Thrombosis and anticoagulant treatment in special populations

Barco, S.L.

THROMBOSIS AND ANTICOAGULANT TREATMENT IN SPECIAL POPULATIONS

STEFANO BARCO

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The same challenge, namely how to practice evidence-based medicine in the absence of firm evidence, applies to groups of patients for whom antithrombotic drugs are or could be indicated: individuals with rare or multiple diseases as well as individuals who had been excluded from or were underrepresented in large clinical trials, such as elderly, children, pregnant women, and patients with unusual site thromboses or cancer, constitute these “special” populations.

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THROMBOSIS AND ANTICOAGULANT TREATMENT IN SPECIAL POPULATIONS

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General introduction and outline of the thesis

GENERAL INTRODUCTION

The generation of physicians who are currently in their 30s has been educated under the flourishing paradigm of evidence-based medicine, defined by its inventors as «the combination of the best research evidence with clinical skills and patient values and preferences»[1]. This revolutionary approach contributed to the development of a number of effective treatments, which improved the course of high-prevalence diseases and, in some cases, were a last resort option for otherwise untreatable conditions. Two examples include anticoagulant treatment for stroke prevention in patients with atrial fibrillation and parenteral nutrition for intestinal failure[2,3].

However, as a consequence of the spread of several novel treatments, physicians face an increasing number of treatment-related complications (e.g., major bleedings in anticoagulated patients and catheter-related thromboembolic events during parenteral nutrition administration) for which the best evidence available is still absent or of low quality.

The same concept, namely how to practice evidence-based medicine in the absence of firm evidence, applies to other groups of patients for whom anticoagulants are or could be indicated. Individuals with rare or multiple diseases as well as individuals who had been excluded or were underrepresented in large clinical trials, such as elderly, children, pregnant women, and patients with unusual site thrombosis or with cancer, constitute these “special” populations.

OUTLINE OF THE THESIS

Reversal of direct oral factor Xa inhibitors with prothrombin complex concentrate

More than 1% of the general population requires anticoagulant treatment for prevention of ischemic stroke and systemic embolism in patients with atrial fibrillation[4]. Vitamin K antagonists have been the mainstay therapy for decades and only in recent years direct oral anticoagulants have become available as a first-line option for these patients. Despite the fact that phase III trials demonstrated that the risk of major bleeding was overall reduced once compared to the standard treatment[5], hemorrhage still represents a frequently occurring and serious complication.

Prothrombin complex concentrate is recommended for vitamin K antagonist-associated major bleeding and contains three or four non-activated vitamin K-dependent coagulation factors (depending on the presence of factor VII) plus a varying amount of natural coagulation inhibitors. Four-factor prothrombin complex concentrate has been the first agent studied *in vivo* for reversing the direct oral anticoagulant rivaroxaban, which specific inhibits factor Xa. At the dosage of 50 IU/kg, prothrombin complex concentrate normalized hemostatic surrogate parameters (thrombin generation and prothrombin time) in healthy volunteers receiving supratherapeutic doses of rivaroxaban[6]. Since prothrombin complex concentrate infusions are associated with a significant risk of thrombotic complications in real-life patients[7,8], we aim to assess the use of two lower dosages (37.5 and 25 IU/kg) compared to placebo for reversing the anticoagulant effects of direct oral factor Xa inhibitors rivaroxaban and apixaban (*Part I*, in **Chapters 2** and **3**). Furthermore, we estimate the intrinsic safety profile of prothrombin complex concentrate in terms of clinical outcomes pooling the results of eight interventional studies enrolling healthy volunteers not having any underlying thrombosis risk factors (**Chapter 4**).

Parenteral nutrition

Patients on parenteral nutrition are a neglected and poorly studied population, especially with respect to research on cardiovascular outcomes. This is partly due to the relative rarity of this condition, which accounts for 1-20 individuals per million inhabitants within Europe[9,10]. Recurrent catheter-related thrombosis is one of the most frequent and relevant complications, ultimately leading to loss of vascular access and a need for intestine transplantation since parenteral nutrition cannot be delivered. In clinical practice, up to two-thirds of adult patients on parenteral nutrition are using anticoagulants, as recommended by parenteral nutrition guidelines[11]. However, several issues regard the pharmacokinetics of oral anticoagulants, particularly in patients with short bowel syndrome, as well as their efficacy and safety.

Part II focuses on the use of anticoagulant agents for the prevention and treatment of thrombotic complications in patients on parenteral nutrition. In **Chapter 5**, we systematically assess the available evidence on efficacy, safety, and feasibility of anticoagulant regimens for preventing and managing catheter-related thrombosis during parenteral nutrition. In **Chapter 6** we analyze data from a large cohort of Dutch patients followed at the Academic Medical Center of Amsterdam for the management of home parenteral nutrition. The rates of symptomatic, objectively diagnosed venous thrombosis, major bleeding, vena cava syndrome and heparin-associated complica-

tions are assessed, as well as predictors of first venous thrombosis. **Chapter 7** reports the results of the phase I interventional crossover PDER PAN study (Pharmacokinetics and -dynamics of Dabigatran Etexilate and Rivaroxaban in short bowel syndrome patients on PArenteral Nutrition).

Venous thrombosis in special populations

Part III includes original studies and reviews on subgroups of patients requiring anticoagulation for venous thrombosis or atrial fibrillation. The first two chapters focus on individuals with cerebral venous thrombosis: in **Chapter 8** we performed a meta-analysis of published observational studies attempting to evaluate the strength of the association between cerebral venous thrombosis and inherited or acquired thrombophilia. In **Chapter 9** an international multicenter cohort study of more than 600 patients is presented and assesses the characteristics of cerebral venous thrombosis and the predictors of recurrences. In **Chapter 10** we provide an update of recent findings regarding the diagnosis and treatment of venous thromboembolism in pregnant women. In **Chapter 11** we present the findings of a multicenter study of 202 consecutive patients with idiopathic or provoked splanchnic vein thrombosis assessed for the presence of a paroxysmal nocturnal hemoglobinuria clone. **Chapter 12** summarizes the risk-benefit profile of the recently approved direct oral anticoagulants in elderly patients on the basis of data from phase III randomized controlled trials on venous thromboembolism and atrial fibrillation. In **Chapter 13** we illustrate the case of a patient with atrial fibrillation in whom the dose of direct oral anticoagulant dabigatran etexilate was chosen (in presence of interfering co-medications) on the basis of pharmacokinetic parameters.

Economic perspective

Part IV (**Chapter 14**) encompasses the first study defining the economical burden of venous thromboembolism and its complications within the European Union-28. Annual individual costs for deep venous thrombosis, pulmonary embolism, bleeding and other potential anticoagulant-related complications are retrieved from the literature and, after adjustments for inflation and purchasing power parity between countries, served to populate a cost-model based on adult incidence-based events. Total, hospital-associated, preventable, and indirect annual costs are finally provided and commented.

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PART I

Reversal of direct oral factor Xa inhibitors with prothrombin complex concentrate

2

In vivo reversal of the anticoagulant effect of rivaroxaban with four-factor prothrombin complex concentrate

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ABSTRACT

Four-factor prothrombin complex concentrate (PCC) 50 IU/kg is able to swiftly restore hemostatic parameters in healthy subjects on rivaroxaban. We hypothesized that lower dosages of PCC may already be sufficient to restore normal hemostasis.

In this double-blind, crossover, placebo-controlled study, we compared the effects of PCC 37.5 IU/kg, PCC 25 IU/kg, and placebo on thrombin generation (endogenous thrombin potential, ETP) and prothrombin time in 6 healthy subjects receiving rivaroxaban 15 mg twice daily for 2.5 days.

Fifteen minutes after infusion of PCC 37.5 IU/kg, ETP increased from 47±16% to 64±22% (p=0.03; pre-rivaroxaban ETP: 92±14%). ETP over 24 hours was higher than after placebo (p=0.001). PCC 25 IU/kg did not modify ETP within 15 minutes (53±11% to 59±12%; p=0.14) and was not different from placebo over 24 hours (p=0.31). ETP reached pre-rivaroxaban levels within 6 hours after PCC 37.5 IU/kg infusion and within 12-24 hours after PCC 25 IU/kg infusion. Both dosages restored rivaroxaban-induced prothrombin time prolongation after 15 minutes (p<0.001). Placebo did not have an effect on coagulation parameters.

37.5 IU/kg of PCC leads to partial restoration of thrombin generation, whereas 25 IU/kg does not. PCC 37.5 IU/kg may be insufficient for immediate full reversal of peak therapeutic rivaroxaban levels.

INTRODUCTION

Rivaroxaban is one of the direct oral factor Xa inhibitors approved for the prevention and treatment of thromboembolism[1,2]. More than 8 million patients have received rivaroxaban worldwide since its registration[3] on the basis of phase III trials comparing it with conventional anticoagulant therapy (heparin followed by vitamin K antagonist).

A concern of treatment with rivaroxaban is the lack of a specific reversal agent that can restore hemostasis in case of major bleeding. Although specific antidotes are under development, regulatory approval is dependent upon ongoing phase III clinical trials[4] and those are not yet available in clinical practice. Meanwhile, patients with major bleeding on rivaroxaban are being managed with supportive measures and non-specific prohemostatic agents, such as non-activated prothrombin complex concentrate (PCC)[5,6]. The latest European Heart Rhythm Association guidance document[6] suggests that patients with life-threatening bleeding on rivaroxaban should receive PCC 25 IU/kg repeated once or twice, although that dosage has never been tested in humans[7,8] and was partially or not effective in animal models[9,10,11].

Two recent *in vivo* studies showed that the anticoagulant effect of rivaroxaban measured by thrombin generation (Endogenous Thrombin Potential, ETP) was normalized within 15 minutes by 4-factor PCC 50 IU/kg Cofact (Sanquin Blood Supply, Amsterdam, the Netherlands)[7] and within 6-8 hours by 4-factor PCC 50 IU/kg Beriplex P/N (CSL Behring, Marburg, Germany)[8]. However, after ETP normalization an increase beyond baseline (e.g. pre-rivaroxaban) values in thrombin generation was observed, suggesting an "overshoot" that may lead to increased thrombosis risk[7]. PCC administration for anticoagulation reversal in patients receiving viamin K antagonists is associated with a low, but quantifiable risk of thromboembolic complications of 0.7-1.8% per administration[12]. Therefore, if a dose-dependent effect of PCC occurs as observed in animal models[9,11], using a lower dosage could reduce the risk of thromboembolic adverse events with a similar efficacy profile and lower costs.

The objective of our study was to assess the effect of a single administration of non-activated 4-factor PCC at the dosages of 37.5 and 25 IU/kg on the anticoagulant effect of steady-state therapeutic-dose rivaroxaban in healthy subjects.

MATERIALS AND METHODS

Study design

The study was performed as a single-center, randomized, double-blind, placebo-controlled, crossover study at the Academic Medical Center of Amsterdam (the Netherlands).

All subjects provided written informed consent prior to screening and were subsequently randomized in blocks for the order of reversal method by an independent investigator. Rivaroxaban 15 mg was prescribed twice daily from day -2 to day 0: the final dose was taken on day 0 in the morning without food consumption, followed after 3 hours by either a single bolus of PCC 37.5 IU/kg, PCC 25 IU/kg, or a similar volume of saline (placebo). Subjects returned to the same prescription of rivaroxaban and crossed-over for the method of reversal after a wash-out period of at least 15 days (Figure 1).

Oral rivaroxaban (Xarelto, Bayer, Leverkusen, Germany) was used in the commercially available formulation[1,2] and adherence to study medication was evaluated at each infusion by checking the blisters. Rivaroxaban was stored in the hospital pharmacy and provided at baseline. The dosage of 15 mg twice daily was chosen as it is the highest approved dosage for the initial treatment of acute venous thromboembolism[1,2,13,14].

Four-factor PCC (Cofact, Sanquin Blood Supply, Amsterdam, the Netherlands) was supplied by the manufacturer. A vial of 500 IU PCC contains 500 IU of factor IX, 280-700 IU of factor II, 140-400 IU of factor VII, 280-700 IU of factor X, 222-780 IU of protein C, 20-160 IU of protein S, and antithrombin, without any heparin added. PCC was reconstituted by an independent researcher in the hospital pharmacy according to manufacturer's instructions and subsequently administered by blinded investigators.

The study was conducted in accordance with the Declaration of Helsinki (59th version Korea, October 2008), the Medical Research Involving Human Subjects Act, and according to guidelines for Good Clinical Practice. The study protocol was reviewed by the institutional review ethics board and registered at the Dutch Trial Registry (NTR3559). Subjects were recruited by public advertisement located at the Academic Medical Center (Amsterdam) and received a financial reimbursement coherent with the time spent on the study.

Study population

Subjects were included after screening if they met the following inclusion criteria: male sex, age between 18 and 50 years, normal laboratory screening tests, no abnormalities at physical examination, normal vital signs, and ability to provide written informed consent. Laboratory screening included renal and hepatic function, hepatitis B, hepatitis C, and human immunodeficiency virus serology, complete blood cells count, prothrombin time, and activated partial thromboplastin time. Exclusion criteria were a history of allergic reaction to blood products, a personal or family history of coagulation disorders, participating in any other investigational interventional study within the past 30 days. During the study, the consumption of nicotine, alcohol, and drugs was recorded.

Study procedures, sample collection and analysis

Blood samples were collected at screening visit, at baseline visit on day -2, at day 0 immediately before the PCC/saline infusion and 15, 30, 60, 120, 240, and 360 minutes after the end of infusion, and at day 1 (T=24 hours, Figure 1).

A peripheral venous catheter was placed to administer PCC or saline, and a second catheter was inserted for blood withdrawal on the infusion days. The lines for blood samples were flushed with saline and the first 5 mL blood discarded at each blood withdrawal. Tubes of 5 mL with 3.2% citrate were used and samples were double spun within 2 hours from withdrawal to produce platelet-poor citrated plasma. Samples were then stored at -80°C until analysis.

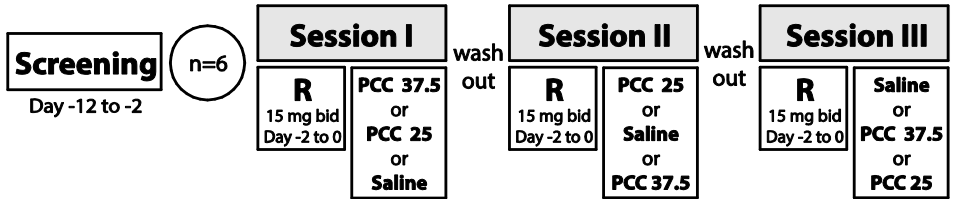

Figure 1. Study design

PCC infusion and blood withdrawal were performed at each session. Time points sampling: T=0 (pre-PCC), 15, 30 minutes, 1, 2, 4, 6, 24 hours after the end of PCC/saline infusion. Abbreviations: R, rivaroxaban; PCC, prothrombin complex concentrate (expressed as IU/kg).

The following measures were used for study outcomes: endogenous thrombin potential (ETP; as a measure of thrombin generation) and prothrombin time[7]. The Calibrated Automated Thrombogram assays the generation of thrombin in clotting plasma using a microtiter plate-reading fluorometer (Fluoroskan Ascent, ThermoLab Systems, Helsinki, Finland) and Thrombinoscope software (Thrombinoscope BV, Maastricht, the Netherlands). The software calculated the ETP, the total amount of thrombin generated in time during the test, normalized to pooled plasma obtained from more than 200 individuals; reference values: 65-146%. The assay was carried out as described by Hemker et al. with 5 pM of tissue factor as initiator of coagulation[15]. Prothrombin time was analyzed with an automated coagulation analyzer (Behring Coagulation System XP), Thromborel S reagent, and protocols from the manufacturer (Siemens Healthcare Diagnostics, Marburg, Germany); reference values: 10.7-12.9 seconds. A calibrated anti-FXa activity (Biophen DiXal anti-FXa assay, Hyphen, Neuville-sur-Oise, France) was used to verify adequate rivaroxaban uptake before PCC administration. For exploratory purposes, additional analyses that were not part of predefined outcomes were performed: results are shown as Supplementary Material. All laboratory analyses were performed by laboratory technicians, who were unaware of the type and the dosage of the reversal method.

Potential adverse events were recorded throughout the study: the final visit was scheduled after 20 days from the last infusion.

Study outcomes

Pre-defined study outcomes were: 1) the effect of PCC administration on coagulation parameters, as reflected by difference in ETP (primary outcome) and prothrombin time between T=0 (pre-PCC) and T=15 for each PCC dosage, and 2) the effect of two different PCC dosages compared to placebo over 24 hours.

Sample size calculation and statistical analysis

Our sample size calculation was based on the primary outcome from the study by Eerenberg et al.[7]: improvement of ETP between T=0 and T=15. In that study[7] pre-rivaroxaban ETP values in the rivaroxaban group were 92±22% (mean ± standard deviation [SD]). Administration of rivaroxaban decreased ETP to 51±21% and PCC increased ETP to 114±26%. Using a paired Student t-test, an *alpha* of 0.05 and a power of 0.8, a sample size of 6 subjects in the present crossover study was

calculated for the primary outcome, the effect of two PCC dosages on ETP, as reflected by differences between T=0 (pre-PCC) and T=15 for each PCC dosage.

Data are presented as mean±SD or median (range), whatever was appropriate. For the immediate effect of 4-factor PCC, paired t-tests were performed. The effect over 24 hours was analyzed with linear mixed models. Two-sided p-values <0.05 were considered to be statistically significant. Data analysis was performed using IBM SPSS v21.0 (IBM Corporation, Armonk NY, United States).

RESULTS

Six subjects were enrolled and all completed the study. Subjects had a median age of 22 years (range 20-50 years), mean height of 180±7 cm, and mean weight of 83±14 kg. All subjects had normal baseline screening tests.

The mean PCC dosage per subject was 2,070±356 IU in the 25 IU/kg dosage session and 3,113±534 IU in the 37.5 IU/kg dosage session.

Effects of rivaroxaban on coagulation before PCC/placebo

After having received twice-daily rivaroxaban 15 mg from day -2 to day 0 (five doses), subjects showed statistically significant changes of both ETP and prothrombin time consistent across sessions (overall, p=0.001 for ETP and p=0.001 for prothrombin time), indicating an effect of rivaroxaban on those parameters.

Mean anti-FXa-derived rivaroxaban peak level at steady-state (day 0, T=0 pre-PCC) was 189±72 ng/mL (range 93-351) with no differences between groups (placebo: 190±52 ng/mL; PCC 25 IU/kg: 184±69 ng/mL; PCC 37.5 IU/kg: 193±102 ng/mL), suggesting rivaroxaban blood levels in line with data from literature[16].

Immediate effect outcomes

Fifteen minutes after infusion of PCC 37.5 IU/kg, ETP increased from 47±16% to 64±22% (p=0.03). After infusion of PCC 25 IU/kg, ETP modified from 53±11% to 59±12% (p=0.14; Figure 2; Table 1).

Fifteen minutes after infusion of PCC 37.5 IU/kg, prothrombin time decreased from 13.4±1.0 seconds to 11.5±0.8 seconds (p<0.0001). After infusion of PCC 25 IU/kg,

prothrombin time decreased from 13.2±1.3 seconds to 11.6±0.9 seconds (p<0.001; Figure 3, Table 1).

No changes in both ETP and prothrombin time were observed over the first 15 minutes after infusion of saline in the placebo group (ETP: 45±17% to 42±6%, p=0.43; prothrombin time: 13.7±1.5 seconds to 13.7±1.3 seconds, p=0.88).

Table 1. Immediate and sustained effect outcomes

ETP (%)	**PCC 37.5**	**p**	**PCC 25**	**p**	**Placebo**	**p**
Pre-rivaroxaban	92±14		98±13		97±16	
T=0	47±16	0.03	53±11	0.14	45±17	0.43
T=15 min	64±22		59±12		42±6	
T=6 h	98±33		86±10		60±10	
T=24 h	125±28		103±18		83±14	
T=0-24 h	*vs* placebo	0.001	*vs* placebo	0.31	-	-
PT (seconds)	**PCC 37.5**	**p**	**PCC 25**	**p**	**Placebo**	**p**
Pre-rivaroxaban	11.3±0.7		11.4±0.8		11.4±0.8	
T=0	13.4±1.0	<0.0001	13.2±1.3	<0.001	13.7±1.5	0.88
T=15 min	11.5±0.8		11.6±0.9		13.7±1.3	
T=6 h	11.2±0.4		11.2±0.8		12.2±0.7	
T=24 h	11.3±0.7		11.0±0.5		11.8±0.7	
T=0-24 h	*vs* placebo	<0.0001	*vs* placebo	<0.0001	-	-

T=0 indicates the pre-PCC infusion timepoint. Abbreviations: ETP, endogenous thrombin potential; PCC, prothrombin complex concentrate (expressed as IU/kg); PT, prothrombin time.

Sustained effect outcomes

ETP levels significantly differed over time between PCC 37.5 IU/kg and placebo during 24 hours after infusion (p=0.001), but not between PCC 25 IU/kg and placebo (p=0.31; Figure 2; Table 1). Mean ETP values reached pre-rivaroxaban levels after about 6 hours from the PCC 37.5 IU/kg dosage infusion and after 12-24 hours from the PCC 25 IU/kg dosage infusion.

Prothrombin time values were significantly different over time between each PCC dosage and placebo during 24 hours after infusion (p<0.0001; Figure 3, Table 1).

Additional data that were not part of predefined outcomes, including anti-FXa-derived rivaroxaban concentration and ETP values in individual subjects, are provided as Supplementary Material (Figures A to D).

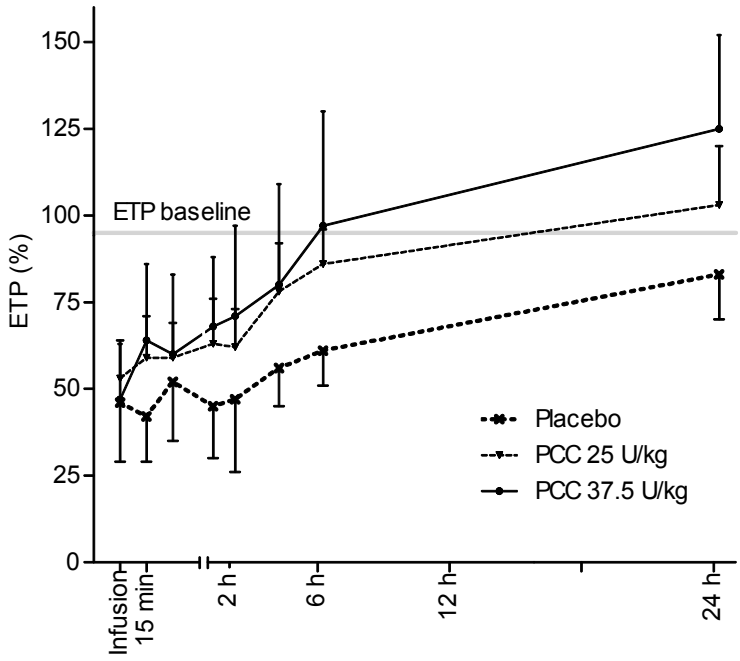

Figure 2. Effect of rivaroxaban followed by two dosages of 4-factor prothrombin complex concentrate or placebo on the endogenous thrombin potential (%)

Immediate effect outcome: differences between T=0 (pre-PCC) and T=15 (15 minutes after PCC infusion). ETP baseline (pre-rivaroxaban ETP): mean ETP value before rivaroxaban administration. Abbreviations: PCC, prothrombin complex concentrate; ETP, endogenous thrombin potential.

Adverse events

Three subjects experienced six adverse events, including two hematomas on the site of peripheral line placement, one recurrent epistaxis, two bleeding episodes after minor skin abrasions, and a vasovagal reaction while placing the peripheral line before infusion.

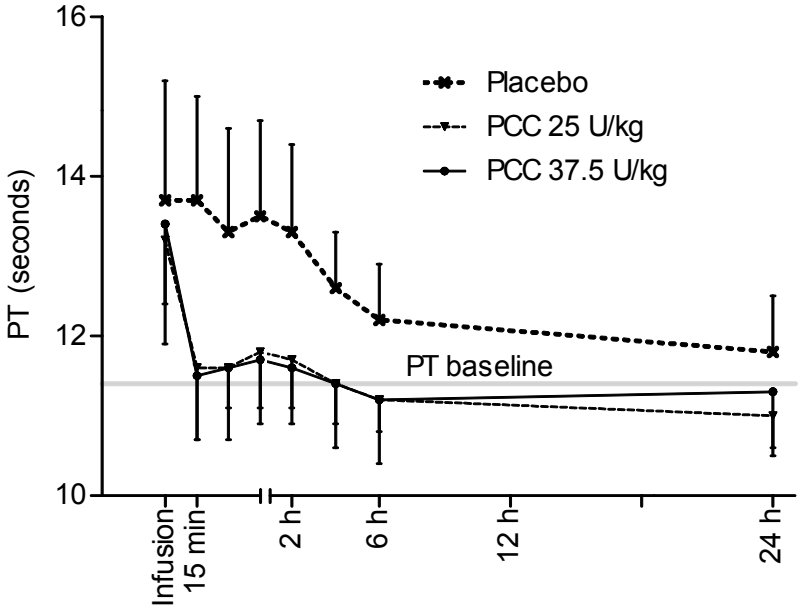

Figure 3. Effect of rivaroxaban followed by two dosages of 4-factor prothrombin complex concentrate or placebo on prothrombin time (seconds)

Immediate effect outcome: differences between T=0 (pre-PCC) and T=15 (15 minutes after PCC infusion). PT baseline (pre-rivaroxaban PT): mean prothrombin time value before rivaroxaban administration. Abbreviations: PCC, prothrombin complex concentrate; PT, prothrombin time.

DISCUSSION

In our crossover study of healthy subjects receiving therapeutic-dose rivaroxaban, we observed that 4-factor PCC 37.5 IU/kg generated a significant but modest increase of thrombin generation within 15 minutes, with pre-rivaroxaban ETP levels reached 6 hours after PCC infusion. PCC 25 IU/kg had no significant immediate or sustained effect on thrombin generation. Both dosages normalized prothrombin time within 15 minutes. Combined with the results of the prior 50 IU/kg PCC study[7], our findings suggest a dose-dependent reversal effect of PCC on the peak anticoagulant effect of rivaroxaban.

The discrepancy between effects of PCC on ETP and prothrombin time might be explained by the intrinsic characteristics of those tests: whereas ETP is a global hemostasis parameter and is influenced by many coagulation factors, prothrombin

time is very sensitive to levels of factor VII present in PCC[15,17]. ETP and prothrombin time as pharmacodynamic outcomes have been studied in *in vivo* and *in vitro* studies on direct oral factor Xa inhibitor reversal with PCCs and activated PCC[7-9,18,19]. Although ETP and prothrombin time might represent acceptable estimates of rivaroxaban anticoagulant effect and rivaroxaban concentrations[20,21], respectively, both have substantial inter-individual variability[22,23,24] and it is uncertain to what extent they are relevant for the ultimate desired effect: restoration of effective hemostasis and improvement of the clinical outcome of bleeding. Nevertheless, this limitation currently applies to most reversal strategies for any form of anticoagulation. Whereas several studies have demonstrated that PCC leads to faster INR normalization over fresh frozen plasma, none have convincingly demonstrated that faster INR normalization *per se* improves clinical outcome of bleeding compared with fresh frozen plasma[25]. Furthermore, the utility of anti-FXa activity as a tool for monitoring low-molecular weight heparins and predicting efficacy and safety outcomes is even less proven.

PCCs have been evaluated in a few consecutive patients with rivaroxaban-associated bleeding[5] and preliminary findings of their *in vivo* effect mostly derive from animal models[9-11]. In these studies, 4-factor PCC showed to be effective for reducing the mesenteric bleeding time in rats receiving PCC 50 IU/kg (but not after PCC 25 IU/kg infusion)[9], for preventing hematoma expansion in mice in a dosage of 50-100 IU/kg (but not after PCC 25 IU/kg infusion)[11], while it was not able to normalize the blood loss and the ear immersion bleeding time in rabbits (PCC 40 IU/kg)[10]. Results from our *in vivo* study are in line with findings from those animal studies with respect to the 25 IU/kg dosage.

Our results apply to this specific 4-factor PCC (Cofact, Sanquin Blood Supply, Amsterdam, the Netherlands) and different PCCs vary in composition with respect to individual coagulation factor levels which could have the effect on coagulation assays[8]. The PCC dose is quantified by the amount of factor IX but non-activated PCCs can be formulated with either four (factors II, VII, IX and X) or three coagulation factors (factors II, IX and X) and also vary with respect to levels of protein C, protein S, antithrombin, and heparin. Nevertheless, consistent data have been recently presented in two *in vivo* studies that studied other 4-factor PCCs at different dosages (50-25-10 IU/kg, and 50 IU/kg, respectively) in healthy subjects receiving either edoxaban[18] or apixaban[19].

While most of the current guidelines recommend PCC for vitamin K antagonist-associated major bleeding reversal[26,27], guidelines for rivaroxaban reversal[6,28,29,30] are based on a few experimental data. Our *in vivo* study reinforces the rationale for the

use of PCC for the management of rivaroxaban-associated major bleeding in clinical practice until approval of a specific antidote for factor Xa inhibitors[4,31]. The administration of 4-factor PCC for rivaroxaban reversal in acute bleeding should be based on its dose-dependent effect on hemostatic parameters. For immediate and full reversal of peak levels of therapeutic-dose rivaroxaban administration of PCC 37.5 IU/kg is likely to be insufficient. Such a lower dose could theoretically still be effective with a longer duration since the last rivaroxaban dose (e.g. more than 12 hours).

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SUPPLEMENTARY MATERIAL

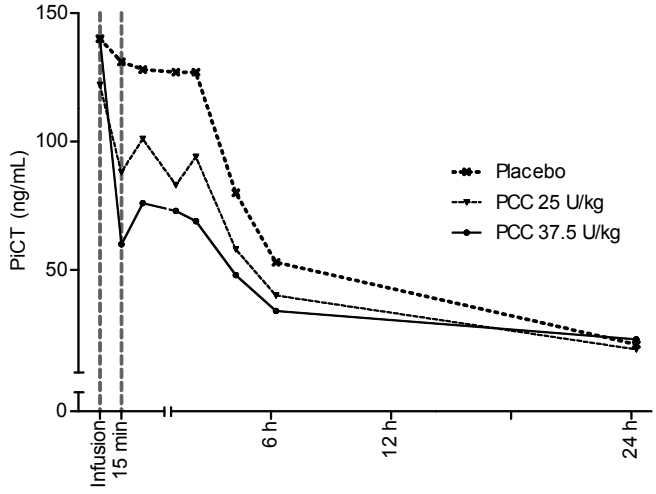

Figure A. Effect of rivaroxaban followed by two different dosages of 4-factor prothrombin complex concentrate or placebo on prothrombinase-induced clotting time-derived rivaroxaban concentration (Pentapharm, Basel, Switzerland)

Abbreviations: PCC, prothrombin complex concentrate; PiCT, prothrombinase-induced clotting time.

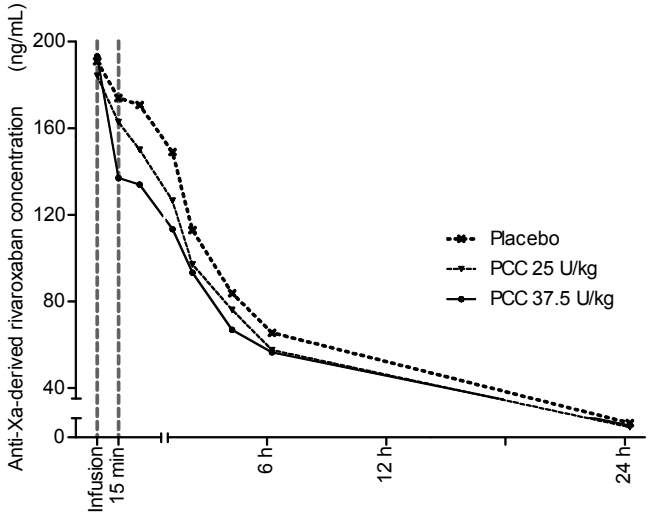

Figure B. Effect of rivaroxaban followed by two different dosages of 4-factor prothrombin complex concentrate or placebo on calibrated anti-FXa-derived rivaroxaban concentration (Biophen DiXal anti-FXa assay, Hyphen, Neuville-sur-Oise, France)

Abbreviations: PCC, prothrombin complex concentrate.

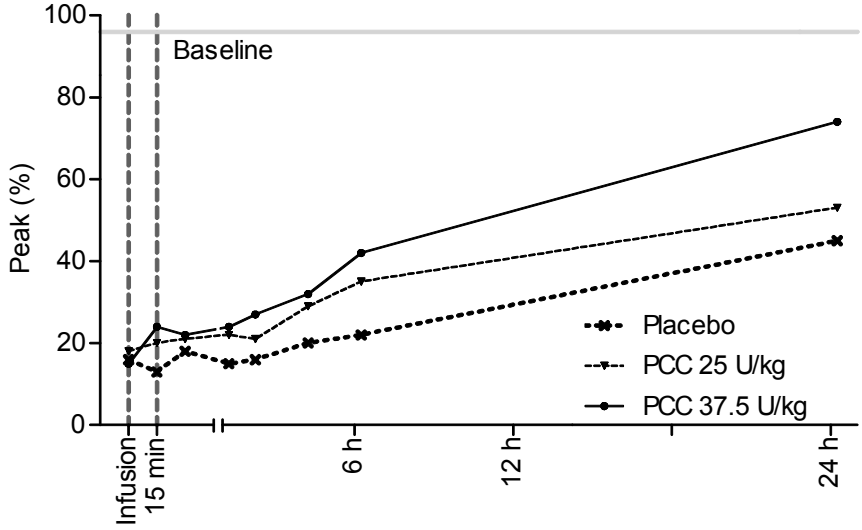

Figure C. Effect of rivaroxaban followed by two different dosages of 4-factor prothrombin complex concentrate or placebo on peak of thrombin generation (%)

Abbreviations: PCC, prothrombin complex concentrate.

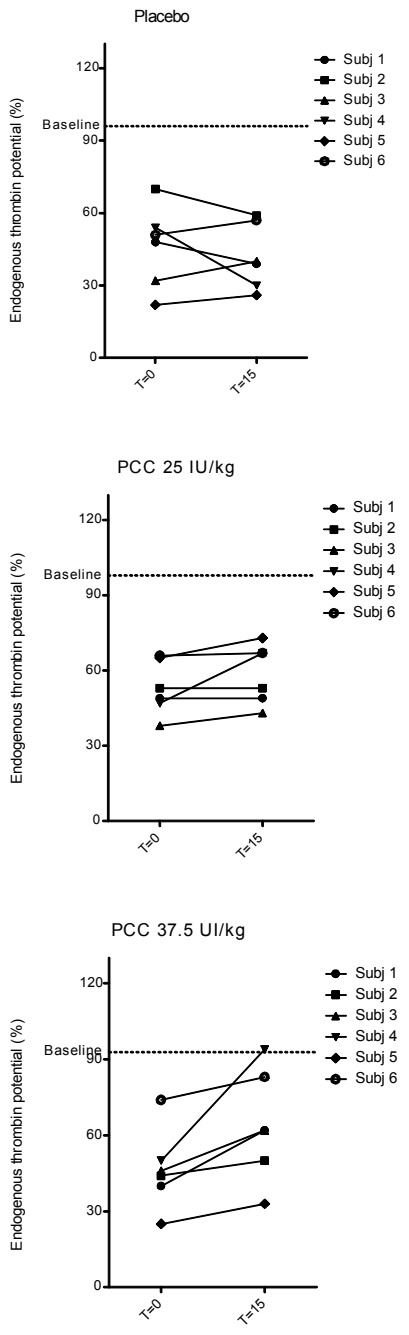

Figure D. Endogenous thrombin potential values (%) in individual subjects at the time of prothrombin complex concentrate infusion (T=0) and 15 minutes (T=15) after infusion

Abbreviations: PCC, prothrombin complex concentrate; Subj., subject.

3

In vivo increase in thrombin generation by four-factor prothrombin complex concentrate in apixaban-treated healthy volunteers

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ABSTRACT

Four-factor prothrombin complex concentrate (PCC) 50 IU/kg increased thrombin generation beyond baseline values in healthy subjects treated with rivaroxaban.

The aim of the present study is to assess whether infusion with doses of 37.5 and 25 IU/kg of PCC reverses the anticoagulant effect of a high therapeutic dose of apixaban, another oral direct factor Xa inhibitor.

In a randomized, double-blind, placebo-controlled, crossover study, 6 healthy subjects received apixaban 10 mg twice daily for 3.5 days followed by either a single bolus of PCC 37.5 IU/kg, PCC 25 IU/kg, or saline (placebo). The primary outcome was the effect of PCC 15 minutes after infusion on thrombin generation (endogenous thrombin potential [ETP]); secondary outcomes were the immediate effect of PCC on prothrombin time and the effect of PCC compared with placebo over 24 hours on ETP and prothrombin time.

Fifteen minutes after infusion of 37.5 IU/kg and 25 IU/kg PCC, ETP increased from 41±11% to 56±23% ($p=0.06$) and from 44±12% to 51±15% ($p=0.03$), respectively. ETP significantly differed over time between PCC 37.5 IU/kg and placebo during 24 hours after infusion ($p<0.01$). Both PCC dosages restored apixaban-induced prothrombin time prolongation after 15 minutes ($p<0.01$) which was sustained over 24 hours. Placebo had no effect on coagulation parameters.

Both PCC 37.5 IU/kg and 25 IU/kg improved coagulation parameters in healthy subjects, indicating partial reversal of the anticoagulant effect of apixaban. This suggests that 4-factor PCC is suitable as a reversal agent for apixaban-treated patients.

INTRODUCTION

Apixaban is one of the oral anticoagulants that directly inhibits factor Xa and is registered for treatment and prevention of venous thromboembolism and for stroke prevention in patients with atrial fibrillation[1]. Apixaban was evaluated in a fixed-dose regimen without the need for frequent laboratory testing and dose adjustments and, thereby, simplifies oral anticoagulant therapy compared with vitamin K antagonists[2,3].

Although anticoagulation is highly effective in the prevention and treatment of thromboembolism, its use is associated with an increased risk of bleeding. Vitamin K antagonist therapy tops the list of drugs of which adverse drug reactions (e.g. bleeding) leads to hospital admission, indicating that anticoagulation associated bleeding is a frequently observed problem[4]. In case of life-threatening bleeding complications, the effect of vitamin K antagonists on coagulation parameters (e.g. INR) can be immediately reversed with prothrombin complex concentrate (PCC)[5].

Specific reversal agents for direct oral factor Xa inhibitors including apixaban are under development but will probably not be available in routine clinical practice in the next few years[6,7]. In the absence of specific reversal agents, non-specific prohemostatic agents could be useful. Previously, 4-factor PCC 50 IU/kg (Cofact, Sanquin Blood Supply, Amsterdam, the Netherlands), containing zymogen factor X, completely reversed coagulation parameter changes in healthy subjects treated with a supra-therapeutic dose of rivaroxaban, another direct oral factor Xa inhibitor[8,9]. However, in that study thrombin generation (expressed as endogenous thrombin potential [ETP]) increased beyond baseline values[8]. Observations from animal models showed that lower PCC doses seem equally effective in rivaroxaban associated bleeding[10-12].

The ability of PCC to reverse the anticoagulant effect of apixaban has not been assessed in humans. However, as both rivaroxaban and apixaban are direct oral factor Xa inhibitors, it is plausible that PCC also reverses the anticoagulant effect of apixaban. If so, the lowest effective dose may be preferred to minimize the risk of prothrombotic complications and to reduce costs.

The objective of our study was to assess the effect of a single administration of non-activated 4-factor PCC at the dosages of 37.5 and 25 IU/kg on the anticoagulant effect of a high therapeutic dose of apixaban in healthy subjects.

MATERIALS AND METHODS

Study design

The study was performed as a single-center, randomized, double-blind, placebo-controlled, crossover study at the Academic Medical Center in Amsterdam. After providing written informed consent, subjects received apixaban 10 mg twice daily from day -3 to day 0 to achieve steady-state drug levels (Figure 1). The final apixaban dose was taken on day 0 in the morning without food consumption. Three hours after the last dose, subjects received either a single bolus of PCC 37.5 IU/kg, PCC 25 IU/kg, or a similar volume of saline (placebo). Subjects were randomized for the order of reversal method. After a wash-out period of 15-30 days, all subjects returned to the same prescription of apixaban and crossed-over for the method of reversal. All subjects subsequently received all the reversal methods (Figure 1). After 20 days from the last visit, patients had the final study visit.

Oral apixaban (Eliquis, Bristol-Myers Squibb/Pfizer, Middlesex, United Kingdom) was given in a dose of 10 mg twice daily, the initial dose for the treatment of acute venous thromboembolism in the phase III AMPLIFY trial[13]. Adherence to study medication was evaluated at each infusion by checking the blisters. Apixaban was stored in the hospital pharmacy and provided at baseline.

Four-factor PCC (Cofact, Sanquin Blood Supply, Amsterdam, the Netherlands) was supplied by the manufacturer. A vial of 500 IU PCC contains 500 IU of factor IX, 280 to 700 IU of factor II, 140 to 400 IU of factor VII, 280 to 700 IU of factor X, 222 to 780 IU of protein C, 20 to 160 IU of protein S, and antithrombin, without any heparin added. PCC was reconstituted by an independent researcher in the hospital pharmacy according to manufacturer's instructions and subsequently administered by blinded investigators.

The study was conducted in accordance with the Declaration of Helsinki (60th version Fortaleza, October 2013) and according to guidelines for Good Clinical Practice. The study protocol was reviewed by the institutional review ethics board and registered at the Dutch Trial Registry (NTR3559). Subjects were recruited by public advertisement located at the Academic Medical Center (Amsterdam) and received a financial reimbursement coherent with the time spent on the study.

Study population

Subjects were included if they met the following inclusion criteria: male sex, age between 18 and 50 years, normal laboratory screening tests, no abnormalities at physical examination, normal vital signs, and ability to provide written informed consent. Laboratory screening included renal and hepatic function, hepatitis B, C, and HIV serology, complete blood cell count, prothrombin time, and activated partial thromboplastin time. Exclusion criteria were a history of allergic reaction to blood products, a personal or family history of coagulation disorders, participation in any other investigational interventional study within the past 30 days, medication use within 14 days before the start of the study, unhealthy use of alcohol, and any concomitant condition that, as judged by the investigator, would have placed the subject at increased risk of harm if he participated in the study. During the study, the consumption of nicotine, alcohol, and recreational drugs was recorded.

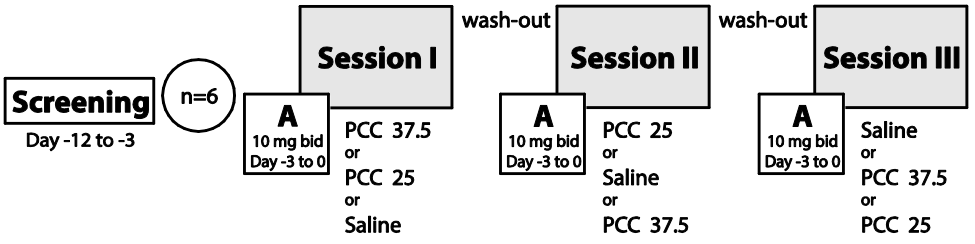

Figure 1. Study design

Infusion and blood withdrawal were performed at each session. Time points sampling: T=0, 15, 30 minutes, 1, 2, 4, 6, 24 hours after the end of PCC/saline infusion. Abbreviations: A, apixaban; PCC, prothrombin complex concentrate (expressed as number of IU/kg); bid, twice daily.

Study procedures, sample collection and analysis

Blood samples were collected at screening visit, at baseline visit on day -3, at day 0 immediately before the PCC/saline infusion and 15, 30, 60, 120, 240, and 360 minutes after the end of infusion, and at day 1 (T=24 hours, Figure 1). A peripheral venous catheter was placed to administer PCC or saline, and a second catheter was inserted for blood withdrawal on the infusion days. The lines for blood samples were flushed with saline and the first 5 mL blood discarded at each blood withdrawal. Tubes of 5 mL with 3.2% sodium citrate were used and samples were double spun within 2 hours from withdrawal to produce platelet-poor citrated plasma. Samples were then stored at -80°C until analysis.

The following assays were used for study outcomes: ETP as a measure of thrombin generation and prothrombin time. The Calibrated Automated Thrombogram assays the generation of thrombin in clotting plasma using a microtiter plate–reading fluorometer (Fluoroskan Ascent, ThermoLab Systems, Helsinki, Finland) and Thrombinoscope software (Thrombinoscope BV, Maastricht, the Netherlands). The software calculated the ETP, the total amount of thrombin formed in time that was normalized to pooled plasma obtained from more than 200 individuals; reference values: 65-146%. The assay was carried out as described by Hemker et al. with 5 pM of tissue factor as initiator of coagulation[14].

Prothrombin time was analyzed with automated coagulation analyzer (Behring Coagulation System XP), Thromborel S reagent, and protocols from the manufacturer (Siemens Healthcare Diagnostics, Marburg, Germany); reference values: 10.7-12.9 seconds. A calibrated anti-FXa activity assay (Berichrom Heparin, Siemens) was used to verify adequate apixaban uptake before PCC administration.

For exploratory purposes, additional analyses that were not part of the predefined outcomes were performed to study the effect of PCC on activated partial thromboplastin time (Pathromtin SL reagent, Siemens Healthcare Diagnostics), and prothrombinase-induced clotting time assay (Pentapharm, Basel, Switzerland): results are shown as Supplemental Material.

All laboratory analyses were performed by laboratory technicians, who were unaware of the type and the dosage of the reversal method.

Study outcomes

Pre-defined study outcomes were: 1) the effect of PCC administration on coagulation parameters, as reflected by difference in ETP (primary outcome) and prothrombin time between T=0 and T=15 minutes for each reversal method, and 2) the effect of two different PCC dosages compared to placebo over 24 hours.

Sample size calculation and statistical analysis

Our sample size calculation was based on the primary outcome from the study by Eerenberg et al.[8]: improvement of ETP between T=0 and T=15. In that study, baseline ETP values in the rivaroxaban group were 92±22% (mean±standard deviation [SD]). Administration of rivaroxaban decreased ETP to 51±21% and PCC increased ETP to 114±26%[8].

Using a paired Student t-test, an *alpha* of 0.05 and a power of 0.80, a sample size of 6 subjects in the present crossover study was calculated for the primary outcome, the effect of two PCC dosages on ETP, as reflected by differences between T=0 and T=15 for each PCC dosage.

Paired t-tests were used to assess the immediate effect (e.g. 15 minutes after infusion) of PCC on ETP and prothrombin time within each treatment group. The effect over 24 hours between treatment groups was analyzed with linear mixed models. Two-sided p-values <0.05 were considered to be statistically significant. Data analysis was performed using IBM SPSS v21.0 (IBM Corporation, Armonk NY, United States).

RESULTS

Six subjects were enrolled and all completed the study. Baseline characteristics were: mean age (± SD) of 26±7 years and weight (± SD) of 75±12 kg. All subjects had normal baseline screening tests. In the 37.5 IU/kg session, the PCC dosage ranged between 2,250 and 3,563 IU. In the 25 IU/kg session, the PCC dosage ranged between 1,500 and 2,375 IU.

Effects of apixaban on coagulation before infusion of prothrombin complex concentrate or placebo

After apixaban 10 mg twice daily from day -3 to day 0 (seven doses), subjects showed statistically significant modifications of both ETP and prothrombin time consistent across sessions (overall, p<0.01 for ETP and p<0.01 for prothrombin time). Mean anti-FXa-derived apixaban peak level at steady state was 330±113 ng/mL with no differences between groups, indicating sufficient oral uptake.

Immediate effect outcomes

Fifteen minutes after infusion of PCC 37.5 IU/kg, ETP increased from 41±11% to 56±23% (p=0.06). After infusion of PCC 25 IU/kg, ETP increased from 44±12% to 51±15% (p=0.03; Figure 2, Table 1).

Fifteen minutes after infusions of both PCC 37.5 IU/kg and PCC 25 IU/kg, the prothrombin time prolongation induced by apixaban normalized (13.6±0.8 seconds to

11.8±0.5 seconds: p<0.01 and 13.1±1.2 seconds to 12.0±0.8 seconds: p<0.01, respectively; Figure 2, Table 1).

No changes in both ETP and prothrombin time were observed over the first 15 minutes after infusion of saline in the placebo group (ETP: 45±10% to 44±11%, p=0.85; prothrombin time: 13.6±1.2 seconds to 13.4±0.8 seconds, p=0.44).

