Real-World Safety of Anticoagulants

Chapter 5

Real-World Safety of Anticoagulants

Niccolò Lombardi, Giada Crescioli and
Alfredo Vannacci

Additional information is available at the end of the chapter

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Abstract

The aim of the present chapter is to characterize the management of anticoagulation in the real-world clinical setting, describing all pharmacological aspects of anticoagulant medications, in particular safety aspects. The chapter is structured into three main sections. Within the first two sections, active principles are classified on the basis of their pharmacological properties (pharmacodynamic and pharmacokinetic characteristic) in old and new molecules (or first and second generation class). In the third one, we discuss safety issues of anticoagulants using available data from postmarketing evidences. Furthermore, we also provide some essential information on the use of this kind of medications in special populations (i.e., elderly, coronary artery disease, diabetics, chronic kidney disease, etc.) in clinical practice.

Keywords: anticoagulants, safety, comorbidity, observational study

1. Introduction

Hemostasis is the physiologic process for the prevention of hemorrhage and bleeding in response to blood vessel damage, while physiologic inhibition of coagulation ensures the fluidity of blood. The ways in which these factors all balance each other can be the difference between hemostasis and thrombosis [1]. Alteration of this balance in favor of coagulation results in thrombosis, a pathological process characterized by the formation of a platelet or fibrin clot, which could occlude both arterial and venous vessels.

This chapter reviews the agents commonly used for controlling blood fluidity, including in particular:

- The coumarin anticoagulants: warfarin, acenocumarol, and phenprocoumon;
- Direct oral anticoagulants (DOACs): dabigatran, rivaroxaban, apixaban, and edoxaban;
- Parenteral anticoagulant heparin and its derivatives.

2. Oral anticoagulants

2.1. Old agents

2.1.1. Warfarin

2.1.1.1. Mechanism of action

Warfarin is a racemic mixture of two optically active isomers, the R and S enantiomers, and it produces its anticoagulant effect by interfering with the cyclic interconversion of vitamin K and vitamin K-2,3-epoxide. Warfarin blocks vitamin K epoxide reductase (VKOR), and the consequent conversion of oxidized vitamin K epoxide into its reduced form, vitamin K hydroquinone [2]. However, warfarin also has a simultaneous procoagulant effect caused by blocking the activation of protein C and S, two endogenous anticoagulants. A rapid depletion of these proteins leads to a transient hypercoagulable state in the first 1 or 2 days of therapy.

2.1.1.2. Indications

Warfarin is prescribed for the treatment and prophylaxis of various thromboembolic diseases such as atrial fibrillation (AF), deep venous thrombosis (DVT), transient ischemic attacks (TIA), pulmonary embolism, and other thromboembolic disorders that may affect carriers of cardiac valvular prosthesis or patients who underwent electric cardioversion [3, 4]. The dose-response relationship of warfarin is influenced by genetic and environmental factors, including mutations in gene coding for cytochrome P (CYP) 450, the hepatic enzyme responsible for oxidative metabolism of warfarin, mutations in gene coding for VKOR [5], concomitant drugs, diet, and various disease states [6]. Although warfarin and other dicumarol derivatives cross the placenta and contribute to fetal bone and central nervous system abnormalities when mothers are treated with warfarin within the first-trimester of pregnancy, there is no evidence that warfarin directly affects bone metabolism when administered to children or adults [7]. Women who will be managed with therapeutic anticoagulation in pregnancy should be treated preferably with a parenteral agent, such as heparin and low-molecular-weight heparin (LMWH), unfractionated heparin (UFH), and fondaparinux [8].

2.1.1.3. Pharmacokinetics

After oral intake, warfarin enantiomers are absorbed rapidly and almost completely from the gastrointestinal tract (100% of bioavailability) and reach their maximal plasma concentration in 90 minutes in healthy people. Racemic warfarin is extensively bound to plasma protein (mainly albumin, 99%) and has a plasma half-life of 36–42 hours [9]. Warfarin is extensively

metabolized in the liver by different CYP enzymes: CYP3A4 and CYP1A2 primarily metabolize the R-enantiomer, whereas the S-enantiomer is mainly metabolized by CYP2C9. The inactive metabolites are excreted with the urine and stool [10].

2.1.1.4. Interactions

Drug interactions that alter the pharmacokinetics of warfarin may include alterations in absorption (e.g., cholestyramine), which would decrease the anticoagulant effect. Reduced plasma-binding because of the presence of excessively albumin-bound drugs causes an increase in free drug plasma concentration and therefore an increase in antithrombotic activity. Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs), and large doses of penicillins inhibit platelet function, prolong bleeding time, and have the potential to increase the risk of warfarin-associated bleeding, especially upper gastrointestinal bleeding due to their gastric erosion effect. Many drug interactions with warfarin are caused by alterations in metabolism either by CYP2C9 enzyme induction [11], which increases warfarin clearance and thereby reducing antithrombotic activity (e.g., phenytoin, rifampin) [12], or stereoselective and nonselective enzyme inhibition (e.g., amiodarone, cimetidine, sulfamethoxazole, metronidazole) [13], which increases its antithrombotic effect (and the INR). Amiodarone is a potent inhibitor of the metabolic clearance of both the S-enantiomer and the R-enantiomer and potentiates warfarin anticoagulation [14]. The anticoagulant effect of warfarin is augmented by second-generation and third-generation cephalosporins, which inhibit the cyclic interconversion of vitamin K, by thyroxine, which increases the metabolism of coagulation factors, by clofibrate and by acetaminophen, by inhibition of VKOR through a toxic metabolite of the drug [2]. The effect of statins or fibrates on the risk of bleeding in patients on VKAs is controversial. The initiation of a fibrate or statin that inhibits CYP3A4 enzymes was reported to increase the risk of gastrointestinal bleeding, whereas statins that are mainly excreted unchanged were not found to be associated with such an increased risk [15, 16]. Furthermore, nutritional supplements and herbal products are particularly problematic in warfarin-treated patients, who often fail to inform physicians and use these products as self-medication. Fluctuating levels of dietary vitamin K derive predominantly from phylloquinones in plant material, i.e., green tea and *natto* [17, 18].

