Anticoagulants
Old and new

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Summary
Anticoagulants are effective in the prevention and treatment of a variety of arterial and venous thrombotic disorders but are associated with an increased risk of serious bleeding complications. Based on well documented studies of patients using vitamin K antagonists the incidence of major bleeding is 0.5%/year and the incidence of intracranial bleeding is 0.2%/year, however, in real life practice this incidence may be even higher. Risk factors for bleeding are the intensity of anticoagulation, the management strategy to keep the anticoagulant effect in the desired range, and patient characteristics. Recently, a new generation of anticoagulants have been developed and is currently evaluated in clinical trials. Initial results show a similar or superior efficacy over conventional anticoagulant agents with a good safety profile. In case of serious bleeding complications in a patient who uses vitamin K antagonists, this anticoagulant treatment can be quickly reversed by administration of vitamin K or coagulation factor concentrates. For the newer anticoagulants, quick reversal strategies are more cumbersome, although some interventions, including prothrombin complex concentrates, show promising results in initial experimental studies.

Keywords
Anticoagulants, vitamin K antagonists, heparin, pentasaccharide, dabigatran, rivaroxaban

Antikoagulanzien, Vitamin-K-Antagonisten, Heparin, Pentasaccharide, Dabigatran, Rivaroxaban

Zusammenfassung

Risk factors
The most important risk factor for haemorrhage in users of anticoagulants is the intensity of the anticoagulant effect (2, 5). Studies indicate that with a target INR of >3.0 the incidence of major bleeding is twice as large as in studies with a target INR of 2.0–3.0 (6, 7). In a metanalysis of studies in patients with prosthetic heart valves, a lower INR target range resulted in a lower frequency of major bleeding and in-
tracranial haemorrhage with a similar anti-thrombotic efficacy (8). A retrospective analysis of outpatients using warfarin who presented with intracranial haemorrhage demonstrated that the risk of this complication doubled for each 1 unit increment of the INR (9). Not only the target INR but also the actual individual INR is strongly associated with the risk of bleeding (10).

In a large cohort of patients on VKAs an INR of >4.5 was the strongest independent risk factor for bleeding (relative risk 5.96, 95% confidence interval (CI) 3.68–9.67) (11). It has been clearly shown that management of anticoagulant treatment by specialized anticoagulation clinics results in higher proportion of patients in the therapeutic target range compared to often less organized conventional care by general practitioners and specialists (12, 13). Computerized warfarin adjustments proved superior to manual regulation in overall control of anticoagulation (14, 15). Patient self-management of anticoagulation has been shown to result in an improvement of the time in the therapeutic range compared with conventional care and a control of anticoagulation that is as good as management by specialized anticoagulation clinics (16).

Patient characteristics constitute another important determinant of the bleeding risk bleeding. Elderly patients have a twofold increased risk of bleeding (17) and the relative risk of intracranial haemorrhage (in particular at higher INR) was 2.5 (95% CI 2.3–9.4) in patients >85 years compared to patients 70–74 years old (18). Recently, genetic factors have been identified that may affect the risk of bleeding. Common polymorphisms in the P450 CYP2C9 enzyme were found to be associated with slow metabolism of VKAs and (possibly) a higher risk of bleeding (2, 19). Other genetic factors that may influence the requirement of VKAs are variants in the vitamin K epoxide reductase complex subunit 1 gene (VKORC1) (20). Co-morbidity, such as renal or hepatic insufficiency, may also significantly increase the risk of bleeding. A case-control study in 1986 patients on VKAs showed that this comorbidity increased the risk of bleeding by about 2.5 (21). Another very important determinant of the risk of bleeding is the use of other medication, in particular agents affecting platelet function. Two metaanalyses, comprising six trials with a total of 3874 patients and ten trials with a total of 5938 patients, found a relative risk of major bleeding when VKAs were combined with aspirin of 2.4 (95% CI 1.2–4.8) and 2.5 (95% CI 1.7–3.7), respectively (22, 23). A population-based case-control study confirmed the high risk of upper gastrointestinal bleeding in patients using VKAs in combination with aspirin and/or clopidogrel (24).