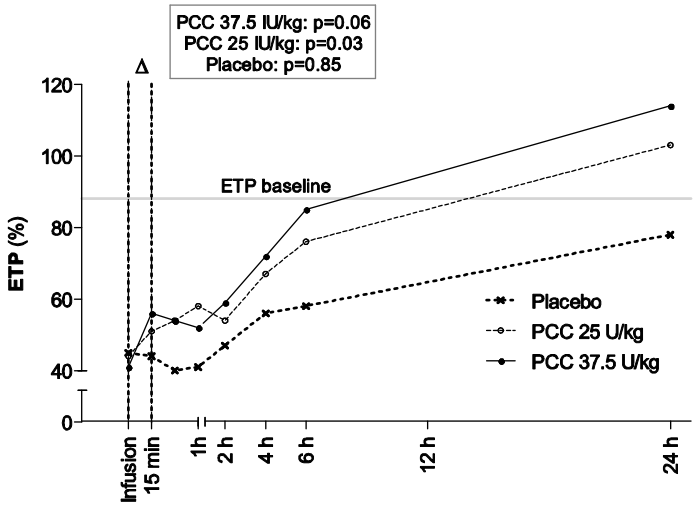

Figure 2. Effect of apixaban followed by two dosages of 4-factor prothrombin complex concentrate or placebo on the endogenous thrombin potential (%)

Immediate effect outcome: differences between T=0 and T=15 (15 minutes after infusion). ETP baseline: mean ETP value before apixaban administration. Abbreviations: PCC, prothrombin complex concentrate; ETP, endogenous thrombin potential.

Sustained effect outcomes

ETP levels significantly differed over time between PCC 37.5 IU/kg and placebo during 24 hours after infusion (p<0.01), but not between PCC 25 IU/kg and placebo (p=0.10; Figure 2, Table 1). Mean ETP values gradually reached pre-study baseline levels after about 6 hours from the PCC 37.5 IU/kg dosage infusion and after 24 hours from the PCC 25 IU/kg dosage infusion.

Prothrombin time values were significantly different over time between each PCC dosage and placebo during 24 hours after infusion (p<0.01; Figure 3, Table 1).

Adverse events

One subject had numbness in his calf several days after infusion of 37.5 IU/kg PCC. An ultrasound to rule out a deep venous thrombosis was not performed as he did not mention discomfort until the next visit, at which time the complaints had completely subsided. Another subject fell on his right hip one day before he started with the third session of apixaban. He developed a hematoma of 3 by 2 centimeter and took apixaban the next day. The hematoma did not expand after apixaban intake and resolved completely. During that session the subject received 25 IU/kg PCC as reversal.

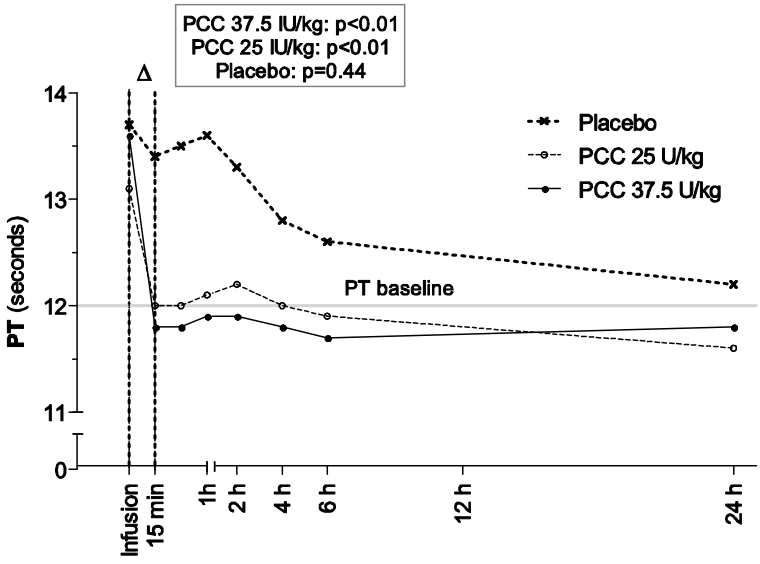

Figure 3. Effect of apixaban followed by two dosages of 4-factor prothrombin complex concentrate or placebo on the prothrombin time (seconds)

Immediate effect outcome: differences between T=0 minutes and T=15 (15 minutes after infusion). PT baseline: mean prothrombin time value before apixaban administration. Abbreviations: PT, prothrombin complex concentrate; PT, prothrombin time.

Table 1. Immediate and sustained effect outcomes

Endogenous thrombin potential (%)	PCC 37.5	PCC 25	Placebo
T=0	41±11	44±12	45±10
T=15 minutes	56±23 p=0.06	51±15 p=0.03	44±11 p=0.85
T=0-24 hours	vs placebo p<0.01	vs placebo p=0.10	-
Prothrombin time (seconds)	**PCC 37.5**	**PCC 25**	**Placebo**
T=0	13.6±0.8	13.1±1.2	13.6±1.2
T=15 minutes	11.8±0.5 p<0.01	12.0±0.8 p<0.01	13.4±0.8 p=0.44
T=0-24 hours	vs placebo p<0.01	vs placebo p<0.01	-

T=0 indicates the pre-PCC infusion timepoint. Abbreviations: PCC, prothrombin complex concentrate (expressed as IU/kg).

DISCUSSION

Our study suggests that 4-factor PCC in a dose of 25 IU/kg and 37.5 IU/kg is able to rapidly increase ETP, whereas prothrombin time normalized, indicating at least partial reversal of the anticoagulant effect of a high therapeutic dose of apixaban at peak concentrations. The effect of PCC was sustained for 24 hours after infusion.

The discrepancy in effects on the coagulation assays might reflect the different intrinsic characteristics of the ETP and prothrombin time assays. The prothrombin time assay is very sensitive to the factor VII levels in PCC whereas ETP is influenced by many coagulation factors. This is demonstrated by a similar 3- and 4-factor PCC study in rivaroxaban treated healthy subjects which observed a greater effect on the prothrombin time by 4-factor PCC compared with 3-factor PCC and a similar effect on ETP[9]. Due to the inconsistencies in coagulation outcomes (partial ETP and full prothrombin time reversal) the extent of reversal is unclear. However it is likely that PCC in a dose of 25 or 37.5 IU/kg at least partially reverses apixaban-induced coagulation changes. In a recently presented conference abstract, a high dose of 50 IU/kg PCC restored ETP to baseline values within 3.5 hours, faster than in our study, suggesting a more potent effect of a higher dose[15].

This is the first study that investigated the potential of 4-factor PCC to reverse the anticoagulant effect of apixaban in healthy human subjects. The enrolment of healthy, non-bleeding male subjects and the use of coagulation assays as a surrogate outcome limit the generalizability to bleeding patients. However, the strength of the present study is that it evaluated the effects on human hemostasis and that the dosages used are applicable to routine patient care. In animal models a 25-50 IU/kg dose of PCC was able to improve clinical outcome in dabigatran and rivaroxaban treated rats with intracranial hemorrhage even without correction of the coagulation tests[10,16]. The combined results of animal and healthy volunteer studies suggest that PCC could act as a reversal agent for factor Xa inhibitor associated bleeding.

Our study investigated one particular type of 4-factor PCC, e.g. Cofact. Differences in composition of other 4-factor PCCs may cause other effects on coagulation parameters. Nevertheless, it is unlikely that these small differences would lead to substantially different effects.

Ultimately, the goal of apixaban reversal in bleeding patients is to improve the outcome of bleeding. Although restoration of hemostasis would intuitively improve clinical outcome, the approval of PCC for reversal of vitamin K antagonist associated bleeding was based on studies with coagulation parameters as primary outcome. The beneficial effect of PCC on the clinical outcome of vitamin K antagonist associated bleeding has never been firmly established[5,17-22].

The European Heart Rhythm Association guidance document suggests PCC or activated PCC as potential reversal agents in patients with direct oral anticoagulants-associated bleeding[23]. Our study strengthens the suggestion to use PCC as a reversal agent until other specific antidotes become available[6,7]. Moreover, the risk of thrombotic complications of PCC is probably smaller than of activated factor concentrates such as activated PCC or recombinant factor VIIa[24,25].

The results of our study indicate that 4-factor PCC at dosages of 37.5 and 25 IU/kg (at least partially) restores normal hemostasis in apixaban-treated healthy subjects. This suggests that PCC is a suitable reversal agent for apixaban-treated patients that present with bleeding or the need for emergency invasive procedures. However the effect of PCC on clinical outcome should be confirmed in acute real-world patients.

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SUPPLEMENTARY MATERIAL

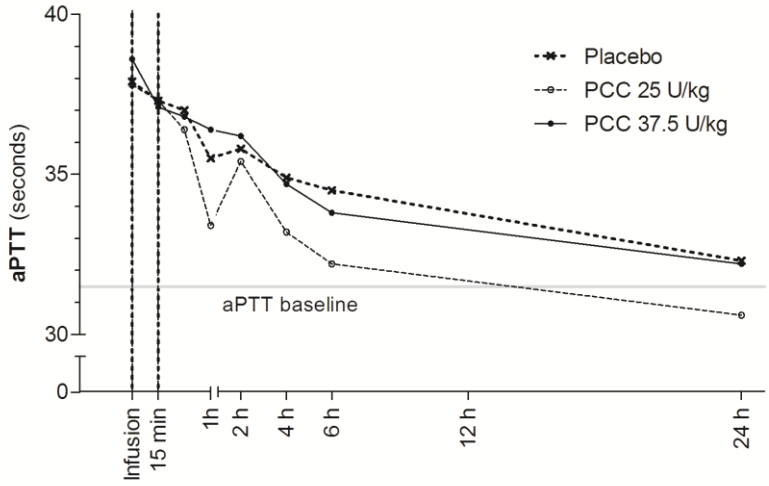

Figure A. Effect of apixaban followed by two different dosages of four-factor prothrombin complex concentrate or placebo on activated partial thromboplastin time (seconds)

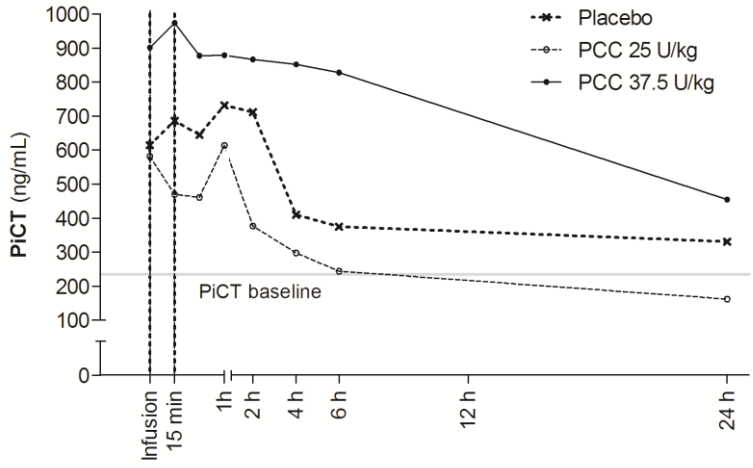

Figure B. Effect of apixaban followed by two different dosages of four-factor prothrombin complex concentrate or placebo on prothrombinase-induced clotting time-derived apixaban concentrations (ng/mL)

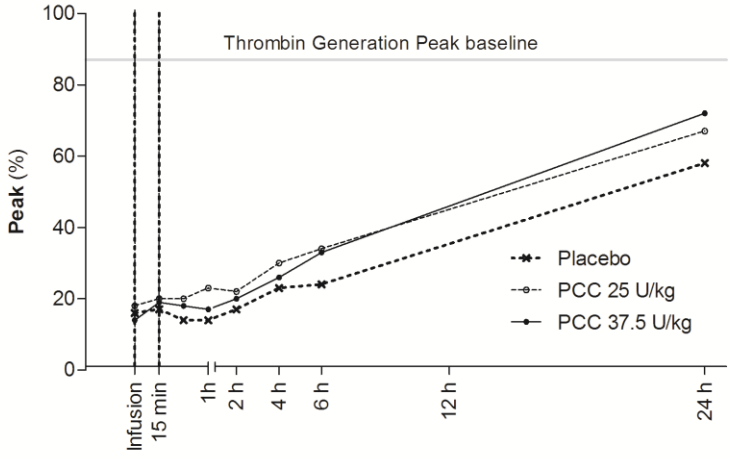

Figure C. Effect of apixaban followed by two different dosages of four-factor prothrombin complex concentrate or placebo on thrombin generation peak (%)

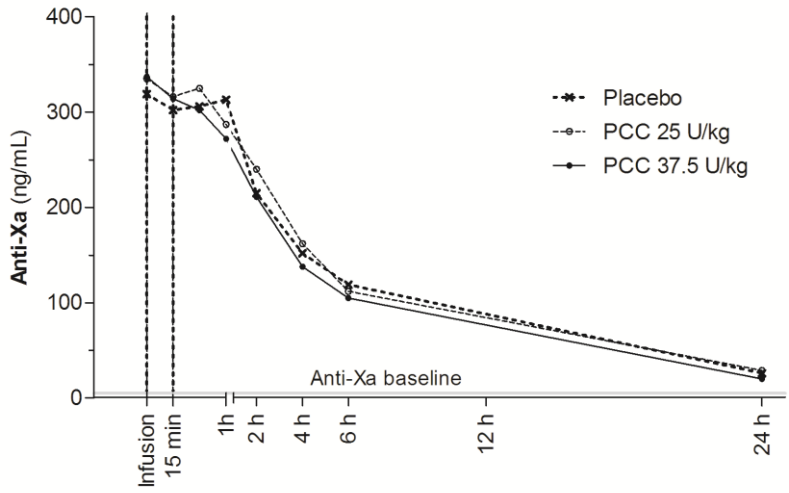

Figure D. Effect of apixaban followed by two different dosages of four-factor prothrombin complex concentrate or placebo on anti-fXa-derived apixaban concentrations (ng/mL)

4

Safety of prothrombin complex concentrate in healthy subjects

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INTRODUCTION

Prothrombin complex concentrate (PCC) is recommended as first-line treatment for vitamin K antagonist-associated major bleeding. Different PCC formulations contain non-activated vitamin K-dependent coagulation factors plus varying amount of coagulation inhibitors. Three-factor PCC contains factors II, IX, and X, but little or no factor VII, which is conversely contained in significant amounts into 4-factor PCC. PCC is effective in reversing vitamin K antagonist activity as it instantly replaces the deficient zymogens in vitamin K antagonist-anticoagulated patients, restoring the physiological hemostatic response to injury.

However, the use of PCC in emergency situations has been associated with thrombotic events[1,2]. It is unclear whether the risk of thrombosis is caused by the PCC itself or by the concomitant withdrawal of anticoagulants in patients with an underlying condition that requires anticoagulation. A pooled analysis of two recent randomized controlled trials enrolling patients with acute vitamin K antagonist-associated bleeding or scheduled with urgent invasive procedures found that 2.6% of 191 patients enrolled in the 4-factor PCC arm had a thrombotic event within 7 days from infusion, rising to 7.3% at 60 days[2]. Rates were similar in the comparator group receiving fresh frozen plasma[2]. A prior meta-analysis of 27 studies evaluated the safety of reversal with PCC in 1,032 patients with vitamin K antagonist-associated bleeding or the need for urgent invasive procedure: 12 events occurred within 4 days from PCC infusion (1.4%; 95% Confidence Interval [95%CI]: 0.8-2.1)[1]. The risk of thrombosis was numerically higher with 4-factor PCC than with 3-factor PCC (1.8%; 95%CI: 1.0-3.0 *vs* 0.7%; 95%CI: 0.0-2.4)[1].

We aimed to systematically review the literature to estimate the proportion of thrombotic events after PCC infusion in healthy subjects since these individuals do not have any underlying condition requiring anticoagulation and, therefore, thrombotic events would be caused by the PCC itself.

METHODS

MEDLINE (via PubMed) and EMBASE (via Ovid) were searched up to October 25th 2015 without language restriction (search strategy available upon request). The search was supplemented by reviewing additional articles from the reference lists of retrieved studies, and by hand-searching.

Prospective interventional studies enrolling more than 5 adult healthy subjects who received PCC were considered eligible. Thrombotic events were defined according to criteria adopted by the authors of the prior meta-analysis[1] and included deep vein thrombosis or pulmonary embolism, myocardial infarction or acute coronary syndrome, ischemic stroke, transient ischemic attack or arterial thrombosis of a limb. Studies were included in the meta-analysis if safety outcomes regarding potential thrombotic adverse events were reported and if the mean follow-up after PCC infusions was longer than 7 days, as the infused coagulation factors have half-lives ranging from 6 to 72 hours. A two-step selection process was applied in parallel (CP, AT) and data regarding study population, intervention, outcomes and type of study were then abstracted (SB, CP, AT). The weighted mean proportion of thromboembolic events (number of thrombotic events divided per number of PCC infusions) is presented together with Wilson score 95%CI.

RESULTS AND DISCUSSION

Our literature search identified 5,142 studies (1,336 from MEDLINE and 3,806 from EMBASE). Fourteen studies were evaluated in full text for eligibility and 8 were included (Table 1)[3-10], of which one is available as conference abstract only[9]. The main goal of these studies was testing the hemostatic changes induced by PCC infusion in healthy subjects previously exposed[3-7,9,10] or not[8] to oral anticoagulants. Safety outcomes on thrombotic complications after PCC administration were recorded. We identified a total of 234 infusions in 194 subjects. No thromboembolic events were documented (0.0%; 95%CI: 0.0-1.6). Due to the lack of events, we did not investigate any subgroup of subjects.

Overall, PCC was able to increase thrombin generation[3-7,9,10], but seemed to be associated with a very low risk of thrombotic complications in healthy subjects. The upper limit of the 95%CI still exceeds the point estimate of the prior meta-analysis (1.4%; 95%CI: 0.8-2.1)[1], but is lower than the 2.6% 7-day rate observed in two randomized controlled trials studying 4-factor PCC[2].

Table 1. Characteristics of the included studies evaluating prothrombin complex concentrate infusion in healthy subjects

Study	n	Age; male sex	Anticoagulant before PCC infusion	PCC type	n infus.	Dose
Barco[4]	6	22 (20-50); 6 (100)	Rivaroxaban 15 mg bid for 2.5 days	4F Cofact	6	37.5
					6	25
Brown[3]	24	31 (6.6); 20 (83)	Edoxaban 180 mg single-dose	3F Bebulin	11	50
					10	25
			Edoxaban 60 mg single-dose		12	50
					12	25
Cheung[5]	6	26 (7); 6 (100)	Apixaban 10 mg bid for 3.5 days	4F Cofact	6	37.5
					6	25
Perlstein[9]	15	33 (7); n.a.	Apixaban 10 mg bid for 3.5 days	4F Cofact	15	50
				4F Beriplex	15	
Zahir[10]	93	30.6; 64 (68.8)	Edoxaban 60 mg single-dose	4F Beriplex	27	50
					28	25
					30	10
Levi[7]	23	42 (11.5); 5 (42)	Rivaroxaban 20 mg bid for 4.5 days	3F Profilnine	12	50
		43.3 (8.3); 7 (64)		4F Beriplex	11	
Eerenberg[6]	12	24 (4); 12 (100)	Rivaroxaban 20 mg bid for 2.5 days	4F Cofact	6	50
			Dabigatran 150 mg bid for 2.5 days		6	
Ostermann[8]	15	41 (13); 8 (53)	None	4F Beriplex	15	50
Total	194	22-43 (means); 128 (72)	n=41 (Rivaroxaban) n=42 (Apixaban) n=6 (Dabigatran) n=130 (Edoxaban) n=15 (none)	n=177 (4F) n=57 (3F)	n=234	50: n=130

Age expressed as mean (standard deviation or range). Proportion of males expressed as n (%). Abbreviations: PCC, prothrombin complex concentrate; 4F, 4-factor PCC; 3F, 3-factor PCC; IU, international unit; n.a., not available; N Infus., number of infusions; bid, twice daily.

In our analysis, subjects were relatively young, with no personal (and often familiar) history of thrombosis, and no relevant co-morbidities. Patients on anticoagulants, apart from having an underlying condition for which the anticoagulants are prescribed, are usually older, have more co-morbidities leading to a higher baseline risk of thrombosis and present transient risk factors associated with acute bleeding or invasive procedures. On the other hand, the PCC dosage used in most of these

studies is higher than the dosage required for reversal of vitamin K antagonists (median 25 IU/kg, range 15.5-50.5 IU/kg), although the thrombotic risk after PCC might not be proportional to PCC dosage as one would expect[2].

PCC administration in healthy subjects leads to a negligible risk of thrombotic events and appears to be safe for research purposes. This suggests that the increased thrombosis risk after emergency PCC infusion in acute patients is due to the withdrawal of anticoagulation and underlying prothrombotic conditions. Further studies need to dissect whether specific factors are boosting the potential PCC-associated thrombogenicity.

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PART II

Parenteral nutrition

5

Anticoagulants for the prevention and treatment of catheter-related thrombosis in adults and children on parenteral nutrition: systematic review and critical appraisal

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Submitted

ABSTRACT

Patients on parenteral nutrition require a central venous access and are at risk for catheter-related thrombosis, pulmonary embolism and vena cava syndrome. Parenteral nutrition guidelines suggest anticoagulation for the primary prevention of catheter-related thrombosis during long-term parenteral nutrition.

The objective of the present work is to systematically review the efficacy, safety and feasibility of anticoagulant use for preventing and treating catheter-related thrombosis during parenteral nutrition.

We searched for interventional and observational studies on adults and children receiving systemic anticoagulants during either short- or long-term parenteral nutrition delivered via central venous access. Primary outcomes were: objectively-confirmed catheter-related thrombosis, pulmonary embolism and bleeding. Secondary outcomes were heparin-induced thrombocytopenia, prevalence of anticoagulation, and quality of INR management in vitamin K antagonist-treated patients.

We identified 1,199 studies, of which 23 were included. Seven interventional studies of short-term parenteral nutrition (adult population, n=5) were classified as low-quality: in those, intravenous unfractionated heparin did not prevent catheter-related thrombosis if compared to saline. No interventional studies were conducted in patients on long-term parenteral nutrition. Observational data were sparse, rarely focusing on anticoagulation, and overall of low-quality. The reported use of anti-coagulants was comprised between 22% and 66% in recent multicenter cohorts.

The amount and quality of data in this area are very suboptimal: most studies are outdated and involved heterogeneous populations. Currently, there is insufficient evidence to allow conclusions regarding the efficacy and safety of anticoagulants in this setting: therefore, recommending their routine use for primary prevention does not appear justified.

INTRODUCTION

Parenteral nutrition is indicated for patients with intestinal failure unable to maintain their nutritional balance by oral or enteral intake. Usually requiring central venous access, these patients are at risk of catheter-related thrombosis, pulmonary embolism, and vena cava syndrome. Moreover, recurrent catheter-related thrombosis can causes progressive loss of vascular access, leading to inability to continue parenteral nutrition and leaving intestinal transplantation as a last resort solution.

Patients on parenteral nutrition represent a highly heterogeneous group accounting for different thrombotic risk factors, such as cancer, recurrent bacterial infections, autoimmune and inflammatory bowel diseases, and often starting on parenteral nutrition due to acute splanchnic thrombosis. They also share parenteral nutrition-specific risk factors, including hyperosmolar formulation-mediated endothelial injury, need of a long-term venous access, and development of renal and hepatic failure[1,2].

National and international parenteral nutrition guidelines suggest or recommend various anticoagulant regimens for the prevention and treatment of catheter-related thrombosis during long-term parenteral nutrition[3-9]. Overall, recommendations are inconsistent with those presented in the guidelines involving other populations of adult patients with a central venous access (Table 1)[10-13].

The aim of this systematic review is to evaluate the efficacy and safety of anticoagulants for preventing and managing catheter-related thromboembolic complications in patients on both short- and long-term parenteral nutrition administered via a central access. Prevalence and quality of anticoagulant treatments, as well as heparin-induced thrombocytopenia, are evaluated as secondary outcomes.

Table 1. Overview of the international guidelines on catheter-related thrombosis prevention and treatment

PN guidelines	Primary CRT prevention	Treatment of CRT and prevention of recurrent CRT	
DGEM (2009)[6]	Low-dose oral prophylactic anticoagulant during long-term PN (grade B)	Urokinase/tPA	-
ESPEN (2009)[7]	Once-daily LMWH 100 IU/kg in high-risk patients on long-term PNa (grade C)	Removal of the catheter if infected, malpositioned or obstructed (grade B); local/systemic urokinase/tPA for acute symptomatic CRT within 24 h from symptoms onset (grade C)	Long-term LMWH or LMWH followed by VKA for subacute symptomatic CRT (grade C); LMWHs are preferred (grade C)
AuSPEN (2008)[5]	-	Low-dose tPA within 3-4 days of symptoms onset. Stenting of the partially occluded superior vena cava to enable reinsertion of a CVC (consensus)	In case of CRT resolution, the catheter can remain in place and anticoagulation therapy considered. Long-term warfarin is preferred (consensus)
SINPE (2002)	Low-dose VKA or LMWH during long-term PNb (grade C)	-	-
ASPEN (2002)[3]	Low-dose anticoagulant during long-term PNb (grade B)	-	-
Other guidelines	Primary CRT prevention	Treatment of CRT and prevention of recurrent CRT	
ISTH (2014) [10]	No routine CRT prophylaxis/heparin flushes (adult cancer patients)	Anticoagulation with LMWH and no catheter removal in cancer patients; removal if infected or malpositioned; anticoagulation for incidental CRT; anticoagulation over thrombolysis (adult cancer patients)	3-6 months of anticoagulation; LMWH over warfarin; long-term anticoagulation in patients with persistent need of central venous access (adult cancer patients)
ACCP (2012) [11]	No routine CRT prophylaxis (grade 2C)	No catheter removal if it is functional (grade 2C); if proximal veins are involved, anticoagulation for 3 months (adults; grade 2C)	After catheter removal, anticoagulation for 3 months (adults; grade 2C); if no catheter removal, long-term anticoagulation (adults; grade 1C)
ACCP (2012) [47 50]	UFH infusion at 0.5 IU/kg for catheter patency over no prophylaxis in neonates (grade 1A)	Catheter removal; either anticoagulation or radiologic monitoring; LMWH or UFH followed-by LMWH for 6 weeks-3 months (neonates and children; grade 2C)	If catheter is still in place on completion of therapeutic anticoagulation, prophylactic anticoagulation until catheter is removed (neonates and children; grade 2C)
GCPG (2013) [13]	No routine CRT prophylaxis in cancer patients	Anticoagulation for 3 months, no catheter removal if it is functional, LMWH preferred (adult cancer patients)	No recommendations for the length of anticoagulation (adult cancer patients)

[a]Patients with cancer, chronic inflammatory disease, or family/personal history of idiopathic venous thrombosis. bLong-term PN, no contraindication to receive anticoagulants.
Abbreviations: DGEM, Deutsche Gesellschaft für Ernährungsmedizin; ESPEN, European Society for Clinical Nutrition and Metabolism; AuSPEN, Australasian Society for Parenteral and Enteral Nutrition; GCPG, Good Clinical Practices Guidelines; SINPE, Società Italiana di Nutrizione Artificiale e Metabolismo; ASPEN, American Society for Parenteral and Enteral Nutrition; LMWH, low-molecular weigth heparin; CRT, catheter-related thrombosis; tPA, tissue plasminogen activator; VKA, vitamin K antagonist; CVC, central venous catheter; IU, units.

MATERIALS AND METHODS

Study identification

We systematically searched MEDLINE (January 1966 to November 23, 2015; *via* PubMed) and EMBASE (January 1980 to November 23, 2015; *via* OVID). We developed the search strategy without language restrictions after having selected seminal articles for relevant keywords or assigned MeSH subjects (strategy available as Supplementary Material). We supplemented this search by manually reviewing reference lists of retrieved articles, nutrition journals databases, relevant review papers, guidelines/guidance documents, and grey literature. Authors were contacted if there was ambiguity about original data. No review protocol was registered.

Study selection and outcome definitions

Two reviewers (SB and JJA) performed study selection in duplicate and calculated the inter-observer agreement (Cohen's k): disagreements were solved by a third reviewer (MC). We included peer-reviewed papers if they met the following criteria:

- Population: in- and outpatients (n≥5) on parenteral nutrition requiring a central vascular access. No age restriction was applied. If patients on parenteral nutrition represented a subgroup of a bigger cohort, we excluded the paper if insufficient data were provided.
- Intervention: use of systemic anticoagulant regimens at any specified dose for primary prevention, secondary prevention or treatment of catheter-related thrombosis compared to lower dosage of anticoagulant or no anticoagulant. Heparin locks and heparin-bonded catheters were not considered systemic anticoagulation.
- Primary outcomes (at least one of the following): a) efficacy outcomes (rates of objectively confirmed first or recurrent catheter-related thrombosis and/or pulmonary embolism), and b) safety outcome (rate of major or clinically significant bleeding), expressed as cumulative incidence and/or incidence rate (number of events/patient-time). Accepted diagnostic tests for catheter-related thrombosis included: Doppler or compression ultrasonography, venography, visual central venous catheter inspection after removal in symptomatic patients. Accepted diagnostic tests for pulmonary embolism included: computer

tomographic angiography, ventilation/perfusion lung scan, or autopsy findings. Bleeding events were classified according with the definition in the original paper.

- Secondary outcomes: prevalence of anticoagulant administration for any indications reported in either cross-sectional studies or observational longitudinal studies at baseline; pharmacokinetics and –dynamics analyses, quality of anticoagulant treatment (INR time-in-therapeutic-range in patients receiving vitamin K antagonists); rate of heparin-induced thrombocytopenia.
- Study design: no limitations.

Two reviewers (SB and JJA) retrieved the following data in duplicate: author identification, country, year of publication, patient baseline demographics, study setting, study design, catheter type, sample size, pharmacological regimen, length of follow-up, rate of primary outcomes, and presence of any of the secondary outcomes.

Assessment of study quality and risk of bias

Assessment of the risk of bias of interventional studies and of the validity of observational studies was independently performed by two reviewers (SB and JJA).

The scheme recommended by the Cochrane Collaboration for Cochrane systematic reviews on interventions served for interventional studies. Assessment comprised a description and a judgment in a “Risk of bias” table for entry addressing specific features of the study.

For observational studies, the quality assessment derived from the Newcastle-Ottawa Scale (www.ohri.ca/programs/clinical_epidemiology/oxford.asp): a 'star system' aims to evaluate the selection and comparability of study groups, and the ascertainment of either the exposure or outcome of interest. Studies were considered of high quality standard if they received at least 7 stars out of 9. No quality assessment was performed for cross-sectional studies as they were considered only for prevalence of anticoagulant administration.

Statistical methods

We calculated odds ratios and 95% confidence intervals (95%CI) for interventional studies using random-effect Mantel-Haenszel methods. We planned to pool data across interventional studies of adequate quality using the random-effect models due to identifiable reasons for heterogeneity being likely to occur. Heterogeneity of the

results among studies was tested with the quantity I^2, which describes the percentage of total variation across studies that is due to heterogeneity rather than chance (I^2 values >50% indicate a substantial level of heterogeneity).

We calculated crude incidence rates and 95%CI for observational studies under the Poisson assumptions on the basis of the available data, if not reported in the original papers: they equal the total number of events divided by the population-time-at-risk (patient-year) or catheter life-time-at-risk (catheter-year).

Review Manager 5.0 (Cochrane Collaboration, the Netherlands), StatsDirect 2.7.8 (StatsDirect Ltd, United Kingdom) and Confidence Interval Analysis 2.0 (Trevor Bryant, University of Southampton, United Kingdom) were used for analyses.

Current ongoing studies and grey literature

We made a systematic attempt to search for current ongoing studies on the topic and for the grey literature (abstracts, theses, conferences), exploring the following sites on November 2015: www.clinicaltrials.gov, www.greylit.org, and www.google.com (first 500 results; keywords: “parenteral nutrition” and “anticoagulant”).

RESULTS

Study identification and selection

We identified 1,199 studies with our literature search strategy: 392 from MEDLINE and 807 from EMBASE. After first selection on the basis of pre-specified criteria, 31 papers were retrieved in full text and forty-four additional studies were obtained from cross-references and relevant reviews/guidelines. After full text evaluation, 23 studies were included and 52 excluded ((Figure 1). The inter-observer agreement (Cohen’s k) for the study selection showed an optimal agreement between authors with regard to interventional studies (k=1.00) and a substantial agreement for observational studies (k=0.80).

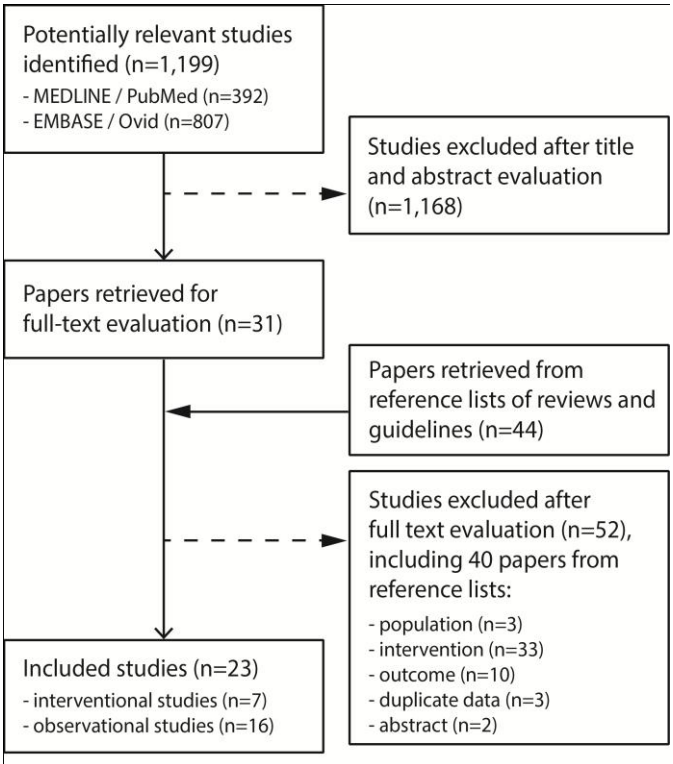


Figure 1. Overview of the included and excluded papers

Study characteristics

We classified studies into interventional studies (n=7, Table 2)[14-20] and observational studies (n=16, Table A, Supplementary material)[21-36]. Study populations consisted of adult patients in 5 interventional[14-16,18,19] and 10 observational studies[22-24,26,27,30,31,33-35], while 2 observational studies had mixed-age populations[25,28]. The size of interventional study ranged from 34 to 49 adults (n total=206) and from 68 to 239 pediatric patients (n total=307). Observational study sizes ranged from 8 to 1010 patients.

At least one primary efficacy or safety outcome was reported in all interventional studies[14-20] and in 9 out of 16 observational studies[22,24-28,32,35,36].

The types of central venous access varied largely among studies consistent with the time when the studies were conducted. These are summarized in Tables 2 and A (Supplementary Material), as well as the length of follow-ups and patients' main

baseline characteristics. Patients´ indications for parenteral nutrition in each study are presented in Table B (Supplementary Material).

Risk of bias and study quality assessment

All the interventional studies were classified as low quality due to their high or unclear risk of bias[14-20] (Figures 2a and 2b): therefore, no meta-analysis of results was performed. Importantly, the external validity of all the older studies is limited, as some of the described protocols of catheter, anticoagulant and parenteral nutrition management are outdated[14-16,18,19,22,25,27,28,32,35].

Only one prospective observational study in children[29] was classified as a high-quality study according to the Newcastle-Ottawa Scale (7 or more stars) and evaluated one of the secondary outcomes (quality of anticoagulant treatment). Due to their cross-sectional design, the Newcastle-Ottawa Scale could not be applied to 3 studies[21,31,34].

Primary outcomes

Catheter-related thrombosis

Five interventional studies in adults and two in neonates randomized patients on short-term parenteral nutrition to receive either intravenous prophylactic intravenous unfractionated heparin or no anticoagulant[14-20]. None of the studies demonstrated a statistical difference between groups (Table 3). All studies were of low quality and therefore no further meta-analysis was performed.

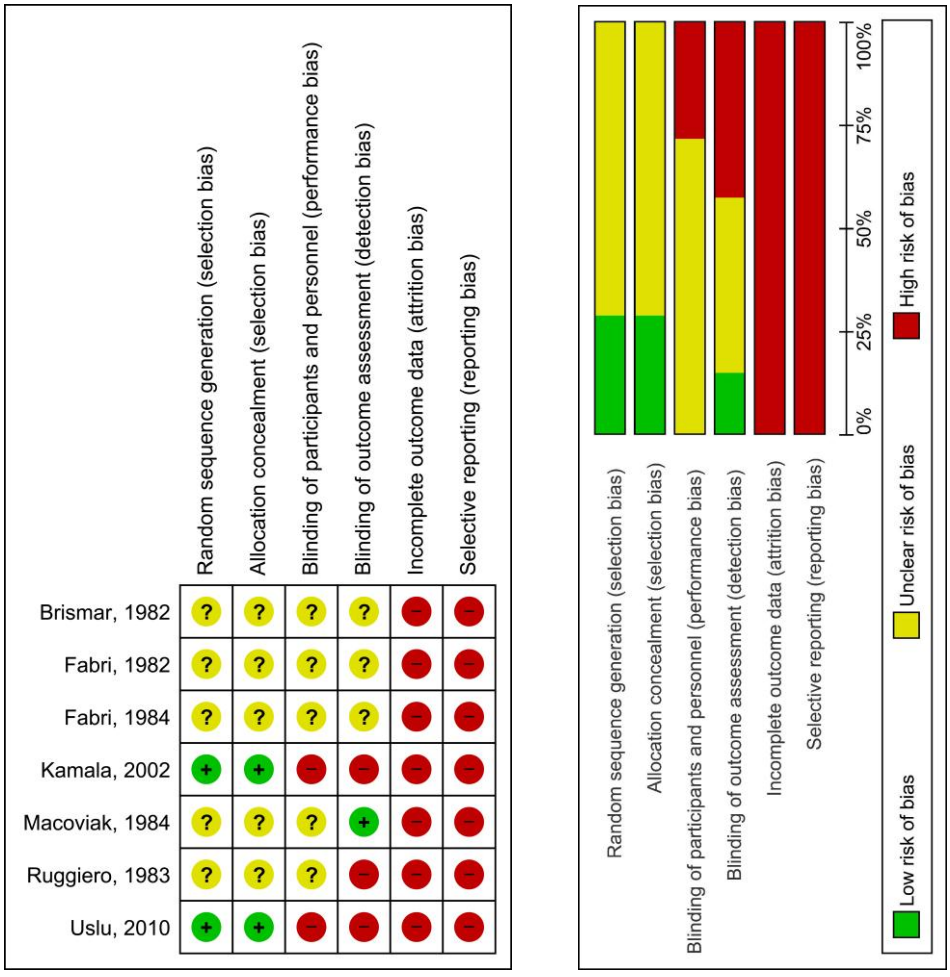
For most of the observational studies, the strategies of warfarin or parenteral nutrition management and the definitions of study outcomes are not comparable to the current standards of care, or the study design presents major limitations: therefore, no useful information can be retrieved from them.

Only one observational study in children[36] adopted a (partly) prospective design and aimed to compare the effect of nadroparin or acenocumarol treatment *vs* no treatment for the prevention of catheter-related thrombosis (n=32). Anticoagulation was associated to a decrease of catheter-related thrombosis rate from 0.12 events/patient-year (95%CI: 0.06-0.23) to 0.03 events/patient-year (95%CI: 0.001-0.14) over time, improving the cumulative thrombosis-free survival at 5 years (93% *vs* 48%, p=0.047).

Table 2. Baseline characteristics of interventional studies in short-term parenteral nutrition

Study	Population, n	Venous access	Intervention	Comparator	Outcome	Follow-up
Fabri 1982[15]	24 (adults)	Subclavian CVC	UFH 3,000 IU/L PN solution	No UFH	(a)symptomatic CRT	Not available
Brismar 1982[14]	49 (adults)	External jugular CVC	UFH 5,000 IU/6 hours	No UFH	(a)symptomatic CRT	<8 weeks
Ruggiero 1983[19]	34 (adults)	Subclavian CVC	UFH 1,000 IU/L PN solution	No UFH	(a)symptomatic CRT	<8 weeks
Fabri 1984[16]	40 (adults)	Subclavian CVC	UFH 3,000 IU/L PN solution	No UFH	(a)symptomatic CRT	<8 weeks
Macoviak 1984[18]	37 (adults)	Subclavian CVC	UFH 1 IU/mL PN solution	No UFH	(a)symptomatic CRT	<8 weeks
Kamala 2002[17]	68 (neonates)	PICC	UFH 1 IU/mL PN solution	No UFH	(a)symptomatic CRT; IVH	<6 weeks
Uslu 2010[20]	239 (neonates)	PICC	UFH 0.5 IU/kg/h PN solution	No UFH	(a)symptomatic CRT; IVH	<4 weeks

All the studies evaluated UFH for primary prevention of asymptomatic CRT. Abbreviations: PICC, peripherally inserted central venous catheter; PN, parenteral nutrition; RCT, randomized controlled trial; aPTT, activate partial thromboplastin time; IVH, intraventricular hemorrhage; CVC, central venous catheter; CRT, central venous catheter thrombosis; UFH, unfractionated heparin sodium; DIC, disseminated intravascular coagulation; AC, anticoagulant; ICU, intensive care unit.



Figures 2a and 2b. Summary of risk of bias in interventional studies

Table 3. Primary outcomes in interventional studies including patients on short-term parenteral nutrition, n/N (%)

Study	CRT (UFH group)	CRT (saline)	Odds Ratio (95% CI)	Bleeding* (UFH group)	Bleeding* (saline)	Odds Ratio (95% CI)
Neonatal Populations						
Kamala[17]	5/36 (13.9%)	7/32 (21.9%)	0.58 (0.16-2.04)	4/23 (17.4%)	4/20 (20%)	0.84 (0.18-3.92)
Uslu[20]	2/118 (1.7%)	5/121 (4.1%)	0.40 (0.08-2.10)	21/118 (17.8%)	23/121 (19%)	0.92 (0.48-1.78)
Adult Populations						
Brismar[14]	0/23 (0.0%)	1/26 (3.8%)	0.36 (0.01-9.32)	-	-	-
Fabri[15]	2/24 (8.3%)	7/22 (31.8%)	0.19 (0.04-1.17)	-	-	-
Ruggiero[19]	0/20 (0.0%)	0/20 (0.0%)	Not estimable	-	-	-
Fabri[16]	0/17 (0.0%)	0/17 (0.0%)	Not estimable	-	-	-
Macoviak[18]	2/17 (11.8%)	1/20 (5%)	2.53 (0.21-30.68)	-	-	-

*Bleeding: developing/worsening of intraventricular hemorrhage in neonates admitted at the intensive care unit. Abbreviations: CRT, central venous catheter thrombosis; PE, pulmonary embolism; UFH, unfractionated heparin; 95% CI, 95% confidence interval.

Pulmonary embolism

In one retrospective longitudinal study[25], 6 symptomatic (17.6%) and 4 asymptomatic pulmonary embolism events (11.8%) were recorded in 32 children on long-term parenteral nutrition. The authors concluded that unfractionated heparin did not prevent pulmonary embolism: however, data analysis is not reported in the original paper and therefore no conclusions can be drawn[25].

Bleeding

Interventional studies in adults did not report bleeding rates[14-16,18,19]. No difference in developing/worsening of intraventricular hemorrhage was found in two randomized trials comparing intravenous prophylactic unfractionated heparin to saline in neonates (Table 2)[17,20].

No bleedings were reported in an observational pediatric study (0 events/39 patient-years in anticoagulant group *vs* 0/74.2 patient-years in controls)[36] and in a retrospective study including adults with acquired immunodeficiency syndrome (0 events/5.2 patient-years in warfarin group *vs* 0 events/19.4 patient-years in controls)[26].

Secondary outcomes

Prevalence of anticoagulant administration

The prevalence of patients receiving systemic anticoagulation for any indication was reported in 15 observational studies (Table 4)[21-28,30-36]. The proportion of patients receiving systemic anticoagulants varied considerably among studies, ranging from 1% to 100% in cohort studies, and from 22% to 42% in cross-sectional studies. A recent multicenter prospective study published in 2015 showed that 41 out of 62 patients on home parenteral nutrition (66%) were receiving various anticoagulant regimens at the start of parenteral nutrition (low-dose vitamin K antagonist 45%; INR 2.0-2.5-adjusted vitamin K antagonist 8%; low-molecular-weight heparin 13%) with differences among countries[24].

Table 4. Proportion of patients receiving an anticoagulant treatment

Study	Population (n)	Type of anticoagulant	Proportion (%)
Ladefoeged, 1981[28]	Children and adults (70)	UFH, P	100
Imperial, 1982[27]	Adults (1,010)	UFH	98
Bern, 1986[a,22]	Adults (23)	UFH flushes	100
		W	56
Schmidt, 1989[32]	Children and adults (35)	UFH	100
Dollery, 1994[25]	Children (34)	UFH	25
Veerabagu, 1995[35]	Adults (90)	UFH	100
		W	49
Andrew, 1995[b,21]	Children (12)	W	42
Duerksen, 1996[26]	Adults (47)	W	19
Cowl, 2000[23]	Adults (102)	UFH flushes	100
		Other systemic AC	1
Van Gossum, 2001[b,34]	Adults (228)	Therapeutic-dose AC	26
		Prophylactic-dose AC	12
Vegting, 2012[36]	Children (32)	LMWH or acenocumarol	25
Puiggrós, 2012[b],31	Adults (49)	UFH flushes	69
		Systemic AC	22
Olthof, 2014[c],[30]	Adults (212)	Systemic AC	53-55
Tourè, 2014[c],[33]	Adults (196)	VKA or LMWH	25-26
Cuerda, 2015[24]	Adults (62)	Low-dose VKA	45
		VKA (INR target 2.0-2.5)	8
		LMWH	13
		Total	66

[a]Patients receiving other AC regimens were excluded. [b]Cross-sectional. [C]Prevalence of AC administration per catheter.
Abbreviations: UFH, unfractionated heparin; P, phenprocoumon; W, warfarin; AC, anticoagulant, LMWH, low-molecular-weight heparin.

Pharmacological studies and quality of anticoagulant treatment

A prospective observational study in 8 children on home parenteral nutrition[29] analyzed the mean dose of warfarin required to achieve a target INR of either 2.0 to 3.0 (0.33 mg/kg/day; range: 0.125-0.65 mg/kg/day) or 1.3 to 2.0 (0.26 mg/kg/day; range: 0.16-0.37 mg/kg/day). The percentage of INR tests achieving the target therapeutic range was 51.9% in the first group (INR target: 2.0-3.0) and 69.4% in the second group (INR target: 1.3-2.0). The median duration of treatment was 817 days (range: 186-1,025 days) and INR was monitored every 6.6 days as an average.

Heparin-induced thrombocytopenia

No studies evaluated the proportion of patients developing heparin-induced thrombocytopenia during parenteral nutrition. To our knowledge, only 5 cases of medium-high probability heparin-induced thrombocytopenia have been described[37-41].

Current ongoing studies and grey literature

The grey literature did not provide further data and we did not identify protocols of ongoing interventional studies on anticoagulants. A crossover phase I study has just concluded the enrolment of patients with short bowel syndrome treated with at least 3 consecutive months of parenteral nutrition and exposed to the direct oral anticoagulants dabigatran etexilate and rivaroxaban (NTR4192).

DISCUSSION

Our results indicate that a crucial gap of knowledge exists with regard to efficacy, safety and feasibility of anticoagulant treatment for catheter-related thrombosis prevention in adults and children on parenteral nutrition. Two recent interventional studies indicate a non-significant protective effect of prophylactic unfractionated heparin in neonates on short-term parenteral nutrition[17,20], but the low quality of these studies precludes any objective interpretation (Figures 2a and 2b). Nonetheless, since up to 22-66% of patients on parenteral nutrition still receive systemic anticoagulation, the subject of the present review represents an urgent and relevant issue to address[24,30,31,33,34].

The dramatic improvement in parenteral nutrition management observed over the past 20 years involved catheter size, type and technique for placement,

characteristics of parenteral nutrition solutions, and strategies for preventing infectious complications. These new approaches have likely represented the main risk reducing factors for catheter-related thrombosis, contributing to decrease its rate of almost two orders of magnitude (from about 0.2-1.0 events of catheter-related thrombosis per patient-year in 1980-1995[14-16,18,19,22,27,28,35,42] to 0.01-0.04 in recent studies[24,36,43]). For this reason, the results presented in older papers cannot be translated to the current practice: vice versa, the current strategies showed to have drastically improved the survival of patients requiring parenteral nutrition and will hopefully contribute to reduce the heterogeneity among studies.

Parenteral nutrition-focused guidelines on catheter-related thrombosis have been published between 2002 and 2009 (reviewed in Table 1), but are mostly based on papers older than 20 years. Due to the lack of evidence in this setting, studies and evidence-based guidelines on anticoagulants for catheter-related thrombosis prevention in other (non-parenteral nutrition) adult and children populations should be considered (Table 1). In adult cancer patients, both heparins and vitamin K antagonists *vs* placebo or no anticoagulant might prevent only asymptomatic catheter-related thrombosis[44] and it is not clear if that corresponds to an overall clinical benefit. In neonates, prophylactic intravenous unfractionated heparin (0.5 IU/kg/h) did not show a significant difference for the risk of peripherally inserted central catheters-thrombosis, but was associated with a prolonged duration of catheter patency[45]. Only a single study reported imprecise effects of low-molecular-weight heparin for the risk of catheter-related thrombosis in children with central venous catheter[46], while no effect of systemic anticoagulation for the risk of (a)symptomatic thromboembolic events was demonstrated in pediatric cancer patients with a tunneled catheter[47].