2.1.1.5. Therapy management

Patients at low risk of thrombosis (i.e., AF) do not require heparin treatment at the beginning of warfarin therapy and a low initial dose regimen starting with 3 mg warfarin is recommended. The time taken to reach a therapeutic International Normalized Ratio (INR) is not critical; INR values should be monitored weekly on day 1 (baseline), day 8 and day 15, especially in older people who respond more slowly with changes to the INR. All patients who are sensitive to warfarin effects should monitor their INR more frequently (i.e., every 3–4 days). Patients at high risk of thrombosis (i.e., DVT) should be treated with heparin or LMWH when starting warfarin therapy for a minimum of 5 consecutive days. For initiation, a starting dose of 5 mg warfarin with daily INR monitoring for a minimum of 5 days is recommended. After day 4, clinicians should continue regular INR monitoring every 3 to 4 days until stabilized, and if the patient is still on heparin or LMWH, review the ongoing need for these additional anticoagulants. If INR values change of 0.5 over 3 days or 1.0 over 7 days, the INR is considered

unstable. After INR stabilization, clinicians should adopt a maintenance dosing. The weekly dose can be prescribed using a range of dosing regimens (i.e., alternate day dosing or dose regimens with different doses for weekdays compared to the weekend).

Dose modification should be taken into account following the INR monitoring:

- INR < 1.5: weekly dose should be increased by 20%;
- INR between 1.5 and 1.9: weekly dose should be increased by 10% only if the alteration of the values persists for more than a week;
- INR between 2 and 3: no change in dose regimen;
- INR between 3.1 and 3.9: weekly dose should be decreased by 10–20% only if the alteration of the values persists for more than a week;
- INR between 4 and 4.9: the patient has to omit one dose, decrease the weekly dose by 10–20%, and monitor INR in 2–5 days.

For INR values greater than or equal to 5, there is a significantly increased risk of bleeding and vitamin K administration should be evaluated. Patients have to cease warfarin therapy and restart with a reduced dose, when INR is minor than 5 [19].

In case of switch to another anticoagulant agent and in particular to a direct oral anticoagulant agents (DOACs) in patients with atrial fibrillation, INR should be strictly monitored. Apixaban and dabigatran should be started when INR < 2, rivaroxaban when INR < 3, and edoxaban when INR ≤ 2.5 [20].

2.1.1.6. Real-world safety aspects

Vitamin K antagonists reduce stroke and systemic embolism by 64% and all-cause mortality by 26%, compared to placebo in patients with atrial fibrillation [21]. However, as already mentioned above, VKAs have many drug and food interactions and require routine INR monitoring, and these limitations result in under-treatment for 30–50% of AF patients [22].

The $CHADS_2$ scoring system [23] is a simple system that can be used to assess the annual risk of stroke in AF. In the $CHADS_2$ scoring system, each point increases the annual risk of stroke by a factor of 1.5. Treatment with warfarin is recommended for a $CHADS_2$ or CHA_2DS_2VASc scores of equal to or greater than 2. While the $CHADS_2$ score is simple, it does not include many common stroke risk factors. The CHA_2DS_2VASc score is inclusive of the most common stroke risk factors in everyday clinical practice and has been validated in multiple cohorts; the accumulated evidence shows that CHA_2DS_2VASc is better at identifying "truly low-risk" patients with AF and is as good as, and possibly better than, scores such as $CHADS_2$ in identifying patients who develop stroke and thromboembolism [24]. Direct comparison between the effects of warfarin and aspirin has been undertaken in several studies, demonstrating that warfarin was significantly superior, with a relative risk (RR) reduction of stroke of 39%.

In clinical practice, the risk of stroke should be weighed against the risk of bleeding to assess appropriateness of anticoagulant therapy. Warfarin causes major bleedings in 1–2% of people treated and intracranial bleeding in 0.1–0.5% of patients each year of treatment [25]. The highest rate of major

bleeding occurs in the first 3 months of treatment [26]. In comparison, aspirin causes major bleeding in 1.3% of patients [26]. Absolute risk increases for intracranial hemorrhage with warfarin compared to aspirin is only 0.2% per year [27]. Risk of bleeding can be assessed using the HAS-BLED scoring system, where a bleeding risk score of equal to or greater than 3 indicates high risk. There are other bleeding risk assessment tools available including HEMORR$_2$HAGES [23]. Assessment may identify reversible risks that can be managed prior to initiation of warfarin. In general, clinicians should be cautious and conduct regular review of the patient if initiating warfarin [24].

2.1.2. Acenocoumarol and phenprocoumon

Like warfarin, acenocoumarol and phenprocoumon also exist as optical isomers that have different stereochemical characteristics. R-acenocoumarol has an elimination half-life of 9 h; it is primarily metabolized by CYP2C9 and CYP2C19 and is more potent than S-acenocoumarol. In fact, S-acenocoumarol has a faster clearance (elimination half-life of 0.5 h), it is primarily metabolized by CYP2C9 and undergoes extensive first pass metabolism. The treatment dose may vary between patients up to 10-fold, ranging from 1 to 9 mg daily [28, 29].

Phenprocoumon is a much longer acting agent, with both the R- and the S-isomers with elimination half-lives of 5.5 days. Both are metabolized by CYP2C9, and S-phenprocoumon is 1.5–2.5 times more potent than R-phenprocoumon. It is administered in daily maintenance doses of 0.75–9 mg [29, 30].

As for warfarin, allelic variants of CYP2C9, CYP2C9*2, and CYP2C9*3 could lead to bleeding complications, especially if they code for enzymes with approximately 12 and 5% of the enzymatic activity of the wild type genotype CYP2C9*1 [31].

2.2. Novel oral anticoagulants

2.2.1. Direct oral anticoagulants

Four non–vitamin K antagonist oral anticoagulants (NOACs), or direct oral anticoagulants (DOACs), are widely used as alternatives to warfarin: dabigatran etexilate, rivaroxaban, apixaban, and edoxaban. In contrast with warfarin, DOACs have a more predictable therapeutic effect, do not require routine INR monitoring, and have fewer potential drug-drug interactions and no restriction on dietary consumption of vitamin K–containing food [32].

2.2.1.1. Mechanism of action

DOACs act through direct inhibition of thrombin or inhibition of activated factor X (factor Xa). Dabigatran etexilate mesylate is a competitive direct thrombin inhibitor. Rivaroxaban, apixaban, and edoxaban inhibit factor Xa and prothrombinase activity, thus inhibiting the conversion of prothrombin to thrombin and decreasing thrombus formation.

