne tissue factor: another view of thrombosis. *Proc Natl Acad Sci U S A,* 96**,** 2311-5.

GIUGLIANO, R. P., WIVIOTT, S. D., STONE, P. H., SIMON, D. I., SCHWEIGER, M. J., BOUCHARD, A., LEESAR, M. A., GOULDER, M. A., DEITCHER, S. R., MCCABE, C. H. & BRAUNWALD, E. 2007. Recombinant nematode anticoagulant protein c2 in patients with non-ST-segment elevation acute coronary syndrome: the ANTHEM-TIMI-32 trial. *J Am Coll Cardiol,* 49**,** 2398-407.

GLADSTONE, D. J., GEERTS, W. H., DOUKETIS, J., IVERS, N., HEALEY, J. S. & LEBLANC, K. 2015. How to Monitor Patients Receiving Direct Oral Anticoagulants for Stroke Prevention in Atrial Fibrillation: A Practice Tool Endorsed by Thrombosis Canada, the Canadian Stroke Consortium, the Canadian Cardiovascular Pharmacists Network, and the Canadian Cardiovascular Society. *Ann Intern Med,* 163**,** 382-5.

GODIER, A., MICLOT, A., LE BONNIEC, B., DURAND, M., FISCHER, A. M., EMMERICH, J., MARCHAND-LEROUX, C., LECOMPTE, T. & SAMAMA, C. M. 2012. Evaluation of prothrombin complex concentrate and recombinant activated factor VII to reverse rivaroxaban in a rabbit model. *Anesthesiology,* 116**,** 94-102.

GOSSELIN, R. C., ADCOCK, D. M., BATES, S. M., DOUXFILS, J., FAVALORO, E. J., GOUIN-THIBAULT, I., GUILLERMO, C., KAWAI, Y., LINDHOFF-LAST, E. & KITCHEN, S. 2018. International Council for Standardization in Haematology (ICSH) Recommendations for Laboratory Measurement of Direct Oral Anticoagulants. *Thromb Haemost,* 118**,** 437-450.

GOSSELIN, R. C., FRANCART, S. J., HAWES, E. M., MOLL, S., DAGER, W. E. & ADCOCK, D. M. 2015. Heparin-Calibrated Chromogenic Anti-Xa Activity Measurements in Patients Receiving Rivaroxaban: Can This Test Be Used to Quantify Drug Level? *Ann Pharmacother,* 49**,** 777-83.

GRAETZ, T. J., TELLOR, B. R., SMITH, J. R. & AVIDAN, M. S. 2011. Desirudin: a review of the pharmacology and clinical application for the prevention of deep vein thrombosis. *Expert Rev Cardiovasc Ther,* 9**,** 1101-9.

GRANGER, C. B., LOPES, R. D., HANNA, M., ANSELL, J., HYLEK, E. M., ALEXANDER, J. H., THOMAS, L., WANG, J., BAHIT, M. C., VERHEUGT, F., LAWRENCE, J., XAVIER, D. & WALLENTIN, L. 2015. Clinical events after transitioning from apixaban versus warfarin to warfarin at the end of the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) trial. *Am Heart J,* 169**,** 25-30.

GRAY, D. R., GARABEDIAN-RUFFALO, S. M. & CHRETIEN, S. D. 2007. Cost-justification of a clinical pharmacist-managed anticoagulation clinic. *Ann Pharmacother,* 41**,** 496-501.

GREENBERG, C. S., ACHYUTHAN, K. E. & FENTON, J. W., 2ND 1987. Factor XIIIa formation promoted by complexing of alpha-thrombin, fibrin, and plasma factor XIII. *Blood,* 69**,** 867-71.

GREINACHER, A., VÖLPEL, H., JANSSENS, U., HACH-WUNDERLE, V., KEMKES-MATTHES, B., EICHLER, P., MUELLER-VELTEN, H. G. & PÖTZSCH, B. 1999. Recombinant hirudin (lepirudin) provides safe and effective anticoagulation in patients with heparin-induced thrombocytopenia: a prospective study. *Circulation,* 99**,** 73-80.

GRIFFIN, J. H., MOSNIER, L. O., FERNANDEZ, J. A. & ZLOKOVIC, B. V. 2016. 2016 Scientific Sessions Sol Sherry Distinguished Lecturer in Thrombosis: Thrombotic Stroke: Neuroprotective Therapy by Recombinant-Activated Protein C. *Arterioscler Thromb Vasc Biol,* 36**,** 2143-2151.

GRIGAS, D., HODOUS, T., WELTON, W. & PEARSON, D. 1978. Medical grand rounds from the West Virginia University Hospital. Sarcoidosis. *W V Med J,* 74**,** 242-7.

GROSSER, T., FRIES, S. & FITZGERALD, G. A. 2006. Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities. *J Clin Invest,* 116**,** 4-15.

GRUBER, A., MARZEK, U. M., BUETEHORN, U. & AL., E. 2009. Management of bleeding in patients receiving direct oral anticoagulants. *Haematologica,* 92.

GRÜTTER, M. G., PRIESTLE, J. P., RAHUEL, J., GROSSENBACHER, H., BODE, W., HOFSTEENGE, J. & STONE, S. R. 1990. Crystal structure of the thrombin-hirudin complex: a novel mode of serine protease inhibition. *Embo j,* 9**,** 2361-5.

GSKSOURCE. 2014. Available: https://www.gsksource.com/gskprm/htdocs/documents/ARGATROBAN.PDF [Accessed November 03, 2019].

GUERTIN, K. R. & CHOI, Y. M. 2007. The discovery of the Factor Xa inhibitor otamixaban: from lead identification to clinical development. *Curr Med Chem,* 14**,** 2471-81.

GULSETH, M. P., WITTKOWSKY, A. K., FANIKOS, J., SPINLER, S. A., DAGER, W. E. & NUTESCU, E. A. 2011. Dabigatran etexilate in clinical practice: confronting challenges to improve safety and effectiveness. *Pharmacotherapy,* 31**,** 1232-49.

HA, J. T., NEUEN, B. L., CHENG, L. P., JUN, M., TOYAMA, T., GALLAGHER, M. P., JARDINE, M. J., SOOD, M. M., GARG, A. X., PALMER, S. C., MARK, P. B., WHEELER, D. C., JHA, V., FREEDMAN, B., JOHNSON, D. W., PERKOVIC, V. & BADVE, S. V. 2019. Benefits and Harms of Oral Anticoagulant Therapy in Chronic Kidney Disease: A Systematic Review and Meta-analysis. *Ann Intern Med,* 171**,** 181-189.

HAGEDORN, I., SCHMIDBAUER, S., PLEINES, I., KLEINSCHNITZ, C., KRONTHALER, U., STOLL, G., DICKNEITE, G. & NIESWANDT, B. 2010. Factor XIIa inhibitor recombinant human albumin Infestin-4 abolishes occlusive arterial thrombus formation without affecting bleeding. *Circulation,* 121**,** 1510-7.

HALL, S. W., NAGASHIMA, M., ZHAO, L., MORSER, J. & LEUNG, L. L. 1999. Thrombin interacts with thrombomodulin, protein C, and thrombin-activatable fibrinolysis inhibitor via specific and distinct domains. *J Biol Chem,* 274**,** 25510-6.

HALLAK, H. O., WEDLUND, P. J., MODI, M. W., PATEL, I. H., LEWIS, G. L., WOODRUFF, B. & TROWBRIDGE, A. A. 1993. High clearance of (S)-warfarin in a warfarin-resistant subject. *Br J Clin Pharmacol,* 35**,** 327-30.

HALTER, J. B., OUSLANDER, J. G., STUDENSKI, S., HIGH, K. P., ASTHANA, S., SUPIANO, M. A., RITCHIE, C., HAZZARD, W. R. & WOOLARD, N. F. 2017. *Hazzard's Geriatric Medicine and Gerontology*. 7 ed.: McGraw-Hill Medical Education.

HANSSON, K. & STENFLO, J. 2005. Post-translational modifications in proteins involved in blood coagulation. *J Thromb Haemost,* 3**,** 2633-48.

HARRINGTON, R. A., BECKER, R. C., CANNON, C. P., GUTTERMAN, D., LINCOFF, A. M., POPMA, J. J., STEG, G., GUYATT, G. H. & GOODMAN, S. G. 2008. Antithrombotic therapy for non-ST-segment elevation acute coronary syndromes: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest,* 133**,** 670s-707s.

HARRISON, P., SAVIDGE, G. F. & CRAMER, E. M. 1990. The origin and physiological relevance of alpha-granule adhesive proteins. *Br J Haematol,* 74**,** 125-30.

HAUEL, N. H., NAR, H., PRIEPKE, H., RIES, U., STASSEN, J. M. & WIENEN, W. 2002. Structure-based design of novel potent nonpeptide thrombin inhibitors. *J Med Chem,* 45**,** 1757-66.

HENEGHAN, C., ALONSO-COELLO, P., GARCIA-ALAMINO, J. M., PERERA, R., MEATS, E. & GLASZIOU, P. 2006. Self-monitoring of oral anticoagulation: a systematic review and meta-analysis. *Lancet,* 367**,** 404-11.

HENEGHAN, C. J., GARCIA-ALAMINO, J. M., SPENCER, E. A., WARD, A. M., PERERA, R., BANKHEAD, C., ALONSO-COELLO, P., FITZMAURICE, D., MAHTANI, K. R. & ONAKPOYA, I. J. 2016. Self-monitoring and self-management of oral anticoagulation. *Cochrane Database Syst Rev,* 7**,** Cd003839.

HIRSH, J., BAUER, K. A., DONATI, M. B., GOULD, M., SAMAMA, M. M. & WEITZ, J. I. 2008. Parenteral anticoagulants: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest,* 133**,** 141s-159s.

HIRSH, J. & LEVINE, M. N. 1992. Low molecular weight heparin. *Blood,* 79**,** 1-17.

HIRSH, J. & WEITZ, J. I. 1999. New antithrombotic agents. *Lancet,* 353**,** 1431-6.