Interventional studies did not report bleeding rate, which appeared to be low in two observational studies[26,36], while no data on heparin-induced thrombocytopenia are available. In the absence of adequate data of harm and of a demonstrated efficacy, recommending primary thromboprophylaxis does not seem justified. Updates are urgently required and there is a strong need for multicenter prospective interventional studies involving both parenteral nutrition experts and coagulation specialists. Future studies not only need to include bleeding, but also less frequent complications, including pulmonary embolism, vena cava syndrome, and heparin-induced thrombocytopenia.

Several issues regard the pharmacokinetics and –dynamics of anticoagulants during parenteral nutrition, especially when patients with short bowel syndrome receive oral compounds. Erratic vitamin K antagonist and vitamin K absorption, bacterial

overgrowth, vitamin K supplementation contributed from fat emulsion in the feeding solution, interfering concomitant medications and hepatic impairment represent important factors complicating vitamin K antagonist management[42]. The adverse effects of long-term intravenous unfractionated heparin include osteoporosis and the formation of precipitates with lipids[2], while the use of low-molecular-weight heparins in patients on parenteral nutrition is not recommended with concomitant severe renal dysfunction and the subcutaneous way of administration could reduce patients' compliance. Two recent reports of 4 patients with short bowel syndrome treated with the direct oral anticoagulant rivaroxaban suggest that it could represent a therapeutic alternative in selected patients[48,49]. A randomized crossover phase I study has just concluded the enrolment of patients with short bowel syndrome exposed to the direct oral anticoagulants dabigatran etexilate and rivaroxaban (NTR4192).

In conclusion, there is insufficient evidence to allow conclusions regarding the efficacy and safety of anticoagulants used to prevent catheter-related thrombosis in adults and children on short- and long-term parenteral nutrition and well-designed studies are urgently needed.

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SUPPLEMENTARY MATERIAL

Literature search strategy: MEDLINE

(accessed via PubMed on Nov 23, 2015)

("anticoagulants"[All Fields] OR "heparin*"[All Fields] OR "heparin*"[tiab] OR "heparin"[MeSH Terms] OR "fondaparinux"[tiab] OR "anticoagulant*"[tiab] OR "warfarin"[tiab] OR "acenocumarol"[tiab] OR "coumarins"[MeSH Terms] OR "phenprocoumon"[tiab] OR "rivaroxaban"[tiab] OR "dabigatran"[tiab] OR "apixaban"[tiab]) AND ("parenteral nutrition, total"[MeSH Terms] OR "total parenteral nutrition"[All Fields] OR "parenteral nutrition"[tiab] OR "parenteral nutrition"[MeSH Terms] OR "parenteral nutrition"[All Fields])

Table A. Baseline characteristics of observational studies

Study	Population	Central venous access	Patients, n (♂, age)[a]	Treatment	Follow-up	Outcome
Dollery, 1994 (r)[25]	Children	Hickman or Broviac CVC	32 (na; 0-13)	UFH 1 unit/mL (n=8), no heparin (n=24)	Range 0.4-7 years	(A)symptomatic PE, AC prevalence
Andrew, 1995 (cs)[21]	Children	Single-lumen, thick-walled silicone rubber	12 (6; 0-6)	Warfarin (different length and INR target)	Not available	AC prevalence
Newall, 2003 (p)[29]	Children	Not available	8 (na; 0-17)	INR-adjusted warfarin (INR target 2.0-3.0 or 1.3-2.0; n=8)	15.2 warfarin years (mean 691.6 days; median 817; range 186-1025)	Pharmacological study, quality of AC treatment
Vegting, 2012 (r-p)[36]	Children	Subcutaneous tunneled single- or double-lumen central lines and ports	32 (na; 0-17)	Nadroparin 80 IU/kg or acenocoumarol (n=18); no AC (n=27)	Median 25 months (range 2-167)	(A)symptomatic CRT, bleeding, AC prevalence
Ladefoged, 1981 (r)[28]	Adults and children	Polyvinyl chloride or Broviac CVC	70 (33; 46, 6-69)	Phenprocoumon or UFH 5,000 IU bid	Median 4.5 months (range 1-63)	Symptomatic CRT, AC prevalence
Schmidt-S, 1990 (r)[32]	Adults and children	Broviac CVC (different venous access)	35 (na; 0-23)	UFH (1 UI/mL PN solution)	Mean 577 days (range 58-2.633)	Symptomatic PE, AC prevalence
Imperial, 1983 (r)[27]	Adults	Hickman CVC	1010	UFH<6,000 UI/day (n=129); 6,000 UI/day (n=858); no AC (n=23)	Mean 25 days (2-56) in low-dose UFH; mean 14 days (2-42) in high-dose UFH	(A)symptomatic CRT, AC prevalence
Bern, 1986 (p)[22]	Adults	Silicone rubber Hickman or Broviac CVC	26	UFH 2000 IU/day flushes (all patients). Warfarin 2.0 mg qd (n=13); no warfarin (n=13)	Warfarin 3235 days; no warfarin 2763)	Symptomatic CRT, AC prevalence
Veerabagu, 1994 (r)[35]	Adults	Not available	90 (47; 50)	Minidose warfarin 1-2 mg/day (n=53), INR-adjusted warfarin (n=18), no warfarin (n=46); UFH 6,000 IU/day (all patients)	1312 months (mini-dose), 619 months (INR-adjusted), 931 months (no warfarin)	Symptomatic CRT, AC prevalence
Duerksen, 1996 (r)[26]	Adults	Permanent CVC (Hickman n=38, venous access discs n=9)	47 (43; 36)	Warfarin 1 mg/day vs (n=9), no warfarin (n=40)	Mean 6.3 months	Symptomatic CRT, bleeding, AC prevalence
Cowl, 2000 (p)[23]	Adults	PICC vs CVC	102 (56; 21-88)	UFH flushes with 3 ml (UFH 100 IU/ml), systemic AC	Not available	AC prevalence
v Gossum, 2001 (cs)[34]	Adults	CVC	228 (na; 0-23)	Any AC	12 months	AC prevalence
Puiggros, 2012 (cs)[31]	Adults	Tunnelled CVC (77.6%), implanted port (22.4%)	49 (16; 52)	Any AC (22%); flushes with UFH (69%)	Mean 57.4 months (range 1-286)	AC prevalence
Olthof, 2014 (r)[30]	Adults	Hickman, port-a-cath	212 (102; 48)	Any systemic AC	Total: 600 catheter-year	AC prevalence
Tourè, 2014 (p)[33]	Adults	Broviac (n=133), 77 PICC (n=77)	196 (77, 56)	VKA, heparins	Total: 134.6 catheter-year	AC prevalence
Cuerda (p), 2015[24]	Adults	Tunnelled (n=52), implanted port (n=10)	62 (31, 50)	LMWH, low-dose VKA, VKA (target INR 2.0-2.5)	Median: 1 year	AC prevalence

[a]Data are expressed as n total (n male, age or age range): age and age range are expressed in years, age is expressed as mean or median according to data available from original papers.
Abbreviations: p, prospective study; r, retrospective study; cs, cross-sectional study; PICC, peripherally inserted central venous catheter; PN, parenteral nutrition; RCT, randomized controlled trial; aPTT, activate partial thromboplastin time; IVH, intraventricular hemorrhage; CVC, central venous catheter; CRT, central venous catheter thrombosis; UFH, unfractionated heparin sodium; DIC, disseminated intravascular coagulation; AC, anticoagulant.

Table B. Indications for parenteral nutrition in the included studies, n (%)

Study	SBS	Cancer	IBDs	Inf./Thr.	Dismotility	AIDS	Radiation	Surgery	Other	Total n
Brismar[14]	-	16 (32)	17 (34)	-	-	-	-	-	17 (34)	50
Fabri[16]	-	-	-	-	-	-	-	-	-	46
Ruggiero[19]	-	-	-	-	-	-	-	-	-	34
Fabri[15]	-	-	-	-	-	-	-	-	-	40
Macoviak[18]	-	-	-	-	-	-	-	-	-	37
Kamala[17]	-	-	-	-	-	-	-	-	-	68
Uslu[20]	-	-	-	-	-	-	-	-	-	239
Ladefoged[28]	26 (37)	-	-	-	-	-	-	-	44 (63)	70
Schmidt[32]	8 (23)	-	26 (74)	-	1 (3)	-				35
Dollery[25]	-	-	15 (44)	-	7 (21)	-	-	10 (29.4)	2 (6)	34
Andrew[21]	3 (25)	-	-	-	1 (8)	-	-	-	8 (67)	12
Newall[29]	-	-	2 (25)	-	1 (12)	-	-	-	5 (62)	8
Vegting[36]	10(22)	-	-	-	15 (33)	-	-	-	20 (44)	45[a]
Imperial[27]	-	-	-	-	-	-	-	-	-	1,010
Bern[22]	-	1 (4)	9 (39)	5 (22)	1 (4)	-	4 (17)	-	3 (13)	23
Veerabagu[35]	-	12 (13)	17 (19)	21 (23)	-	-	6 (7)	8 (9)	26 (29)	90
Duerksen[26]	-	-	-	-	-	47 (100)	-	-	-	47
Cowl[23]	10 (10)	16 (16)	12 (12)	1 (1)	-	-	-	17 (17)	56 (55)	102
v Gossum[34]	-	-	75 (33)	57 (25)	18 (78)	-	-	43 (19)	23 (10)	228
Puiggros[31]	-	8 (16)	1 (2)	10 (20)	10 (20)	-	10 (20)	4 (8)	6 (12)	49
Olthof[30]	(58-60)	-	-	-	(28-37)	-	-	-	(4-5)	212
Tourè[33]	101 (52)	32 (16)	13 (7)	55 (28)	12 (6)	-	22 (11)	84 (43)	52 (27)	196
Cuerda[24]	-	-	8 (13)	13 (21)	11 (18)	-	10 (16)	8 (13)	12 (19)	62

[a]Thirteen patients were included in both groups (N=32). Abbreviations: SBS, short bowel syndrome, IBD, inflammatory bowel disease; AIDS, acquired immunodeficiency syndrome; Inf./Thr., infarction/splancnic thrombosis.

6

Home parenteral nutrition-associated thromboembolic and bleeding events: results of a cohort study of 236 individuals

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ABSTRACT

Home parenteral nutrition is necessary for patients with intestinal failure. Recurrent catheter-related thrombosis is common, leading to infectious complications, pulmonary embolism, vascular access loss and intestinal transplantation. Efficacy and safety of anticoagulants is unknown in this setting and based on sparse and low-quality observational data.

Our aim was to estimate the incidence of thromboembolic, bleeding and anticoagulant-related complications in home parenteral nutrition patients, and evaluate risk factors for first venous thrombosis.

This retrospective cohort study included all adult patients followed for long-term home parenteral nutrition at our center between 1986 and 2014. Primary outcomes were symptomatic objectively-diagnosed venous thrombosis, encompassing catheter-related thrombosis and venous thromboembolism, and major bleeding. Secondary outcomes were: vena cava syndrome and heparin-induced thrombocytopenia or hypersensitivity.

Two-hundred-thirty-six patients were included (median home parenteral nutrition duration: 17 months) and 136 received anticoagulants at parenteral nutrition onset (57.6%). Overall, the annual incidence of first venous thrombosis was 11.4% (95% Confidence Interval [95%CI]: 8.6-14.7): the strongest predictor of venous thrombosis was personal history of thrombosis (adjusted Hazard Ratio 2.45; 95%CI: 1.15-5.22), while anticoagulation seemed to account only for a mild protection (adjusted Hazard Ratio 0.66; 95%CI: 0.32-1.37). The annual incidence of major bleeding was 4.3% on *vs* 1.8% off anticoagulants. Vena cava syndrome developed in 20.7% of patients with venous thrombosis. One patient had isolated heparin-induced thrombocytopenia (0.6% of exposed) and 4 had heparin hypersensitivity (2.5% of exposed).

Patients on home parenteral nutrition have a significant risk of venous thrombosis, major bleeding, and vena cava syndrome. Anticoagulants might reduce thrombosis risk, but several safety concerns remain in this population.

INTRODUCTION

Parenteral nutrition was introduced four decades ago[1] and represents the first-line treatment for patients with intestinal failure unable to absorb nutrients and fluids after oral or enteral intake. The prevalence of patients on long-term home parenteral nutrition is low, ranging from 1 to 20 individuals per million inhabitants in Europe with dramatically improved survival rates since its introduction[2,3].

Home parenteral nutrition requires permanent central venous access for intravenous administration of parenteral nutrition solution, which represents an independent risk factor of catheter-related thrombosis in patients with a central venous access due to its composition and high osmolarity[4-6]. Recurrent catheter-related thrombosis is one of the most common complications and may trigger bacterial infections, sepsis, pulmonary embolism, or vena cava syndrome, leading to a progressive loss of vascular access and the inability to further continue parenteral nutrition with the need for intestinal transplantation[7,8].

Various anticoagulant regimens at different dosages are recommended for primary prevention of catheter-related thrombosis by home parenteral nutrition-focused guidelines on the basis of moderate or low level of evidence[7-13]. Conversely, thrombosis-focused guidelines or guidance documents do not recommend their routine use in adults, not even in cancer patients with a central venous catheter for intravenous chemotherapy, arguably one of the most hypercoagulable patient groups[14-17]. Evidence on the efficacy and safety of anticoagulants in this setting is lacking since no interventional studies ever focused on home parenteral nutrition. Low-quality observational data are sparse and mostly deriving from papers that are obsolete with regard to the type of parenteral nutrition solutions, venous catheters and placement techniques available at the time they were performed[18-20]. Moreover, no pharmacokinetic studies have been performed in patients on home parenteral nutrition (especially those with short bowel syndrome), while the choice of the best anticoagulant is particularly challenging due to unpredictable absorption rates. The management of vitamin K antagonists is difficult due to several reasons, including variable vitamin K and vitamin K antagonist absorption (e.g., related to the underlying intestinal disease or to the presence of short bowel syndrome), bacterial overgrowth, variations in vitamin K supplementation, drug interactions with components of the parenteral nutrition solution, interfering co-medications and parenteral nutrition-related hepatic impairment possibly interfering with coagulation factor synthesis[21-24]. Intravenous unfractionated heparin may lead to osteoporosis and to the formation of precipitates with lipids[24]. Low-molecular-weight heparins are not recommended with

concomitant severe renal function and long-term subcutaneous administration or skin side effects[25] may reduce compliance, while the risk of heparin-induced thrombocytopenia has never been estimated in individuals on home parenteral nutrition.

Using an observational study design, we aimed to estimate the rate of symptomatic objectively-diagnosed venous thrombosis (encompassing catheter-related thrombosis, deep venous thrombosis, and pulmonary embolism), major bleeding, vena cava syndrome, and heparin complications in a large cohort of consecutive individuals on home parenteral nutrition, as well as assess predicting variables and the effect of anticoagulant treatment on first venous thrombosis.

MATERIAL AND METHODS

Study design, setting and participants

Our observational retrospective study included consecutive individuals on home parenteral nutrition followed at the outpatient clinic of the Academic Medical Center (Amsterdam, the Netherlands). The hospital is a tertiary university institution and one of the two national centers that manage individuals on home parenteral nutrition, which includes patient education, counseling, management of complications, and routine follow-up on the basis of international guidelines recommendations.

All patients aged 16 years or older who started with long-term home parenteral nutrition between January 1986 and January 2014 were screened for inclusion. Individuals on home parenteral nutrition for less than three consecutive months, with peripheral venous catheters or with incomplete charts were excluded. The reporting of this study follows guidelines set out for observational studies (STROBE)[26].

Study outcomes and source data

The primary outcomes were 1) symptomatic objectively-diagnosed venous thrombosis, considered as a composite outcome of catheter-related thrombosis, deep venous thrombosis and pulmonary embolism, and 2) major bleeding. The secondary outcomes were vena cava syndrome, acute heparin-induced thrombocytopenia, and heparin hypersensitivity.

All individuals were monitored for clinical signs and symptoms of thrombosis according to good clinical practice: imaging tests used to be performed either in presence of symptoms suggesting the presence of thrombosis or before the placement of a new venous access. No routine screening imaging tests were performed in asymptomatic individuals. Accepted tests for catheter-related thrombosis/deep venous thrombosis were conventional angiography, compressive ultrasound or echo-color Doppler, computed tomography venography, magnetic resonance imaging with magnetic resonance venography. The diagnosis of pulmonary embolism was accepted if reported by the treating physician on the basis of symptoms and after testing the patient with either helical computed tomography or ventilation/perfusion lung scan. Bleedings were retrospectively classified on the basis of data from medical charts as "major bleeding" accordingly with the International Society on Thrombosis and Haemostasis (ISTH) criteria for either surgical or non-surgical patients[27,28]. The temporal link between anticoagulant exposure and primary outcomes was necessarily based on the description of the events made by the treating physicians at the time of occurrence and on the subsequent decision taken with regard to the anticoagulant treatment.

Two institutional databases of both in- and outpatients candidate to receive home parenteral nutrition were manually reviewed and inclusion criteria applied. Medical charts, radiological data, individuals´ personal documentation, and specialists´ medical reports served as source data for baseline characteristics, systemic anticoagulant treatment and outcomes. Heparin-induced thrombocytopenia and heparin hypersensitivity data were primarily obtained from permanent alerts generated by the electronic patient medical chart once the adverse drug event was first detected by the treating physician. Two independent investigators retrieved the data (BS, CH) and two other researchers assessed the quality of the process (SB, MC). The primary outcomes were adjudicated by a co-author (MB), who independently accessed the source data and verified each event for primary study outcomes, the presence of symptoms, and whether an accepted imaging test was performed or not.

The following patients' variables were recorded for this study: age, sex, underlying diseases and co-morbidities, estimated length of remaining bowel after surgical intervention, duration of home parenteral nutrition and indication, type and duration of anticoagulant treatment, intestinal transplantation, study outcomes, and death.

The choice of site of central access was based on the international guidelines available at the time of catheter placements, preferring single- or double-lumen permanent tunnelled central catheters placed accessing the right jugular/subclavian veins as a first option. Alternative access sites, including contralateral neck veins,

femoral veins, hepatic vein, and peripheral-inserted central catheters, were subsequently used in case of local neck vein complications.

Statistical methods

The absolute risks of first venous thrombosis, recurrent venous thrombosis and major bleeding events were calculated after home parenteral nutrition onset: the time unit of statistical analysis was patient-year and the observation time start was set at parenteral nutrition onset. Individuals were right-censored at the time of home parenteral nutrition discontinuation, death, if they were lost to follow-up or if they moved to a different center for clinical follow-up.

The annual incidence rates of complications were calculated by dividing the number of events by the time to first event or censoring: rates were expressed as number of events/100 patient-years. The rates of first and second venous thrombosis recurrences were calculated by dividing the number of venous thrombosis events by the time starting from prior venous thrombosis. The 95% Confidence Interval (95%CI) for incidence rate was based on the exact approximation of the Poisson's distribution, while 95%CI for proportion was based on bivariate distribution (Wilson score method).

Descriptive analyses of the baseline characteristics used counts and percentages for categorical data, while continuous data were expressed as mean and standard deviation (SD) or median and interquartile range (IQR), according to the distribution of data. Baseline characteristics of the group of individuals receiving anticoagulants at parenteral nutrition onset were compared to the group not receiving anticoagulants. Differences between groups were evaluated by Student t-test or Mann-Whitney U test for continuous variables, and by Fisher's exact test for categorical data, where appropriate.

To explore the potential role of anticoagulant therapy on the risk of first and recurrent venous thrombosis, separate data during exposure or no exposure to anticoagulants were provided. First venous thrombosis-free survival is described using the Kaplan-Meier curve, aiming to minimize time-immortal bias by adjusting for anticoagulant exposure, which has been introduced as a time-varying factor [29]. Furthermore, multivariable Cox regression model was fit to estimate hazard ratios (HRs) and corresponding 95%CIs for the risk of first venous thrombosis in patients on *vs* off anticoagulation. Use of anticoagulants served as a time-varying variable. Adjustments for clinically relevant risk factors at baseline were made according with 1) predefined standard risk factors for venous thrombosis (age, sex), 2) estimated remaining bowel

length after surgery since it represents a relevant confounder (due to its intrinsic imprecision, it was included as a categorical variable), and 3) to covariates paralleling the supposed risk factors provided by the recommendations from the European Society for Clinical Nutrition and Metabolism (ESPEN)[8] for considering primary catheter-related thrombosis prevention: prior thrombotic events (encompassing deep venous thrombosis, pulmonary embolism and arterial/venous splanchnic thrombosis), inflammatory bowel diseases, active cancer (or chemotherapy) during home parenteral nutrition or in the prior 5 years. Covariates of interest were entered in one step into a parsimonious regression model if proportional hazard assumptions were confirmed; potential correlations were assessed to ensure absence of collinearity. Univariable analysis is provided as Supplementary Material. Annual incidence rates of major bleeding in groups of patients receiving or not anticoagulants were compared by calculating incidence rate ratios with the corresponding 95%CIs. Differences were considered statistically significant if $p<0.05$. The software SPSS v. 21.0 (IBM Corporation, Armonk NY, United States) was used for the analysis.

RESULTS

A total of 267 adult individuals were evaluated by the treating physicians for treatment with home parenteral nutrition and have been retrospectively screened for the purpose of the present work: 31 have been excluded from the study as either they were not treated with parenteral nutrition (n=4), stopped parenteral nutrition after hospital discharge with no subsequent home parenteral nutrition (n=23), or had incomplete medical charts (n=4).

236 patients fulfilled the inclusion criteria: the vast majority (91.2%) was started with home parenteral nutrition after 1998. Median home parenteral nutrition duration was 17 months (IQR, 8-44 months) with a total follow-up of 684.1 patient-years. Patients´ baseline characteristics are summarized in Table 1.

Table 1. Patients' baseline characteristics

	Total	AC at HPN onset	No AC at HPN onset
Number of patients	236	136	100
HPN duration (months)	17 (8-44)	17 (9-43)	18 (8-49)
Male sex	94 (39.8)	61 (44.9)	33 (33.0)
Age (years)	54 (44-63)	55 (46-64)	53 (43-63)
Year of HPN start			
2008-2013	127 (53.8)	74 (54.4)	53 (53.0)
1998-2007	86 (36.4)	50 (36.8)	36 (36.0)
1986-1997	23 (9.7)	12 (8.8)	11 (11.0)
Estimated post-resection bowel length (cm)[a]	75 (45-130)	65 (40-110)	105 (70-150)
Indication for HPN			
Short bowel syndrome[a]	137 (58.1)	92 (67.6)	45 (45.0)
Dismotility	64 (27.1)	30 (22.1)	34 (34.0)
Radiation enteritis	20 (8.5)	9 (6.6)	11 (11.0)
Enterocutaneous fistula	45 (19.1)	26 (19.1)	19 (19.0)
Other	18 (7.6)	8 (5.9)	10 (10.0)
Co-morbidities			
Prior acute coronary syndrome or ischemic stroke	21 (8.9)	14 (10.3)	7 (7.0)
Autoimmune disease	38 (16.1)	18 (13.2)	20 (20.0)
Inflammatory bowel diseases	41 (17.4)	22 (16.2)	19 (19.0)
Crohn's disease	36 (15.3)	17 (12.5)	19 (19.0)
Ulcerative colitis	5 (2.1)	5 (3.7)	0 (0)
Amyloidosis	2 (0.8)	1 (0.7)	1 (1.0)
Cancer (prior 5 years)	65 (27.5)	35 (25.7)	30 (30.0)
Bowel resection (any)	172 (72.9)	105 (77.2)	67 (67.0)
Arterial splanchnic thrombosis[a]	40 (16.9)	37 (27.2)	3 (3.0)
Venous splanchnic thrombosis[a]	18 (7.6)	15 (11)	3 (3.0)
Prior venous thromboembolism[b]	33 (14.0)	22 (16.2)	11 (11.0)
Atrial fibrillation[a]	14 (5.9)	13 (9.6)	1 (1.0)
Know HIV seropositivity	2 (0.8)	0 (0)	2 (2.0)

Data are expressed as number (%) or median (interquartile range).

[a]The difference between the groups of patients receiving or not anticoagulants at HPN onset is statistically different. [b]Venous thromboembolism encompasses deep venous thrombosis and pulmonary embolism. Abbreviations: HPN, home parenteral nutrition; AC, anticoagulant; HIV, human immunodeficiency virus.

Patients who received anticoagulants at the time of initiation of parenteral nutrition more often had short bowel syndrome and a history of splanchnic thrombosis or atrial fibrillation than patients not receiving anticoagulants (Table 1). One-hundred-thirty-six patients were on anticoagulants at the time of initiation of parenteral nutrition (57.6%) increasing to 181 patients during the course of home parenteral nutrition (76.7%) with a total follow-up time on anticoagulants of 452.4 patient-years. Table 2 summarizes the characteristics of anticoagulant regimens: 69 patients (38.1%) were treated with long-term low-molecular-weight heparins, while 97 received long-term vitamin K antagonists (53.6%), of whom 18 (9.9%) were switched between different vitamin K antagonists, including intravenous warfarin (n=17, 9.4%). Thirty-one patients received anticoagulant together with a concomitant antiplatelet agent.

Data regarding the characteristics of each inserted catheter and catheter-related infections were incomplete and hence could not be collected.

Venous thrombosis

Eighty-three objectively-diagnosed symptomatic venous thrombosis events were recorded in 58 of 236 patients (24.6%; 95%CI: 19.5-30.4). The rate of first venous thrombosis during home parenteral nutrition was 11.4/100 patient-years (95%CI: 8.6-14.7), of which 72.4% were isolated catheter-related thrombosis, 19.0% catheter-related thrombosis-associated pulmonary embolism and 8.6% pulmonary embolism or deep venous thrombosis events not associated to the presence of a catheter. The observed annual rates of first venous thrombosis were 10.1/100 patient-years (n=31; 95%CI: 19.1-25.9) on, and 13.3/100 patient-years (n=27; 95%CI: 15.4-29.8) off anticoagulants, respectively (Table 3).

The rates of first and second recurrent venous thrombosis events were 13.7/100 patient-years (95%CI, 8.0-22.0/100 patient-years) and 24.5/100 patient-years (95%CI: 9.0-53.3), respectively (Table 3; Figure A, Supplementary Material).

Table 2. Duration and characteristics of anticoagulant treatments

	Total	Anticoagulants	No anticoagulants
Before PN start, no. (% of total)	236	52 (22)	184 (78)
Observation period			
At PN onset, no. (%)	236	136 (57.6)	100 (42.4)
Before first venous thrombosis		162 (68.6)	74 (31.4)
During PN (any time)		181 (76.7)	55 (23.3)[a]
Time to first VT or censoring (total follow-up) in patient-years	509.7 (684.1)	306.7 (452.4)	203.0 (231.7)
Starting dose	162		
Prophylactic, no. (%)		59 (36.4)	
Therapeutic	-	101 (62.3)	-
Unknown		2 (1.3)	
Anticoagulant drugs	181		
Unfractionated heparin, no. (%)		6 (3.3)	
LMWH (all exposures)		160 (88.4)	
Long-term LMWH only[b]		69 (38.1)	
Fondaparinux		2 (1.1)	
Vitamin K antagonist		97 (53.6)	
More than 2 vitamin K antagonists	-	18 (9.9)	-
Warfarin (intravenous)		17 (9.4)	
Acenocumarol		76 (42.0)	
Phenprocumon		26 (14.4)	
Rivaroxaban		1 (0.6)	
Danaparoid		1 (0.6)	
Antiplatelet agent use, no.	47	31	16

[a]Fifty-five patients have not been exposed to systemic anticoagulation. [b]Sixty-nine patients have been exposed to LMWH only. Abbreviations: PN, parenteral nutrition; LMWH, low-molecular-weight heparin; NA, not applicable.

The cumulative incidence of venous thrombosis-free survival is shown in Figure 1, while an overview of the types of anticoagulant exposure is provided in Table 3. The strongest risk factor for acute objectively diagnosed symptomatic first venous thrombosis during the first 3 years from home parenteral nutrition onset was a personal history of thrombosis prior to home parenteral nutrition (adjusted HR 2.45; 95%CI: 1.15-5.22; after adjustment for age, sex, time of exposure to anticoagulants, cancer, estimated remaining bowel length after surgery and presence of inflammatory bowel diseases) (Table 4). Anticoagulation, at any regimen, was associated with a statistically non-significant risk reduction of venous thrombosis (adjusted HR 0.66; 95%CI: 0.32-1.37). In exploratory analyses, year of parenteral nutrition onset did not

influence the point estimates, also not when used as a categorical variable (before or after year 2010, corresponding to the spread of the latest ESPEN international guidelines in the clinical practice; data not shown)[8].

Table 3. Thromboembolic complications during home parenteral nutrition (HPN)

	First venous thrombosis during HPN		
	Total	*Anticoagulants*[a]	*No anticoagulants*[a]
No. of patients	236	162	124
Patient-years	509.7	306.7	203.0
Median FUP, years (IQR)	1.1 (0.6-2.8)	0.9 (0.4-2.2)	0.8 (0.4-1.9)
No. of events (%; 95%CI)	58 (24.6; 19.5-30.4)	31 (19.1; 13.8-25.9)	27 (21.8; 15.4-29.8)
Annual incidence, % (95%CI)	11.4 (8.6-14.7)	10.1 (6.9-14.3)	13.3 (8.8-19.4)
CRT, no. (%)	42 (72.4)	22 (71.0)	20 (74.1)
Venous thromboembolism	11 (19.0)	6 (19.4)	5 (18.5)
CRT-associated PE	5 (8.6)	3 (9.7)	2 (7.4)
	First recurrence[b]	**Second recurrence**[c]	
No. of patients	58	17	
Patient-years	123.7	24.5	
Median FUP, years (IQR)	1.0 (0.4-3.3)	1.1 (0.6-2.8)	
No. of events (%; 95%CI)	17 (29.3; 19.2-42.0)	6 (35.3; 17.3-58.7)	
Annual incidence, % (95%CI)	13.7 (8.0-22.0)	24.5 (9.0-53.3)	
CRT, no. (%)	14 (82.4)	5 (83.3)	
Venous thromboembolism	3 (17.6)	1 (16.7)	

Prevalence is calculated as the proportion of patients with thrombosis. Annual incidence is calculated as the number of events per 100 patient-years. Venous thromboembolism encompasses deep venous thrombosis and pulmonary embolism not associated with catheter-related thrombosis. [a]Fifty patients were switched from no anticoagulant to anticoagulant or vice-versa: therefore, their observation times have been included in both groups. [b]Exposure to anticoagulants between first venous thrombosis and first recurrence could not be precisely determined. [c]All the patients were on anticoagulants after first recurrence. Abbreviations: HPN, home parenteral nutrition; IQR, interquartile range; 95%CI, 95% confidence interval; HPN, home parenteral nutrition; CRT, catheter-related thrombosis; PE, pulmonary embolism.

Table 4. Association between anticoagulation or potential risk factors and first venous thrombosis during home parenteral nutrition

Variable	Adjusted Hazard Ratio	95% Confidence Interval
Age (hazard per unit increase)	1.00	0.98-1.03
Male sex	0.55	0.26-1.15
Anticoagulation	0.66	0.32-1.37
Prior thrombosis[a]	2.45	1.15-5.22
Cancer[b]	1.07	0.48-2.34
Bowel length (> than 150 cm)	1.73	0.81-3.73
Inflammatory bowel diseases	0.90	0.39-2.06

Covariates of interest were entered in one step into a parsimonious multivariable Cox regression model if proportional hazard assumptions were confirmed. Correlations were assessed to ensure absence of collinearity. First venous thrombosis served as dependent variable. Univariable analysis is provided as Supplementary Material.

[a]Prior thrombotic events encompass deep venous thrombosis, pulmonary embolism and arterial/venous splanchnic thromboses. [b]Cancer has been defined as active cancer, chemotherapy treatment ongoing, or a diagnosis of cancer in the prior 5 years from parenteral nutrition onset.

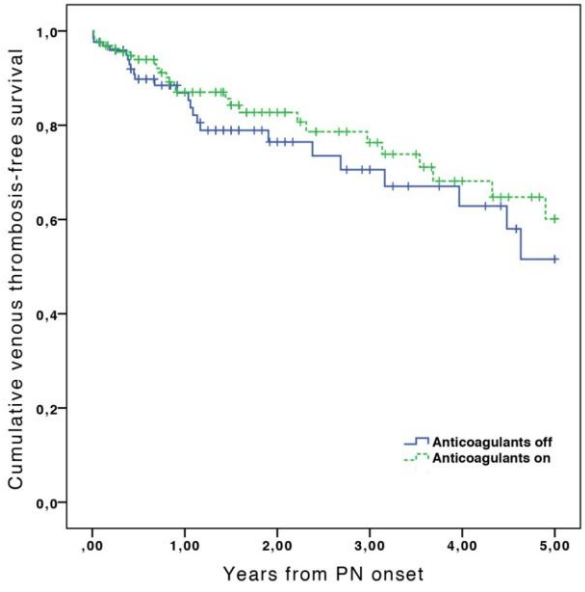

Figure 1. Cumulative incidence of first venous thrombosis-free survival during home parenteral nutrition

Multiple exposures have been adjusted for time-varying periods of anticoagulant administration[29]. Abbreviation: PN, parenteral nutrition.

Major bleeding

Twenty-three major bleeding events occurred in 22 patients (9.2%; 95%CI: 6.2-13.7). The annual incidence rate of a first episode of major bleeding was 3.4/100 patient-years (95%CI: 2.1-5.2). Five events were related to surgery performed at the time of parenteral nutrition onset and 3 of them occurred in the first 10 days of parenteral nutrition (Table 5). Eighteen bleeding events occurred while on anticoagulant treatment (4.3/100 patient-years; 95%CI: 2.5-6.8), while 4 occurred in patients not receiving anticoagulants (1.8/100 patient-years; 95%CI: 0.5-4.6). The incidence rate ratio was 2.4 (95%CI: 0.9-8.3) on *vs* off anticoagulants.

Major bleeding was fatal in 3 patients, all of whom were on anticoagulants (leg compartment syndrome after femoral catheter placement, retroperitoneal bleeding and intracranial bleeding).

Table 5. Major bleeding events during home parenteral nutrition

	Total	Anticoagulants	No anticoagulants
No. of patients	236	181	127
Patient-years[a]	643.1	419.4	223.7
No. of events (%; 95%CI)	22 (9.2; 6.2-13.7)	18† (9.9; 6.4-15.2)	4[b] (3.1; 1.2-7.8)
Annual incidence, % (95%CI)	3.4 (2.1-5.2)	4.3 (2.5-6.8)	1.8 (0.5-4.6)

Annual incidence (95%CI: 95% Confidence Interval) is expressed as the number of events per 100 patient-years.
[a]Fifty patients were switched from no anticoagulant to anticoagulant or vice-versa: therefore, observation times have been included in both groups. [b]Gastrointestinal (n=7), compartment syndrome after femoral vein catheter placement (2), intracranial (2), retroperitoneal (2), mediastinal (1), enterocutaneous fistula (1), left flank (1), spinal cord (1), abdominal (1). Three bleeding events were fatal. ‡Intracranial (n=2), abdominal (1), unspecified (1).

Other complications

Vena cava syndrome was diagnosed in 12 patients (5.1% of the total cohort; 95%CI: 2.9-8.7), representing 20.7% of those patients who developed venous thrombosis during home parenteral nutrition (95%CI: 12.3-32.8).

One patient developed acute isolated heparin-induced thrombocytopenia (with no associated thrombosis) during low-molecular-weight heparin treatment and received danaparoid as alternative anticoagulant. The total number of initiations of low-

molecular-weight heparin was not available: therefore, the proportion of diagnosed heparin-induced thrombocytopenia has been conservatively calculated using the minimum number of patients certainly exposed to low-molecular-weight heparin as denominator (n=160). The maximum expected rate of diagnosed heparin-induced thrombocytopenia is 0.6% per treatment (95%CI: 0.1-3.5).

Four patients had clinical manifestations of low-molecular-weight heparin hypersensitivity (2.5%; 95%CI: 1.0-6.3), including local or generalized skin reactions, and heparin was discontinued in all of them.

Intestinal transplantation, end of follow-up and mortality rate

Two patients (0.8%) underwent intestinal transplantation. One had recurrent thrombotic/infectious events and was transplanted 13 years after home parenteral nutrition onset because of loss of venous access: he died 15 months after transplantation. The second intestinal transplantation was performed in a patient 6 years after home parenteral nutrition onset aiming to improve quality of life and the patient is still off parenteral nutrition 30 months later.

Home parenteral nutrition was stopped in 78 patients (33.1%) after a median of 11 months (IQR: 8-24.25) because of intestinal adaptation resulting in adequate feeding status on either oral or enteral nutrition. Seventy-five patients (31.8%) were still treated with home parenteral nutrition at the time of data extraction. Seventy-one patients died (30.1%) after a median of 19 months from parenteral nutrition onset (IQR: 11-49). Four patients (1.7%) were transferred to another medical center, while 8 patients (3.4%) were lost to follow-up after median home parenteral nutrition duration of 10.5 months (IQR: 3.25-16.25).

DISCUSSION

Patients on home parenteral nutrition have a significant risk of both venous thrombosis and major bleeding. In this large cohort of 236 patients on long-term home parenteral nutrition, the absolute annual incidence of first objectively diagnosed venous thrombosis was 11.4% (n=58 events). Most of the events were related to the presence of a central venous catheter (isolated catheter-related thrombosis: 72.4%; catheter-related thrombosis-associated PE: 8.6% of events) and complicated by vena cava syndrome in 20.7% of cases (5.1% of the entire cohort).

The annual rate of major bleeding was 4.3% and 1.8% on and off anticoagulants, respectively.

Our data suggest a modest reduction of first venous thrombosis in patients receiving anticoagulation, but this was statistically not significant (adjusted HR 0.66; 95%CI: 0.32-1.37). No subgroup analysis of different anticoagulant regimens was possible due to the frequent changes of dosage and drug compound secondary to hospitalisations, new concomitant drugs, adverse events, worsening of hepatic/renal function, or difficult INR management in patients receiving vitamin K antagonists. Expectably, most of the documented events were catheter-related thromboses and it is known that the efficacy of anticoagulants for prevention of catheter-related thrombosis is less than in other clinical settings[14,30,31]. A recent Cochrane meta-analysis of randomized trials in cancer patients with a central venous catheter found that prophylactic-dose heparin was associated with a reduction in symptomatic catheter-related thrombosis (risk ratio 0.48; 95%CI: 0.27-0.86) when compared to no heparin, and a similar but non-significant risk ratio of 0.51 (95%CI: 0.21-1.22) for vitamin K antagonists [32]. Moreover, a meta-analysis of 15 trials of patients with central venous access showed a significant risk reduction in catheter-related thrombosis rate associated with the administration of any-type anticoagulants[31]. The effect of anticoagulants on prevention of venous thromboembolism could not be evaluated due to the relatively low number of venous thromboembolic events.

In our cohort, the annual incidence of recurrent venous thrombosis events increased to 13.7% after first venous thrombosis and to 24.5% after second venous thrombosis. The observed increased risk of recurrent catheter-related thrombosis could be explained by subsequent catheter placement in more thrombogenic sites (e.g. femoral or deep abdominal veins) if neck veins are not suitable after thrombotic or infectious complications[30,33]. The observed rate of major bleeding was 2.4-fold increased during any-dose anticoagulant treatments (95%CI: 0.9-8.3) and 3 fatal bleedings out of 18 events were recorded during anticoagulation. No recent data on home parenteral nutrition patients are available in the literature for a meaningful comparison. An evaluation of bleeding risk factors was not possible due to the low number of events and to the lack of information on relevant covariates, such as length of exposure to antiplatelet agents, number of surgical procedures, organ function and individual hemostatic profile. Nevertheless, the annual rate of major bleeding during any-dose anticoagulation (4.3/100 patient-years; 95%CI: 2.5-6.8) appears significantly higher than what is observed in patients receiving therapeutic-dose oral anticoagulants for atrial fibrillation[34].

The proportion of patients developing vena cava syndrome is comparable to that from the sole study which evaluated it in a mixed-age cohort of 527 parenteral nutrition patients treated between 1973 and 1991 (overall proportion: 4%; annual incidence: 2%)[35]. In our cohort, one patient with recurrent thrombotic events and vena cava syndrome underwent intestinal transplantation due to the loss of available vascular access.

The other secondary outcomes have never been explored within a cohort of home parenteral nutrition patients: the maximum expected proportion of diagnosed acute heparin-induced thrombocytopenia was less than 1% per heparin treatment and is comparable to other subgroups of patients receiving low-molecular-weight heparins[36]. Heparin hypersensitivity represented a reason for discontinuation and switch to another drug compound in 2.5% of patients treated (95%CI: 1.0-6.3), similarly to what is described in literature[25].

To our knowledge, the present study is the first focusing entirely on the burden of thromboembolic and bleeding complications in patients on home parenteral nutrition. Some recent studies reported the rate of catheter-related thrombosis in this setting, but only 3 of them included symptomatic objectively-diagnosed events, and reported rates of catheter-related thrombosis ranging from 2.2 to 4.5/100 patient-years[37-39]. Nevertheless, the role of anticoagulants in home parenteral nutrition has been poorly investigated in the past two decades for the prevention of catheter-related thrombosis and venous thromboembolism, and current home parenteral nutrition-focused guidelines are necessarily based on studies published more than 20 years ago[7-13]. A crossover pharmacokinetics study has just concluded the enrolment of patients with short bowel syndrome treated with the direct oral anticoagulants rivaroxaban and dabigatran etexilate (NTR4192).

We acknowledge that our study has several limitations, starting from the retrospective study design and likely presence of residual confounders. Confounding of indication for anticoagulation was only partially minimized by adjusting for relevant covariates, such as a prior thrombotic event, which represents the sole statistically significant risk factor for future venous thrombosis. Anticoagulation was erratic in several patients, who were often switched between vitamin K antagonists and heparins due to difficult INR management, need of invasive procedures, organ failure, lack of compliance, or drug-related adverse events. Therefore, our findings cannot apply to a specific class of drugs or dosage; this would have required both a prospective design and a larger study population. Another limitation of our study regards the lack of information of the type, the number and the site of central venous access. However, all patients were

managed following the same internal protocol based on international guidelines and no systematic deviations are likely to have occurred.

In conclusion, our study shows that patients on home parenteral nutrition have a significant risk of both thrombosis and major bleeding events, of about 10% and 4% per year of anticoagulation respectively. The occurrence of prior thrombosis represents a predictor for future venous thrombosis events. The prescribed anticoagulants might reduce this risk: however, no conclusions can be drawn from our observational data. Several concerns regarding safety of anticoagulants in this patient population suggest caution. A prospective study is needed to evaluate to efficacy and role of anticoagulation in patients on home parenteral nutrition.

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SUPPLEMENTARY MATERIAL

Table A. Association between anticoagulation or potential risk factors and first venous thrombosis during home parenteral nutrition (univariable analysis, Cox regression model)

Variable	Adjusted Hazard Ratio	95% Confidence Interval
Age (per unit of year)	1.01	0.98-1.03
Male sex	0.55	0.28-1.10
Anticoagulation	0.83	0.45-1.53
Prior thrombosis[a]	1.60	0.87-2.93
Cancer[b]	0.97	0.47-1.97
Bowel length (> than 150 cm)	1.33	0.69-2.56
Inflammatory bowel diseases	0.97	0.45-2.11

[a]Prior thrombosis encompasses deep venous thrombosis, pulmonary embolism and arterial/venous splanchnic thromboses. [b]Cancer has been defined as active cancer, chemotherapy treatment ongoing, or a diagnosis of cancer in the prior 5 years from parenteral nutrition onset.

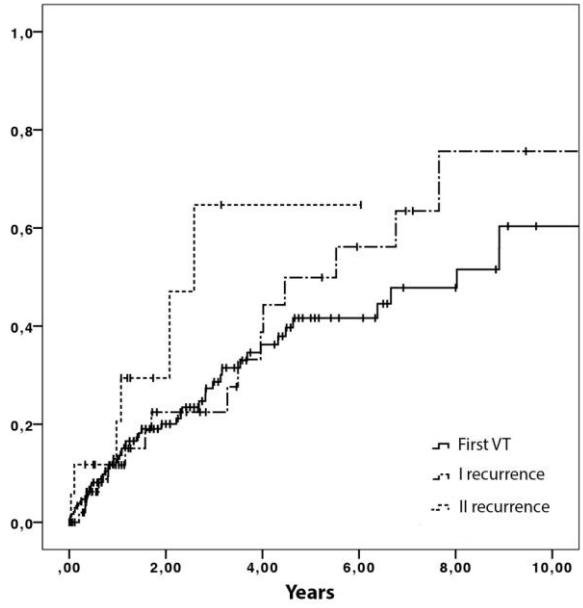

Figure A. Cumulative incidence of first venous thrombosis-free and recurrent venous thrombosis-free survival during home parenteral nutrition

Abbreviations: VT, venous thrombosis (encompassing catheter-related thrombosis, deep venous thrombosis and pulmonary embolism).

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Pharmacokinetics and -dynamics of dabigatran etexilate and rivaroxaban in short bowel syndrome patients treated with parenteral nutrition: the PDER PAN study

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ABSTRACT

Patients on parenteral nutrition for short bowel syndrome often receive anticoagulants for the prevention or treatment of venous thromboembolism. However, reduced or erratic enteral absorption of vitamin K antagonists can preclude their use, while parenteral heparin administration is burdensome and may reduce compliance. Direct oral anticoagulants dabigatran etexilate and rivaroxaban may represent ideal agents, since they are mostly absorbed in the stomach and proximal bowel, which are usually maintained in patients with short bowel syndrome.

The aim of this randomized crossover study was to explore steady state pharmacokinetics and pharmacodynamics (PK/PD) parameters of dabigatran etexilate 150 mg twice daily and rivaroxaban 20 mg once daily in patients who required long-term parenteral nutrition for short bowel syndrome. We enrolled 6 adult patients with a remaining bowel length ≤170 cm after Treitz ligamentum, normal renal and hepatic function and no prior gastrectomy and obtained an extensive 10 time points PK profile, which was compared with reference PK parameters from phase III trials in patients with atrial fibrillation.

The observed-to-reference ratios of mean dabigatran $Cmax_{ss}$ and $Cthrough_{ss}$ were 0.57 and 0.52, and 0.73 and 0.76 for mean rivaroxaban AUC_{ss} and $Cmax_{ss}$. Dabigatran etexilate and rivaroxaban PK parameters were within reference values (5^{th} to 95^{th} percentile) in 33% (2 of 6) and 67% (4 of 6), demonstrating adequate absorption. Absorption was negligible in one patient with ultra-short bowel (15 cm).

Rivaroxaban and dabigatran etexilate might be attractive alternatives to vitamin K antagonists and heparin injections in selected patients with short bowel syndrome, although initial monitoring may be required to verify adequate absorption.

INTRODUCTION

Short bowel syndrome is a rare multicausal disorder also leading to inadequate absorption by oral or enteral intake. In adults, it usually develops after massive or multiple sequential bowel resections[1]. Enteral nutrition is preferred, whenever possible, to promote intestinal recovery. However, in case of severe short bowel syndrome, parenteral nutrition is required for maintaining a sufficient nutritional status and volemic balance, which depend on the length of remaining bowel and on the extent of post-surgical adaptive changes of the bowel surface[1,2]. Reduced bowel length affects the bioavailability of oral drugs in short bowel syndrome patients[2]. However, several other concomitant factors add to reduced drug absorption and metabolism, such as mucosal integrity, intestinal motility, site of drug absorption, type of formulation, co-morbidities, pH and parenteral nutrition-associated metabolic changes[2].

In recent cross-sectional studies, 25% to 66% of parenteral nutrition patients require systemic anticoagulation[3-6]. Common indications for their use are venous thromboembolism, atrial fibrillation and management of catheter-related thrombosis with an incidence of 2.2-4.5 per 100 patient-years in adult parenteral nutrition patients[3,7,8]. However, no interventional studies on PK parameters, efficacy and safety of anticoagulant agents were conducted in short bowel syndrome. In these patients, dosing of vitamin K antagonists to therapeutic INR levels can be difficult due to drug malabsorption, fluctuating vitamin K levels, bacterial overgrowth and hepatic impairment. Although low-molecular-weight heparin can be prescribed for long-term anticoagulant treatment[3,5,9], it is burdensome since it is given as daily subcutaneous injections, it often leads to skin hypersensitivity reactions[10], and carries the risk of heparin-induced thrombocytopenia and osteoporosis[11,12].

In recent years, direct oral anticoagulants have emerged as a first-line option for the treatment and prevention of venous thromboembolism and for stroke prevention in patients with atrial fibrillation[13]. In contrast with vitamin K antagonists, direct oral anticoagulants have a more stable PK profile, less interaction with co-medication, food, and vitamin K status, and routine laboratory monitoring is not recommended[13,14]. Being largely absorbed proximally in the gastrointestinal tract[14-16], the direct oral anticoagulants dabigatran etexilate and rivaroxaban could represent a therapeutic option in patients with short bowel syndrome. In the PDER PAN study, we aimed to determine whether dabigatran etexilate and rivaroxaban are sufficiently orally absorbed in patients with short bowel syndrome.

MATERIALS AND METHODS

The study was performed as an investigator-initiated, single center, randomized, open-label, interventional crossover phase I study. The primary objective was to determine PK parameters of therapeutic-dose dabigatran etexilate and rivaroxaban at steady state in patients with short bowel syndrome. The study protocol was registered at the Dutch Trial Register (NTR4192).