2.2.1.2. Indications

DOACs indications comprehend thromboembolic prevention in patients with nonvalvular atrial fibrillation (AF), DVT, and pulmonary embolism. For each one of these conditions, dose regimen has to be adjusted. Creatinine clearance (CrCl) values should be checked for dose management.

2.2.1.3. Pharmacokinetics

Dabigatran etexilate has a bioavailability of 3–7%, and it is bound to plasma proteins for a total amount of 35%. Its plasma half-life ranges between 12 and 17 hours but increases to 18–28 hours in case of mild to severe renal impairment. Dabigatran is converted into active dabigatran through hepatic and plasma hydrolysis and hepatic glucuronidation. Excretion occurs through the kidney after i.v. administration, while after oral administration, 7% of drug is recovered in urine and 86% is excreted in feces.

Rivaroxaban reaches the peak of plasma concentration in 2–4 hours, and its bioavailability ranges between 66% (20 mg) and 80–100% (10 mg); 92–95% of the drug administered is bound to plasma proteins for a total volume of distribution of 50 L. Hepatic oxidation by CYP3A4/5, CYP2J2, and hydrolysis converts rivaroxaban to inactive metabolites excreted through kidney (66%) and feces (28%).

Apixaban has a bioavailability of 50% and reaches plasma peak concentration in 3–4 hours. It is bound for 87% to plasmatic proteins, and it is metabolized by CYP3A4/5, 1A2, 2C9, 2C19, 2 J2, O-demethylation, and hydroxylation in the liver. Excretion occurs through the kidney (27%) and intestinal and biliary tract.

Edoxaban reaches the peak of plasma concentration in 1–2 hours, has a bioavailability of 62%, and it is bound for 55% to plasma protein. Its plasma half-life ranges from 10 to 14 hours. Edoxaban is converted through CYP3A4, hydrolysis, conjugation, and oxidation. Metabolites are excreted through kidneys (50%) and biliary/intestinal excretion [33].

2.2.1.4. Interactions

All DOACs are substrate of P-glycoprotein (P-gp), and apixaban and rivaroxaban are substrates for CYP3A4 metabolism. Thus, concomitant medications that are inducers or inhibitors of these pathways should be evaluated for potential interactions [34]. In particular, concomitant use of rifampin (P-gp inducer), carbamazepine, phenytoin (both P-gp and CYP3A4 inducers) and cyclosporine, ketoconazole, verapamil, and HIV protease inhibitors (both P-gp and CYP3A4 inhibitors) should be avoided or needs an adjustment of DOACs treatment [34, 35].

2.2.1.5. Therapy management

The main advantage of DOACs is a more rapid onset and offset of action that eliminates the necessity of routine INR monitoring. In case of acute care or perioperative settings, when there is uncertainty about the timing of last ingestion, renal function, and gastrointestinal absorption, there are several values that can be checked to assess DOACs effect, such as activated partial thromboplastin time (aPPT), prothrombin time (PT), and antifactor Xa activity. During rivaroxaban treatment renal function (CrCl), complete blood count (CBC) tests and hepatic function monitoring are required periodically, at least annually.

Dabigatran treatment of nonvalvular AF provides:

- 150 mg twice daily if CrCl>30 mL/min;
- 75 mg twice daily if CrCl is between 15 and 30 mL/min;
- 75 mg twice daily if CrCl is between 30 and 50 mL/min with concomitant P-gp inhibitors.

Rivaroxaban is administered with the evening meal on the basis of CrCl values:

- 20 mg daily if CrCl<50 mL/min;
- 15 mg daily if CrCl is between 15 and 50 mL/min.

Apixaban is given at the dose of 5 mg twice daily in patients with nonvalvular AF and reduced to 2.5 mg twice daily if there are at least two of the following conditions: body weight ≤ 60 kg, creatinine ≥ 1.5 mg/dL, age ≥ 80 years. Apixaban is not recommended in case of severe hepatic impairment.

Edoxaban is administered on the basis of CrCl:

- 60 mg daily if CrCl is between 50 and 95 mL/min;
- 30 mg daily if CrCl is between 15 and 50 mL/min.

2.2.1.6. Real-world safety aspects

2.2.1.6.1. Dabigatran

A systematic review and meta-analysis was performed with the aim of summarizing all available evidence from high-quality real-world observational studies regarding efficacy and safety of non–vitamin-K oral anticoagulants compared with vitamin-K antagonists in patients with atrial fibrillation [36]. Compared with warfarin, dabigatran is associated with lower risk for intracranial hemorrhage in several studies that included 606.855 patients (hazard ratio (HR), 0.42; 95% confidence interval (CI), 0.37–0.49) and lower risk for death in 319.486 patients (HR, 0.63; 95% CI, 0.52–0.76). There is no statistical difference between dabigatran and warfarin for the outcomes of ischemic strokes (HR, 0.96; 95% CI, 0.80–1.16); ischemic stroke or systemic embolism (HR, 1.17; 95% CI, 0.92–1.50); any stroke or systemic embolism (HR, 0.93; 95% CI, 0.77–1.14); major hemorrhage (HR, 0.83; 95% CI, 0.65–1.05); and myocardial infarction (HR, 0.96; 95% CI, 0.77–1.21). Authors identified 10 studies that included 537.770 patients which assessed the outcome of gastrointestinal hemorrhage and reported higher risk of dabigatran compared with warfarin (HR, 1.20; 95% CI, 1.06–1.36). Authors also reported the presence of a significant heterogeneity in all analyses with the exception of the outcome of intracranial hemorrhage and any stroke or systemic embolism.

2.2.1.6.2. Rivaroxaban

As reported in the study authored by Ntaios and Colleagues [36], compared with warfarin, rivaroxaban is associated with lower risk for intracranial hemorrhage in several real-world studies that included 136,221 patients (HR, 0.64; 95% CI, 0.47–0.86). There is no statistical difference between rivaroxaban and warfarin for the outcomes of ischemic stroke (HR, 0.89; 95% CI, 0.76–1.04); ischemic stroke or systemic embolism (HR, 0.73; 95% CI, 0.52–1.04); any stroke or systemic embolism (HR, 0.87; 95% CI, 0.71–1.07); major hemorrhage (HR, 1.00; 95% CI, 0.92–1.08); myocardial infarction (HR, 1.02; 95% CI, 0.54–1.89); and death (HR, 0.67; 95% CI, 0.35–1.30). Authors identified four studies that included 71.368 patients which assessed the

outcome of gastrointestinal hemorrhage and reported higher risk of rivaroxaban compared with warfarin (HR, 1.24; 95% CI, 1.08–1.41). Authors reported a significant heterogeneity for the outcomes of intracranial hemorrhage and death.

