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MANAGEMENT OF HEMORRHAGIC COMPLICATIONS ASSOCIATED WITH ANTITHROMBOTIC THERAPY

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Management of Hemorrhagic Complications Associated With Antithrombotic Therapy

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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ABSTRACT

Anticoagulants are used for the prevention and treatment of arterial and venous thromboembolism. Such therapy is always associated with an increased risk of bleeding. Intracranial hemorrhage is the most serious form of anticoagulants-related bleeding and caries a high mortality rate. Many treating physicians and patients are reluctant to restart anticoagulation after such an event. For patients with continued increased risk of cardioembolism, the decision of when to restart anticoagulation is challenging and few small, single-center studies and case reports are the only available source of information.

Prothrombin complex concentrates (PCC) have been shown to rapidly correct the prolonged prothrombin time associated with vitamin K antagonist (VKA) therapy. The clinical efficacy of such treatment for controlling bleeding or preventing post-operative hemorrhage in patients treated with VKA is uncertain. Thromboembolism is a potential side effect of PCC and there are limited data on the risk of such complications.

New direct oral anticoagulants are approved as alternatives for VKA. These agents lack antidote and the management and outcome of patients with major bleeding on these agents is uncertain. In this thesis different aspects of the management of anticoagulants-associated bleeding complications are studied and discussed.

A cohort of 234 patients from Sweden and Canada with VKA-related intracranial hemorrhage was analyzed to establish the optimal time point for resumption of anticoagulation. Early resumption carries a high risk of recurrent bleeding, whereas the risk of thromboembolism increases with delayed start. According to the result of our study, the optimal timing of resuming

anticoagulation after VKA-associated intracranial hemorrhage seems to be between week 10 and 30 after the event.

A subgroup of the above-mentioned cohort was combined with another cohort from the Netherlands. A total of 135 patients were investigated to assess the effect of PCC - compared to plasma - on survival after VKA-related intracerebral hemorrhage. Unadjusted analysis showed a benefit for PCC treatment in reducing the 30-day all-cause mortality. After adjustment for differences in prognostic factors between the two treatment groups, especially the volume of intracerebral hematoma, the beneficial effect of PCC was attenuated and became non-significant.

The safety of reversal of VKA with PCC was assessed in a third cohort of 160 Swedish patients with a major bleeding event or before emergency surgery. Six patients (3.8%) developed thromboembolic events (5 arterial and 1 venous) within a week after the administration of PCC. In these patients, the unmasking of the increased risk for thromboembolism by the reversal of anticoagulation and the activation of the coagulation system by the bleeding or surgery, seem to be important contributing factors, while PCC seem to increase the risk only minimally, if any.

A cohort of 1121 patients with major bleeding on dabigatran or warfarin, derived from 5 phase III trials, was used to study the management and outcomes of these bleeding events in the absence of an antidote to dabigatran. The patients who suffered major bleeding on dabigatran were older, had more renal impairment and were more frequently treated with antiplatelet agents or non-steroidal anti-inflammatory drugs compared to those on warfarin, yet the outcome of dabigatran bleeding was better as reflected by a shorter stay by 1 day in the

intensive care unit and lower 30-day all-cause mortality compared to the warfarin group.

In summary, PCC seem to be safe for the treatment of VKA-associated bleeding, but their beneficial effect over plasma in case of intracerebral hemorrhage is questionable. The absence of a reversal agent for dabigatran does not seem to impact negatively on the outcome after major bleeding. When intracranial hemorrhage occurs on VKA resumption of anticoagulation should be delayed for 10-30 weeks.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Antikoagulantia, även kallade blodförtunnande, används för att förebygga och behandla en rad olika tillstånd som innebär en ökad risk för blodproppsbildning i artär- eller vensida. Användning av dessa medel innebär en ökad risk för allvarliga blödningar. Intrakraniell blödning (hjärnblödning) är den allvarligaste varianten av dessa blödningar och medför en hög dödlighet. Många behandlande läkare och patienter är tveksamma till att sätta in blodförtunnande igen efter en sådan händelse, men för patienter med fortsatt ökad risk för blodproppsbildning, måste blodförtunning återinsättas vid någon tidpunkt efter blödningen. Enstaka små studier och fallbeskrivningar är den enda informationskälla som finns tillgänglig som stöd för beslut om återinsättning av dessa läkemedel.

Vid behov av snabbt upphävande av warfarineffekten har ett läkemedel, protrombinkomplexkoncentrat, visat sig kunna snabbt korrigera blodprovavvikelser orsakade av warfarinbehandling, men den kliniska effekten av sådan reversering för att stoppa blödningen eller förhindra en sådan efter operation på warfarinbehandlade patienter är inte studerad. Behandling med protrombinkomplexkoncentrat kan teoretiskt leda till en ökad risk för blodproppsbildning, och det finns några rapporter och små studier om sådana komplikationer efter behandling med dessa medel.

Några av de nya blodförtunnandemedlen är registrerade som alternativ till warfarin. Till skillnad från warfarin saknar dessa mediciner modemedel som kan upphäva deras effekt och prognosen för patienter med större blödningar på dessa medel är fortfarande okänd.

Denna avhandling berör olika aspekter av hantering av blödningskomplikationer av blodförtunnande läkemedel.

Studie I

I denna studie analyserades 234 patienter från Sverige och Kanada med warfarin orsakad hjärnblödning för att fastställa den optimala tidpunkten för återinsättning av warfarin. Att sätta in warfarin tidigt efter en blödning innebär en hög risk för en ny blödning, medan att vänta lång tid med warfarin ökar risken för blodproppsbildning. I vår studie har vi noterat att den optimala tidpunkten för att återinsätta warfarin efter hjärnblödning förefaller vara mellan 10 och 30 veckor efter blödningen.

Studie II

I denna studie har vi analyserat data från totalt 135 patienter från Sverige, Kanada och Holland, som har drabbats av en speciell variant av hjärnblödning, intracerebral blödning, under warfarinbehandling. Vid en sådan blödning kan man ge antingen protrombinkomplexkoncentrat eller plasma för att upphäva warfarin effekten. Data analyserades för att bedöma effekten av protrombinkomplexkoncentrat jämfört med plasma för att förbättra överlevnaden. Den initiala analysen visade en fördel för behandling med protrombinkomplexkoncentrat med minskning av dödligheten under de första 30 dagarna efter blödningen. Korrigering för skillnader mellan de två behandlingsgrupperna, framförallt volymen av hjärnblödningen, försvagade den gynnsamma effekten av protrombinkomplexkoncentrat. Protrombinkomplexkoncentrat verkar således inte minskar 30-dagars dödlighet

jämfört med plasma behandling för patienter med warfarinrelaterad intracerebral blödning.

Studie III

Säkerheten av behandling med protrombinkomplexkoncentrat utvärderades i en tredje grupp av 160 patienter i Sverige som fick detta läkemedel för att upphäva warfarineffekten på grund av en större blödningar eller före akut operation. Totalt drabbades 6 patienter av blodproppar (5 på artärsidan och 1 på vensidan) inom en vecka efter behandling med protrombinkomplexkoncentrat. Sådana patienter har redan en ökad risk för blodproppsbildning utan blodförtunnande läkemedel, och dessutom bidrar aktivering av koagulationssystemet pga. blödningen eller operationen till den ökade risken. Behandling med protrombinkomplexkoncentrat tycks öka risken för blodproppar endast minimalt.

Studie IV

En stor grupp av 1121 patienter med allvarliga blödningar på ett nytt blodförtunnande läkemedel (dabigatran) eller warfarin studerades för att bedöma utfallet av dessa blödningar i frånvaro av ett motmedel mot dabigatran. De patienter som drabbats av större blödningar på dabigatran var äldre, hade sämre njurfunktion och var oftare behandlade med andra mediciner som påverkar blodets levringsförmåga jämfört med warfaringruppen. Prognosen av blödningar på dabigatran var bättre i form av kortare vistelse på intensivvården samt lägre 30-dagars dödlighet jämfört med warfaringruppen.

Sammanfattningsvis verkar protrombinkomplexkoncentrat vara säkert för behandling av warfarin relaterade blödningar, men deras fördel över plasma vid intracerebral blödning kan ifrågasättas. Frånvaron av ett motmedel för dabigatran verkar inte inverka negativt på utfallet av allvarliga blödningar. När en hjärnblödning inträffar hos en warfarin behandlad patient bör återinsättning av warfarin ske först efter 10-30 veckor.

LIST OF SCIENTIFIC PAPERS INCLUDED IN THE THESIS

I. **Majeed A**, Kim YK, Roberts RS, Holmstrom M, Schulman S. Optimal timing of resumption of warfarin after intracranial hemorrhage. ***Stroke***. 2010;41(12):2860-6.

II. **Majeed** A, Meijer K, Larrazabal R, Arnberg F, Luijckx GJ, Roberts RS, Holmström M, Schulman S. Mortality in vitamin K antagonist-related intracerebral bleeding treated with plasma or 4-factor prothrombin complex concentrate. ***Thrombosis Haemostasis***. 2014;111(2):233-9

III. **Majeed A**, Eelde A, Agren A, Schulman S, Holmstrom M. Thromboembolic safety and efficacy of prothrombin complex concentrates in the emergency reversal of warfarin coagulopathy. ***Thrombosis Research***. 2012;129(2):146-51.

IV. **Majeed A**, Hwang HG, Connolly SJ, Eikelboom JW, Ezekowitz MD, Wallentin L, Brueckmann M, Fraessdorf M, Yusuf S, Schulman S. Management and outcomes of major bleeding during treatment with dabigatran or warfarin. ***Circulation***. 2013;128(21):2325-32.

CONTENTS

LIST OF ABBREVIATIONS

AF	Atrial fibrillation
APCC	Activated prothrombin complex concentrate
APTT	Activated partial thromboplastin time
CI	Confidence interval
COX2	Cyclooxygenase 2
DOAC	Direct oral anticoagulant
DTI	Direct thrombin inhibitor
DVT	Deep venous thrombosis
eGFR	estimated glomerular filtration rate
GCS	Glasgow Coma Scale
GI	Gastrointestinal
ICeH	Intracerebral hemorrhage
ICH	Intracranial hemorrhage
INR	International Normalized Ratio
ISI	International Sensitivity Index
LMWH	Low molecular weight heparin
MI	Myocardial infarction
mRS	Modified Rankin score
NSAIDs	Non-steroidal anti-inflammatory drugs
OR	Odds ratio
P-gp	permeability glycoprotein
PCC	Prothrombin complex concentrate
PHV	Prosthetic heart valve

PT	Prothrombin time
RCT	Randomized controlled trial
rFVIIa	Recombinant activated factor VII (NovoSeven®)
SAH	Subarachnoidal hemorrhage
SDH	Subdural hemorrhage
SPAF	Stroke prophylaxis in atrial fibrillation
VKA	Vitamin K antagonist
VKORC1	Vitamin K epoxide reductase C1 subunit
VTE	Venous thromboembolism
WHO	World Health Organization

1 INTRODUCTION

Antithrombotic agents are used for the prevention and treatment of venous and arterial thromboembolism, which are important causes of morbidity and mortality. There are two classes of antithrombotic agents: antiplatelet agents and anticoagulants. Antiplatelet agents are most effective in preventing and treating arterial thromboembolism, where high sheer conditions lead to the development of thrombi rich in platelets and with only a small amount of fibrin. Anticoagulants, on the other hand, inhibit one or more factors in the coagulation cascade (Fig 1), leading to a reduction in fibrin formation, and are therefore most effective for venous thrombi, which are mainly composed of fibrin and red blood cells.

ANTICOAGULANTS

Anticoagulants are further divided according to the mode of administration into oral and parenteral agents. Parenteral anticoagulants can be either direct or indirect agents. The indirect parenteral anticoagulants include heparin, low molecular weight heparins (LMWH), fondaparinux and danaparoid. The effect of these agents is facilitated by endogenous plasma co-factors, the most important of which is antithrombin. Antithrombin inhibits the activity of several coagulation factors in the coagulation system, especially factor X and factor II. In the presence of LMWH, the inhibitory effect of antithrombin on factor X and II is greatly increased. The direct parenteral agents, on the other hand can directly inhibit coagulation factors without the need for other cofactors. Most of the available direct parenteral agents target, including hirudins, bivalirudin, and argatroban, inhibit thrombin.

Vitamin K antagonists have been the only available oral anticoagulants for over 60 years. Recently, several oral agents have been developed that directly and selectively inhibit different factors in the coagulation system. This thesis focuses on the management of bleeding events complicating two different classes of oral anticoagulants: vitamin K antagonists and direct thrombin inhibitors.

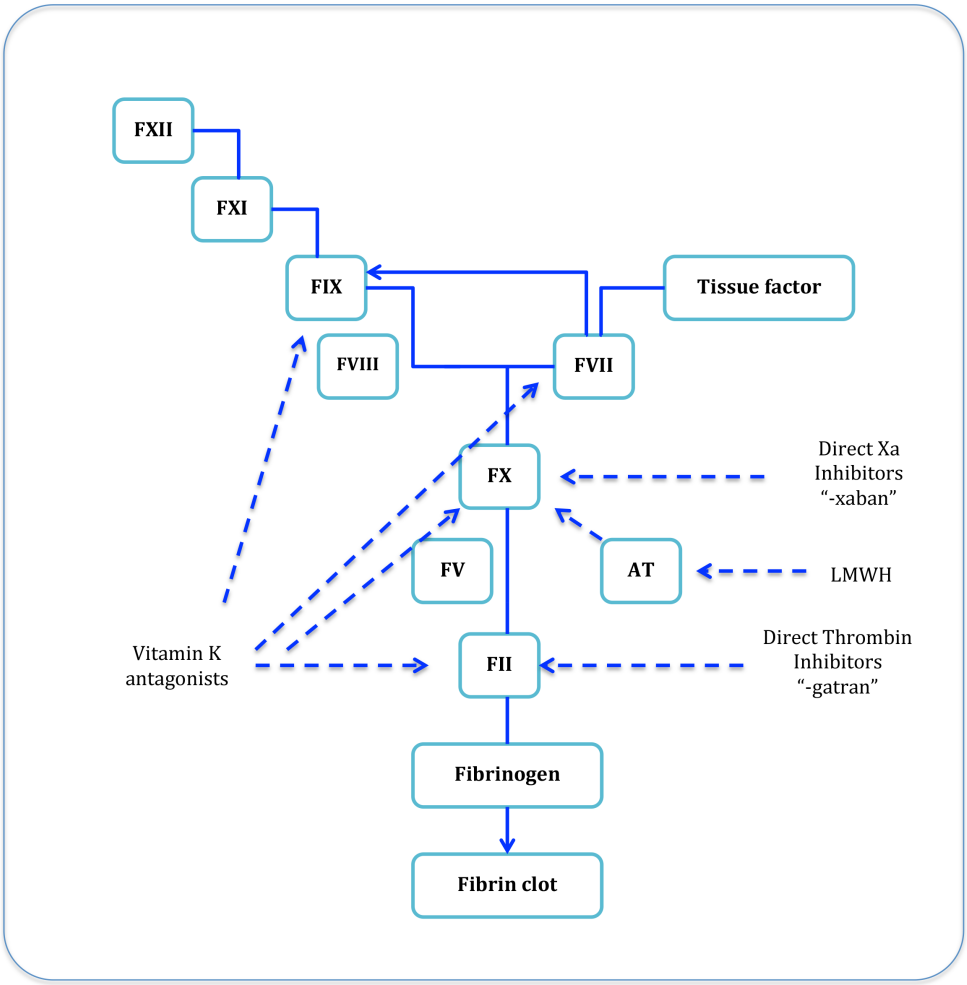

Figure.1 Site of action of the different anticoagulants

VITAMIN K ANTAGONISTS

Vitamin K antagonists (VKA), also known as the coumarins, have been the only available oral anticoagulants for more than six decades. They were the mainstay for the prevention and treatment of arterial and venous thromboembolism. The efficacy of VKAs has been shown in several studies and for a wide range of indications[1-3].

Despite the long experience with VKAs and the wealth of evidence supporting their efficacy, they still have several drawbacks and many physicians still find them challenging to use[4]. First, there is considerable inter-patient dose response variability due to genetic and environmental factors[5]. Second, these agents have a narrow therapeutic window. Another factor is that VKAs exhibit a wide range of drug and diet interactions[6]. These factors combined necessitate regular laboratory monitoring and dose adjustment, which in turn is difficult to maintain with a good level of quality.

Mechanism of action of VKA

VKA act by inhibiting the C1 subunit of vitamin K epoxide reductase (VKORC1) enzyme complex. The enzyme is important for the cyclic conversion between vitamin K and the 2,3 vitamin K epoxide. This reduces the regeneration of vitamin K1 epoxide and results in a condition of an acquired vitamin K deficiency, which in turn interferes with the γ-carboxylation of glutamate residues (Gla) on the N-terminal regions of vitamin K-dependent proteins[7-9]. This γ-carboxylation is important for the procoagulant activity of vitamin K dependent factors (factor II, VII, IX, and X). The end result of treatment with VKA is the production of partially γ-carboxylated proteins with reduced

coagulant activity[10,11].

The same process results in the production of defective vitamin K dependent anticoagulant proteins C, S and Z. Reduction of the levels of these anticoagulant proteins is seen earlier than most pro-coagulant vitamin K dependent proteins due to the longer half-life of the latter (especially factor II). Treatment with VKAs is therefore potentially thrombogenic in the early treatment phase[12], but the anticoagulant effect dominates later on.

Monitoring VKAs anticoagulant activity

Historically, the prothrombin time (PT) was used to monitor the intensity of anticoagulation during treatment with vitamin K antagonists. The PT, when measured with the Owren method commonly used in Scandinavian countries, is sensitive to the plasma levels of three vitamin K dependent coagulation factors (Factor II, VII and X), the functional level of which is reduced by VKAs. Prothrombin Time is either expressed in seconds, or as a ratio of the patient's plasma value to that of plasma from a healthy control subject, or as a percentage of diluted normal plasma. The resulting PT is not standardized and is dependent on the responsiveness of the thromboplastin added in the test to the reduction of activatible vitamin K dependent factors and the type of instrument used, resulting in difficulties in comparing and monitoring the intensity of VKA anticoagulation when the test is performed by different laboratories or by using different reagents. The solution was to standardize the reporting of PT using an "International Sensitivity Index" (ISI) specific for each thromboplastin reagent, that reflects the responsiveness of the given thromboplastin to the reduction of activatible vitamin K-dependent coagulation factors compared with the primary

World Health Organization (WHO) international reference preparations[13]. The resulting International Normalized Ratio (INR) is calculated according to the following formula:

$$INR = (\text{patient PT}/\text{mean normal PT})^{ISI}$$

Optimal therapeutic intensity

The goal of finding an optimal therapeutic intensity is to find a therapeutic window with maintained efficacy while causing the least side effect, the most important of which in case of anticoagulants is bleeding. Higher INR values are associated with an increased risk of bleeding[14-16]. The optimal therapeutic range may not be the same for all VKA indications. Depending on the underlying disease, characteristics of the patient and the balance between bleeding and thromboembolic risk, it may sometimes be necessary to adjust the INR target area upwards, thereby gaining efficacy while offering some safety, or vice versa. In venous thromboembolism, a "moderate-intensity" INR range of 2.0-3.0 was found to be as efficacious as a higher intensity one (3-0-4.0) while causing lower risk of bleeding[17]. Similarly, in patients with atrial fibrillation (AF), dose adjusted warfarin therapy to an INR of 2.0-3.0 is both effective and safe[18-20]. Conversely, lower intensity INR (<2.0) was found to be less effective than a moderate intensity INR regimen (2.0-3.0)[21-26]. The scenario is somewhat different in patients with prosthetic heart valves (PHV) and especially those with mitral mechanical heart prosthesis where experts (especially in Europe) recommend a higher intensity INR of up to 3.5[27,28].

THE NEW ANTICOAGULANTS

During the past decade, several new agents have been discovered or molecularly engineered to either inhibit the initiation of coagulation by targeting the factor VIIa/tissue factor complex, or to inhibit the propagation of coagulation by blocking individual coagulation factors, like factor V, IX, or X, or by inhibiting the formation of fibrin by blocking the last enzymatic step in the coagulation cascade at the level of thrombin. Of these drugs, inhibitors of factor Xa and of thrombin are the two groups that have reached furthest in clinical trials and several agents in these groups are now registered worldwide. These new direct oral anticoagulants (DOACs) still have their own limitations and management of bleeding complications in the absence of a specific antidote has been a hot topic addressed by several recent publications. Very recently, the results from a pre-clinical study on a humanized antibody that blocks the active form of FXII have shown promising antithrombotic effect without an increased bleeding risk[29], but this dissociation remains to be demonstrated in the clinical setting.