Study population

All patients who were or had been treated at the Department of Endocrinology and Metabolism of the Academic Medical Center (Amsterdam, the Netherlands) for the management of parenteral nutrition were screened between July 2013 and February 2015. Our institution is a tertiary university hospital in Amsterdam and one of the two national centers that manages patients on home parenteral nutrition.

Inclusion criteria were: diagnosis of short bowel syndrome and prior or current use of parenteral nutrition for at least 3 months, clinically stable, age 18 years or over, body weight 50 to 110 kg, total bowel length of 170 cm or less after Treitz ligamentum irrespective of the presence of colon.

Exclusion criteria were: partial or total gastrectomy, duodenum resection, moderate to severe renal or hepatic impairment, recent major bleeding, ongoing anticoagulant treatment for a condition considered to pose a high risk of thrombosis, cytochrome P450 (CYP)3A4- or P-glycoprotein-dependent co-medication use in the prior 14 days, chronic antiplatelet treatment, participation in another interventional study in the prior 30 days, pregnancy, significant hemostatic abnormalities, and presence of any condition that would place the subject at increased risk of harm.

More specific details regarding eligibility criteria are provided as Supplementary Material.

Study design and drugs

The dosages of dabigatran etexilate and rivaroxaban used in our study were those approved for non-valvular atrial fibrillation and treatment of venous thromboembolism: dabigatran etexilate 150 mg twice daily and rivaroxaban 20 mg once daily[13-16]. According to calculated half-lives in healthy volunteers[14-16], steady-state levels are reached after 3 days for dabigatran, and after 2 days for rivaroxaban.

A 5-day administration was chosen to ensure steady state of both drug concentrations.

At the screening visit, the eligibility criteria were verified, including a personal medical history, baseline screening tests and co-medications. On day 0, patients were randomized in two groups: subjects in group one started taking rivaroxaban 20 mg once daily (8 a.m.) from day 0 to day 4 (five doses in total), while patients in the other group received dabigatran etexilate 150 mg twice daily (8 a.m. - 8 p.m.) from day 0 to day 4 (nine doses in total) (Figure 1). After a wash-out period of at least 4 days, patients in both groups switched to the other direct oral anticoagulant and the procedure was repeated. As some patients with short bowel syndrome, despite needing parenteral nutrition, can ingest and absorb variable amount of food, all patients were asked to come on days 0 and days 4 in a fasting state to limit the inter-individual variability related to food exposure.

Randomization lists were automatically generated and were available to patients, study physicians and laboratory technicians. Study drugs were obtained through the pharmacy of our institution and administered in the commercially available formulation.

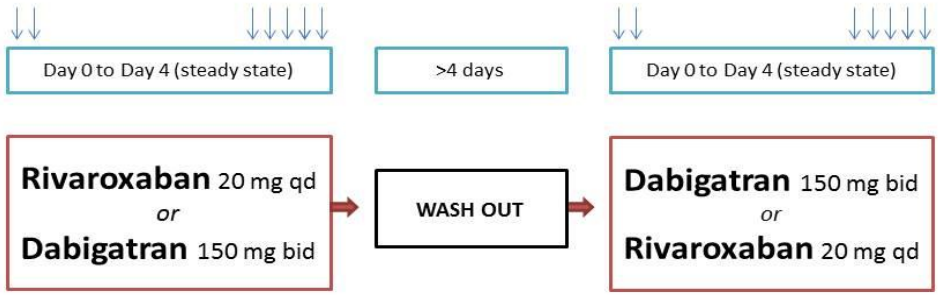


Figure 1. Study design

Rivaroxaban and dabigatran etexilate were administered for 5 days from Day 0 to Day 4 (in total, five daily doses of rivaroxaban and nine twice daily doses of dabigatran etexilate). Arrows indicate blood withdrawal (on Day 0, at before the first dose and 3 hours following the first dose; on Day 5, at T=0 [=trough] and 1, 2, 3, 4, 5, 6, 8, 10, 12 [dabigatran session only], 24 [rivaroxaban session only] hours following the last dose). Abbreviations: qd, once daily; bid, twice daily.

Blood sampling and procedures

One EDTA 4.5 mL tube and one citrated 3.2% 4.5 mL tubes were collected in all patients at the following time points:

- day 0: T=0 (=Cblank) and T=3 hours following the first dose of dabigatran etexilate or rivaroxaban;
- day 4: T=0 (=pre-dose or $Ctrough_{ss}$), T=1, 2, 3, 4, 5, 6, 8, 10, 12 (dabigatran etexilate session only), 24 (rivaroxaban session only) hours following the last dose of the study drugs. On days 4, a peripheral venous line for withdrawing blood was temporarily placed.

Citrated tubes were centrifuged 3000 *g* for 15 minutes at 15°C within one hour after withdrawal, while EDTA tubes were centrifuged at the same speed for 10 min at 4°C within 30 minutes after withdrawal. Samples were stored at -20°C until analysis.

Study parameters

Plasma drug concentrations (PK) were determined by ultra-performance liquid chromatography tandem mass-spectrometry (UPLC-MS/MS) as described elsewhere[17].

PD of dabigatran was measured through activated partial thromboplastin time (Actin FS, Siemens Healthcare, Marburg, Germany) and diluted thrombin time (Hemoclot thrombin inhibitor assay, HYPHEN BioMed, Neuville-sur-Oise, France). PD of rivaroxaban was measured by testing Neoplastin Plus prothrombin time (Diagnostica Stago, Asnières, France) and calibrated quantitative anti-factor Xa assay (STA Rotachrom, Diagnostica Stago, Asnières-sur-Seine, France).

Safety

Patients who were receiving an anticoagulant before the study period were bridged with low-molecular-weight heparin before, after and in between the study sessions. The timing of last low-molecular-weigth dose was chosen to not interfere with PK parameters of dabigatran and rivaroxaban (see Supplementary Material for detailed description).

Vital signs were measured at baseline and at each scheduled visit, and concomitant medications prescribed during the study period were systematically recorded. Adherence to study medication was evaluated by checking the blister packaging on day 4. Adverse events were recorded along with degree of severity, timing and onset.

Ethical considerations

The study protocol was approved by the Medical Ethics Committee of the Academic Medical Center. Each subject provided written consent to the participation in the study. The study was conducted in accordance with the Declaration of Helsinki (59th version Korea, October 2008), the Medical Research Involving Human Subjects Act (WMO) and according to guidelines for GCP. Subjects received financial compensation consistent with the amount of time required by the study and travel costs were reimbursed.

Statistical analysis

Baseline characteristics are presented as means (standard deviation, SD) or medians (interquartile range, IQR), and as numbers (percentages), where appropriate.

PK parameters were calculated by non-compartimental analysis and the following parameters obtained: $Tmax_{ss}$ (time to maximal concentration), $Cmax_{ss}$ (maximal concentration), $AUC_{0\text{-}t}$ (area under the plasma concentration *vs* time curve; dabigatran: 0-12 hours, rivaroxaban: 0-24 hours), adjusted CL/F (oral clearance), adjusted volume of distribution (Vd) and $T1/2_{ss}$ (elimination half-life) at steady state. The primary PK parameters were area under the concentration–time curve (AUC), maximum plasma concentration (Cmax), and half-life at steady state. PK data are presented as geometric means, coefficients of variation and 90% confidence intervals (90%CI) or as medians and interquartile range. PK parameters were compared to published reference PK data obtained in phase III trials[18;19] by calculating mean observed-to-reference ratios. Levels between the 5th and 95th percentile of phase III were considered adequate[18;19]. Pearson´s coefficient correlation was used to analyze the inter-assay comparisons for drug concentrations between ultra-performance liquid chromatography tandem mass-spectrometry and calibrated PD assays (calibrated quantitative anti-factor Xa assay for rivaroxaban and dTT for dabigatran). Calculations were made with PK Solver[20], SPSS version 21 (IBM, US), Graph Pad Prism 5 (GraphPad Software, US) and Microsoft Excel 2010 (Microsoft, US).

RESULTS

Baseline characteristics and laboratory variables

The medical charts of 153 individuals who were followed for parenteral nutrition management between 1986 and 2015 were screened for inclusion: of those, 75 patients were still receiving parenteral nutrition. Six patients fulfilled the eligibility criteria and were enrolled between July 2013 and February 2015. All subjects completed the study. Individual demographic and baseline characteristics are summarized in Table 1. Four patients were male; mean age, weight and remaining bowel length were 56 years (range 40-77), 79 kg (62-110) and 97 cm (15-170), respectively.

Pharmacokinetic parameters

Dabigatran

In 2 of 6 patients, the steady state curves of ultra-performance liquid chromatography tandem mass-spectrometry showed a clear increase in dabigatran plasma concentrations, while in the remaining four patients a peak was less pronounced (Figure 2a).

All calculated PK parameters and individual values are summarized in Table 2 and Figures 2a. $Tmax_{ss}$ was 2.5 hours (IQR 2.0-4.0), while median terminal $T1/2_{ss}$ was 6.5 hours (IQR 5.5-9.5). $Cmax_{ss}$ and $Cthrough_{ss}$ were 87.9 ng/mL (90%CI: 56.1-137.9) and 39.0 ng/mL (90%CI: 23.2-65.6), respectively. AUC_{∞} was 958.1 ng/ml*h (635.2-1,445). Oral clearance and volume distribution were 234.4 L/h (155.3-353.7) and 2,305 L (1,511-3,515), respectively (Table 2).

$Cmax_{ss}$ and $Cthrough_{ss}$ were lower than observed in patients with atrial fibrillation enrolled in the phase III RE-LY trial: mean $Cmax_{ss}$ ratio was 0.57 (range 0.26-1.08) and mean $Cthrough_{ss}$ was 0.52 (range 0.19-1.30)[19]. Two out of 6 (33%) short bowel syndrome patients had values that fit between the 5th and 95th percentile of reference geometric means[19].

Rivaroxaban

In 5 of 6 patients, the curves of ultra-performance liquid chromatography tandem mass-spectrometry showed an increase in rivaroxaban plasma concentrations at steady state (Figure 2b). Patient 3, who had the shortest bowel length (15 cm), showed a negligible increase.

All individual values and calculated PK parameters are summarized in Table 3. Median terminal $T1/2_{ss}$ was 4.9 hours (IQR 3.1-8.6), while median $Tmax_{ss}$ was 3.5 hours (IQR 2.0-5.0). $Cthrough_{ss}$ and $Cmax_{ss}$ geometric means were 9.3 ng/mL (90%CI: 1.2-71.5) and 167.3 ng/mL (90%CI: 101.6-275.6), respectively. AUC_{∞} geometric mean was 1,723 ng/mL*h (90%CI: 899.2-3,300). The coefficient of variations of $Cmax_{ss}$ and AUC_{∞} were 45% and 44%, respectively (Table 3).

If compared to atrial fibrillation reference patients, mean AUC_{ss} ratio was 0.73 (range 0.12-1.03) and mean $Cmax_{ss}$ was 0.76 (range 0.21-1.20). If compared to reference patients with deep venous thrombosis, mean AUC_{ss} ratio was 0.65 (range 0.57-0.92) and mean $Cmax_{ss}$ ratio was 0.70 (range 0.21-1.11)[18]. AUC_{∞} and $Cmax_{ss}$ of individual patients were between the 5th and 95th percentile in 5 and 3 patients, respectively[18].

Table 1. Summary of baseline characteristics of the subjects included in the study

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age, years	40	61	50	77	42	63
Sex	Male	Female	Male	Female	Male	Male
Weight, kg	76	62	72	60.5	110	74
Height, cm	187	162	174	165	176	192
eGRF, mL/min	>60	>60	>60	52	>60	>60
Bowel length, cm	140	50	15	35	170	170
Colon in continuity	No	Yes	No	Yes	No	Yes
Stoma	Ileostomy	Jejuno-ileostomy	Jejunostomy	No stoma	Ileostomy	No stoma
AST/ALT, U/L	140/-	48/71	30/32	29/49	59/50	40/39
PPIs use	Yes	Yes	Yes	Yes	Yes	No
Antidiarrheals use	No	Yes	No	Yes	Yes	No
Cause of intestinal resection	Ileus due to radiation enteritis (desmoid tumor)	Small bowel ischemia due to bowel torsion, carcinoid	Diverticulitis, bowel perforation, art. mes. thrombosis	Superior mesenteric vein thrombosis	Crohn disease	Small bowel ischemia due to bowel torsion
Months from bowel resection	53	34	59	38	32	17
Other comorbidities	FAP	None	Peripheral artery disease treated with PTAs	Protein S deficiency	APS, superior VCS, recurrent venous thrombosis	Esophagus carcinoma
AC ongoing	No	No	Warfarin i.v.	Rivaroxaban	Phenprocoumon, LMWH	No
AC length, months	NA	NA	59	38	13	No
Ongoing HPN (n infusions/week)	Yes (7)	Yes (6)	Yes (6)	No	No	No

The following co-medications were ongoing during the study treatment: Questran, Creon, PCM, Oxazepam, vitamin D (Patient 2); Vitamin D (Patient 3); Ranitidine Calcium, Vitamin D, Vitamin B12 (Patient 4); Metronidazole, Plaquenil, Codeine, Vitamin K, Tramadol, Vitamin D, Magnesium, NaCl Glucose (Patient 5); Ritalin, Vitamin D (Patient 6). Abbreviations: eGRF, estimated glomerular filtration rate; AST, ALT; FAP, familial adenomatous polyposis; APS, antiphospholipid syndrome; VCS, vena cava syndrome; PPI, proton pump inhibitor; LMWH, low-molecular-weight hepari; HPN, home parenteral nutrition; NA, not applicable; dd/w, days per week.

Table 2. Dabigatran etexilate 150 mg twice daily pharmacokinetic parameters

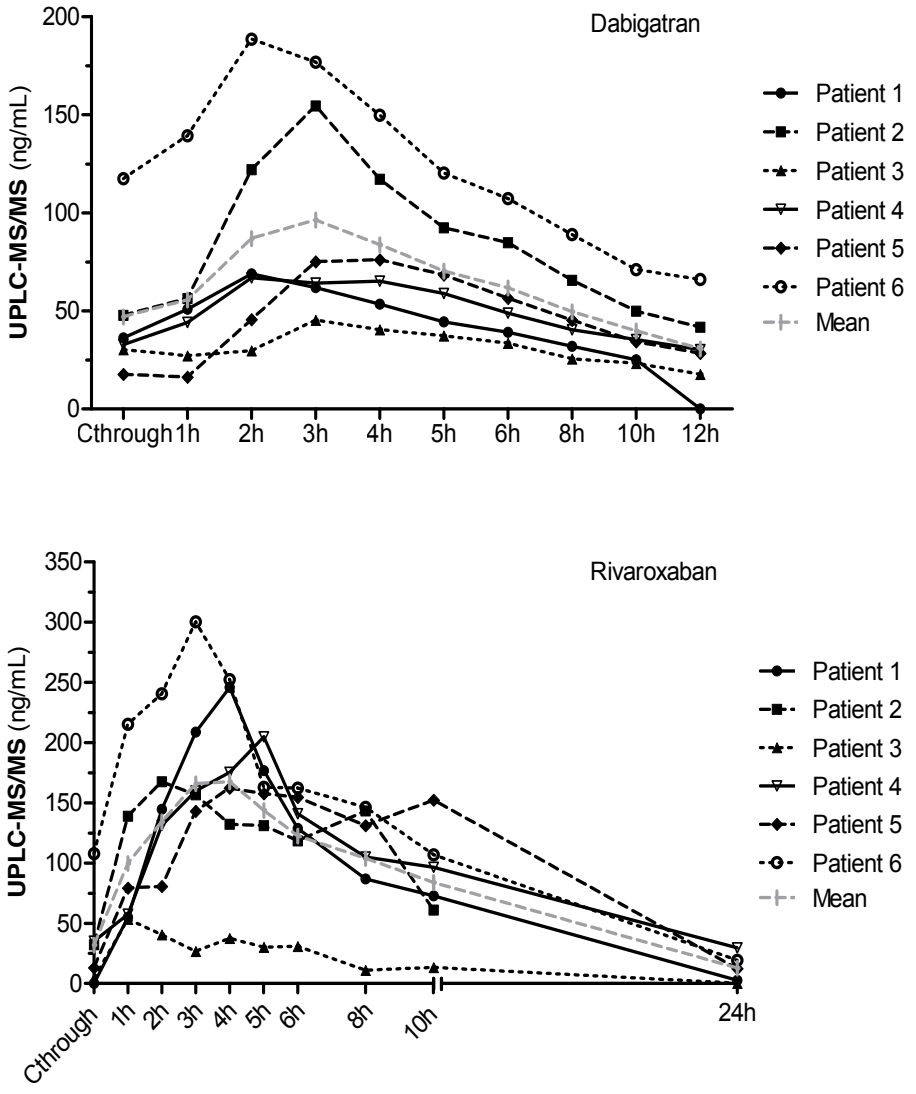
Dabigatran	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Total
C3h$_{firstdose}$ (µg/L)	90.4	37.6	28	65.6	59.0	56.9	52.5 (39, 37.2-74.0), 58
Cthrough$_{ss}$ (µg/L)	36.2	47.7	30.2	32.8	17.6	117.4	39.0 (76, 23.2-65.6), 34.5
Cmax$_{ss}$ (µg/L)	68.9	154.4	45.3	66.9	76.1	188.5	87.9 (57, 56.1-137.9), 72.5
AUC$_{\infty}$ (µg*h/L mL)	669.1	1318.3	532	985.8	787.8	2122.1	958.1 (55, 635.2-1445), 886.8
T1/2 (h)	6.2	5.9	6.8	9.5	5.5	7.8	6.5 (5.5-9.5)
Tmax (h)	2.0	3.0	3.0	2.0	4.0	2.0	2.5 (2.0-4.0)
CL [0-12] (L/h)	336.7	155.3	416.1	262.8	266.8	108.7	234.4 (44, 155.3-353.7), 264.8
Vd (L)	3000.7	1313.6	4057.3	3613.0	2120.4	1222.6	2305 (47, 1511-3515), 2560.6

In the last column, values are expressed as (geometric mean [coefficient of variation, 90% CI], median) for drug concentration after 3 hours from the first dose (C3h$_{firstdose}$), through concentration at steady state (Cthrough$_{ss}$), peak concentration at steady state (Cmax$_{ss}$), area under the curve (AUC$_{\infty}$), clearance (CL [0-12]) and volume of distribution (Vd). Half-life (t1/2) and Tmax are described by median and interquartile range.

Table 3. Rivaroxaban 20 mg once daily pharmacokinetic parameters

Rivaroxaban	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Total
$C3h_{firstdose}$ (µg/L)	131.9	180.9	21.4	164.9	197.2	249.3	126.7 (49, 60.6-264.8), 172.9
$Cthrough_{ss}$ (µg/L)	0	32.2	4.2	35.1	13.0	107.7	9.3 (124, 1.2-71.5), 22.6
$Cmax_{ss}$ (µg/L)	245.8	167.6	53.3	204.8	162.3	300.4	167.3 (45, 101.6-275,6), 186.2
AUC_{∞} (µg*h/L)	1798.0	2239.3	354.8	2515.8	2503.5	2904.8	1723 (44, 899.2-3300), 2371
T1/2 (h)	3.15	8.4	4.4	8.6	4.3	5.5	4.9 (3.1-8.6)
Tmax (h)	4.0	2.0	2.0	5.0	4.0	3.0	3.5 (2.0-5.0)
CL [0-24] (L/h)	11.1	10.6	73.7	9.3	8.2	7.3	13 (131, 6.4-26.5), 10
Vcorr (L)	50.5	128.7	46.3	115.1	51.6	58.2	68.6 (49, 47.3-99.4), 54.9

In the last column, values are expressed as (geometric mean [coefficient of variation, 90% CI], median) for drug concentration after 3 hours from the first dose ($C3h_{firstdose}$), through concentration at steady state ($Cthrough_{ss}$), peak concentration at steady state ($Cmax_{ss}$), area under the curve (AUC_{∞}), clearance (CL [0-12]) and volume of distribution (Vd). Half-life (t1/2) and Tmax are described by median and interquartile range.



Figures 2a and 2b. Steady-state dabigatran and rivaroxaban plasma concentrations and arithmetic means

Drug concentrations derived from pharmacodynamic measurements

The concentrations of the drugs calculated on the basis of PD parameters correlated well with the ultra-performance liquid chromatography tandem mass-spectrometry with a R^2 of 0.9 for both dilute thrombin time (dabigatran) and calibrated anti-factor Xa assay (rivaroxaban).

The arithmetic means and standard deviations of drugs plasma concentration, prothrombin time (rivaroxaban) and activated partial thromboplastin time (dabigatran) values are shown in Figures A and B (Supplementary Material). Mean $aPTT_{peak}$ ratio (dabigatran) and PT_{peak} ratio (rivaroxaban) (value at peak concentration / value at baseline) were 1.5 and 2.0, respectively.

Safety

At inclusion, 3 patients were receiving an anticoagulant treatment for prior thrombotic events (intravenous warfarin, rivaroxaban and phenprocumon being switched to heparin) and were bridged to the study drug without complications. None of the patients experienced adverse events during the study period.

DISCUSSION

In this study, we demonstrated that rivaroxaban and, to a lesser extent, dabigatran etexilate have substantial oral bioavailability in patients with short bowel syndrome treated with long-term parenteral nutrition. We observed large inter-individual variability which can be attributed to the small sample size as well as to the clinical heterogeneity of enrolled patients. Compared to PK data from phase III trials with therapeutic-dose dabigatran etexilate and rivaroxaban[18,19], 33% of patients had dabigatran etexilate blood levels between the 5th and 95th percentiles (reference range), and 67% had rivaroxaban levels within reference range. In one patient with very short remaining bowel length (15 cm) the absorption of both drugs was negligible.

It is yet unknown what the target levels are for dabigatran and rivaroxaban that ensure the optimal balance between risk of thrombosis and bleeding. We therefore used the reference ranges obtained from the phase III trials that led to regulatory

approval. Targeting reference ranges of plasma concentrations from those trials seems the best approach to reach a similar efficacy and safety profile of these drugs. For dabigatran, Cmaxss and Cthroughss have been shown to correlate with bleeding risk, and to a lesser extent, with stroke risk19. In the selected reference studies, patients were older (mean ages were 56 years[21] in venous thromboembolism and 73 years[22] in atrial fibrillation patients) and there was a relevant proportion of individuals with moderate renal failure or using interfering co-medications[21,22], contributing to increasing the plasma concentrations of both drugs. AUC_{∞} and Cmaxss geometric means of short bowel syndrome patients were comparable to those observed in two phase I studies on rivaroxaban 20 mg once daily enrolling healthy male volunteers with mean ages of 34-42 years (Studies 12359 and 10999 for FDA approval, available at www.accessdata.fda.gov).

The observed-to-reference blood level ratio was lower for dabigatran than for rivaroxaban. This may be explained by the low oral bioavailability of dabigatran. Furthermore, rivaroxaban, but not dabigatran etexilate, is mainly absorbed in the stomach[14], and short bowel syndrome patients had bowel resections only after the Treitz ligamentum. Therefore, dabigatran etexilate absorption might be more affected than rivaroxaban absorption, although results have to be interpreted with caution due to the small number of patients. Another factor that might have contributed to the apparent difference in drug absorption is co-administration of a proton pump inhibitor, recorded in 5 out of 6 patients of our cohort. Proton pump inhibitors are known to reduce Cmax and AUC of dabigatran etexilate by about 12.5%[16,23], while they do not modify PK parameters in rivaroxaban-treated patients[24].

The half-life of dabigatran in our study patients seems shorter than what is known from literature[14]. Both the oral clearance and volume distribution were significantly higher in the dabigatran etexilate sessions, since they depend on dabigatran plasma concentrations (and consequently low AUCss), which were very low due to reduced absorption. In this study we did not calculate the bioavailability of both drugs as we deemed it not ethical to expose patients to both oral and intravenous direct oral anticoagulants administration. However, based on the high oral clearance rates and distribution volumes we assumed a low bioavailability of dabigatran in patients with short bowel syndrome. Data in patients with short bowel syndrome suggest that half-life of rivaroxaban (4.9 hours; IQR 3.2-8.6) was shorter than what has been described in phase I studies in healthy volunteers, while both oral clearance and volume distribution were similar[14].

Although direct oral anticoagulants do not require routine monitoring, this might be different in short bowel syndrome due to the risk of lower exposures. The

concentrations of the drugs calculated on the basis of PD parameters had a good correlation with the ultra-performance liquid chromatography tandem mass-spectrometry assay and therefore dilute thrombin time-derived dabigatran concentrations and calibrated anti-FXa activity-derived rivaroxaban concentrations represent useful tools for this purpose.

The main limitation of our study is the small sample size and the residual high heterogeneity of the included individuals, despite the strict eligibility criteria. Patients with short bowel syndrome exposed to long-term parenteral nutrition represent a rare population, with a prevalence of 2 to 20 individuals on parenteral nutrition per million inhabitants in Europe[25,26]. Hence, enrolling a higher number of patients at a single center was deemed unfeasible. However, our study is the first PK study conducted in adults with short bowel syndrome treated with anticoagulants and one of the first observations of direct oral anticoagulants administration in this setting. To our knowledge, only 4 patients with short bowel syndrome receiving a direct oral anticoagulant have been described[27,28]. All of them showed adequate plasma concentrations of rivaroxaban if compared to reference values[27,28], while one of them had inadequate dabigatran concentrations before being switched to rivaroxaban[28]. The strength of our study is represented by the cross-over exposure to both direct oral anticoagulants, which allows a direct comparison within a single patient, and by the fact that the number of interfering factors has been minimized by strict eligibility criteria, leaving bowel-related factors as the main responsible of the observed results.

We conclude that in patients with short bowel syndrome, rivaroxaban and, to a lesser extent, dabigatran etexilate might be attractive alternatives to low-molecular-weight injections, but confirmation of adequate blood levels may be required.

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SUPPLEMENTARY MATERIAL

Amendment

The protocol was amended after the enrolment of the first patient. The amendment was done based on the feasibility to include patients with short bowel syndrome. Differences from the original protocol are represented by the inclusion of one subject aged 77 years (prior limit was set at 75 years old), of one subject with a body weigth of 110 kg (prior limit was set at 100 kg) and of two subjects with an estimate bowel length of 170 cm (prior limit was set at 150 cm). With respect to the latter point, it is known that the estimate of remaining bowel length is highly and intrinsically imprecise, since it is calculated from the supposed total length of bowel minus the length of bowel resected or from imaging tests performed in the patient during post-surgery follow-up.

Study population: exclusion criteria

- Moderate to severe renal impairment was defined as glomerular filtration rate (estimated with CKD-EPI formula) <50 mL/min); moderate to severe hepatic impairment was defined as Child-Pugh score B or C,
- Major bleeding events were defined according to the International Society on Thrombosis and Haemostasis definition for non-surgical patients: symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intra- articular or pericardial, or intramuscular with compartment

syndrome, and/or bleeding causing a fall in hemoglobin level of 20 g/L or more, or leading to transfusion of two or more units of whole blood or red cells.

- Cytochrome P450 3A4 and/or P-gp-dependent co-medications included: verapamil, azoles, amiodarone, dronedarone, azithromycin, erythromycin, clarythromycin, quinidine, ritonavir, cyclosporine, propafenone, isoniazid, rifampin, rifapentine, primidone, St. John's wort, carbamazepine, oxcarbazepine, phenobarbital, pentobarbital, nevirapine, nafcillin, fosphenonytoin.
- Chronic treatment with either non-aspirin non-steroidal anti-inflammatory drugs, aspirin at a dosage higher than 100 mg/day, or dual antiplatelet therapy were considered exclusion criteria.
- Ongoing anticoagulant treatment for a condition considered to pose a high risk of thrombosis was an exclusion criterion and included treatment for a recent thrombotic event.
- Bleeding and thrombotic events were considered recent if occurred in the prior 6 months.
- Patients with remaining bowel longer than 170 cm were excluded. According with the most recent ESPEN guidelines (2009): "Parenteral nutrition is likely to be needed if the remaining small bowel length is very short (e.g., less than 100 cm with a jejunostomy and less than 50 cm with a remaining colon in continuity). With longer lengths parenteral nutrition, water and electrolytes may be needed until oral/enteral intake is adequate to maintain nutrition, water and electrolyte status. […] Assuming strict compliance with dietary/water and electrolyte advice, after 2 years, dependency on parenteral nutrition is likely to be long-term.". On the basis of that, 170 cm has been considered a reasonable cutoff balancing the relative rarity of these patients, the intrinsic imprecision of bowel length estimate, and the grey area of patients with bowel length comprised between 100 and 170 cm for whom home parenteral nutrition administration varies throughout time according with bowel residual function and recovery after the first years from surgical resection.

Bridging schemes

The following general schemes are adapted according to individual patient anticoagulant indication and preferences.

Prior to receive the study drug (first dose on Day 0)

	DAY -6	DAY -5	DAY -4	DAY -3	DAY -2	DAY -1
LMWH	✓	✓	✓	✓	✓	No drug
Fondaparinux	✓	✓	✓	✓	✓	No drug
Acenocumarol	✓	✓	LMWH once-daily	LMWH once-daily	LMWH once-daily	No drug
Warfarin	✓	No drug	LMWH once-daily	LMWH once-daily	LMWH once-daily	No drug
Rivaroxaban	✓	✓	✓	✓	✓	✓
Dabigatran etexilate	✓	✓	✓	✓	✓	✓

Wash-out between the two sessions

The period length for wash-out/bridging is no shorter than 4 days:

Patients on low-molecular-weight heparin before enrollment receive the same low-molecular-weight heparin regimen starting 24 hours after the last dose of direct oral anticoagulant (I session). Low-molecular-weight heparin is discontinued 24 hours before the subsequent session;

Patients on vitamin K antagonists before enrollment receive once-daily low-molecular-weight heparin starting 24 hours after the last dose of direct oral anticoagulant (I session): low-molecular-weight heparin is discontinued 24 hours before the subsequent session.

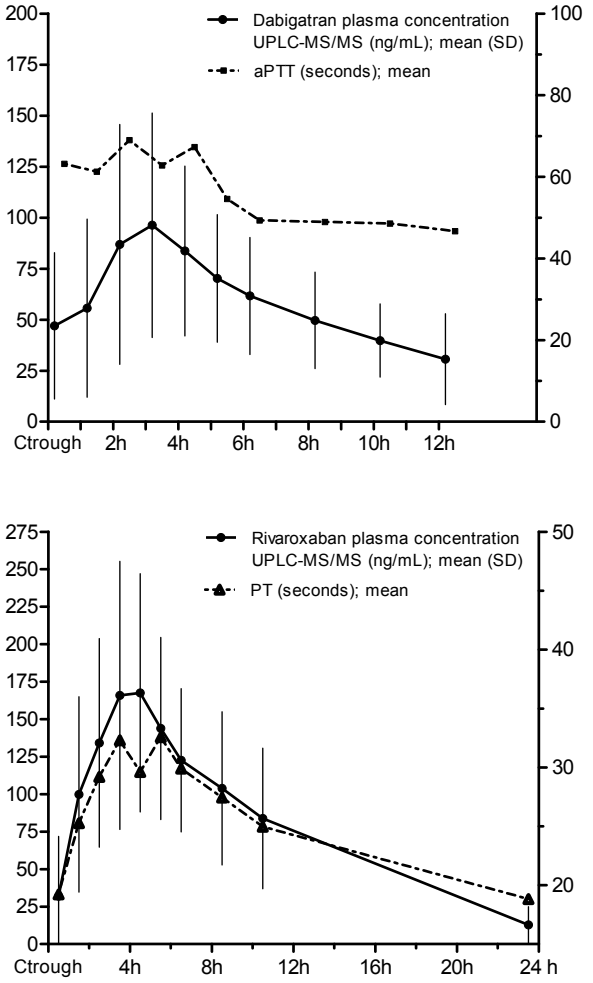
End of the study

Patients previously on low-molecular-weight heparin are prescribed with low-molecular-weight heparin at the same dose starting 24 hours after the last dose of direct oral anticoagulant.

Patients previously on vitamin K antagonist are prescribed with vitamin K antagonist, possibly bridged with once-daily low-molecular-weight heparin until a therapeutic INR

target was reached, in line with the usual clinical practice and accordingly with anticoagulant indication;

Patients previously on rivaroxaban are prescribed with rivaroxaban at the same dose starting 24 hours after the last dose of direct oral anticoagulant.



Figures A and B. Arithmetic means of UPLC-MS/MS concentration, prothrombin time (rivaroxaban) and activated partial thromboplastin time (dabigatran) values

PART III

Venous thrombosis in special populations

8

Cerebral venous thrombosis and thrombophilia: a systematic review and meta-analysis

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ABSTRACT

Cerebral venous thrombosis is a rare manifestation of venous thromboembolism and stroke.

The aim of our systematic review was to provide an updated summary of the strength of association between cerebral venous thrombosis and thrombophilia and to explore the relevance of thrombophilia for recurrence of cerebral venous thrombosis or other venous thromboembolism, or other outcome variables.

MEDLINE (via PubMed), EMBASE (via Ovid) and CENTRAL were systematically searched, including references of retrieved articles. Cohort studies of ≥40 patients and case-control studies comparing the prevalence of thrombophilia in patients with cerebral venous thrombosis and unrelated controls were eligible. Two reviewers independently selected studies, assessed quality and extracted data. A meta-analysis was performed for high quality case-control studies with unselected cases and healthy controls. Odds ratios with 95% confidence intervals were calculated and pooled.

We included 23 cohort studies and 33 case-control studies. A significant association was demonstrated between cerebral venous thrombosis and all inherited thrombophilic factors, as well as increased levels of homocysteine. Inconclusive results were found on the relevance of thrombophilia for recurrent cerebral venous thrombosis or other venous thromboembolism.

Although there is a strong association between cerebral venous thrombosis and thrombophilia, the clinical relevance of thrombophilia testing in patients with cerebral venous thrombosis seems limited, similarly to other forms of venous thromboembolism.

INTRODUCTION

Cerebral venous thrombosis is a rare manifestation of venous thromboembolism and stroke, accounting for <1% of all strokes[1]. Its incidence is estimated at 2-5 cases per 1,000,000 individuals[2,3], although a recent cross-sectional study suggests it may be higher than previously recognized (1.32 per 100,000 person-years)[4]. A significant proportion of cerebral venous thrombosis occurs secondary to local risk factors such as surgery, infections, trauma or brain tumors, and systemic conditions such as inflammatory diseases or malignancies. Cerebral venous thrombosis may also be associated with the use of oral contraceptives, pregnancy or the postpartum period[2,3,5,6]. In the last two decades, several inherited and acquired thrombophilic factors were discovered. Large studies have confirmed the association between, for example, the factor V Leiden mutation, the prothrombin G20210A mutation and an increased risk of deep vein thrombosis of the leg or pulmonary embolism[7,8]. Hence, thrombophilia is often tested for in patients with venous thromboembolism. Recent studies indicate that thrombophilia testing only serves a limited purpose[9,10]. Guidelines thus advise against routine thrombophilia testing for idiopathic venous thromboembolism[11].

Due to the rarity of cerebral venous thrombosis, neither the association with thrombophilia, nor the relevance of thrombophilia testing has been as extensively examined as in deep venous thrombosis or pulmonary embolism.

The aim of our systematic review was to provide an updated summary of the strength of association between cerebral venous thrombosis and inherited or acquired thrombophilia. We also aimed to evaluate the strength of this association compared with patients with non-cerebral venous thromboembolism, and to explore the relevance of thrombophilia for recurrence of cerebral venous thrombosis or other venous thromboembolism, or other outcome variables.

We considered the following thrombophilic factors: factor V Leiden mutation, prothrombin G20210A mutation; antithrombin, protein C or protein S deficiency; high factor VIII levels; JAK2 V617F mutation; antiphospholipid syndrome (presence of anticardiolipin antibodies, antiphospholipid or anti-β2 glycoprotein antibodies, and/or lupus anticoagulant); PAI-1 polymorphisms or hyperhomocysteinemia.

MATERIAL AND METHODS

Study identification

We systematically searched MEDLINE (January 1966 to January 2013, week 2; via PubMed), EMBASE (January 1980 to January 2013, week 2; via OVID) and the Cochrane Central Register of Controlled Trials (CENTRAL, the Cochrane Library, Issue 1 2013). The search strategy was developed in accordance with professional librarian instructions; we did not apply language restrictions and used a wide number of keywords and subject headings (full strategy available upon request). We also manually reviewed references of retrieved articles.

Study selection

Studies were included if they met all of the following criteria:

1) diagnosis of cerebral venous thrombosis was objectively confirmed by accepted imaging methods (computed tomographic -venography, magnetic resonance imaging with magnetic resonance-venography, or conventional angiography);

2) patients were compared with a control group of non-genetically related subjects, or patients were analyzed in a moderate to large-size (n ≥40) retrospective or prospective cohort study (selection criteria for inclusion were recorded);

3) prevalence of at least one of the following thrombophilic factors was assessed: factor V Leiden mutation; prothrombin G20210A mutation; antithrombin, protein C or protein S deficiency; high factor VIII levels; JAK2 V617F mutation; antiphospholipid syndrome (presence of anticardiolipin antibodies, antiphospholipid or anti-β2 glycoprotein antibodies, and/or lupus anticoagulant); PAI-1 polymorphisms; or hyperhomocysteinemia; and

4) inherited or acquired thrombophilic factors were measured in a commonly accepted manner.

Studies on other variants of thrombophilic mutations or MTHFR mutations were considered outside the scope of this review. Observational studies that assessed prevalence of cerebral venous thrombosis and thrombophilia, and clinical interventional studies for cerebral venous thrombosis, such as anticoagulation treatment, were eligible. Conference abstracts were only considered if sufficient data on study design, participants, events and outcome measures were available for data extraction. Duplicate reports of the same study and studies with considerable

(population) overlap (> 50%) were excluded to avoid publication bias; the most recent full-text articles were used. If necessary, authors were contacted for more information and verification of overlap between study populations in different papers. Two authors (SB and MNL) independently assessed the eligibility of articles for inclusion in the review. Discrepancies were resolved through discussion or with the opinion of a third author (SM).

Study assessment

Although the use of quality scales for observational studies is controversial[12, 13], we used the Newcastle-Ottawa Scale (NOS) for assessment of risk of bias and quality of evidence of case-control studies[14]. A meta-analysis was performed only with studies that a) obtained the highest scores on this scale (8 or 9 stars) and b) included consecutive unselected patients.

Data extraction

Data were systematically and independently extracted using a standardized data extraction form by two reviewers (MNL and SB). Discrepancies were resolved through discussion or with the opinion of a third author (SM).

The following parameters of studies were collected: study characteristics, baseline characteristics of study population (and controls), outcome measures, interventions and results. Number and proportion of patients (and controls) for one or more of the predefined assessed thrombophilic factors (see study selection) were collected of each study. If necessary, study authors were contacted for more information. Each study is summarized according to its unadjusted odds ratio (OR).

Statistical analysis

We used Review Manager (RevMan; version 5.2 for Windows; Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2012) to pool data for each risk factor for case-control studies included in the meta-analysis, by using the Mantel-Haenszel method and a random-effects model to correct for potential heterogeneity. Data were only pooled if a thrombophilic factor was investigated in at least three case-control studies. Statistical heterogeneity between studies was assessed by using the I^2 statistic ($I^2 \leq 30\%$ low heterogeneity, $I^2 > 30\%$ moderate heterogeneity, $I^2>50\%$ substantial heterogeneity). We made funnel plots of effect size (OR) against standard

error for meta-analyses with more than 5 studies to assess presence of publication bias. Pooled results were reported as ORs and presented with 95% confidence interval (95%CI) and two-sided p-values, with $p<0.05$ considered statistically significant.

RESULTS

Study identification and selection

We identified 1,355 studies using our search strategy: 516 from PubMed, 761 from EMBASE and 78 from CENTRAL. We excluded 1,222 studies after screening for duplicates and evaluation of titles and abstracts using the predefined inclusion and exclusion criteria. We retrieved 133 studies in full-text for detailed evaluation and verification of overlaps in study populations. An additional 16 studies were obtained from manually reviewing references of retrieved articles for full-text evaluation. In total, 93 studies were subsequently excluded: 54 studies did not meet the inclusion criteria, 17 studies presented duplicate data or had overlapping populations, and 20 studies did not report crucial data. Two articles were excluded because of our inability to translate from Chinese. Cohort and case-control studies were considered as separate groups; overlaps in populations between them were not further evaluated. Finally, we included 23 cohort studies[15-37] and 33 case-control studies[38-70] in our systematic review (Figure 1).

To evaluate the strength of association between thrombophilia and cerebral venous thrombosis in comparison with deep venous thrombosis or pulmonary embolism (Table 2b), data from one case-control study, that was not included in further analyses due to overlap in study populations[71], were extracted. To explore the relevance of thrombophilia for recurrence of cerebral venous thrombosis or other venous thromboembolism, or other outcome variables (Table 3a and 3b), data from two other studies (one cohort[72] and one case-control[73]) were extracted, which were not included in other analyses due to overlap in study populations.

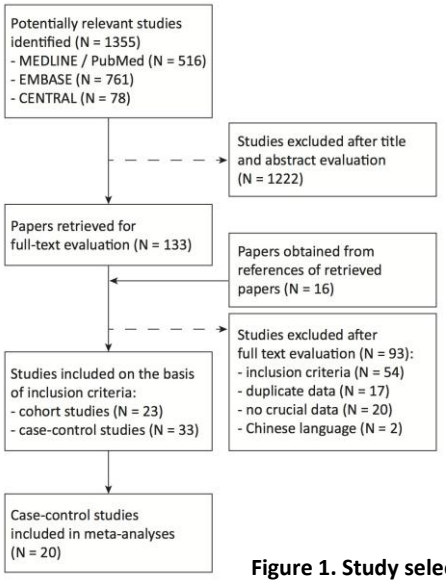


Figure 1. Study selection

Study characteristics

The majority of included studies investigated adult patients or a mixed population, while one cohort study 15 and three case-control studies[47,48,64] evaluated only children younger than 18 years. In all cohort and case-control studies, patients were investigated for presence of one inherited or acquired thrombophilic factor only, and not for combined abnormalities or interactions between multiple factors. If combined thrombophilic abnormalities were present in a study population, we recorded this in the Tables. For studies on hyperhomocysteinemia, we only extracted data regarding fasting levels of homocysteine.

Cohort studies

The 23 cohort studies included 3,345 patients[15-37] with the number of cerebral venous thrombosis cases per study ranging from 40 to 706. Tables 1a and 1b summarize the baseline characteristics and results of the cohort studies, divided in studies with unselected patients or without specified inclusion criteria (Table 1a), and studies with specific selection criteria for inclusion, respectively.

Table 1a. Prevalence of thrombophilia in cohort studies with unselected patients with cerebral venous thrombosis or without specified inclusion criteria

Study		Pts. n (♀)	Age[a]	Source	FV[b]	FII[b]	AT[b]	PC[b]	PS[b]	JAK2[b]	LAC[b]	aCL[b]	APLs[b]	HH[b]
deVeber 2001[15]	p	160 (74)	(0-18)	multicenter	3/123 (2.4)	1/123 (0.8)	7/123 (5.7)	9/123 (7.3)	5/123 (4.1)	-	-	10/123 (8.1)	-	-
Camargo 2005[16]	p	50 (35)	35 (8-81)	one center	2/49 (4)	4/49 (8)	0/49 (0)	6/49 (12)	2/49 (4)	-	-	-	5/49 (10.2)	-
Terazzi 2005[17]	r	48 (38)	45 (19-77)	multicenter	5/48 (19.2)[c]	-	1/48 (3.8)[c]	2/48 (7.7)[c]	2/48 (7.7)[c]	-	-----1/48 (3.8)[c]-----		-	3/48 (11.5)[c]
Wasay 2008[18]	rp	182 (109)	38 (13-82)	multicenter	1/35 (2.8)	-	1/92 (1.1)	3/115 (2.6)	4/115 (3.5)	-	----------7/94 (4)----------			9/89 (10.1)
Coutinho 2009[19]	p	624 (465)	34 F 42 M	multicenter	33/440 (7.5)	37/438 (8.4)	4/431 (0.9)	20/436 (4.5)	33/439 (7.5)	-	-	-	-	-
Algahtani 2011[20]	r	111 (78)	30 (1-80)	one center	1/111[d] (0.9)	-	1/111[d] (0.9)	1/111[d] (0.9)	5/111[d] (4.5)	-	-	-	15/111[d] (13.5)	-
Dentali 2012[21]	r	706 (520)	40 (±16.3)	multicenter	51/560 (9.1)	105/551 (19.1)	11/564 (2)	18/552 (3.3)	18/550 (3.3)	17/131 (13.0)	28/554 (5.1)	18/556 (3.3)	-	69/546 (12.6)
Narayan 2012[22]	r	428 (198)	32 (8-65)	one center	-	-	22/428 (5.1)	39/428 (9.1)	53/428 (12.3)	-	-	31/428 (7.2)	-	78/428 (18.2)
Uzar 2012[23]	r	47 (31)	30 (5-65)	one center	4/47 (8.5)	2/47 (4.3)	2/47 (4.3)	2/47 (4.3)	4/47 (8.5)	-	6/47 (12.8)	-------2/47 (4.3)-------		3/47 (6.4)
Total		**2356 (1548)**												

(FV, heterozygosity for the Factor V Leiden mutation; FII, heterozygosity for mutation G20210A of the prothrombin gene; AT, antithrombin deficiency; PC, protein C deficiency; PS, protein S deficiency; FVIII; high factor VIII levels; JAK2, JAK2 V617F mutation; LAC, lupus anticoagulant; aCL, anticardiolipin antibodies; APLs, antiphospholipid syndrome; HH, hyperhomocysteinemia; r, retrospective study; p, prospective study; rp, retroprospective study; F, female; M, male; CVT, cerebral venous thrombosis)

a. Age is expressed as mean age (age range) or (±SD)
b. Data are expressed as n, number of patients with thrombophilia/N, total number of patients (%)
c. Percentages calculated for the 26 patients in whom all factors were investigated
d. Personal communication from the author

Table 1b. Prevalence of thrombophilia in cohort studies with specific selection criteria for the included study population

Study		Patients n (♀)	Age[a]	Exclusion criteria	FV[b]	FII[b]	AT[b]	PC[b]	PS[b]	FVIII[b]	JAK2[b]	LAC[b]	aCL[b]	APLs[b]	HH[b]
Deschiens 1996[24]	r	40 (30)	36 (19-71)	delay in thrombophilia tests	4/40 (10)	-	0/40 (0)	1/40 (2.5)	1/40 (2.5)	-	-	-	3/40 (7.5)	-	-
de Bruijn 2001[25]	p	59 (50)	37 (18-80)	contraindication to heparin, pregnancy, surgery	7/51 (14)	-	-	2/51 (4)	-	-	-	-------6/42 (14)-------		-	6/15 (40)
Breteau 2003[26]	r	55 (42)	39 (16-68)	isolated cavernous sinus thrombosis	1/55 (1.8)	3/55 (5.4)	-	2/55 (3.6)	4/55 (7.2)	-	-	-	-	-	-
Amberger 2004[27]	p	56 (44)	41	no compliance or no relatives	9/56 (16)[e]	3/56 (5.3)[e]	-	1/56 (1.8)	-	-	-	-	-	-	-
Stolz 2005[28]	r p	79 (61)	43 (17-78)	no compliance, inpatients (FUP)	4/58 (6.9)	6/58 (10.3)	-	2/58 (3.4)	2/58 (3.4)	-	-	4/58 (6.9)	-	-	-
De Stefano 2007[29]	r	45 (36)	35 (19-80)	overt cancer, MPN (JAK2 analysis only)	5/45 (11.1)	4/45 (8.8)	---------3/45 (6.6)[d]---------			-	2/41 (4.8)	------3/45 (6.6)[d]------		-	0/45 (0)
Bellucci 2008[30]	r	87 (71)	35 (16-77)	MPN	11/87 (12.6)	2/87 (2.2)[c]	---------7/87 (2.2)---------			-	1/87 (1.1)	-	-	6/87 (6.6)	-
Zubkov 2009[31]	r	56 (37)	40 (3-84)	underlying brain lesions, no images	8/56 (14.2)	2/56 (3.6)	-	-	2/56 (3.6)	-	-	3/56 (5.4)	-	-	-
Alonso-Canovas 2009[32]	r	79 (43)	46 (2-82)	intravenous device-related CVT	3/79 (3.8)	10/79 (12.7)	-	2/78 (2.5)	-	-	-	4/79 (5.1) APLs I 3/79 (3.8) APLs II			-
Martinelli 2010[33]	r	145 (106)	33 (11-89)	cancer, AC therapy, short FUP	12/145 (8)	27/145 (19)	---------13/145 (9)[d]---------			-		----------13/145 (9)[d]----------			-
Aaron 2010[34]	p	41 (41)	23 (22-40)	nonpregnancy-related CVT	3/41 (7.3)[f]	0/41 (0)[f]	1/41 (2.4)	2/41 (4.8)	4/41 (9.7)	6/41 (14.6)	-	5/41 (12)	0/41 (0)	-	14/41 (34)
Yokus 2010[35]	r	43 (30)	36 (16-50)	provoked CVT	13/43 (30.2)[g]	4/43 (9.3)	0/43 (0)	2/43 (4.7)	8/43 (18.6)	2/43 (4.7)	-	8/43 (18.3)	-	-	2/43 (4.7)
Sanz-Gallego 2011[36]	r	52 (37)	47 (18-87)	provoked CVT partially excluded	2/52 (3.8)	4/52 (7.6)	0/52 (0)	-	2/52 (3.8)	-	-	2/52 (3.8)	-	-	4/52 (7.6)
Passamonti 2012[37]	r	152 (112)	35 (16-79)	cancer, no DNA, CVT >12 months, MPN (JAK2 analysis only)	17/152 (11.2)	33/152 (27.7)	---------7/152 (4.6)---------			-	4/146 (2.7)	2/52 (3.8)	-----11/112 (7.2)-----		34/152 (22.4)
Total		**989 (740)**													

(FV, heterozygosity for the Factor V Leiden mutation; FII, heterozygosity for mutation G20210A of the prothrombin gene; AT, antithrombin deficiency; PC, protein C deficiency; PS, protein S deficiency; FVIII, high factor VIII levels; JAK2, JAK2 V617F mutation; LAC, lupus anticoagulant; aCL, anticardiolipin antibodies; APLs, antiphospholipid syndrome; HH, hyperhomocysteinemia; r, retrospective study; p, prospective study; AC, anticoagulant; MPN, myeloproliferative neoplasm; FUP, follow-up; CVT, cerebral venous thrombosis)

a. Age is expressed as mean age (age range) or (±SD)
b. Data are expressed as n, number of patients with thrombophilia/N, total number of patients (%)
c. One further patient was found homozygous for prothrombin mutation (1/87, 1.1%)
d. Including AT, PC, PS deficiency, APL antibodies, and combined abnormalities (defined as severe thrombophilia)
e. One further patient was found to have a double heterozygosity for both the Factor V Leiden and prothrombin G20210A mutation
f. One further patient was found homozygous for the Factor V Leiden mutation (1/41, 2%)

Two further patients were found homozygous for the Factor V Leiden mutation (2/43, 4.6%)

The cohort studies showed that thrombophilia was present in a considerable proportion of patients with cerebral venous thrombosis; the highest prevalence was found for heterozygosity for the factor V Leiden and prothrombin G20210A mutation, and the acquired factor hyperhomocysteinemia. High factor VIII levels and the JAK2 V617F mutation were only assessed in two[34,35] and four studies[21,29,30,37], respectively, which investigated thrombophilia in a specific subselection of patients with cerebral venous thrombosis.