2.2.1.6.3. Apixaban

Compared with warfarin, apixaban is associated with lower risk for intracranial hemorrhage in several real-world studies that included 66.482 patients (HR, 0.45; 95% CI, 0.31–0.63); lower risk for gastrointestinal hemorrhage in 2 studies that included 33.323 patients (HR, 0.63; 95% CI, 0.42–0.95); and lower risk for major hemorrhage in 4 studies that included 89.036 patients (HR, 0.55; 95% CI, 0.48–0.63) [36]. Authors identified only one study of 41.785 patients which assessed death; it reported lower risk with apixaban compared with warfarin (HR, 0.65; 95% CI, 0.56–0.75). Also, they identified only one study of 15.390 patients which assessed the outcome of any stroke or systemic embolism; it reported lower risk of apixaban compared with warfarin (HR, 0.67; 95% CI, 0.46–0.98). There is neither a statistical difference between apixaban and warfarin for the outcomes of ischemic stroke (HR, 0.95; 95% CI, 0.75–1.19) nor for ischemic stroke or systemic embolism (HR, 1.07; 95% CI, 0.87–1.31). Authors reported a significant degree of heterogeneity in the analysis of the outcomes of gastrointestinal hemorrhage but not for the other outcomes.

2.2.2. Betrixaban

Betrixaban is an oral factor Xa inhibitor whose extended duration treatment reduced a composite of asymptomatic DVT, symptomatic DVT, nonfatal PE, and venous thromboembolism (VTE)–related death compared to enoxaparin, without an increase in major bleeding [37]. It acts by competitively binding to the active site of Xa, preventing its ability to convert prothrombin to thrombin. Betrixaban is rapidly absorbed with mean peak concentrations occurring within 3–4 hours after oral administration. Excretion is mostly unchanged through the bile with renal excretion accounting for 5–7% of the orally administered dose. Betrixaban is not a substrate for major cytochrome P450 enzymes, but it is a substrate for P-gp. Potent inhibitors of P-gp (i.e., ketoconazole, amiodarone, and diltiazem) increase betrixaban concentrations around twofold [38]. A recent network meta-analysis aimed to comprehensively analyze the thromboprophylactic drugs that are used to prevent thrombosis and reduce bleeding risk. Results showed that sudoxicam, FXI-ASO (factor XI antisense oligonucleotide), and betrixaban were likely to be associated with the lowest risk of all-cause bleeding after major joint surgery. Furthermore, betrixaban, dalteparin, and warfarin were associated with the lowest risk of major bleeding/nonmajor clinically relevant bleeding events [39].

The FDA (Food and Drug Administration) approved betrixaban on June 2017 for the prophylaxis of VTE in adult patients hospitalized for an acute medical illness, who are at risk for thromboembolic complications due to moderate or severe restricted mobility and other risk factors for VTE. The recommended dose of betrixaban is an initial single dose of 160 mg starting on day 1, followed by 80 mg once daily taken for 35–42 days at the same time each day with food. Dose should be reduced in case of severe renal and hepatic impairment or

concomitant treatment with P-gp inhibitors. The most common adverse reactions (≥5%), observed in the APEX trial considered for approval, were related to bleeding.

2.2.3. Sulodexide

Sulodexide belongs to a class of substances known as glycosaminoglycans (GAGs), composed of a fast-moving heparin fraction (80%) with affinity for antithrombin III and a dermatan sulfate fraction (20%) with affinity for heparin cofactor II.

2.2.3.1. Mechanism of action

Sulodexide exerts a strong antithrombotic activity by simultaneously potentiating the antiprotease activities of both antithrombin III and heparin cofactor II. Sulodexide also has a profibrinolytic effect (through activation of tissue plasminogen activator and inhibition of plasminogen activator inhibitor), an antiproliferative effect on smooth muscle cells, and antilipemic and antiatherosclerotic effects [40, 41].

2.2.3.2. Indications

Several clinical studies have demonstrated the efficacy of sulodexide in the treatment or prevention of vascular diseases associated with increased thrombotic risk, such as peripheral arterial occlusive diseases, post-myocardial infarction, recurrent DVT, and postthrombotic syndrome [42]. Other clinical applications are treatment of venous ulcers, cerebrovascular disorders, diabetic nephropathy, and other diabetic complications (nephropathy and macular edema).

2.2.3.3. Pharmacokinetics

Sulodexide is available for both intravenous and intramuscular administration, but it can be taken also orally. By the oral route, Sulodexide is absorbed within 1–2 hours and behaves as a mono-compound, reaching the time to peak in about 4 hours with a dose of 50–100 mg. Similar concentrations are maintained at least up to 18 hours. The distribution volume is very large, due to a higher affinity of sulodexide for the extensive surface area of the endothelium rather than for plasma proteins. Metabolism is liver dependent and based on N-desulfation, while excretion is mostly kidney dependent [40, 43].

2.2.3.4. Therapy management

Nowadays, sulodexide 250 LSU (lipasemic unit) capsules or 600 LSU parenteral preparation is approved only in Europe (Italy) for venous chronic ulcers in adults (250 LSU twice daily, away from meals).

2.2.3.5. Safety

Oral administration could lead to disorders of the gastrointestinal system with nausea, vomiting, and heartburn.

3. Parenteral anticoagulants

3.1. Heparins and low-molecular-weight heparins

Heparan sulfate (HS) proteoglycans play vital functions in many biological processes in the animal kingdom, and the GAG moiety is essential for these functions. Heparin is synthesized from UDP-sugar precursors as a polymer of alternating D-glucuronic acid and N-acetyl-D-glucosamine residues [44]. Unfractionated heparin (UFH) is a glycosaminoglycan consisting of heterogeneous mixture of polysaccharide chains with alternating residues of D-glucosamin and uronic acid, glucoronic acid, or iduronic acid, with a molecular weight range of 3000–30,000 Da. Low-molecular-weight heparins (LWMHs) are fragments of UFH produced by controlled enzymatic or chemical depolymerization processes with a mean molecular weight of about 5000 Da [45].