Oral thrombin inhibitors

Thrombin has a central role in the coagulation system, and drugs targeting thrombin for their anticoagulant effect were the first to be developed. These drugs act by directly and reversibly blocking the active site of thrombin hence their name direct thrombin inhibitors (DTI). Several agents were developed in this group. The first, ximelagatran, showed promising results in phase III studies[30] but was then withdrawn from the market in 2006 because of an increased risk of idiosyncratic hepatic toxicity[31,32].

Dabigatran etexilate was the second oral DTI to be developed and tested in large-scale trials. After oral administration, it has a relatively low bioavailability of 6.5% with an inter-individual variability of CV≈30%[33], reaching top plasma concentration after 1.5-3 hours. The prodrug is converted by unspecific ubiquitous esterases in the plasma to the active drug form, dabigatran, and is not dependent on cytochrome p450 for its metabolism [34]. It is, however a substrate for the permeability-glycoprotein (P-gp) transporter system, which is responsible for the efflux of dabigatran into the intestinal tract, and drugs that induce or inhibit the P-gp system have the potential to interact with dabigatran. About 35% of circulating dabigatran is bound to plasma proteins[34]. The half-life of dabigatran is 14-17 hours in patients with normal renal function. Eighty percent of the active drug is eliminated unchanged through the renal route[33]. Because of this predominant renal elimination, the half-life of dabigatran is prolonged in patients with renal impairment and there is a risk of drug accumulation in these patients.

Oral factor Xa inhibitors

This group of anticoagulants includes a larger number of drugs than the thrombin inhibitors. Several of these agents (rivaroxaban, apixaban and edoxaban) are now licensed in different parts of the world for a number of indications. These agents are small molecules that bind directly and reversibly to the active form of factor X, thereby blocking the coagulation system at the confluence of the intrinsic and extrinsic pathways. These drugs have a higher bioavailability (rivaroxaban: 80%, apixaban: 60%, edoxaban: 50%), a similar time to peak plasma concentration (≈1-4 hours) a shorter half-life (≈6-14 hours), are more protein bound in the

circulation, and less dependent on renal excretion (apixaban: 25%, rivaroxaban: 66%, edoxaban 35%) than dabigatran [35,36].

TREATMENT INDICATIONS

Atrial fibrillation

Atrial fibrillation is the commonest cardiac arrhythmia. It is an independent risk factor for the development of cardiac thromboembolism and is associated with a fivefold increase in the risk of ischemic stroke without antithrombotic therapy [37], corresponding to annual rates of about 5%[38]. During an episode of atrial fibrillation, turbulent and slow blood flow in the atria, especially in the left atrial appendage, contributes to the increased risk of thrombus formation and subsequent embolization. The exact incidence of ischemic stroke in patients with AF varies widely depending on several other associated conditions, including age, sex, diabetes, hypertension, heart failure, previous ischemic neurological events and possibly arterial vascular disease in general [39,40].

Several studies, including randomized controlled trials (RCT) have shown that treatment with VKAs reduces the risk of non-fatal ischemic stroke by two thirds. These agents were also shown to reduce the risk of fatal stroke by about 25%[18-20,38,41,42]. Vitamin K antagonists have become the standard therapy for stroke prophylaxis in AF (SPAF) with increased risk of thromboembolism, but due to the limitations of VKAs and especially the fear of bleeding complications, the uptake of VKAs for this indication is still suboptimal. Large studies have shown that compared to warfarin, several of the DOACs (dabigatran 110mg[43], rivaroxaban[44], apixaban[45] and edoxaban[46]) were non-inferior or even superior

(dabigatran 150mg)[43] for the ischemic stroke prevention in patients with AF. As with VKAs, treatment with these agents is associated with an increased risk of non-major and major bleeding events[18-20,38,41-46] but the rates are somewhat lower for the DOACs than for warfarin[43-46].

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Venous thromboembolism

Vitamin K antagonists have been shown to be effective for the treatment of deep venous thrombosis in the extremities as well as pulmonary embolism[1]. Results from the only one RCT that compared the use of anticoagulants to placebo in patients with pulmonary thromboembolism showed a significant reduction of mortality and venous thromboembolism (VTE) recurrence rate as well as reduced VTE related mortality[47]. Typically, treatment is initiated with LMWH parallel with VKAs, due to the observation of higher recurrence rates in patients receiving VKAs only[48]. A therapeutic range INR of 2.0-3.0 has been shown to be the optimal treatment intensity for patients on VKA for VTE[49,50]. A similar approach of initial combination with LMWH was used in the dabigatran VTE treatment studies, with comparable efficacy and better safety[51-53]. Two factor Xa inhibitors were, however, shown to be as effective when given at an increased dose for the first 1-3 weeks without the need of an initial treatment period with LMWH[54,55], simplifying the management of VTE patients. The optimal duration of anticoagulation in VTE is still discussed, and several factors are argued to affect this decision including age, sex, body mass index, the site of VTE, whether the patient has had a previous VTE or not, the presence or absence of provoking factors, thrombophilia, and other persistent risk factors as cancer[1] The DOACs are also as effective as LMWH in preventing VTE after major orthopedic surgery[56-62]. Dose adjusted warfarin therapy has also been shown to

be effective in reducing the incidence of post-operative venous thromboembolism[63].

Prosthetic heart valves

Mechanical prosthetic heart valves (PHV) are associated with an increased risk of both valvular thrombosis and stroke/systemic thromboembolism[64]. Most of the data available in the literature is based on follow-up of patients with older PHV models, and the risk of thromboembolic complications with the new models is likely to be lower[65]. Treatment with VKAs appears to drastically reduce the risk of these events by up to 90% at the price of increased bleeding risk[66]. Patients with combined prosthetic aortic and mitral valves appear to be at the highest risk of thromboembolism, followed by implants at the mitral and then the aortic positions[65,67]. Consequently, a higher anticoagulation intensity (INR range 2.5-3.5) is generally recommended for patients with mitral valve prosthesis[67]. Vitamin K antagonists seem to be more effective than low molecular weight heparins (LMWH) for long term anticoagulation of patients with PHV, but the evidence supporting this comes mainly from pregnant women with PHV[68]. The use of LMWH during pregnancy is advocated by some due to the increased risk of congenital malformations related to the teratogenic effect of VKA[69,70]. Recently a study comparing DTI dabigatran at doses adjusted according to plasma concentration with standard VKA therapy for patients with PHV was terminated prematurely du to an excess of thromboembolic and bleeding complications in the dabigatran arm[71].

Other indications

Warfarin is efficacious in a number of other conditions. In patients with acute coronary syndrome the use of warfarin as single therapy or in combination with aspirin is beneficial[72,73] leading to more substantial risk reduction compared with the addition of clopidogrel to aspirin. The high bleeding risk seen with combinations of warfarin and antiplatelet agents has probably let to limited use of such therapy compared to the aspirin-clopidogrel combination.

Warfarin is also used in patients with primary pulmonary hypertension to prevent the formation of small thrombi in the pulmonary arterial tree that might lead to further disease progression[74]. However, the evidence for this indication for warfarin is not strong[75]

BLEEDING COMPLICATIONS

Bleeding is the most serious complication of treatment with anticoagulants. Generally, bleeding complications are reported in clinical studies as minor, clinically relevant but non-major, major or fatal bleeding. There were no standardized definitions for these types of bleedings making it difficult to perfom indirect comparisons between different anticoagulants[76]. Later on, the International Society on Thrombosis and Hemostasis (ISTH), developed standardized criteria for the definition of major bleeding in clinical trials[77], and these criteria have been used in most non-surgical studies on the DOACs but in the surgical studies there were important differences in the definition.

Incidence of major bleeding complications on anticoagulants:

As mentioned above, the definitions of "major" and "minor" bleeds were somewhat different in the studies. This together with difference in treatment indication and patient's characteristics contributes to the variation in bleeding rates reported. It is also important to keep in mind the background risk of major bleeding in the respective patient group, i.e. the risk of bleeding events in comparable patients who are not on treatment with anticoagulants.

A review of published studies reported annual rates of fatal, major, and major/minor bleeding of 0.6%, 3.0%, and 9.6% respectively[78]. In a pooled analysis of five trials on the effect of warfarin in patients with AF, the annual incidence of major bleeding on warfarin was 1.3%, which represents a modest increase of the risk of bleeding compared to the control arm (1%) with an annual attributable risk of only 0.3%[38]. Recently, a meta-analysis including data from over 300,000 patients reported a pooled estimate of the rate of major bleeding of 2.51 (99% confidence interval: 2.03–3.11) bleeds per 100 patient-years[79]. Another meta-analysis of studies on warfarin treatment due to VTE, that compared bleeding rates in a group who discontinued warfarin after "short-term" treatment to those that discontinued warfarin after "long term" treatment" focusing on the period from the discontinuation of warfarin in the first group to the discontinuation in the second group reported almost a doubling of the bleeding rates in the group that continued treatment (0.6% vs. 1.1%)[80]

Intracranial hemorrhage (ICH) is the most feared type of anticoagulants related bleeding events, with mortality rates as high as 68% and considerable morbidity in patients surviving the bleeding event[81]. In a pooled analysis of the five major AF trials, the annual rate of ICH in the active VKA treatment group was 0.3%, compared to 0.1% in the control group[38], but another study showed a more

drastic increase in the risk[82]. Specifically intracerebral hemorrhage (ICeH) was shown to have the highest mortality rates of all types of ICH while subdural hematoma (SDH) has the highest recurrence rates[83]. One of the main advantages of the DOACs is the significant reduction of rates of ICH across all the phase III trials on these agents[43-46].

Gastrointestinal bleeding (GI bleeding) is the commonest type of major bleeding complicating treatment with anticoagulation. In one study GI bleeding in VKA treated patients occurred with an annual rate of 4.5% and was associated with a significant increase in morbidity and mortality[84]. The DOACs seem to be associated with higher rates of GI bleeding[43-46], probably due to higher concentration of these drugs in the GI tract, caused partly be the lower bioavailability and partly by the fact that excretion of these drugs into the GI lumen through the P-gp system is one of the elimination routes.

One detailed review of studies including patients who were carefully monitored for VKA treatment concluded that VKAs increase the annual risk of extra-cranial bleeding by 0.3-0.5% and of ICH by 0.2% compared to controls[85].

Predictors of bleeding events on oral anticoagulants

Several factors have been associated with an increased bleeding risk in patients on anticoagulants, including the intensity of anticoagulation, the characteristics of the patient, other interacting/potentiating drugs and the duration of treatment.

The intensity and stability of anticoagulation

a. The target INR

Randomized trials have repeatedly shown that higher target INR with VKA treatment is associated with an increased bleeding risk, regardless if the patient was on VKA for VTE[17], mechanical heart valves[86-90]or AF[91-93]. Targeting an INR of >3.0 was associated with doubling or the risk of major bleeding [17,87,89,90], and specifically, the risk of ICH doubled with each 1 unit increase in the targeted INR[14].

b. Actual INR

The actual INR intensity has been strongly related to the bleeding risk[94], and especially to the risk of ICH, which seems to increase sharply above INR of 4[65,95].

c. Time in therapeutic range (TTR)

The percent time in therapeutic INR range is a way of summarizing the quality of anticoagulation over time[96]. Patients with low TTR, reflecting a large variation in the INR over time, are at a higher risk of VKA related major bleeding, probably because they spend longer time in the supra-therapeutic range compared to patients with high TTR [16,97-99]

Duration of treatment

Several studies have reported a higher incidence of bleeding events early in the phase of introducing VKAs compared to the later treatment period[15,16,100,101].

First month bleeding rates of up to 3%, dropping to only 0.8% per month for the rest of the first year, and thereafter down to 0.3% per month were reported in one study[100].

Patient characteristics

a. Age

Most of the evidence for the effect of age on the bleeding risk while on anticoagulants comes from SPAF studies. A number of studies identified increased bleeding risk with age[98,102-105]. Age above 75 years was found to be an independent predictor of increased bleeding risk both in patients on warfarin (Relative risk, 6.6; 95% confidence interval [CI], 1.2–37; p = 0.032)[105] and in those on DOACs like dabigatran[106]. There also seems to be an interaction between supratherapeutic INR and old age, especially for ICH, resulting in a more significant increase in the risk of ICH for a given supratherapeutic INR as compared to younger patients[107,108]. The odds ratio (OR) for ICH in patients older than 85 years is 1.3 (95% CI, 0.8 –2.2) compared to those 70-74 years old at an INR range of 2.0-3.0; however the OR for the same comparison increases significantly to 2.5 (95% CI, 2.3–9.4) at an INR range of 3.5-3.9[95].

b. Gender

Studies have reported conflicting results on the effect of sex on the bleeding risk with the oral anticoagulants[109,110]. Women appear to have a higher incidence of minor bleeding while on oral anticoagulants[98], but the incidence of major bleeding seems to be similar in both sexes[98,105]

c. Previous bleeding

A history of previous bleeding has been shown to be a risk factor for new bleeding events[111-113]. Specifically a history of gastrointestinal (GI) bleeding predicts a new GI bleeding in the setting of anticoagulants [114], but this could not be confirmed in other studies[91,107,115]. It is also unclear if the changing panorama of peptic ulcer disease with the introduction of eradication therapy for helicobacter pylori would affect this risk factor. A history of a non-bleeding peptic ulcer has not been shown to increase the risk of recurrent GI bleeding[16,115]. For ICH, SDHs have been shown to carry a high recurrence rate[83].

d. Renal impairment

Patients with renal impairment represent a challenge for treatment with oral anticoagulants, both with the old and the new agents. Several guidelines list impaired renal function as a risk factor for bleeding on anticoagulants[85,116], although this association was only weak in other studies[16,20,107]. A systematic analysis of published data on the bleeding rates in patients with renal impairment and hemodialysis did not identify studies with comparison arm including patients with warfarin therapy and normal renal function, and all comparisons were made with historical data limiting the quality of the evidence[117]. To date, no randomized controlled trials have addressed this question properly.

The DOACs are all more or less dependent on renal function for elimination[36]. Therefore, renal impairment would at least theoretically increase the risk of drug accumulation and thereby increasing the bleeding risk. Indeed, renal impairment (estimated glomerular filtration rate [eGFR] less than 30 or 25 ml/minute) was an exclusion criterion in all phase III trials investing these agents. This trend of increased bleeding risk was shown in subgroup analysis of all the phase III

trials[118]. However, in one post-hoc analysis of major bleeding events in the RELY trial, which compared dabigatran and warfarin for SPAF, impaired renal function was not an independent risk factor for bleeding[106] Similarly, in the SPORTIF trial that compared the oral thrombin inhibitor ximelagatran and warfarin for SPAF, renal impairment was not an independent risk factor for bleeding after multivariate analysis. In one study with the factor Xa inhibitor rivaroxaban, patients with moderate renal impairment had increased bleeding risk as compared to those with normal renal function[119].

In another population-based trial, patients with AF and renal impairment, had increased rates of bleeding, regardless if they were treated with VKA or not; furthermore, the increase in bleeding risk seemed to be similar in both groups[120]. This illustrates the importance of having a comparison arm and can at least explain some of inconsistencies between the different trials.

In summary, there is no strong evidence that renal impairment is an independent risk factor for bleeding in patients treated with anticoagulants, especially for those on vitamin K antagonists.

e. Other comorbidities

Hypertension was found to be associated with an increased bleeding risk, especially ICH[100], with an associated relative risk of 3.4, p=0.05. In this study, the incidence of ICH was 7% in patients with a history of blood pressure >160mmHg compared to only 1% in patients with a history of blood pressure 140-159mmHg. Other studies have also found a similar association between major bleeding (regardless of the location) and hypertension [121], including even "treated hypertension"[85].

Hospitalization for alcohol-related diagnosis within the past 18 months was found to be an independent risk factor for readmission for bleeding in one study (relative hazard 2.6, 95% CI: 1.4 to 4.8)[113]. Other risk factors identified by studies include cerebrovascular disease[107], ischemic stroke[20,102], and serious heart disease[107]. A history of diabetes has been associated with an increased bleeding risk on anticoagulants[85], though this has been challenged by a more recent study[109].

The presence of malignancy has been strongly associated with an increased bleeding risk in several studies[113,115,122], and is related to the stage of the disease[123] but not to the degree of anticoagulation[25,123]. Indeed, the finding of higher bleeding rates and lower efficacy of VKAs compared to LMWH for patients with VTE and cancer has been the basis for recommending against the use of VKAs in cancer patients[124].

f. Pharmacogenetic factors

The cytochrome P450 CYP2C9 enzyme is responsible for the metabolism and the hydroxylation of the S-enantiomer of warfarin[125], and polymorphism of this enzyme have been identified. Two variants of the enzyme CYP2C9*2 and CYP2C9*3 seem to be associated with a slower metabolism of VKA and the need for lower doses to achieve therapeutic INR[126] and were also associated with higher incidence of major and minor bleeding in some studies[127-129]. Similarly, a polymorphism in the VKORC1 enzyme has also been shown to be associated with the need for lower dose of VKA[130], which might also be associated with an increased bleeding risk.

Another type of polymorphism affecting platelet glycoprotein IIb/IIIa, a receptor for fibrinogen that is important in platelet activation, has been associated with a

significantly increased risk of bleeding in a cohort of patients with VKA and antiplatelet therapy for ventricular assist device, even though the VKA dose, the INR levels and the platelet tests were not affected by this polymorphism[131]

g. Other concomitant drugs

Vitamin K antagonists are known to interact with a large number of drugs[132,133], affecting the pharmacokinetics and pharmacodynamics of VKAs, and necessitating more frequent control of INR when one of these drugs is administered during VKA therapy.

While VKAs affect the "plasma coagulation", antiplatelet agents further inhibit the coagulation process by blocking the primary hemostatic system. In a Danish study investigating the risk of non-fatal or fatal bleeding in combination therapy with warfarin, the risk of bleeding was doubled with the addition of aspirin, tripled when adding clopidogrel, and quadrupled when adding both aspirin and clopidogrel to warfarin therapy[134]. A meta-analysis found similar results[135]. In another meta-analysis, the risk of ICH doubled (RR, 2.4; 95% CI, 1.2– 4.8; p=0.02) when warfarin was combined with aspirin[136]. Data from several other studies were consistent with the above results of an increased risk of major or minor bleeding when antiplatelet therapy was added to VKAs[85].

In a recent post-hoc analysis of data from the RELY study, looking at the bleeding risk when antiplatelet agents were combined with the new agent dabigatran, a similar trend of doubling of the bleeding risk was noticed[137]. Similar results were seen in a pooled analysis of phase I and II studies in patients with myocardial infarction and combination therapy with antiplatelet and the DOACs[138].

Non-steroidal anti-inflammatory drugs (NSAIDs) are associated with an increased risk of bleeding, specifically upper GI bleeding and peptic ulcer bleeding[139]. Several studies[140], including registry studies[141,142], have shown that the addition of NSAIDs to VKAs increased the risk of upper GI bleeding by more than three times. Data on the concomitant use of NSAIDs and the DOACS are scarce with some studies reporting an increased bleeding risk[143], while others did not show such an increased risk[144]

Selective cyclooxygenase 2 (COX2) inhibitors are theoretically associated with lower rates of bleeding, and specifically lower GI bleeding rates, since they selectively inhibit COX2 resulting in less platelet inhibition and less gastric mucosal injury. Studies investigating the bleeding risk in patients receiving concomitant COX2 inhibitors and VKAs yielded diverging results, with some showing a similar, 2-3 fold increase, risk of GI bleeding compared to NSAID[145], and others failing to show an increased bleeding risk with such combinations[146,147].

There is currently a wealth of evidence that the use of paracetamol with VKAs causes a modest increase in the INR values[148,149], and is a risk factor for excessive anticoagulation[150]. One study reported an excess of upper GI bleeding in patients taking paracetamol and VKAs[141], although adjustment for comorbidities necessitating the intake of paracetamol in this group was not done, which limits the quality of the evidence.

Impact of bleeding on patient outcome

Bleeding carries the risk of increased immediate morbidity and mortality when occurring in the setting of anticoagulation. In addition, it seems to be

associated with poorer long-term outcome[151]. There are several explanations to these results; first, such bleeding events are usually managed by discontinuation of anticoagulation, either temporarily or permanently, which exposes the patient to an increased risk of thromboembolism[152]. Second, many of the factors that precipitate or predict a bleeding event are at the same time predictors of increased morbidity and mortality[153]. Third, anemia resulting from the bleeding leads to poorer outcome independent of the direct effect of bleeding itself[154]. Last but not least, transfusion of blood products has been shown to carry increased risk of adverse events, including volume overload, acute lung injury, infection transmission and is generally associated with poorer prognosis[155-157], and not always associated with improved survival[158-160].