HODIVALA-DILKE, K. M., MCHUGH, K. P., TSAKIRIS, D. A., RAYBURN, H., CROWLEY, D., ULLMAN-CULLERE, M., ROSS, F. P., COLLER, B. S., TEITELBAUM, S. & HYNES, R. O. 1999. Beta3-integrin-deficient mice are a model for Glanzmann thrombasthenia showing placental defects and reduced survival. *J Clin Invest,* 103**,** 229-38.

HOLBROOK, A. M., PEREIRA, J. A., LABIRIS, R., MCDONALD, H., DOUKETIS, J. D., CROWTHER, M. & WELLS, P. S. 2005. Systematic overview of warfarin and its drug and food interactions. *Arch Intern Med,* 165**,** 1095-106.

HOLLOPETER, G., JANTZEN, H. M., VINCENT, D., LI, G., ENGLAND, L., RAMAKRISHNAN, V., YANG, R. B., NURDEN, P., NURDEN, A., JULIUS, D. & CONLEY, P. B. 2001. Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature,* 409**,** 202-7.

HONICKEL, M., MARON, B., VAN RYN, J., BRAUNSCHWEIG, T., TEN CATE, H., SPRONK, H. M., ROSSAINT, R. & GROTTKE, O. 2016. Therapy with activated prothrombin complex concentrate is effective in reducing dabigatran-associated blood loss in a porcine polytrauma model. *Thromb Haemost,* 115**,** 271-84.

HOYLAERTS, M., RIJKEN, D. C., LIJNEN, H. R. & COLLEN, D. 1982. Kinetics of the activation of plasminogen by human tissue plasminogen activator. Role of fibrin. *J Biol Chem,* 257**,** 2912-9.

HTUN, K. T., MCFADYEN, J. & TRAN, H. A. 2014. The successful management of dabigatran-associated critical end-organ bleeding with recombinant factor VIIa. *Ann Hematol,* 93**,** 1785-6.

HUANG, Z. F., HIGUCHI, D., LASKY, N. & BROZE, G. J., JR. 1997. Tissue factor pathway inhibitor gene disruption produces intrauterine lethality in mice. *Blood,* 90**,** 944-51.

HULL, R. D., RASKOB, G. E., ROSENBLOOM, D., LEMAIRE, J., PINEO, G. F., BAYLIS, B., GINSBERG, J. S., PANJU, A. A., BRILL-EDWARDS, P. & BRANT, R. 1992. Optimal therapeutic level of heparin therapy in patients with venous thrombosis. *Arch Intern Med,* 152**,** 1589-95.

HULSE, M. L. 1996. Warfarin resistance: diagnosis and therapeutic alternatives. *Pharmacotherapy,* 16**,** 1009-17.

HUR, W. S., MAZINANI, N., LU, X. J., BRITTON, H. M., BYRNES, J. R., WOLBERG, A. S. & KASTRUP, C. J. 2015. Coagulation factor XIIIa is inactivated by plasmin. *Blood,* 126**,** 2329-37.

HUSTED, S., DE CATERINA, R., ANDREOTTI, F., ARNESEN, H., BACHMANN, F., HUBER, K., JESPERSEN, J., KRISTENSEN, S. D., LIP, G. Y., MORAIS, J., RASMUSSEN, L. H., SIEGBAHN, A., STOREY, R. F. & WEITZ, J. I. 2014. Non-vitamin K antagonist oral anticoagulants (NOACs): No longer new or novel. *Thromb Haemost,* 111**,** 781-2.

HUSTED, S. & LIP, G. Y. 2014. Response to Ansell et al. "Non-vitamin K antagonist oral anticoagulants (NOACs): no longer new or novel". (Thromb Haemost 2014; 112: 841). *Thromb Haemost,* 112**,** 842.

IQBAL, M. B., JOHNS, M., CAO, J., LIU, Y., YU, S. C., HYDE, G. D., LAFFAN, M. A., MARCHESE, F. P., CHO, S. H., CLARK, A. R., GAVINS, F. N., WOOLLARD, K. J., BLACKSHEAR, P. J., MACKMAN, N., DEAN, J. L., BOOTHBY, M. & HASKARD, D. O. 2014. PARP-14 combines with tristetraprolin in the selective posttranscriptional control of macrophage tissue factor expression. *Blood,* 124**,** 3646-55.

ISERMANN, B., HENDRICKSON, S. B., ZOGG, M., WING, M., CUMMISKEY, M., KISANUKI, Y. Y., YANAGISAWA, M. & WEILER, H. 2001. Endothelium-specific loss of murine thrombomodulin disrupts the protein C anticoagulant pathway and causes juvenile-onset thrombosis. *J Clin Invest,* 108**,** 537-46.

IVANCIU, L., KRISHNASWAMY, S. & CAMIRE, R. M. 2014. New insights into the spatiotemporal localization of prothrombinase in vivo. *Blood,* 124**,** 1705-14.

JAFFER, I. 2014. Antithrombotic Therapy.

JENNINGS, I., KITCHEN, D., KEELING, D., FITZMAURICE, D. & HENEGHAN, C. 2014. Patient self-testing and self-management of oral anticoagulation with vitamin K antagonists: guidance from the British Committee for Standards in Haematology. *Br J Haematol,* 167**,** 600-7.

JESTY, J., SPENCER, A. K. & NEMERSON, Y. 1974. The mechanism of activation of factor X. Kinetic control of alternative pathways leading to the formation of activated factor X. *J Biol Chem,* 249**,** 5614-22.

JESTY, J., WUN, T. C. & LORENZ, A. 1994. Kinetics of the inhibition of factor Xa and the tissue factor-factor VIIa complex by the tissue factor pathway inhibitor in the presence and absence of heparin. *Biochemistry,* 33**,** 12686-94.

JONES, M., MCEWAN, P., MORGAN, C. L., PETERS, J. R., GOODFELLOW, J. & CURRIE, C. J. 2005. Evaluation of the pattern of treatment, level of anticoagulation control, and outcome of treatment with warfarin in patients with non-valvar atrial fibrillation: a record linkage study in a large British population. *Heart,* 91**,** 472-7.

JORDAN, R. E., OOSTA, G. M., GARDNER, W. T. & ROSENBERG, R. D. 1980. The kinetics of hemostatic enzyme-antithrombin interactions in the presence of low molecular weight heparin. *J Biol Chem,* 255**,** 10081-90.

JOVE, M., MASLANKA, M., MINKOWITZ, H. S. & JAFFER, A. K. 2014. Safety of desirudin in thrombosis prevention after total knee arthroplasty: the DESIR-ABLE study. *Am J Ther,* 21**,** 496-9.

JOWETT, S., BRYAN, S., MURRAY, E., MCCAHON, D., RAFTERY, J., HOBBS, F. D. & FITZMAURICE, D. 2006. Patient self-management of anticoagulation therapy: a trial-based cost-effectiveness analysis. *Br J Haematol,* 134**,** 632-9.

JUURLINK, D. N. 2007. Drug interactions with warfarin: what clinicians need to know. *Cmaj,* 177**,** 369-71.

KAHN, M. L., ZHENG, Y. W., HUANG, W., BIGORNIA, V., ZENG, D., MOFF, S., FARESE, R. V., JR., TAM, C. & COUGHLIN, S. R. 1998. A dual thrombin receptor system for platelet activation. *Nature,* 394**,** 690-4.

KALAFATIS, M., BERTINA, R. M., RAND, M. D. & MANN, K. G. 1995. Characterization of the molecular defect in factor VR506Q. *J Biol Chem,* 270**,** 4053-7.

KAMIKUBO, Y., MENDOLICCHIO, G. L., ZAMPOLLI, A., MARCHESE, P., ROTHMEIER, A. S., ORJE, J. N., GALE, A. J., KRISHNASWAMY, S., GRUBER, A., OSTERGAARD, H., PETERSEN, L. C., RUF, W. & RUGGERI, Z. M. 2017. Selective factor VIII activation by the tissue factor-factor VIIa-factor Xa complex. *Blood,* 130**,** 1661-1670.

KANE, W. H., LINDHOUT, M. J., JACKSON, C. M. & MAJERUS, P. W. 1980. Factor Va-dependent binding of factor Xa to human platelets. *J Biol Chem,* 255**,** 1170-4.

KEARON, C., COMP, P., DOUKETIS, J., ROYDS, R., YAMADA, K. & GENT, M. 2005. Dose-response study of recombinant human soluble thrombomodulin (ART-123) in the prevention of venous thromboembolism after total hip replacement. *J Thromb Haemost,* 3**,** 962-8.

KEARON, C., KAHN, S. R., AGNELLI, G., GOLDHABER, S., RASKOB, G. E. & COMEROTA, A. J. 2008. Antithrombotic therapy for venous thromboembolic disease: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest,* 133**,** 454s-545s.

KHO, S., BARBER, B. E., JOHAR, E., ANDRIES, B., POESPOPRODJO, J. R., KENANGALEM, E., PIERA, K. A., EHMANN, A., PRICE, R. N., WILLIAM, T., WOODBERRY, T., FOOTE, S., MINIGO, G., YEO, T. W., GRIGG, M. J., ANSTEY, N. M. & MCMORRAN, B. J. 2018. Platelets kill circulating parasites of all major Plasmodium species in human malaria. *Blood,* 132**,** 1332-1344.

KIMMEL, S. E., FRENCH, B., KASNER, S. E., JOHNSON, J. A., ANDERSON, J. L., GAGE, B. F., ROSENBERG, Y. D., EBY, C. S., MADIGAN, R. A., MCBANE, R. B., ABDEL-RAHMAN, S. Z., STEVENS, S. M., YALE, S., MOHLER, E. R., 3RD, FANG, M. C., SHAH, V., HORENSTEIN, R. B., LIMDI, N. A., MULDOWNEY, J. A., 3RD, GUJRAL, J., DELAFONTAINE, P., DESNICK, R. J., ORTEL, T. L., BILLETT, H. H., PENDLETON, R. C., GELLER, N. L., HALPERIN, J. L., GOLDHABER, S. Z., CALDWELL, M. D., CALIFF, R. M. & ELLENBERG, J. H. 2013. A pharmacogenetic versus a clinical algorithm for warfarin dosing. *N Engl J Med,* 369**,** 2283-93.