3.1.1. Mechanism of action

Both UFH and LMWHs exert their anticoagulant activity by inhibiting thrombin-activated conversion of fibrinogen to fibrin [46]: binding of a unique pentasaccharide to antithrombin causes a conformational change in antithrombin that accelerates its interaction with thrombin and factor Xa by about 1000 times. Binding of the pentasaccharide to antithrombin results directly in inhibition of factor Xa, and the pentasaccharide also blocks the activation of factor IX and neutralizes factor Xa by activating factor X inhibitor.

3.1.2. Indications

Heparins, and in particular LMWHs, indications comprehend: DVT prophylaxis during perioperative or postoperative period of general/orthopedic surgery or in bedridden patients; DVT treatment in patients affected by pulmonary embolism; instable angina and non-ST-elevation myocardial infarction (NSTEMI) prophylaxis with concomitant use of acetylsalicylic acid; coagulation prevention in patients undergoing dialysis; and symptomatic VTE (proximal DVT and/or pulmonary embolism) to reduce the recurrence of VTE in patients with solid tumor cancers.

3.1.3. Pharmacokinetics

Heparin is not absorbed orally and therefore is administered parenterally. The two preferred routes of administration are continuous intravenous infusion or subcutaneous injection. If administered subcutaneously, the dose of heparin should be higher than the usual intravenous dose because subcutaneous administration is associated with reduced bioavailability [46]. UFH does not have predictable pharmacokinetics, and it has a small volume of distribution and a relatively short half-life of about 0.5–1 hour [47]. After injection heparin rapidly disappears from blood: the rapid, saturable elimination phase is thought to reflect UFH binding to vascular endothelial cells, macrophages, and reticuloendothelial cells, where it is internalized and metabolized into smaller and less sulfated forms [48]. At higher doses, the cellular binding sites are saturated, and heparin is cleared predominantly by renal elimination [49]. UFH binds also to plasma proteins, changing its pharmacokinetic profile and reducing its

anticoagulant activity. Because of the unpredictable anticoagulant response, careful/close monitoring is essential, when UFH is given in therapeutic doses [48].

LMWHs do not bind to endothelial cells, macrophages, or reticuloendothelial cells and have a 2–4 times longer half-life compared to UFH (3–6 hours). Furthermore, LMWHs have much lower affinity for heparin-binding plasma proteins and are mainly removed by nonsaturable renal filtration, and thus, their clearance is independent of dose and plasma concentration [50].

3.1.4. Interactions

Heparin use should be avoided in case of concomitant treatment with antiplatelet drugs (NSAIDs, diclofenac, piroxicam, ketorolac, nimesulide, and acetylsalicylic acid), anticoagulant agents (warfarin), and glucocorticoids, due to increase in bleeding risk. Furthermore, LMWH should not be used in patients with previous heparin-induced thrombocytopenia/thrombosis (HITT), known hypersensitivity or adverse reaction to LMWH (dalteparin or enoxaparin), severe renal impairment, active bleeding, severe or uncontrolled hypertension, active peptic ulcerations, hemophilia, and severe liver disease.

3.1.5. Therapy management

Heparin therapy for VTE treatment is typically administered by continuous intravenous infusion, but adjusted dose and fixed dose subcutaneous injections can also be utilized. Obese patients clear LMWHs faster than nonobese patients due to hyperfiltration, and their dose should be adjusted on total body weight or LMWHs should be substituted with UFHs [51]. Therapy monitoring comprehends regular CrCl, platelet count, and antifactor Xa assay measurements.

In case of VTE prophylaxis, dosing and duration of LMWHs follow this pattern:

- dalteparin 5000 units subcutaneously once daily for 5–10 days or enoxaparin 40 mg subcutaneously once daily for 7–10 days in patients undergoing surgery interventions, who had previously VTE or are bedridden;
- dalteparin 2500 units subcutaneously once daily or enoxaparin 20 mg subcutaneously once daily in case of renal impairment.

Dosing recommendations for treatment of NSTEMI with enoxaparin take into account renal functions and concomitant therapies with antiplatelet agents. In particular:

- enoxaparin 1 mg/kg subcutaneously every 12 hours if CrCl is >50 mL/min;
- enoxaparin 1 mg/kg subcutaneously every 12 hours if CrCl is between 30 and 50 mL/min with continue; monitoring of renal function and antifactor Xa levels;
- heparins use is not recommended if CrCl is <30 mL/min.

In case of thrombolysis, anticoagulation is generally given in addition to dual antiplatelet therapy at doses adjusted on patient's age:

- age < 75 years, 30 mg i.v. bolus immediately prior to thrombolysis followed within 15 minutes by 1 mg/kg subcutaneously every 12 hours (each dose should not exceed 100 mg);
- age > 75 years, 0.75 mg/kg subcutaneously every 12 hours (each dose should not exceed 75 mg in the first two administrations);
- UFHs in patients with CrCl is <30 mL/min.

Intravenous anticoagulation with UFHs in addition to antiplatelet therapy is recommended for all patients undergoing primary percutaneous coronary intervention (PCI). Anticoagulant therapy is selected according to patient's ischemic and bleeding risks (70–100 units/kg i.v. bolus or 50–70 units/kg i.v. bolus with GPIIb/IIIa inhibitor). Bivalirudin or intravenous enoxaparin (0.5 mg/kg i.v.) may be used as alternatives to UFH.

3.1.6. Real-world safety aspects

Safe and effective use of heparin requires maintaining a delicate balance: dosing low enough to minimize the risk of bleeding, yet high enough to treat or prevent thrombosis. Achieving a therapeutic level of heparin within 24 hours significantly reduces the risk for recurrent VTE [52]. However, non-protocol-driven practice achieves this outcome only 40% of the time [53].

Bleeding is the primary untoward effect of heparin. Major bleeding occurs in 1–5% of patients treated with intravenous heparin for venous thromboembolism [54]. The incidence of bleeding is somewhat less in patients treated with LMWH for this indication, although the risk of bleeding appears to increase with higher total daily doses of heparin.