Reversal of anticoagulation

In certain situations, as in the case of major bleeding or before an emergency invasive procedures or surgery, a rapid reversal of the effect of anticoagulants is needed to limit the bleeding or to allow safe surgery. Several reversal methods are available, and can be classified as non-specific supportive measures or specific reversal strategies. The choice of strategy depends on several factors including the intensity, type of and the indication for anticoagulation, the use of other hemostatic agents that can affect the coagulation system, the rapidity with which reversal is needed, patient characteristics, the severity and location of bleeding or the type of intervention needed. One of the risks of these reversal strategies is pushing the balance in the coagulation system towards the thrombotic side exposing the patients to an increased risk of thromboembolism. Therefore a careful choice of the reversal strategy is needed to avoid any adverse

events.

The “specific” reversal strategies available for the reversal of anticoagulants can be further subgrouped. Few anticoagulants have “specific antidotes”. Vitamin K can be used to competitively overcome the VKAs, allowing for the synthesis of functional vitamin K dependent coagulation factors. Protamin is a strong basic substance that binds to and inactivates the acidic heparin. Other specific reversal strategies involve administration of readily available coagulation factors, e.g. prothrombin complex concentrate (PCC) or plasma for the reversal of VKA effect. A third group includes the bypassing agents recombinant activated factor VII (rFVIIa), and activated PCC (APCC). Finally, compensatory pro-hemostatic agents to improve coagulation (desmopressin) or to reduce fibrinolytic breakdown of the hemostatic clots (e.g. tranexamic acid) can be utilized as adjuncts.

Assessment of the intensity of anticoagulation

In case of vitamin K antagonists, the standardized method for measuring the degree of anticoagulation is the PT, expressed as INR which can be performed as a point of care analysis in many emergency department, allowing for instantaneous assessment of the intensity of anticoagulation, or at a central laboratory. INR is standardized based on the ISI of the thromboplastin used in the local laboratory as discussed before[13].

Measurement of the degree of anticoagulation with the DOACs is more challenging: to start with, there are several drugs and classes of DOACs, and the different agents affect the coagulation system differently. There is no single standard test that can be used for all these agents, which makes prior information

on the specific drug used by patient in question essential for the measurement. Most of the tests that can measure the level of anticoagulation in patients on DOACs are still not widely available, and the non-specific tests (PT, INR, activated partial thromboplastin time [APTT], thrombin time, non-standardized anti-Xa) are affected differently by DOACs and normal results of these analyses do not completely exclude the presence of therapeutic concentrations of the DOACs[161,162]. Considering the relatively short half-life of the DOACs, the level of anticoagulation, and subsequently the effect on the coagulation system and laboratory tests, is also dependent on the time since the last intake of the DOAC.

In case of the DTI dabigatran, the best commercially available test to assess therapeutic or toxic levels of the drug is diluted thrombin time (Hemoclot®)[163]. A number of additional laboratory tests can also be used for accurate measurement of dabigatran level[163], but these tests are less available. Other tests give a more semi-quantitative evaluation; APTT has a linear correlation with the plasma concentration of dabigatran until therapeutic levels, beyond which it flattens out[161]. A normal APTT is therefore useful to exclude clinically important concentrations of dabigatran, but APTT is not useful to measure supratherapeutic levels.

For the factor Xa inhibitors (rivaroxaban, apixaban, edoxaban, and betrixaban), the use of chromogenic anti-Xa assay with drug-specific calibrators offers a quantitative way of assessing the level of anticoagulation[164,165]. The PT was found to be sensitive for the plasma concentration of rivaroxaban[166], but the result should not be transformed into INR for measuring the level of the Xa inhibitors, since INR is specifically designed for use with VKAs, and this calibration makes it less sensitive to the Xa inhibitors. A later study concluded that APTT was more sensitive than PT for rivaroxaban levels, but the effect of

low levels of rivaroxaban on the APTT was weak, therefore normal APTT cannot be used to exclude therapeutic concentrations of the oral Xa inhibitors[162]. Prothrombin time is less sensitive for apixaban than rivaroxaban and should not be used to assess the level of the former[167].

Non-specific measure

General supportive measures should be employed for all patients presenting with bleeding, regardless the cause or drug being used by the patients. They generally aim at giving circulatory support, reducing the level of drug exposure, and improving the hemostasis. Anticoagulants, and even concomitant antiplatelet drugs, should be temporarily discontinued in the event of a major bleeding.

General support of the circulation involves securing vascular access. Very careful consideration of the use of plasma expanders is encouraged as these agents might further disturb the coagulation by dilution of the different components of the hemostatic system. The administration of red cell concentrates, plasma and platelets according to a pre-specified ratio (4:4:1) has been seen to reduce mortality in massive hemorrhage in trauma, probably by preventing hemodilution and consumption coagulopathy[168].

Reduction of absorption of the ingested drug through the administration of activated charcoal can be used in cases of intoxication if the drug intake was within 2-3 hours before presentation[169,170]. Gastric lavage can also be used in this setting, though it caries a greater risk of aspiration in a patient with depressed level of consciousness. Enhancing drug elimination buy ensuring adequate diuresis is of particular importance in case of the DOACs which are more dependent on renal route of elimination than VKAs. Diuresis dose not reduce

drug exposure nor increase glomerular filtration, but it may reduce tubular reabsorption of these drugs[36].

Dabigatran has a low plasma protein binding (35%) and therefore, in case of serious bleeding or intoxication, hemodialysis can be used to remove the drug. Animal models have shown that the use of activated charcoal perfusion dialysis can reduce the drug level 75-80% after 1 hour of dialysis[169]. In another study and based on drug concentration in inlet and outlet dialysis lines, 62% and 68% of the drug was removed after 1 and 2 hours of dialysis respectively[171]. Several case reports have demonstrated the efficacy of this method in managing life threatening bleeding on dabigatran[172-175]. Dialysis is not effective in removing the Xa-inhibitors, which are highly bound to plasma proteins.

Several non-specific measures can be used to improve the hemostasis. Mechanical measures can be used to reduce blood loss, including compression of the bleeding vessel, surgical intervention to ligate bleeders, or endoscopically managing bleeding site, e.g. by adrenalin injection to a bleeding ulcer. Thrombin or fibrin glue can also be applied locally to reduce blood loss. When bleeding sites are not easily accessible, coiling of the bleeding vessel can be used.

Antifibrinolytic agents, such as tranexamic acid and epsilon amino-caproic acid, act by competitively binding to and blocking the active site of plasminogen, preventing its conversion to plasmin. These agents have been shown to be effective in reducing the blood loss in cases of trauma[176] and different types of surgery[177,178] without an increased risk or thromboembolism. Local use of tranexamic acid is effective in preventing excessive blood loss after tooth extraction in patients on warfarin therapy[179]. No studies have specifically looked at the role of antifibrinolytic agents in case of bleeding related to anticoagulants,

but their use in this setting should be encouraged due to their safety.

Acidosis, hypocalcemia, anemia, and hypothermia have all been shown to affect the hemostatic system negatively, and should be corrected promptly in the bleeding patient[180].

Specific hemostatic agents and antidotes

1. Vitamin K

Vitamin K_1 (phytonadione, phytomenadione) and vitamin K_2 (menaquinone) occur naturally in certain foods and vitamin K_2 variants are also produced by some of the intestinal flora. It can either be administered orally or intravenously and can reverse the effect of VKAs by replenishing the vitamin K depots. Intravenous administration is rarely (3/100 000) complicated by anaphylactic reactions[181]. Oral administration is not recommended in patients with malabsorption or biliary obstruction. Full effect is only achieved after 12-24 hours, making vitamin K unsuitable as the only treatment for patients with serious bleeding related to VKAs. Doses of 0.5-1mg seem to be enough to correct supratherapeutic INR to therapeutic range faster than only adjusting VKA dose and is associated with minimal risk of overcorrection[182]. In one study, treatment with low doses of vitamin K also led to lower bleeding rates[183]. Higher doses might be associated with "warfarin resistance" up to a week. However, such high doses of 5-10mg are recommended for patients with a serious bleeding event, with very high INR or with liver diseases[5].

2. Plasma

The use of plasma for the reversal of the effect of warfarin is still debated[184]. Plasma transfusion for the reversal of anticoagulation carries the risk of infection transmission, acute lung injury, volume overload, and incomplete anticoagulation reversal[155-157]. It is time consuming due to the need of ABO blood group compatibility, thawing and the need for transfusing a large volume for effective reversal[155]. In one study, the mean time to correction of INR with plasma transfusion for warfarin related intracranial hemorrhage was 30 hours[185], making it a less than ideal choice for the reversal of anticoagulation. Plasma, however, has a physiological, balanced combination of coagulation factors when administered as a fresh product. It is mainly useful in patients with large loss of volume or when the initial bleeding is complicated by hemodilution and consumption coagulopathy due to volume substitution with iv fluids[186,187]. No trials have investigated the use of plasma as the only reversal agent for the DOACs.

3. Prothrombin complex concentrates (PCC)

Prothrombin complex concentrates are lyophilized, heat or solvent-detergent treated, nano-filtrated concentrates of vitamin K dependent coagulation factors (factor II, VII, IX, and X) produced from human plasma. They can be divided into APCC containing both non-activated and low levels of activated vitamin K dependent factors, and non-activated PCC. Another way of classifying PCC depending on their level of factor VII, is 3-factor PCC containing low levels of FVII or 4-factor PCC with higher level of FVII. Some brands of PCC contain also anticoagulant proteins C, S and Z, as well as antithrombin, a low

concentration of heparin and sodium citrate. The addition of these substances aims at reducing the thromboembolic risk associated with the administration of PCC[49]. PCC are standardized according to their factor IX levels. Compared to plasma, PCC have a 25 times higher concentration of vitamin K dependent factors, thereby reducing the necessary volume to achieve similar effect by 25 times[188], PCCs are non-blood group specific, readily available at room temperature without the need of thawing, and these properties mean that PCC can be given more rapidly than plasma to treat serious bleeding. Several PCCs are available in different countries for the reversal of VKAs[157].

In patients with acquired deficiency of vitamin K dependent factors, the administration of PCC rapidly corrects the plasma levels of these factors. In a controlled trial, the INR was corrected to under 1.3 at 30 minutes after the end of infusion in 62% of patients on VKA treated with PCC compared to only 9.6% who received plasma[189]. It is, however, unclear if this more rapid reduction of INR can be translated into an improvement in morbidity and mortality, especially in patients with ICeH, where the more rapid reversal of anticoagulation can theoretically limit the expansion of the hematoma, which has been shown to be related to the disability and mortality after such bleeding[190-195]. There is a fear of the use of PCC because of the risk of thromboembolic events complicating their use[157], but the magnitude of this risk is still not well determined.

Data on the use of PCC for the reversal of the DOACs are emerging. In one trial in healthy individuals given dabigatran or rivaroxaban, PCC was able to correct laboratory abnormalities induced by rivaroxaban but not by dabigatran[196]. Case reports on patients with dabigatran related bleeding complications treated with PCC have been conflicting regarding its efficacy[172,197].

Activated prothrombin complex concentrates are mainly used for the treatment of hemophilia patients with acquired inhibitors to coagulation factors and are probably associated with higher risk of thromboembolic complications than PCC[198]. They are not used for the reversal of VKAs, but recent publications have shown promising results for the reversal of the DOACs, especially dabigatran, with APCC[199-201]. In a recent publication, four patients with life-threatening bleeding were managed successfully with APCC[202].

4. Recombinant activated factor VII

Recombinant FVIIa (rFVIIa, NovoSeven®, NovoNordisk, Denmark) was initially developed for the management of bleeding in patients with hemophilia and acquired coagulation factor inhibitors, but its off-label use has rapidly expanded. Several studies have demonstrated the efficacy of rFVIIa in the setting of VKA-associated bleeding, at least for correcting INR rapidly[203-206]. The use of rFVIIa for this indication is, however, not recommended due to the significantly increased risk of arterial thromboembolic events[207].

The limited experience from the use of rFVIIa for dabigatran bleeding has not been encouraging. It was not effective in limiting the bleeding in animal models[208], nor in correcting laboratory abnormalities induced by another DTI, melagatran, in healthy individuals[209]. It has not either shown convincing evidence of efficacy in a few case reports[172,173].

The use of rFVIIa for the reversal of factor Xa inhibitors is very limited, and most data available come from animal models, which have shown correction of laboratory abnormalities without a clear effect on the bleeding volume[210,211].

5. Specific antidotes

Specific antidotes have been developed recently to reverse the anticoagulant action of the DTI dabigatran and the Xa inhibitors using different approaches. A highly selective humanized monoclonal antibody fragment (Fab) against dabigatran was developed by Boehringer Ingelheim, Germany, and is currently in phase II studies in patients with dabigatran-related bleeding or who are in need of emergency surgery. The antibody binds selectively to dabigatran and has no other effects on the coagulation system. It has a rapid dose-dependent effect lasting up to 6 hours after intravenous injection and has been shown to reverse the anticoagulant effect of dabigatran ex-vivo as well as reduce the blood loss in a rat-tail injury model[212].

A recombinant modified factor Xa (PRT4445) that has a high affinity to the Xa inhibitors has been developed as an antidote to several oral and parenteral Xa inhibitors. By binding to these anticoagulants, it prevents them from binding to the native active factor X. The structural modification in this recombinant Xa decoy renders it inactive in the coagulation pathway. PRT4445 was shown to be well tolerated in healthy volunteers and with rapid (5min) and sustained (3 hours) effect. In preclinical studies, PRT4445 was shown to be effective in reversing the effect of betrixaban, fondaparinux and enoxaparin[36].

Recently, a universal anticoagulant-reversal agent has been developed by Perosphere; PER997 is a small molecule that can bind to and inactivate several of the novel oral agents, including dabigatran, rivaroxaban, apixaban and edoxaban. Rat-tail injury models have shown the ability of PER997 to reduce blood loss in animals treated with the above anticoagulants. It dose not affect

commonly used coagulation test and has no effect on the coagulation system in patients not taking anticoagulants. Clinical trials are now underway for the evaluation of the efficacy of PER997[36].

Restarting anticoagulation after bleeding

In patients suffering a major bleeding event while on anticoagulants, and in spite of the underlying increased risk of thromboembolism that warranted anticoagulation in the first place, the use of anticoagulants is usually discontinued. Depending on the indication for anticoagulation and the severity of the bleeding event, this discontinuation is either permanent or temporary. In the latter case the patient is sometimes started first on other forms of anticoagulation (e.g. prophylactic dose of LMWH) before oral anticoagulation is resumed. In many cases of VTE, treatment can either be discontinued permanently or the patient can be switched to LMWH, which is as effective as and seem to carry lower bleeding risk than VKAs. Patients with cardio-embolic indications for anticoagulation represent a special challenge, however; LMWH has not been systematically evaluated for the prevention of thromboembolism in patients with AF and this indication is not approved for LMWH. In patients with PHV LMWH are known to be less effective in preventing valvular thrombosis and systemic embolization, as discussed previously. The decision about whether to and when to restart anticoagulation must balance the risk of recurrent bleeding if the anticoagulation is restarted early after the bleeding, and the risk of a thromboembolic event if anticoagulation is delayed. A few number of studies have tried to address the question of when to restart anticoagulation after a major bleeding event[213-220], generally including patients form one center with limited

follow-up, and the question remains inadequately answered in the absence of large controlled trials or RCTs.

2 METHODS

STUDY POPULATION AND END POINTS:

Study I and II focused on different aspects of the management of patients with VKA-related ICH; hence some patients included in the Study I were also included in the Study II, and the two study populations were partly identical. Study III investigated the incidence of a particular adverse event of PCC, namely thromboembolism. Study IV assessed the outcome of patients receiving non-specific supportive therapy for major bleeding related to dabigatran, in the absence of a specific reversal agent. The population of Study I and II was therefore different from Study III, and a different population was used in the Study IV from the other three.

Study I and II

Several centers were needed to identify a sufficient number of patients for a reliable analysis. The fact that PCC were-at the time of the study- not registered for use in Canada and that plasma was the main modality for the acute reversal of VKA made it possible to compare PCC and plasma for the reversal of VKA by including patients from Europe and Canada.

All of the patients in the Study I (and most of those included in Study II) were recruited from 3 tertiary care hospitals: Karolinska University Hospital in Solna-Stockholm, and Karolinska University Hospital in Huddinge-Stockholm, both in Sweden; and Hamilton Health Sciences-General Hospital, in Ontario, Canada. Study II also included patients from The Netherlands who received PCC for the

treatment of VKA- related ICeH. The Dutch patients included in Study II were recruited from a tertiary referral center at the University Medical Centre Groningen, Groningen.

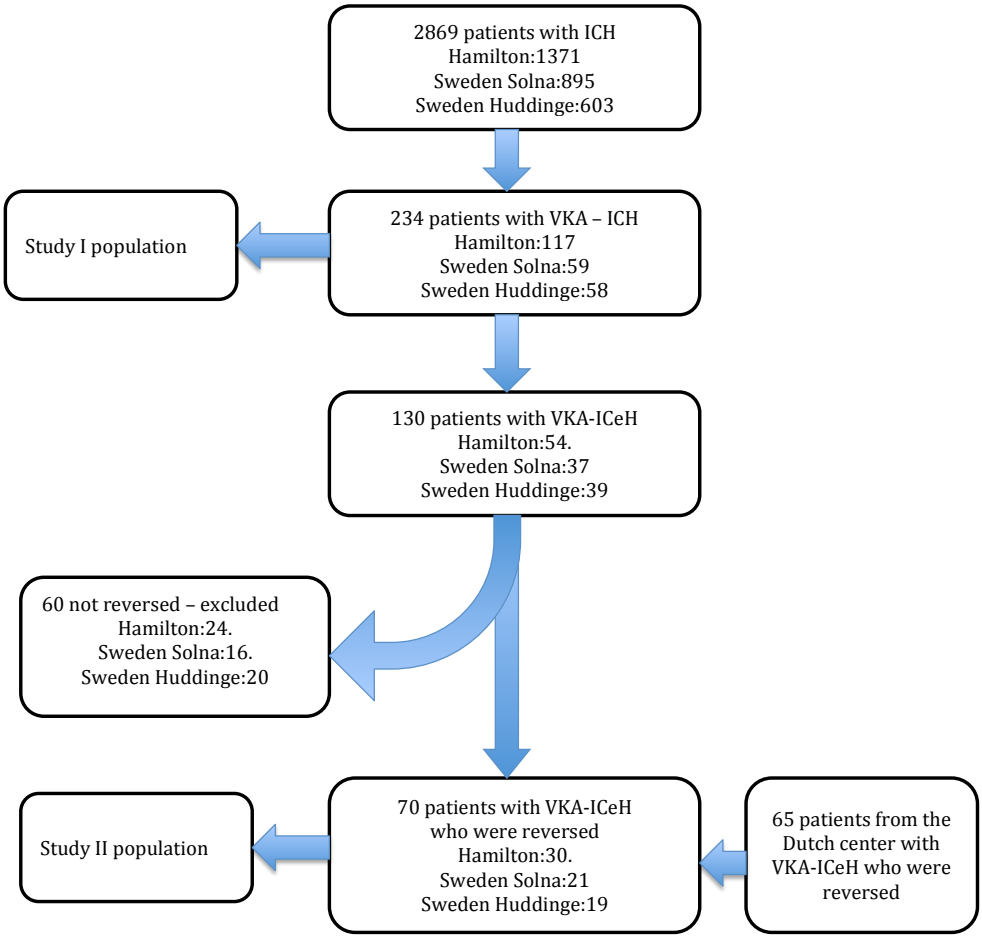


Figure 2: Population of Study I and II

Inclusion and exclusion criteria

The patients were eligible for inclusion if they had a radiologically verified ICH, were on VKA treatment and had an INR of more than 1.5 at the time of bleeding. For Study II, the ICH had to be an ICeH and an additional criterion was that the patient must have received either PCC or plasma for the reversal of VKA effect to be included. Any combination of ICeH with SDH or subarachnoid hemorrhage (SAH) was classified as ICeH. Patients with multi-trauma, or with hemorrhagic transformation of ischemic stroke were excluded from both studies.

Data extraction

Medical charts were manually reviewed for all patients admitted between January 2002 and December 2007 in Canada, and between January 2004 and December 2008 in Sweden, with a diagnosis of ICH (ICD-10 diagnosis code I600-I629). Data from patients with VKA-associated ICH underwent a final review by a third investigator. In The Netherlands, the medical charts of consecutive patients with VKA-related ICeH admitted between January 2003 and December 2010 were reviewed for inclusion in Study II.

Data regarding patient characteristics, indication for VKA treatment, risk score for stroke in patients with AF according to the CHADS2 score[39], additional antithrombotic treatment, INR at presentation, cause of bleeding (spontaneous or traumatic), location of bleeding according to radiological investigations, and neurosurgical intervention were collected.