Non-steroidal anti-inflammatory agents (NSAIDs) are also associated with an enhanced risk of gastrointestinal bleeding. The combined use of VKAs and NSAIDs may result in an 11-fold higher risk of hospitalization for gastro-intestinal bleeding as compared to the general population (25). This risk is not significantly lower when using selective inhibitors of COX-2 (26).

Clinical trial evidence and real life practice

Patients that use anticoagulants in real life clinical practice are often different from the population in clinical trials with their inclusion and exclusion criteria (27). In six pivotal trials that demonstrated the superiority of warfarin over placebo in the prevention of thromboembolic complications in patients with atrial fibrillation, 28,787 patients were screened but only 12.6% of these patients were included in the study. Similarly, only 14,614 out of 36,945 patients (23%) with acute coronary syndromes were included in five major trials on the use of VKAs (21).

In studies the average incidence of major bleeding is approximately 0.5%/year (2), whereas in three real-life surveys this incidence varied from 1.35%/year to 3.4%/year (11, 29, 30). The incidence of intracranial haemorrhage was 0.2%/year in the clinical trials compared to 0.4–0.6%/year in the unscreened samples. In a case-control study in 993 patients on VKAs with major bleeding and 993 non-bleeding controls less than 70% of patients using VKAs would have been eligible for the clinical trials (21).

In the group of patients that presented with haemorrhage, the proportion with exclusion criteria was considerably greater (40%; 95% CI 37.4–43%) than in the control group (23%; 95% CI 21.0–26%). The bleeding risk increased sharply with having more exclusion criteria as compared to none: having two versus none increased the risk 4-fold (OR 3.8; 95% CI 2.7–5.2) while in subjects with three or more the risk was 15 times increased (OR 14.9; 95% CI 4.7–46). Hence, in patients not fulfilling the criteria for studies a more individual consideration should be made regarding the expected efficacy and the estimated risk of bleeding.

Management of bleeding in patients using VKAs

In case of major bleeding it may be required to reverse the anticoagulant effect of the various agents (Tab. 1) (31). When interrupting the administration of VKAs important differences in the half-lives of the various agents need to be taken into account (12):

- 9 hours foracenocoumarol,
- 36–42 hours for warfarin, and
- 90 hours for phenprocoumon.

The most straightforward intervention to counteract the effect of VKAs is the administration of vitamin K (32). There is quite some debate on the use of vitamin K in patients with a too high INR but no signs of bleeding. However, a recent randomized controlled trial did not find any difference in bleeding or other complications in non-bleeding patients with INR values of 4.5 to 10 that were treated with vitamin K or placebo (33). In patients with clinically significant bleeding administration of vitamin K is crucial to reverse the anticoagulant effect of VKA’s. Vitamin K can be given orally and intravenously, whereas the parenteral route has the advantage of a more rapid onset of the treatment (34). After the administration of i.v. vitamin K, within 2 hours the INR will start to drop and will be
Anticoagulants completely normalized within 12–16 hours (35), whereas after oral administration it will take up to 24 hours to normalize the INR (32). Intramuscular injections of vitamin K should be avoided in patients who are anticoagulated and subcutaneous administration of vitamin K results in a less predictable bioavailability (34). When the INR is below 7 a dose range of 2.5–5 mg vitamin K has been advocated whereas with higher INR’s a dose of 5 to 10 mg is required to correct the INR. Higher doses of vitamin K are equally effective but may lead to VKA resistance for more than a week, which may hamper long-term management (36). A potential concern with the use of parenteral vitamin K is the occurrence of anaphylactic reactions, although the incidence of this complication is very low, in particular with the more modern micelle preparations (37).