KOJIMA, H., NEWTON-NASH, D., WEISS, H. J., ZHAO, J., SIMS, P. J. & WIEDMER, T. 1994. Production and characterization of transformed B-lymphocytes expressing the membrane defect of Scott syndrome. *J Clin Invest,* 94**,** 2237-44.

KOLEV, K. & MACHOVICH, R. 2003. Molecular and cellular modulation of fibrinolysis. *Thromb Haemost,* 89**,** 610-21.

KONIGSBERG, W., KIRCHHOFER, D., RIEDERER, M. A. & NEMERSON, Y. 2001. The TF:VIIa complex: clinical significance, structure-function relationships and its role in signaling and metastasis. *Thromb Haemost,* 86**,** 757-71.

KORNBERG, A., RAO, N. N. & AULT-RICHE, D. 1999. Inorganic polyphosphate: a molecule of many functions. *Annu Rev Biochem,* 68**,** 89-125.

KOTHARI, H., NAYAK, R. C., RAO, L. V. & PENDURTHI, U. R. 2010. Cystine 186-cystine 209 disulfide bond is not essential for the procoagulant activity of tissue factor or for its de-encryption. *Blood,* 115**,** 4273-83.

KRISHNASWAMY, S. 2013. The transition of prothrombin to thrombin. *J Thromb Haemost,* 11 Suppl 1**,** 265-76.

KROLL, M. H. & SCHAFER, A. I. 1989. Biochemical mechanisms of platelet activation. *Blood,* 74**,** 1181-95.

KUANG, S. Q., HASHAM, S., PHILLIPS, M. D., WOLF, D., WAN, Y., THIAGARAJAN, P. & MILEWICZ, D. M. 2001. Characterization of a novel autosomal dominant bleeding disorder in a large kindred from east Texas. *Blood,* 97**,** 1549-54.

LAKATOS, B., STOECKLE, M., ELZI, L., BATTEGAY, M. & MARZOLINI, C. 2014. Gastrointestinal bleeding associated with rivaroxaban administration in a treated patient infected with human immunodeficiency virus. *Swiss Med Wkly,* 144**,** w13906.

LAMBOURNE, M. D., ELTRINGHAM-SMITH, L. J., GATAIANCE, S., ARNOLD, D. M., CROWTHER, M. A. & SHEFFIELD, W. P. 2012. Prothrombin complex concentrates reduce blood loss in murine coagulopathy induced by warfarin, but not in that induced by dabigatran etexilate. *J Thromb Haemost,* 10**,** 1830-40.

LÄMMLE, B., WUILLEMIN, W. A., HUBER, I., KRAUSKOPF, M., ZÜRCHER, C., PFLUGSHAUPT, R. & FURLAN, M. 1991. Thromboembolism and bleeding tendency in congenital factor XII deficiency--a study on 74 subjects from 14 Swiss families. *Thromb Haemost,* 65**,** 117-21.

LANE, D. A., PHILIPPOU, H. & HUNTINGTON, J. A. 2005. Directing thrombin. *Blood,* 106**,** 2605-12.

LAU, W. C., CHAN, E. W., CHEUNG, C. L., SING, C. W., MAN, K. K., LIP, G. Y., SIU, C. W., LAM, J. K., LEE, A. C. & WONG, I. C. 2017a. Association Between Dabigatran vs Warfarin and Risk of Osteoporotic Fractures Among Patients With Nonvalvular Atrial Fibrillation. *Jama,* 317**,** 1151-1158.

LAU, W. C. Y., WONG, I. C. K. & CHAN, E. W. 2017b. Osteoporotic Fractures Associated With Dabigatran vs Warfarin-Reply. *Jama,* 318**,** 91.

LAULICHT, B., BAKHRU, S., JIANG, X., CHEN, L., PAN, D., GROSSO, M., MORISHIMA, Y., BROWN, K., MASUMOTO, H., COSTIN, J. & STEINER, S. 2013. Antidote for new oral anticoagulants: Mechanism of action and binding specificity of PER977. *ISTH Abstract.*

LAUX, V., PERZBORN, E., HEITMEIER, S., VON DEGENFELD, G., DITTRICH-WENGENROTH, E., BUCHMÜLLER, A., GERDES, C. & MISSELWITZ, F. 2009. Direct inhibitors of coagulation proteins - the end of the heparin and low-molecular-weight heparin era for anticoagulant therapy? *Thromb Haemost,* 102**,** 892-9.

LECHTENBERG, B. C., MURRAY-RUST, T. A., JOHNSON, D. J., ADAMS, T. E., KRISHNASWAMY, S., CAMIRE, R. M. & HUNTINGTON, J. A. 2013. Crystal structure of the prothrombinase complex from the venom of Pseudonaja textilis. *Blood,* 122**,** 2777-83.

LEE, A., AGNELLI, G., BÜLLER, H., GINSBERG, J., HEIT, J., ROTE, W., VLASUK, G., COSTANTINI, L., JULIAN, J., COMP, P., VAN DER MEER, J., PIOVELLA, F., RASKOB, G. & GENT, M. 2001. Dose-response study of recombinant factor VIIa/tissue factor inhibitor recombinant nematode anticoagulant protein c2 in prevention of postoperative venous thromboembolism in patients undergoing total knee replacement. *Circulation,* 104**,** 74-8.

LEFKOVITS, J. & TOPOL, E. J. 1994. Direct thrombin inhibitors in cardiovascular medicine. *Circulation,* 90**,** 1522-36.

LEFRERE, J. J., GUYON, F., HORELLOU, M. H., CONARD, J. & SAMAMA, M. 1986. [Resistance to vitamin K antagonists. 6 cases]. *Ann Med Interne (Paris),* 137**,** 384-90.

LEGER, A. J., COVIC, L. & KULIOPULOS, A. 2006. Protease-activated receptors in cardiovascular diseases. *Circulation,* 114**,** 1070-7.

LEHMAN, S. J. & CHEW, D. P. 2006. Bivalirudin in percutaneous coronary intervention. *Vasc Health Risk Manag,* 2**,** 357-63.

LEUNG, L. 2019. Hemostatic control mechanisms and termination of clotting. UpToDate, Inc. .

LEUNG, L. & MORSER, J. 2012. Hemostasis and Vascular Inflammation. *In:* HOFFMAN, G. S., WEYAND, C. M., LANGFORD, C. A. & AL., E. (eds.) *Inflammatory Diseases of Blood Vessels.* Oxford, UK: Wiley-Blackwell.

LEUNG, L. L., MYLES, T., NISHIMURA, T., SONG, J. J. & ROBINSON, W. H. 2008. Regulation of tissue inflammation by thrombin-activatable carboxypeptidase B (or TAFI). *Mol Immunol,* 45**,** 4080-3.

LEVI, M., VAN DER POLL, T. & SCHULTZ, M. 2012. Systemic versus localized coagulation activation contributing to organ failure in critically ill patients. *Semin Immunopathol,* 34**,** 167-79.

LEVY, J. H., AGENO, W., CHAN, N. C., CROWTHER, M., VERHAMME, P. & WEITZ, J. I. 2016. When and how to use antidotes for the reversal of direct oral anticoagulants: guidance from the SSC of the ISTH. *J Thromb Haemost,* 14**,** 623-7.

LIAKONI, E., RÄTZ BRAVO, A. E., TERRACCIANO, L., HEIM, M. & KRÄHENBÜHL, S. 2014. Symptomatic hepatocellular liver injury with hyperbilirubinemia in two patients treated with rivaroxaban. *JAMA Intern Med,* 174**,** 1683-6.

LIANG, H. P. H., KERSCHEN, E. J., BASU, S. & AL., E. 2018. Coagulation factor V mediates inhibition of tissue factor signaling by activated protein C in mice. *Blood,* 131**,** 2509.

LIANG, Y., FU, Y., QI, R., WANG, M., YANG, N., HE, L., YU, F., ZHANG, J., YUN, C. H., WANG, X., LIU, J. & KONG, W. 2015. Cartilage oligomeric matrix protein is a natural inhibitor of thrombin. *Blood,* 126**,** 905-14.

LIMDI, N. A., LIMDI, M. A., CAVALLARI, L., ANDERSON, A. M., CROWLEY, M. R., BAIRD, M. F., ALLON, M. & BEASLEY, T. M. 2010. Warfarin dosing in patients with impaired kidney function. *Am J Kidney Dis,* 56**,** 823-31.

LINCOFF, A. M., MEHRAN, R., POVSIC, T. J., ZELENKOFSKE, S. L., HUANG, Z., ARMSTRONG, P. W., STEG, P. G., BODE, C., COHEN, M. G., BULLER, C., LAANMETS, P., VALGIMIGLI, M., MARANDI, T., FRIDRICH, V., CANTOR, W. J., MERKELY, B., LOPEZ-SENDON, J., CORNEL, J.

H., KASPRZAK, J. D., ASCHERMANN, M., GUETTA, V., MORAIS, J., SINNAEVE, P. R., HUBER, K., STABLES, R., SELLERS, M. A., BORGMAN, M., GLENN, L., LEVINSON, A. I., LOPES, R. D., HASSELBLAD, V., BECKER, R. C. & ALEXANDER, J. H. 2016. Effect of the REG1 anticoagulation system versus bivalirudin on outcomes after percutaneous coronary intervention (REGULATE-PCI): a randomised clinical trial. *Lancet,* 387**,** 349-356.

LISMAN, T., MOSNIER, L. O., LAMBERT, T., MAUSER-BUNSCHOTEN, E. P., MEIJERS, J. C., NIEUWENHUIS, H. K. & DE GROOT, P. G. 2002. Inhibition of fibrinolysis by recombinant factor VIIa in plasma from patients with severe hemophilia A. *Blood,* 99**,** 175-9.

LOPES, R. D., HOROWITZ, J. D., GARCIA, D. A., CROWTHER, M. A. & HYLEK, E. M. 2011. Warfarin and acetaminophen interaction: a summary of the evidence and biologic plausibility. *Blood,* 118**,** 6269-73.

LÓPEZ, J. A., ANDREWS, R. K., AFSHAR-KHARGHAN, V. & BERNDT, M. C. 1998. Bernard-Soulier syndrome. *Blood,* 91**,** 4397-418.