The CT scans of all patients with ICeH that were included in the Study II were retrieved and the volume of the ICeH was calculated using 5-mm non-contrast

CT images, which were loaded into OsiriX Imaging Software, v.4.1.1 (OsiriX Foundation, Geneva, Switzerland). The volume of the hematoma was estimated by manual tracing of the perimeter with abnormal high attenuation on each CT slice. After all CT slices were evaluated, OsiriX Imaging Software combined the separate hemorrhage areas and rendered a three-dimensional representation from which the total hemorrhage volume was computed. One radiologist in Canada used this method to calculate the hematoma volume for patients from Canada and The Netherlands, and another radiologist in Sweden performed the corresponding calculations for the Swedish patients. To assess the inter-observer correlation, CT scans from 14 randomly selected patients were sent to both radiologists to independently measure the ICeH volume. The Pearson's r-correlation coefficient was then calculated from the volume measurements.

Another variable of importance in Study II was the time to PCC administration; which was calculated as the difference between the time of debut of ICeH symptoms – as documented in the patient's medical notes – and the time when PCC was given as noted in the transfusion records or medical notes. Since the administration of plasma takes longer time than PCC due to the volume of plasma needed for the reversal, the time to administration in the plasma group was calculated as the time from the onset of ICeH symptoms until the time when the transfusion of plasma was completed, as maximum effect of transfused plasma on the INR would be expected first when the whole volume of plasma was given.

End points

From the follow-up period after ICH, we collected data on the resumption of

warfarin, recurrent ICH, ischemic stroke, systemic embolism, transient ischemic attacks, heart valve thrombosis, VTE, death, and the timing of these events in relation to the index ICH. Data were retrieved from electronic hospital records and by contacting each patient's family physician.

Recurrent ICH was based on the radiological finding of a new ICH or expansion of the previously known ICH, performed due to new symptoms or deterioration of neurological status. Hence, the diagnosis of a recurrent ICH required a combination of new symptoms and new radiological findings. This was done to mimic the clinical setting where regular follow-up imaging is not always done without new symptoms. Similarly, the diagnosis of ischemic stroke had to be based on new neurological symptoms together with supportive radiological findings. We assessed the burden of recurrent ICH and ischemic stroke to be fairly similar when analyzing the optimal timing of resumption of anticoagulation. The occurrence of systemic embolization had to be objectively verified by imaging.

We used the 30-day all-cause mortality in the analyses of Study II, and did not use the cause-specific mortality as such classification is not always very reliable nor easy to do when the medical notes are reviewed retrospectively.

Study III

The population of Study III was recruited from centers spread over half of Sweden between February 2002 and October 2010. The patients were considered for inclusion when the Coagulation Unit at Karolinska University Hospital was contacted for advice on the dose and administration of PCC.

Dose recommendations for PCC were based on body weight, the actual INR and the severity of the bleeding/type of intended intervention. Dose calculation was according to the method described in a previous publication[221], but the actual given dose and any additional reversal with vitamin K or plasma was always decided by the local treating physician.

The investigator at the Coagulation Unit recommended checking the INR 30 minutes after the administration of PCC. In reality, not all the patients had their INR taken exactly 30 minutes after PCC infusion, and some others had several INRs after PCC administration e.g. in case of incomplete correction of INR to the desired level. We also recommended daily INRs after the successful correction.

Inclusion and exclusion criteria

Patients were included in the study if there was documentation in their medical journal of the transfusion of PCC for the reversal of VKA effect either because of a serious bleeding event or before urgent surgery. Treatment with PCC for other coagulopathies was an exclusion criterion.

Data extraction

The medical journals of patients included were requested a month after the major bleeding event. Data on the patient characteristics, indication of VKA, the bleeding location and severity, actual dose of PCC given, the use of different blood products, or vitamin K for the reversal of VKA effect, INR before and after given PCC, type of surgery performed-if any, length of stay in hospital, the

effect of given PCC in reversing the effect of VKA and controlling bleeding or preventing bleeding after a surgical intervention, the occurrence of thromboembolic events and death were collected from the charts.

End points

The main safety endpoint was the occurrence of arterial (stroke, myocardial infarction or peripheral thromboembolism) or VTE (DVT or pulmonary embolism) with 7 days after PCC administration, and depending on the identification of such events, the safety of PCC was judged as "good" or "poor". The event had to be objectively verified, and two investigators independently reviewed the medical journals for the occurrence of these events. We used this "7-day rule" to identify events that were ***likely*** to be directly related to PCC administration and the rationale for using the 7 days cut-off was based on the half-life of the different coagulation factors included in PCC and to allow for some time for the event to be diagnosed.

A secondary outcome in the study was the assessment the hemostatic effect of PCC. Two different measures were used, the first and main one was assessment of the clinical effect of PCC in stopping the bleeding, as judged by changes in hemoglobin level, the need for transfusion of blood products and intravenous fluid, or cessation of continued bleeding clinically or radiologically. In case of emergency surgery, the effect of PCC was judged depending on the amount of peri-operative bleeding documented versus the expected bleeding, and the actual need of blood transfusion versus the expected from the type of surgery performed. Three levels of "clinical efficacy" were used; "good efficacy" if the bleeding stopped shortly after PCC infusion and without the need of further

blood transfusion, “poor efficacy” if the bleeding and the need of blood transfusion continued after PCC or if the bleeding resulted in death or disability; “moderate efficacy” was used for cases falling between these two definitions. The other efficacy measure was the total number of cases successfully reversed to the desired INR level after PCC administration. The main focus was, however, on the clinical outcome rather than the surrogate laboratory end point.

Study IV

The efficacy of dabigatran as an anticoagulant was assessed in a number of phase III trials. The long term use of dabigatran versus warfarin was studied for SPAF (the RE-LY trial)[43] and for treatment of VTE (RE-COVER I[52] and RE-COVER II [222] for the acute treatment, and RE-MEDY[51] and RE-SONATE[223] for the extended treatment of VTE). In total, these trials included more than 16,755 patients on dabigatran and 10,002 patients on warfarin at several hundred centers worldwide. The RE-LY trial enrolled patients between December 22, 2005, and December 15, 2007. The different VTE trials included patients from April 2006 to October 2010. These five trials constituted the basis from which cases with major bleeding events that met the inclusion and exclusion criteria were included in our study.

Inclusion and exclusion criteria

Eligible cases were those with centrally adjudicated major bleeding event, defined according to the ISTH definition of major bleeding[77], and which occured either on treatment or within three days after the last dose of the study drug. This “three-day rule” was specifically used, after taking into consideration the half-

life of dabigatran and warfarin, and to ensure that sufficient amount of the study drug was still in the circulation to be attributable for the bleeding event. The three-day rule was applied for any - temporary or permanent - discontinuation of the drug. We extended this rule to include one patient in the dabigatran arm who had a major bleeding event 4 days after discontinuing the drug, because the patient had severe renal impairment and abnormal coagulation laboratory tests suggestive of clinically significant circulating dabigatran level even at 4 days after the major bleeding event.

Data extraction

Serious adverse event narratives and bleeding event narratives for all eligible patients who suffered a major bleeding event were obtained from Boehringer Ingelheim. The major bleeding event narratives were generated automatically from the database at Boehringer Ingelheim using a template, while the life threatening bleeding narratives had been prepared manually by a medical writer.

Two investigators examined and extracted data from the narratives on the patient characteristics, the use of antiplatelet agents or NSAID, the location and type of bleeding, treatment given for the bleeding including transfusion of blood products and/or other hemostatic agents, duration of hospitalization and death after the major bleeding event. These data were compared with and verified against the data in the database of each of the five phase III trials

Renal function was assessed with the Cockroft-Gault formula for calculating the eGFR, using the creatinine on admission for the bleeding event. Creatinine levels were sometimes unavailable from the hospital records during the major bleeding event, and in that case the most recent creatinine prior to the event was used. We

also adopted a similar classification of traumatic vs. non-traumatic ICH to that published by Heart et al, based on any description in the case report form (CRF) of injury that could reasonably cause the ICH[224]

End points

The total resources needed for the management of the bleeding events in this study were assessed using data collected from the RE-LY database at the Population Health Research Institute (Hamilton, Canada). This database is based on information in the CRFs. Data collected include the number of units of the different blood products given for each patient and proportion of all bleeds treated with the different blood products, the proportion of events that necessitated hospital admission, the length of stay in intensive care and in step-down unit, the decrease in hemoglobin from baseline before bleeding to the first obtained hemoglobin level during the event and from then to the lowest level in the event. Similar information was collected for the patients in the VTE trials, with focus on the utilization of the different blood products. We also registered information on death and the timing of death after the major bleeding event.

Patients suffering ICH while on anticoagulants rarely require transfusion of large volume of different blood products, unless the ICH is a part of other bleeding events, e.g in patients with multiple trauma. We therefore also captured data on the degree of and resources needed for the management of disability complicating ICH. Information was obtained from the study database on the modified Rankin Scale(mRS) both initially on admission for the ICH and then during follow-up. Information on discharge destination was also collected as an indirect measure of the degree of disability after ICH.

STATISTICAL ANALYSES

Variables in the different studies were tested for normality using skewedness, and QQ plots. We used mean values and standard deviations to describe data that was normally distributed and median and interquartile range for data that was skewed.

For continuous variables, groups were compared using two-tailed Student t-test or Mann-Whitney test depending on the distribution of the sample. Categorical variables and proportions were compared using Chi square or Fisher's exact test.

When comparing two groups for a dichotomous outcome, e.g. death-yes/no, effect-good/poor, several methods exist for correcting for any imbalances between the groups in confounders that can bias the outcome of interest. These measures may be implemented at the design stage, such as randomization, or at the analysis stage as stratification or multivariate analysis. In our studies, we used both multivariate analysis (Study II and IV) and stratification (Study IV) to correct for such imbalances.

In Study III, a modified intention-to-treat analysis was used including all patients who actually received PCC for the reversal of warfarin effect. The Exact method was used to calculate the confidence intervals (CIs) of proportions, which gives an asymmetrical interval with a higher upper limit than the conventional Wald method, but ensures better 95% coverage.

In the Study II, a step-wise logistic regression analysis was used to assess the effect of reversal strategy on the mortality of patients with ICeH and at the same time to adjust for the imbalance of important prognostic factors between the two

treatment groups. The initial model was constructed with only the reversal method as an independent variable and the 30-day all cause mortality as the dependent variable. The odds ratio (OR) obtained at this stage represent the crude (unadjusted) OR for mortality in the plasma and PCC-treated groups. In the following steps, the other prognostic variables were added in a forward step-wise manner. Adding any variable to the model corrected for the imbalance in the variable between the plasma and the PCC-treated group, and the resulting OR from the model will then be adjusted for the included variable(s). The associated p-value with the newly included variable is based on the Wald test and reflects if the newly added variable significantly impacts on the OR. The process of adding new variables will continue until there is no significantly important variable.

Logistic regression was also used in Study IV to adjust for the variable differences between the warfarin group and the dabigatran group and the impact of these differences on the 30-day mortality in both groups.

The effect of time-dependent variables was studies in the Study I and IV. In Study I, the effect of the timing of warfarin resumption after warfarin-related ICH on the risk of recurrent ICH or thromboembolic events was assessed using Cox-proportional hazards model, which also allows for the adjustment for difference in other variables in a similar way as the logistic regression. A model was fitted separately for recurrent ICH and another model for ischemic stroke. The time variable was divided into “periods” with reasonably constant risk of the event of interest. These periods and the associated risk differed between the recurrent bleeding model and the model for ischemic events because of the difference in the nature of these two outcomes. The two models were then combined to obtain a “total risk” and determine the interval when such total risk

is lowest and therefore would correspond to the optimal period of warfarin resumption. The assumption in the Cox-model was that warfarin resumption at any time results in a constant proportional increase of the risk of ICH and a constant proportional reduction in the risk of ischemic events. This assumption was also tested for and verified in post-hoc tests.

Kaplan Meier analysis was used to assess the unadjusted difference in the 30-day all cause mortality in the dabigatran and the warfarin arm, and to test for statistical significance (Figure 1 in the fourth study). The Kaplan Meier analysis was also used to test for the differences in the 30-day mortality after PCC treatment for ICeH with and without adjustment for the hematoma volume.

A P value of < 0.05 was considered statistically significant.

Different statistical software was used during the course of these studies for the analyses including SAS 9 and 9.3 (SAS Institute Inc., Cary, NC, USA), STATA 12 and Microsoft Excel 2003.

ETHICAL CONSIDERATIONS

All studies in this thesis complied with the Declaration of Helsinki. Study I and II were approved by the institutional ethics committees in Sweden and in Canada. These ethics committees waived the need for patients consent to access the data in the medical records. For Study II, legislation in The Netherlands and local hospital medical ethics committee do not require approval or review of the protocol for retrospective studies in Dutch patients. Study III was approved by the institutional ethics committee in Sweden. For Study IV, all 5 phase III studies were approved by the local ethics committee in the countries where the studies

were conducted, and for our analysis of the major bleeds, the Research Ethics Board of McMaster University Faculty of Health Sciences-Hamilton Health Sciences approved the project without the need for patient consent. All data analyses were done on anonymous data without the possibility of tracing back the individual records to the patients.

3 RESULTS

STUDY I

Baseline characteristics

A total of 3287 admissions for ICH in 2869 consecutive patients were identified at the three study centers during the study period. After manual screening of the medical records of all admissions, 234 (8.2%) patients were found to fulfill the inclusion and exclusion criteria. Baseline characteristics of the patients and their distribution according to the study center are shown in table 1 and table 2, respectively in the original paper.

The indication for anticoagulation in 58% of the patients was AF. This is also reflected by the median age of the patients (76 years).

Follow-up and mortality

Fifty-seven patients died within the first week after the ICH, most of them (54 patients) because of direct effect of the ICH. The remaining three suffered arterial thromboembolic events (2 ischemic strokes and 1 myocardial infarction) that resulted in fatal outcome. Intracerebral hemorrhage had the highest mortality within the first week (47 patients, representing 36% of all ICeH), which was significantly higher than first week mortality in other types of ICH (bleeding was fatal in the first week in 3 patients [14%] with SAH, and 7 patients [8%] with SDH). Mortality was higher in the ICeH group throughout the follow-up (59%) compared to patients with SDH (32%), and this difference was statistically

significant (Fig 3. Kaplan–Meier analysis, P<0.001). This group of patients with a fatal outcome within the first week was excluded from the analysis of the optimal timing for the resumption of warfarin because restarting warfarin within the first week is rarely considered, if at all, due to the presumed high rate of recurrent bleeding.

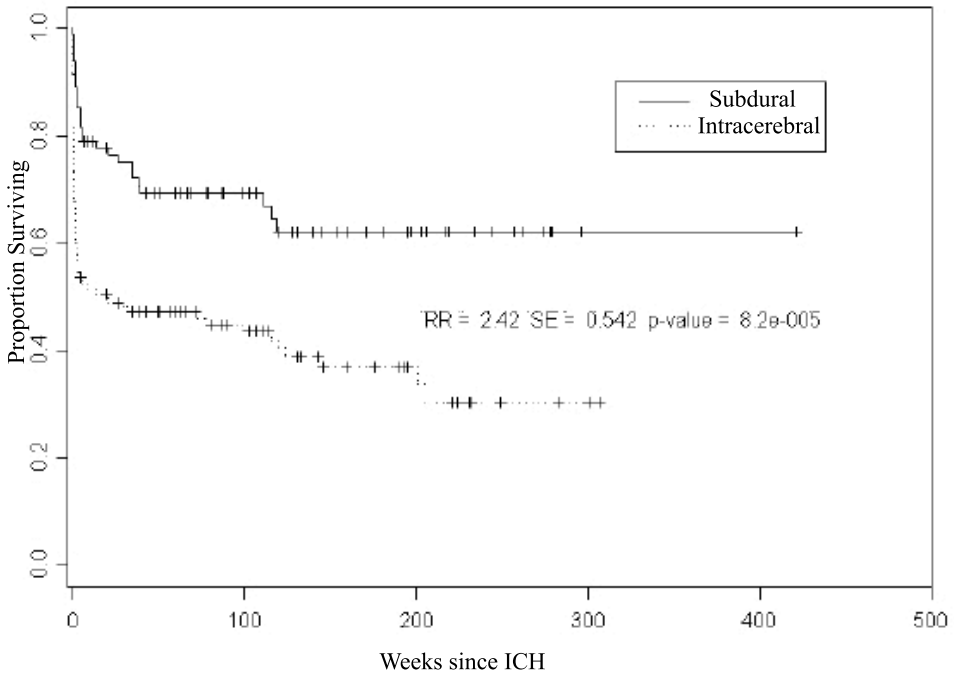


Figure 3: Mortality according to the bleeding site (intracerebral vs. subdural)

The remaining 234 patients were followed for a median of 34 weeks (IQR 1–115), and during this period 113 patients (48%) died, 57 of whom within the first week as mentioned above, corresponding to a median survival of 4.5 years after the ICH (figure 4). This high early mortality shortened the median follow-up. However, by excluding patients with fatal outcome in the first week as mentioned above, the median follow-up increased to 69 weeks (IQR 19 –144). The remaining 177 (first week survivors) constituted the basis of our risk-benefit analysis group.

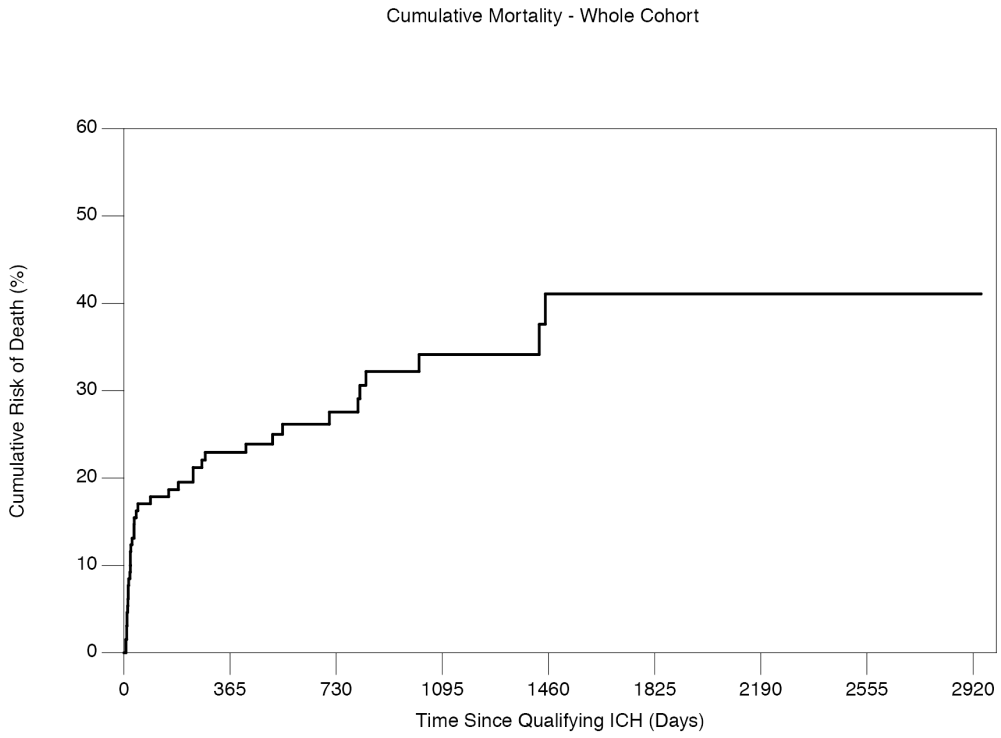


Figure 4: Mortality during follow-up in the entire cohort

Restarting warfarin after the ICH event

Of the 177 patients surviving the first week, 59 patients (33%) were restarted on warfarin after a median of 5.6 weeks (IQR 2.6–17). The median age of those who restarted warfarin was 70 years (IQR 63–77), which was significantly younger than those who did not restart warfarin (78 years, IQR 70.5–72). A significantly higher proportion ($p<0.001$) of patients with mechanical heart valves (15 [79%] of the patients with mechanical aortic valve and 7 [77%] of the patients with mechanical mitral valve) were restarted on warfarin after ICH as compared to those with AF (22 patients representing 22% of all AF patients in the study) or VTE (8 patients comprising 27% of VTE patients at baseline). Patients with PHV also resumed warfarin after a shorter period (4.6 weeks [IQR 2.3–15] for patients with mechanical aortic valve, 3.1 weeks [IQR 2.2–14] for patients with mechanical mitral valve) than the 22 patients with AF who resumed warfarin after a median of 9.2 weeks (IQR 5.6–34). Unexpectedly, the 8 patients who resumed warfarin for VTE did so after only a median of 2.3 weeks (IQR 1.7–19).