Case-control studies

The 33 case-control studies included 1,639 patients and 6,201 controls[38-70], with the number of cerebral venous thrombosis cases per study ranging from 7 to 163. In 31 of 33 studies, unrelated healthy subjects were used as controls[38-68], whereas two studies compared cerebral venous thrombosis patients to non-cerebral venous thromboembolism cases (deep venous thrombosis or pulmonary embolism)[69,70]. Table 2a summarizes the baseline characteristics of case-control studies comparing cerebral venous thrombosis cases with unrelated healthy subjects in the control group. Table 2b summarizes the baseline characteristics of studies comparing cerebral venous thrombosis patients with non-cerebral venous thromboembolism cases.

Meta-analyses of case-control studies

The 31 case-control studies with unrelated healthy subjects as controls were assessed for risk of bias and quality of evidence using the NOS for case-control studies. A total of 20 case-control studies obtained the highest score on the (8 or 9 stars) and included consecutive unselected patients; these studies were included in our meta-analyses[38,40,44,46-51,53,55,56,59-65,67]. We did not perform a meta-analysis on the following thrombophilic factors due to the limited number of available studies: high factor VIII levels (n=1), JAK2 V617F mutation (n=1), antiphospholipid syndrome (presence of anticardiolipin antibodies (n=2), antiphospholipid (n=1) or anti-β2 glycoprotein antibodies (n=0), and/or lupus anticoagulant (n=2)) and PAI-1 polymorphisms (n=1).

Table 2a. Case-control studies using healthy subjects as control group

Study		Cases n (♀)	Age[a]	Inclusion criteria	Exclusion criteria	Controls n (♀)	Age[a]	Inclusion criteria	Tests[*]
Martinelli 1996[38]	r	25 (20)	32 (21-64)	patients with CVT referred for coagulation screening	local infection-related CVT episode	75	-	age- and sex-matched healthy controls	FV (AT, PC, PS, APLA)
Zuber 1996[39]	r	19 (15)	-	patients with CVT from a single center	-	57	-	age- and sex-matched healthy controls	FV (AT, PC, PS, APLA)
Lüdemann[c] 1998[40]	r	55 (40)	40 (11-83)	patients with nonfatal CVT from two German centers	-	272	(18-55)	healthy subjects from the same geographical region	FV (PC, PS, aCL)
Hillier 1998[41]	r	15 (13)	(17-76)	patient with CVT from a single center	-	300	-	healthy subjects from the South Wales population	FV, FII, AT, PC, PS, LAC
Weih 1998[42]	r	12 (10)	34 (21-60)	patients with nonfatal CVT from one center	septic venous thrombosis, neoplasm-related CVT, or of non-German descent	187 (112)	-	age and sex-matched healthy subjects	FV (AT, PC, PS)
Christopher 1999[43]	r	31 (26)	28 (±8)	patients with CVT admitted to one center	patients with infection-, trauma-, or surgery-related CVT	31 (26)	27 (±8)	age- and sex-matched healthy volunteers	aCL
Madonna 2000[44]	r	10 (6)	35	consecutive patients referred to one center for a history of CVT	-	259 (144)	37	healthy subjects from the same ethnic background	FV, FII
Voetsch 2000[45]	c s	14 (10)	25 (16-31)	first nonfatal CVT episode, consecutive patients	PC/PS/AT deficiency, APLA, systemic disease, cancer	225 (126)	34 (16-50)	matched healthy subjects working in the hospital	FV, FII
Bombeli 2002[46]	r	51 (37)	37 (17-61)	unselected patients admitted for thrombophilia screening	-	120 (72)	37 (19-62)	healthy volunteers (hospital staff)	FV, FII, AT, PC, PS
Bonduel 2003[47]	p	23 (3)	7.2 (0-16)	consecutive children with first onset of cerebral thromboembolism	patients with congenital heart diseases and vascular abnormalities	102 (42)	7.1 (0-16)	healthy potential bone marrow donors or undergoing minor surgery	FV, FII (AT, PC, PS, LAC)
Heller 2003[48]	p	149 (65)	6.0 (0-18)	consecutive admitted term neonates and children with first CVT episode	preterm infants, older than 18 years at onset, non-Caucasian, incomplete workup	149	6.2 (0-18)	age- and sex-matched patients, matched for underlying diseases	FV, FII, PC, PS, aCL (AT)
Martinelli 2003[49]	r	121 (91)	33 (12-64)	unrelated patients with CVT	incomplete thrombophilia screening, previous VTE	242 (182)	36 (13-62)	age- and sex-matched healthy individuals	FV, FII, AT, PC, PS, APLA, HH
Boncoraglio 2004[50]	p	26 (19)	43 (21-73)	consecutive unrelated patients with a first episode of CVT	-	100 (72)	41 (21-72)	healthy hospital employees	FV, FII, HH (AT, PC, PS, LAC, aCL)

Cantu 2004[51]	r	45 (38)	28 (14-55)	consecutive patients with nonfatal first CVT	patients lost to FUP	90 (67)	28 (16-53)	healthy matched subjects	-	HH (FV, FII, AT, PC, PS, LAC, aCL)
Ventura 2004[52]	r	30 (14)	35 (16-49)	idiopathic CVT from the same ethnic background (white)	provoked CVT	40 (18)	34 (18-51)	age- and sex-matched healthy subjects from the hospital staff	cancer, cardiac, renal, hematological, or liver diseases	FV, FII (AT, PC, PS, HH)
Kenet 2004[54]	r	38 (15)	5.6 (±4)	consecutive children from newborn period to 18 years	preterm infants	112 (52)	6.3 (±5.8)	healthy subjects	cancer, acute infectious diseases, known coagulopathies	FV, FII, AT, PC, PS
Rodrigues 2004[55]	r	42 (28)	28	consecutive patients	-	134 (80)	34	healthy unrelated subjects	-	FV, FII
Le Cam-Duchez 2005[56]	r	26 (18)	41 (2-75)	consecutive patients	no consent for participating in study	84 (50)	36 (20-62)	age- and sex-matched healthy volunteers	family or personal history of VTE	FV, FII (AT, PC, PS, APLA)
Gadelha 2005[57]	r	26 (21)	28 (3-46)	patients with CVT younger than 50 years	cancer, diabetes mellitus, APLA, infectious or collagen disease	217 (134)	29 (15-62)	healthy sex-, age-, racial background-matched subjects	related patients with history of thrombosis, pregnancy	FV, FII
Tufano 2005[53]	r	20 (12)	34 (±12)	consecutive patients	age > 50 years	328 (195)	37 (±10)	age- and sex-matched apparently healthy volunteers	different ethnic background	FV, FII
Dindagur 2006[58]	r	86 (86)	24 (±4)	patients with CVT within 4 weeks after delivery tested for FV	provoked CVT (sepsis, cancer, blood cell disorders), renal/hepatic dysfunction	86 (86)	24 (±4)	age-matched healthy women	complications during puerperium	FV, FII
Lichy 2006[59]	r	77 (60)	38	consecutive nontraumatic CVT	-	203 (118)	36	healthy volunteers randomly selected	history of cerebrovascular disease	FV, FII (AT, PC, PS, APLA, HH)
Bugnicourt 2007[60]	r	16 (13)	47 (20-73)	consecutive patients with CVT	lost to FUP (n = 5), refused interview (n = 3), dead from nonvascular cause (n = 1)	64	-	age and sex-matched healthy blood donors	-	FVIII (FV, FII, PC, PS, LAC, HH)
Colaizzo 2007[61]	r	45 (31)	40 (13-55)	Caucasian patients referred for thrombophilia workup	anticoagulant treatment	286 (163)	44 (21-73)	healthy Italian hospital employees without personal history of VTE	-	FV, FII, JAK2[b] (AT, PC, PS, APLA)
Stolz 2007[62]	p	121 (93)	43 (18-85)	infectious-related CVT	healthy	120 (90)	43 (22-82)	age- and sex-matched healthy volunteers from hospital staff and medical students	-	FV, FII, PC, PS, LAC

Cesarman-Maus 2010[63]	r	40 (33)	28 (14-61)	consecutive patients with CVT	-	145 (145)	37 (22-63)	subjects who met standard criteria for blood donation	history of thrombosis	FV, AT, PC, PS (APLA)
Laugesaar 2010[64]	r	7	-	patients with neonatal and perinatal stroke (including sinovenous thrombosis)	major head trauma	400 (193)	-	newborns born consecutively in all delivery departments of Estonia	-	FV, FII (AT, PC, LAC)
Rahimi 2010[65]	r	24 (17)	37 (±12)	Kurd patients with CVT from Western Iran	-	100 (50)	36 (±13)	age- and sex-matched healthy subjects	-	FV, FII
Ringelstein 2012[66]	r	136 (103)	41 (16-85)	consecutive Caucasians or Western European patients	provoked CVT, related patients	1054 (561)	55 (25-74)	healthy participants to a population-based survey	history of stroke	FV, FII, PAI-1
Ashjazad 2012[67]	r	57 (38)	34 (±9)	patients with CVT from Fars province, Southern Iran	-	50 (35)	-	healthy subjects matched for age, sex, ethnicity	family history of thrombosis	FV, FII
Ben-Salem Berrabah 2012[68]	r	26 (21)	38 (15-72)	patients with CVT from a single center (Tunis)	-	197 (71)	31 (14-54)	healthy subjects	-	FV, FII
Total		**1413 (968)**				**5829**				

(FV, heterozygosity for the Factor V Leiden mutation; FII, heterozygosity for mutation G20210A of the prothrombin gene; AT, antithrombin deficiency; PC, protein C deficiency; PS, protein S deficiency; FVIII, high factor VIII levels; JAK2, JAK2 V617F mutation; LAC, lupus anticoagulant; aCL, anticardiolipin antibodies; APLA, antiphospholipid antibodies; APLs, antiphospholipid syndrome; PAI-1, plasminogen activator inhibitor-1 polymorphisms; HH, hyperhomocysteinemia; r, retrospective study; p, prospective study; cs, cross-sectional study; AC, anticoagulant; MPN, myeloproliferative neoplasm; FUP, follow-up; VTE, venous thromboembolism; DVT, deep venous thrombosis; PE, pulmonary embolism; CVT, cerebral venous thrombosis)

a. Age is expressed as mean age (age range) or (±SD)

b. JAK2 V617F mutation was not found in both groups (0/45 vs 0/286)

c. Overlapping population with another paper (Junker et al)[81], evaluating PAI-1

*Tests within parentheses were only performed in cases

Table 2b. Case-control studies using patients with venous thromboembolism at a non-cerebral site as control group

Study		Cases n (♀)	Age[a]	Inclusion criteria	Exclusion criteria	Contr. (♀)	Age[a]	Inclusion criteria	Thrombophilia tests	Case n/N[b]	Contr. n/N[b]	OR
Martinelli 1998[71]	r	40 (31)	31 (15-64)	unrelated patients with a first episode of idiopathic CVT referred for coagulation screening	infection-related CVT, post-traumatic cerebral fistula	80 (62)	30 (13-62)	age- and sex-matched patients with first episode of lower-extremity proximal DVT	FV	6/40	15/80	0.76 (0.27-2.15)
									FII	8/40	14/80	1.18 (0.45- 3.10)
									AT, PC, PS, APLA	1/40	13/80	0.13 (0.02-1.05)
Colaizzo 2007[61]	r	45 (31)	40 (13-55)	Caucasian patients referred for coagulation screening	AC treatment	110 (51)	49 (12-81)	DVT in one leg	FV	1/45	14/110	0.16 (0.02-1.22)
									FII	8/45	9/110	2.43 (0.87-6.76)
									JAK2	0/45	0/110	-
Libourel 2007[69]	r	63 (50)	35 (16-71)	patients with CVT from one center	-	209 (107)	59 (18-94)	age- and sex-matched consecutive patients with prior DVT or PE	FV	11/52	12/52	0.91 (0.36-2.32)
									FII	2/52	0/52	-
									AT	0/52	0/52	-
									PC	3/52	1/52	3.12 (0.31-31.0)
									PS (type I+III)	11/52	13/52	0.80 (0.32-2.01)
									FVIII	30/52	31/52	0.92 (0.42-2.02)
									LAC	2/52	1/52	2.04 (0.18-23.22)
									HH	9/52	7/52	1.35 (0.46-3.93)
Wysokinska 2008[70]	r	163 (100)	40 (±20)	patients with CVT tested for thrombophilia	-	163 (85)	50 (±17)	randomly selected group of patients with lower-extremity DVT	FV	17/163	39/163	0.37 (0.20-0.69)
									FII	14/122	8/163	0.52 (0.27-0.99)
									AT	1/163	4/163	0.30 (0.03-2.72)
									PC	0/163	7/163	-
									PS	6/163	3/163	2.04 (0.50-8.29)
									LAC	3/163	9/163	0.32 (0.09-1.21)
									aCL (IgM/IgG)	22/163 6/163	6/163 4/163	4.08 (1.61-10.36) 1.52 (0.42-5.49)
									HH	7/163	3/163	2.39 (0.61-9.42)
Total		**311 (212)**				**562 (305)**						

(FV, heterozygosity for the Factor V Leiden mutation; FII, heterozygosity for mutation G20210A of the prothrombin gene; AT, antithrombin deficiency; PC, protein C deficiency; PS, protein S deficiency; FVIII, high factor VIII levels; JAK2, JAK2 V617F mutation; LAC, lupus anticoagulant; aCL, anticardiolipin antibodies; APLA, antiphospholipid antibodies; APLs, antiphospholipid syndrome; HH, hyperhomocysteinemia; r, retrospective study; p, prospective study; cs, cross-sectional; AC, anticoagulant; MPN, myeloproliferative neoplasm; FUP, follow-up; VTE, venous thromboembolism; DVT, deep venous thrombosis; PE, pulmonary embolism; CVT, cerebral venous thrombosis)

a. Age is expressed as mean age (age range) or (±SD)

b. Data are expressed as n, number of patients with thrombophilia/N, total number of patients (%)

Factor V Leiden mutation

Overall 18 studies evaluating the role of the factor V Leiden mutation for the risk of cerebral venous thrombosis included 919 cases and 3,168 healthy controls[38, 40,44,46-50,53,55,56,59,61-65,67]. Compared with controls, the pooled OR for cerebral venous thrombosis was 2.89 (95%CI: 2.10-3.97; p<0.001; Figure 2). There was no heterogeneity between studies (I^2=0%; p=0.67). The funnel plot (not shown) appeared symmetric, suggesting that there was no publication bias.

Prothrombin G20210A mutation

Overall 15 studies considered the association between the prothrombin G20210A mutation and cerebral venous thrombosis, including 776 cases and 2,636 healthy controls[44,46-50,53,55,56,59,61,62,64,65,67]. The pooled OR for cerebral venous thrombosis was 6.05 (95%CI: 4.12-8.90; p<0.001; Figure 3). Heterogeneity was very low (I^2=5%; p=0.39). The funnel plot (not shown) appeared symmetric, suggesting absence of a publication bias.

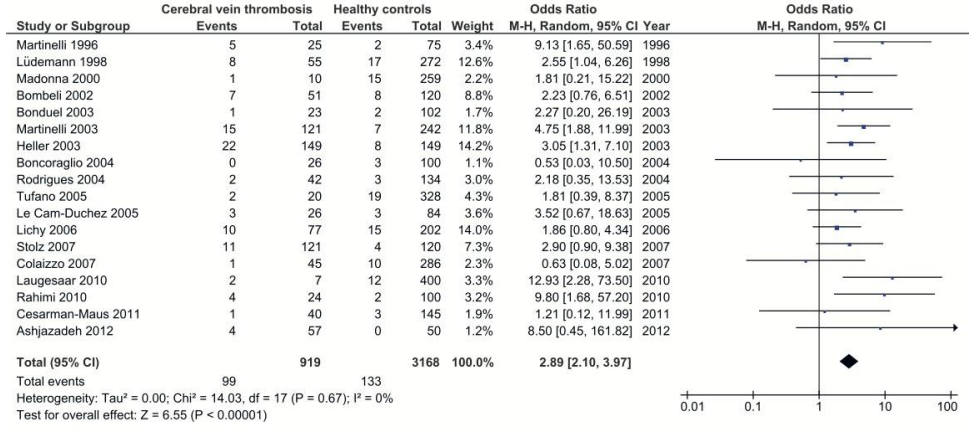


Figure 2. Forest plot for the association between cerebral venous thrombosis and the factor V Leiden mutation with odds ratios and pooled odds ratio (Mantel-Haenszel method with random-effects model)

Antithrombin, protein C and protein S deficiency

A total of three antithrombin[46,49,63], five protein C[46,48,49,62,63], and five protein S deficiency[46,48,49,62,63] case-control studies were evaluated, respectively. For antithrombin deficiency, the pooled OR for cerebral venous thrombosis was 3.75 (95%CI: 1.02-13.82; p=0.05; Figure 4a). For protein C deficiency, the pooled OR for cerebral venous thrombosis was 8.35 (95%CI: 2.61-26.67; p<0.001; Figure 4b), and the pooled OR for cerebral venous thrombosis and protein S deficiency was 6.45 (95%CI: 1.89-22.03; p=0.003; Figure 4c). There was no heterogeneity between studies in any of the meta-analyses (I^2=0%; p=0.41; I^2=0%; p=0.89; I^2=0%; p=0.88, respectively).

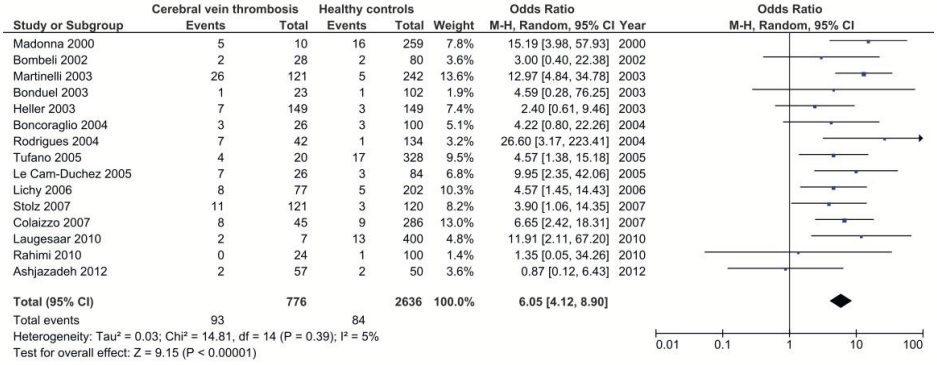


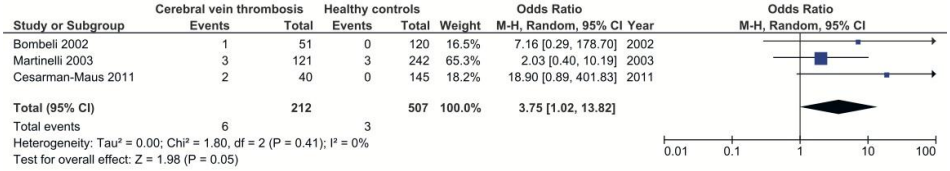
Figure 3. Forest plot for the association between cerebral venous thrombosis and the prothrombin G20210A mutation with odds ratios and pooled odds ratio (Mantel-Haenszel method with random-effects model)

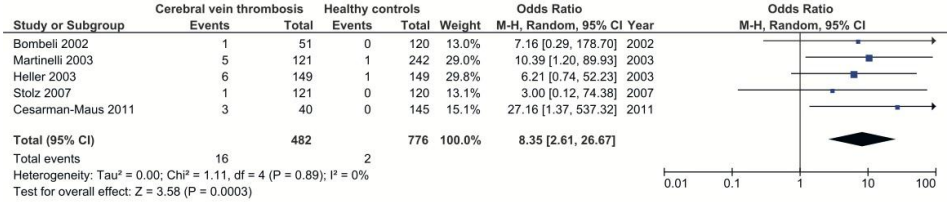
Hyperhomocysteinemia

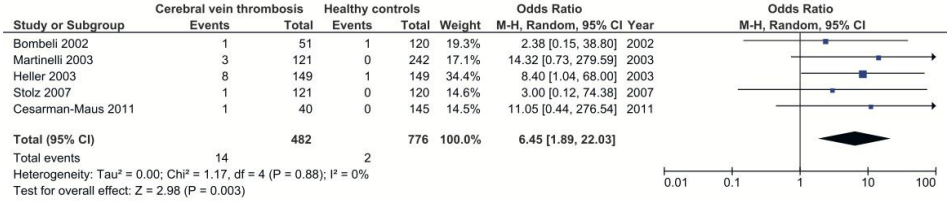
Four case-control studies investigated hyperhomocysteinemia in 212 cases with cerebral venous thrombosis and 760 controls[49-51,53]. Fasting hyperhomocysteinemia was defined as levels >95th percentile of the sex-specific normal population in three studies[49,50,53], and >90th percentile in one study[51]. The pooled OR for cerebral venous thrombosis compared to healthy controls was 2.99 (95%CI: 1.32-6.75; p=0.009; Figure 5). There was substantial heterogeneity between studies (I^2=71%; p=0.02).

Association between cerebral venous thrombosis and thrombophilia in comparison with non-cerebral venous thromboembolism

Four case-control studies[61,69-71] compared the association between thrombophilia and cerebral venous thrombosis (311 cases) to non-cerebral venous thromboembolism (562 controls with deep venous thrombosis of the leg or pulmonary embolism). In total, data of 19 ORs could be extracted. A meta-analysis was not performed as there were considerable differences in design and inclusion criteria for cerebral venous thrombosis cases and controls with non-cerebral venous thromboembolism between studies.







Figures 4a (top of the three), b and c. Forest plot for the association between cerebral venous thrombosis and antithrombin deficiency (4a), protein C deficiency (4b) and protein S (4c) with odds ratios and pooled odds ratio (Mantel-Haenszel method with random-effects model)

Relevance of thrombophilia for recurrence of cerebral venous thrombosis or other venous thromboembolism, or other outcome variables

Six cohort studies[21,31,33,36,37,72] and two case-control studies[55,73] evaluated the association between thrombophilia and recurrence of cerebral venous thrombosis or other venous thromboembolism, or other outcome variables (Table 3a and 3b). Presence of antithrombin, protein C or protein S deficiency, antiphospholipid antibodies, or combined abnormalities was significantly associated with an increased risk of recurrent venous thromboembolism in one cohort study[33]. However, no association was found in another cohort study[21] and in the only case-control study on the relevance of thrombophilia for recurrence of venous thromboembolism[73].

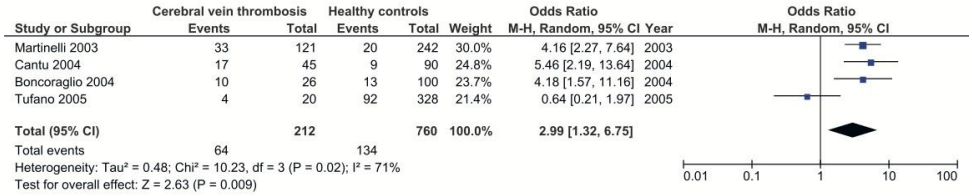


Figure 5. Forest plot for the association between cerebral venous thrombosis and hyperhomocysteinemia with odds ratios and pooled odds ratio (Mantel-Haenszel method with random-effects model)

Table 3a. Prediction of recurrence of cerebral venous thrombosis or other venous thromboembolism, or other outcome variables (cohort studies)

Study	Predictors	Description	
Zubkov 2009[31]	parenchymal lesions	Age, sex, and acquired or congenital thrombophilia did not influence the likelihood of presenting with parenchymal lesions	
		Category	*Hazard Ratio (95% confidence interval)*
Martinelli 2010[33]	risk of recurrent VTE associated with thrombophilia	No thrombophilia	1 (reference)
		Mild thrombophilia[a]	0.86 (0.23-3.23)
		Severe thrombophilia[b]	4.13 (1.24-13.7)
	risk of recurrent VTE associated with thrombophilia (adjusted for sex)	No thrombophilia	1 (reference)
		Mild thrombophilia[a]	1.25 (0.32-4.79)
		Severe thrombophilia[b]	5.02 (1.49-17.0)
	VTE recurrence associated with sex	Female	1 (reference)
		Male	7.01 (2.34-21.0)
		Category	*Recurrence Rate, %pt-yr (95% confidence interval)*
	recurrence rate of VTE after a first episode of CVT (crude incidence estimates)	No thrombophilia	1.35 (0.56-2.47)
		Factor V Leiden mutation	2.47 (0.21-7.19)
		Prothrombin G20210A mutation	0.46 (0-1.85)
		Severe thrombophilia[b]	6.50 (1.62-14.6)
		Category	*Prevalence*
Girot[72] ISCVT 2007	early intracranial hemorrhage (E-ICH)	With E-ICH	54/245 (22%) acquired thrombophilia
		Without E-ICH	44/379 (12%) no acquired thrombophilia (P < 0.05)
		With E-ICH	54/245 (22%) genetic thrombophilia
		Without E-ICH	86/379 (23%) no genetic thrombophilia (NS)
Sanz-Gallego 2010[36]	venous infarction (whole study population)	Infarction	9/22 (40.9%) thrombophilia
		No infarction	5/30 (16.7%) no thrombophilia (NS)
	venous infarction (females)	Infarction	5/22 (31.2%) thrombophilia and OC
		No infarction	2/30 (9.6%) no thrombophilia and OC (p<0.05)
Dentali 2012[21]	VTE recurrence	Thrombophilia and severe thrombophilia were not associated with an increased recurrence of VTE	
Passamonti 2012[37]	MPN-free survival	MPN-free survival after CVT was significantly different in patients with and without the JAK2 V617F mutation, in favor of patients without the mutation (log-rank test chi^2: 159; p < 0.0001).	

(VTE, venous thromboembolism; MPN, myeloproliferative neoplasm; CVT, cerebral venous thrombosis; NS, not significant; OC, oral contraceptives)

a. Mild thrombophilia was defined as presence of heterozygosity for the Factor V Leiden mutation, heterozygosity for mutation G20210A of the prothrombin gene, and dysfibrinogenemia

b. Severe thrombophilia was defined as presence of antithrombin, protein C or protein S deficiency, antiphospholipid antibodies, or combined abnormalities

Table 3b. Prediction of recurrence of cerebral venous thrombosis or other venous thromboembolism, or other outcome variables (case control studies)

Study	CC	Predictors	
Rodrigues 2004[55]	case, n = 42 control, n = 134	clinical outcome	no relationship between the Factor V Leiden mutation, prothrombin mutation and clinical outcome; 21% of patients without limitations carried thrombophilic mutations versus 25% of patients with sequelae
Gosk-Bierska 2006[a73]	case, n =154 control, n = 300	venous thrombosis	no covariates associated with recurrent VTE; recurrence rates were nearly identical in cases and controls

(CVT, cerebral venous thrombosis; VTE, venous thromboembolism)

a. Population overlapping with Wysokinska (2008);[70] study has been excluded from selection of case-control studies and was used only for data on prediction of recurrent VTE

DISCUSSION

Our results demonstrate strong associations between cerebral venous thrombosis and all inherited thrombophilias, as well as increased levels of homocysteine. For the inherited defects, meta-analyses among several studies were homogeneous, whereas for hyperhomocysteinemia results were substantially heterogeneous, which appeared to be due to one outlier[53].

The association between thrombophilia and venous thromboembolism has been previously established, particularly for deep venous thrombosis and pulmonary embolism[7,8]. We compared the association between cerebral venous thrombosis and thrombophilia to patients with non-cerebral venous thromboembolism (deep venous thrombosis or pulmonary embolism). One study suggested that anticardiolipin antibodies were significantly more associated with cerebral venous thrombosis than deep venous thrombosis or pulmonary embolism[70], but in general associations between thrombophilia and cerebral venous thrombosis, and deep venous thrombosis or pulmonary embolism seemed similar.

Other reviews on the association between cerebral venous thrombosis and thrombophilia showed similar results[74-77]. Our study is one of the most comprehensive systematic reviews, evaluating 23 cohort studies and 33 case-control studies, including adults and children of all ancestries. We also systematically assessed statistical heterogeneity and addressed potential publication bias. However, several limitations are applicable to our study. Our systematic review and meta-analyses are based on cohort and case-control studies, and the application of meta-analyses to observational studies is controversial[13]. To minimize potential bias, we included only studies with highest scores (8 or 9 stars) on the Newcastle-Ottawa Scale for case-control studies and with unselected consecutive patients in our meta-analyses[14]. Heterogeneity between studies, calculated using the I^2 statistic, was generally low, indicating that the analyzed studies were indeed comparable. Generated funnel plots appeared symmetric, suggesting that there was no publication bias. However, not all included studies strictly matched their controls for age and sex. Also, due to the low prevalence of antithrombin, protein C or protein S deficiency in healthy controls, confidence intervals of these meta-analyses are relatively wide. In studies evaluating the antiphospholipid syndrome (n=5) in cerebral venous thrombosis patients, different definitions were used for the antiphospholipid syndrome, and presence of various antibodies was used as a surrogate. Hence, valid meta-analyses could not be performed for these thrombophilic factors. Furthermore, for anticardiolipin or antiphospholipid antibodies, lupus anticoagulant and homocysteine levels, timing of

sample collection (acute phase of cerebral venous thrombosis or during follow-up) and performance of confirmation sampling were not always described. Finally, several studies included pregnant and postpartum women, or those using oral contraceptives or hormone replacement therapy. These hormonal factors have been shown to individually increase thrombotic risk and can interact with inherited thrombophilia in a multiplicative manner[78], as has also been shown in studies on cerebral venous thrombosis, thrombophilia and use of oral contraceptives[49,74,76,79].

The clinical importance of the association between cerebral venous thrombosis and thrombophilia is driven by its impact on outcome, e.g. recurrence of cerebral venous thrombosis or other venous thromboembolism, or other outcome variables. A recent Cochrane review showed that no controlled clinical trials are available assessing the benefit of thrombophilia testing to predict the risk of recurrent venous thromboembolism in patients with a prior episode of venous thromboembolism[80]. Also for cerebral venous thrombosis, observational data only are available on this topic. In the evaluated cohort and case-control studies, inconclusive results were found for the relevance of antithrombin, protein C or protein S deficiency, presence of antiphospholipid antibodies or combined abnormalities on the risk of recurrent venous thromboembolism, while presence of heterozygosity for the factor V Leiden or prothrombin G20210A mutation were not associated with an increased risk of recurrent cerebral venous thrombosis or other venous thromboembolism. Hence, similarly to deep venous thrombosis or pulmonary embolism[9,10], thrombophilia is likely to have a limited clinical relevance for cerebral venous thrombosis.

To summarize, although our meta-analyses show strong associations between cerebral venous thrombosis and all inherited thrombophilias, as well as increased levels of homocysteine, the relevance of thrombophilia testing in patients with cerebral venous thrombosis seems limited, similarly to other forms of venous thromboembolism.

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Long-term outcomes of patients with cerebral vein thrombosis: a multicenter study

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ABSTRACT

Little information is available on the long-term clinical outcome of cerebral vein thrombosis.

In an international, retrospective cohort study, we assessed the long-term rates of mortality, residual disability and recurrent venous thromboembolism in a cohort of patients with a first episode of cerebral vein thrombosis.

Seven hundred and six patients (73.7% females) with cerebral vein thrombosis were included. Patients were followed for a total of 3,171 patient-years. Median follow-up was 40 months (range 6-297 months). At the end of follow-up, 20 patients had died (2.8%). The outcome was generally good: 89.1% of patients had a complete recovery (modified Rankin Score 0-1) and 3.8% had a partial recovery and were independent (modified Rankin Score 2). Eighty-four per cent of patients were treated with oral anticoagulants and the mean treatment duration was 12 months. Cerebral vein thrombosis recurred in 31 patients (4.4%), and 46 patients (6.5%) had a venous thromboembolism in a different site, for an overall incidence of recurrence of 23.6 events per 1,000 patient-years (95% Confidence Interval [CI]: 17.8-28.7) and of 35.1 events/1,000 patient-years (95%CI: 27.7-44.4) after anticoagulant therapy withdrawal. A previous venous thromboembolism was the only significant predictor of recurrence at multivariable analysis (hazard ratio [HR] 2.70; 95%CI: 1.25-5.83).

The long-term risk of mortality and recurrent venous thromboembolism appears to be low in patients who survived the acute phase of cerebral vein thrombosis. A previous venous thromboembolism history independently predicts recurrent events.

INTRODUCTION

Cerebral vein thrombosis has long been considered a rare disease with important long-term morbidity and mortality rates[1,2]. In the last decade, new non-invasive diagnostic techniques have increased the frequency with which this disease is diagnosed and there has been increasing evidence that an early diagnosis and a timely start of anticoagulant treatment significantly reduced morbidity from cerebral vein thrombosis and improved survival[3].

Recent guidelines recommend the use of unfractionated heparin or low-molecular-weight heparin followed by at least 3-6 months of oral anticoagulant therapy with vitamin K antagonists for most patients with a first episode of cerebral vein thrombosis[4,5]. However, the optimal duration of anticoagulant treatment is not established because little information is available on the long-term rate of recurrent cerebral vein thrombosis or the rate of recurrence of venous thromboembolic events in other sites after the discontinuation of anticoagulant drugs. These rates have been reported to be low in a large prospective study[6], and these findings were confirmed by a subsequent meta-analysis that included 19 studies for a total of about 1,500 patients[7]. However, these results were based on relatively short periods of follow-up. Furthermore, almost all published studies were too small to evaluate potential risk factors for recurrence. More recently, a large cohort study from a single center confirmed the substantially low rate of recurrences after a longer-term follow-up[8].

Thus, to better estimate the long-term recurrence rates of cerebral vein thrombosis and to accurately identify risk factors for recurrence, we conducted a large, international, multicenter, retrospective cohort study in a population of patients with a first episode of cerebral vein thrombosis.

METHODS

The study involved 27 centers from Italy, Czech Republic and the USA. All centers were Thrombosis Units, Anticoagulation Clinics, or Neurology Clinics. These centers are routinely involved in the management of cerebral vein thrombosis patients from the time of diagnosis and the start of anticoagulant treatment or are subsequently involved in the monitoring of oral anticoagulant therapy and the evaluation of specific risk factors, such as thrombophilia, after the first days of acute treatment. All involved centers regularly perform long-term follow-up of these patients.

At each participating center, data on consecutive cases of patients with a first episode of objectively diagnosed cerebral vein thrombosis were collected. All eligible patients have been regularly followed by the local anticoagulation clinics and/or by neurology clinics/stroke units. As the main aim of the study was to evaluate the clinical history of cerebral vein thrombosis, only patients with an available follow-up of at least 6 months or with an outcome event occurring within the first 6 months (death, recurrence of cerebral vein thrombosis and occurrence of venous thromboembolism) were included in the study. However, to explore the presence of potential differences in the population with and without follow-up, their baseline characteristics were compared.

Case report forms were prepared by the coordinating center (Varese, Italy) and were sent to all participating centers. Local investigators were asked to fill out the form and to send it back to the coordinating center. All data were cross-checked and validated centrally at the end of the follow-up period.

Demographic data, site of thrombosis, medical history focusing on potential risk factors for thrombosis, treatment and clinical outcome were gathered. Furthermore, information on family and a personal history of venous thromboembolism was also collected. A positive personal history of venous thromboembolism was adjudicated if the patient had a previous objectively assessed episode of a deep vein thrombosis, pulmonary embolism, splanchnic vein thrombosis or cerebral vein thrombosis. A positive family history of venous thromboembolism was adjudicated when one or more first-degree relatives had an objectively assessed episode of venous thromboembolism.

Cerebral vein thrombosis was defined as secondary in the presence of one of the following risk factors: cancer, infections, trauma, oral contraceptive use, pregnancy, puerperium, hormone replacement therapy, neurosurgery and myeloproliferative neoplasms. In the absence of the aforementioned predisposing factors, the cerebral vein thrombosis episode was defined as unprovoked. Information on thrombophilic abnormalities, including antithrombin, proteins C, protein S, factor V Leiden, and G20210A mutations, homocysteine, lupus anticoagulant, anticardiolipin antibodies, anti-beta2-glycoprotein I antibodies were collected when available.

Information on clinical events during follow-up was first collected using the computerized database of each anticoagulation clinic or neurology department, where data on patients' outcomes are regularly collected. Furthermore, for the purpose of this study and in order to guarantee the most accurate and updated information, investigators were requested to contact all included patients by means of a visit at the

center, a telephone contact, or a mailed questionnaire if this was not scheduled during their regular clinical activity. At the time of contact, information on cerebral vein thrombosis recurrence, on the occurrence of venous thromboembolism in other sites, and on the death of the patient was collected and was added to the information stored at each center database. For all reported events, accurate evaluation of source documentation was requested. Only objectively diagnosed events were considered. Accepted tests were the following: magnetic resonance imaging with magnetic resonance venography, computed tomography venography, or conventional angiography for the diagnosis of recurrent cerebral venous thrombosis; compressive B-mode ultrasound or echo-color Doppler for the diagnosis of lower or upper extremity deep vein thrombosis; perfusion or ventilation/perfusion lung scan or helical-computed tomography for the diagnosis of pulmonary embolism. Adjudication of cerebral vein thrombosis recurrence after objective testing was performed locally at each participating center.

Finally, information on the cause of death was requested. The following data were also recorded for this study: the presence of residual disability at the time of the last patient contact (defined according to the modified Rankin Scale)[9] and current and previous antithrombotic treatments. Residual disability was classified according to the modified Rankin Score as complete recovery (modified Rankin Score 0–1); partial recovery, independent (modified Rankin Score 2); dependent (modified Rankin Score 3–5); and death (modified Rankin Score 6).

The Institutional Review Board approved the study, which was carried out and is reported according to the Strengthening the Reporting of observational Studies in Epidemiology (STROBE) guidelines for observational studies[10].

Continuous variables were expressed as mean plus or minus the standard deviation (SD) or as median with minimum and maximum values when data did not have a normal distribution; categorical data are given as counts and percentages. The annual incidence of recurrent thrombosis was calculated for the whole group of venous thromboses and then separately for cerebral vein thrombosis and venous thromboembolism in other sites by dividing the number of events by the total number of patient-years followed until the venous thromboembolism or death. To explore the potential role of anticoagulant therapy on the risk of recurrent thrombosis, separate data of the risk of recurrence during anticoagulation and after anticoagulation discontinuation were provided. The 95% confidence intervals (CIs) were based on the exact approximation of the Poisson distribution. Because of the low recurrence rates of venous thromboembolism, when the analysis was limited to specific subgroups, only crude estimates of the incidence were given. Recurrence-free survival was

calculated using the Kaplan–Meier method[11]. The role of potential risk factors for thrombosis recurrence was evaluated using the Cox proportional-hazard model[12]. All the potential risk factors for venous thromboembolism were introduced in the Cox model. The impact of different severities of thrombophilia was analyzed.

Severe thrombophilia was defined as the presence of antithrombin, protein C or protein S deficiency, antiphospholipid antibodies and by the concomitant presence of more than one abnormality[13]. As a first step, the risk of thrombosis recurrence in patients with thrombophilia was compared with that of patients without thrombophilia. Subsequently, the risk of thrombosis recurrence in patients with severe thrombophilia was compared with that of patients without severe thrombophilia. We first gave unadjusted hazard ratios (HRs) estimates and then we adjusted the estimates for other possible confounders. A p-value of <0.05 was chosen as the cut off for statistical significance. All statistical analyses were performed with SPSS 11.0 for Windows (IBM Corporation, Armonk NY, United States).

RESULTS

Seven hundred and 41 patients with a first episode of cerebral vein thrombosis were considered for inclusion during the study period. For 35 patients (4.9%) the duration of follow-up was insufficient (<6 months) and these patients were excluded from the analysis. Baseline characteristics of patients with and without a sufficient follow-up were not different (data not shown). Hence, 706 patients (mean age 40.0 ± 16.3 years) were included in the study. Baseline demographic, clinical characteristics and potential risk factors for cerebral venous thrombosis are listed in Table 1.

Four hundred and two patients (55.8%) had at least one risk factor, whereas in 304 patients (44.2%) cerebral venous thrombosis was idiopathic. Significantly more women had at least one risk factor compared with men (61.0% *vs* 45.7%; $p<0.05$). As not all thrombophilic abnormalities were tested in all of the patients, we provided separate results for each thrombophilic abnormality.

Table 1. Baseline demographic, clinical characteristics and potential risk factors for recurrence of included patients

Total number	n=706
Male gender, *n* (%)	186 (26.3)
Mean age, years (± SD)	40.0 (16.3)
Principal sites of thrombosis, *n* (%)	Superior sagittal sinus 267 (37.8) Left lateral sinus 281 (39.8) Right lateral sinus 225 (31.9)
Concomitant intracranial hemorrhage, n (%)	
Risk factors at first CVT, n (%)	Infections 59 (8.3) Trauma 18 (2.5) OC or HRT 278 (39.4) Pregnancy/puerperium 55 (7.8) Cancer or MPD 52 (7.4) Thrombophilic abnormalities (one at least) 290 (41.1) Severe thrombophilic abnormalities 83 (11.7) Unprovoked 312 (44.2)
Personal history of VTE, n (%)	54 (7.6)
Family history of VTE, n (%)	109 (15.4)
Acute antithrombotic therapy, n (%)	LMWH 443 (62.7) UFH 155 (21.9) Thrombolysis 11 (1.5) None 97 (13.7)
Post acute antithrombotic therapy, n (%)	Oral anticoagulants 590 (83.6) LMWH 37 (5.2) Acetyl salicylic acid 14 (2.0) None 62 (6.9)

Abbreviations: CVT, cerebral vein thrombosis; HRT, hormone replacement therapy; LMWH, low-molecular-weight heparin; MPD, myeloproliferative disease; OC, oral contraceptives; UFH, unfractionated heparin; VTE, venous thromboembolic events.

The median duration of anticoagulant treatment was 12 months and 134 patients (19.0%) were still on OAT at the time of the last contact. The median duration of follow-up was 40 months (range 1–297 months) for a total follow-up of 3171 patient-years. Total follow-up during anticoagulation was 1,143 patient-years and after anticoagulation discontinuation was 2,028 patient-years.

The outcome of these patients was generally good: 89.1% of patients had a complete recovery (modified Rankin Score 0–1) and 3.8% had a partial recovery and were independent (modified Rankin Score 2). Over the time period of this study, there were 20 deaths in patients with cerebral venous thrombosis for a mortality rate of 2.8%. The cause of death was most commonly malignancy related (n=12). Other causes

included consequences of cerebral venous thrombosis (n = 2), arterial stroke (n=2), fatal arrhythmia (n=1), hepatic failure (n=1) and sepsis (n=1). In one patient the cause of death was unknown. Three deaths occurred within 1 month. Patients with a concomitant intracranial hemorrhage at presentation had a slightly, but not significant worse prognosis (modified Rankin Score ≥ 3) at the end of follow-up in comparison with patients without concomitant intracranial hemorrhage at presentation (9.1% vs. 7.1%; p=0.42). The estimated survival was 98% at 1 year and 95% at 5 years.

Seventy-five patients (10.6%) were diagnosed with recurrent, non-fatal venous thromboembolism. Clinical characteristics of patients with venous thromboembolism recurrence are listed in Table 2. Recurrent events included recurrent cerebral venous thrombosis in 31 patients (4.4%) and venous thromboembolism in other sites in the remaining 46 patients (6.5%). Two patients had a venous thromboembolism and a concomitant recurrence of cerebral venous thrombosis. Venous thromboembolism in other sites occurred in the lower limbs in 30 patients, in the pulmonary arteries in 6 patients and in the lower limbs and pulmonary arteries at the same time in 7 patients. Finally, 3 patients had a splanchnic vein thrombosis and 1 had a thrombosis of the upper limbs. Recurrence-free survival using the Kaplan–Meier method is represented in Figure 1. The overall incidence of recurrent venous thromboembolism was 23.6 per 1,000 patient-years (95%CI 17.8–28.7).

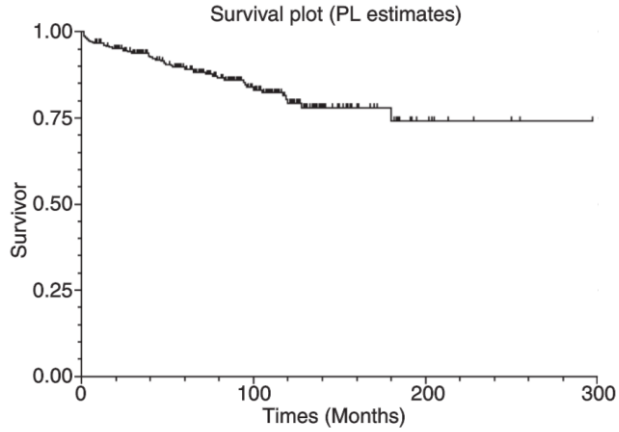

Figure 1. Recurrence-free survival using the Kaplan–Meier method

Most events occurred after anticoagulation discontinuation for an incidence of recurrence in this group of 35.1 events/1,000 patient-years (95% CI, 27.7-44.4). The

recurrence rate was similar in patients with unprovoked cerebral venous thrombosis and in patients with cerebral venous thrombosis secondary to a known risk factors (22.8 events/1,000 patient-years, 95% CI 15.9, 32.6 vs. 27.0 events/1,000 patient-years, 95% CI 20.4-36.0).

Table 2. Characteristics of patients with recurrent venous thromboembolism

Recurrent VTE	n=75
Mean Age, year (± SD)	36.9 (13.8)
Male gender, n (%)	22 (29.3)
CVT recurrence, n (%)	31 (4.4)
VTE (splanchnic thrombosis, deep venous thrombosis, pulmonary embolism) recurrence, n (%)	46 (6.5)
Principal sites of thrombosis, n (%)*	Superior sagittal sinus 40 (53.3) Left lateral sinus 29 (38.7) Right lateral sinus 26 (34.7)
Concomitant intracranial haemorrhage, n (%)	19 (25.3)
Risk factors at first CVT, n (%)	Infections 4 (5.3) Trauma 7 (9.3) OC or HRT 26 (34.7) Pregnancy/puerperium 8 (10.7) Cancer or MPD 8 (10.7) Thrombophilic abnormalities (one at least) 35 (46.7) Severe thrombophilic abnormalities (14.7) Idiopathic 30 (37.3)
Personal history of VTE, n (%)	14 (18.7)
Family history of VTE, n (%)	14 (18.7)

Two patients had both the recurrences. CVT, cerebral vein thrombosis; HRT, hormone replacement therapy; LMWH, low-molecular-weight heparin; MPD, myeloproliferative disease; OC, oral contraceptives; UFH, unfractionated heparin; VTE, venous thromboembolic events. *More than one site was involved in some patients.