LU, G., DEGUZMAN, F. R., HOLLENBACH, S. J., KARBARZ, M. J., ABE, K., LEE, G., LUAN, P., HUTCHALEELAHA, A., INAGAKI, M., CONLEY, P. B., PHILLIPS, D. R. & SINHA, U. 2013. A specific antidote for reversal of anticoagulation by direct and indirect inhibitors of coagulation factor Xa. *Nat Med,* 19**,** 446-51.

MAHAFFEY, K. W., WOJDYLA, D. M., CARROLL, K., BECKER, R. C., STOREY, R. F., ANGIOLILLO, D. J., HELD, C., CANNON, C. P., JAMES, S., PIEPER, K. S., HORROW, J., HARRINGTON, R. A. & WALLENTIN, L. 2011. Ticagrelor compared with clopidogrel by geographic region in the Platelet Inhibition and Patient Outcomes (PLATO) trial. *Circulation,* 124**,** 544-54.

MAJEED, A., AGREN, A., HOLMSTROM, M., BRUZELIUS, M., CHAIRETI, R., ODEBERG, J., HEMPEL, E. L., MAGNUSSON, M., FRISK, T. & SCHULMAN, S. 2017. Management of rivaroxaban- or apixaban-associated major bleeding with prothrombin complex concentrates: a cohort study. *Blood,* 130**,** 1706-1712.

MAMMEN, E. F. 1998. Antithrombin: its physiological importance and role in DIC. *Semin Thromb Hemost,* 24**,** 19-25.

MANDAL, S. K., PENDURTHI, U. R. & RAO, L. V. 2006. Cellular localization and trafficking of tissue factor. *Blood,* 107**,** 4746-53.

MANN, K. G., BRUMMEL-ZIEDINS, K., ORFEO, T. & BUTENAS, S. 2006. Models of blood coagulation. *Blood Cells Mol Dis,* 36**,** 108-17.

MANN, K. G., KRISHNASWAMY, S. & LAWSON, J. H. 1992. Surface-dependent hemostasis. *Semin Hematol,* 29**,** 213-26.

MANN, K. G., NESHEIM, M. E., HIBBARD, L. S. & TRACY, P. B. 1981. The role of factor V in the assembly of the prothrombinase complex. *Ann N Y Acad Sci,* 370**,** 378-88.

MANNUCCI, P. M. 2013. Thromboprophylaxis in the oldest old with atrial fibrillation: between Scylla and Charybdis. *Eur J Intern Med,* 24**,** 285-7.

MARCUM, J. A., MCKENNEY, J. B. & ROSENBERG, R. D. 1984. Acceleration of thrombin-antithrombin complex formation in rat hindquarters via heparinlike molecules bound to the endothelium. *J Clin Invest,* 74**,** 341-50.

MARGETIC, S., CELAP, I., DELIC BRKLJACIC, D., PAVLOVIC, N., SUPRAHA GORETA, S., KOBASIC, I., LOVRENCIC-HUZJAN, A. & BASIC KES, V. 2020. Chromogenic anti-FXa assay calibrated with

low molecular weight heparin in patients treated with rivaroxaban and apixaban: possibilities and limitations. *Biochem Med (Zagreb),* 30**,** 010702.

MARLU, R., HODAJ, E., PARIS, A., ALBALADEJO, P., CRACOWSKI, J. L. & PERNOD, G. 2012. Effect of non-specific reversal agents on anticoagulant activity of dabigatran and rivaroxaban: a randomised crossover ex vivo study in healthy volunteers. *Thromb Haemost,* 108**,** 217-24.

MARTIN, K., BEYER-WESTENDORF, J., DAVIDSON, B. L., HUISMAN, M. V., SANDSET, P. M. & MOLL, S. 2016. Use of the direct oral anticoagulants in obese patients: guidance from the SSC of the ISTH. *J Thromb Haemost,* 14**,** 1308-13.

MASUKO, S. & LINHARDT, R. J. 2012. Chemoenzymatic synthesis of the next generation of ultralow MW heparin therapeutics. *Future Med Chem,* 4**,** 289-96.

MEHTA, S. R., BASSAND, J. P., CHROLAVICIUS, S., DIAZ, R., EIKELBOOM, J. W., FOX, K. A., GRANGER, C. B., JOLLY, S., JOYNER, C. D., RUPPRECHT, H. J., WIDIMSKY, P., AFZAL, R., POGUE, J. & YUSUF, S. 2010. Dose comparisons of clopidogrel and aspirin in acute coronary syndromes. *N Engl J Med,* 363**,** 930-42.

MEHTA, S. R., YUSUF, S., PETERS, R. J., BERTRAND, M. E., LEWIS, B. S., NATARAJAN, M. K., MALMBERG, K., RUPPRECHT, H., ZHAO, F., CHROLAVICIUS, S., COPLAND, I. & FOX, K. A. 2001. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. *Lancet,* 358**,** 527-33.

METLAY, J. P., HENNESSY, S., LOCALIO, A. R., HAN, X., YANG, W., COHEN, A., LEONARD, C. E., HAYNES, K., KIMMEL, S. E., FELDMAN, H. I. & STROM, B. L. 2008. Patient reported receipt of medication instructions for warfarin is associated with reduced risk of serious bleeding events. *J Gen Intern Med,* 23**,** 1589-94.

MILLS, I., CVITAŠ, T., HOMANN, K., KALLAY, N. & KUCHITSU, K. 1993. Quantities, Units and Symbols. *Physical Chemistry, Green Book.* International Union of Pure and Applied Chemistry

MINNEMA, M. C., FRIEDERICH, P. W., LEVI, M., VON DEM BORNE, P. A., MOSNIER, L. O., MEIJERS, J. C., BIEMOND, B. J., HACK, C. E., BOUMA, B. N. & TEN CATE, H. 1998. Enhancement of rabbit jugular vein thrombolysis by neutralization of factor XI. In vivo evidence for a role of factor XI as an anti-fibrinolytic factor. *J Clin Invest,* 101**,** 10-4.

MOLL, S., LINDLEY, C., PESCATORE, S., MORRISON, D., TSURUTA, K., MOHRI, M., SERADA, M., SATA, M., SHIMIZU, H., YAMADA, K. & WHITE, G. C., 2ND 2004. Phase I study of a novel recombinant human soluble thrombomodulin, ART-123. *J Thromb Haemost,* 2**,** 1745-51.

MOLTON, S., DUNOIS, C. & AMIRAL, J. Neutralization of heparin interferences in DOAC measurement with Anti-Xa chromogenic assays. ISTH 2019, 2019 Melbourne, Australia.

MONCADA, S. & HIGGS, A. 1993. The L-arginine-nitric oxide pathway. *N Engl J Med,* 329**,** 2002-12.

MOORE, K. T. & KRÖLL, D. 2017. Influences of Obesity and Bariatric Surgery on the Clinical and Pharmacologic Profile of Rivaroxaban. *Am J Med,* 130**,** 1024-1032.

MOORE, T. J., COHEN, M. R. & MATTISON, D. R. 2014. Dabigatran, bleeding, and the regulators. *Bmj,* 349**,** g4517.

MORALES-VIDAL, S., SCHNECK, M., FLASTER, M. & BILLER, J. 2012. Direct thrombin inhibitors and factor Xa inhibitors in patients with cerebrovascular disease. *Expert review of neurotherapeutics,* 12**,** 179-89; quiz 190.

MORRISON, S. A. & JESTY, J. 1984. Tissue factor-dependent activation of tritium-labeled factor IX and factor X in human plasma. *Blood,* 63**,** 1338-47.

MORRISSEY, J. H. 2001. Tissue factor: an enzyme cofactor and a true receptor. *Thromb Haemost,* 86**,** 66-74.

MORRISSEY, J. H., CHOI, S. H. & SMITH, S. A. 2012. Polyphosphate: an ancient molecule that links platelets, coagulation, and inflammation. *Blood,* 119**,** 5972-9.

MORROW, D. A., BRAUNWALD, E., BONACA, M. P., AMERISO, S. F., DALBY, A. J., FISH, M. P., FOX, K. A., LIPKA, L. J., LIU, X., NICOLAU, J. C., OPHUIS, A. J., PAOLASSO, E., SCIRICA, B. M., SPINAR, J., THEROUX, P., WIVIOTT, S. D., STRONY, J. & MURPHY, S. A. 2012. Vorapaxar in the secondary prevention of atherothrombotic events. *N Engl J Med,* 366**,** 1404-13.

MOSESSON, M. W. 1992. The roles of fibrinogen and fibrin in hemostasis and thrombosis. *Semin Hematol,* 29**,** 177-88.

MOSNIER, L. O., LISMAN, T., VAN DEN BERG, H. M., NIEUWENHUIS, H. K., MEIJERS, J. C. & BOUMA, B. N. 2001a. The defective down regulation of fibrinolysis in haemophilia A can be restored by increasing the TAFI plasma concentration. *Thromb Haemost,* 86**,** 1035-9.

MOSNIER, L. O., MEIJERS, J. C. & BOUMA, B. N. 2001b. Regulation of fibrinolysis in plasma by TAFI and protein C is dependent on the concentration of thrombomodulin. *Thromb Haemost,* 85**,** 5-11.

MUNOZ, E. M. & LINHARDT, R. J. 2004. Heparin-binding domains in vascular biology. *Arterioscler Thromb Vasc Biol,* 24**,** 1549-57.

NAGLER, M., RADDATZ-MULLER, P., SCHMID, P., BACHMANN, L. M. & WUILLEMIN, W. A. 2013. Accuracy of the point-of-care coagulometer CoaguChek XS in the hands of patients. *J Thromb Haemost,* 11**,** 197-9.

NAITO, K. & FUJIKAWA, K. 1991. Activation of human blood coagulation factor XI independent of factor XII. Factor XI is activated by thrombin and factor XIa in the presence of negatively charged surfaces. *J Biol Chem,* 266**,** 7353-8.

NESHEIM, M. 1998. Fibrinolysis and the plasma carboxypeptidase. *Curr Opin Hematol,* 5**,** 309-13.