When analyzed according to bleeding localization, warfarin was restarted in a higher proportion of patients with SAH (7 patients, 39% of first week SAH survivals) and SDH (29 patients, 38% of SDH patients who survived the first week) as compared to patients with ICeH (23 patients, 28% of ICeH first week survivals), although this difference was not statistically significant. Conversely, resumption of anticoagulation was earlier, after a median of 4.4 weeks (IQR 2.3–14), in the ICeH group as compared to a median of 6.4 weeks (IQR 3.6 –26) after SDH.

There was no difference in the proportion of patients with previous stroke who restarted warfarin (10 patients, 31%) compared to the proportion of those

restarted without previous stroke (49 patients, 34%).

Recurrent ICH

Of the 59 patients who restarted warfarin after ICH, 8 patients developed a recurrent ICH, as compared to 10 recurrences in the 118 patients who never resumed anticoagulation. The Kaplan-Meier analysis showed a trend (p=0.07) towards higher recurrence rate in the patients with SDH (16%) compared to ICeH (8.4%)(fig 5). None of the patients with SAH had recurrent bleeding. In one patient with ICeH at baseline, recurrence was as SDH. Another patient had an opposite sequence of presentation. The remaining ICH recurrences were all in the index presentation site. The cumulative risk of recurrent ICH without restarted anticoagulation or from the time point of resumption of anticoagulation is shown in Figure 1A and 1B, respectively, in Study I.

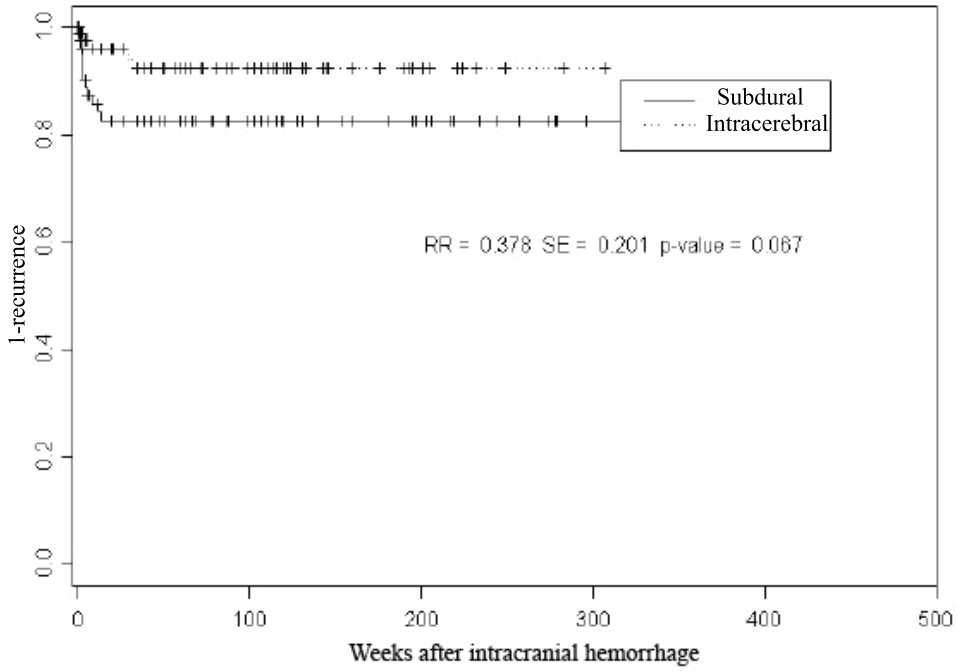


Figure 5. Recurrent ICH according to localization (intracerebral vs. subdural)

Thromboembolic events

In total, there were 21 arterial and 11 venous thromboembolic events during the study period, regardless the status of warfarin resumption. Most of the arterial events (n=18) were ischemic strokes, occurring in 12 (12%) of all survivors after the first week in the AF group, 4 (14%) in the mechanical heart valves group, 1 (3%) in the VTE group, and 1 (6%) in the group who had other indication for warfarin. The remaining 3 events were 1 transient ischemic attack, one systemic embolism to the brachial artery and one to the femoral artery. The group with

previous stroke had a significantly higher risk of recurrent ischemic event (28%) compared with those without (8.6%); (P=0.004). Of the 11 VTE events in the study, only 4 had VTE as the indication of anticoagulation before the ICH, and these events tended to occur early, after median of 2 weeks (IQR, 1.5–5) from the ICH.

Risk modeling of recurrent ICH versus thromboembolic events

Of the 177 patients who survived the first week after ICH, 45 patients had VTE as indication for anticoagulation. Since other management alternatives (either permanent discontinuation of anticoagulation or shift to LMWH, which is as effective for VTE treatment as warfarin, possibly with lower bleeding risk) are more frequently considered in patients in the VTE group versus those with cardiac indication for anticoagulation (AF or mechanical heart valves) we decided to do a risk modeling including only patients in the latter group or those who had a previous stroke.

Restarting warfarin at any time after the ICH must take into account the increased risk of recurrent bleeding after warfarin resumption (relative to the risk of recurrent bleeding without warfarin resumption) and to balance that against the risk of an ischemic event after the index ICH and before warfarin resumption, during which the patient is left without anticoagulation (relative to the reduction of that risk after the point of warfarin resumption). In this analysis, we assumed that any recurrent ICH has the same clinical burden or disutility as an ischemic event, though this approach might be criticized (see methods and discussion).

We aimed to build a statistical model that takes into consideration the observed

rates of recurrent ICH and of thromboembolism, according to warfarin resumption status and depending on the time point of warfarin resumption after the ICH. The model would then be used to make inferences about predicted rates of these events in the restarted and in the non-restarted group and for each proposed "warfarin resumption interval", given a sufficiently large cohort of patients.

A Cox proportional hazards model, with a time dependent variable that represented the point of warfarin resumption, revealed that the "risk" of recurrent ICH after an initial warfarin related ICH, increased dramatically by an average factor of 5 (hazard ration [HR] 5.57; 95% CI, 1.80–17.25; P=0.0029) after warfarin was restarted. At the same time, the risk of an ischemic event, specifically the risk of an ischemic stroke was significantly reduced by about 90% (HR 0.11; 95% CI, 0.14–0.87; P=0.036). The assumption of proportionality of the rates was demonstrated by the relatively constant HR during the different follow up intervals, as can be seen in table 1. The HR representing the increased risk of ICH if warfarin was restarted before day 35 (HR=4.13) is comparable to that when warfarin is restarted between days 36-63 (4.46). Even though no cases of recurrent ICH were observed after day 63 when warfarin was not restarted by that time, this is most certainly explained by a lack of sufficient number of patients in this group and the relatively limited follow-up time rather than an actual absence of bleeding events in this period. To correct for this, the observed baseline bleeding rates in the group that restarted warfarin after day 64 and the assumed constant HRs in the model (HR=5.57) can be used to predict and replace the missing information on the bleeding rates in the group that did not start after day 64. A similar picture of "missing events" in the analysis of the thromboembolic events is seen in table 1 and can be corrected for using the same strategy of calculating predicted rates using the observed rates of

thromboembolism in the group that did not restart warfarin and with the proportional HR for the reduction of the risk of thromboembolism calculated from the Cox model (HR=0.11). This allowed us to build a complete picture on the risk of recurrent bleeding and on the risk of ischemic events before and after warfarin resumption and for different time points of warfarin resumption after the ICH. This final model uses therefore a combination of observed rates and Cox model predicted rates.

Table 1.1: Cox proportional hazards model for recurrent intracranial hemorrhage at different time intervals with and without resumption of warfarin

Warfarin Status	Risk of intracranial hemorrhage per day Events/Days of observation (rate)			
	1-35 days	36-63 days	64-217 days	>218 days
Observed rates				
No	7/3829 (0.18%)	1/2250 (0.044%)	0/10146 (0.00%)	0/32208 (0.00%)
Yes	2/265 (0.75%)	1/504 (0.20%)	2/4008 (0.049%)	2/29056 (0.0069%)
Hazard ratio	4.13	4.46	∞	∞
Rates used in prediction model*				
No	0.18%	0.044%	0.0090%†	0.0012%†
Yes	1.02%‡	0.25%‡	0.049%	0.0069%

Table 1.2: Cox proportional hazards model for ischemic event at different time intervals with and without resumption of warfarin

Warfarin Status	Risk of ischemic stroke per day Events/Days of observation (rate)		
	1-77 days	78-329 days	≥330 days
Observed rates			
No	5/7301 (0.068%)	6/15360 (0.039%)	4/24112 (0.017%)
Yes	0/1152 (0.00%)	0/6830 (0.00%)	1/28325 (0.0035%)
Hazard ratio	0.00	0.00	0.21
Rates used in prediction model*			
No	0.068%	0.039%	0.017%
Yes	0.0075%§	0.0043%§	0.0018%§

*The Cox proportional hazard model provided a Warfarin Hazard Ratio for recurrent intracranial hemorrhage of 5.57 (95% CI 1.80-17.25) *P*=0.0029 and for ischemic stroke of 0.11 (95% CI 0.0139-0.868) *P*=0.036. The rates used in the prediction model were based on these hazard ratios as follows: †Observed rate on warfarin/5.57, ‡Observed rate without warfarin x 5.57, §Observed rate without warfarin x 0.11. The remaining proposed rates are those actually observed

The data presented in the paper, including the observed and predicted rates presented in table 1, show a relatively higher initial rate of recurrent ICH compared to later periods in the group that did not restart warfarin, and this initial rate is multiplied by a factor of 5 when warfarin is restarted after ICH. With time, the risk of recurrent ICH in the group that did not restart warfarin decreased successively, and even though restarting warfarin at any time would still increase the risk by a factor of 5; the declining rates of bleeding in the absence of warfarin meant that the risks of recurrent bleeding with warfarin also declined. The rates decline rapidly in the first 10 weeks from the index ICH event.

Similarly, data show a much higher initial rate of thromboembolic events immediately after the ICH, declining with time after the index event. Adding warfarin at any time reduced the risk of a new ischemic event, but unlike recurrent ICH, the rates of ischemic events continued to be relatively high with time as shown in figure 6.

To determine the optimal timing of resumption of warfarin after an ICH we looked at the "total risk" which is the cumulative risk of recurrent ICH, and ischemic events before and after warfarin resumption through the intended treatment period. Given that the overall survival in our material is 4.5 years, we looked at the "total risk" in different treatment periods or horizons. This total risk gives similar weight to a recurrent ICH and ischemic event. Even though the point of intersection between the curve for recurrent ICH and ischemic event varied according to the treatment period, the "total" risk remained constant, and showed a much higher initial total risk if warfarin is started early after ICH,

driven by the high incidence of recurrent ICH, and similarly a high total risk if warfarin is restarted late, driven by the cumulative risk of ischemic events (Figure 6). The optimal period of resumption of warfarin, which carried the lowest "total risk" for the combined recurrent ICH and ischemic stroke before and after the time of resumption appears to be between 10 to 30 weeks after the index warfarin-related ICH.

Similar results were obtained when the analysis was not restricted by the inclusion of patients with previous stroke or cardiac indication for anticoagulation.

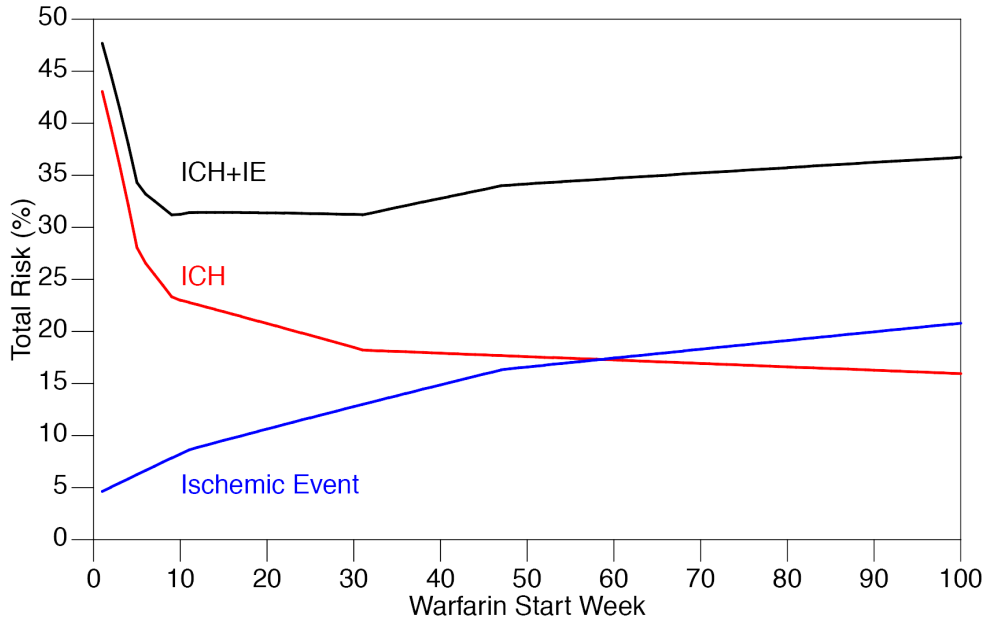


Figure 6: Risk of recurrent ICH and ischemic stroke according to VKA resumption time

STUDY II

Baseline characteristics

A total of 144 patients were included in the study at the three centers but 9 were excluded from further analysis because their CT scans were not available for calculation of the ICeH volume, leaving 135 patients from whom data were included in the statistical analysis. All of the Dutch patients (n=65) and most of the Swedish patients (35 of 40) received treatment with PCC, while all of the Canadian patients (n=30) and some Swedish patients (5 of 40) received plasma. There were some imbalances in the prevalence of prognostic factors between the plasma-treated group and the PCC-treated group, including larger ICeH hematoma (64.5 cm^3 vs. 36.0 cm^3, p=0.021)(figure 7), more frequent intraventricular extension of the ICeH hematoma (60% vs. 32%, p=0.004), more use of antiplatelet agents together with anticoagulants (26% vs. 7%, p=0.008) and higher incidence of diabetes (40% vs. 18%, p=0.008) in the plasma-treated group. The median time to administration was also – as expected – significantly longer in the plasma group compared to the PCC group (15.5hrs vs. 4hrs, p<0.001). No significant differences were found between the groups regarding age, sex, cause of ICeH (traumatic or spontaneous), INR on presentation, bleeding localization, surgical evacuation of the hematoma, or INR on presentation (2.9 vs. 3.0) .The characteristics of the included patients are shown in table 1 of the original paper.

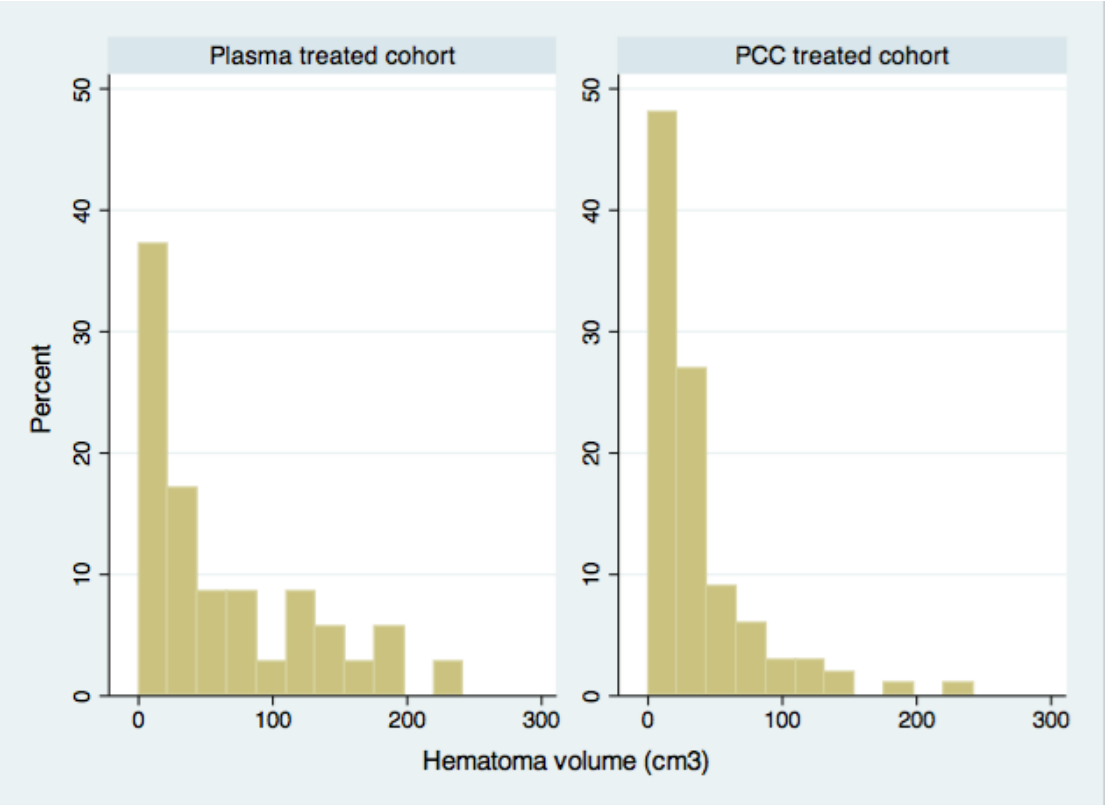


Figure 7: Distribution of intracerebral hematoma volume according to reversal group.

When comparing the Swedish and Dutch centers, where most of the patients were treated with PCC, there was no significant difference in the initial hematoma volume (31.1 cm^3 vs. 38.6 cm^3, p=0.39) nor in the presenting INR (2.8 and 3.1).

The Pearson's r coefficient for the correlations between the measurements from the two radiologists was 0.99 indicating very similar results by both radiologists for the measurement of the ICeH volume.

The distribution of INR on presentation is shown in figure 8. Sixty-two patients (47%) had therapeutic INR on presentation, and exactly the same number of patients had supratherapeutic INR, whereas the remaining patients (n=10, 6%) had subtherapeutic INR on presentation.

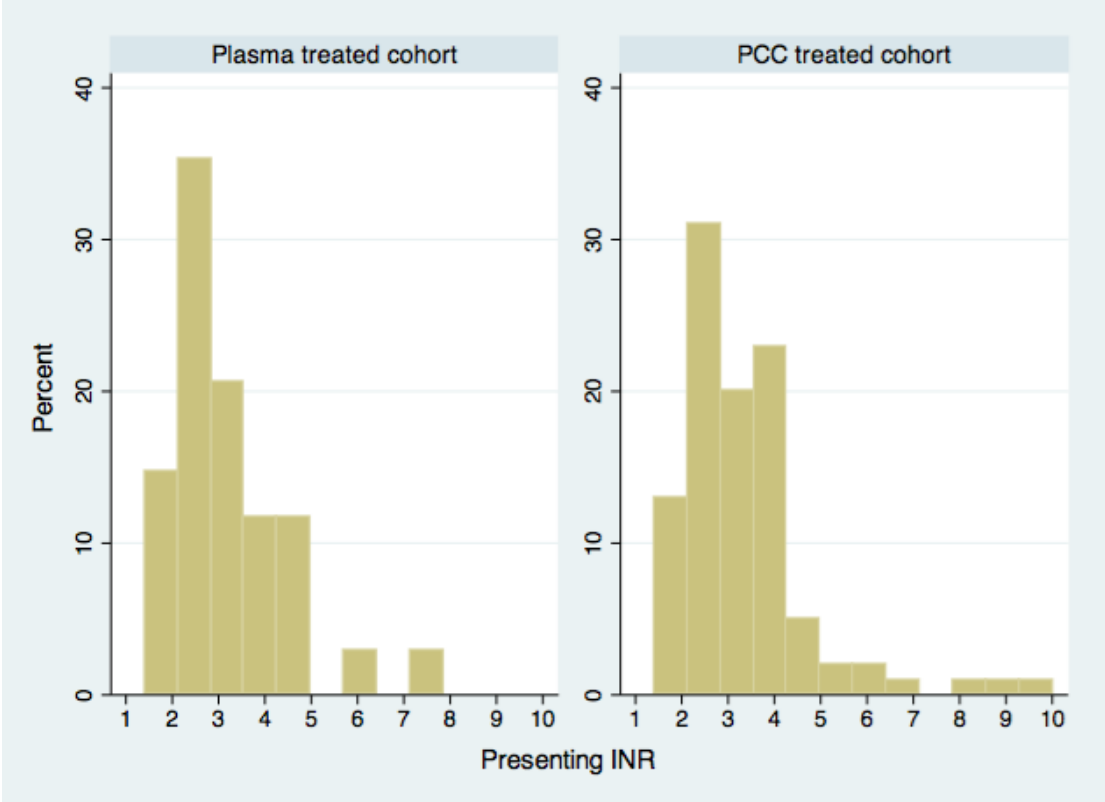


Figure 8:Distribution of initial INR according to reversal group

VKA reversal

Four different types of PCC were used in the study, all of which were 4-factor PCC. All Dutch patients received Cofact® (Sanquin BV, n=65 [65%]). The Swedish patients received either Ocplex® (also called Octaplex®, Octapharma,

n=25 [25%]), Prothromplex-T (Baxter, n=6 [6%]), or Beriplex (CSL Behring, n=1 [1%]). In three cases (3%) the type of PCC given was not documented. The patients received a median dose of 1750 IU, corresponding to a median of 22.5 IU/kg (interquartile range [IQR] 20–26). Seventy-one patients (71%) received an "adequate" dose of PCC, defined as a dose corresponding to at least 75% of the dose calculated to be sufficient to correct the initial INR to a level of 1.5 or less.