Cox proportional hazards models were fitted to identify covariates associated with time to venous thromboembolism in patients with cerebral venous thrombosis. In the univariate model, a personal history of venous thromboembolism (HR 2.73, 95%CI 1.53–4.89; p<0.001), recent head trauma (HR 4.20, 95%CI 1.93–9.15; p<0.001) and cancer (HR 2.57, 95%CI 0.91, 3.95; p<0.012) were associated with recurrent venous thromboembolism. Thrombophilia and severe thrombophilia were not associated with an increased risk of venous thromboembolism recurrence. As a result

of the controversial role of hyperhomocysteinaemia, we repeated the analysis excluding this thrombophilic abnormality; however, the results did not change (data not shown). Indefinite OAT was not associated with improved event free survival (Table 3). When all potential risk variables were included in a multivariable model, only a personal history of venous thromboembolism remained significantly associated with recurrent cerebral venous thrombosis or venous thromboembolism in other sites (HR 2.70; 95%CI: 1.25–5.83; p<0.011).

Table 3. Association of baseline characteristics and potential risk factor for recurrent venous thromboembolism at univariable analysis

	HR	95% Confidence Interval	p
Age at diagnosis	1,00	0.98–1.01	0.578
Sex	1,37	0.83–2.25	0,221
Personal history of VTE	2,73	1.53–4.89	0,001
Family history of VTE	1,14	0.63–2.00	0,669
Unprovoked presentation	1,02	0.61–1.69	0,926
Thrombophilic abnormalities	1,11	0.71–1.75	0,648
Severe thrombophilic abnormalities	1,16	0.61–2.20	0,654
Oral contraceptives/hormone replacement therapy	0,72	0.45–1.14	0,161
Cancer	2,57	0.91–3.95	0,012
Recent neurosurgery	1,71	0.24–12.4	0,594
Recent head trauma	4,20	1.93–9.15	<0,001
Local or systemic infection	0,67	0.21–1.60	0,436
Myeloproliferative disease	2,03	0.28–14.60	0,483
Pregnancy/puerperium	1,5	0.48–2.28	0,911
Long-term anticoagulant therapy	1,13	0.65–1.95	0,664

Abbreviations: HR, hazard ratio; VTE, venous thromboembolism.

DISCUSSION

To our knowledge, this is the largest multicenter cohort study with an adequately long follow-up to evaluate the clinical history of patients with a first episode of cerebral vein thrombosis.

The principal finding of this study is in the estimate of anticipated rates of recurrent venous thromboembolic events, either cerebral vein thrombosis or venous thromboembolism in other sites. During more than 3,000 patient-years of follow-up,

there were 31 episodes of cerebral vein thrombosis recurrence and 46 episodes of venous thromboembolism in other sites, with an overall rate of 23.6 per 1,000 patient-years and this rate was similar in patients with unprovoked cerebral vein thrombosis and in patients with cerebral vein thrombosis secondary to known risk factors. When recurrences were assessed only after oral anticoagulant therapy was stopped, the incidence rate was only slightly higher (35.1 events/1,000 patient-years). These results provide a robust confirmation of previous observations[6,8]. In the International Study on Cerebral Vein and Dural Sinus Thrombosis (ISCVT), the investigators observed 14 recurrent cerebral vein thrombosis events (2.2%) and 19 lower limb deep vein thrombosis or pulmonary embolism (3.0%) over a median follow-up period of 16 months[6]. More recently, Martinelli et al.[8] found an event rate of 2.03 per 100 patient-years in a population of 145 patients with a first episode of cerebral vein thrombosis. Although only a minority of patients was treated life-long, substantially low rates of recurrent events were observed in this study. After multivariable analysis, a previous venous thromboembolism, but not the duration of oral anticoagulant therapy, was associated with the risk of recurrence suggesting that long-term oral anticoagulant therapy may be not necessary in most cerebral vein thrombosis patients. Furthermore, in this study the presence of thrombophilia did not appear to be associated with an increased risk of venous thromboembolism recurrence. This may be as a result of a marginal effect of thrombophilic abnormalities on the risk of venous thromboembolism recurrence, although other potential confounding factors, such as a different duration of anticoagulation in patients with specific thrombophilic abnormalities, could not be excluded. Thus, the role of thrombophila in cerebral vein thrombosis patients remains to be established. Many recurrent venous thromboembolism occurred in women in which the first cerebral vein thrombosis occurred during pregnancy or puerperium or was secondary to hormone replacement therapy or oral ontraceptive use. However, neither female gender nor pregnancy/puerperium or the use of hormone replacement therapy/ oral ontraceptive appeared to be an independent risk of venous thromboembolism recurrence. Two previous studies have evaluated potential risk factors for recurrence in cerebral vein thrombosis patients[8,14]. In the study by Gosk-Bierska et al.[14], no variable was significantly associated with recurrent venous thrombosis in these patients, whereas in the study conducted by Martinelli et al.[8], risk factors for recurrent venous thrombosis were male gender and, for recurrence of venous thromboembolism in other sites only, severe thrombophilia. These differences across studies may be because of differences in patient selection and to the smaller sample of the previous studies. For example, in the single center study by Martinelli et al.[8], all patients were referred for thrombophilia work-up and for counseling on the secondary

prevention of venous thromboembolism. In our study, participating centers had more heterogeneous roles in the management of cerebral vein thrombosis patients. Furthermore, taken together, the two studies by Gosk-Bierska and Martinelli enrolled a total of approximately 300 patients and may be underpowered to detect potential risk factors for recurrence.

This study also confirmed that most patients with cerebral vein thrombosis have a more benign prognosis than previously suspected: only 2.8% of patients had died at the end of the follow-up period and most surviving patients recovered completely or had only mild functional or cognitive deficit. This may be as a consequence of several factors. First, more sensitive diagnostic techniques have undoubtedly led to the detection of smaller thrombi, which probably have better prognosis. Second, older series included a higher proportion of patients with infection-associated cerebral vein thrombosis; these events are now less and less common as a result of the widespread use of antibiotics. Third, the widespread use of anticoagulant drugs for the acute and the long-term treatment of cerebral vein thrombosis have certainly contributed to improve the natural history of this disease. During follow-up, most patients had died as a result of underlying conditions such as cancer.

Although a formal comparison was not possible, cerebral vein thrombosis patients appear to have a lower risk of venous thromboembolism recurrence in comparison to patients with deep venous thrombosis or pulmonary embolism[15]. This low risk may explain why many established risk factors for venous thromboembolism recurrence in patients with a deep venous thrombosis or pulmonary embolism are not significant in cerebral vein thrombosis patients. Furthermore, these patients seem to have a more benign prognosis compared with patients with usual site thrombosis[15]. Differences in the baseline characteristics and in the concomitant diseases among these two populations may explain these results.

The present study has some limitations. First, the design of the study is retrospective. However, to overcome at least some of the limitations that are intrinsic to retrospective studies, we only involved centers where patients are regularly monitored and followed up, and to avoid misleading results we paid meticulous attention in the ascertainment of the reported outcome events and only patients with adequate quality of data or with available sources of documentation to complete missing information were considered eligible for inclusion. Second, a referral bias could not be excluded as we mainly enrolled patients who were started on oral anticoagulant therapy. Thus, the results of this study on the long-term outcome of the disease may not completely apply to the whole population of patients with cerebral vein thrombosis, as patients who died during the acute phase of the disease and patients

who were deemed ineligible for long-term secondary prevention with anticoagulant drugs may be insufficiently represented. However, based on the results of previous studies[6,7] these patients should represent a small minority of cerebral vein thrombosis patients. Third, screening for thrombophilia was not systematically performed in all the patients. Thus, although the risk of recurrence in patients with mild thrombophilia did not appear to be increased, the results of this study on the role of thrombophilia should be interpreted with caution. Fourth, we have no reliable data on the recanalization rate in the whole group of cerebral vein thrombosis patients. Thus, we could not comment on its potential influence on the risk of venous thromboembolism recurrence. Last and more important, a minority of patients with massive hemorrhages or other severe presentations may not have been included in this study as these patients may not be referred to the Thrombosis Units or Anticoagulation or Neurological Clinics. Thus, our population may have a slightly better prognosis compared with the general population of patients with cerebral vein thrombosis.

On the other hand, study strengths include the long duration of follow-up, both during anticoagulant treatment and after anticoagulant treatment withdrawal, and the large sample size of this cohort of patients, who had cerebral vein thrombosis diagnosed with objective methods and who were followed up homogeneously in a high number of centers.

In conclusion, the long-term risk of cerebral vein thrombosis recurrence and of venous thromboembolism in other sites appears to be low in patients with a first episode of cerebral venous thrombosis, regardless of the duration of treatment. This risk appeared to be increased only in patients with previous venous thromboembolism. The long-term prognosis of cerebral vein thrombosis in terms of mortality and residual disability is favorable in patients who survive the acute phase of disease.

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Pregnancy and venous thromboembolism

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ABSTRACT

Pregnancy and the postpartum period are associated with an increased risk of venous thromboembolism, which complicates 1 to 2 of 1,000 pregnancies and represents a leading cause of mortality during pregnancy in developed countries. Strong evidence for the management of pregnancy-related venous thromboembolism is missing, mostly because pregnant women have been excluded from all major trials investigating different diagnostic tools and treatment regimens. Nevertheless, proper evaluation of the involved risk factors is mandatory to reduce the incidence of pregnancy-related venous thromboembolism and improve outcomes. Low-molecular-weight heparins are considered as a first-line option in the management of pregnancy-related venous thromboembolism.

With regard to future research, there is a need for methodologically strong studies in pregnant women, especially with respect to risk stratification, optimal heparin doses, usefulness of anti-FXa levels and their correlation with clinical outcomes, and correct management of anticoagulation during delivery.

INTRODUCTION

Venous thromboembolism includes two interrelated clinical conditions, which can be both symptomatic at the time of the diagnosis or develop as an isolated manifestation of the disease: deep vein thrombosis and pulmonary embolism. Pregnancy and the postpartum period are associated with a 4- to 10-fold increased risk of venous thromboembolism compared with the risk in women of fertile age, which is approximately 1 to 5 per 10,000 women per year[1,2]. Pregnancy-related venous thromboembolism represents a leading cause of morbidity and mortality in developed countries, accounting for 20 to 30% of all deaths in the pregnant population[3,4].

Although some issues on the management of pregnancy-related venous thromboembolism remain to be further evaluated, many of these deaths are preventable. As for the non-pregnant population, the clinician should focus both on treating patients at risk with appropriate prophylaxis, and urgently initiating optimal anticoagulant therapy if a pregnancy-related venous thromboembolism is diagnosed by objective diagnostic techniques.

EPIDEMIOLOGY

Venous thromboembolism complicates 1 to 2 of 1,000 pregnancies and the risk of developing deep venous thrombosis or pulmonary embolism in pregnancy is at least four times as great as the risk in the non-pregnant population[1,5,6]. Although antepartum venous thromboembolism is more frequent than postpartum venous thromboembolism, the daily absolute risk is highest during the 6 weeks postpartum. Most studies suggest that the incidence of venous thromboembolism events is similar during the trimesters[7,8,9]. Nevertheless, a recent study indicates that the absolute risk of venous thromboembolism per 10,000 pregnancy per year increases throughout the trimesters from 4.1 (95% confidence interval [CI], 3.2-5.2), to 5.7 (95% CI, 4.6-7.2), to 15.6 (95% CI, 12.3-19.8)[10]. The highest risk of venous thromboembolism has been observed immediately after the delivery (60.0; 95% CI, 47.2-76.4), declining to 3.8 (95% CI, 2.5-5.8) 5 to 6 weeks after delivery[10]. These results must be interpreted cautiously according to the retrospective nature of the study. Moreover, International Classification of Disease codes were used and these may underestimate the incidence of symptomatic venous thromboembolism[11]. A recent article, analyzing a large, prospective primary care database of 972,683 women aged 15 to 44 years, showed an incidence rate ratio compared with non-pregnant women of 1.6 (95% CI,

0.9-2.8) during the first, 2.1 (95% CI, 1.3-3.4) during the second, and 6.1 (95% CI, 4.7-7.9) during the third trimesters[12]. The incidence rate ratio during the postpartum period was 22.1 (95% CI, 18.1-27.1), compared with non-pregnant women[12].

The distribution between symptomatic deep venous thrombosis and symptomatic PE events in pregnant women is quite different from the non-pregnant population. Deep venous thrombosis accounts for 85% of pregnancy-related symptomatic venous thromboembolism, mostly occurring antepartum. On the contrary, the majority of PE events in pregnancy (85%) are diagnosed postpartum, often in association with cesarean delivery[8].

RISK FACTORS

Apart from the prothrombotic state that characterizes the physiology of pregnancy, the risk of developing venous thromboembolism is dependent on both inherited and acquired risk factors, which include personal risk factors and pregnancy-associated risk factors. The known risk factors present in the general population could also represent risk factors for pregnancy-associated venous thromboembolism, although not completely investigated. Venous thromboembolism, immobilization, overweight, cigarette smoking, sickle cell disease, surgery, age, cancer, and inflammatory disorders are general risk factors for venous thromboembolism that can also occur in pregnant women. Moreover, pregnancy-specific risk factors have been described in different studies, suggesting assisted reproduction technique, multiple pregnancies, intrauterine fetal growth restriction, preeclampsia, emergency cesarean section, postpartum hemorrhage, infection, and parity as the main pregnancy-specific risk factors[1,7,13,14]. However, their odds ratios (ORs) largely differ between studies and between ante- and postpartum periods.

The presence of inherited and acquired thrombophilia increases the risk of pregnancy-related venous thromboembolism. Table 1 shows the relative and absolute risks of pregnancy-related venous thromboembolism in the presence of specific thrombophilic defects. Women with homozygosity for factor V Leiden or for the prothrombin G20210A mutation have the highest relative risk for developing venous thromboembolism (OR, 34.4; 95% CI, 9.9-120.1, and 26.4; 95% CI, 1.2-559.3, respectively) with an absolute risk of pregnancy-related venous thromboembolism of approximately 4%[15,16]. Heterozygosity for factor V Leiden and for the prothrombin G20210A mutation and other inherited abnormalities, such as deficiencies of the

natural anticoagulants antithrombin, protein S and protein C, are associated with an absolute lower risk. Deficiency of one of the natural anticoagulants have historically been considered as high risk on the basis of older studies that evaluated severely affected families with likely coinheritance of other thrombophilias[15-17]. Furthermore, often also recurrent venous thromboembolism was considered[18].

A positive family history of venous thromboembolism (deep venous thrombosis or pulmonary embolism in at least one first-degree relative) increases the risk for pregnancy-related venous thromboembolism from two- to fourfold independently from the presence of a concomitant thrombophilia[19]. Furthermore, data from retrospective studies indicate that in women with a personal history of venous thromboembolism, the risk of recurrences during pregnancy increases at least by three- to fourfold in comparison with the risk of recurrences outside pregnancy[20,21].

Data on acquired thrombophilia as a risk factor for pregnancy-related venous thromboembolism are inadequate to allow conclusions. One case–control study in pregnant women showed that the presence of lupus anticoagulant, anticardiolipin, or anti-β_2 glycoprotein 1 antibodies increased the risk of venous thromboembolism by 1.4-fold (95% CI, 0.8-2.5), but the validity of the results is limited by the fact that the presence of acquired thrombophilia was determined several years after the pregnancy-related venous thromboembolism[22].

Table 1 Risk of pregnancy-related venous thromboembolism in thrombophilic women stratified by family history for thromboembolism

Thrombophilic defect	Prevalence (population), %[73-77]	Estimated relative risk (OR, 95%CI)	Absolute risk of VTE[a], % of pregnancies (95%CI)[a]	
			Family studies	*Nonfamily studies*
FV Leiden (heterozygous)	2.0–7.0	8.3 (5.4–12.7)[17]	3.1 (2.1–4.6)[78,79]	1.2 (0.8–1.8)
FV Leiden (homozygous)	0.2–0.5	34.4 (9.9–120.1)[17]	14.0 (6.3–25.8)[80,81]	4.8 (1.4–16.8)
Prothrombin G20210A mutation (heterozygous)	2.0	6.8 (2.5–18.8)[17]	2.6 (0.9–5.6)[82]	1.0 (0.3–2.6)
Prothrombin G20210A mutation (homozygous)	Very rare	26.4 (1.2–559.3)[17]	–	3.7 (0.2–78.3)
Antithrombin deficiency	<0.1–0.6	4.7 (1.3–17.0)[17]	3.0 (0.08–15.8)[83]	0.7 (0.2–2.4)
Protein C deficiency	0.2–0.3	4.8 (2.2–10.6)[17]	1.7 (0.4–8.9)[83]	0.7 (0.3–1.5)
Protein S deficiency	<0.1–0.1	3.2 (1.5–6.9)[17]	6.6 (2.2–14.7)[83]	0.5 (0.2–1.0)
Compound heterozygosity, FV Leiden, and prothrombin mutation	–	–	1.8 (0.5–6.3)[84]	–
Lupus anticoagulants (persistent)[b]	Inconsistent data	2–10 (wide CIs)	–	0.3–1.4 (95% CI uncertain)

[a]Observed in family studies, estimated from multiplying the baseline risk of 1.40 per 1,000 by the relative risk in non-family studies. [b]Risk increase is stronger for lupus anticoagulant than for anticardiolipin or anti-β_2 glycoprotein 1 antibodies. Data are limited; hence, the estimated absolute risk should be interpreted with caution.

Abbreviations: CI, confidence interval; FV, factor V; OR, odds ratio; VTE, venous thromboembolism.

Pathophysiology

The increased risk of pregnancy-related venous thromboembolism during pregnancy is primarily because of physiological changes that predispose women to a procoagulant state. These modifications are probably a physiological evolutionary adaptation to decrease the bleeding induced by the placental separation at delivery.

The increase in diameter of the capacitance vessels and the presence of venous stasis are also assumed to be important predisposing factors for venous thromboembolism in the first trimester, and both seem to be induced by the increased blood levels of progesterone typical at that gestational time[23,24]. The compression of the pelvic veins by the gravid uterus becomes more important in conditioning an increased venous stasis in the last two trimesters of pregnancy[16]. Pregnancy is characterized by a marked increase of progesterone and estrogens, and these changes are responsible for the higher incidence of venous thromboembolism during pregnancy, causing a physiologically switch in the global hemostatic balance characterized by an acquired and transient state of hypercoagulability. Pregnancy is characterized by an increase in procoagulants (plasma levels of factors V, VII, VIII, IX, X, XII, fibrinogen, von Willebrand factor) and a decrease in anticoagulant activity due to a decrease in protein S concentration and an increase in activated protein C resistance[25,26]. Fibrinolytic activity is decreased because of an increase in plasminogen activator inhibitor 1 and 2 activities and a decrease in tissue plasminogen activator. Furthermore, markers of thrombin generation, such as D-dimer, the prothrombin fragment F1 + 2, and thrombin–antithrombin complex are also increased[26-27]. The direction of changes in hormones and coagulation proteins are depicted in Figure 1. Although normal pregnancy is characterized by increased levels of platelet-derived and endothelial-derived microparticles, a cause–effect relationship with venous thromboembolism has never been investigated[28].

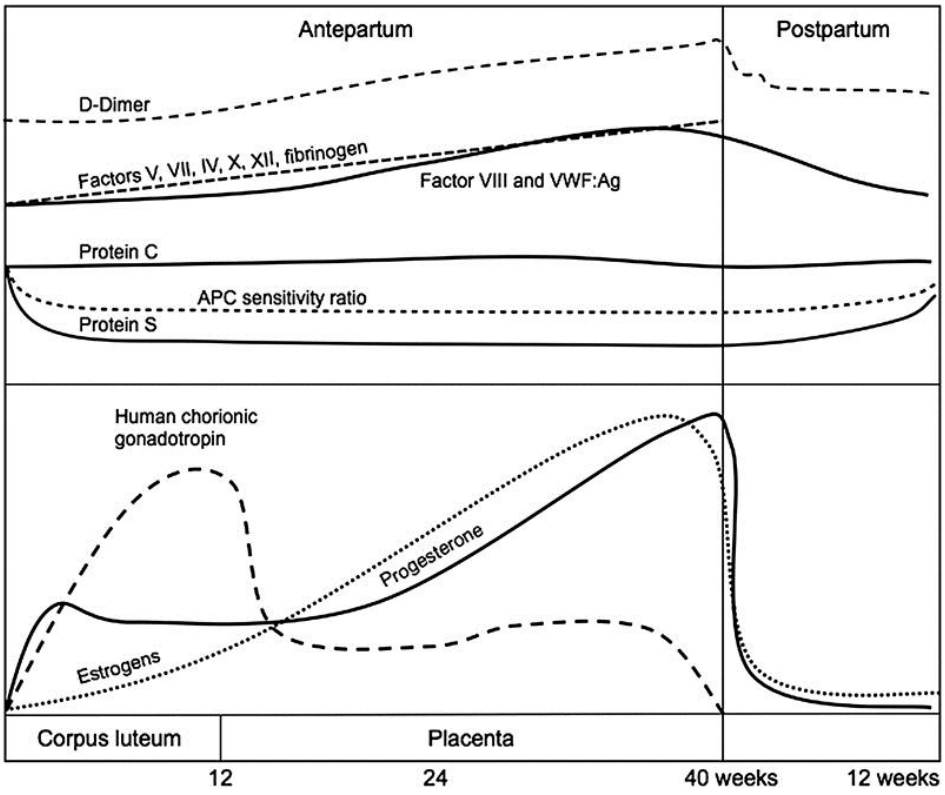

Figure 1. Qualitative levels and hemostatic direction of hormone changes during normal pregnancy

Abbreviations: APC, activated protein C; VWF:Ag, von Willebrand factor antigen[25,26,71,72].

The peculiarity of pregnancy-specific risk factors might be responsible for the different localizations of the thrombus along the deep venous system as compared with the non-pregnant deep venous thrombosis population. In pregnancy-related deep venous thrombosis, the thrombus is more likely to become symptomatic in the left leg (90 vs. 60% in non-pregnant patients) probably because of the presence of the gravid uterus and the consequent compression of the left iliac vein by the right iliac artery at their crossing[8,9,29]. Furthermore, in contrast to non-pregnant patients, deep venous thrombosis is often restricted to the more proximal veins without extension to the more distal veins: more than 60% of cases of deep venous thrombosis is confined to the iliac and/or femoral vein, and 17% of all deep venous thrombosis during pregnancy is diagnosed as an isolated iliac vein thrombosis[29].

DIAGNOSIS

The diagnosis of venous thromboembolism in the non-pregnant population derives from a combination of different strategies, which include clinical probability assessed by patient history, clinical findings, D-dimer assay, and imaging tests. An accurate diagnosis of a new acute symptomatic venous thromboembolism during pregnancy is imperative, not only because of venous thromboembolism -related mortality and bleeding risk of anticoagulant treatment but also because it alters the management of subsequent pregnancies, and forms a contraindication to the use of further hormonal therapy, for instance oral contraceptives. However, studies on the diagnostic strategies of venous thromboembolism have excluded pregnant women, and the extrapolation of diagnostic imaging algorithms for venous thromboembolism in non-pregnant patients is not adequate when applied in pregnancy.

First of all, clinical findings of venous thromboembolism in the pregnant women can be non-specific and the typical venous thromboembolism signs and symptoms (leg swelling, tachycardia, tachypnea, and dyspnea) may be normal findings in pregnancy because of the physiological cardiovascular and metabolic changes[30]. Clinical decision rules using these signs and symptoms are therefore probably not useful in pregnancy and have never been validated in pregnant patients. Moreover, although D-dimer assays have a high sensitivity in the non-pregnant population and are increasingly used for the exclusion of venous thromboembolism, D-dimer levels are often increased during normal pregnancy and this reduces the test's specificity. For these reasons, the use of clinical decision rules combined with D-dimer testing is not recommended to rule out pregnancy-related venous thromboembolism.

The cornerstone of diagnosing pregnancy-related venous thromboembolism is demonstrating the presence of the clot with the use of objective imaging tests, such as compression ultrasound, computed tomography scans, perfusion / ventilation) scintigraphy, and angiography. However, all of these tests show some pregnancy-specific limitations which should be acknowledged.

For diagnosis of deep venous thrombosis (Figure 2), compression ultrasound may be less reliable in the pregnant population because of its reduced sensitivity in diagnosing isolated pelvic and iliac vein thrombosis, as often is seen during pregnancy[31]. For this purpose, the use of serial two- or three-point compression ultrasound in case of high suspicion of proximal deep venous thrombosis is not recommended. Although duplex Doppler ultrasonography can suggest isolated iliac vein thrombosis detecting an absent waveform on modulation of respiration, this indirect finding is influenced by anatomic and operator-dependent elements, and both

false-positive and false-negative results might occur. Finally, in case of a high suspicion of iliac vein thrombosis and a negative duplex Doppler, venography or an angio-computed tomography scan could be considered in these situations to diagnose very proximal deep venous thrombosis.

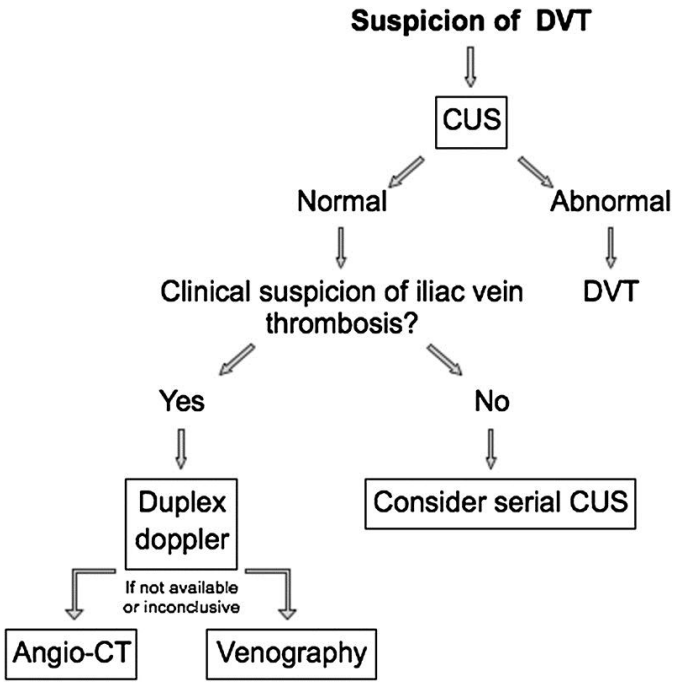

Figure 2. Algorithm of a clinical suspicion of deep venous thrombosis in pregnancy

Abbreviations: CT, computed tomography; CUS, compression ultrasonography; DVT, deep vein thrombosis.

The choice of imaging for a diagnosis of pulmonary embolism (Figure 3) is strongly dependent on the local availability and expertise. Pulmonary embolism is a life-threatening condition and any diagnostic procedure should be performed urgently in case of significant suspicion. Performing a CT scan as a first-line imaging test gives the possibility to reach a reliable pulmonary embolism diagnosis, and to demonstrate an alternative diagnosis if pulmonary embolism is excluded. High probability perfusion scintigraphy is diagnostic for pulmonary embolism and delivers lower maternal radiation dose compared with CT scan: for these reasons, it is recommended as the first-line imaging test by the recent guidelines by the American Thoracic Society[32].

Normal or low probability perfusion scintigraphy rules out pulmonary embolism, but the most important disadvantage is the non-diagnostic result demonstrated by the majority of scans[32-34]. In these patients, a further imaging test, such as CT scan, is mandatory with a consequent delay in diagnosis. Furthermore, perfusion scintigraphy is not widely available in an emergency setting.

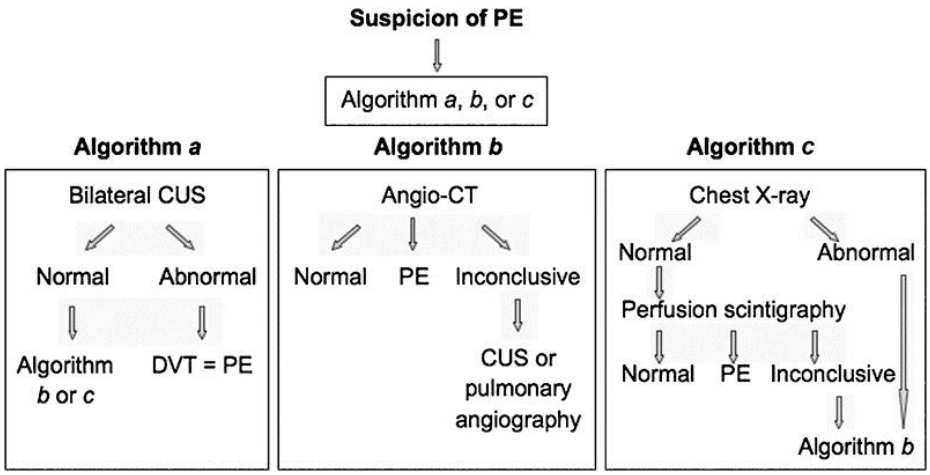

Figure 3. Algorithms of a clinical suspicion of pulmonary embolism in pregnancy

Abbreviations: Angio-CT, angio-computed tomography; CUS, compression ultrasonography; DVT, deep vein thrombosis; PE, pulmonary embolism.

The fetal radiation exposure rates for most of the imaging tests are much lower than the threshold dose for induction of malignancies (100 mSv), justifying their use for excluding a potentially fatal pulmonary embolism[35]. All imaging tests can be used in pregnancy as long as a maximum effort is done to achieve the lowest dose as possible. Furthermore, the iodinated contrast agents used in computed tomography scan and angiography cross the placenta: although no effect on fetal thyroid function has ever been reported, the newborns should be screened for hypothyroidism. In case of a pregnancy-related pulmonary embolism, it is a reasonable approach to avoid radiation exposure and to perform a compression ultrasound as a first-line imaging test to evaluate the presence of an asymptomatic deep venous thrombosis. However, it is a debated approach as it may result in false-positive compression ultrasound results, and it should not delay the prompt evaluation with an objective diagnostic testing of the lungs[36]. Hence, we recommend the use of compression ultrasound as a

surrogate of pulmonary embolism imaging tests in pregnant women only if signs or symptoms of deep venous thrombosis are present.

USE OF ANTICOAGULANT DRUGS DURING PREGNANCY

Pregnancy is a peculiar condition in medicine because every decision on the treatment contemplates an effect and risk not only for the health of the mother but also for the fetus. The balance between risks and benefits of any anticoagulant administration during pregnancy is often challenging because of the lack of well-sized observational or interventional studies about therapeutic options[15]. Pregnant women have been excluded from all major phase III trials investigating the efficacy and safety of different anticoagulant regimens. The low grade of evidence is reflected by the low strength of the recommendations in the guidelines from several scientific societies, such as the American College of Chest Physicians (ACCP) or the ATS[15,32].

The main complications of anticoagulant therapy during pregnancy can involve the woman (bleeding, heparin-induced thrombocytopenia, heparin-associated osteoporosis, bruising, and local allergic reactions for heparin-related compounds) or the fetus (congenital malformations, fetal bleeding). Bleeding is mostly related to the delivery and the cesarean section. Hence anticoagulation should be stopped before delivery and restarted after 12 to 24 hours, depending on the amount of blood loss and the estimated risk of recurrent venous thromboembolism in absence of anticoagulation[35]. Furthermore, anticoagulant treatment is a contraindication to the use of neuraxial anesthesia because of a small risk of neuraxial hematoma and a discontinuation of the anticoagulation is mandatory, that is, at least 24 hours for low-molecular-weight heparin or 6 hours for unfractionated heparin.

In the pregnant population, heparins are preferred over vitamin K antagonists, which cross the placenta and can inflict damage to the fetus and cause vitamin K antagonist-related embryopathy. In the third trimester and at delivery, vitamin K antagonists increase the bleeding risk for the fetus due to the physiological fetal low levels of vitamin K-dependent coagulation factors. The largest amount of data comes from studies on vitamin K antagonist warfarin: the incidence of warfarin-related embryopathy in the first trimester of pregnancy is 6.4% (95% CI: 4.6-8.9), according to a systematic review that considered 549 patients from 24 studies[37]. It characteristically presents as nasal hypoplasia, stippled epiphyses, or limb hypoplasia. The risk of embryopathy can be minimized if warfarin is switched to

heparin between 6th and 12th weeks of gestation. Also, the use of vitamin K antagonists in the second and third trimester has been associated with a twofold increased risk of minor neurologic dysfunction or a lower than 80 intelligence quotient (OR 2.1; 95% CI: 1.2–3.8)[38,39].

Among heparins, low-molecular-weight heparins should be considered the first choice drugs in pregnant women for both prophylaxis and therapy and their safety has been evaluated in pregnant women by a systematic review and several observational studies[40-43]. low-molecular-weight heparins do not cross the placenta and show preferable characteristics, such as better bioavailability than unfractionated heparin, longer plasma half-life, more predictable dose response, no need of activated partial thromboplastin time monitoring, and improved maternal safety profile with respect to osteoporosis and heparin-induced thrombocytopenia[40,41,44]. Some important issues about the use of both prophylactic and therapeutic doses of low-molecular-weight heparin in pregnant women remain controversial, such as the determination of the appropriate dose on the basis of body weight, which increases during pregnancy, and the increase of volume of distribution and glomerular filtration, which modify the renal clearance of low-molecular-weight heparin. These controversies on the most appropriate dose of low-molecular-weight heparin in pregnancy have led to suggestions of monitoring the anticoagulant effect using anti-FXa levels. Nevertheless, no large studies demonstrate that there is an optimal therapeutic anti-FXa range on the basis of clinical end points, the appropriate target anti-FXa is uncertain and there is only partial accuracy and reliability of the measurement[45]. The latest ACCP guidelines do not overtly recommend an anti-FXa monitoring in pregnant women unless they have a prosthetic valve[15]. On the contrary, some experts indicate the need of a periodic monitoring in pregnant women and a dose escalation of low-molecular-weight heparin to maintain anti-FXa activity levels on the basis of some small studies[46-48]. Anti-FXa levels in women treated with therapeutic doses of low-molecular-weight heparin can be monitored 4 hours after the last subcutaneous injection and target to an anti-FXa level of 0.8 to 1.6 with a once-daily regimen of low-molecular-weight heparin (0.6 to 1.0 unit/mL if a twice-daily regimen is used) at periodic intervals[35].

When low-molecular-weight heparin administration is problematic or contraindicated because of allergic reactions, or in women with renal dysfunction, unfractionated heparin can be used both intravenously and subcutaneously and monitored as in the non-pregnant population, although it is known that activated partial thromboplastin time monitoring is less reliable in pregnancy[44].

Heparin-induced thrombocytopenia is a transient autoimmune prothrombotic syndrome, which may develop during anticoagulant treatment with any kind of heparin[49]. The description of heparin-induced thrombocytopenia in pregnant women is anecdotal and pregnancy is classified as a low-risk condition for heparin-induced thrombocytopenia: this low incidence in pregnancy is probably due, as for other autoimmune diseases, to the protective effect of estrogen hormones on the immune system[50,51]. Heparin-induced thrombocytopenia is transient and potentially life threatening: its management is based on the immediate discontinuation of heparin and on the administration of an alternative non-heparin anticoagulant at full dose[50].

The description of synthetic pentasaccharide fondaparinux administration in pregnancy derives from some case reports suggesting that it may be a safe option in women with allergic reactions to low-molecular-weight heparin or administered off-label to patients with acute heparin-induced thrombocytopenia, if no other alternative approved drugs are available[52-54]. However, routine use is not recommended because of its ability to cross the placenta in humans and excretion in the milk of lactating rats[55-56].

There are no studies assessing the safety and efficacy of new oral anticoagulants (e.g. direct thrombin inhibitors and factor Xa inhibitors) in pregnancy and breast-feeding. Moreover, they appear to cause animal toxicity and to be secreted into breast milk. Their use is therefore not recommended in pregnancy as well as during lactation[57,58].

PREVENTION OF PREGNANCY-RELATED VENOUS THROMBO-EMBOLISM

There are two main general categories of pregnant women for whom thromboprophylaxis should be considered: women with a history of venous thromboembolism and women without a history of venous thromboembolism, but with multiple risk factors, such as thrombophilia and cesarean section. The optimal low-molecular-weight heparin dosage in these two categories is not completely established, and prophylactic low doses, intermediate doses, or full therapeutic doses are possible options for the prophylaxis in different clinical settings, based on the patient's personal history. There are neither high quality prospective trials nor randomized clinical trials that compared different doses of thromboprophylaxis in pregnant women with prior venous thromboembolism. Some of the observational studies evaluating the risk of recurrent venous thromboembolism stratify patients

according to the perceived risk of recurrence which was demonstrated to range from 0 to 15%, although 15% is probably an overestimation, because venous thromboembolism had not been diagnosed with objective testing[59-61]. In the absence of high quality direct evidence, all the therapeutic regimens have to balance the calculated risk of recurrences against the risks of adverse effects. The incidence of overall bleeding with the use of prophylactic low-molecular-weight heparin dosage in pregnancy is approximately 2% (95% CI: 1.50-2.57), considering antepartum, postpartum, and wound hematoma[40].

In the balance of the risk of antepartum recurrent venous thromboembolism, one should consider the circumstances present during the prior venous thromboembolism episode: an unprovoked venous thromboembolism or a venous thromboembolism provoked by an oral contraceptive pill, or pregnancy, or in combination of thrombophilia seem to confer the highest risk of recurrence[21,60,62]. In these settings, an antepartum prophylactic or intermediate dose of low-molecular-weight heparin could be a reasonable option. As the risk of venous thromboembolism during pregnancy seems to increase starting from the first trimester, antepartum prophylaxis should be initiated as soon as possible.

While the risk of venous thromboembolism for asymptomatic women with thrombophilia has been well quantified over the past years, no intervention studies have compared different prophylactic regimes. Furthermore, it seems rational to include the presence of a family history in the recommendations for prophylaxis. Based on the risk profile of thrombophilia, ACCP guidelines suggest that only (1) homozygous carriers of the factor V Leiden or prothrombin gene mutations (regardless of family history) and (2) women with the other low-risk inherited thrombophilias with a family history of venous thromboembolism should be considered for thrombophrophylaxis[15]. For women with thrombophilia other than homozygous factor V Leiden or prothrombin gene mutations without a family history of venous thromboembolism, ACCP guidelines suggest clinical surveillance instead of thromboprophylaxis both antepartum and postpartum. Table 2 presents a summary of recommendations to prevent a first or recurrent pregnancy-related venous thromboembolism in relation to personal and familiar history, thrombophilia, and cesarean section.

Women with deficiencies of the natural anticoagulants without prior venous thromboembolism seem to have a similar risk than women with other low-risk thrombophilia. However, it is important to emphasize that, in absence of conclusive evidence, there is considerable disagreement among experts about the indications for thromboprophylaxis. Finally, elastic stockings for inpatients are safe adjunctive

options to be considered during low-molecular-weight heparin prophylaxis in women with a high-risk profile and multiple risk factors for venous thromboembolism.

Table 2. Summary of recommendations to prevent a first or recurrent pregnancy-related venous thromboembolism[a]

Antepartum[b] and postpartum prophylaxis	Postpartum only prophylaxis during 6 weeks[c]	No pharmacologic prophylaxis[c]
Women with a single unprovoked episode of VTE, or provoked by the use of oral contraceptives, pregnancy, or postpartum	Women with a history of a single episode of VTE related to a major nonhormonal transient risk factor	General population
Women with a history of recurrent VTE	Women with hereditary thrombophilia and a positive family history[d] of VTE	Women with a positive family history[d] of VTE
Women who are homozygous for factor V Leiden or prothrombin mutation who have a positive family history[d] of VTE	Women who are homozygous for factor V Leiden or prothrombin mutation who do not have a positive family history[d] of VTE	Women who are heterozygous for factor V Leiden or prothrombin mutation who do not have a positive family history[d] of VTE
-	Women undergoing a cesarean section with multiple risk factors that persist following delivery[15]	Women undergoing cesarean section without additional thrombosis risk factors[15]

[a]Recommendations are weak, based on a low level of evidence leaving room for individualized prophylactic; strategies based on the patient's preferences, on the concomitant diseases, and on the presence of other risk factors of venous thromboembolism. [b]Antepartum prophylaxis should ideally start as soon as possible after conception. [c]Unless women can be categorized into one of the more aggressive prophylactic strategies in this table. [d]A positive family history is defined as having a first-degree relative with venous thromboembolism.
Abbreviation: VTE, venous thromboembolism.

THERAPY OF PREGNANCY-RELATED VENOUS THROMBOEMBOLISM

In the non-pregnant population, low-molecular-weight heparin administration for the treatment of acute venous thromboembolism is firmly established with similar efficacy of once- *vs* twice-daily regimens[43]. In pregnant patients, a weight-adjusted dosing regimen is also used regardless of the number of daily administrations. The need for an increase of the dose in proportion to the change of weight throughout the pregnancy is controversial[63].

When low-molecular-weight heparins at therapeutic doses are used, the incidence of major bleeding varies according to different studies. In a systematic review of 15 studies, including 174 women treated for acute venous thromboembolism, it was estimated to be 1.72% (95% CI: 0.36-5.00)[40]. In a prospective evaluation of 126 patients with acute venous thromboembolism, the observed clinical relevant bleeding during pregnancy was 6%[43]. With regard to the postpartum hemorrhage (defined as 500 mL of blood loss or more), the risk varies between 2 and 18%[43,64,65]. In a cohort of pregnant patients treated with therapeutic doses of low-molecular-weight heparin in the Academic Medical Center in Amsterdam, the incidence of postpartum hemorrhage in women receiving therapeutic doses of low-molecular-weight heparin was high, but not statistically different from a cohort of women who delivered in the same hospital and who were not treated with anticoagulants (18 vs. 22%; RR, 0.8, 95% CI: 0.5-1.4)[65].

Switching from low-molecular-weight heparin to unfractionated heparin may be considered in case of acute venous thromboembolism within 2 weeks of delivery, a high risk of bleeding, or a high risk of recurrent venous thromboembolism. Ideally, unfractionated heparin should be discontinued 4 to 6 hours before elective induction or a cesarean section, to maintain as long as possible an adequate level of anticoagulation. In addition, if an acute venous thromboembolism occurs close to the expected date of delivery, a retrievable inferior vena cava filter can be inserted and removed postpartum. However, experience is limited and the risk of filter migration and inferior vena cava perforation may be increased during pregnancy and has to be considered[66].

The maternal and placental effects of thrombolytic therapy have not been adequately studied. The number of women treated with thrombolytic therapy, mostly streptokinase, is small and its use should be reserved for life-threatening maternal thromboembolism only[16].

PREVENTION OF POST-THROMBOTIC SYNDROME

The post-thrombotic syndrome is a well-known complication of acute venous thromboembolism in 25 to 50% of the non-pregnant population[67]. The clinical findings (swelling, pain, itching, skin discoloration, skin ulcers) develop after months or years from the acute venous thromboembolism event and the use of elastic compression stockings has been shown to reduce the risk of developing post-thrombotic syndrome by approximately 50%[68,69]. It is likely that post-thrombotic syndrome is more frequent in pregnant women because of the more proximal deep venous thrombosis occurrence. A recent article describes the long-term follow-up of women with pregnancy-related deep venous thrombosis, and 42% developed post-thrombotic syndrome with 7% having a severe form. Severe post-thrombotic syndrome was defined as the presence of a venous ulcer of the lower limb or a high clinic score based on signs and symptoms throughout two consecutive visits at least 3 months apart. Unfortunately, no information is given in the article about the use of elastic compression stockings[70].

CURRENT CLINICAL CHALLENGES IN THE MANAGEMENT OF PREGNANCY-RELATED VENOUS THROMBOEMBOLISM

The level of evidence for the diagnosis and management of pregnancy-related venous thromboembolism is low: virtually all diagnostic or management studies evaluated only small cohorts of women and often methodological limits do not permit any firm conclusion.

The management of venous thromboembolism in the non-pregnant adult population is being characterized by an increasing number of evidence-based diagnostic algorithms and by the development of new anticoagulant drugs for multiple indications. Pregnant women have been excluded by most of the large trials performed in the last decade, from which we derive the tools for the management of venous thromboembolism. By consequence, the management of venous thromboembolism in this setting remains a serious challenge for clinicians.

With regard to future research, there is a need for methodologically strong studies in pregnant women, especially concerning some debated issues, such as risk stratification and the role of thrombophilia, optimal low-molecular-weight heparin dosages for prevention and treatment of venous thromboembolism, utility of anti-FXa

levels and their correlation with clinical outcomes, and the correct management of the bridging therapy before and after the delivery. A pragmatic approach is the best when we have to deal with this special population of pregnant women.

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Clonal populations of hematopoietic cells with paroxysmal nocturnal hemoglobinuria phenotype in patients with splanchnic vein thrombosis

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ABSTRACT

Splanchnic vein thrombosis is a serious complication in patients with paroxysmal nocturnal hemoglobinuria. Mutant paroxysmal nocturnal hemoglobinuria clones can be associated with an increased risk of splanchnic vein thrombosis even in the absence of overt disease, but their prevalence in non-selected patients with splanchnic vein thrombosis remains unknown.

Patients with objective diagnosis of splanchnic vein thrombosis and without known paroxysmal nocturnal hemoglobinuria were tested for the presence of paroxysmal nocturnal hemoglobinuria clone using high-sensitivity flow cytometric analysis.

A total of 202 patients with splanchnic vein thrombosis were eligible, 58.4% were males, mean age was 54.6 years (range 17-94), site of thrombosis was portal in 103 patients, mesenteric in 67, splenic in 37, and supra-hepatic in 10. Splanchnic vein thrombosis was associated with JAK2 V6167F in 28 of 126 (22.2%) screened patients, liver cirrhosis in 15.3% patients, recent surgery in 10.9%, and myeloproliferative neoplasm in 10.6%, whereas in 34.6% of patients neither permanent nor transient risk factors were detected. None of the patients had a clearly demonstrable paroxysmal nocturnal hemoglobinuria clone, but in two patients (0.99%, 95% Confidence Interval 0.17%-3.91%) we observed very small paroxysmal nocturnal hemoglobinuria clones (size 0.014% and 0.16%) confirmed in two independent samples. One patient had portal vein thrombosis and no associated risk factors, the second had superior mesenteric vein thrombosis and inflammatory bowel disease.

Very small paroxysmal nocturnal hemoglobinuria clones can be detected in patients with splanchnic vein thrombosis and no clinical manifestations of disease. Future studies are needed to explore the potential role of this finding in the pathogenesis of splanchnic vein thrombosis.

INTRODUCTION

Venous thromboembolism is one of the most frequent complications in patients with paroxysmal nocturnal hemoglobinuria and a major cause of mortality, with the majority of events occurring in the splanchnic veins[1]. Although the mechanism of thrombosis in patients with paroxysmal nocturnal hemoglobinuria remains poorly understood, a number of potential complement-mediated prothrombotic conditions have been described, including endothelial cell damage, deficiency of tissue factor pathway inhibitor, enhanced platelet activation, formation of procoagulant microparticles, and impaired fibrinolysis[2].

Because thrombosis can be the first manifestation of paroxysmal nocturnal hemoglobinuria, the presence of mutant paroxysmal nocturnal hemoglobinuria clones could be associated with an increased risk of splanchnic vein thrombosis even in the absence of overt disease, but the prevalence of these clones in patients with splanchnic vein thrombosis has never been investigated in Caucasian patients.

MATERIALS AND METHODS

In a multicenter, cross-sectional study, patients with objective diagnosis of splanchnic vein thrombosis (portal, mesenteric, splenic, or supra-hepatic) within the previous 2 years were investigated for the presence of paroxysmal nocturnal hemoglobinuria clones. Known or clinically suspected paroxysmal nocturnal hemoglobinuria and ongoing treatments potentially interfering with the laboratory assessment of paroxysmal nocturnal hemoglobinuria clone (e.g. recent chemotherapy, treatment with hypomethylant drugs or immunosuppressive therapy) were exclusion criteria.

After the obtainment of written informed consent, patients underwent blood sampling and information was collected on demographic characteristics, site of splanchnic vein thrombosis and presence of risk factors. The presence of paroxysmal nocturnal hemoglobinuria clone was centrally assessed at the laboratory of the Hematology Unit of the S. Bortolo Hospital in Vicenza, Italy, using multiparameter flow cytometric analysis. A six-colour multiparameter approach was designed to perform high-sensitivity assay on at least $3x10^5$ granulocytes, able to identify an abnormal population of >0.01% paroxysmal nocturnal hemoglobinuria cells[3]. Monocytes were also tested as an internal positive control. To increase specificity, a multiparameter gating strategy was used by combining light scatter cell properties and CD45

expression with two different lineage markers, bright CD15 for granulocytes and bright CD33 for monocytes. Furthermore, the expression of two different GPI-linked molecules (fluorescein-labeled proaerolysin FLAER/CD24 on granulocytes and FLAER/CD14 on monocytes) was studied. All samples were tested using a single 6-colour reagent combination as follows: FLAER (fluorescein-labeled proaerolysin), CD24-PE, CD45-PercP, CD33 PE-cy7, CD15 APC, CD14 APC-H7.

All samples were processed within 24 hours of their arrival to the central laboratory (24 to 48 hours from blood sampling). An automated WBC cell count was performed before staining procedure to decide the appropriate volume of blood sample and the number of the test tubes in order to achieve a target cell of 5-6x10^5neutrophils. To preserve the light scatter properties of the cells, a stain-then-lyse procedure was performed. Samples with a neutrophil count <1,000/μL were pre-lysed and then stained in order to reduce the number of test tubes and consumption of antibodies. Because of the high-sensitivity technique used, samples with FLAER negative events >95th percentile underwent a second evaluation to conservatively reduce the rate of false-positive results. This second evaluation was performed on cases with a first positive test, matched (in a 1:3 ratio) with controls with a first negative test. Cases and controls were reanalyzed at the central laboratory by the same operator (IG) blinded to the initial result of the first tests for each new sample.

