Organ-specific bleeding patterns of anticoagulant therapy: lessons from clinical trials

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Summary
Anticoagulants are effective at preventing and treating thrombosis, but can cause bleeding. For decades, vitamin K antagonists (VKAs) have been the only available oral anticoagulants. The development of non-VKA oral anticoagulants (NOACs), which inhibit either factor Xa or thrombin stoichiometrically, has provided alternatives to VKAs for several indications. The results of recent large-scale randomised controlled trials comparing NOACs with VKAs for the prevention of stroke in patients with non-valvular atrial fibrillation (AF) have produced some unexpected results. As a group, NOACs showed similar efficacy as warfarin, but a reduced risk of major bleeding. The reduction in bleeding with NOACs was greatest with intracranial hemorrhage. In contrast, the relative risk of gastrointestinal bleeding was increased with some NOACs. In this review, we explore the potential mechanisms as well as the implications of these organ-specific bleeding patterns.

Keywords
Venous thrombosis, stroke prevention, thrombosis

Introduction
Anticoagulants are effective for the prevention and treatment of thrombosis, but since they act by either reducing thrombin generation or inhibiting thrombin activity, they are associated with bleeding. The normal generation of thrombin at sites of vascular injury both reduces post-traumatic bleeding and prevents subclinical micro-bleeds from enlarging and forming clinically relevant bleeds. Both categories of bleeds are increased by anticoagulants.

In the late 1980s and 1990s, several randomised controlled trials (RCTs) demonstrated that vitamin K antagonists (VKAs) in doses adjusted to target an international normalised ratio (INR) between 2.0 and 3.0 were effective in reducing the risk of cardio-gentic embolism, primarily embolic strokes, in patients with non-valvular atrial fibrillation (AF) (1). These trials showed consistent risk reductions of about two-thirds in the incidence of stroke with VKAs compared to no treatment in intention-to-treat populations, and of about 80–85% in patients with excellent INR control. VKAs doubled the risk of major bleeding, but the trials had insufficient numbers of events to identify organ-specific patterns of bleeding (2).

Although VKAs are effective in reducing stroke risk in patients with AF, they require routine laboratory monitoring (3). To obviate the need for monitoring, a new generation of non-VKA oral anticoagulants (NOACs) with fewer drug and food interactions and more predictable pharmacokinetics has been evaluated. Over the past five years, several direct inhibitors of factor Xa or factor IIa (thrombin) have been evaluated in large RCTs (2, 4–8). Together, these trials enrolled more than 80,000 patients and to date (August, 2014), three compounds have been approved by regulatory authorities for prevention of stroke and systemic embolism in patients with non-valvular AF: two factor Xa inhibitors – rivaroxaban and apixaban, and one thrombin inhibitor – dabigatran. A fourth compound, the factor Xa inhibitor edoxaban, has recently been evaluated in a large RCT and could be approved shortly. In each of the recent trials, a NOAC was compared with aspirin or a VKA and each trial showed impressive efficacy and bleeding results for the NOAC (2, 4–8). Although these favourable results are likely, in part, due to careful selection of drug dose regimens, the greater variability of anticoagulant response to warfarin is also important; there were similar bleeding rates in NOAC- and warfarin-treated patients in centres with good INR control (9, 10).

Although major bleeding rates in the AF trials were low, the number of patients included in the trials was large, thereby providing a large absolute number of bleeding events. Further analysis revealed unexpected patterns of organ-related bleeding. In this paper, we summarise the relative risks of bleeding with NOACs compared with VKAs in different organs and explore possible mechanisms for the unexpected findings.

Organ-specific patterns of bleeding
Pooled data from the four trials comparing NOACs with VKAs (84,540 patients and 4,781 bleeding events) indicate that NOACs
were associated with a 23% reduction in the relative risk (RR) of major bleeding (RR 0.77, 95% confidence interval [CI] 0.73–0.82) (▶Figure 1 A).

Further analysis of the overall major bleeding rates revealed two contrasting “organ-specific” bleeding patterns (▶Figure 1 B).

First, intracranial haemorrhage (ICH) was uniformly reduced by NOACs, with median relative risk reduction of about 60% (median RR 0.42, range 0.34–0.52). This large effect is very important, as ICH is associated with a mortality rate of up to 40% and a high risk of permanent neurological deficits in survivors.