The plasma-treated group (all 30 Canadian patients and 5 of the Swedish patients) received a median dose of 4 units (IQR 3-6 units), corresponding to about 1000ml, with a wide variation from 1 unit up to 20 units of plasma.

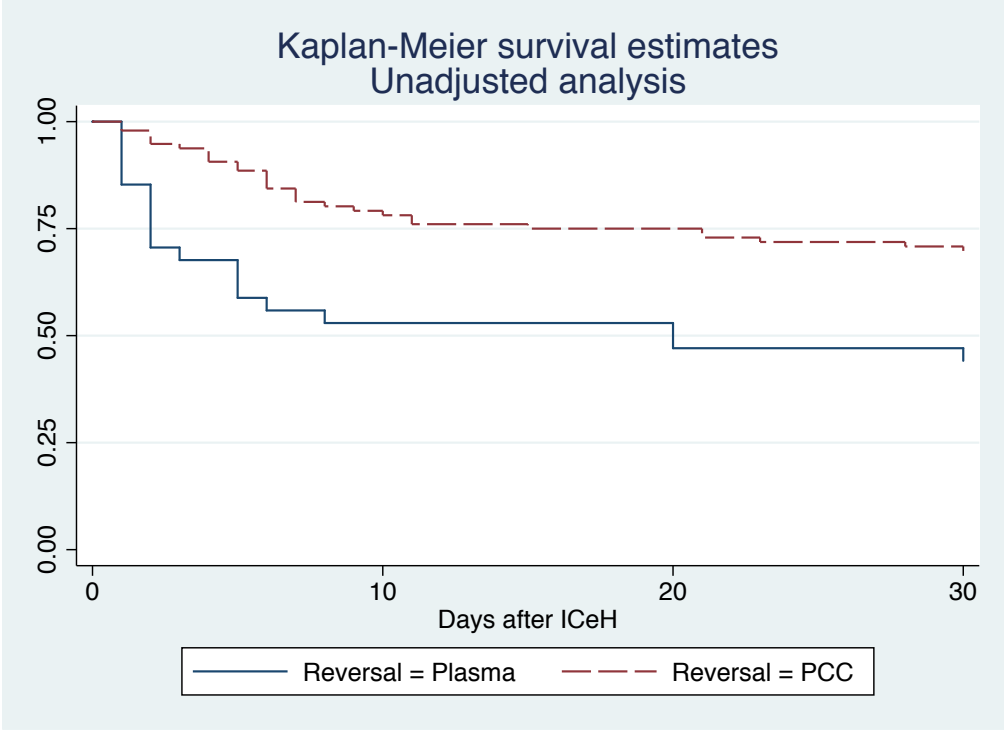
In addition to plasma or PCC, patients with major bleeding were usually given 5-10mg of vitamin K intravenously.

Effect of plasma and PCC on mortality.

We decided to consider the 30-day all-cause mortality for the comparison of the effect of plasma and PCC on the outcome of ICeH. Of all the patients included in the study, 51 (38%) died during the first month. When looking at the two treatment groups, 19 (54%) of all patients who received plasma died within the first month compared to 32(32%) in the PCC group. These data indicate a strong effect of PCC on the reduction of 30-day mortality after warfarin related ICeH, with a statistically significant crude OR of 0.40 (95% CI, 0.18–0.87, p=0. 021).

Because of the differences between the two treatment groups in the prognostic variables at baseline, a multivariate analysis was done using step-wise logistic regression. Three variables had a significant impact on the mortality of the

patients, the strongest of which was the volume of the ICeH ($p<0.001$), then the bleeding localization ($p=0.0049$) and then age ($p=0.044$). Adjusting for these three variables, and especially for the hematoma volume, in the regression model reduced the beneficial effect of PCC on mortality as compared to plasma to an OR of 0.49 (95% CI, 0.19 to 1.24), which was not statistically significant with p-value 0.13 (Figure 9). Further adjustment for other prognostic variables, and even when taking into consideration the difference in the time to administration between the PCC and plasma group, did not impact the results significantly.


Kaplan-Meier survival estimates
Unadjusted analysis
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10
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30
Days after ICeH
Reversal = Plasma
Reversal = PCC

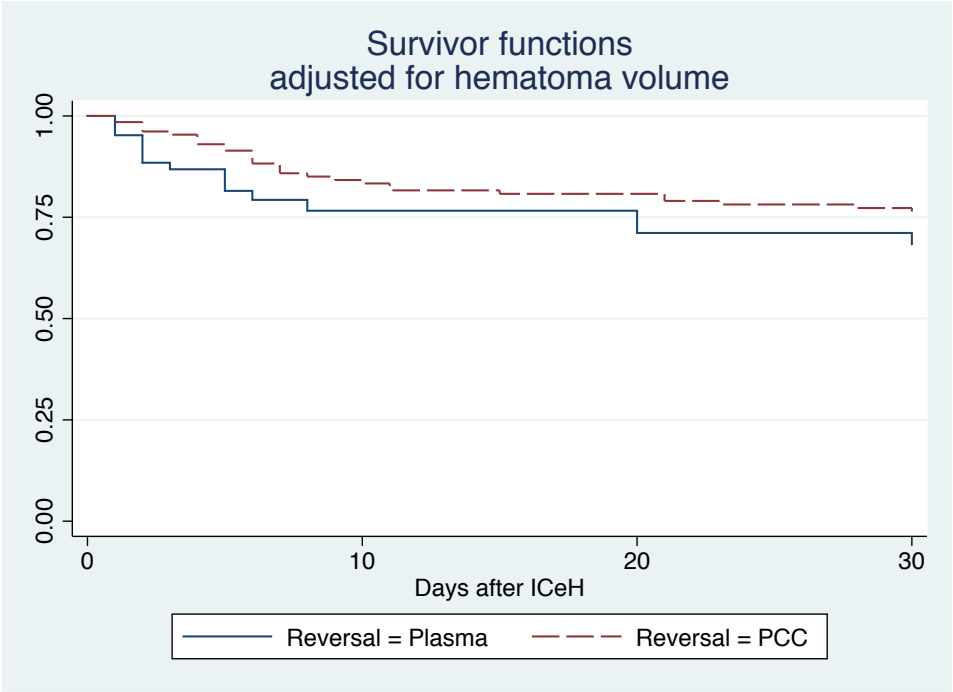

Figure 9: The 30-day mortality after warfarin -related intracerebral hemorrhage without and with adjustment for hematoma volume

STUDY III

Baseline characteristics

Two hundred and ten patients were included during the study period, however 50 patients were later excluded from further analysis (in 35 cases PCC was given for other indication than VKA reversal, and in 15 patients no clear documentation of treatment with PCC, despite recommendation for such treatment, was found in the medical notes). The demographics of the remaining

160 patients are shown in table 1 in the original paper. Even though the study was planned to include patients from centers in two thirds of the country, about two thirds of the actually included patients (62.5%) were at three tertiary referral hospitals in Stockholm. The most common indication for VKA treatment in this study, as in the previous two, was AF (n=73, 46%), and the median age was 75 years (IQR 65–80). Two thirds of the patients were males (n=109, 68%) and the median weight was 76kg (IQR 70-85).

The initial INR in 95 patients (59.4%) in the study was supratherapeutic, 57 (35.7%) had therapeutic INR and the remaining 7 patients (4.4%) had subtherapeutic INR values. The distribution of INR according to the indication for reversal is presented in table 2.

Table 2: Distribution of INR before and after treatment according to PCC indication

	All patients*	Bleeding	Emergency surgery*
	N=160	N=116	N=44
Initial INR, median (IQR)	3.5 (2.6-5.4)	3.5 (2.6-5.4)	3.6 (2.8-5.1)
Sub-therapeutic (%)	7 (4.4)	2 (1.7)	5 (11.4)
Therapeutic (%)	57 (35.7)	47 (40.5)	10 (22.7)
Supra-therapeutic (%)	95 (59.4)	67 (57.8)	28 (63.6)
INR after PCC, median (IQR)	1.4 (1.2-1.6)	1.3 (1.2-1.6)	1.5 (1.3-1.7)
INR <1.5	91 (56.9)	72 (62.1)	19 (43.2)
INR 1.5-1.9	45 (28.1)	27 (23.3)	18 (40.1)
INR >1.9	12 (7.5)	8 (6.9)	4 (9.1)
No INR available	12 (7.5)	9 (7.8)	3 (6.8)

PCC – prothrombin complex concentrate; INR – International Normalized Ratio. IQR–interquartile range

* Initial INR not available for one patient in the emergency surgery group

The indication for treatment with PCC in most of the cases was reversal of VKA effect because of a bleeding event (n=116, 73%), while the remaining patients (n=44, 28%) received PCC for urgent VKA reversal preoperatively. The most common type of bleeding treated with PCC was ICH (n=59, 37%) followed by GI bleeding (n=21, 13%). In addition to PCC, 74% of the patients received treatment with vitamin K intravenously at doses between 2-10mg.

Prothrombin complex concentrates

Different types of PCC were used in the study, depending on the local availability, but most of the patients (96.3%) received either Prothromplex (Baxter), or Ocplex (elsewhere called Octaplex, Octapharma) or Beriplex (CSL Behring). Doses ranged from 8 IU/kg (600IU) to 63 IU/kg (3500IU), the lowest was given to a patient with ICH presenting with an INR of 1.5, and the highest to another ICH patient with INR 6.3, and resulting in lowering of the INR to 1.2 and 1.7 respectively. The median dose of PCC given in the study was 1800IU (1200–2000IU) corresponding to 24 IU/kg (IQR 18–29). Most of the patients (n=118, 74%) received vitamin K and a third (n=55) received plasma in addition to PCC.

Safety assessment

Two investigators independently reviewed the charts of all 160 patients, but 2 patients were excluded from further analysis because no discharge notes were available to allow for complete safety assessment. In the remaining 158 patients, the two investigators agreed on the absence of thromboembolic events in 149 cases, and on the occurrence of thromboembolic events in 9 cases, with a

"possible" relationship to PCC treatment. In three of these 9 patients, the initial clinical suspicion of a thromboembolic event (1 stroke and 2 pulmonary embolisms) was not objectively verified leaving only 6 cases of objectively verified thromboembolic events occurring within a week after PCC administration where a possible causal relationship was identified in the study (table 3). These included 5 arterial events (3 with stroke, 1 myocardial infarction [MI], and 1 systemic embolism to the spleen) and 1 DVT. Two of the patients with stroke had AF as VKA indication, and the third stroke patient was on warfarin due to a PHV.

Table 3. Characteristics of patients with thromboembolic events within the first week after treatment with PCC

Age	Sex	VKA indication	Reversal indication	PCC dose	Vit K	Plasma	INR before PCC	INR after PCC	TE	Time (d)	Outcome
85	F	PHV+AF	Hematuria	1000	No	2 units	2.2	N/A	MI	?	Death after 2yrs
59	M	PHV	ICH	2000	Yes	4 units	2.2	1.2	Stroke	1	Death after 7d
67	M	AF	Hemoragic stroke	2000	Yes	No	2.0	1.4	DVT	4	Recovered
79	M	AF	ICH	1500	Yes	No	2.6	1.3	Stroke	2	Recovered
71	M	AF	GI bleed	1800	Yes	1 unit	2.5	1.4	Splenic Infarct	6	Recovered
83	M	AF	Pre-op	2000	Yes	No	3.4	1.2	Stroke	2	Death after 5d

In two cases, the two investigators disagreed on whether the patient had suffered a thromboembolic event or not, these two cases were resolved after review by a third independent investigator. Overall the agreement between the two investigators was very good with a kappa coefficient of inter-observer agreement of 0.88 (95% CI 0.72- 1.04).

Mortality

Ten patients died within the first week after treatment with PCC; the details of these patients are available in table 4. Seven patients (6.1%) had VKA-related bleeding (5 of these patients had warfarin-related ICH on admission, one had retroperitoneal bleeding, one had post-operative bleeding) and 3 (6.7%) were in the emergency surgery group. Thromboembolism was judged to be a contributing factor to the death of one patient (patient 10, table 4) who presented initially with bacterial meningitis and high INR that was reversed with PCC before a lumbar puncture; the patient developed an ischemic stroke 2 days after PCC administration and the final cause of death as recorded in the death certificate was a combination of infectious meningitis and ischemic stroke.

Table 4: Causes of death within the first week after PCC treatment

Patient	Age	Sex	Indication for VKA	Indication for reversal	Death after PCC (d)	Death Cause
1	87	F	PHV	ICH	5	ICH
2	83	F	PHV	Surgical bleeding	4	Pneumonia, heart failure
3	80	F	VTE	Emergency surgery	7	Septicemia
4	81	M	AF	Retroperitoneal bleeding	0	Hypovolemia
5	74	M	PHV	ICH	0	ICH
6	61	F	PHV	ICH	1	ICH
7	79	F	Stroke	ICH	1	ICH
8	79	M	AF	Emergency surgery	2	Septicemia, organ failure
9	85	M	PHV	ICH	0	ICH
10	83	M	AF	Emergency surgery	5	Septicemia, stroke

Assessment of efficacy

Clinical assessment of the outcome of the patients, in addition to the effect on the INR was used in the efficacy assessment. Overall, complete data for efficacy evaluation was available for 156 patients; 146(91%) of whom were adjudicated by the two investigators in the study to have a good effect of given PCC.

The effect of PCC was adjudicated as poor in 4(3%) patients, all of whom had ICH on admission and after receiving PCC in doses of 20-31 IU/kg, the INR was reversed to between 1.2-1.5 in all 4 cases. Three of these cases were also given vitamin K and one was given plasma. The outcome of the bleeding was fatal in 3 of the 4 patients shortly after admission.

For 6 patients the effect of PCC was adjudicated as moderate (4 with ICH and 2 with GI bleeding); only 3 of these 6 patients attained an INR of 1.5 or lower after

PCC, and the PCC dose given to 2 of these patients was estimated as suboptimal for full reversal.

There was no significant difference between the post-PCC treatment INR of patients with ICH and good effect of PCC versus those with moderate and poor effect of PCC (1.3 vs. 1.35, P=0.423). The details of the patients with suboptimal effect of PCC are shown in table 5.

Table 5: Characteristics of patients with sub-optimal effect of PCC for the reversal of warfarin.

Patient	Age	Sex	Indication For VKA	Indication for PCC	PCC dose (IU/kg)	Vit-K	Plasma	INR before PCC	INR after PCC	Effect	Outcome
1	63	M	AF	ICH	2400 (29)	No	No	5.2	1.3	Moderate	Death after 5 yrs
2	87	F	carotid stenosis	ICH	600 (12)	Yes	1 unit	2.1	1.2	Moderate	Alive
3	69	F	PHV	SDH	1800 (25)	Yes	3 units	3.1	1.6	Moderate	Death after 3 w
4	75	F	AF+stroke	SDH	600 (8)	No	1 unit	2.7	1.4	Moderate	Death after 4 yrs
5	74	M	PHV	GI bleeding	2000 (28)	?	3 units	3.6	1.4	Moderate	Death after 3 yrs
6	76	F	VTE	GI bleeding	1000 (20)	Yes	No	4.7	1.6	Moderate	Death after 7 m
7	74	F	VTE	ICH	2400 (30)	No	No	4.0	1.5	Poor	Death after 4 m
8	61	F	VTE	ICH	1200 (20)	Yes	No	2.4	NA	Poor	Death after 1 d
9	87	F	PHV	SDH	?	Yes	No	3.0	1.5	Poor	Death after 5 d
10	75	M	PHV	SDH	2500 (31)	Yes	1 unit	2.7	1.2	Poor	Alive

PCC – prothrombin complex concentrate; INR – International Normalized Ratio; AF – atrial fibrillation; ICH – intracranial hemorrhage; SDH – subdural hematoma; PHV – prosthetic heart valve; GI – gastrointestinal; VTE – venous thromboembolism. VKA– vitamin K antagonists

STUDY IV

Baseline demographics

A total of 1034 patients, who suffered a centrally adjudicated major bleeding event in the 5 phase III trials on dabigatran, met the inclusion criteria for our study. Some of these patients suffered more than one major bleeding event, so the total number of bleeding events in the study (1121) was higher. The number of patients randomized in the 5 phase III trials and the demographics of the subgroup included in our study are summarized in tables 1 and 2 in the original paper.

Altogether, we had data on 262 major bleeding events in 6,015 patients treated with dabigatran 110mg bid, 365 major bleeds in 10,740 patients treated with dabigatran 150mg bid, and 408 major bleeding events in 10,002 patients treated with warfarin. Patients who had major bleeding event on dabigatran were significantly older (mean age in the dabigatran 110mg bid was 75.9, in the dabigatran 150mg bid 75.1, and in the warfarin group 71.7, dabigatran vs. warfarin $p < 0.0001$), had more impaired renal function (mean creatinine clearance in the dabigatran 110mg BID was 51, in the dabigatran 150mg bid 55, and in the warfarin group 62 mL/min, dabigatran vs. warfarin $p < 0.0001$) and had more frequent use of aspirin (dabigatran 110mg bid 35.1%, dabigatran 150mg bid 27.4%, and warfarin group 24.8%, dabigatran vs. warfarin $p < 0.038$) and of NSAIDs (dabigatran 110mg bid 16.4%, dabigatran 150mg bid 12.1%, and warfarin group 8.3%, dabigatran vs. warfarin $p < 0.006$).

Transfusion of blood products

About one third of the patients in our study (n=365, 33%) did not receive blood transfusion or any other blood products, (dabigatran 110 mg – 100 of 293 events [34%], dabigatran 150 mg – 126 of 403 events [31%], warfarin – 139 of 425 events [33%]). Transfusion of red cells only (ie, without any other blood products, coagulation factors, vitamin K, or local hemostatic intervention), was given to a significantly larger number of patients in the dabigatran arm (35%; dabigatran 110 mg – 137 of 293 bleeds [47%], dabigatran 150 mg – 173 of 403 bleeds [43%], warfarin – 85 of 425 bleeds [20%]), however, the difference in the median number of units transfused per patient in the three arms was not statistically significant. The details and comparisons are summarized in table 3 in the publication.

When looking at the hemoglobin levels taken during different time points in the RE-LY study, we noticed a drop in hemoglobin for all patients in the studies, regardless the type of anticoagulation and regardless if they had a bleeding event. In patients who suffered a major bleeding event, the drop of hemoglobin from the time of randomization until the bleeding event was significantly larger in the dabigatran arm (dabigatran, 38.0 g/L, warfarin, 30.7 g/L; P=0.02). Similarly, in patients who did not suffer any bleeding event, the drop in hemoglobin during 12 months was significantly larger in both dabigatran groups compared to the warfarin treated group (0.6g/L [0.14–1.06] greater for dabigatran 110mg bid, p=0.011, and 1.1 g/L [0.63–1.57] greater for the dabigatran 150mg bid, p<0.0001).

Transfusion of hemostatic agents

Data were available on the transfusion of plasma, vitamin K, factor concentrates, cryoprecipitate, or platelets from the CRFs and the event reports, mainly from the RE-LY trial. Most of the patients with major bleeding events were either managed conservatively without any transfusion or only transfused with red cells. Consequently, only a small fraction of the major bleeding events were managed with transfusion of hemostatic agents.

The proportion of patients transfused with plasma was significantly smaller in the dabigatran groups (61[17.8%] patients in the dabigatran 110mg, 86 [21.6%] patients in the dabigatran 150mg versus 127 [30.2%] than in the warfarin arm (p value significant for all comparisons with warfarin)

There was a low frequency of utilization of other factor concentrates, cryoprecipitate, or platelets without a significant difference between the study drugs. There were very few patients who received treatment with rVIIa or PCC for dabigatran- or warfarin-associated bleeding, and none of the patients in all the studies received APCC.

Other treatment modalities

Vitamin K was mainly given to warfarin- related major bleeding events in the phase III trials: in 59 bleeds (14%) it was given as the only treatment while in 73 bleeds (17%) it was given in conjunction with other hemostatic agents. Fewer patients in the dabigatran 110mg or 150mg groups received vitamin K in the

event of a major bleeding, corresponding to 18 (2.6%) and 39 bleeds (5.6%) respectively.