NUTESCU, E., CHUATRISORN, I. & HELLENBART, E. 2011. Drug and dietary interactions of warfarin and novel oral anticoagulants: an update. *J Thromb Thrombolysis,* 31**,** 326-43.

O'BRIEN, L. M., MASTRI, M. & FAY, P. J. 2000. Regulation of factor VIIIa by human activated protein C and protein S: inactivation of cofactor in the intrinsic factor Xase. *Blood,* 95**,** 1714-20.

OSTERUD, B. & RAPAPORT, S. I. 1977. Activation of factor IX by the reaction product of tissue factor and factor VII: additional pathway for initiating blood coagulation. *Proc Natl Acad Sci U S A,* 74**,** 5260-4.

PAN, J., DINH, T. T., RAJARAMAN, A., LEE, M., SCHOLZ, A., CZUPALLA, C. J., KIEFEL, H., ZHU, L., XIA, L., MORSER, J., JIANG, H., SANTAMBROGIO, L. & BUTCHER, E. C. 2016. Patterns of expression of factor VIII and von Willebrand factor by endothelial cell subsets in vivo. *Blood,* 128**,** 104-9.

PASSING, H. & BABLOK, W. 1983. A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry, Part I. *J Clin Chem Clin Biochem,* 21**,** 709-20.

PERRY, D. J. 1994. Antithrombin and its inherited deficiencies. *Blood Rev,* 8**,** 37-55.

PERZBORN, E., GRUBER, A., TINEL, H., MARZEC, U. M., BUETEHORN, U., BUCHMUELLER, A., HEITMEIER, S. & LAUX, V. 2013. Reversal of rivaroxaban anticoagulation by haemostatic agents in rats and primates. *Thromb Haemost,* 110**,** 162-72.

PFIZER. 2014. Available: http://packageinserts.bms.com/pi/pi_eliquis.pdf [Accessed November 03, 2019].

PFIZER. 2016. Available: http://www.pfizer.ca/sites/g/files/g10028126/f/201607/ELIQUIS_PM_184464_16June2016_E_marketed.pdf [Accessed November 03, 2019].

PHILLIPS, D. R., CONLEY, P. B., SINHA, U. & ANDRE, P. 2005. Therapeutic approaches in arterial thrombosis. *J Thromb Haemost,* 3**,** 1577-89.

PIRAN, S., KHATIB, R., SCHULMAN, S., MAJEED, A., HOLBROOK, A., WITT, D. M., WIERCIOCH, W., SCHUNEMANN, H. J. & NIEUWLAAT, R. 2019. Management of direct factor Xa inhibitor-related major bleeding with prothrombin complex concentrate: a meta-analysis. *Blood Adv,* 3**,** 158-167.

PIRMOHAMED, M., BURNSIDE, G., ERIKSSON, N., JORGENSEN, A. L., TOH, C. H., NICHOLSON, T., KESTEVEN, P., CHRISTERSSON, C., WAHLSTROM, B., STAFBERG, C., ZHANG, J. E., LEATHART, J. B., KOHNKE, H., MAITLAND-VAN DER ZEE, A. H., WILLIAMSON, P. R., DALY, A. K., AVERY, P., KAMALI, F. & WADELIUS, M. 2013. A randomized trial of genotype-guided dosing of warfarin. *N Engl J Med,* 369**,** 2294-303.

PISANO, J. J., FINLAYSON, J. S. & PEYTON, M. P. 1968. Cross-link in fibrin polymerized by factor 13: epsilon-(gamma-glutamyl)lysine. *Science,* 160**,** 892-3.

PITTMAN, D. D. & KAUFMAN, R. J. 1988. Proteolytic requirements for thrombin activation of anti-hemophilic factor (factor VIII). *Proc Natl Acad Sci U S A,* 85**,** 2429-33.

POLLACK, C. V., JR., REILLY, P. A., EIKELBOOM, J., GLUND, S., VERHAMME, P., BERNSTEIN, R. A., DUBIEL, R., HUISMAN, M. V., HYLEK, E. M., KAMPHUISEN, P. W., KREUZER, J., LEVY, J. H., SELLKE, F. W., STANGIER, J., STEINER, T., WANG, B., KAM, C. W. & WEITZ, J. I. 2015. Idarucizumab for Dabigatran Reversal. *N Engl J Med,* 373**,** 511-20.

POLLACK, C. V., JR., REILLY, P. A., VAN RYN, J., EIKELBOOM, J. W., GLUND, S., BERNSTEIN, R. A., DUBIEL, R., HUISMAN, M. V., HYLEK, E. M., KAM, C. W., KAMPHUISEN, P. W., KREUZER, J., LEVY, J. H., ROYLE, G., SELLKE, F. W., STANGIER, J., STEINER, T., VERHAMME, P., WANG, B., YOUNG, L. & WEITZ, J. I. 2017. Idarucizumab for Dabigatran Reversal - Full Cohort Analysis. *N Engl J Med,* 377**,** 431-441.

POULSEN, B. K., GROVE, E. L. & HUSTED, S. E. 2012. New oral anticoagulants: a review of the literature with particular emphasis on patients with impaired renal function. *Drugs,* 72**,** 1739-53.

POWELL, J. R. 2015. Are new oral anticoagulant dosing recommendations optimal for all patients? *Jama,* 313**,** 1013-4.

PRAGST, I., ZEITLER, S. H., DOERR, B., KASPEREIT, F. J., HERZOG, E., DICKNEITE, G. & VAN RYN, J. 2012. Reversal of dabigatran anticoagulation by prothrombin complex concentrate (Beriplex P/N) in a rabbit model. *J Thromb Haemost,* 10**,** 1841-8.

PRANDONI, P. 2001. Heparins and venous thromboembolism: current practice and future directions. *Thromb Haemost,* 86**,** 488-98.

PRESTA, L., SIMS, P., MENG, Y. G., MORAN, P., BULLENS, S., BUNTING, S., SCHOENFELD, J., LOWE, D., LAI, J., RANCATORE, P., IVERSON, M., LIM, A., CHISHOLM, V., KELLEY, R. F.,

RIEDERER, M. & KIRCHHOFER, D. 2001. Generation of a humanized, high affinity anti-tissue factor antibody for use as a novel antithrombotic therapeutic. *Thromb Haemost,* 85**,** 379-89.

QIU, C., MUCHANT, D., BEIERWALTES, W. H., RACUSEN, L. & BAYLIS, C. 1998. Evolution of chronic nitric oxide inhibition hypertension: relationship to renal function. *Hypertension,* 31**,** 21-6.

RAK, J. 2014. New checkpoint of the coagulant phenotype. *Blood,* 124**,** 3511-3.

RAO, R. B. 2014. Regarding the effect of dabigatran plasma concentrations. *J Am Coll Cardiol,* 63**,** 2885.

RAPAPORT, S. I. & RAO, L. V. 1995. The tissue factor pathway: how it has become a "prima ballerina". *Thromb Haemost,* 74**,** 7-17.

REDLITZ, A., TAN, A. K., EATON, D. L. & PLOW, E. F. 1995. Plasma carboxypeptidases as regulators of the plasminogen system. *J Clin Invest,* 96**,** 2534-8.

REILLY, P. A., CONNOLLY, S. J., YUSUF, S., EIKELBOOM, J. W., EZEKOWITZ, M. D. & WALLENTIN, L. 2014a. Reply: regarding the effect of dabigatran plasma concentrations. *J Am Coll Cardiol,* 63**,** 2885-6.

REILLY, P. A., LEHR, T., HAERTTER, S., CONNOLLY, S. J., YUSUF, S., EIKELBOOM, J. W., EZEKOWITZ, M. D., NEHMIZ, G., WANG, S. & WALLENTIN, L. 2014b. The effect of dabigatran plasma concentrations and patient characteristics on the frequency of ischemic stroke and major bleeding in atrial fibrillation patients: the RE-LY Trial (Randomized Evaluation of Long-Term Anticoagulation Therapy). *J Am Coll Cardiol,* 63**,** 321-8.

REINHARDT, C., VON BRÜHL, M. L., MANUKYAN, D., GRAHL, L., LORENZ, M., ALTMANN, B., DLUGAI, S., HESS, S., KONRAD, I., ORSCHIEDT, L., MACKMAN, N., RUDDOCK, L., MASSBERG, S. & ENGELMANN, B. 2008. Protein disulfide isomerase acts as an injury response signal that enhances fibrin generation via tissue factor activation. *J Clin Invest,* 118**,** 1110-22.

RENNÉ, T., POZGAJOVA, M., GRUNER, S., SCHUH, K., PAUER, H. U., BURFEIND, P., GAILANI, D. & NIESWANDT, B. 2005. Defective thrombus formation in mice lacking coagulation factor XII. *J Exp Med,* 202**,** 271-81.

REUNER, K. H., JENETZKY, E., ALEU, A., LITFIN, F., MELLADO, P., KLOSS, M., JÜTTLER, E., GRAU, A. J., RICKMANN, H., PATSCHEKE, H. & LICHY, C. 2008. Factor XII C46T gene polymorphism and the risk of cerebral venous thrombosis. *Neurology,* 70**,** 129-32.

ROEHRIG, S., STRAUB, A., POHLMANN, J., LAMPE, T., PERNERSTORFER, J., SCHLEMMER, K. H., REINEMER, P. & PERZBORN, E. 2005. Discovery of the novel antithrombotic agent 5-chloro-N-({(5S)-2-oxo-3- [4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl}methyl)thiophene- 2-carboxamide (BAY 59-7939): an oral, direct factor Xa inhibitor. *J Med Chem,* 48**,** 5900-8.

ROSE, A. J., OZONOFF, A., BERLOWITZ, D. R., HENAULT, L. E. & HYLEK, E. M. 2009. Warfarin dose management affects INR control. *J Thromb Haemost,* 7**,** 94-101.

ROSEN, E. D., GAILANI, D. & CASTELLINO, F. J. 2002. FXI is essential for thrombus formation following FeCl3-induced injury of the carotid artery in the mouse. *Thromb Haemost,* 87**,** 774-6.

ROSING, J., VAN RIJN, J. L., BEVERS, E. M., VAN DIEIJEN, G., COMFURIUS, P. & ZWAAL, R. F. 1985. The role of activated human platelets in prothrombin and factor X activation. *Blood,* 65**,** 319-32.