Second, the effect on gastro-intestinal (GI) bleeding varied by NOAC compared with VKAs; rivaroxaban and the higher doses of dabigatran (150 mg bid) and edoxaban (60 mg od) increased the risk, apixaban and the lower dose of dabigatran (110 mg bid) produced a similar risk, and the lower dose of edoxaban (30 mg od) reduced the risk of GI bleeding.

Potential causes of the patterns of organ specific bleeding

Intracranial bleeding

Although the quality of INR control (lower time in therapeutic range, TTR) is a risk factor for warfarin-associated bleeding (11, 12), most major bleeds, including ICH, occur while patients are within therapeutic range. In a recent study of 260 warfarin-associated cases of ICH, the majority of patients (78%) presented with INR results of 2.5–3.0, while only 13.5% had INR results higher than 3.0 (13). Furthermore, NOACs compared with warfarin were associated with a lower relative risk of ICH in centres with optimal INR control as well as in those with poor INR control (14), whereas a reduction in the relative risk of major bleeding was most evident in centers with poor INR control (9).

These findings support the hypothesis that additional mechanisms, other than quality of INR control, are responsible for the large and consistent reductions in the risk of ICH with NOACs compared with VKAs. We propose two such mechanisms: 1) the

![Figure 1: Overall and organ-specific relative risks of bleeding. Mean relative bleeding risk and 95% confidence interval for all major bleeding (A) and by bleeding subtype (Gastrointestinal, haemorrhagic stroke, or other, B). Horizontal bars represent 95% confidence interval of the pooled data.](image)
different mechanisms of action of NOACs and VKAs; and 2) the unique physiology of the brain.

Drug-related factors
When a blood vessel wall is disrupted, blood comes into contact with tissue factor (TF), which activates coagulation, ultimately leading to formation of a thrombus or a haemostatic plug. By interfering with vitamin K metabolism, VKAs lower blood concentrations of four vitamin K–dependent clotting factors (II, VII, IX, and X) (15), thereby effectively damping down thrombin generation following tissue injury. NOACs are stoichiometric inhibitors that selectively target the individual coagulation proteins, factor Xa (rivaroxaban, apixaban, edoxaban) and factor IIa (dabigatran). At concentrations seen with clinical use, stoichiometric inhibitors might be less effective than VKAs at blocking the high local concentrations of factor Xa or factor IIa generated in response to intense TF-mediated coagulation activation. TF is exposed to flowing blood at sites of rupture of a cerebral blood vessel (Fig. 2). The factor Xa or IIa molecules that escape neutralization are then able to generate more thrombin locally and limit the extent of bleeding, preventing it from becoming clinically overt (16).

Brain-specific regulation of haemostasis
The endothelial lining of brain capillaries is surrounded by pericytes and astrocytes, which produce a specialised subendothelial basal membrane that is rich in TF (17). It has been proposed that this TF-rich basal membrane acts as a protective mechanism against spontaneous intracerebral haematoma formation (18). Potentially further limiting the risk of bleeding is the low expression of tissue factor pathway inhibitor (TFPI) in the brain (18).

Autopsy and imaging studies have shown that asymptomatic capillary brain bleeds are found in 5–10 % of persons over the age of 60 in the general population. The prevalence of these microbleeds increases with age (19), as do other risk factors typically associated with ICH, such as hypertension and amyloid angiopathy. By impairing physiological haemostasis, anticoagulant therapy could promote the transformation of a subclinical “micro-bleed” into a clinically apparent “macro-bleed”, a notion that is supported by the observation that the presence of cerebral micro-bleeds markedly increases the risk of intracerebral haemorrhage in VKA-treated patients (20, 21).

We propose that NOACs are associated with lower frequencies of ICH than VKAs, because the two proposed mechanisms act in consort. Thus, the effects of the stoichiometric inhibitors can be overwhelmed by high concentrations of their target clotting enzymes and high concentrations of these clotting enzymes are generated locally by the high concentration of TF in the cerebrovascular micro-circulation. Our hypothesis is supported by results of several animal studies. In a murine model of induced intracerebral haematoma, treatment with VKA led to large expansions in hematoma volume and a poorer functional outcome (22, 23), whereas concentrations of dabigatran or rivaroxaban seen with clinical use of these agents did not increase hematoma volume (24, 25). A second murine model used highly focused laser beams to induce punctiform vascular lesions reminiscent of intracerebral micro-bleeds found in the human brain. In control animals the localised vascular lesions did not progress, whereas in mice pre-treated with a VKA, these small lesions led to macro-haemorrhages. In contrast, much like the control animals, mice treated with dabigatran did not develop macro-haemorrhages (25). Furthermore, pooled data from smaller trials with NOACs in patients with venous thromboembolism showed a similar 70 % relative risk reduction in intracranial bleeding.