Heparin-induced thrombocytopenia (platelet count <150,000/mL or a 50% decrease from the pre-treatment value) occurs in ~0.5% of medical patients 5–10 days after initiation of therapy with heparin [55]. Although the incidence may be lower, thrombocytopenia also occurs with LMWHs and fondaparinux and platelet counts should be monitored. Thrombotic complications that can be life-threatening or lead to amputation occur in about one-half of the affected heparin-treated patients and may precede the onset of thrombocytopenia. The incidence of heparin-induced thrombocytopenia and thrombosis is higher in surgical patients than in medical patients. Women are twice as likely as men to develop this condition.

3.2. Fondaparinux, idraparinux, and idrabiotaparinux

Fondaparinux is a synthetic heparin-like compound that acts as indirect factor Xa inhibitor [50]. Idraparinux and idrabiotaparinux are second and third generation pentasaccharides derived from fondaparinux.

After subcutaneous administration, fondaparinux is cleared by kidneys and has an elimination half-life of 17 h in healthy volunteers and 29 h in patients with moderate renal insufficiency, and therefore, it is contraindicated in patients with severe renal insufficiency. In this specific condition, laboratory monitoring is performed with anti-Xa assay, aPTT, and PT [50]. Fondaparinux is approved as an alternative for heparin or LMWH in the initial treatment of VTE in conjunction with a vitamin K antagonist (VKA) and, in Europe, for ACS in patients for whom PCI is not indicated [56].

Idraparinux has a longer elimination half-time of 120 h after a single administration and accumulation does occur and after more than 6 months of treatment, the elimination half-time is increased up to 60 days. It is administered once weekly and is thereby suitable for long-term anticoagulation [57]. Idrabiotaparinux was evaluated for treatment of VTE and for stroke prevention in patients with AF, resulting as safe and efficient as idraparinux [58].

3.3. Irudin, lepirudin, desirudin, bivalirudin, and argatroban

Hirudin is a direct thrombin inhibitor (DTI) derived from the salivary secretions of leech (*Hirudo medicinalis*). Lepirudin and desirudin are two forms of recombinant hirudin, structurally identical except for minute differences in the amino-terminus sequence [59]. Unlike heparin and others anticoagulants, DTIs do not need antithrombin to perform its anticoagulant action: epirudin and desirudin form a bivalent irreversible complex with thrombin, while hirudin binds to both the active site as well as to exosite I on thrombin.

Lepirudin and desirudin have been evaluated for the prevention and treatment of VTE and in patients with acute coronary syndrome (ACS). These drugs are generally more effective than heparin in prevention of thrombosis but lead to more bleeding complications possibly related to their irreversible binding to thrombin [60]. Their indications of use comprehend: treatment and prevention of suspected or proven HIT; VTE prophylaxis after hip or knee arthroplasty. Lepirudin and desirudin are administered intravenously or subcutaneously and have a half-life of 80 and 60–120 minutes, respectively. The excretion is totally renal. Due to their narrow therapeutic range, therapy monitoring should be done regularly through aPTT, activated clotting time (ACT), and anti-Xa assay evaluation.

Bivalirudin is used in treatment of patients with unstable angina undergoing PCI even if at risk of HIT. This is a synthetic analog of hirudin that forms a reversible, high-affinity complex with thrombin [61]. Consequently, bivalirudin has a shorter half-life (25 minutes) and is a weaker thrombin inhibitor compared to hirudin, with a potentially larger therapeutic window. Its clearance is for 80% enzymatic and for 20% renal. Bivalirudin is now one of the preferred drugs for patients undergoing PCI in American and European guidelines, and it has become one of the most widely used antithrombotics in the USA for PCI [62].

Argatroban is a small, univalent competitive inhibitor of thrombin. It binds selectively and reversibly to the active site of thrombin and has a short elimination half-life of 50 min through hepatobiliary clearance. Since it is metabolized in the liver, it should be used with caution in patients with liver failure. Argatroban has been approved for the prevention and treatment of VTE in patients with HIT [46, 47] and for patients with (a history of) HIT who need to undergo PCI [46, 63].

Author details

Niccolò Lombardi, Giada Crescioli and Alfredo Vannacci*

*Address all correspondence to: alfredo.vannacci@unifi.it

Department of Neurosciences, Psychology, Drug Research and Child Health, Section of Pharmacology and Toxicology, University of Florence, Florence, Italy

References

[1] Tran R, Myers DR, Ciciliano J, Trybus Hardy EL, Sakurai Y, Ahn B, et al. Biomechanics of haemostasis and thrombosis in health and disease: From the macro- to molecular scale. Journal of Cellular and Molecular Medicine. 2013;**17**:579-596

[2] Ageno W, Gallus AS, Wittkowsky A, Crowther M, Hylek EM, Palareti G. Oral anticoagulant therapy: Antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest. 2012;**141**:e44S-e88S

[3] Di Minno A, Frigerio B, Spadarella G, Ravani A, Sansaro D, Amato M, et al. Old and new oral anticoagulants: Food, herbal medicines and drug interactions. Blood Reviews. 2017;**31**:193-203

[4] Hawkins D. Limitations of traditional anticoagulants. Pharmacotherapy. 2004;**24**:62S-65S

[5] Geisen C, Watzka M, Sittinger K, Steffens M, Daugela L, Seifried E, et al. VKORC1 haplotypes and their impact on the inter-individual and inter-ethnical variability of oral anticoagulation. Thrombosis and Haemostasis. 2005;**94**:773-779

[6] Hirsh J, Fuster V, Ansell J, Halperin JL, American Heart Association/American College of Cardiology F. American Heart Association/American College of Cardiology Foundation guide to warfarin therapy. Journal of the American College of Cardiology. 2003;**41**: 1633-1652

[7] Hall JG, Pauli RM, Wilson KM. Maternal and fetal sequelae of anticoagulation during pregnancy. The American Journal of Medicine. 1980;**68**:122-140

[8] Fogerty AE. Challenges of anticoagulation therapy in pregnancy. Current Treatment Options in Cardiovascular Medicine. 2017;**19**:76

[9] Hirsh J. Oral anticoagulant drugs. The New England Journal of Medicine. 1991;**324**: 1865-1875

[10] Jacobs LG. Warfarin pharmacology, clinical management, and evaluation of hemorrhagic risk for the elderly. Cardiology Clinics. 2008;**26**:157-167

[11] Cropp JS, Bussey HI. A review of enzyme induction of warfarin metabolism with recommendations for patient management. Pharmacotherapy. 1997;**17**:917-928