ROSS, B., MILLER, M. A., DITCH, K. & TRAN, M. 2014. Clinical experience of life-threatening dabigatran-related bleeding at a large, tertiary care, academic medical center: a case series. *J Med Toxicol,* 10**,** 223-8.

RUIZ, F. A., LEA, C. R., OLDFIELD, E. & DOCAMPO, R. 2004. Human platelet dense granules contain polyphosphate and are similar to acidocalcisomes of bacteria and unicellular eukaryotes. *J Biol Chem,* 279**,** 44250-7.

RYAN, F., BYRNE, S. & O'SHEA, S. 2009. Randomized controlled trial of supervised patient self-testing of warfarin therapy using an internet-based expert system. *J Thromb Haemost,* 7**,** 1284-90.

RYDEL, T. J., RAVICHANDRAN, K. G., TULINSKY, A., BODE, W., HUBER, R., ROITSCH, C. & FENTON, J. W., 2ND 1990. The structure of a complex of recombinant hirudin and human alpha-thrombin. *Science,* 249**,** 277-80.

SALOMON, O., STEINBERG, D. M., KOREN-MORAG, N., TANNE, D. & SELIGSOHN, U. 2008. Reduced incidence of ischemic stroke in patients with severe factor XI deficiency. *Blood,* 111**,** 4113-7.

SAMAMA, M. M. 2011. The mechanism of action of rivaroxaban--an oral, direct Factor Xa inhibitor--compared with other anticoagulants. *Thromb Res,* 127**,** 497-504.

SAMIS, J. A., RAMSEY, G. D., WALKER, J. B., NESHEIM, M. E. & GILES, A. R. 2000. Proteolytic processing of human coagulation factor IX by plasmin. *Blood,* 95**,** 943-51.

SAOKAEW, S., PERMSUWAN, U., CHAIYAKUNAPRUK, N., NATHISUWAN, S. & SUKONTHASARN, A. 2010. Effectiveness of pharmacist-participated warfarin therapy management: a systematic review and meta-analysis. *J Thromb Haemost,* 8**,** 2418-27.

SAPOSNIK, B., RENY, J. L., GAUSSEM, P., EMMERICH, J., AIACH, M. & GANDRILLE, S. 2004. A haplotype of the EPCR gene is associated with increased plasma levels of sEPCR and is a candidate risk factor for thrombosis. *Blood,* 103**,** 1311-8.

SAVAGE, B., SALDIVAR, E. & RUGGERI, Z. M. 1996. Initiation of platelet adhesion by arrest onto fibrinogen or translocation on von Willebrand factor. *Cell,* 84**,** 289-97.

SAVAGE, B., SHATTIL, S. J. & RUGGERI, Z. M. 1992. Modulation of platelet function through adhesion receptors. A dual role for glycoprotein IIb-IIIa (integrin alpha IIb beta 3) mediated by fibrinogen and glycoprotein Ib-von Willebrand factor. *J Biol Chem,* 267**,** 11300-6.

SCHELLEMAN, H., BILKER, W. B., BRENSINGER, C. M., WAN, F., YANG, Y. X. & HENNESSY, S. 2010. Fibrate/Statin initiation in warfarin users and gastrointestinal bleeding risk. *Am J Med,* 123**,** 151-7.

SCHIELE, F., VUILLEMENOT, A., KRAMARZ, P., KIEFFER, Y., ANGUENOT, T., BERNARD, Y. & BASSAND, J. P. 1995. Use of recombinant hirudin as antithrombotic treatment in patients with heparin-induced thrombocytopenia. *Am J Hematol,* 50**,** 20-5.

SCHULMAN, S. & CROWTHER, M. A. 2012. How I treat with anticoagulants in 2012: new and old anticoagulants, and when and how to switch. *Blood,* 119**,** 3016-23.

SCHWARTZ, B. S. 1994. Differential inhibition of soluble and cell surface receptor-bound single-chain urokinase by plasminogen activator inhibitor type 2. A potential regulatory mechanism. *J Biol Chem,* 269**,** 8319-23.

SERRUYS, P. W., VRANCKX, P. & ALLIKMETS, K. 2006. Clinical development of bivalirudin (Angiox): rationale for thrombin-specific anticoagulation in percutaneous coronary intervention and acute coronary syndromes. *Int J Clin Pract,* 60**,** 344-50.

SHATTIL, S. J., KASHIWAGI, H. & PAMPORI, N. 1998. Integrin signaling: the platelet paradigm. *Blood,* 91**,** 2645-57.

SHEEHAN, J. P. & SADLER, J. E. 1994. Molecular mapping of the heparin-binding exosite of thrombin. *Proc Natl Acad Sci U S A,* 91**,** 5518-22.

SHEN, J., SAMPIETRO, S., WU, J., TANG, J., GUPTA, S., MATZKO, C. N., TANG, C., YU, Y., BRASS, L. F., ZHU, L. & STALKER, T. J. 2017. Coordination of platelet agonist signaling during the hemostatic response in vivo. *Blood Adv,* 1**,** 2767-2775.

SIEBENHOFER, A., BERGHOLD, A. & SAWICKI, P. T. 2004. Systematic review of studies of self-management of oral anticoagulation. *Thromb Haemost,* 91**,** 225-32.

SIEBENHOFER, A., RAKOVAC, I., KLEESPIES, C., PISO, B. & DIDJURGEIT, U. 2008. Self-management of oral anticoagulation reduces major outcomes in the elderly. A randomized controlled trial. *Thromb Haemost,* 100**,** 1089-98.

SIEGAL, D., LU, G., LEEDS, J. M., KARBARZ, M., CASTILLO, J., MATHUR, V., HUTCHALEELAHA, A., SINHA, U., KITT, M., MCCLURE, M., HOLLENBACH, S. J., CURNUTTE, J. T., CONLEY, P. B. & CROWTHER, M. 2017. Safety, pharmacokinetics, and reversal of apixaban anticoagulation with andexanet alfa. *Blood Adv,* 1**,** 1827-1838.

SIEGAL, D. M., CURNUTTE, J. T., CONNOLLY, S. J., LU, G., CONLEY, P. B., WIENS, B. L., MATHUR, V. S., CASTILLO, J., BRONSON, M. D., LEEDS, J. M., MAR, F. A., GOLD, A. & CROWTHER, M. A. 2015. Andexanet Alfa for the Reversal of Factor Xa Inhibitor Activity. *N Engl J Med,* 373**,** 2413-24.

SIEGAL, D. M. & KONKLE, B. A. 2014. What is the effect of rivaroxaban on routine coagulation tests? *Hematology Am Soc Hematol Educ Program,* 2014**,** 334-6.

SIXMA, J. J., VAN ZANTEN, G. H., HUIZINGA, E. G., VAN DER PLAS, R. M., VERKLEY, M., WU, Y. P., GROS, P. & DE GROOT, P. G. 1997. Platelet adhesion to collagen: an update. *Thromb Haemost,* 78**,** 434-8.

SMITH, W. L. 1989. The eicosanoids and their biochemical mechanisms of action. *Biochem J,* 259**,** 315-24.

SOUSA, H. R., GASPAR, R. S., SENA, E. M., DA SILVA, S. A., FONTELLES, J. L., ARAUJO, T. L., MASTROGIOVANNI, M., FRIES, D. M., AZEVEDO-SANTOS, A. P., LAURINDO, F. R., TROSTCHANSKY, A. & PAES, A. M. 2017. Novel antiplatelet role for a protein disulfide isomerase-targeted peptide: evidence of covalent binding to the C-terminal CGHC redox motif. *J Thromb Haemost,* 15**,** 774-784.

SOUTHWORTH, M. R., REICHMAN, M. E. & UNGER, E. F. 2013. Dabigatran and postmarketing reports of bleeding. *N Engl J Med,* 368**,** 1272-4.

SPEARMAN, C. 1904. The Proof and Measurement of Association between Two Things. *The American Journal of Psychology,* 15**,** 72-101.

SPRENGERS, E. D. & KLUFT, C. 1987. Plasminogen activator inhibitors. *Blood,* 69**,** 381-7.

STANGIER, J., RATHGEN, K., STÄHLE, H. & MAZUR, D. 2010. Influence of renal impairment on the pharmacokinetics and pharmacodynamics of oral dabigatran etexilate: an open-label, parallel-group, single-centre study. *Clin Pharmacokinet,* 49**,** 259-68.

STASSENS, P., BERGUM, P. W., GANSEMANS, Y., JESPERS, L., LAROCHE, Y., HUANG, S., MAKI, S., MESSENS, J., LAUWEREYS, M., CAPPELLO, M., HOTEZ, P. J., LASTERS, I. & VLASUK, G. P. 1996. Anticoagulant repertoire of the hookworm Ancylostoma caninum. *Proc Natl Acad Sci U S A,* 93**,** 2149-54.

STEG, P. G., MEHTA, S. R., POLLACK, C. V., JR., BODE, C., COHEN, M., FRENCH, W. J., HOEKSTRA, J., RAO, S. V., RUZYLLO, W., RUIZ-NODAR, J. M., SABATE, M., WIDIMSKY, P., KISS, R. G., NAVARRO ESTRADA, J. L., HOD, H., KERKAR, P., GUNERI, S., SEZER, M., RUDA, M., NICOLAU, J. C., CAVALLINI, C., EBRAHIM, I., PETROV, I., KIM, J. H., JEONG, M. H., RAMOS LOPEZ, G. A., LAANMETS, P., KOVAR, F., GAUDIN, C., FANOUILLERE, K. C., MININI, P., HOFFMAN, E. B., MORYUSEF, A., WIVIOTT, S. D. & SABATINE, M. S. 2013. Anticoagulation with otamixaban and ischemic events in non-ST-segment elevation acute coronary syndromes: the TAO randomized clinical trial. *Jama,* 310**,** 1145-55.

STEIN, C. M., BROWN, N., VAUGHAN, D. E., LANG, C. C. & WOOD, A. J. 1998. Regulation of local tissue-type plasminogen activator release by endothelium-dependent and endothelium-independent agonists in human vasculature. *J Am Coll Cardiol,* 32**,** 117-22.