Hemodialysis was used in one patient in the RE-LY trial, who was on dabigatran treatment, and who developed moderate renal impairment with dabigatran accumulation before major surgery (coronary artery bypass grafting), resulting in massive postoperative bleeding with transfusions of blood, plasma, platelets, cryoprecipitate, 5 doses of rFVIIa, and fibrin glue without successful control of the bleeding despite this massive transfusion. After 6 hours of dialysis, the thrombin time was shortened from 128 to 65 seconds and the bleeding was controlled.

Hospitalization and discharge destinations

Data on hospitalization and the discharge destination were available mainly for patients in the RE-LY trial. The proportion of patients requiring hospitalization after a major bleeding event was comparable in all the three treatment groups (150 [43.9%] in the dabigatran 110mg group, 199 [49.9%] in the dabigatran 150mg group and 198 [47.0%] in the warfarin group, p value not significant for all between-groups comparisons). Similarly, there were no significant differences in the between-groups comparisons of the length of hospitalization (mean days [SD]; 7.0 [7.0] for the dabigatran 110mg group, 7.0 [8.0] for the dabigatran 150mg and 7.0 [8.0] for the warfarin group), or of the number of nights in the step-down units (mean days [SD]; 0.8 [2.1] for the dabigatran 110mg group, 1.0 [2.7] for the dabigatran 150mg and 1.0 [2.7] for the warfarin group). However, the length of stay in the intensive care unit was significantly shorter for both dabigatran treatment groups than that of warfarin (mean days

[SD]; 1.2 [3.3] for the dabigatran 110mg group, 2.5 [6.1] for the dabigatran 150mg and 3.2 [7.0] for the warfarin group, p-value significant for all comparisons of dabigatran with warfarin). There was also a trend toward lower number of patients with bleeding events requiring surgery or resulting in death in the dabigatran groups (56 [16.4%] in the dabigatran 110mg group, 76 [19.0%] in the dabigatran 150mg group and 94 [22.3%] in the warfarin group, p-value for comparison between dabigatran and warfarin treatment groups=0.06)

Information on discharge destination was available for 710(52%) of all patients in the RE-LY trial who required hospitalization after a major bleeding event. The destination was defined in the medical records as one of three categories: home, long-term facility, or other hospital. The details of the number and proportion of patients discharged to these three destinations are summarized in table 4 of the publication; there was no significant difference in the proportion of patients discharged to the different destinations in any of the comparisons between the treatment groups.

Disability

Data on the disability after ICH, measured with mRS both at admission and upon discharge, was available for 78 (55%) patients with ICH. At presentation, the dabigatran 150mg group had a significantly higher mRS than the warfarin group (median [IQR]; 6[1] for the dabigatran 150mg, 5[3] for the warfarin group, p=0.03). However mRS at follow-up and the differences between the initial and the final mRS for all the three treatment groups were not significant (see table 4 in the published study).

Mortality after major bleeding

Mortality after the first major bleeding event, pooled from all the 5 phase III trials, was significantly lower at day 7 in the combined dabigatran treatment group as compared to warfarin (5.3% vs. 8.4% respectively, p=0.045). At 30 days, the crude rates showed a strong trend to lower mortality in the dabigatran group (9.1% vs. 13.0% p=0.057). Similarly, the Kaplan-Meier curves showed a strong trend to better survival in the combined dabigatran treatment group (p=0.052) (fig 10).

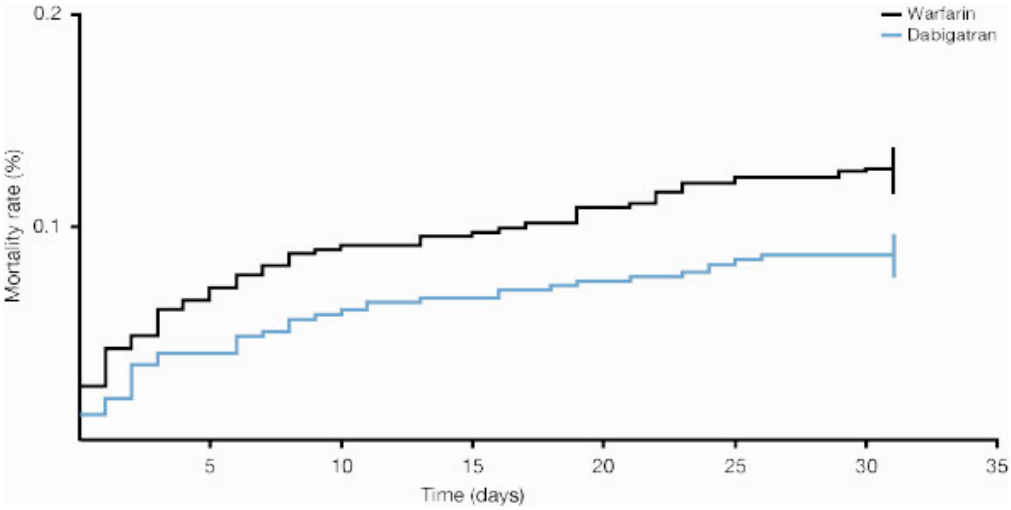

Figure 10. Kaplan-Meier curve for the 30-day mortality after a major bleeding event.

Because the baseline characteristics of the dabigatran and the warfarin-treated groups were different at baseline, adjustment was done using logistic regression. After adjustment for age, sex, body weight, renal function at the time of the bleed, and additional antithrombotic therapy, the OR for the 30-day all-cause mortality in the dabigatran treated group was 0.66 (95% CI, 0.44–1.00; P=0.051). When stratified by dabigatran dose, the adjusted ORs were 0.65 (95% CI, 0.38–1.11) and 0.68 (95% CI, 0.42–1.08) for the 110mg and the 150mg respectively.

There was a significant difference in the occurrence of concomitant cancer between the AF (RE-LY) trial and the 4 combined VTE trials (5% versus 36%; P=0.003). Therefore, a further analysis was done for the RE-LY study alone. Corresponding adjusted OR for 30-day mortality in RE-LY for the combined dabigatran treatment doses was 0.56 (95% CI, 0.36–0.86; P=0.009), for

dabigatran 150 mg it was 0.52 (95% CI, 0.31–0.88), and for dabigatran 110 mg it was 0.60 (95% CI, 0.35– 1.03) indicating mortality benefits in the combined dabigatran groups and for the 150mg dose compared to warfarin.

4 DISCUSSION

METHODOLOGICAL ISSUES

General design considerations

Study I and II were conducted with a retrospective design, while in Study III the information was collected prospectively. Study IV is an ad-hoc analysis, ie retrospective analysis of data collected prospectively to answer a question (outcome of bleeding) different from the primary one (efficacy of dabigatran versus warfarin). Randomized controlled trials are the golden standard in study design because of their ability to control for known and unknown confounders, and since patients are followed prospectively, they can also minimize the risk for any spurious causality and bias. However, it is sometimes not feasible to use RCT design to address a certain question, either because such a design would be ethically questionable (e.g. using an RCT to answer the question in Study III), or because the RCT would be costly and run for many years when the outcome is a rare event (e.g. using RCT instead of retrospective design for Study I and II). In fact, an RCT with similar design to Study II has been running since 2009[225], but according to personal communication with the principle investigator of the study, "recruitment has been slow". In such situations, retrospective studies or prospective non-randomized trials can be an alternative. Including consecutive patients improves the study internal validity in this case, as we did in Study I and II. Having a control arm allows calculating the relative risk or OR depending on the study design and gives additional information, but even a control arm is sometimes not possible (e.g. Study III as discussed in individual study results below). Considering the questions we wanted to answer, the design chosen for

each of the four studies can be considered the best for limiting the time frame and with the resources available.

Internal validity

a. Selection bias

Selection bias occurs when individuals selected for inclusion in a study, or their likelihood of completing the study (i.e subject retention in the study) leads to results that are different from those obtained if the entire population at risk would have been included. Such bias might cause the exposure-outcome distribution in the sample selected to be different from that in the general population at risk, leading to spurious estimate of the exposure-outcome relationship.

In Study I and II, a certain "selection bias" might have been introduced by including patients from tertiary referral centers only, where more advanced and skilled management possibilities exist, leading to a possible selection of more complicated cases with ICH which are usually referred to highly experienced centers with possibilities of neurosurgical intervention. However, most of the services provided in these four hospitals are actually based on the catchment area and to a much lesser extent on the severity of the condition of the patient. In addition, we endeavored to identify and include consecutive cases of VKA-related ICH. The ICD-10 codes were used to identify the population with ICH during the study period in the three hospitals in Sweden and Canada, and these journals were then scrutinized to identify VKA-related ICH according to a strict

definition. This system is based on the assumption that cases of ICH are correctly coded for with the ICD-10 system. Since all cases of ICH are managed as in-patients, it is unlikely that cases will be missed due to incorrect ICD coding at the emergency unit or the outpatient clinic, and any cases that might be wrongly coded would only result in a random rather than a systemic error.

In Study III, our intention was to include patients consecutively, however, this could not be achieved as an unknown number of cases were missed by the hematologist on-call. This process occurred randomly without any specific pattern, and is therefore unlikely to cause a systematic error. On the other hand, it is also possible that a consulting physician would contact the coagulation center at Karolinska University Hospital to discuss more complicated cases, with fragile bleeding-thromboembolism balance, and higher risk of thromboembolism regardless of treatment administered. Moreover, there is an "indication bias" in this group; patients receive PCC because they are on warfarin due to an increased risk of thromboembolism, and they are either bleeding or in need of emergency surgery, both of which inherently increase the risk of thromboembolism, i.e. PCC is given to a group that already has an increased risk of the outcome of interest (thromboembolism in this case). The two last mentioned factors could potentially result in an "overestimation" of the risk of thromboembolic events after PCC administration; however, such overestimation is clinically relevant since PCC is usually given to similar patient groups. The rates we found in our study were comparable to other published reports[226-228]. The optimal design to measure the PCC-attributable risk increase would be to administer PCC to healthy patients given warfarin just to assess the safety of reversal with PCC, a design that is neither ethical nor possible to do on a large scale, even though a study with comparable design was published in a prestigious journal recently[196]. The method we used reflects the real world

scenario in a better way. When assessing the safety of a treatment, it might be also be argued that it is preferable to choose a design that would optimize the chance of detecting adverse events (even if it results in an overestimation of the outcome of interest) than one that underestimates the risks associated with the treatment.

In Study IV, the source population of the five phase III trials was selected according to rigorous inclusion and exclusion criteria and, as in many other RCTs, there is always a risk of the study sample being different from the larger patient population by recruiting generally healthier, more compliant individuals thereby introducing some Healthy Worker Effect. This is a known issue with RCTs that is difficult to overcome.

When it comes to the issue of subject retention, this is more important to consider in prospective trials. In Study III we had two patients of the 160 included that had to be excluded from the safety analysis because discharge notes were not available, however, there was no mentioning of any thromboembolic events in the available medical notes. We had complete one-week follow-up of the remaining 158 patients, enabling us to assess the occurrence of any thromboembolic event during the pre-specified time frame of 1 week after PCC treatment. A small number of patients died before completing the first week of follow-up, but we had access to the cause of death as reported in the death certificate. Study IV included patients from one open-label RCT (RE-LY) and 4 double blinded RCTs (RE-COVER, RE-COVER II, RE-MEDY and RE-SONATE). Although the phase III studies had some drop outs, more in the double blinded trials than the open label as expected due to the complexity of the protocols of blinded studies, this did not affect our subgroup analysis. Our starting point was all patients who developed a major bleeding event on

treatment, and since such bleeds are generally treated as in-patients, and followed closely as part of the original study, with transfusions of blood products recorded according to specific forms, it is unlikely that subject retention bias affected the results of our study.

b. Confounders

One important co-variant that we were unable to adjust for in Study II is the Glasgow Coma Scale (GCS). The GCS at presentation was related to the mortality of ICH in a number of studies[229,230]. Our data on the important prognostic covariates were collected retrospectively from medical records. In many cases the admitting physician did not record the GCS and in some cases an alternative system called the RLS-85 (Reaction Level Scale), popular in Scandinavia[231], was used instead to document the level of consciousness of the patient. This system cannot be translated directly to GCS. In most of the cases where GCS was not recorded, the information available in the medical records was insufficient to calculate the GCS. More importantly, the volume of the ICeH, which we have adjusted for in all patients, is a better predictor of the 30-day mortality than GCS[232].

c. Assessment of exposure variables

In Study I and II, we examined the medical records and hospital medications to ensure that the patients were receiving anticoagulation with VKA at the time of the bleeding. We also reviewed the laboratory results and included only patients with INR over 1.5 to ensure that there was an antihemostatic effect of VKA at

the time of the event. All ICHs had to be objectively verified by imaging (Computerized Tomography or Magnetic Resonance Imaging). Laboratory parameters and other covariates were collected from records at the time of the bleeding event. Both hospital medication charts and medical notes were used to confirm the dose and administration of PCC or plasma. Time from onset of symptoms to transfusion of PCC or plasma was calculated from the information available in the medical records, introducing some element of uncertainty depending on the accuracy of this documentation. There is of course a question of the quality and representativeness of the data registered in the medical journals, which is always a limitation in retrospective study. We tried to confirm the data collected in the study by checking the same information from different sources (e.g. medical notes, laboratory results, hospital medication notes, reports of diagnostic imaging etc). When the time for onset of symptoms was recorded as a range we used the mid-point in our estimation of the treatment delay.

For Study III, the data was collected prospectively, hospital medication charts were examined to confirm that the patient actually received PCC and to verify the dose given. We also reviewed the nursing notes regarding the administration of PCC and the INR before and after the stated administration of PCC to make sure that the patient received the treatment. In Study IV, information on the study individuals was available from the 5 phase III trials according to pre-specified CRFs and collected in the study databases. The RE-LY trial databases were maintained both by the sponsor (Boehringer Ingelheim) and by the trial management (Population Health Research Institute) in different formats. We had two independent investigators extract the information from the serious adverse events narratives and the bleeding event narratives, any discrepancy was re-evaluated and the final data set was further checked against the study databases.

d. Assessment of outcome variables

The outcome variables in Study I, II and IV were chosen as hard endpoints and were objectively verified. Recurrent ICH or ischemic events were based on the development of new neurological symptoms but also a radiological verification was required in study I. Since this study was done retrospectively, there was no protocol in place to determine the point of VKA resumption or recommend radiological investigations at specific time points to assess for asymptomatic recurrent ICH or ischemic stroke. The design we used mimics, however, closely the every day clinical setting and could be considered as strength rather than a limitation of the study. In Study II and IV, another hard end point (30-day all-cause mortality) was used and this data was available for all the patients included in the study. The rationale for using this rather than cause-specific mortality has been discussed previously in the methods section. The primary outcome in Study III was safety, and for that the occurrence of a thromboembolic event had to be objectively verified. In the efficacy assessment two investigators independently reviewed the medical records according to pre-defined criteria to increase the level of objectiveness.

In summary, we tried to use as objective assessment as possible for both exposure and outcome variables to improve the data quality and the internal validity of the study

External validity

External validity of a study refers to the generalizability of the results of the

study. A good internal validity (which has been discussed previously) is a requirement for external validity. Other important aspects of a study that influence its external validity are if the individuals in the study, the time period of the study, the settings, and the places where the study was conducted are similar to the group(s) we want to generalize the results to. All the four studies included patients from several centers ("places"), and inclusion was not limited to specific age groups, sex, race or other individual factors. We have even included patients with a spectrum of VKA indications (SPAF and VTE in all four studies, PHV and previous stroke in study I, II, III, since dabigatran in Study IV has not been approved for PHV). In the studies on ICH we included both spontaneous and traumatic (although not severe multitrauma where ICH has a different natural course), deep and lobar ICeH, all subsets of ICH in Study I (ICeH, SAH, SDH) and with INR in the subtherapeutic (1.5-1.9), therapeutic (usually 2.0-3.0) and supraterapeutic range. The setting of VKA reversal in Study III included both patients with bleeding and those requiring emergency surgery and used several types of PCC as a reversal agent. Study IV was not limited by a specific bleeding localization or cause, and the bleeding was managed in different types of hospitals, primary to tertiary center. The rigorous inclusion and exclusion criteria in the 5 phase III trials may limit the generalizability both of the results of all the phase III trials and of our subgroup analyses. The four studies in this thesis included patients during different periods within the last 10 years, during which the indications of VKA, the diagnosis and management of major bleeding events, and the use of plasma or PCC have not changed much from current ones. These factors altogether imply that our studies have good external validity.

DISCUSSION OF THE INDIVIDUAL PAPERS

Study I: Restarting warfarin after ICH

Main results

In this study, we reported a risk-benefit analysis of the optimal timing for resumption of anticoagulation after warfarin-associated ICH. The multicenter design, the relatively large number of included patients and of events in the study, and the modeling of the risk of bleeding and ischemic events before and after anticoagulation allows us to provide a better estimate of the optimal resumption interval. In contrast to previous studies, our data have shown that the optimal timing of resumption is between 10-30 weeks after the warfarin-related ICH. The span of 20 weeks allows for individualization of the exact point of anticoagulation resumption depending on the risk of recurrent bleeding or thromboembolism. According to this discussion, patients with a higher risk of recurrent bleeding, e.g. those with SDH, can be restarted later in this interval and those with higher risk of thromboembolism, e.g. PHV can be restarted around week 10 after ICH. This seemingly long delay of at least 10 weeks until resumption of anticoagulation might seem prohibitive for a patient with PHV. Taken in perspective, the risk of thromboembolism without anticoagulation has been estimated at 8.6% per year without any anticoagulation[66], which would correspond to 1.7% during 10 weeks. This might be higher after a bleed and might be reduced somewhat by giving low-dose aspirin in the meantime, which does not appear to increase the risk of recurrent ICH. More importantly, with the risk of recurrent ICH in case of early resumption of VKA as high as 13%, it is not only much higher than the early risk of ischemic stroke but with a recurrence

the VKA will need to be discontinued again, probably for a much longer period. It should be investigated whether serial echocardiograms during the 10-week interval can identify small deposits of clots before they result in serious thromboembolism.

Strength

Our study comprises one of the largest published cohorts, where patients were included from several centers and with the longest follow-up of patients with warfarin-related ICH. Compared to other studies, we did not limit the inclusion to patients with PHV, but we included other high-risk groups, e.g. with cardiac indication for anticoagulation due to AF with additional risk factors for stroke, patients with myocardial infarction developing intramural thrombus, and patients who had a previous stroke. We also had a larger number of events in our study that enabled us to build a model for the risk of recurrent ICH vs. the risk of ischemic stroke. In this model, we took into consideration for the first time the risk of bleeding and of ischemic events before and after the point of resumption of warfarin after an ICH and calculated a "total risk" of these combined events depending on the time of resumption and the treatment horizon with warfarin.

Limitations

The main limitation is the retrospective design without a study protocol to guide the exact timing of warfarin resumption and to optimize data collection. The point of resumption of VKA treatment was decided by the physician in charge and may have been biased by the fear for ischemic events or recurrent ICH. This is evident by the fact that patients with PHV were resumed on anticoagulation

earlier than those with other indications for anticoagulation. However, patients with previous stroke, who had an up to 28% risk of recurrent ischemic event in our material, were not restarted earlier than those without previous stroke, and patients with SDH, who in our material had a higher recurrence rate than other subtypes of ICH, were not restarted on VKA later or less frequently than patients with ICeH or SAH. Even though younger patients were restarted more often on VKA than older ones, we found no interaction between the risk of recurrence and age.

Other limitations include the relatively low number of events in our study, even though we had larger number of events than all previously published studies. We also had a shorter follow-up of those who never resumed (median = 39 weeks) compared to those who resumed anticoagulation, but all the events of interest should have been captured by that follow-up duration.

The best study design to answer the question of when to resume anticoagulation would have been an RCT. Such a design is not feasible due to the low rate of events that are eligible for inclusion in the study and the resulting long recruitment time and high cost. We had to include patients from three tertiary referral centers and to extend to a period of 5 years to reach the number of patients needed for the modeling in our study. Although we did not perform any formal power analysis, the size of our cohort and the inclusion from 3 centers over a 5-year period of an event that is relatively rare and carries a high mortality rate, together with the survival analysis makes our results the best available assessment of the optimal resumption time of warfarin after an ICH.

Comparison with other studies

In one systematic review 120 patients with PHV as the indication for anticoagulation were pooled from 6 studies, all of which were of small size and had limited follow-up. The authors concluded that it was safe to resume anticoagulation after only 2 weeks from the ICH[233]. There were very few events in this analysis (2 recurrent ICH and 4 ischemic strokes). In another systematic review pooling data on 492 patients, the authors concluded that it was safe to restart anticoagulation within 72 hours, but this analysis had a number of methodological limitations, of which the main was the large number of single-case reports with high risk of bias toward successful cases[234].