[12] Greenblatt DJ, von Moltke LL. Interaction of warfarin with drugs, natural substances, and foods. Journal of Clinical Pharmacology. 2005;**45**:127-132

[13] Breckenridge A, Orme M, Wesseling H, Lewis RJ, Gibbons R. Pharmacokinetics and pharmacodynamics of the enantiomers of warfarin in man. Clinical Pharmacology and Therapeutics. 1974;**15**:424-430

[14] O'Reilly RA, Trager WF, Rettie AE, Goulart DA. Interaction of amiodarone with racemic warfarin and its separated enantiomorphs in humans. Clinical Pharmacology and Therapeutics. 1987;**42**:290-294

[15] Schelleman H, Bilker WB, Brensinger CM, Wan F, Yang YX, Hennessy S. Fibrate/statin initiation in warfarin users and gastrointestinal bleeding risk. The American Journal of Medicine. 2010;**123**:151-157

[16] Douketis JD, Melo M, Bell CM, Mamdani MM. Does statin therapy decrease the risk for bleeding in patients who are receiving warfarin? The American Journal of Medicine. 2007;**120**:369 e9-369 e14

[17] Holbrook AM, Pereira JA, Labiris R, McDonald H, Douketis JD, Crowther M, et al. Systematic overview of warfarin and its drug and food interactions. Archives of Internal Medicine. 2005;**165**:1095-1106

[18] Schurgers LJ, Teunissen KJ, Hamulyak K, Knapen MH, Vik H, Vermeer C. Vitamin K-containing dietary supplements: Comparison of synthetic vitamin K1 and natto-derived menaquinone-7. Blood. 2007;**109**:3279-3283

[19] Guidelines for warfarin management in the community. Available at: https://www.health.qld.gov.au/clinical%20practice/guidelines-procedures/medicines?a=165945

[20] Kovacs RJ, Flaker GC, Saxonhouse SJ, Doherty JU, Birtcher KK, Cuker A, et al. Practical management of anticoagulation in patients with atrial fibrillation. Journal of the American College of Cardiology. 2015;**65**:1340-1360

[21] Hart RG, Pearce LA, Aguilar MI. Adjusted-dose warfarin versus aspirin for preventing stroke in patients with atrial fibrillation. Annals of Internal Medicine. 2007;**147**:590-592

[22] De Caterina R, Husted S, Wallentin L, Andreotti F, Arnesen H, Bachmann F, et al. Vitamin K antagonists in heart disease: Current status and perspectives (section III). Position paper of the ESC working group on thrombosis—Task force on anticoagulants in heart disease. Thrombosis and Haemostasis. 2013;**110**:1087-1107

[23] Gage BF, Yan Y, Milligan PE, Waterman AD, Culverhouse R, Rich MW, et al. Clinical classification schemes for predicting hemorrhage: Results from the National Registry of Atrial Fibrillation (NRAF). American Heart Journal. 2006;**151**:713-719

[24] European Heart Rhythm A, European Association for Cardio-Thoracic S, Camm AJ, Kirchhof P, Lip GY, Schotten U, et al. Guidelines for the management of atrial fibrillation: The task force for the management of atrial fibrillation of the European Society of Cardiology (ESC). European Heart Journal. 2010;**31**:2369-2429

[25] Gallus AS, Baker RI, Chong BH, Ockelford PA, Street AM. Consensus guidelines for warfarin therapy. Recommendations from the Australasian Society of Thrombosis and Haemostasis. The Medical Journal of Australia. 2000;**172**:600-605

[26] van Walraven C, Hart RG, Singer DE, Laupacis A, Connolly S, Petersen P, et al. Oral anticoagulants vs aspirin in nonvalvular atrial fibrillation: An individual patient meta-analysis. JAMA. 2002;**288**:2441-2448

[27] Hart RG, Pearce LA, Aguilar MI. Meta-analysis: Antithrombotic therapy to prevent stroke in patients who have nonvalvular atrial fibrillation. Annals of Internal Medicine. 2007;**146**:857-867

[28] Godbillon J, Richard J, Gerardin A, Meinertz T, Kasper W, Jahnchen E. Pharmacokinetics of the enantiomers of acenocoumarol in man. British Journal of Clinical Pharmacology. 1981;**12**:621-629

[29] Verhoef TI, Redekop WK, Daly AK, van Schie RM, de Boer A, Maitland-van der Zee AH. Pharmacogenetic-guided dosing of coumarin anticoagulants: Algorithms for warfarin, acenocoumarol and phenprocoumon. British Journal of Clinical Pharmacology. 2014;**77**:626-641

[30] Haustein KO. Pharmacokinetic and pharmacodynamic properties of oral anticoagulants, especially phenprocoumon. Seminars in Thrombosis and Hemostasis. 1999;**25**:5-11

[31] Rettie AE, Wienkers LC, Gonzalez FJ, Trager WF, Korzekwa KR. Impaired (S)-warfarin metabolism catalysed by the R144C allelic variant of CYP2C9. Pharmacogenetics. 1994;**4**: 39-42

[32] Levy JH, Faraoni D, Spring JL, Douketis JD, Samama CM. Managing new oral anticoagulants in the perioperative and intensive care unit setting. Anesthesiology. 2013;**118**:1466-1474

[33] Raval AN, Cigarroa JE, Chung MK, Diaz-Sandoval LJ, Diercks D, Piccini JP, et al. Management of patients on non-vitamin K antagonist oral anticoagulants in the acute care and periprocedural setting: A scientific statement from the American Heart Association. Circulation. 2017;**135**:e604-e633

[34] Piccini JP, Hellkamp AS, Washam JB, Becker RC, Breithardt G, Berkowitz SD, et al. Polypharmacy and the efficacy and safety of rivaroxaban versus warfarin in the prevention of stroke in patients with nonvalvular atrial fibrillation. Circulation. 2016;**133**:352-360

[35] Ruff CT, Giugliano RP, Braunwald E, Morrow DA, Murphy SA, Kuder JF, et al. Association between edoxaban dose, concentration, anti-factor Xa activity, and outcomes: An analysis of data from the randomised, double-blind ENGAGE AF-TIMI 48 trial. Lancet. 2015;**385**:2288-2295