STEINHUBL, S. R., BERGER, P. B., MANN, J. T., 3RD, FRY, E. T., DELAGO, A., WILMER, C. & TOPOL, E. J. 2002. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. *Jama,* 288**,** 2411-20.

SUGIYAMA, T. 2017. Osteoporotic Fractures Associated With Dabigatran vs Warfarin. *Jama,* 318**,** 90-91.

SUN, Y. & GAILANI, D. 1996. Identification of a factor IX binding site on the third apple domain of activated factor XI. *J Biol Chem,* 271**,** 29023-8.

SUZUKI, J., UMEDA, M., SIMS, P. J. & NAGATA, S. 2010. Calcium-dependent phospholipid scrambling by TMEM16F. *Nature,* 468**,** 834-8.

SUZUKI, K., DAHLBÄCK, B. & STENFLO, J. 1982. Thrombin-catalyzed activation of human coagulation factor V. *J Biol Chem,* 257**,** 6556-64.

SWAN, S. K. & HURSTING, M. J. 2000. The pharmacokinetics and pharmacodynamics of argatroban: effects of age, gender, and hepatic or renal dysfunction. *Pharmacotherapy,* 20**,** 318-29.

TAYLOR, F. B., JR., PEER, G. T., LOCKHART, M. S., FERRELL, G. & ESMON, C. T. 2001. Endothelial cell protein C receptor plays an important role in protein C activation in vivo. *Blood,* 97**,** 1685-8.

THALJI, N. K., IVANCIU, L., DAVIDSON, R., GIMOTTY, P. A., KRISHNASWAMY, S. & CAMIRE, R. M. 2016. A rapid pro-hemostatic approach to overcome direct oral anticoagulants. *Nat Med,* 22**,** 924-32.

THORELLI, E., KAUFMAN, R. J. & DAHLBÄCK, B. 1999. Cleavage of factor V at Arg 506 by activated protein C and the expression of anticoagulant activity of factor V. *Blood,* 93**,** 2552-8.

TIRADO, I., SORIA, J. M., MATEO, J., OLIVER, A., SOUTO, J. C., SANTAMARIA, A., FELICES, R., BORRELL, M. & FONTCUBERTA, J. 2004. Association after linkage analysis indicates that homozygosity for the 46C-->T polymorphism in the F12 gene is a genetic risk factor for venous thrombosis. *Thromb Haemost,* 91**,** 899-904.

TOPPER, J. N., CAI, J., FALB, D. & GIMBRONE, M. A., JR. 1996. Identification of vascular endothelial genes differentially responsive to fluid mechanical stimuli: cyclooxygenase-2, manganese superoxide dismutase, and endothelial cell nitric oxide synthase are selectively up-regulated by steady laminar shear stress. *Proc Natl Acad Sci U S A,* 93**,** 10417-22.

TOTI, F., SATTA, N., FRESSINAUD, E., MEYER, D. & FREYSSINET, J. M. 1996. Scott syndrome, characterized by impaired transmembrane migration of procoagulant phosphatidylserine and hemorrhagic complications, is an inherited disorder. *Blood,* 87**,** 1409-15.

TRACY, P. B., EIDE, L. L., BOWIE, E. J. & MANN, K. G. 1982. Radioimmunoassay of factor V in human plasma and platelets. *Blood,* 60**,** 59-63.

TRACY, P. B., EIDE, L. L. & MANN, K. G. 1985. Human prothrombinase complex assembly and function on isolated peripheral blood cell populations. *J Biol Chem,* 260**,** 2119-24.

TRACY, P. B., GILES, A. R., MANN, K. G., EIDE, L. L., HOOGENDOORN, H. & RIVARD, G. E. 1984. Factor V (Quebec): a bleeding diathesis associated with a qualitative platelet Factor V deficiency. *J Clin Invest,* 74**,** 1221-8.

TRICOCI, P., HUANG, Z., HELD, C., MOLITERNO, D. J., ARMSTRONG, P. W., VAN DE WERF, F., WHITE, H. D., AYLWARD, P. E., WALLENTIN, L., CHEN, E., LOKHNYGINA, Y., PEI, J., LEONARDI, S., RORICK, T. L., KILIAN, A. M., JENNINGS, L. H., AMBROSIO, G., BODE, C., CEQUIER, A., CORNEL, J. H., DIAZ, R., ERKAN, A., HUBER, K., HUDSON, M. P., JIANG, L., JUKEMA, J. W., LEWIS, B. S., LINCOFF, A. M., MONTALESCOT, G., NICOLAU, J. C., OGAWA, H., PFISTERER, M., PRIETO, J. C., RUZYLLO, W., SINNAEVE, P. R., STOREY, R. F., VALGIMIGLI, M., WHELLAN, D. J., WIDIMSKY, P., STRONY, J., HARRINGTON, R. A. & MAHAFFEY, K. W. 2012. Thrombin-receptor antagonist vorapaxar in acute coronary syndromes. *N Engl J Med,* 366**,** 20-33.

TRUJILLO-SANTOS, J., DI MICCO, P., DENTALI, F., DOUKETIS, J., DIAZ-PEROMINGO, J. A., NÚÑEZ, M. J., CAÑAS, I., MASTROIACOVO, D., SARAIVA DE SOUSA, M. & MONREAL, M. 2017. Real-life treatment of venous thromboembolism with direct oral anticoagulants: The influence of recommended dosing and regimens. *Thromb Haemost,* 117**,** 382-389.

TURPIE, A. G. 1998. Pharmacology of the low-molecular-weight heparins. *Am Heart J,* 135**,** S329-35.

TURPIE, A. G. 2008. New oral anticoagulants in atrial fibrillation. *Eur Heart J,* 29**,** 155-65.

VALNICKOVA, Z. & ENGHILD, J. J. 1998. Human procarboxypeptidase U, or thrombin-activable fibrinolysis inhibitor, is a substrate for transglutaminases. Evidence for transglutaminase-catalyzed cross-linking to fibrin. *J Biol Chem,* 273**,** 27220-4.

VAN DER POLL, T. & LEVI, M. 2012. Crosstalk between inflammation and coagulation: the lessons of sepsis. *Curr Vasc Pharmacol,* 10**,** 632-8.

VAN MEIJER, M. & PANNEKOEK, H. 1995. Structure of plasminogen activator inhibitor 1 (PAI-1) and its function in fibrinolysis: an update. *Fibrinolysis,* 9**,** 263-276.

VAN PELT, L. J., LUKENS, M. V., TESTA, S., CHATELAIN, B., DOUXFILS, J. & MULLIER, F. 2018. The DaXa-inhibition assay: A concept for a readily available, universal aXa assay that measures the direct inhibitory effect of all anti-Xa drugs. *Thromb Res,* 168**,** 63-66.

VAN RYN, J., BARUCH, L. & CLEMENS, A. 2012. Interpretation of point-of-care INR results in patients treated with dabigatran. *Am J Med,* 125**,** 417-20.

VAN RYN, J., STANGIER, J., HAERTTER, S., LIESENFELD, K. H., WIENEN, W., FEURING, M. & CLEMENS, A. 2010. Dabigatran etexilate--a novel, reversible, oral direct thrombin inhibitor: interpretation of coagulation assays and reversal of anticoagulant activity. *Thromb Haemost,* 103**,** 1116-27.

VAN SPALL, H. G., WALLENTIN, L., YUSUF, S., EIKELBOOM, J. W., NIEUWLAAT, R., YANG, S., KABALI, C., REILLY, P. A., EZEKOWITZ, M. D. & CONNOLLY, S. J. 2012. Variation in warfarin dose adjustment practice is responsible for differences in the quality of anticoagulation control between

centers and countries: an analysis of patients receiving warfarin in the randomized evaluation of long-term anticoagulation therapy (RE-LY) trial. *Circulation,* 126**,** 2309-16.

VAN THIEL, D. H., GEORGE, M. & FAREED, J. 2001. Low levels of thrombin activatable fibrinolysis inhibitor (TAFI) in patients with chronic liver disease. *Thromb Haemost,* 85**,** 667-70.

VAN ZONNEVELD, A. J., VEERMAN, H. & PANNEKOEK, H. 1986. Autonomous functions of structural domains on human tissue-type plasminogen activator. *Proc Natl Acad Sci U S A,* 83**,** 4670-4.

VANHOUTTE, P. M., SHIMOKAWA, H., FELETOU, M. & TANG, E. H. 2017. Endothelial dysfunction and vascular disease - a 30th anniversary update. *Acta Physiol (Oxf),* 219**,** 22-96.

VAZQUEZ-GARZA, E., JERJES-SANCHEZ, C., NAVARRETE, A., JOYA-HARRISON, J. & RODRIGUEZ, D. 2017. Venous thromboembolism: thrombosis, inflammation, and immunothrombosis for clinicians. *J Thromb Thrombolysis,* 44**,** 377-385.

VAZQUEZ, S. R. 2018. Drug-drug interactions in an era of multiple anticoagulants: a focus on clinically relevant drug interactions. *Blood,* 132**,** 2230-2239.

VERHAMME, P., TANGELDER, M., VERHAEGHE, R., AGENO, W., GLAZER, S., PRINS, M., JACQUEMIN, M. & BÜLLER, H. 2011. Single intravenous administration of TB-402 for the prophylaxis of venous thromboembolism after total knee replacement: a dose-escalating, randomized, controlled trial. *J Thromb Haemost,* 9**,** 664-71.

VERHOEF, T. I., RAGIA, G., DE BOER, A., BARALLON, R., KOLOVOU, G., KOLOVOU, V., KONSTANTINIDES, S., LE CESSIE, S., MALTEZOS, E., VAN DER MEER, F. J., REDEKOP, W. K., REMKES, M., ROSENDAAL, F. R., VAN SCHIE, R. M., TAVRIDOU, A., TZIAKAS, D., WADELIUS, M., MANOLOPOULOS, V. G. & MAITLAND-VAN DER ZEE, A. H. 2013. A randomized trial of genotype-guided dosing of acenocoumarol and phenprocoumon. *N Engl J Med,* 369**,** 2304-12.