Gastro-intestinal bleeding
NOACs may predispose to GI bleeding
Given the reduction in all non-GI bleeding, it is surprising that the risk of GI bleeding is increased with three regimens and not reduced with the other two. A likely explanation lies in the variable amounts of active drug that accumulate in the GI tract, which has the potential to induce local bleeding.

After being absorbed from the GI tract, VKAs target the hepatic enzyme, vitamin K epoxide reductase. In contrast, the NOACs directly target coagulation proteins and because they are incompletely absorbed by the GI tract, part of the oral dose remains in the lumen of the gut (26) (Table 1). In contrast to VKAs, which can only cause GI bleeding via a systemic anticoagulant effect, NOACs have the potential to cause GI bleeding through their both systemic and local effects on the GI mucosa.

Risk of GI bleeding differs among different NOAC regimens
In contrast to the risk of ICH, which is consistently lower with NOACs compared with VKAs, the relative risk for GI bleeding varies considerably among different NOACs, from a lower relative risk with lower dose of edoxaban to a higher relative risk with rivaroxaban and high-dose dabigatran and edoxaban. As shown in Figure 1, the different effects on GI bleeding of

<table>
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<th>Drug</th>
<th>Bioavailability</th>
<th>Active drug in the gut?</th>
<th>Intraluminal levels of active drugs</th>
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<tr>
<td>Warfarin</td>
<td>95–100 %</td>
<td>No</td>
<td>&lt;5 % (inactive)</td>
</tr>
<tr>
<td>Dabigatran</td>
<td>7 %</td>
<td>Yes (prodrug, activated by gut esterases)</td>
<td>~ 80 % (28)</td>
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<tr>
<td>Rivaroxaban</td>
<td>66 %</td>
<td>Yes</td>
<td>~ 30 % (30)</td>
</tr>
<tr>
<td>Apixaban</td>
<td>50 %</td>
<td>Yes</td>
<td>~35 % (29)</td>
</tr>
<tr>
<td>Edoxaban</td>
<td>62 %</td>
<td>Yes</td>
<td>~50 % (27)</td>
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Figure 2: Anticoagulant effect of direct anticoagulants and VKA at low and high tissue factor levels. A) Left panel: Tissue factor (TF), released as a result of tissue damage, activates factor VII to factor VIIa, which results in the activation of factor X to Xa, and finally in the generation of active thrombin (IIa). Warfarin (mid panel) reduces the functional levels of the coagulation factors II, VII, IX and X. This reduces the rate of thrombin generation upon activation by TF. NOACs are direct inhibitors of either factor Xa (rivaroxaban, apixaban, edoxaban) or thrombin (dabigatran). The right panel illustrates the effect of thrombin inhibition by dabigatran. Each molecule of dabigatran (green) inhibits a single molecule of thrombin. B) Intense activation of coagulation by a high local concentration of tissue factor (TF) increases the thrombin generation (left panel). Compared to warfarin (mid panel), which reduces the rate of thrombin generation, the relative anticoagulant effect of dabigatran becomes smaller as large numbers of thrombin overwhelm the available dabigatran molecules.

the NOACs are largely responsible for the differences in overall major bleeding. Although all NOACs are associated with a significant reduction in bleeding when GI bleeding is excluded (Figure 1B), the reduction in overall major bleeding is no longer statistically significant for dabigatran 150 mg and rivaroxaban because the risk of GI bleeding is increased with both regimens. Several factors might explain their different effects of NOACs on GI bleeding.
Mechanism of GI bleeding with NOACs