In case of very serious or even life-threatening bleeding, immediate correction of the INR is mandatory and can be achieved by the administration of vitamin K-dependent coagulation factors. Theoretically, these factors are present in fresh frozen plasma, however, the amount of plasma that is required to correct the INR is very large, carries the risk of fluid overload, and will probably take hours to administer (38). Therefore, prothrombin complex concentrates (PCCs), containing all vitamin K-dependent coagulation factors, are more useful. Although PCCs can indeed be given using fixed dose schemes, it has been shown that individualized dosing regimens based on INR at presentation and body weight are more effective (39). In a prospective cohort study of patients on VKAs who presented with major bleeding patients PCCs were effective in reducing the INR below 2 in 56 out of 58 patients (40). Another prospective study in patients using VKA and presenting with bleeding also found that PCCs resulted in at least satisfactory and sustained haemostasis in 98% (41). In recent years the safety of PCCs, in particular regarding the transmission of blood-borne infectious diseases, has markedly improved owing to several techniques, such as pasteurization, nanofiltration, and addition of solvent detergent. The risk of disseminated intravascular coagulation (DIC) due to traces of activated coagulation factors in PCCs comes from older literature and modern PCCs seem not to be associated with eliciting DIC (42).

Another option for immediate correction of the INR in patients using VKAs is the administration of recombinant factor VIIa, although this treatment is not officially approved for this indication. Correction of the INR after administration of rVIIa may be a “cosmetic” effect since the prothrombin time is very sensitive towards traces of VIIa in plasma, however, a small clinical study found that rVIIa at a dose of 16 μg/kg resulted in satisfactory haemostasis in 14 out of 16 patients who presented with major bleeding while using VKAs (43). A series of six patients with central nervous system bleeding due to treatment with VKAs showed successful arrest of bleeding in all patients after administration of rVIIa (44).

**Heparin, low molecular weight heparins and pentasaccharides**

Heparin and its derivatives act by binding to antithrombin and thereby about 1000-fold potentiating the anticoagulant effect of this endogenous inhibitor towards thrombin and factor Xa (and some other coagulation factors). Heparin is an effective anticoagulant and is widely used for prevention and treatment of both arterial and venous thromboembolism. Therapeutic doses of heparin are associated with a substantially increased risk of (major) hemorrhage and also low dose prophylactic heparin may cause bleeding complications (45). Heparin has a relatively short half-life of about 60–90 minutes and therefore the anticoagulant effect of therapeutic doses of heparin will be mostly eliminated at 3–4 hours after termination of continuous intravenous administration (46). The anticoagulant effect of high dose subcutaneous heparin, however, will take a longer time to abolish. If a more immediate neutralization of heparin is required, intravenous protamine sulphate is the antidote of choice.

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**Tab. 1 Reversion of the anticoagulant effect**

<table>
<thead>
<tr>
<th>anticoagulant</th>
<th>time until restoration of haemostasis after cessation of therapeutic dose</th>
<th>antidote</th>
<th>remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>heparin</td>
<td>3–4 h</td>
<td>protamine sulphate 25–30 mg → immediate reversal</td>
<td>1 mg of protamin per 100 anti-Xa units given in the last 2–3 h</td>
</tr>
<tr>
<td>LMWH</td>
<td>12–24 h</td>
<td>(partially) protamine sulphate 25–50 mg → immediate reversal</td>
<td>1 mg of protamine per 100 anti-Xa units given in the last 8 h</td>
</tr>
</tbody>
</table>
| pentasaccharides | • fondaparinux 24–30 h  
|               | • idraparinux 5–15 days                        | recombinant factor VIIa 90 μg/kg body weight → immediate thrombin generation | based on laboratory end-points, no systematic experience in bleeding patients |
| vitamin K antagonists | • acenocoumarol 18–24 h  
|               | • warfarin 60–80 h                             | vitamin K i. v → reversal in 12–16 h  
|               | • phenprocoumon 8–10 days                      | vitamin K orally → reversal in 24 h | dosage of vitamin K or PCC depends on INR and bodyweight |
| oral thrombin and factor Xa inhibitors | usually within 12 h, depends on compound | PCC or recombinant factor Xa for Xa inhibitors → unsure for factor IIa inhibitors | based on laboratory end-points, no systematic experience in bleeding patients |