A sample of convenience of at least 200 patients was deemed sufficient to detect at least one positive sample if the true prevalence of paroxysmal nocturnal hemoglobinuria clones in asymptomatic subjects was 2%. Descriptive statistics were used for the purpose of this study. The study was conducted in accordance with the Declaration of Helsinki and Ethics Committee approval was obtained at each participating center. Study data were collected and managed using REDCap electronic data capture tools hosted at the Vicenza Hematology Department[4].

RESULTS

The study enrolled 202 patients: demographic characteristics, site of thrombosis, and risk factors are reported in Table 1. Median time elapsed between the index event and blood testing was 349 days.

Table 1. Demographic characteristics, site of splanchnic vein thrombosis and risk factors.

Total number	n=202
Age (mean, range)	54.6 years (17–94)
Male gender (number, %)	118 (58.4%)
Portal vein thrombosis (number, %)	103 (51.0%)
Superior Mesenteric vein thrombosis (number, %)	66 (32.7%)
Inferior Mesenteric vein thrombosis (number, %)	8 (4.0%)
Splenic vein thrombosis (number, %)	39 (19.3%)
Supra-hepatic thrombosis (number, %)	10 (4.9%)
Multiple vein thrombosis (number, %)	64 (31.7%)
Personal history of venous thrombosis (number, %)	25 (12.4%)
Family history of venous thrombosis (number, %)	26 (12.9%)
Antithrombin deficiency (number, %)	7/141 (5.0%)
Protein C deficiency (number, %)	9/129 (7.0%)
Protein S deficiency (number, %)	6/130 (4.6%)
Factor V Leiden (number, %)	14/151 (9.3%)
Prothrombin G20210A (number, %)	20/145 (13.8%)
Lupus anticoagulant (number, %)	7/142 (4.9%)
Hyperomocysteinemia (number, %)	25/141 (17.7%)
JAK2 V617F (number, %)	28/126 (22.2%)
No associated risk factors (number, %)	70 (34.6%)
Cancer (number, %)	17 (8.4%)
Inflammatory/infectious disease (number, %)	14 (6.9%)
Myeloproliferative neoplasm (number, %)	21 (10.4%)
Surgery (number, %)	22 (10.9%)
Liver cirrhosis (number, %)	31 (15.3%)
Hormonal therapy (number, %)	13 (15.5%)
Other risk factors (number, %)	37 (18.3%)

None of the patients had a clearly demonstrable paroxysmal nocturnal hemoglobinuria clone (e.g., >1% of the target granulocyte population), therefore ruling out the presence of “classic” paroxysmal nocturnal hemoglobinuria in our study population. By using a high sensitivity assay to evaluate the possible presence of smaller paroxysmal nocturnal hemoglobinuria clones, upon analysis on at least $3x10^5$ granulocytes, definite clusters of FLAER-negative cells were unambiguously identified in 9 patients, with the size of the paroxysmal nocturnal hemoglobinuria clone ranging from 0.01% to 0.16%. All nine patients were contacted for a second

assessment: 8 were available for testing, 1 died before the second test because of liver cirrhosis. As an internal control, 24 patients with an initially negative test for the paroxysmal nocturnal hemoglobinuria clone also underwent a second blood sampling. The mean time elapsed between testing was 14.5 months (range 1 to 21 months). After the second assessment, performed by the same laboratory operator who was blinded to the result of the previous clone size, a definite cluster of FLAER-negative cells was confirmed in 2 patients (clone sizes of 0.014% and 0.16%, respectively), for an observed prevalence of small paroxysmal nocturnal hemoglobinuria clones of 0.99% (95%: CI 0.17%-3.91%) in our study sample. No clustering of FLAER-negative cells was observed in the remaining 6 patients with a first positive sample or in the 24 patients with a negative sample at first examination. Time between diagnosis of splanchnic vein thrombosis and the first test in the two positive patients was 2 and 24 months, respectively. The first patient had portal vein thrombosis and no associated major risk factors, the second had superior mesenteric vein thrombosis and inflammatory bowel disease as a potential co-existing risk factor.

DISCUSSION

Paroxysmal nocturnal hemoglobinuria is a rare acquired disorder with three main clinical features: complement-mediated hemolysis, bone marrow failure, and a pro-thrombotic tendency. The clinical spectrum at presentation is highly variable, and clinical indications for paroxysmal nocturnal hemoglobinuria testing are in general limited to the patient presenting with unexplained hemoglobinuria and/or with Coombs-negative hemolytic anemia[3]. In these patients, a significant paroxysmal nocturnal hemoglobinuria clone is usually manifest, with paroxysmal nocturnal hemoglobinuria cells accounting for more than 1% of the granulocyte population. However, since other forms of paroxysmal nocturnal hemoglobinuria do not have hemolysis, paroxysmal nocturnal hemoglobinuria screening with high sensitivity assays has been proposed for other patient groups such as patients with aplastic anemia or the myelodysplastic disorder refractory anemia[5,6]. In these patients, the detection of smaller paroxysmal nocturnal hemoglobinuria clones is considered clinically important since it likely represents bone marrow failure that is immune mediated, with obvious therapeutic implications in favour of immunosuppressive therapy.

Whether patients with thrombosis at unusual sites should also be screened (and if so, whether the use of high sensitivity assays to detect small clones is advisable) remains

uncertain. It was suggested that testing for paroxysmal nocturnal hemoglobinuria, in particular in patients with Budd-Chiari syndrome, should be part of the standard etiology-screening as diagnostic signs of paroxysmal nocturnal hemoglobinuria may not be specific[5,7]. Indeed, the prothrombotic state may not only be determined by underlying hemolysis, but the presence of paroxysmal nocturnal hemoglobinuria clones, even of small size, may suggest the existence of a complement-mediated endothelial cell damage to GPI-deficient endothelial cells[2]. In our study we were unable to demonstrate the presence of a significant paroxysmal nocturnal hemoglobinuria clone in the 202 enrolled patients. However, in about 1% of patients with splanchnic vein thrombosis we detected the presence of very small paroxysmal nocturnal hemoglobinuria clones. This prevalence is similar to that reported in the two previous study conducted in this setting in Asian patients[8-10], and is much lower than the prevalence of paroxysmal nocturnal hemoglobinuria clones found in patients with aplastic anemia or myelodysplasia[11,12].

Whether our finding may have clinical implications remains difficult to establish. To provide the most accurate epidemiological information on the prevalence of paroxysmal nocturnal hemoglobinuria clones in this setting, we decided to include unselected patients with splanchnic vein thrombosis regardless of the presence of concomitant risk factors. Thus, it remains possible that a higher prevalence could have been detected if only patients with otherwise unexplained events were studied. However, if we only consider the 70 patients without co-existing risk factors, the prevalence remains similar to that observed in the whole study cohort (1.43%; 95%CI: 0.07%-8.77%). Because paroxysmal nocturnal hemoglobinuria is particularly associated with the Budd-Chiari syndrome[7], we cannot exclude that screening this population only could have led to different results. Due to the rarity of this disease, only 10 patients in our cohort had Budd-Chiari syndrome and none resulted positive at the assay.

The absence of a control group is a limitation of our study since little information exists on the prevalence of small paroxysmal nocturnal hemoglobinuria clones in healthy control subjects. Despite the difficulties inherent to the analysis of rare events using flow cytometry, we are confident that our findings are solid for a number of reasons. First, the reported frequency of paroxysmal nocturnal hemoglobinuria cells in normal subjects is 22–50 per million, or 1 to 2.5 in 50,000 cells, a frequency that is one or two logarithms lower than the clone sizes observed in our patients[11-13], and the prevalence of clones of >0.01%, although unknown, could be expected to be extremely rare. Second, we tried to exclude the presence of transient clones by repeated, blinded analysis in a second sample. It is essentially unknown if and how

very small clones may vary in size in the general population; however, small clones identified in normal individuals have been reported to be transient, and disappeared in subsequent samples[11]. Third, the assay we employed is considered highly sensitive and specific[3]. Yet, the high discrepancy between the rates of positive results here obtained in sequential assays deserves further investigation on the stability of clones of minimal sizes and on their clinical meaning. Finally, whether the presence of a paroxysmal nocturnal hemoglobinuria clone of minimal size can be considered a risk factor for thrombosis remains to be established; although the size of clone clearly correlates with the thrombotic risk, a significant rate of thrombosis has been reported also in patients with clones of small size[14].

In conclusion, in an adequately sized multicenter study, we were able to exclude that the classic paroxysmal nocturnal hemoglobinuria phenotype with a significant (>1%) clone size is a risk factor for splanchnic vein thrombosis, but we detected very small paroxysmal nocturnal hemoglobinuria clones in few of these patients. Our results discourage the use of high-sensitivity flow cytometry for paroxysmal nocturnal hemoglobinuria in patients with splanchnic vein thrombosis without any sign of hemolysis. Future studies should explore the potential role of these small paroxysmal nocturnal hemoglobinuria clones in the pathogenesis of splanchnic vein thrombosis by assessing the presence of an underlying complement-mediated prothrombotic state in these patients.

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12

New oral anticoagulants in elderly patients

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ABSTRACT

The new direct oral anticoagulants dabigatran etexilate, rivaroxaban, and apixaban have been extensively studied for prevention and treatment of venous thrombo-embolic disease and for stroke prevention in atrial fibrillation. Elderly patients have the highest incidence of thrombotic complications but also have the highest risk of anticoagulant associated bleeding. In this review we critically examine the balance between risks and benefits of direct oral anticoagulants compared with vitamin K antagonists in elderly patients enrolled in phase III randomized controlled trials for the management of venous thrombosis and stroke prevention in atrial fibrillation. Results show that the favourable balance between risks and benefits of direct oral anticoagulants is preserved in the elderly population.

INTRODUCTION

Increasing age is an important risk factor for arterial and venous thromboembolic disease. The risk of ischemic stroke increases 1.5-fold for every 10 years of age increase[1] with estimated incidences of 14 and 29 per 1,000 person-years for people aged 75 to <85 years and people 85 years or over, respectively[2]. The increased incidence is in part explained by the increased prevalence of stroke risk factors in the elderly, such as hypertension, heart failure and atrial fibrillation. While atrial fibrillation is uncommon in patients below 65 years of age (<2%), the prevalence is approximately 10% in patients aged 85 years or over[3]. Thereby, almost 25% of ischemic strokes in patients over 80 years of age are attributable to atrial fibrillation[4]. Oral anticoagulant therapy with vitamin K antagonists reduces the risk of ischemic stroke by 64% in patients with atrial fibrillation, and due to the higher incidence of stroke in the elderly, the absolute risk reduction is higher in elderly than in younger patients[5]. The incidence of venous thromboembolism rises similarly with age. The incidence of a first episode of deep vein thrombosis or pulmonary embolism is below 1 per 1,000 person-years in people under 50 years of age and rises to 6 per 1,000 person-years in patients over 80 years of age[6].

Although vitamin K antagonist therapy is highly effective for prevention and treatment of arterial and venous thromboembolism, anticoagulants can cause bleeding complications. The risk per year of major bleeding in patients treated with vitamin K antagonists is estimated at 2–3% with another 14% of patients experiencing minor bleeding complications[7]. The risk of anticoagulant associated bleeding is age-dependent and increases by approximately 40% per 10 years of age increase[7]. Concern about the risk of anticoagulant associated bleeding contributes to the underuse of vitamin K antagonists in patients with atrial fibrillation. Surveys from Europe and North-America have consistently shown that vitamin K antagonists are used in only 50-60% of patients with atrial fibrillation[8-10] and in only 35% of those 85 or older[8].

Direct oral anticoagulants are small molecules designed to specifically target individual clotting factors. Due to lower propensity for food and drug interactions, the anticoagulant effects of direct oral anticoagulants compared with vitamin K antagonists are much more predictable allowing them to be given in fixed doses without routine coagulation monitoring. The direct thrombin inhibitor, dabigatran etexilate, and the direct factor Xa inhibitors, rivaroxaban and apixaban, have undergone extensive testing in phase III randomized controlled trials for prevention and treatment of venous thromboembolism, for stroke prevention in patients with

atrial fibrillation and as secondary prevention after acute coronary syndromes[11-26]. In the venous thromboembolism treatment and atrial fibrillation trials direct oral anticoagulants were either non-inferior or superior to monitored vitamin K antagonist treatment, with similar or reduced rates of major bleeding[13,15,16,19,22-25]. Moreover, in patients with atrial fibrillation, each of the direct oral anticoagulants were associated with a 30–70% reduction in intracranial hemorrhage compared with warfarin[15,19,22].

Clinicians have questioned the generalizability of the results comparing direct oral anticoagulants with warfarin to elderly patients at highest risk of thrombosis and of bleeding. Elderly patients generally have more co-morbidities and concomitant medication use and a higher prevalence of chronic kidney disease than younger patients. Impaired renal function could lead to increased blood levels of direct oral anticoagulants because these agents are partially renally cleared (dabigatran 80%, rivaroxaban 33%, apixaban 25%), whereas vitamin K antagonists are 100% non-renally cleared[27-29]. Since direct oral anticoagulants are used at a fixed dose, more variability of the drug blood levels may be expected in the elderly and it is uncertain if this variability may unfavourably alter the balance between risks and benefits of treatment. In a recent case-series of 44 patients with dabigatran associated bleeding complications, 67% of patients were aged 80 years or over, raising concerns about the safety of direct oral anticoagulants in the elderly[30].

In this review we describe the safety and efficacy of direct oral anticoagulants in elderly patients. We focus on subgroup analyses of the phase III randomized controlled trials in which the direct oral anticoagulants were compared with vitamin K antagonists in patients with venous thromboembolism or atrial fibrillation.

METHODS

All phase III randomized controlled trials comparing direct oral anticoagulants with vitamin K antagonist therapy for initial and prolonged treatment of venous thromboembolism and for stroke prevention in atrial fibrillation were included. From these trials, all available analyses for age subgroups were obtained from the original publications of the specific trials, from their online supplements, from congress abstracts and from data product monographs on which the regulatory approval for these drugs was based. Hazard ratios (HRs) and concomitant 95% confidence intervals (95%CIs) of the direct oral anticoagulant *vs* vitamin K antagonist therapy for each available age category are presented. Consistency of the HRs among the various

age categories was assessed by the p-value for interaction between age category and treatment. The interaction was considered statistically significant when the interaction p-value was 0.05 or lower. Interaction p-values were calculated based on the HRs and 95%CIs in case they were not provided by the publication.

RESULTS

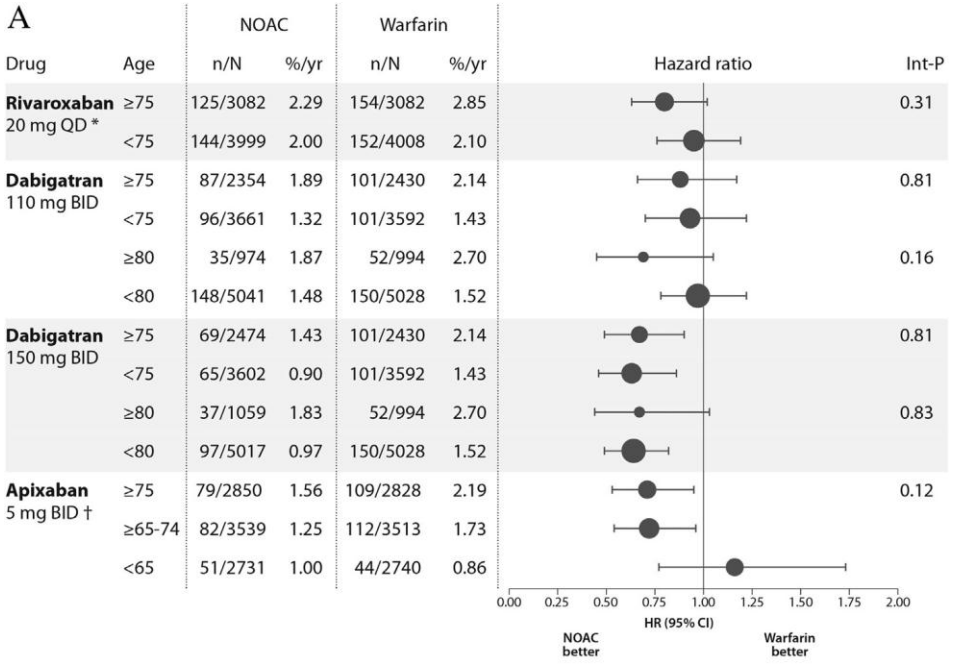
Direct oral anticoagulants in elderly patients with atrial fibrillation

Dabigatran etexilate

The efficacy and safety of two doses of the thrombin inhibitor dabigatran etexilate *vs* warfarin was studied in the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) trial[15,16]. This trial included 18,113 patients who were randomized to receive dabigatran etexilate 150 mg twice daily (n=6,076), 110 mg twice daily (n=6,015) or warfarin (n=6,022). The 150 mg twice daily dose, compared to warfarin, demonstrated a 35% relative risk reduction of the primary outcome stroke or systemic embolism (HR 0.65; 95%CI 0.52–0.81) and a similar risk of major bleeding (HR 0.93; 95%CI: 0.81-1.07)[16]. The 110 twice daily dose was non-inferior to warfarin with respect to efficacy (HR 0.90; 95%CI: 0.74-1.10), but showed a significant 20% relative risk reduction of major bleeding (HR 0.80; 95%CI: 0.70–0.93)[16]. Both doses of dabigatran etexilate demonstrated a highly significant reduction in intracranial hemorrhage compared with warfarin of 69% (110 mg twice daily) and 60% (150 mg twice daily)[15].

Two subgroup analyses have addressed the effects of dabigatran etexilate *vs* warfarin in elderly patients (75 years or over *vs* below)[31] and in very elderly patients (80 years or over *vs* below)[32]. In the RE-LY trial 7,258 patients were aged 75 years or over (40%). The event-rates for elderly *vs* younger patients were higher for both stroke and systemic embolism, as well as for anticoagulant associated bleeding (Figures 1a and 1b). In these patients, the superiority of dabigatran 150 mg twice daily *vs* warfarin with regard to stroke prevention was similar as in patients below 75 years of age (HR 0.67; 95%CI: 0.49-0.90, and HR 0.63; 95%CI: 0.46-0.86, respectively, p-interaction = 0.81, figure 1a)[31]. The efficacy of the 110 mg twice daily dose vs. warfarin was similarly consistent between elderly and younger patients (HR 0.88; 95%CI: 0.66–1.17, and HR 0.93; 95%CI: 0.70–1.23, respectively, p-value for interaction 0.81, Figure 1a)[31]. With respect to major bleeding, a significant interaction

between age and treatment was found. The point estimates of the hazard ratios for bleeding of both doses of dabigatran etexilate *vs* warfarin were higher in elderly patients (HR 1.18; 95%CI: 0.98–1.42 for 150 mg twice daily, and HR 1.01; 95%CI: 0.83-1.23 for 110 mg twice daily) than in younger patients (HR 0.70; 95%CI: 0.57-0.86 for 150 mg twice daily, p-interaction <0.001, and HR 0.62; 95%CI: 0.50-0.77 for 110 mg twice daily, p-interaction <0.001, Figure 1b)[31]. This shows that the incidence of bleeding in patients treated with dabigatran etexilate rises more steeply with age than the incidence in patients treated with warfarin. However, the large reductions of intracranial hemorrhage observed in patients treated with dabigatran etexilate *vs* patients treated with warfarin were preserved in the elderly patients (Figure 2). In the analysis of the 3,027 very elderly patients (aged 80 years or over; 17% of the RE-LY cohort), the results were similar[32]. The incidence of bleeding in the dabigatran etexilate groups rose more steeply with age than in the warfarin group with an HR in the very elderly group of 1.35 (95%CI: 1.03-1.77) for 150 mg twice daily *vs* warfarin and an HR of 1.13 (95%CI: 0.85–1.50) for 110 mg twice daily *vs* warfarin (Figure 1b)[32]. The hazard ratios for efficacy and intracranial hemorrhage were again quite similar between very elderly and younger patients (Figures 1a and 2a)[32].



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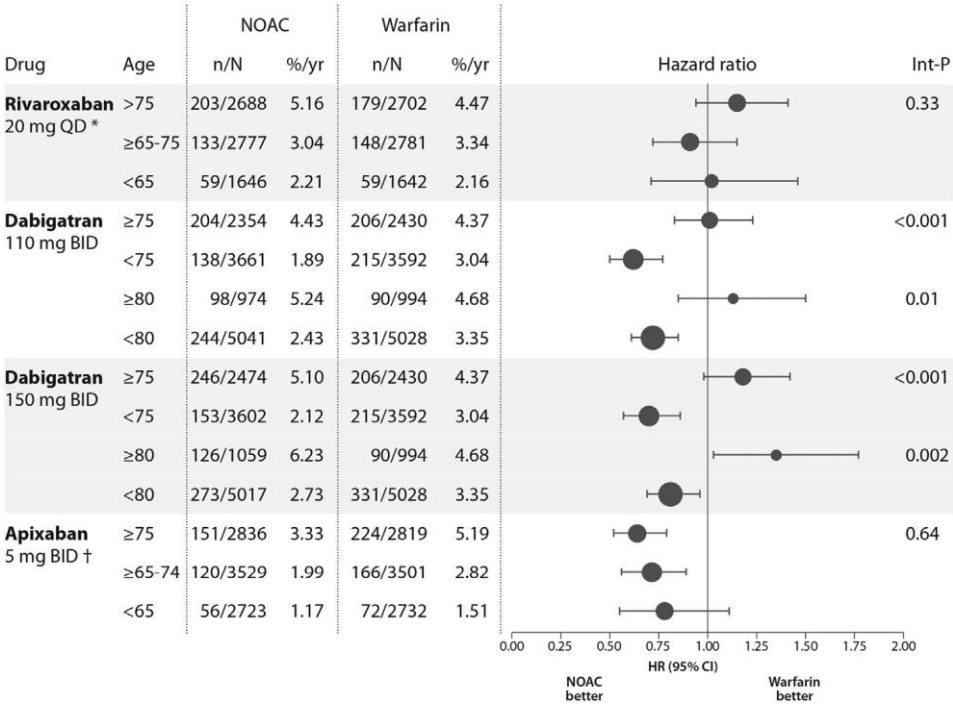


Figures 1a and 1b. A. Primary efficacy outcome of stroke or systemic embolism in phase III randomized controlled trials comparing a new direct oral anticoagulant (NOAC) with vitamin K antagonists in patients with atrial fibrillation. B. Major bleeding in phase III randomized controlled trials comparing a NOAC with vitamin K antagonists in patients with atrial fibrillation.
NOAC indicates New direct Oral Anticoagulant; QD, once daily; bid, twice daily; n, number of patients with events; N, the total number of patients in the subgroup; %/yr, event-rate expressed as the number of events per 100 patient-years of follow-up; Int-P, p-value for interaction between age category and treatment; Hazard ratios and concomitant 95% confidence interval are from the Cox proportional hazard model with treatment group as a covariate. *A reduced dose of rivaroxaban 15 mg once daily was used for patients with a estimated Glomerular Filtration Rate 30–50 mL/min. †A reduced dose of apixaban 2.5 mg twice daily was used for patients with 2 of the following criteria: age ≥80 years, body weight ≤60 kg, and serum creatinine ≥ 133 µmol/L (1.5 mg/dL)[19,31-35].

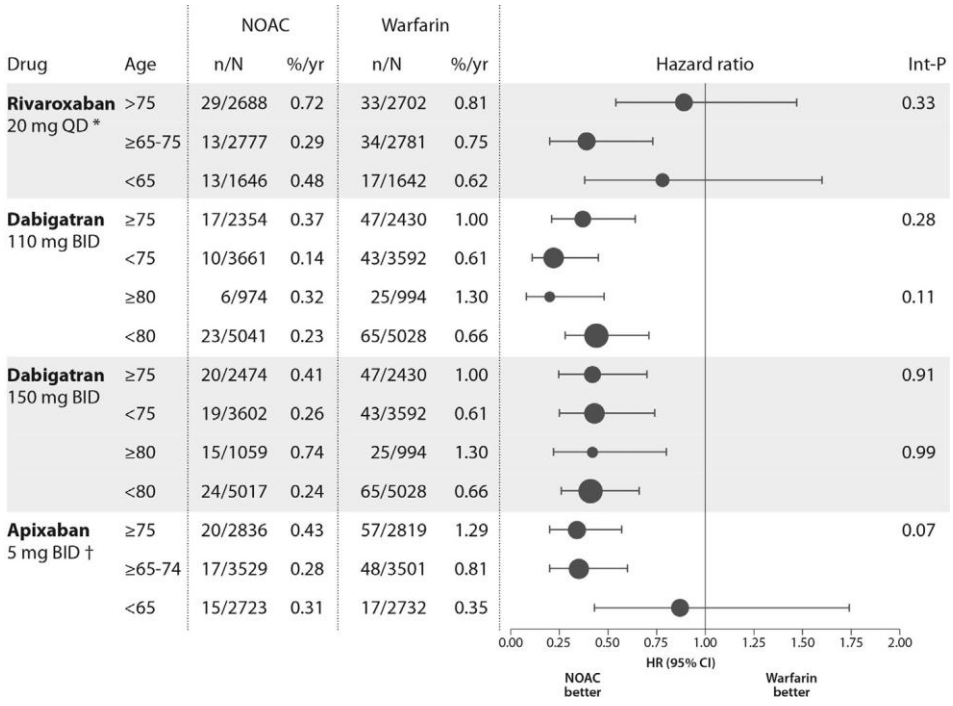


Figure 2. Intracranial hemorrhage in phase III randomized controlled trials comparing a NOAC with vitamin K antagonists in patients with atrial fibrillation

NOAC indicates New direct Oral Anticoagulant; QD, once daily; BID, twice daily; n, number of patients with events; N, the total number of patients in the subgroup; %/yr, event-rate expressed as the number of events per 100 patient-years of follow-up; Int-P, p-value for interaction between age category and treatment; Hazard ratios and concomitant 95% confidence interval are from the Cox proportional hazard model with treatment group as a covariate. *A reduced dose of rivaroxaban 15 mg once daily was used for patients with an estimated Glomerular Filtration Rate 30–50 mL/min. †A reduced dose of apixaban 2.5 mg BID was used for patients with 2 of the following criteria: age ≥80 years, body weight ≤60 kg, and serum creatinine ≥133 µmol/L (1.5 mg/dL). Presented data is obtained from References[31-35].

<u>Rivaroxaban</u>

The oral direct factor Xa inhibitor rivaroxaban was compared to warfarin in patients with atrial fibrillation in the Rivaroxaban Once Daily Oral Direct factor Xa Inhibition Compared with Vitamin K Antagonism for Prevention of Stroke and Embolism Trial in Atrial Fibrillation (ROCKET-AF)[22]. This phase III randomized controlled trial included 14,264 patients, of whom 7,131 patients were treated with rivaroxaban. Patients with moderate renal insufficiency (estimated Glomerular Filtration Rate: 30–50 mL/min) received a reduced dose of 15 mg once daily and consisted of 21% of the patients

randomized to treatment with rivaroxaban; the remainder received a 20 mg once daily dose[22]. In the intention-to-treat analysis, rivaroxaban was similarly effective at preventing stroke or systemic embolism (HR 0.88; 95%CI: 0.75–1.03) and similarly safe (HR for major bleeding 1.04; 95%CI: 0.90–1.20)[22]. Rivaroxaban decreased the risk of intracranial hemorrhage by 33% compared with warfarin (HR 0.67; 95%CI: 0.47–0.93)[22].

The efficacy subgroup analysis of rivaroxaban *vs* warfarin for stroke prevention has been presented for the 6,229 patients aged 75 years or over (44%) *vs* below 75 years[33]. For both treatment arms, the event-rates for stroke and for anticoagulant associated bleeding were higher in the elderly patients than in younger patients (Figures 1a and 1b). The results of these subgroup analyses were very consistent with the trial's main outcome. Rivaroxaban was non-inferior to warfarin at preventing stroke or systemic embolism in patients aged 75 years or over (HR 0.80; 95%CI: 0.63–1.02), as well as in younger patients (HR 0.95, 95%CI: 0.76–1.19, p-interaction=0.31, Figure 1a)[33]. The subgroup analyses for elderly patients with respect to major bleeding and intracranial hemorrhage were published in the product monograph and are available online[34]. The HRs for major bleeding in patients treated with rivaroxaban *vs* warfarin were similar in the three age groups (<65 years, ≥65 to <75 years, and ≥75 years, Figure 1b)[34]. In the elderly rivaroxaban was non-inferior to warfarin with an HR of 1.15 (95%CI: 0.94–1.41)[34]. With respect to intracranial hemorrhage, there was no significant interaction between age and treatment, although there was some variation in the point estimates for the HR in the subgroups (Figure 3).

Apixaban

Apixaban was compared with warfarin in the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) trial[19]. This trial included 18,201 patients of whom 9,120 received treatment with apixaban. Most patients randomized to apixaban received a dose of 5 mg twice daily, but a reduced dose of 2.5 mg twice daily was used for patients who met two of the three following criteria: age 80 years or over, body weight of 60 kg or below, and creatinine level of 133 µmol/L (1.5 mg/dL) or higher. The main outcome of the ARISTOTLE trial was that apixaban compared with warfarin was superior in the prevention of stroke or systemic embolism (HR 0.79; 95%CI: 0.66–0.95), and also superior with respect to safety (HR for major bleeding 0.69; 95%CI: 0.60–0.80)[19].

The subgroup analyses by age have been published in the main trial publication, as well as in the Product Monograph[19,35]. A total of 2,850 patients (31%) included in the

trial were aged 75 years or over. The event-rates for stroke and anticoagulant associated bleeding were higher in elderly patients than in younger patients (Figures 1a and 1b). Despite some variation in the point estimate of the HR, there was no significant interaction between age category (<65 years, ≥65 to <75 years, and ≥75 years) and randomized treatment with respect to prevention of stroke or systemic embolism (p-interaction=0.12, Figure 1a). In patients 75 years or over, the efficacy HR was 0.71 (95%CI: 0.53–0.95)[19,35]. For major bleeding, there was again no significant interaction between age and randomized treatment (p-interaction 0.64, Figure 1b)[19,35].

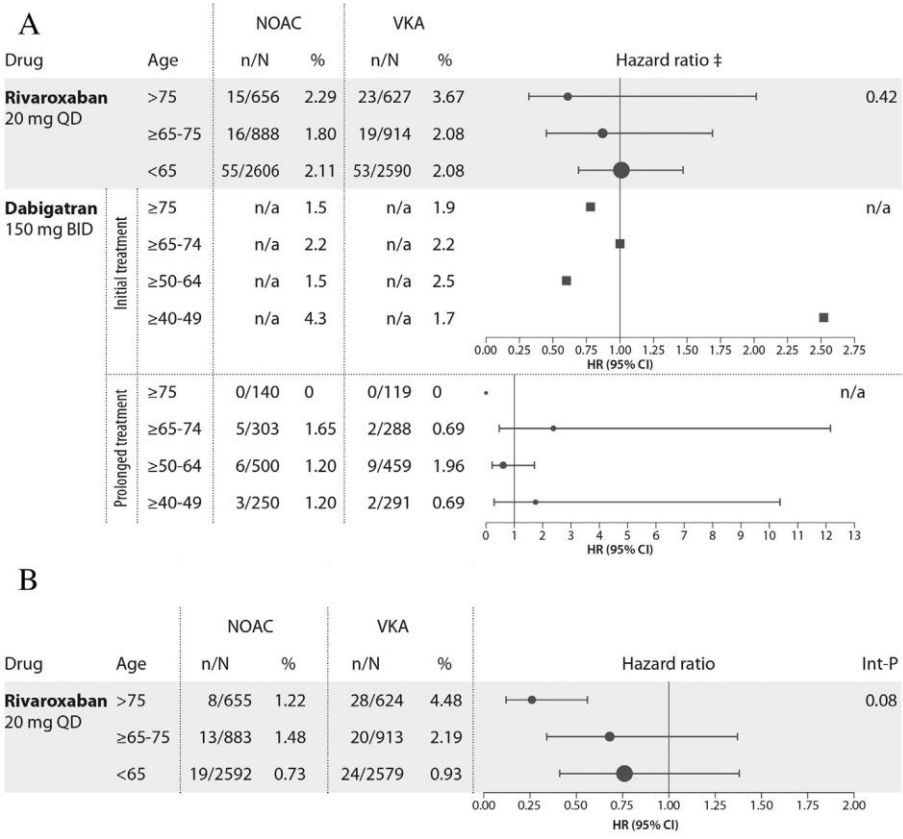
Direct oral anticoagulants for venous thromboembolism therapy in elderly patients

Dabigatran etexilate

Dabigatran etexilate was compared with warfarin in three phase III randomized controlled trials. The RE-COVER and RE-COVER II trials included 5,107 patients with acute deep venous thrombosis or pulmonary embolism and randomized to treatment with dabigatran etexilate at a dose of 150 mg twice daily or warfarin, after an initial course of heparin or low-molecular weight heparin for both treatment arms[23,36]. The RE-MEDY trial included 2,856 patients with venous thromboembolism who had completed 3–12 months of anticoagulant treatment with vitamin K antagonist or dabigatran etexilate[24]. In this trial for prolonged treatment of venous thromboembolism, patients were randomized to treatment with dabigatran 150 mg twice daily or warfarin. In all three trials dabigatran etexilate, compared with warfarin, was non-inferior with respect to recurrent venous thromboembolism and with respect to major bleeding[37]. In the composite safety outcome major or clinically relevant non-major bleeding, dabigatran etexilate was superior to warfarin with a relative risk reduction of approximately 40%[37].

Limited data on elderly patients is available for the RE-COVER and RE-MEDY trials through the online supplements of the main publications[23,24]. This is in part explained by the mean age of patients in venous thromboembolism treatment trials which is much lower than in atrial fibrillation trials (55 yrs *vs* 72 yrs[15,23,24]), and only 12% of patients (n=259) in the venous thromboembolism treatment trials were aged 75 years or over[23,24]. Figure 3 presents the available subgroup analyses for the RE-COVER (initial treatment) and RE-MEDY (prolonged treatment) trials. Although the cumulative incidence of recurrent venous thromboembolism in the subgroup of patients 75 years or over seems to be in line with the overall trials' results, no firm

conclusions can be drawn. Furthermore, subgroup analyses by age for bleeding outcomes have not yet been published. A pooled analysis of the RE-COVER, RE-COVER II and RE-MEDY trials may provide useful insights, but has thus far not been published.



Figures 3a and 3b. A. Primary efficacy outcome of recurrent venous thromboembolism in phase III trials that compared a NOAC with vitamin K antagonist therapy in patients with venous thromboembolism. B. Major bleeding in phase III trials that compared a NOAC with vitamin K antagonist therapy in patients with venous thromboembolism.

NOAC indicates New direct Oral Anticoagulant; VKA, vitamin K antagonist; QD, once daily; BID, twice daily; n, number of patients with events; N, the total number of patients in the subgroup; %, proportion of patients with an event or cumulative incidence; Int-P, p-value for interaction between age category and treatment. ‡ Hazard ratios for rivaroxaban *vs* VKA and concomitant 95% confidence intervals are from the Cox proportional hazard model with treatment group as a covariate for rivaroxaban. Hazard ratios for dabigatran *vs* VKA is the ratio of the cumulative incidence with the 95% confidence interval estimated with Confidence Interval Analysis (version 2.2.0, University of Southampton, United Kingdom). Presented data is obtained from references[23,24,38].

Rivaroxaban

For the initial treatment of venous thromboembolism, rivaroxaban was compared with warfarin in the EINSTEIN program. The EINSTEIN-DVT trial included 3,449 patients with acute deep venous thrombosis and the EINSTEIN-PE trial included 4,832 patients with acute PE[13,25]. In these trials, rivaroxaban was non-inferior with respect to prevention of recurrent venous thromboembolism and with respect to bleeding complications[13,25]. In the EINSTEIN-PE trial, treatment with rivaroxaban was associated with a 35% reduction in major bleeding[25].

Recently, a pooled analysis of both EINSTEIN trials for various subgroups, including age, was presented (Figure 3)[38]. In total, 18% of included patients was aged over 75 years (n=1,283). Interestingly, when rivaroxaban was compared with warfarin in the three age groups, the point estimates suggest that rivaroxaban *vs* VKAs may be relatively safer in elderly patients than in younger patients, although the interaction was not statistically significant (interaction p-value=0.07, Figure 3b)[38].

Apixaban

The effects of apixaban compared with vitamin K antagonist therapy for initial treatment of venous thromboembolism is currently under investigation in the AMPLIFY trial (ClinicalTrial.gov, NCT00643201). In this trial, patients with acute venous thromboembolism are randomized to treatment with apixaban (10 mg twice daily for 7 days, followed by 5 mg twice daily for 6 months) or warfarin, initially combined with enoxaparin. The results of this trial are expected in 2013.

DISCUSSION

The subgroup analyses of the phase III randomized controlled trials in which a direct oral anticoagulant was compared to vitamin K antagonist therapy confirm that the absolute risks of both thrombotic events and bleeding rise with advancing age. However, the increase in risk is similar with direct oral anticoagulants and warfarin in most cases. Therefore, the relative effects of the direct oral anticoagulant *vs* vitamin K antagonist therapy are very consistent among elderly patients, indicating that the main trial results will also apply to those patients. Moreover, the absolute risk reductions will be higher in elderly than in younger patients due to the higher absolute risks in this population, preserving the favourable balance between risks and benefits

of treatment seen in younger patients. Interpretations of subgroup analyses should always be made with caution, since the trials, by definition, are insufficiently powered for subgroups. However, the proportion of patients 75 years or over is consistently over 40% in the trials for patients with atrial fibrillation, and the total number of patients aged 75 years or over is over 19,000[15,16,19,22] which provides considerable power to explore age subgroups. Compared with patients with atrial fibrillation, the data are less reliable for patients with venous thromboembolism, because the trials were smaller and the proportion of patients aged 75 years of over was lower (12–18% over 75,154 patients in total).

The only exception to the consistency of the trial result in the elderly patients seems to be that the risk of major bleeding increases more steeply with age in patients treated with dabigatran etexilate than in vitamin K antagonist treated patients. It would seem plausible that moderate renal insufficiency, more common in the elderly, provides an explanation, since dabigatran etexilate is eliminated by the kidneys to a larger extent than other direct oral anticoagulants and vitamin K antagonists. However, renal insufficiency does not seem to be the main determinant, since another subgroup analysis did not show an interaction between eGFR and treatment for bleeding outcomes[31]. Moreover, the exception can in part be explained by differences in trial design. In the ROCKET-AF and ARISTOTLE trials, a reduced dose of rivaroxaban or apixaban was used for patients with specific characteristics, including renal insufficiency, old age and low body weight. In the RE-LY trial, all patients received the dabigatran etexilate dose to which they were randomized, irrespective of kidney function or age. Because of the increased bleeding risks in the elderly, some regulatory authorities have recommended the lower 110 mg twice daily dose in patients over 80 years of age and recommend considering the lower dose in those aged 75–80 years[39].

An important consideration is to what extent the results of these trials apply to 'real-world' patients in daily clinical practice. It was previously shown that the vitamin K antagonist associated bleeding risk is higher in patients who would have been excluded from the randomized controlled trials than in patients who meet all the in- and exclusion criteria[40]. Moreover, frail elderly patients (assessed by surrogate markers such as gait speed or impaired cognitive function), although not formally excluded from trials, may not be adequately represented by the more healthy elderly in the trials. This highlights the importance of post-marketing registries, such as GLORIA-AF (www.gloria-af.com) and GARFIELD (www.tri-london.ac.uk/garfield) that continue to collect data on the outcome of 'real-world' patients. Very recently, the Food and Drug Administration provided the first post-marketing report of bleeding

complications of dabigatran etexilate *vs* warfarin derived form insurance-claim data and administrative data[41]. This report provides no evidence that post-marketing rates of bleeding with dabigatran etexilate are higher than those observed with warfarin. Until further data from becomes available, this report provides some reassurance about the external validity of the trial results in 'real-world' patients.

In conclusion, the subgroup analyses of the phase III randomized controlled trials of direct oral anticoagulants compared with vitamin K antagonist therapy show that trial results are largely consistent in elderly patients. Moreover, observed relative benefits of direct oral anticoagulants over vitamin K antagonist therapy can lead to larger absolute risk reductions, due to the higher event rates in elderly patients. After the first reassuring report on the bleeding risks in 'real-world' patients, further post-marketing registries will continue to update us on the specific risks of the elderly patients.

PRACTICE POINTS

- The direct oral anticoagulants dabigatran etexilate, rivaroxaban, and apixaban have been extensively studied in large phase III trials for prevention and treatment of venous thromboembolism and for stroke prevention in atrial fibrillation.
- The results of the randomized controls trials indicate that direct oral anticoagulants are at least as effective and safe as vitamin K antagonists and offer significant simplification of anticoagulant therapy because routine coagulation monitoring is unnecessary.
- The use of anticoagulants in elderly patients is of particular concern because they are at highest risk of thrombotic and bleeding events
- Subgroup analyses of the phase III trials in which direct oral anticoagulants were compared with vitamin K antagonists confirm that the favourable balance between risks and benefits observed in younger patients also applies to the frail elderly population.
- Ongoing post-marketing registries will further inform the efficacy and safety of direct oral anticoagulants in 'real-world' patients in general, and in specific high-risk populations such as the elderly.

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13

Successful co-administration of dabigatran etexilate and protease inhibitors ritonavir-lopinavir in a patient with atrial fibrillation

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The direct oral anticogulant dabigatran etexilate has been approved for the prevention of stroke and systemic embolism in individuals with atrial fibrillation, demonstrating to be safe and effective at the dosages of either 110 or 150 mg twice daily[1]. After oral intake, dabigatran etexilate is converted to its active form dabigatran, and hydrolyzed by non-specific ubiquitous esterases[2,3]. Dabigatran etexilate, but not dabigatran, is a substrate of the intestinal efflux transporter P-glycoprotein. Phase I studies showed that co-administration of P-glycoprotein inhibitors increases dabigatran bioavailability from 1.5-fold (amiodarone) up to 2.5-fold (ketoconazole)[2]. High trough concentrations were associated with an increased risk of bleeding in the phase III RE-LY trial[3].

Human immunodeficiency virus protease inhibitors ritonavir and lopinavir are prescribed in many antiretroviral regimens and ritonavir-mediated Cytochrome P4503A inhibition serves to increase lopinavir bioavailability. Ritonavir is also a strong P-glycoprotein inhibitor interfering with many drugs and it may be expected to increase dabigatran exposure. Therefore, their co-administration requires caution[2,3] and is not recommended in some countries[5]. A study conducted by the National Institutes of Health is ongoing to characterize dabigatran pharmacokinetics in combination with ritonavir (NCT01896622).

We present the case of a 64-year-old male on ritonavir/lopinavir requiring periprocedural anticoagulation for atrial fibrillation ablation and with a perceived intolerance to vitamin K antagonists. Routine screening tests were within the normal values. The estimated creatinine clearance was 69 mL/min (Cockroft-Gault equation) and 80 mL/min/1.73 m^2 (4-variable Modification of Diet in Renal Disease Study equation). Patient's personal history included: human immunodeficiency virus-1 infection (1989), chronic asthma, acute coronary syndrome and paroxysmal atrial fibrillation (2006), and two episodes of suspected transient ischemic attacks three years prior to presentation. $CHADS_2$ and CHA_2DS_2-VASC scores for atrial fibrillation stroke risk were 2 and 3, respectively. He had been treated with vitamin K antagonists since the diagnosis of atrial fibrillation and then switched to nadroparin due to poor International Normalized Ratio control and extreme lethargy, which he ascribed to both acenocumarol and phenprocumon. Co-medications were: ritonavir/lopinavir 400/100 mg twice daily, tenofovir, lamivudine, zidovudine, raltegravir, salmeterol inhalation 25 µg twice daily, metoprolol, carbasalate calcium. Apart from P-glycoprotein inhibitors ritonavir and salmeterol[6], co-medications were not known to exert any relevant P-glycoprotein activity. After careful consideration, dabigatran etexilate was chosen for periprocedural anticoagulation on the basis of efficacy and safety profiles comparable to warfarin[7].

Due to the co-administration of two P-glycoprotein inhibitors, we first prescribed the off-label dose of dabigatran etexilate 75 mg twice daily[8], paralleling the United States recommendation for patients with moderate renal impairment receiving either the P-glycoprotein inhibitor dronedarone or ketoconazole[3]. Although no therapeutic range is available, a wide target blood concentration range was derived from the RE-LY trial: 28.2-215 ng/mL for trough, and 52-383 ng/mL for peak concentrations[4].

Dabigatran etexilate intake was scheduled one hour after ritonavir/salmeterol at their expected highest inhibitory influence[9]. After five days on dabigatran etexilate, blood samples were taken to generate pharmacokinetic and pharmacodynamic curves at steady state. Blood samples were collected from a peripheral line and centrifuged twice (3,000 *g*, 15 min, 25°C). The following assays were used: dabigatran plasma concentration (liquid chromatography-mass spectrometry; Acquity UPLC BEH C8 column, Acquity TQ Detector, Waters), diluted thrombin time (Hemoclot, Hyphen BioMed), activated partial thromboplastin time (Actin FS, Siemens Healthcare), thrombin time (Thromboclotin, Siemens Healthcare), and activated clotting time (Hemochron Signature Elite, International Technidyne Corporation). After review of the results and 11-day wash-out period from dabigatran etexilate, the patient was started on the approved dose of 110 mg twice daily and the pharmacokinetic and pharmacodynamic curves were repeated.

Plasma steady state trough values after dabigatran etexilate 75 mg twice daily were 29 ng/mL (liquid chromatography-mass spectrometry), 16 ng/mL (diluted thrombin time), and 33 seconds (activated partial thromboplastin time), while peak values were 96 ng/ml, 109 ng/mL, and 41 seconds, respectively. Thrombin time was greater than 120 seconds at all timepoints (Figure 1, Table 1).

After dabigatran etexilate 110 mg twice daily, plasma steady state trough values increased to 51 ng/mL (liquid chromatography-mass spectrometry), 52 ng/mL (diluted thrombin time), and 35 seconds (activated partial thromboplastin time), while peak values were 89 ng/mL, 113 ng/mL, and 40 seconds, respectively. Time to peak concentration was 2 hours for both doses. Activated clotting time showed substantial agreement with plasma concentrations (Figure 1, Table 1).

Atrial fibrillation ablation was performed after 30 days. The patient has been receiving dabigatran etexilate for six months with no complications reported.

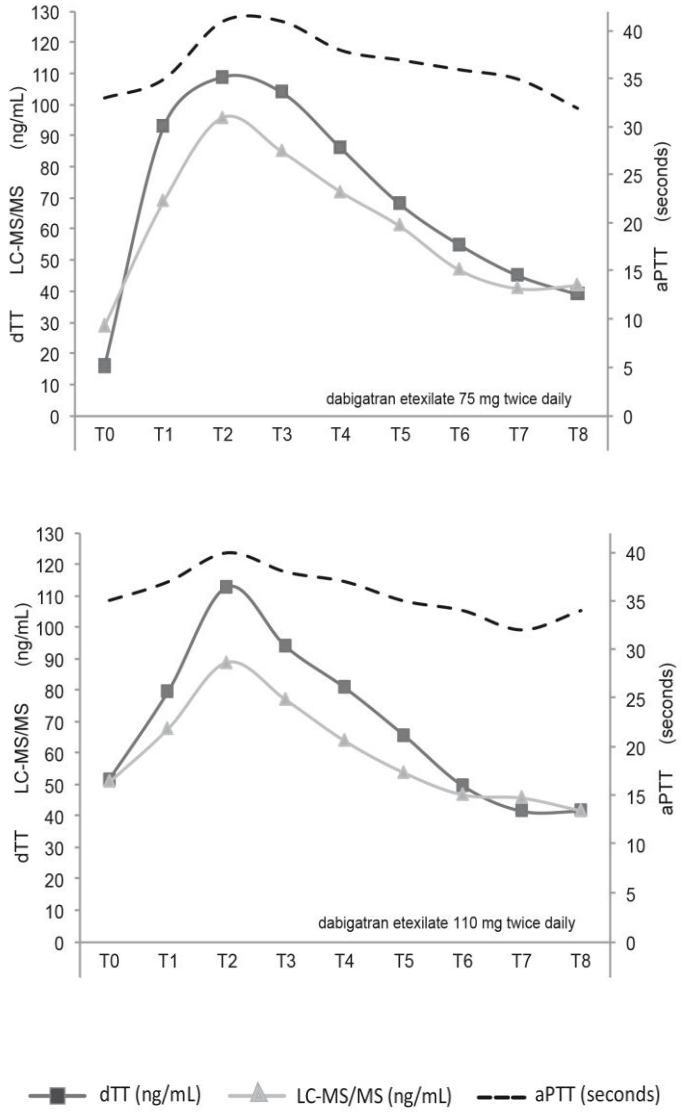

Figure 1. Pharmacokinetic and pharmacodynamic curves of dabigatran etexilate 75 and 110 mg twice daily at steady state

Abbreviations: dTT, diluted Thrombin Time (ng/mL); LC-MS/MS, Liquid Chromatography-Mass Spectrometry/tandem Mass Spectrometry (ng/mL); aPTT, activated Partial Thromboplastin Time (seconds); PT, prothrombin time (seconds).