Differential absorption of NOACs

The fraction of non-absorbed drug depends on the bio-availability of the NOAC, which varies from very low (dabigatran etexilate), to about 60–70% for edoxaban and rivaroxaban (Table 1) (27–30). Dabigatran is administered as an inactive prodrug, dabigatran etexilate, which is converted to active dabigatran by esterases in the blood. However, the non-absorbed dabigatran etexilate is also converted into active metabolites in the GI tract, as evidenced by undetectable prodrug levels in contrast to high levels of the active drug in feces. Therefore, its formulation as an inactive pro-drug does not prevent accumulation of high local concentrations of active anticoagulant in the GI tract (28). It is not known where conversion from pro-drug to active drug occurs, but it is speculated that esterases in the gut bacterial flora are involved. This would explain why dabigatran is the only anticoagulant evaluated in the AF studies that has been reported to have a relatively higher ratio of lower GI bleeds compared to upper GI bleeds (31).

Different dosing and relative anticoagulant intensity of NOACs

Variation in the intensity of anticoagulation produced at the mucosal surface by different NOACs and different NOAC dosing regimens may also contribute to variation in GI bleeding risk. In the two studies that compared two doses of a NOAC with warfarin (5, 6), the incidence of GI bleeding was greatest with the higher than the lower dose of dabigatran and edoxaban, respectively. Furthermore, peak drug levels in the gut would be expected to be higher with a once-daily than a twice-daily dosing regimen, thereby explaining the relative increase in GI bleeding of rivaroxaban and high-dose edoxaban, compared to apixaban, which is administered twice daily.

Differences in study populations

An additional potential explanation for the observed differences in relative risk of GI bleeding among the NOACs may come from the differences between the patient populations included in the trials. Patients in ENGAGE and ROCKET were slightly older than patients in RE-LY and ARISTOTLE and had higher mean CHADS2 scores (5–8). Older populations have a higher prevalence of asymptomatic GI mucosal lesions, which are more likely to bleed in the presence of high local anticoagulant levels. Indeed, in the apixaban trial, GI bleeding represented only 25% of all major bleeding episodes in the control (VKA) arm, whereas in the rivaroxaban trial, GI bleeding accounted for 40% of all major bleeds.

Implications of organ-specific patterns of bleeding

There are two main implications.

• First, ICH is associated with a very high mortality, and can result in major disability. The finding that NOACs consistently reduce ICH compared to VKAs may lower the threshold for prescribing anticoagulants, thus increasing the overall use of these potentially life-saving drugs in AF.

• Second, the increase in GI bleeding with three of the NOACs regimens has led to the evaluation in subsequent randomized trials of the use of concomitant treatment with proton pump inhibitors to reduce GI bleeding. Patients with known mucosal lesions prone to GI bleeding or a history of GI bleeding may benefit from lower doses of NOACs or selection of NOACs not associated with increased GI bleeding. A detailed report of bleeding subtypes, both in clinical trials, and in post-marketing registries or observational studies, should yield important further information on the impact of these organ-specific bleeding risks.

Conclusion

In the AF trials, compared to VKAs, NOACs reduced overall non-GI bleeding, markedly reduced ICH, but three regimens increased GI bleeding. We speculate that these patterns of bleeding might be explained by different pharmacological properties of the anticoagulants.

NOACs inhibit single coagulation factors (Xa or IIa) in a stoichiometric manner. As a result, compared to VKAs, the effects of these anticoagulants can be readily overcome by TF-mediated activation of blood coagulation. Blood is exposed to TF at sites of vascular damage; the brain vasculature is particularly rich in TF. These observations could account for the reduction in overall non-GI bleeding and even larger reduction in ICH with NOACs compared to VKAs. The pattern of GI bleeding with certain NOACs can be explained by high levels of active drug within the GI tract, which could account for both the increase (3 NOAC regimens) and the variability (2 NOAC regimens similar, 1 NOAC regimen decreased) in the RR of GI bleeding compared to VKAs.

Recognising these patterns of bleeding risk has important management implications, particularly for patients who are vulnerable to GI bleeding.

Conflicts of interest

John Eikelboom has received consulting fees and/or honoraria from Astra-Zeneca, Bayer, Boehringer-Ingelheim, BMS, Daiichi Sankyo, Eli Lilly, GSK, Pfizer, Janssen, Sanofi-Aventis and grants and/or in-kind support from Astra-Zeneca, Bayer, Boehringer-Ingelheim, BMS, GSK, Pfizer, Janssen and Sanofi-Aventis. None of the other authors declares any conflicts of interest.

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