[36] Ntaios G, Papavasileiou V, Makaritsis K, Vemmos K, Michel P, Lip GYH. Real-world setting comparison of nonvitamin-K antagonist oral anticoagulants versus vitamin-K antagonists for stroke prevention in atrial fibrillation: A systematic review and meta-analysis. Stroke; a Journal of Cerebral Circulation. 2017;**48**:2494-2503

[37] Cohen AT, Harrington RA, Goldhaber SZ, Hull RD, Wiens BL, Gold A, et al. Extended thromboprophylaxis with betrixaban in acutely ill medical patients. The New England Journal of Medicine. 2016;**375**:534-544

[38] Cohen AT, Harrington R, Goldhaber SZ, Hull R, Gibson CM, Hernandez AF, et al. The design and rationale for the Acute Medically Ill Venous Thromboembolism Prevention with Extended Duration Betrixaban (APEX) study. American Heart Journal. 2014;**167**:335-341

[39] Wang Z, Zheng J, Zhao Y, Xiang Y, Chen X, Jin Y. Effectiveness and tolerability of anticoagulants for thromboprophylaxis after major joint surgery: A network meta-analysis. Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology. 2017;**42**:1999-2020

[40] Coccheri S, Mannello F. Development and use of sulodexide in vascular diseases: Implications for treatment. Drug Design, Development and Therapy. 2013;**8**:49-65

[41] Borawski J, Dubowski M, Rydzewska-Rosolowska A, Mysliwiec M. Intravenous and oral sulodexide versus coagulation activation markers in humans. Clinical and Applied Thrombosis/Hemostasis: Official Journal of the International Academy of Clinical and Applied Thrombosis/Hemostasis. 2009;**15**:596-598

[42] Elleuch N, Zidi H, Bellamine Z, Hamdane A, Guerchi M, Jellazi N, et al. Sulodexide in patients with chronic venous disease of the lower limbs: Clinical efficacy and impact on quality of life. Advances in Therapy. 2016;**33**:1536-1549

[43] Marchi E, Barbanti M, Milani R, Breccia A, Fini A, Gattavecchia E. Organ glycosaminoglycan distribution after intravenous and oral administration in rats. Seminars in Thrombosis and Hemostasis. 1994;**20**:297-300

[44] Sugahara K, Kitagawa H. Heparin and heparan sulfate biosynthesis. IUBMB Life. 2002;**54**:163-175

[45] Hirsh J, Levine MN. Low molecular weight heparin. Blood. 1992;**79**:1-17

[46] Garcia DA, Baglin TP, Weitz JI, Samama MM. Parenteral anticoagulants: Antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest. 2012;**141**:e24S-e43S

[47] Middeldorp S. Heparin: From animal organ extract to designer drug. Thrombosis Research. 2008;**122**:753-762

[48] Leentjens J, Peters M, Esselink AC, Smulders Y, Kramers C. Initial anticoagulation in patients with pulmonary embolism: Thrombolysis, unfractionated heparin, LMWH, fondaparinux, or DOACs? British Journal of Clinical Pharmacology. 2017;**83**:2356-2366

[49] Boneu B, Caranobe C, Sie P. Pharmacokinetics of heparin and low molecular weight heparin. Baillière's Clinical Haematology. 1990;**3**:531-544

[50] Donat F, Duret JP, Santoni A, Cariou R, Necciari J, Magnani H, et al. The pharmacokinetics of fondaparinux sodium in healthy volunteers. Clinical Pharmacokinetics. 2002;**41**(Suppl 2):1-9

[51] Bazinet A, Almanric K, Brunet C, Turcotte I, Martineau J, Caron S, et al. Dosage of enoxaparin among obese and renal impairment patients. Thrombosis Research. 2005;**116**:41-50

[52] Anand SS, Bates S, Ginsberg JS, Levine M, Buller H, Prins M, et al. Recurrent venous thrombosis and heparin therapy: An evaluation of the importance of early activated partial thromboplastin times. Archives of Internal Medicine. 1999;**159**:2029-2032

[53] Wheeler AP, Jaquiss RD, Newman JH. Physician practices in the treatment of pulmonary embolism and deep venous thrombosis. Archives of Internal Medicine. 1988;**148**: 1321-1325

[54] Hirsh J, Anand SS, Halperin JL, Fuster V, American Heart Association. Guide to anticoagulant therapy: Heparin: A statement for healthcare professionals from the American Heart Association. Circulation. 2001;**103**:2994-3018

[55] Warkentin TE. Heparin-induced thrombocytopenia. Hematology/Oncology Clinics of North America. 2007;**21**:589-607

[56] Greinacher A. Heparin-induced thrombocytopenia. The New England Journal of Medicine. 2015;**373**:1883-1884

[57] Harenberg J, Jorg I, Vukojevic Y, Mikus G, Weiss C. Anticoagulant effects of idraparinux after termination of therapy for prevention of recurrent venous thromboembolism: Observations from the van Gogh trials. European Journal of Clinical Pharmacology. 2008;**64**:555-563

[58] Equinox I. Efficacy and safety of once weekly subcutaneous idrabiotaparinux in the treatment of patients with symptomatic deep venous thrombosis. Journal of Thrombosis and Haemostasis. 2011;**9**:92-99

[59] Di Nisio M, Middeldorp S, Buller HR. Direct thrombin inhibitors. The New England Journal of Medicine. 2005;**353**:1028-1040

[60] Effects of recombinant hirudin (lepirudin) compared with heparin on death, myocardial infarction, refractory angina, and revascularisation procedures in patients with acute myocardial ischaemia without ST elevation: A randomised trial. Organisation to Assess Strategies for Ischemic Syndromes (OASIS-2) Investigators. Lancet. 1999;**353**:429-438

[61] Maraganore JM, Bourdon P, Jablonski J, Ramachandran KL, Fenton JW 2nd. Design and characterization of hirulogs: A novel class of bivalent peptide inhibitors of thrombin. Biochemistry. 1990;**29**:7095-7101

[62] Task Force on the management of STseamiotESoC, Steg PG, James SK, Atar D, Badano LP, Blomstrom-Lundqvist C, et al. ESC guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. European Heart Journal. 2012;**33**:2569-2619

[63] Lewis BE, Wallis DE, Leya F, Hursting MJ, Kelton JG, Argatroban I. Argatroban anticoagulation in patients with heparin-induced thrombocytopenia. Archives of Internal Medicine. 2003;**163**:1849-1856