VERRET, L., COUTURIER, J., ROZON, A., SAUDRAIS-JANECEK, S., ST-ONGE, A., NGUYEN, A., BASMADJIAN, A., TREMBLAY, S., BROUILLETTE, D. & DE DENUS, S. 2012. Impact of a pharmacist-led warfarin self-management program on quality of life and anticoagulation control: a randomized trial. *Pharmacotherapy,* 32**,** 871-9.

VINCENT, L. M., TRAN, S., LIVAJA, R., BENSEND, T. A., MILEWICZ, D. M. & DAHLBÄCK, B. 2013. Coagulation factor V(A2440G) causes east Texas bleeding disorder via TFPIalpha. *J Clin Invest,* 123**,** 3777-87.

VON DEM BORNE, P. A., BAJZAR, L., MEIJERS, J. C., NESHEIM, M. E. & BOUMA, B. N. 1997. Thrombin-mediated activation of factor XI results in a thrombin-activatable fibrinolysis inhibitor-dependent inhibition of fibrinolysis. *J Clin Invest,* 99**,** 2323-7.

VON DEM BORNE, P. A., MEIJERS, J. C. & BOUMA, B. N. 1995. Feedback activation of factor XI by thrombin in plasma results in additional formation of thrombin that protects fibrin clots from fibrinolysis. *Blood,* 86**,** 3035-42.

WALKER, F. J. 1981. Regulation of activated protein C by protein S. The role of phospholipid in factor Va inactivation. *J Biol Chem,* 256**,** 11128-31.

WALLENTIN, L., BECKER, R. C., BUDAJ, A., CANNON, C. P., EMANUELSSON, H., HELD, C., HORROW, J., HUSTED, S., JAMES, S., KATUS, H., MAHAFFEY, K. W., SCIRICA, B. M., SKENE,

A., STEG, P. G., STOREY, R. F., HARRINGTON, R. A., FREIJ, A. & THORSEN, M. 2009. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med,* 361**,** 1045-57.

WALLENTIN, L., YUSUF, S., EZEKOWITZ, M. D., ALINGS, M., FLATHER, M., FRANZOSI, M. G., PAIS, P., DANS, A., EIKELBOOM, J., OLDGREN, J., POGUE, J., REILLY, P. A., YANG, S. & CONNOLLY, S. J. 2010. Efficacy and safety of dabigatran compared with warfarin at different levels of international normalised ratio control for stroke prevention in atrial fibrillation: an analysis of the RE-LY trial. *Lancet,* 376**,** 975-83.

WANG, L. & ESSEX, D. W. 2017. A new antithrombotic strategy: inhibition of the C-terminal active site of protein disulfide isomerase. *J Thromb Haemost,* 15**,** 770-773.

WARD, A., TOMPSON, A., FITZMAURICE, D., SUTTON, S., PERERA, R. & HENEGHAN, C. 2015. Cohort study of Anticoagulation Self-Monitoring (CASM): a prospective study of its effectiveness in the community. *Br J Gen Pract,* 65**,** e428-37.

WARKENTIN, T. E., GREINACHER, A. & KOSTER, A. 2008. Bivalirudin. *Thromb Haemost,* 99**,** 830-9.

WATSON, S. P. 1999. Collagen receptor signaling in platelets and megakaryocytes. *Thromb Haemost,* 82**,** 365-76.

WEIDMANN, H., HEIKAUS, L., LONG, A. T., NAUDIN, C., SCHLUTER, H. & RENNE, T. 2017. The plasma contact system, a protease cascade at the nexus of inflammation, coagulation and immunity. *Biochim Biophys Acta Mol Cell Res,* 1864**,** 2118-2127.

WEITZ, J. I. 1997. Low-molecular-weight heparins. *N Engl J Med,* 337**,** 688-98.

WEITZ, J. I. 2003. Heparan sulfate: antithrombotic or not? *J Clin Invest,* 111**,** 952-4.

WEITZ, J. I., HUDOBA, M., MASSEL, D., MARAGANORE, J. & HIRSH, J. 1990. Clot-bound thrombin is protected from inhibition by heparin-antithrombin III but is susceptible to inactivation by antithrombin III-independent inhibitors. *J Clin Invest,* 86**,** 385-91.

WERFEN GMBH 2017. HemosIL® Liquid Anti-Xa Wefen GmbH.

WHITE, R. H. & GINSBERG, J. S. 2003. Low-molecular-weight heparins: are they all the same? *Br J Haematol,* 121**,** 12-20.

WIJELATH, E., NAMEKATA, M., MURRAY, J., FURUYASHIKI, M., ZHANG, S., COAN, D., WAKAO, M., HARRIS, R. B., SUDA, Y., WANG, L. & SOBEL, M. 2010. Multiple mechanisms for exogenous heparin modulation of vascular endothelial growth factor activity. *J Cell Biochem,* 111**,** 461-8.

WITT, D. M., NIEUWLAAT, R., CLARK, N. P., ANSELL, J., HOLBROOK, A., SKOV, J., SHEHAB, N., MOCK, J., MYERS, T., DENTALI, F., CROWTHER, M. A., AGARWAL, A., BHATT, M., KHATIB, R., RIVA, J. J., ZHANG, Y. & GUYATT, G. 2018. American Society of Hematology 2018 guidelines for management of venous thromboembolism: optimal management of anticoagulation therapy. *Blood Adv,* 2**,** 3257-3291.

WIVIOTT, S. D., BRAUNWALD, E., MCCABE, C. H., HORVATH, I., KELTAI, M., HERRMAN, J. P., VAN DE WERF, F., DOWNEY, W. E., SCIRICA, B. M., MURPHY, S. A. & ANTMAN, E. M. 2008. Intensive oral antiplatelet therapy for reduction of ischaemic events including stent thrombosis in patients with acute coronary syndromes treated with percutaneous coronary intervention and stenting in the TRITON-TIMI 38 trial: a subanalysis of a randomised trial. *Lancet,* 371**,** 1353-63.

WIVIOTT, S. D., BRAUNWALD, E., MCCABE, C. H., MONTALESCOT, G., RUZYLLO, W., GOTTLIEB, S., NEUMANN, F. J., ARDISSINO, D., DE SERVI, S., MURPHY, S. A., RIESMEYER, J.,

WEERAKKODY, G., GIBSON, C. M. & ANTMAN, E. M. 2007. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med,* 357**,** 2001-15.

WONG, H. & KEELING, D. 2014. Activated prothrombin complex concentrate for the prevention of dabigatran-associated bleeding. *Br J Haematol,* 166**,** 152-3.

WONG, J. W. & HOGG, P. J. 2010. Analysis of disulfide bonds in protein structures. *J Thromb Haemost*.

WONG, P. C., CRAIN, E. J., XIN, B., WEXLER, R. R., LAM, P. Y., PINTO, D. J., LUETTGEN, J. M. & KNABB, R. M. 2008. Apixaban, an oral, direct and highly selective factor Xa inhibitor: in vitro, antithrombotic and antihemostatic studies. *J Thromb Haemost,* 6**,** 820-9.

WRIGHT, R. S., ANDERSON, J. L., ADAMS, C. D., BRIDGES, C. R., CASEY, D. E., JR., ETTINGER, S. M., FESMIRE, F. M., GANIATS, T. G., JNEID, H., LINCOFF, A. M., PETERSON, E. D., PHILIPPIDES, G. J., THEROUX, P., WENGER, N. K., ZIDAR, J. P. & JACOBS, A. K. 2011. 2011 ACCF/AHA Focused Update of the Guidelines for the Management of Patients With Unstable Angina/ Non-ST-Elevation Myocardial Infarction (Updating the 2007 Guideline): a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation,* 123**,** 2022-60.

XIAN, Y., WANG, T. Y., MCCOY, L. A., EFFRON, M. B., HENRY, T. D., BACH, R. G., ZETTLER, M. E., BAKER, B. A., FONAROW, G. C. & PETERSON, E. D. 2015. Association of Discharge Aspirin Dose With Outcomes After Acute Myocardial Infarction: Insights From the Treatment with ADP Receptor Inhibitors: Longitudinal Assessment of Treatment Patterns and Events after Acute Coronary Syndrome (TRANSLATE-ACS) Study. *Circulation,* 132**,** 174-81.

YAMAMOTO, C., KAJI, T., SAKAMOTO, M., KOZUKA, H. & KOIZUMI, F. 1994. Calcium regulation of tissue plasminogen activator and plasminogen activator inhibitor-1 release from cultured human vascular endothelial cells. *Thromb Res,* 74**,** 163-8.

YANG, H., KIM, A., DAVID, T., PALMER, D., JIN, T., TIEN, J., HUANG, F., CHENG, T., COUGHLIN, S. R., JAN, Y. N. & JAN, L. Y. 2012. TMEM16F forms a Ca2+-activated cation channel required for lipid scrambling in platelets during blood coagulation. *Cell,* 151**,** 111-22.

YUSUF, S., ZHAO, F., MEHTA, S. R., CHROLAVICIUS, S., TOGNONI, G. & FOX, K. K. 2001. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med,* 345**,** 494-502.

ZAHIR, H., BROWN, K. S., VANDELL, A. G., DESAI, M., MAA, J. F., DISHY, V., LOMELI, B., FEUSSNER, A., FENG, W., HE, L., GROSSO, M. A., LANZ, H. J. & ANTMAN, E. M. 2015. Edoxaban effects on bleeding following punch biopsy and reversal by a 4-factor prothrombin complex concentrate. *Circulation,* 131**,** 82-90.

ZHANG, H., LÖWENBERG, E. C., CROSBY, J. R., MACLEOD, A. R., ZHAO, C., GAO, D., BLACK, C., REVENKO, A. S., MEIJERS, J. C., STROES, E. S., LEVI, M. & MONIA, B. P. 2010. Inhibition of the intrinsic coagulation pathway factor XI by antisense oligonucleotides: a novel antithrombotic strategy with lowered bleeding risk. *Blood,* 116**,** 4684-92.

ZHOU, Q., SIMS, P. J. & WIEDMER, T. 1998. Expression of proteins controlling transbilayer movement of plasma membrane phospholipids in the B lymphocytes from a patient with Scott syndrome. *Blood,* 92**,** 1707-12.