In one of the largest published cohorts of patients with warfarin-related ICH, no recurrent ICH was observed when 35 patients (out of 141 patients) resumed warfarin, which could well be explained be the short follow-up of only 30 days[218]. In another cohort with 23 of 52 patients resuming anticoagulation, 3 cases of recurrent ICH and 3 ischemic strokes were observed during a 43-month follow-up, but the low number of events makes it challenging to perform any statistical analysis or draw conclusions on the when to resume anticoagulation[217].

In another analysis, using a mathematical Markov model to assess the risk of recurrence, the authors suggested a difference in the rate of recurrence of deep hemispheric versus lobar types of ICeH, being higher in the latter, which led the authors to conclude that patients with the lobar subtype of ICeH should not resume anticoagulation[235]. This analysis is limited by the inclusion of only patients with AF having no additional risk factor for stroke. Other indications for VKA, e.g. high risk AF patients, patients with PHV or those with previous stroke have a much higher risk of ischemic stroke that may warrant resumption of anticoagulation even in patients with lobar bleeding localization. We were not

able to stratify our analysis of ICH according to the classification above because of insufficient number of events.

Study II: Mortality in VKA-related ICeH treated with plasma or 4-factor PCC

Main findings:

In this study, we are reporting results from the hitherto largest study comparing the 30-day mortality of warfarin-related ICeH treated with either plasma or 4-factor PCC. The rationale of this study was that the more rapid reversal of the anticoagulant effect of warfarin by PCC (through supplementing vitamin K dependent factors and restoring normal hemostatic activity as evident by correction of prolonged INR), would lead to less expansion of the ICeH than in patients treated with plasma, which takes much longer to correct hemostasis. This difference would be mirrored by a lower 30-day mortality rate in the PCC group.

We did observe significantly lower 30-day all-cause mortality in the PCC treated group (OR 0.4, p=0.021), however, much of that difference was due to differences in the baseline characteristics of patients in the two treatment groups, specifically, due to the larger volume of ICeH in the plasma group. Even though there were numerically fewer deaths in the PCC group, correcting for the imbalances in those characteristics caused the OR to increase to 0.49, eradicating the significant difference between the groups.

One plausible explanation is that the main hematoma expansion and damage to

the brain tissue occurs early after the onset of the ICeH. The average time to administration of PCC in our study was 4 hours, and if the potentially fatal damage has already occurred during this initial period, subsequent administration of PCC will have minimal impact on the survival of patients. Even though the time to PCC administration was significantly shorter than plasma (mainly due to the need of plasma thawing, blood-group matching and infusion of a much larger volume), correction for this time difference did not influence the results of the study.

Strength

The main strength of the study is the relatively large number of patients (compared to previously published studies), the inclusion of consecutive cases and the multicenter design. Furthermore, we included information on 11 potentially important prognostic covariates: age, sex, diabetes, concomitant antiplatelet therapy, cause of bleeding (provoked or spontaneous), INR before reversal, hematoma volume (ml) at presentation, bleeding localization, intraventricular hematoma expansion, time to administration of the reversal agent and surgical hematoma evacuation. There was also a very high agreement between the volume measurements of the ICeH by the two radiologists. The use of logistic regression allowed for the statistical correction of differences between basal characteristics that might influence the outcome.

Limitations

There are several limitations in our study. First, the data was collected retrospectively. The optimal design would have been a randomization of patients

with warfarin-related ICeH to either treatment with plasma or PCC. There are several problems with conducting such an RCT; first, there is an ethical issue that the randomization process itself might incur some further delay in the administration of the necessary reversal of anticoagulation, and such a delay by itself might lead to worsening of the patient's outcome. Second, the annual rates of warfarin related ICeH are low and this would result in an unreasonably long study or a substantial increase in the cost to engage many centers to recruit the necessary number of patients.

Another limitation is the sample size; the adjusted 30-day mortality analysis yielded an OR of 0.49 (95% CI, 0.19 to 1.24) with p-value 0.13. This result does not confirm any advantage of treatment with 4-factor PCC, yet the relatively small sample size has lead to somewhat wide confidence intervals and a benefit of treatment with 4-factor PCC cannot completely be ruled out, especially that a formal power analysis has not been done before conducting this study. However, we needed to review 5 years of data on patients with warfarin-related ICeH from four centers to obtain the sample size in this study as ICeH, a subset of ICH, is a rare event during treatment with warfarin and rates of VKA related ICH have been reported to be as low as 0.3%[236].

Another limitation of our study is that we did not have data on the GCS, which is correlated to the mortality in ICeH. We have, however, corrected for several other prognostic factors, including the most important one, namely ICeH volume.

Comparison with other studies

Similar results were published in a Canadian study [194], where a median dose of 1000 IU of PCC was administered to patients with warfarin-related ICH, including patients with ICeH, resulting in correction to INR below 1.5 in 72% of the cases within 1 hour. However, hematoma expansion was seen in 46% of the patients despite PCC and mortality was not reduced in comparison to historical data from patients receiving plasma for the reversal of warfarin-related ICeH.

On the other hand, a study from New Zealand showed improved survival in patients with warfarin-related ICeH when using reversal protocols that included PCC[237]. This study had several limitations; first, the study did not directly compare PCC to plasma treatment, since most of the PCC treated patients (15 of 23) received also plasma and the control arm of the study included patients who were treated with either plasma, vitamin K, or both or who received no treatment at all, which might improve the odds in the PCC treatment group. Second, even when plasma was used for reversal of warfarin in the study, the median dose of plasma given (2 units) is substantially lower than the dose needed for the adequate reversal of warfarin effect. Third, there were a number of differences in the baseline characteristics between the treatment groups, including the use of antiplatelet agents, which was not corrected for in the study analysis.

Study III: Thromboembolic safety and efficacy of PCC in the emergency reversal of warfarin coagulopathy

Main findings

The main focus in our study was on the safety of treatment with PCC assessed by the occurrence of arterial or venous thromboembolic events within a week the administration of PCC. We found in our study that there was a low rate of thromboembolic events following treatment with PCC of 3.8% (95% CI, 1.4-8.0%), most of which were arterial events. When limiting the analysis to patients who had cardiac indication for VKA (i.e. AF or PHV) or who had previous stroke, the rate of stroke after PCC administration was even lower at 2.5%. It is important to recognize that patients who were anticoagulated with warfarin in this study had an underlying increased risk of thromboembolism without anticoagulation. Thus, the interruption of anticoagulation, as in the case of a serious bleeding or in surgery, would expose these patients to an increased risk of thromboembolism, even without PCC administration. In addition, the surgical intervention can activate coagulation and lead to an increased risk of VTE[238] or myocardial infarction[239]. Any causality between PCC and the observed thromboembolic events should therefore be interpreted with caution.

In terms of efficacy, most of the patients were adjudicated to have good effect of PCC and only 10 (6%) patients had poor to moderate effect. Eight of these 10 patients had warfarin-related ICH, which has high mortality[83,218] and treatment with PCC might not, as discussed above, improve the outcome of these patients [240], Additionally, 2 of these 8 patients received suboptimal doses of PCC and 3 of them did not receive vitamin K with PCC. In fact, the INR in 4 of the 8 patients after treatment with PCC ranged between 1.4 to 1.6 suggesting a

rebound effect of VKA caused by the lack of vitamin K administration or the low PCC dose. The remaining 2 of the 10 patients with suboptimal effect of PCC had GI bleeding where other local factors (e.g. arterial bleeding from an ulcer or esophageal variceal bleeding) could have played an important role in the continued bleeding despite of the correction of coagulation with PCC.

Only 57% of all patients had an INR of less than 1.5 after PCC administration in our study. Several factors might have contributed to this relatively low proportion of INR correction. First, there was in several instances limited local supply of PCC resulting in some of the patients receiving lower doses than recommended by us. Second, in patients with emergency - although minor - surgical intervention, the INR was sometimes reversed intentionally to only 1.6-1.8 depending on the type of surgery performed. However, the clinical assessment showed good effect of PCC treatment in 94% of all patients. This begs the question whether the weight- and INR-based dosing of PCC leads to overtreatment. In a recent study a low, fixed dose of PCC seemed effective to manage VKA-associated bleeding[241].

Strength

The main strength in this study is the prospective multicenter design with an initial formal sample size calculation according to the rule of three[242]. The cohort in our study represents one of the largest published with PCC treated patient. Additionally, we used both a clinical end point of efficacy and a laboratory endpoint (INR) for the overall assessment of the treatment efficacy. We relied on two independent investigators to add to the objectivity of efficacy assessment. We included both patients who needed reversal with PCC because of a serious

VKA-related bleeding and those on VKA requiring emergency surgery. For the safety endpoint, we focused on thromboembolic events occurring up to 7 days after PCC administration, as shorter or longer follow-up would mean that we either missed cases that were related to PCC or included events that were highly unlikely to be caused by PCC.

Limitations

The main limitation is that we failed to include all cases treated with PCC from the different centers consecutively. However, this occurred randomly rather than systematically and should not affect the validity of the study. Another limitation is that we did not have a control arm to enable us to assess the PCC-attributable increased risk of thromboembolism. Such a design is hardly feasible and has many ethical issues since PCC is regarded as the first line of reversal for VKA in the event of a serious bleeding or emergency surgery in Sweden.

Comparison with other studies

Four previously published studies, including 100 patients each, reported thromboembolism rates after PCC treatment of 0-2%[243-246], which is comparable to the results of our study when taking into consideration the confidence interval (3.8%, [95% CI, 1.4-8.0%]). The incidence of stroke in patients with cardiac indication for VKA or previous stroke (2.5%) is comparable to that reported in two studies on bridging with LMWH peri-operatively after withholding VKA without active reversal[227,228,242], supporting the minimal-if any- PCC-attributable risk increase for thromboembolism. Most of the events seen in our study were arterial, which is consistent with the findings in another study[226].

The mortality rate within one week after VKA reversal with PCC in our study (6.3%) is comparable to the 7% in another published cohort[247]. Much higher mortality rates (28%) have been reported in an other study on the reversal of VKA with PCC[248], which could be explained by difference in the characteristics of patients included, such as the higher prevalence (62%) of patients with ICH in the latter study and a different follow-up period.

Study IV: Management and Outcomes of Major Bleeding During Treatment With Dabigatran or Warfarin

Main findings

We are here presenting data from the largest cohort of patients with dabigatran-related major bleeding, and who are on anticoagulation either for SPAF or for VTE. We reported a number of important results. First the patients who suffered a major bleeding on dabigatran were significantly older, had more severe renal impairment, and more frequent concomitant use of antiplatelet agents and NSAIDs. This indicates that patients who suffered a major bleeding on dabigatran were higher risk patients, where other factors than only dabigatran itself could have contributed to the bleeding. This brings up the possibility of lowering the bleeding risk by supporting the use of a lower dabigatran dose, as suggested by other guidelines[116], or by avoiding concomitant use of antiplatelet agents or NSAIDs. With respect to the management of major bleeding in the phase III trials, almost all of the dabigatran related bleeding events were managed by supportive care and transfusion of blood and blood products whereas very few patients received coagulation factor concentrates. There was more frequent use of red cell transfusions in the dabigatran than in the warfarin group, which can be explained by the higher incidence of GI bleeding in the

dabigatran arm. Furthermore, a larger proportion of GI bleeds was transfused with red cells among the dabigatran-treated patients than the warfarin-treated patients (71% versus 54% transfused, respectively). This was balanced by the more frequent use of plasma in the warfarin arm, which probably reflects the effort to reverse the anticoagulant effect of this drug.

The higher incidence of GI bleeding in the dabigatran arm together with the lower incidence of ICH might also explain the greater reduction in the hemoglobin level from the baseline level at the time of randomization to the time of admission for the bleeding event. A possible higher incidence of occult GI bleeding in the dabigatran arm might also explain the reduction of hemoglobin seen in patients who had no major bleeding event. The drop was very modest (1.1 g/L per 12 months for the dabigatran 150 mg group), which is reassuring.

The most important finding in our study is, that despite the unavailability of a specific reversal agent for dabigatran in case of a major bleeding, the outcome of patients who suffered this on dabigatran was better than when on warfarin, as evident by the significantly shorter period of stay in the intensive care unit, and the trend to lower 30-day all-cause mortality in the dabigatran arm, which becomes statistically significant when patients in the AF trial (RE-LY) are analyzed separately.

Strength

The main strength of the study is the large sample size, where we included information on consecutive bleeding events, collected both from databases and from serious adverse events- and major bleeding narratives from all the 5 long-term treatment phase III trials. Even though the data was collected through

retrospective review of the charts, the information was originally recorded prospectively in the specific CRFs of the phase III trials, The inclusion of only centrally adjudicated major bleeding events adds to the strength of the study, and it is such bleeds that have the greatest impact on the general health system and on individual patient management. We analyzed events on-treatment or up to 3 days after the last study drug dose to ensure that the bleeding was related to the drug itself. The data were reviewed by 2 independent investigators, and then validated against the central databases to ensure the validity and completeness of information. Finally, the use of quantitative and hard end points, like length of stay in the intensive care unit and mortality, adds to the strength of the study.

Limitations

There are a number of limitations in this study. First, the source population was derived from 5 phase III trials where patients were enrolled using strict inclusion and exclusion criteria and followed according to a rigorous pre-specified protocol, which differs from the real world. Therefore resources and outcomes in clinical practice might differ from the results presented in our study. It should be recognized that patients treated with dabigatran or warfarin were selected according to the same criteria, minimizing the influence of "selection bias" on the results. Second, we included all the patients with major bleeding events in the trials without doing a sample size and power analysis, but the large number of patients in the source population, where more than 16000 patients were treated with dabigatran (almost double the current number of patients on all the DOACs in Sweden) means that the clinical significance of any effect – found or not found in the study- would be substantial in the real world. Third, the data recorded in the major bleeding events and in the adverse event narratives were

intended for reporting the event itself and no specific protocol was used to guide the management of the bleeding event. The decision on how to treat the bleeding was at the discretion of the local investigator, and this resulted in wide variation of treatments given. Such differences are likely to reflect the real world picture.

We were not able to draw any conclusion on the efficacy of coagulation factor concentrates in the management of dabigatran associated bleeding, because of the small number of patients treated with these agents.

One issue that needs to be considered carefully is the unexpected low rate of anticoagulation reversal in the warfarin treated group. In our study, only a third of patients with warfarin-related major bleeding received any reversal, and almost all reversal was with plasma while only 12 received rapid reversal (10 patients received PCC and 2 received rFVIIa). Furthermore, only 31% received vitamin K. This low rate of reversal apparently represents the real world scenario, as mentioned above, since the treatment of any major bleeding was left to the local physician and carried out according to local guidelines in the hospitals. The utilization of plasma instead of PCC can be explained by the inclusion of patients from several centers where plasma is the first line of reversal because PCC is either unavailable or too costly. Using plasma instead of PCC means that it takes significantly longer time for INR correction, and one might argue that this could influence patient's outcome, including mortality – although not necessarily for ICH, as discussed in Study II.

Finally, the RELY trial had an open design and all our data collection from the bleeding events was done through retrospective review, but as mentioned above, the information was recorded prospectively into specifically designed forms during the studies and neither this nor the open study design would affect the

hard endpoints that our conclusions are based on.

Comparison with other studies

The number of bleeding events reported in our study is lower than that of the combined bleeding events reported in the 5 individual phase III studies (1121 vs 1507), this is because we only analyzed data from patients who had a major bleeding while on or within 3 days after the last study drug to include strictly the events that could be related to the study drug.

In our study, patients suffering a major bleeding on dabigatran had lower renal function than those on warfarin. In an analysis of the RELY trial, both age and renal function were found to be risk factors for bleeding but there was no interaction between the renal function and study drug[106], contrary to what is expected from a drug dependent on renal elimination like dabigatran. Yet renal function was an independent risk factor for bleeding on LMWHs, which are also dependent on renal function[249]. We also found that concomitant treatment with antiplatelet agents increased the bleeding risk on dabigatran, similar to previously published findings on combination of antiplatelet agents with warfarin or dabigatran [134,137]

In our study, we presented data on the 30-day all-cause mortality as opposed to another published analysis from the RE-LY trial, where fatal bleeding events, i.e. bleeding events that resulted in death, without specific consideration to the time from the last intake of study medicine, were presented[106]. In this study, rates of fatal bleeding on warfarin were not statistically different from those on dabigatran 150mg but significantly higher than those on dabigatran 110mg. A separate subgroup analysis of ICH in the RE-LY trial has also been published

and showed significantly lower number of fatal ICH with both dabigatran doses compared to warfarin [224]. These two studies used different definitions and selected specific subgroups for their analysis and are difficult to compare with the results of our study.

FUTURE PERSPECTIVES ON THE MANAGEMENT OF HEMORRHAGIC COMPLICATIONS OF ANTITHROMBOTICS

Bleeding, especially ICH, is the most serious complication of treatment with anticoagulants. All the anticoagulants available on the market today have bleeding as a side effect, although a recent work on a factor XIIa inhibitory antibody in an animal model involving extracorporeal circulation showed very promising results with thromboprotective effect without associated increase in bleeding risk[29]. It is unlikely that this antibody will be marketed within the near future, and the problem of bleeding on anticoagulants will continue to be a challenge to manage. Vitamin K antagonists have been available since six decades and PCC can normalize the prolongation of INR induced by VKA but data on the clinical efficacy is still scarce. Several classes of DOACs are currently either licensed or in the final stages of clinical trials These agents have different bleeding risk profiles from each other and from VKA and there are no commercially available antidotes yet. Specifically, these new agents have been shown to be associated with significantly lower rates of ICH. It is very intriguing to see if findings of Study I apply to DOACs and a study with a similar design but including patients on the different DOACs would be the next logical step.

Another question of interest would be to see if there is any subgroup of patients with ICeH who would benefit significantly from treatment with PCC as compared to plasma. In our study the median time to administration of PCC was

4 hours, and the question is how the results would be if we managed to give patients PCC much earlier after the onset of symptoms. Today, patients on warfarin are excluded from the stroke-thrombolysis protocols if they have an INR ≥1.7. It is, however, interesting to establish a broader "stroke" protocol that ensures rapid care and radiological investigation of all patients with new neurological symptoms, where the finding of ICH and elevated INR would lead to quick administration of PCC. In such settings, comparison of PCC administered shortly after symptoms with either plasma or historical data might give further insights in the utility of PCC.

One study has shown that PCC is safe and efficacious in the reversal of rivaroxaban[196]. A consequence of Study III would be a large-scale prospective study that evaluated the safety and efficacy of PCC in the reversal of several DOACs, especially the Xa inhibitors. Parallel with that, it would also be interesting to see if managing the major bleeding events on the Xa inhibitors by supporting the patients hemodynamically without the administration of a specific reversal agent leads to comparable results to those seen in Study IV. The large registries available in Sweden, including the Swedish Prescribed Drug Register and the Inpatient Register, allow for unique research possibilities with the high quality data collected in these registers, and allow for answering complicated questions with relatively limited resources. This is an area that should be explored further.

It also of importance that our studies, especially Study I, II and IV, present data that were not previously available in other publications, but additional studies are needed to confirm our findings.

5 CONCLUSIONS

- In patients suffering ICH while on warfarin treatment, the optimal timing for resumption of warfarin seems to be, contrary to previous studies and recommendations, between weeks 10 and 30 after the bleeding. This 20-week span allows for individual risk benefit assessment of recurrent ICH versus thromboembolism to select the suitable point of resumption.

- Treatment with PCC for patients with VKA-associated ICeH, even though resulting in a more rapid correction of INR, does not seem to reduce the 30-day all-cause mortality.

- Patients treated with PCC to reverse warfarin in case of serious bleeding or before emergency surgery are at an increased risk of developing thromboembolic events, mainly arterial. Several factors might contribute to this increased risk including patient characteristics such as a pre-existing thrombogenic process, for which the patient is anticoagulated, and activation of coagulation by the bleeding or surgery. The contribution of PCC to this increased risk seems to be minimal if any.

- Patients who suffer a major bleeding event on dabigatran are older, have worse renal function and are more frequent concomitant use of antiplatelet agents or NSAID than those who develop major bleeding on warfarin. This indicates that dabigatran may be associated with a higher bleeding threshold than that of warfarin.

- Even in the absence of a specific reversal agent for dabigatran, the outcome of patients with major bleeding on dabigatran treated with supportive

measure seems to be better than those on warfarin, as evident by a shorter stay in the intensive care unit and lower 30-day all-cause mortality in the dabigatran arm.

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