LMWH: low molecular weight heparin; PCC: prothrombin complex concentrate; * experimental treatment
Protamine, derived from fish sperm, binds to heparin to form a stable biologically inactive complex. Each mg of protamine will neutralize approximately 100 units of heparin. Hence, the protamine dose in a patient on a stable therapeutic heparin dose of 1000–1250 U/h should be about 25–30 mg (sufficient to block the amount of heparin given in the last 2–3 hours). The maximum dose of protamine is 50 mg. Since the half-life of protamine is only about 10 minutes, the reversal of therapeutic dose subcutaneous heparin requires a repeated infusion of protamine sulphate (e.g. repeated after one hour). The effect of protamine can be monitored by measuring the activated partial thromboplastin time (aPTT), which should normalize after its administration.

The reversal of low molecular weight heparin (LMWH) is more complex, as protamine sulphate will only neutralize the anti-factor IIa activity and has no or only partial effect on the smaller heparin fragments causing the anti-factor Xa activity of the compound (47, 48). The net effect of protamine reversal of LMWH is not completely clear. There are no clinical studies that have systematically studied this and small case series and experimental animal studies show contradictory results (48–50).

As the aPTT is not useful as a monitoring assay when using LMWH, it can also not be used for the monitoring of the neutralizing effect of protamine. Given the relatively long half-life of LMWH, the lack of an adequate strategy to reverse its anticoagulant action may sometimes cause a problem in clinical situations.

A practical approach is to give 1 mg of protamine per 100 anti-factor Xa units of LMWH given in the last 8 hours (whereas 1 mg of enoxaparin equals 100 anti-factor Xa units). If bleeding continues, a second dose of 0.5 mg per 100 anti-factor Xa units can be given.

The most important adverse effect of protamine is an allergic response, including haemodynamic and respiratory problems (51). Most adverse reactions can be prevented or minimized by slowing the rate of administration of the drug or by pretreatment with steroids and antihistamines.

Risk factors for an adverse reaction are sensitivity to fish (as may occur in traditional fishermen that are often exposed to fish proteins when cutting themselves), a history of vasectomy (which may demolish the blood-testis barrier with consequent formation of anti-semen antibodies) and a history of receiving protamine sulphate containing insulin. Initial reports that the use of protamine sulphate could lead to an increased risk of rebound thrombosis, in particular ischaemic stroke (52, 53) were not confirmed in a recent randomized controlled study (54).

There are some other strategies to reverse (mostly unfractionated) heparin, such as platelet factor-4, heparanase, or extracorporeal heparin-removal devices, but none of these approaches have been properly evaluated and they are not currently approved for clinical use (55–57).

Pentasaccharides

Pentasaccharides are recently developed synthetic compounds that effectively bind and potentiate antithrombin to block factor Xa. Since they lack the additional glycosaminoglycan saccharide residues to bind to thrombin, it has an effect on factor Xa exclusively. The prototype pentasaccharide (and the only one approved for clinical use so far) is fondaparinux. Another pentasaccharide that is currently under study is idraparinux. The main difference between these two agents is the elimination half-life, which is 1–5 hours for fondaparinux and 5.5 days for idraparinux. This means that idraparinux can be administered once weekly, which renders the subcutaneous route of administration less cumbersome. Pentasaccharides were shown to be effective in the prophylaxis and treatment of venous thromboembolism and are currently evaluated in other types of thrombosis (58).

The (very) long half-life of pentasaccharides necessitates the availability of a suitable antidote if major bleeding complicates the treatment, which may especially occur in patients that are treated with therapeutic doses of this type of anticoagulation. So far, there is no antidote for pentasaccharides that has been studied in controlled clinical studies (59).

The only agent that has been systematically evaluated to reverse the anticoagulant effect of pentasaccharides is recombinant factor VIIa (rVIIa). Two randomized placebo-controlled studies in healthy volunteers have tested the hypothesis that rVIIa may be useful as a suitable antidote for pentasaccharide anticoagulation (60,61). In the first study sixteen subjects were treated with therapeutic doses of the pentasaccharide fondaparinux and after two hours (at the time of maximal anticoagulation) challenged with rVIIa or placebo. Injection of rVIIa (90 μg/kg body weight) after fondaparinux normalized the prolonged aPTT and prothrombin time (PT) and reversed the decrease in prothrombin activation fragments 1+2 (F1+2), as observed with fondaparinux alone. Thrombin-generation time and endogenous thrombin potential, which were inhibited by fondaparinux, normalized up to 6 hours after rVIIa injection. In the second study twelve subjects received a single s.c. dose of 7.5 mg idraparinux (which is threefold higher than the currently recommended dose). The inhibition of thrombin generation by idraparinux, as reflected by an increased thrombin generation time (TGT) and decreased level of prothrombin fragment 1+2, was partially reversed by injection of rVIIa 3 hours after idraparinux administration. The administration of rVIIa one week after treatment with idraparinux (when much lower, though still therapeutically, doses of the pentasaccharide were present) resulted in a nearly complete reversal of anticoagulation, reflected by normalization of thrombin generation time and other markers of thrombin generation.

As mentioned, there are no controlled trials in patients who present with pentasaccharide-induced bleeding but there is some anecdotal experience suggesting that rVIIa may indeed stop bleeding in patients anticoagulated with fondaparinux.

New direct factor Xa inhibitors

In recent years a large number of new antithrombotic agents has been developed and tested in clinical trials and many of these new agents will become available for clinical practice in the very near future (62). The need for new anticoagulant agents is quite obvious:
1. The current agents are insufficiently effective. For example 10–15% of patients undergoing major orthopedic surgery develop venous thromboembolism, despite prophylaxis with LMWH (65).

2. The available anticoagulants are relatively unsafe, mostly due to the occurrence of bleeding as discussed.

3. Current anticoagulant agents are often cumbersome with regards to their clinical use, requiring repeated laboratory control and frequent dose adjustments.

Increasing knowledge concerning the function of the haemostatic system in vivo has resulted in a new generation of anticoagulant agents.

Some of these new class of anticoagulants are directed against factor Xa. Prototypes of these agents are rivaroxaban and apixaban, which have shown promising results in initial experimental and clinical studies (64, 65).

- Rivaroxaban was evaluated in a series of trials in patients undergoing major orthopedic surgery (RECORD studies), which showed a higher efficacy of the direct anti-Xa inhibitor compared to enoxaparin and similar bleeding rates (66, 67).
- Apixaban was also compared with enoxaparin in patients undergoing knee replacement surgery and was shown to be equally effective but had significant less bleeding complications (2.9% in the apixaban group compared to 4.3% in the enoxaparin group) (68).

In dose-ranging trials in patients with acute venous thromboembolism rivaroxaban and apixaban were as effective as LMWH but rivaroxaban was associated with a lower incidence of bleeding complications (2.2% versus 8.8%) (69, 70). In a subsequent study rivaroxaban as single agent was more effective for treatment of venous thromboembolism than current standard treatment, i.e. LMWH followed by warfarin (71). Rivaroxaban was also studied in patients with acute coronary syndromes and showed a dose-dependent efficacy but also increased rates of major bleeding at higher doses (72). Similarly, apixaban showed a similar pattern and exhibited 2.5 fold increased bleeding rates, in particular in patients using simultaneous anti-platelet agents (73). Taken together, compared to LMWH direct factor Xa inhibitors result at doses achieving equivalent efficacy a lower bleeding risk and at doses achieving higher efficacy a similar bleeding risk. This means that for some clinical situations these drugs may represent an important improvement, however, the risk of (major) bleeding is still present.

Dependent on the severity of the clinical situation and in view of the half-life of the direct Xa inhibitors, cessation of medication may be sufficient to reverse the anticoagulant effect in case of bleeding. However, if immediate reversal of anticoagulation is required, there is no evidence of any antidote towards the anticoagulant effect of any of these orally available factor Xa inhibitors so far. However, a recent trial in healthy humans showed that administration of prothrombin complex concentrate was able to restore the rivaroxaban-induced inhibition of thrombin generation and normalized the prothrombin time (5). In addition, based on the experience with rVIIa in the reversal of the anticoagulant effect of fondaparinux, one can postulate that rVIIa may be an effective antidote for these agents, however, direct proof has not been demonstrated.

**Direct thrombin inhibitors**

Another important group of new anticoagulants is the class of direct thrombin inhibitors. Thrombin is the central enzyme in the coagulation process, not only mediating the conversion of fibrinogen to fibrin, but also being the most important physiological activator of platelets and various other coagulation factors. Inhibition of thrombin can be achieved by administration of heparin, but in view of the limited capability of the heparin-antithrombin complex to inhibit surface-bound thrombin, new antithrombin-independent anticoagulants have been developed (74). Prototype of these thrombin inhibitors is hirudin, originally derived from the saliva from leeches (*hirudo medicinalis*) and nowadays produced by recombinant technology.

Melagatran is a synthetic thrombin inhibitor, which has predictable pharmacokinetic properties and can thus be used in a fixed dose (75). Moreover, the pro-drug ximelagatran is relatively quickly absorbed after oral ingestion and results in a sufficient systemic availability, rendering this agent suitable for long-term use as oral anticoagulant. Despite clinical trials on prevention and treatment of venous thromboembolism and in patients with atrial fibrillation showing a promising efficacy of (x)melagatran, the compound has been withdrawn by the manufacturer, due to enhanced concentrations of liver enzymes in 6–7% of patients.

Recently, dabigatran, also a direct thrombin inhibitor with good and relatively stable bioavailability after oral ingestion, has been introduced and licensed for prevention of venous thromboembolism after orthopedic surgery. Indeed, clinical trials evaluating dabigatran against LMWH in patients undergoing major orthopedic surgery show similar or slightly better efficacy of the direct thrombin inhibitor and similar bleeding rates (76, 77). The largest group of patients using long-term anticoagulants, however, are those with atrial fibrillation. In these patients dabigatran (150 mg twice daily) showed a significantly lower rate of thromboembolic complications compared with warfarin (relative risk 0.66; 95% confidence interval 0.53–0.82) but also a slightly lower risk of major haemorrhage (3.11% per year in the dabigatran group versus 3.36% per year in the warfarin group) (78). Dabigatran was also shown to be equally effective as VKAs in the treatment of venous thromboembolism (79).

Based on these findings and if confirmed by other ongoing major trials, it may be quite likely that in the future oral anticoagulant treatment with vitamin K antagonists is going to be replaced by treatment with directly acting anticoagulants, such as direct thrombin inhibitors. However, the risk of major bleeding is still relatively large and requires adequate management strategies.

For each of the direct thrombin inhibitors no established antidote is available in case of serious bleeding complicating the anticoagulant treatment. Again, the half life
of most of the agents is relatively short, hence in case of less serious bleeding interruption of treatment will be sufficient to reverse the anticoagulant effect. However, if immediate reversal is required, it is not clear which would be the best strategy. Prothrombin complex concentrate failed to reverse the anticoagulant effect of dabigatran (5). Similarly, in a controlled clinical study in healthy subjects the melagatan-induced effects on aPTT, thrombin generation and platelet activation were not affected by the administration of rVIIa (80).

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