Table 1. Overview of laboratory tests after dabigatran etexilate 75 and 110 mg twice daily at steady state

	T0	T1	T2	T3	T4	T5	T6	T7	T8
Dabigatran etexilate 75 mg twice daily									
dTT	16	93	109	104	86	68	55	45	39
LC-MS/MS	29	69	96	85	72	61	47	41	42
aPTT	33	35	41	41	38	37	36	35	32
ACT	135	160	178	175	170	151	147	146	133
	T0	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**
Dabigatran etexilate 110 mg twice daily									
dTT	52	80	113	94	81	66	50	42	42
LC-MS/MS	51	68	89	77	64	54	47	46	42
aPTT	35	37	40	38	37	35	34	32	34
ACT	156	155	-	167	162	156	143	142	160

T0 indicates Time 0 (pre-dose); T1 indicates one hour following the dose of dabigatran etexilate. Abbreviations: dTT, diluted Thrombin Time (expressed in ng/mL); LC-MS/MS, Liquid Chromatography-Mass Spectrometry/tandem Mass Spectrometry (expressed in ng/mL); aPTT, activated Partial Thromboplastin Time (expressed in seconds); ACT, Activated Clotting Time (expressed in seconds).

In our patient, we were uncertain how to safely dose dabigatran etexilate with co-administration of the P-glycoprotein inhibitors ritonavir and salmeterol. The observed dabigatran trough (pre-dose) concentration for the 110 mg twice daily dosage was in line with findings from the RE-LY trial (median 65.9 ng/mL, 10th-90th percentiles: 28.2-155 ng/mL)[4], while dabigatran etexilate 75 mg twice daily resulted in a low trough concentration, that had been associated with a 50% increased risk of ischemic stroke[4].

Although the interpretation of case reports needs caution due to their intrinsic limitations, in this particular patient the measurement of blood concentrations suggests that ritonavir did not cause dabigatran accumulation. Although no true target blood level range has been established thus far, aiming at concentrations typical for RE-LY trial participants provides some reassurance that the observed ischemic stroke and bleeding rates will apply.

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PART IV

Economic perspective

14

European Union-28: An annualised cost-of-illness model for venous thromboembolism

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ABSTRACT

Annual costs for venous thromboembolism have been defined within the United States demonstrating a large opportunity for cost savings. Costs for the European Union-28 have never been defined.

A literature search was conducted to evaluate European Union-28 cost sources. Median costs were defined for each cost input and costs were inflated to 2014 Euros (€) in the study country and adjusted for Purchasing Power Parity between European Union-28 countries. Adjusted costs were used to populate previously published cost-models based on adult incidence-based events.

In the base model, annual expenditures for total, hospital-associated, preventable, and indirect costs were €1.5-2.2 billion, €1.0-1.5 billion, €0.5-1.1 billion and €0.2-0.3 billion, respectively (indirect costs: 12% of expenditures). In the long-term attack rate model, total, hospital-associated, preventable, and indirect costs were €1.8-3.3 billion, €1.2-2.4 billion, €0.6-1.8 billion and €0.2-0.7 billion (indirect costs: 13% of expenditures). In the multiway sensitivity analysis, annual expenditures for total, hospital-associated, preventable, and indirect costs were €3.0-8.5 billion, €2.2-6.2 billion, €1.1-4.6 billion and €0.5-1.4 billion (indirect costs: 22% of expenditures). When the value of a premature life-lost increased slightly, aggregate costs rose considerably since these costs are higher than the direct medical costs.

When evaluating the models aggregately for costs, the results suggests total, hospital-associated, preventable, and indirect costs ranging from €1.5-13.2 billion, €1.0-9.7 billion, €0.5-7.3 billion and €0.2-6.1 billion, respectively.

Our study demonstrates that costs for venous thromboembolism have a large financial impact upon the European Union-28's healthcare systems and that significant savings could be realized if better preventive measures are applied.

INTRODUCTION

Venous thromboembolism continues to be a major public health issue internationally with suboptimal preventive strategies in many areas of the world[1]. Researchers have recently estimated that 547,596 venous thromboembolism events occur annually among United States adults aged 18 years or older with 348,558 deep vein thrombosis, 277,549 pulmonary embolism, and 78,511 pulmonary embolism with deep venous thrombosis events[2]. Annual burden of venous thromboembolism will continue to rise up to 1.82 million venous thromboembolism events by the year 2050 in the United States[3]. Recent cost estimates suggest that United States venous thromboembolism annual costs range from $13.5 to 69.3 billion (2011 United States Dollars, $) with $4.5 to 39.3 billion of these costs being preventable if improved prophylaxis measures were in place[4]. The European Union-28 also experiences a substantial venous thromboembolism burden: an estimated 684,019 deep venous thrombosis, 434,723 pulmonary embolism, and 610,138 post-thrombotic syndrome events occurr annually[5]. However, to date a European Union-28 cost-of-illness model has not been developed to define annual venous thromboembolism costs.

STUDY OBJECTIVES

The study objectives were to estimate the economic burden of venous thromboembolism in the European Union-28 and subtotals of hospital-associated, preventable, and indirect costs utilizing previously developed cost models and decision trees[4,6], and to discuss differences between these costs and United States costs.

METHODS

Literature search and cost-of-illness sources

A literature search was conducted to identify original studies reporting clinically relevant cost-of-illness source data regarding venous thromboembolism related to either the European Union or a European Union-28 country.

The list of all the 28 countries which are currently part of the European Union (together with their year of entry) includes: Austria (1995), Belgium (1958), Bulgaria (2007), Croatia (2013), Cyprus (2004), Czech Republic (2004), Denmark (1973), Estonia (2004), Finland (1995), France (1958), Germany (1958), Greece (1981), Hungary (2004), Ireland (1973), Italy (1958), Latvia (2004), Lithuania (2004), Luxembourg (1958), Malta (2004), the Netherlands (1958), Poland (2004), Portugal (1986), Romania (2007), Slovakia (2004), Slovenia (2004), Spain (1986), Sweden (1995), and United Kingdom (1973).

Two databases were searched: MEDLINE (January 1994 to June 2014, week 1; accessed *via* www.pubmed.com) and National Health Service Economic Evaluation Database (NHSEED; January 1994 to June 2014, week 1, accessed via www.crd.york.ac.uk) (search terms available in Supplementary Material). Only original peer-reviewed papers were considered eligible. Non-English publications and publications that did not include new venous thromboembolism or venous thromboembolism-associated cost sources were excluded. The titles and abstracts of search results were reviewed to identify potentially eligible studies by two investigators (SB and AW), and the second study selection conducted upon the basis of full-text papers. Potential disagreements regarding the eligibility of a paper were resolved by a third investigator (CEM). The search was supplemented by reviewing additional articles from the reference lists of both retrieved studies and relevant review papers, and by hand-searching.

Quality assessment of studies

Two separate investigators (AW and CEM) applied a quality assessment to the retrieved publications to determine whether and which study data should be included. The quality of the included original studies was evaluated on the basis of the following criteria: clear explanation of cost sources; clear description of the sample that estimates were derived from; whether the sample that generated estimates was generalizable to the European Union population; and whether cost adjustments (inflation/conversion) were appropriately performed. Costs from those papers which did not properly report or apply any of the criteria were not used for the analysis.

Decision trees and cost models

Investigators adapted previously published decision trees to model sequelae and treatment associated with incident deep venous thrombosis and pulmonary

embolism[4,6]. The decision trees contain probability information on patients' characteristics and on possible complications of an incident venous thromboembolism, including hospital- or community-associated deep venous thrombosis/pulmonary embolism events in both inpatient and outpatient settings, readmission and recurrence, minor and major bleeding events, heparin induced thrombocytopenia, post-thrombotic syndrome, and chronic thromboembolic pulmonary hypertension (as described elsewhere[4,6]). Estimated costs of a premature death were considered indirect costs. To calculate hospital-associated or community-associated deep venous thrombosis or pulmonary embolism costs, only the hospital-associated or community-associated branches from the decision tree and cost model were computed as per the previous United States papers[4,6]. In order to calculate the costs of preventable venous thromboembolism, the total hospital-associated costs were multiplied by a range of 50% to 75%: these reduction rates are reflected in the literature for when appropriate in-hospital systems are implemented to avoid preventable venous thromboembolism events[4,6].

High and low estimates of the cost sources were abstracted and adjusted for Purchasing Power Parity within the European Union-28 and for inflation to 2014 Euros (€). Financial data from the same source used in multiple papers (e.g. for United Kingdom: National Health System, NHS) were abstracted from the most recent publication. Corresponding authors were contacted at least twice if there was ambiguity about original data.

Previously published cost models were populated with European Union-28 venous thromboembolism or venous thromboembolism-associated costs and incidences based on the adult population within the European Union-28[4,6]. From all lower and higher cost input estimates that were abstracted for each cost input category, the median lower and higher cost estimate was identified. The lower and higher medians were utilized to populate the cost-of-illness models. The average patients' costs (the sum of each decision tree pathway's probability-weighted costs) were multiplied by low and high estimates of annual incidences of pulmonary embolism and deep venous thrombosis to determine population cost ranges. The low estimates of event probabilities were from incidence rates in Naess et al.[7], while high estimate probabilities were from prevalence rates (long-term attack rates) in the Venous Thromboembolism in Europe (VITAE) study[5], which were used in the previously published models[4]. Prevalence-based rates and long-term attack rates were not overlapped in any model to avoid overinflation. The European Union-28 population considered in our model was 426.8 million and includes inhabitants aged at least 15

years old on January 1st, 2013[8,9]. Calculations were performed using Microsoft Office Excel.

Valuation of the cost of premature death

A cost of premature death due to preventable venous thromboembolism was only reported in one study[10]. Therefore, we aimed to retrieve similar data from studies which used costs of premature death for quantifying the monetary benefit of a specific intervention applied to a large unselected European population.

A study of the mortality benefits of sulfur-dioxide regulation approved within the European Union found that in 20 major European urban centers regulation corresponded to cost per person-year mortality reductions between €25,000 and €133,200[11]. Since the costs of premature death from a pollution study are not entirely applicable to the venous thromboembolism population, which is older than average population affected by pollution, for the baseline cost model, we conservatively utilized the only figure from our literature search of €10,200 (€10,672 inflated to 2014€ and adjusted for Purchasing Power Parity)[10], and increased that value to €25,000 and €50,000 in the later models.

Base cost model and sensitivity analyses

In the base case model, we utilized low and high population incident venous thromboembolism events, low probabilities, and low median costs. In the 'Attack' sensitivity analysis, we utilized low and high venous thromboembolism events, low and high attack probabilities, and low costs. In the final 'Multiway' sensitivity analysis, we included low incidence, high prevalence of venous thromboembolism events from the VITAE study (434,723 pulmonary embolism and 684,019 deep venous thrombosis)[5], low probabilities, and high costs. An overview of the methodology used in base cost model and sensitivity analysis is provided in Table 1.

Finally, we modified the models with increased premature life lost values of €25,000 and €50,000 to vary how they contribute to overall costs as compared to total and direct medical costs.

Table 1. Overview of Base Cost Model and Sensitivity Analyses

Model	Population events used (venous thromboembolism)	Sequelae probabilities used	Costs used
Base Case	low and high incidence	low	low
Attack	low and high incidence	low and high attack	low
Multiway	low incidence and high prevalence	low	high

RESULTS

Study identification and selection

The literature search identified 406 studies: 337 from Medline and 69 from NHSEED. Initially there were 102 papers deemed eligible, including 61 papers selected on the basis of title/abstract and 41 additional papers obtained from references or hand-searching.

Due to the availability of sufficient recent data, only the publications published after 2004 with cost data reflected from 2000 to present were considered for analysis. After full-text review, cost sources from 25 studies were abstracted[10,12-35]. A flowchart with included and excluded studies is available in Supplementary Material (Figure A). A summary with all the costs included in the models reflected in 2014€ is provided in Table 2, while Table 3 shows the median cost inputs calculated from the literature source data and used in the deep venous thrombosis and pulmonary embolism cost models.

Table 2. Included papers and source costs

Paper	Population	Currency (year)	2014-inflated costs (Euros, €)
NHS[35]	United Kingdom	Pound (2014)	A: 637 to 2,812; E: 22,586; I: 1,068 to 3,422
Monreal[33]	France	Euro (2012)	A: 1,094; B: 342.9; C: 404.9; E: 2,209; H: 2,007; I: 3,130; K: 1,795
Monreal[33]	Italy	Euro (2012)	A: 1,221; B: 1,870; C: 1,935; E: 3,149; H: 2,668.2; I: 895; K: 4,771
Monreal[33]	Spain	Euro (2012)	A: 2,037; B: 1,824; C: 1,913; E: 1,163; H: 5,128.4; I: 4,308; K: 5,589
Santos[34]	Portugal	Euro (2012)	A: 2423; B: 2,660; C: 2,423 to 2,660; G: 33,206 to 57,556; H: 566 to 2,582; I: 5,758
NHS[30,33]	Italy	Euro (2012)	A: 2,330; E: 2,104 to 29,893; I: 4,035
Gussoni[32]	Italy	Euro (2010)	L: 225 to 726
Zindel[31]	Germany	Euro (2010)	A: 1,298 to 1,468; B: 2,035; I: 2,307 to 2,765; K: 3,773
Postma[29]	Netherlands	Euro (2010)	F: 3,072; L: 118 to 173
Lecumberri[26]	Spain	Euro (2009)	A: 4,619 to 8,362; I: 7,339 to 10,715; L: 22 to 29
McCullagh[19]	Ireland	Euro (2008)	A: 1,685 to 2,930; B: 3,529 to 3,999; C: 3169; I: 3,891 to 4,360; K: 5,708 to 6,177; M: 132
Migliaccio[28]	United Kingdom	Pound (2008)	A: 2,015; B: 819 to 900; D: 357 to 1,275; E: 1,594 to 14,084; H: 3,557 to 5,642; I: 2,336 to 2,737; K: 384; L: 125 to 275
Norlin[24]	Sweden	Euro (2008)	M: 358 to 512
Wolowacsz[22]	United Kingdom / Europe	Pound (2008)	A: 1,996 to 2,075; B: 511 to 590; D: 114; E: 1,310 to 9,268; F: 374; H: 690 to 3,138; I: 3,202.10; K: 2,205; L: 41 to 177; M: 121 to 271.62
Capri[23]	Italy	Euro (2007)	A: 5,032; C: 5,338; E: 6,478 to 7,878; H: 178 to 902; I: 5,882 to 7,395; L: 17 to 151
Lundkvist[16]	Sweden / Europe	Euro (2006)	A: 960; B: 1,205; D: 18; E: 1,301; H: 586 to 3,719; I: 2,363; K: 3,798; L: 38 to 82; M: 175 to 378
Kroger[27]	Germany	Euro (2006)	I: 9,934 to 14,676
Wilkens[25]	Germany	Euro (2006)	G: 48,403 to 52,004
Elalamy[18]	France	Euro (2005)	F: 1,952 to 3,782
Wilke[21]	Europe	Euro (2005)	F: 2,150 to 8,075; M: 200
Santamaria[15]	Spain	Euro (2004)	C: 2,731; I: 5,300; M: 60 to 839
ten Kate[20]	the Netherlands	Euro (2004)	A: 1,607 to 1,697; E: 4,542 to 12,399; H: 7,004 to 23,015; I: 4,721 to 4,811; M: 181 to 271
Goodacre[14]	United Kingdom	Euro (2004)	D: 803; H: 5,459; M: 93 to 299

McBride[17]	Germany	Euro (2003)	L: 341 to 373
Bjorvatn[13]	Norway	Norwegian Krone (2003)	A: 1,839 to 2,369; B: 2,107 to 2,326; E: 2,188 to 2,774; H: 491 to 3,109; I: 949 to 2,279; K: 2,821 to 4,262
Gomez[10]	Spain	Euro (2002)	D: 518; E: 1,294; F: 518; J: 5,309; N: 10,672
Haentjens[12]	Belgium	Euro (2001)	A: 1,363 to 1,820; E: 1,914; H: 221; I: 4,403; L: 54 to 207

Costs are expressed either as "cost per event" (A, B, C, D, E, F, I, J, K, L, M, N) or as "annual costs" (G, H). The variations in annual costs within the same paper may refer to annual costs in the first year after diagnosis or annual costs for each subsequent year.
Abbreviations: A: deep venous thrombosis (inpatients); B: deep venous thrombosis (outpatients); C: recurrent deep venous thrombosis; D: minor bleeding; E: major bleeding; F: heparin-induced thrombocytopenia; G: chronic thromboembolic pulmonary hypertension; H: post-thrombotic syndrome; I: pulmonary embolism (inpatients); J: recurrent pulmonary embolism; K: pulmonary embolism (outpatients); L: venous thromboembolism (prophylaxis); M, diagnosis of venous thromboembolism; N, cost of a premature death; NHS, National Health Service.

Table 3. Deep venous thrombosis- and pulmonary embolism-associated inputs calculated from literature source costs (2014 Euros, €)

Event or complication	Baseline Low Medians	High Medians
Deep venous thrombosis (inpatients)	1,685	2,330
Deep venous thrombosis (outpatients)	1,614	1,847
Deep venous thrombosis (readmission/recurrence)	2,180	3,999
Pulmonary embolism	3,891	4,197
Pulmonary embolism (readmission/recurrence)	3,773	5,309
Minor bleeding	235	1,039
Major bleeding	2,009	12,399
Heparin-induced thrombocytopenia	1,952	5,928
Post-thrombotic syndrome (annual costs)	690	3,348
Chronic thromboembolic pulmonary hypertension (annual costs)	40,805	54,780
Death (annual costs)	10,682	10,682

Costs are expresses as cost per event if no otherwise specified.

Base case model

In the base case model, venous thromboembolism had estimated €1.5 to 2.2 billion total annual costs, €1.0 to 1.5 billion hospital-associated costs, €0.5 to 1.1 billion

preventable costs and €0.2 to 0.3 billion indirect costs. Indirect costs represented 12% of expenditures (Table 4). In the long-term attack rates model, venous thromboembolism had €1.8 to 3.3 billion total annual costs, €1.2 to 2.4 billion hospital-associated costs, €0.6 to 1.8 billion preventable costs and €0.2 to 0.7 billion indirect costs. Indirect costs represented 13% of expenditures. In the multiway sensitivity analysis, venous thromboembolism had €3.0 to 8.5 billion total annual costs, €2.2 to 6.2 billion hospital-associated costs, €1.1 to 4.6 billion preventable costs and €0.5 to 1.4 billion indirect costs with indirect costs representing 15% of total expenditures.

Table 4. Annual European Union-28 costs (base model) expressed in 2014 billion Euros, €

	Total		Hospital-Associated		Preventable		Total Indirect		% Indirect
	Low	*High*	*Low*	*High*	*Low*	*High*	*Low*	*High*	*Low*
Base	1.5	2.2	1.0	1.5	0.5	1.1	0.2	0.3	12%
Attack	1.8	3.3	1.2	2.4	0.6	1.8	0.2	0.7	13%
Multi-Prob and Costs	3.0	8.5	2.2	6.2	1.1	4.6	0.5	1.4	15%

*Indirect costs werealculated by percentage of low indirect costs of low incident total costs.

Models utilizing €25,000 and €50,000 for value of a premature life lost

In the model utilizing €25,000 for a premature life lost, annual expenditures for total annual costs were €1.7 to 2.6 billion, hospital-associated were €1.1 to 1.7 billion, preventable were €0.6 to 1.3 billion and indirect costs were €0.4 to 0.6 billion. Indirect costs represented 24% of expenditures (Table 5). In the long-term attack rates model for annual expenditures, venous thromboembolism had €2.1 to 3.9 billion total annual costs, €1.4 to 2.8 billion hospital-associated costs, €0.7 to 2.1 billion preventable costs and €0.5 to 0.9 billion indirect costs with indirect costs representing 26% of total expenditures. In the multiway sensitivity analysis for annual expenditures, venous thromboembolism had €3.6 to 10.3 billion total annual costs, €2.6 to 7.4 billion hospital-associated costs, €1.3 to 5.6 billion preventable costs and €1.0 to 3.1 billion indirect costs with indirect costs representing 28% of total expenditures.

Finally in the model utilizing €50,000 for a premature life lost, annual expenditures for total, hospital-associated, preventable and indirect costs further increased to €2.1

to 3.2 billion, €1.4 to 2.2 billion, €0.7 to 1.6 billion and €0.8 to 1.2 billion, respectively, with indirect costs representing 39% of expenditures (Table 6). In the long-term attack rates model for annual expenditures, venous thromboembolism had €2.6 to 4.9 billion total annual costs, €1.8 to 3.6 billion hospital-associated costs, €0.9 to 2.7 billion preventable costs and €1.1 to 1.2 billion indirect costs. Indirect costs represented 41% of expenditures. In the multiway sensitivity analysis for annual expenditures, venous thromboembolism had €4.5 to 13.2 billion total annual costs, €3.3 to 9.7 billion hospital-associated costs, €1.7 to 7.3 billion preventable costs and €2.0 to 6.1 billion indirect costs. Indirect costs represented 44% of expenditures.

Table 5. Annual European Union-28 Costs (premature life lost value = €25,000) expressed in 2014 billion Euros, €

	Total		Hospital-Associated		Preventable		Total Indirect		% Indirect
	Low	*High*	*Low*	*High*	*Low*	*High*	*Low*	*High*	*Low*
Base	1.7	2.6	1.1	1.7	0.6	1.3	0.4	0.6	24 %
Attack	2.1	3.9	1.4	2.8	0.7	2.1	0.5	0.9	26 %
Multi-Prob and Costs	3.6	10.3	2.6	7.4	1.3	5.6	1.0	3.1	28 %

*Indirect costs werealculated by percentage of low indirect costs of low incident total costs.

Table 6. Annual European Union-28 Costs (premature life lost value = €50,000) expressed in 2014 billion Euros, €

	Total		Hospital-Associated		Preventable		Total Indirect		% Indirect
	Low	*High*	*Low*	*High*	*Low*	*High*	*Low*	*High*	*Low*
Base	1.5	2.2	1.0	1.5	0.5	1.1	0.2	0.3	12%
Attack	1.8	3.3	1.2	2.4	0.6	1.8	0.2	0.7	13%
Multi-Prob and Costs	3.0	8.5	2.2	6.2	1.1	4.6	0.5	1.4	15%

*Indirect costs werealculated by percentage of low indirect costs of low incident total costs.

Overview

When evaluating the models aggregately for total, hospital-associated, preventable, and indirect costs, the results suggests total costs ranging from €1.5 to 13.2 billion, hospital-associated costs from €1.0 to 9.7 billion and indirect costs from €0.2 to 6.1.

For the European Union-28, preventable costs of €0.5 to 7.3 per year could likely be saved if venous thromboembolism prevention strategies were better optimized.

DISCUSSION

Our cost-of-illness model for the European Union-28 estimates that total costs attributable to venous thromboembolism range from €1.5 to 13.2 billion. Up to €7.3 billion could be saved on an annual basis if improved venous thromboembolism prevention systems were utilized within the European Union-28. To our knowledge, this is the first study to evaluate total costs related to venous thromboembolism within the European Union-28. We undertook a broad literature search to identify cost inputs and applied stringent inclusion criteria. Cost sources were standardized to permit evaluating European costs as a whole by adjusting for Purchasing Power Parity and inflating to 2014€.

Despite the European Union-28 having a population with approximately 175 million more adults than the United States, European Union-28 costs were estimated to be significantly lower than United States costs[4]. Since the present model is largely derived from the prior United States venous thromboembolism cost model published by our group[4], a difference in modeling approach does not explain the observed gap. The difference is explained in part by the higher cost of a premature death in the United States models, which were based on life-time earnings (weighted average $180,887 to $195,982)[4,6]. Another explanation is costlier model inputs in the United States due to expensive healthcare in the United States compared to the European Union-28[36].

An important concept that was introduced in the United States models is the classification of the omission of venous thromboembolism prophylaxis in at-risk patients as an adverse event or adverse drug event[6]. The same concept can be applied to other disease states, such as antithrombotic use in non-valvular atrial fibrillation and venous thromboembolism treatment, as well as appropriate antiplatelet and beta-blocker use post-myocardial infarction and stent placement. Patient medication adherence, including to the newer direct oral anticoagulants and antiplatelets, is critical for clinics and health-care practitioners to follow up with. In addition, antithrombotic misuse will likely be reduced with the large shift towards risk assessment of patients for venous thromboembolism prevention, venous thromboembolism treatment, and stroke prevention in non-valvular atrial fibrillation.

Thus, unnecessary bleeding caused by antithrombotics that the patient did not require should also be considered an adverse drug event[37,38]. For the first time, ongoing venous thromboembolism prevention studies of direct oral anticoagulants betrixaban[39] and rivaroxaban[40] are utilizing risk assessment strategies for risk stratification of acute medically ill inpatients. This approach will produce a more homogeneous population than in earlier studies of hospitalized medical patients with the aim to better identify patients at higher risk of venous thromboembolism who may require a combination of mechanical and pharmacologic prophylaxis or intensified pharmacologic prophylaxis. It will also identify patients who are at low risk and who may not need thromboprophylaxis or those who may be best managed with mechanical prophylaxis[41,42].

Annual European Union-28 venous thromboembolism costs are comparable to annual costs for inflammatory bowel diseases (€4.6 to 5.6 billion)[43], chronic obstructive pulmonary disease (€23.3 billion), lung cancer (€3.3 billion), pneumonia (€2.2 billion)[44], or cerebrovascular diseases (€19 billion)[44], which have been recently published. Moreover, venous thromboembolism costs account for a relevant portion of the European Union direct costs for cardiovascular diseases (€106 billion, 2009€)[45]: these direct costs represented only 54% of total costs (€196 billion), which included productivity loss due to morbidity, productivity loss due to mortality and informal care costs[45].

Our models are subject to limitations. First the models did not include lost productivity and other indirect costs that could be attributed to the disease; however, addition of these indirect medical costs would only increase aggregate and indirect costs considerably. Lost productivity costs are being more commonly considered in cost-of-illness models for improving tools to allow for cost-benefit and cost-effectiveness analyses to be undertaken[46]. The only indirect cost included was the cost of a premature life lost due to venous thromboembolism. With regard to venous thromboembolism diagnosis-related costs, when they were available, minimum diagnostic costs were included within the total venous thromboembolism costs prior to input of median data. However, diagnostic costs were more often already included in the total venous thromboembolism costs (Table 2). Second, the models did not account for preventable costs that might be saved with nascent management strategies: these include early discharge and home treatment of low-risk patients with acute pulmonary embolism, optimization of deep venous thrombosis outpatient treatment, and single-agent oral approaches instead of treatment with vitamin K antagonists (47-50). Third, the literature search was limited to English publications, so direct source costs from non-English studies were not used in the model. Fourth, it

is well-known that rates of bleeding and venous thromboembolism are higher in the elderly: while we did not identify costs by age subgroups, rates within the long-term attack rates models should account for those higher risks in the full population estimates. Fifth, costs were retrieved from only 13 of 28 European Union countries and from only 3 studies pertaining to the whole European population. Thus, validity of the cost-model is possibly limited by not having cost sources representative of all countries. Lastly, there was limited evidence on the cost of premature death, so a low value from one study pertaining to air pollution was used[10] and two plausible higher values were used (€25,000 and €50,000). Those estimates may not be valid for the cost of venous thromboembolism mortality and were considerably lower than the values used in United States cost model[4], as well as other values noted in other European Union mortality cost modeling studies (Table 3).

Our study demonstrates that the cost of venous thromboembolism has a large financial impact upon the European Union-28's healthcare systems and that significant spending is preventable. Total estimated costs ranged from €1.5 to 13.2 billion per year within the European Union-28 and the results suggest minimum savings of €0.7 to 7.3 billion per year if European hospitals had better appropriate preventive measures in place.

Future cost models should account for the impact of the recently approved oral anticoagulants and of management strategies currently under study, including early discharge and home treatment of low-risk patients with acute pulmonary embolism.

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SUPPLEMENTARY MATERIAL

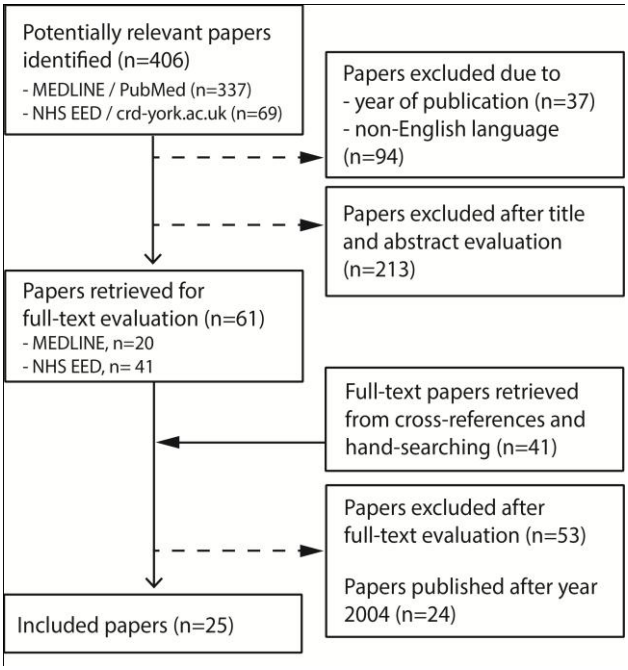

Figure A. Overview of the included and excluded studies

Literature search strategy: National Health Service Economic Evaluation Database (NHS EED) accessed via www.crd.york.ac.uk

("European Union" OR Europe OR Austria OR Belgium OR Bulgaria OR Cyprus OR "Czech Republic" OR Denmark OR Estonia OR Finland OR France OR Germany OR Greece OR Hungary OR Ireland OR Italy OR Latvia OR Lithuania OR Luxembourg OR Malta OR Netherlands OR Poland OR Portugal OR Romania OR Slovakia OR Slovenia OR Spain OR "Great Britain" OR "United Kingdom") AND ("deep-vein thrombosis" OR "pulmonary embolism" OR thromboembolism OR (("deep-vein thrombosis" OR "pulmonary embolism" OR thromboembolism) AND (bleed* OR h*emorrhag*)) OR (thrombocytopenia AND (heparin OR enoxaparin OR dalteparin OR nadroparin)) OR "pulmonary hypertension" OR "post-thrombotic syndrome")

15

Summary and future perspectives

The present work focuses on venous thrombosis and anticoagulant treatment in special (uncommon, neglected, forgotten) groups of patients for whom high-quality evidence is missing. Since «absence of evidence is not evidence of absence»[1], the ultimate goal of all chapters is to provide a rationale for future interventional studies designed to answer meaningful and urgent clinical questions.

The general introduction, **Chapter 1**, highlights the issue of practicing evidence-based medicine in absence of evidence.

The main topic of *Part I* is the reversal of direct oral factor Xa inhibitors rivaroxaban (**Chapter 2**) and apixaban (**Chapter 3**) with prothrombin complex concentrate. Consistent findings between our two studies suggested that coagulation parameters of thrombin generation are modified by prothrombin complex concentrate infusion if compared to placebo and that a dose-dependent reversal effect exists. **Chapter 4** pooled data from 8 studies enrolling healthy volunteers receiving more than 230 infusions of prothrombin complex concentrate in total, the thrombogenicity of which appears negligible. It is therefore likely that not prothrombin complex concentrate itself, but the underlying individual thrombosis risk factors represent the main trigger of thrombotic events in real-life acutely bleeding patients.

Taking our results together with those from a previous study performed in our center[2] and with those from other recent *in vivo* research papers[3-6], we suggest that prothrombin complex concentrate represents an option for managing bleeding events in patients on direct oral factor Xa inhibitors. However, these findings need to be interpreted with caution and confirmed in future studies considering clinical outcomes. This is especially true since randomized controlled trials on vitamin K antagonist reversal in emergency situations teaches us that rapid correction of the coagulation parameter INR (observed with prothrombin complex concentrate but not with the comparator agent fresh frozen plasma) did not translate into a difference in terms of clinical outcomes[7].

Meanwhile, specific antidotes for direct oral factor Xa inhibitors have been developed[8,9] and they are effective in normalizing coagulation parameters within minutes in rivaroxaban and apixaban-treated healthy volunteers. Clinical studies in acutely bleeding patients are currently ongoing (NCT02329327). It remains to be dissected whether specific antidotes will lead to improved clinical outcome, which sites and types of major bleeding would benefit more from an immediate restoration of normal hemostasis, whether *in vivo* mechanisms of clot formation are consistent with results of *in vitro* coagulation tests, what is the relevance of the rebound effect

observed after stopping infusion of specific antidotes and what the economic impact will be.

In *Part II* we presented three studies focusing on thrombotic and bleeding complications, and anticoagulant treatment, in patients on parenteral nutrition. **Chapter 5** shows that no high-quality evidence currently supports the routine use of anticoagulants for primary prevention of catheter-related thrombosis in patients on parenteral nutrition, while their safety in this vulnerable population is largely unknown. In **Chapter 6**, we aimed to fill some of those gaps of knowledge, and concluded that adults on home parenteral nutrition are at a very high risk of both bleeding and thrombotic complications (including vena cava syndrome), and are often switched between various anticoagulant regimens. Moreover, anticoagulants did not seem to reduce the rate of first thrombosis, while their use was associated with a 2.5-fold increase in major bleeding events. **Chapter 7** measured pharmacokinetics parameters of rivaroxaban and dabigatran etexilate in patients with short bowel syndrome and showed that these agents can be considered a reliable therapeutic option in selected patients.

The dramatic improvement in survival rate of patients treated with parenteral nutrition over the past decades has also led to an increased prevalence of individuals experiencing catheter-related complications. These patients are extremely heterogeneous with regard to their baseline characteristics and are at high risk of both thrombotic and bleeding complications. Since conducting a large randomized controlled trial in patients receiving parenteral nutrition may be unfeasible, future prospective studies could focus on two surrogate aspects: 1) Which anticoagulants are most suitable for patients receiving parenteral nutrition with an indication to long-term anticoagulation (e.g. those with prior splanchnic thrombosis), and 2) dissecting which individual risk factors or biomarkers are able to predict first venous thrombosis and therefore enable identifying high-risk patients who may benefit from primary thrombosis prophylaxis.

Part III described several studies on special populations. **Chapter 8** suggests that thrombophilia is strongly associated with cerebral venous thrombosis: however, the clinical relevance of testing seems limited, similarly to other forms of venous thromboembolism. In **Chapter 9** the long-term risk of mortality and recurrent venous thromboembolism appears to be low in patients who survived the acute phase of cerebral venous thrombosis, while a previous history of venous thromboembolism predicts recurrent events. In **Chapter 10** we underline the current unanswered issues of venous thromboembolism and anticoagulation in pregnant women. The results provided in **Chapter 11** indicate that in 1% of patients with any-cause splanchnic

venous thrombosis very small paroxysmal nocturnal hemoglobinuria clones can be detected. In **Chapter 12**, we argue that the favorable balance between risks and benefits of direct oral anticoagulants is preserved in the elderly population. The case described in **Chapter 13** suggests that measuring drug concentrations of direct oral anticoagulants might reassure that certain categories of patients at high-risk of drug over-exposure actually fit into the wide confidence interval derived from phase III trials.

These chapters illustrate that large networks of multidisciplinary researchers are needed to design and conduct adequately sized studies on uncommon diseases. The support of scientific societies in promoting and supporting such studies are therefore crucial.

The results of **Chapter 14** demonstrate that costs of venous thromboembolism have a large financial impact on the healthcare systems of European Union countries and that significant spending is preventable. An important concept underlined in this chapter is the classification of the omission of thromboprophylaxis in at-risk patients as an adverse event, similarly to unnecessary bleeding events associated with anticoagulant treatment prescribed with no stringent indication. Future studies including cost models of venous thromboembolism need to take the impact of direct oral anticoagulants and management strategies currently under study into account.

Evidence-based medicine or its newer complementary tool «precision medicine»[10,11] should serve as founding instrument for improving and spreading cost-effective treatments worldwide. This regards all of us, researchers, physicians, policy makers, and, of course, patients.

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Addenda

SAMENVATTING EN TOEKOMSTPERSPECTIEVEN

Dit proefschrift richt zich op veneuze trombose en de behandeling met antistolling in speciale patiëntengroepen voor wie kwalitatief goede evidence ontbreekt. Aangezien «de afwezigheid van bewijs» niet gelijk staat aan «bewijs van afwezigheid»[1], is het uiteindelijke doel van alle hoofdstukken om een rationale voor toekomstige interventie studies te geven waarmee relevante en urgente klinische vragen beantwoord kunnen worden.

De algemene inleiding, **Hoofdstuk 1**, bediscussieert het beoefenen van evidence-based medicine in afwezigheid van evidence.

Het onderwerp van *Deel 1* is het couperen van directe orale factor Xa-remmers rivaroxaban (**Hoofdstuk 2**) en apixaban (**Hoofdstuk 3**) met protrombinecomplex-concentraat. De resultaten van dit onderzoek suggereren dat trombinegeneratie wordt beinvloed door protrombinecomplex concentraat in vergelijking met placebo en dat dit effect dosis-afhankelijk is. In **Hoofdstuk 4** zijn de resultaten van 8 studies, waarin gezonde vrijwilligers die meer dan 230 infusies van protrombinecomplexconcentraat ondergingen, samengevat, en blijkt de trombogeniciteit ervan te verwaarlozen. Waarschijnlijk is daarom niet het protrombinecomplexconcentraat, maar onderliggende individuele risicofactoren de belangrijkste trigger voor het krijgen van trombose in bloedende patiënten die worden behandeld met protrombinecomplexconcentraat.

Deze resultaten, samen met die van een vorige studie uit ons centrum[2] en met die van andere recente *in vivo* studies[3-6], suggereren dat protrombine-complexconcentraat gebruikt kan worden voor het behandelen van bloedingen bij patiënten die directe orale factor Xa-remmers gebruiken. Deze bevindingen moeten echter nog worden bevestigd in toekomstige studies met klinische eindpunten. Van gerandomiseerde studies over het couperen van vitamine K antagonisten in spoedsituaties weten we namelijk dat een snelle correctie van de stollingsparameter INR, zoals gemeten na toediening van protrombine-complexconcentraat maar niet na Fresh Frozen Plasma, zich niet vertaalt in een verschil in klinische uitkomsten[7].

Inmiddels zijn er specifieke antidota voor directe orale factor Xa-remmers ontwikkeld[8,9], die na toediening bij met rivaroxaban- en apixaban-behandelde gezonde vrijwilligers, stollingsparameters na enkele minuten normaliseerden. Klinische studies hiernaar bij acute bloedende patiënten zijn momenteel gaande (NCT02329327). Het is op dit moment nog niet zeker of specifieke antidota zullen leiden tot een klinisch voordeel, bij welke ernst of lokalisatie van een bloeding

onmiddellijk herstel van de hemostase gunstig is, of mechanismen van stolselvorming *in vivo* overeenkomen met de resultaten van stollingstesten *in vitro*, wat de relevantie is van het rebound effect dat wordt waargenomen na het stoppen van de infusie van de nieuwe specifieke antidota en wat de economische gevolgen zullen zijn.

In *Deel II* beschrijven we drie studies naar trombotische- en bloedingscomplicaties en de behandeling met antistolling bij patiënten die parenterale voeding krijgen. Resultaten in **Hoofdstuk 5** tonen dat er momenteel geen goede evidence is voor het het routinematig gebruik van antistolling ter primaire preventie van catheter-gerelateerde trombose bij patiënten met parenterale voeding, terwijl de veiligheid van antistolling in deze kwetsbare populatie grotendeels onbekend is. In **Hoofdstuk 6** hebben we geprobeerd om een aantal van deze lacunes in de beschikbare kennis te vullen, en concludeerden we dat volwassenen die thuis parenterale voeding krijgen, een zeer hoog risico op zowel bloedings- als trombotische complicaties (inclusief vena cava syndroom) hebben en dat vaak wordt geswitcht tussen de verschillende antistollingregimes. Bovendien lijken anticoagulantia niet de kans op een eerste trombose te verminderen, terwijl het gebruik ervan wel werd geassocieerd met een 2,5-voudige toename van majeure bloedingen. In **Hoofdstuk 7** zijn farmacokinetische parameters van rivaroxaban en dabigatran gemeten bij patiënten met short bowel syndroom en bleek dat deze middelen bij geselecteerde patiënten een goede therapeutische optie kunnen zijn.

De dramatische verbetering van de overleving van de patiënten die worden behandeld met parenterale voeding de afgelopen decennia, heeft tot stijging van de prevalentie van catheter-gerelateerde complicaties geleid. Deze patiëntengroep is zeer heterogeen, en deze patiënten hebben een verhoogd risico op zowel trombotische als bloedingscomplicaties. Aangezien het niet haalbaar is een grote gerandomiseerde studie bij patiënten die parenterale voeding krijgen uit te voeren, zouden toekomstige prospectieve studies zich kunnen richten zich op twee surrogaat aspecten: 1) welk antistollingsmiddel het meest geschikt is voor voor patiënten die parenterale voeding krijgen en een indicatie voor langdurig anticoagulantia hebben (bijvoorbeeld in geval van splanchnische trombose) en 2) welke individuele risicofactoren of biomarkers een eerste veneuze trombose het best voorspellen, om zo hoog-risico patiënten te identificeren die zouden kunnen profiteren van primaire profylaxe.

In *Deel III* worden verschillende hoofdstukken beschreven die speciale patiëntenpopulaties betreffen. Resultaten van **Hoofdstuk 8** suggereren dat trombofilie sterk geassocieerd is met cerebrale veneuze trombose. De klinische relevantie van het testen voor trombofilie lijkt echter beperkt, zoals ook bij andere vormen van

veneuze trombose. Uit **Hoofdstuk 9** blijkt dat het risico op overlijden en recidief veneuze trombo-embolie op lange termijn laag is bij patiënten die de acute fase van cerebrale veneuze trombose overleven, terwijl een voorgeschiedenis van veneuze trombo-embolie wel voorspellend is voor een recidief. In **Hoofdstuk 10** beschrijven we de problemen van veneuze trombo-embolie en antistolling bij zwangere vrouwen. De resultaten in **Hoofdstuk 11** tonen aan dat bij 1% van de patiënten die een veneuze trombose in het splanchnicus gebied (ongeacht de oorzaak) zeer kleine paroxysmale nachtelijke hemoglobinurie klonen kunnen worden gedetecteerd. In **Hoofdstuk 12** stellen we dat de gunstige balans tussen voor- en nadelen van de directe orale anticoagulantia ook aanwezig is in de oudere patiëntenpopulatie. De casus die in **Hoofdstuk 13** beschreven wordt suggereert dat het meten van spiegels van directe orale anticoagulantia bij bepaalde patiënten, waarvan het risico op geneesmiddel over-exposure hoog wordt geacht, nuttig kann zijn en dat spiegels vallen binnen het brede betrouwbaarheidsinterval dat is afgeleid van fase III studies.

De in *Deel IV* van dit proefschrift beschreven hoofdstukken illustreren dat alleen door samenwerking in netwerken van multidisciplinaire onderzoekers, studies kunnen worden opgezet die voldoende groot zijn om deze speciale patiëntenpopulaties te bestuderen. De steun van de wetenschappelijke verenigingen bij het bevorderen van dergelijke netwerken en studies is zeer belangrijk.

De resultaten van **Hoofdstuk 14** tonen aan dat de kosten van veneuze tromboembolie grote financiële gevolgen hebben voor de zorgstelsels van de landen van de Europese Unie en dat aanzienlijke uitgaven zou kunnen worden voorkómen. Een belangrijk concept dat in dit hoofdstuk wordt besproken is het classificeren van het niet-voorschrijven van veneuze tromboembolie profylaxe bij hoog-risico patiënten als een adverse event, net als vermijdbare bloedingen onder antistolling wanneer er geen stricte indicatie voor is. In toekomstige studies, inclusief studies naar de kosten van veneuze trombo-embolie moet rekening worden gehouden met de impact van directe orale anticoagulantia en met behandelstrategieën die momenteel worden onderzocht.

Evidence-based medicine, ofwel de nieuwe aanvulling daarop «precision-medicine»[10,11] moet dienen als instrument voor het verbeteren en het dissemineren van kosten-effectieve behandelingen wereldwijd. Dit geldt voor zowel onderzoekers, artsen, beleidsmakers, en natuurlijk voor patiënten.

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PORTFOLIO

Jul 2012 - Aug 2015

	Year, Credits
Oral and poster presentations	
XXIV International Society for Thrombosis and Haemostasis (ISTH) congress, Amsterdam, the Netherlands; poster presentation: "Thrombophilia and cerebral venous thrombosis: a systematic review and meta-analysis"	2013, 0.5
XXIII Italian Society of Thrombosis and Haemostasis (SISET) congress, Milano, Italy; oral presentations: "In-vivo reversal of the anticoagulant effect of apixaban with 4-factor prothrombin complex concentrate" "In-vivo reversal of the anticoagulant effect of rivaroxaban with 4-factor prothrombin complex concentrate"	2014, 1.0
XXV ISTH congress, Toronto, Canada; poster presentations: "Anticoagulants for prevention of thrombotic complications in patients on parenteral nutrition: a systematic review" "Pharmacokinetics and -dynamics of dabigatran etexilate and rivaroxaban in patients: requiring parenteral nutrition for short bowel syndrome (the PDER PAN study)" "Long-term parenteral nutrition-associated thromboembolic and hemorrhagic complications in 236 single-center outpatients" "The incidence of catheter-related thrombosis in patients on long-term parenteral nutrition: a systematic review and meta-analysis"	2015, 2.0
Congresses and symposia	
XIX International Symposium on Thromboembolism (IST), London, United Kingdom	2012, 0.25
International Conference on Thrombosis and Hemostasis Issues in Cancer (ICTHIC), Bergamo, Italy	2012, 0.5
V Women's Health Issues in Thrombosis and Haemostasis congress, Vienna, Austria	2013, 0.5
XXIV ISTH congress, Amsterdam, the Netherlands	2013, 1.25
ICTHIC, Bergamo, Italy	2014, 0.5
XXIII SISET congress, Milano, Italy	2014, 0.75
XXV ISTH congress, Toronto, Canada	2015, 1.25
Weekly Journal Club, Academic Medical Center, Department of Vascular Medicine, University of Amsterdam (UvA)	2012-2013, 1.0

Courses, seminars, workshops

Graduate School, Academic Medical Center, UvA, Amsterdam, "Practical Biostatistics"	2013, 1.1
Graduate School, Academic Medical Center, UvA, Amsterdam, "Clinical epidemiology"	2013, 1.0
Graduate School, Academic Medical Center, UvA, Amsterdam, "Evidence based searching"	2013, 0.1
Graduate School, Academic Medical Center, Amsterdam, "Systematic reviews and meta-analyses"	2012, 1.0
Weekly department education for residents in Internal Medicine, University of Pavia, Pavia	2013-2015, 1.0
Weekly department journal club and education, Department of Vascular Medicine, Academic Medical Center, UvA, Amsterdam	2012-2013, 0.5

Students monitoring and teaching experience

Bram Salman, Bachelor thesis: "Incidence of catheter-related thrombosis in patients on home parenteral nutrition", UvA	2014, 1.0
Caroline Heuschen, Bachelors' research project: "Home parenteral nutrition-associated thrombotic and bleeding complications: a retrospective cohort study", UvA	2015, 1.0
Marco Corti, Master thesis: "Recurrences and mortality rate in patients with a first episode of isolated distal or proximal deep venous thrombosis", University of Pavia, Italy	2015, 1.0
Seminar: "Reversal of oral anticoagulants", University of Pavia, Italy	2015, 0.2

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"Giuseppe Teresio Bonizzoni award" for young researchers, Ferrata-Storti Foundation, Pavia, Italy	2014, -

Other

Resident in Internal Medicine (2010 to 2015) - University of Pavia, Italy	2012-2015, 180
Member of the 2016 European Congress on Thrombosis and Haemostasis (ECTH) Junior Advisory Board, the Hague, the Netherlands	2016, -
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Publications

Konstantinides SV, Barco S, Lankeit M, Meyer G. Pulmonary embolism: An update. J Am Coll Cardiol 2016, 67:976-90.

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Among all possible lives and epochs, the deepest joy is together with Alice and Agata, wherever we are.

BIO

Born in 1984 in Tortona (Piemonte), to Luisa Ottolini and Angelo Barco, Stefano had a conventional childhood, playing piano and cycling over the hills of Tortonese, before being engaged in a couple of basketball teams of the Northwest of Italy as a semi-pro player.

In 2002, he moved to Pavia and joined the renowned medical school. Consequently, he left piano for blues harp and basketball for streetpoetry. Together with companion Nicola, an agitator from Basilicata, and Manuela, they contributed to script and were pictured in Marina Spada's biopic "Poesia che mi guardi", on the life of poet Antonia Pozzi, which was presented out of competition at 66th Venice International Film Festival. During his university years, he was intensively active in the editorial board of Edizioni OMP, the first Italian company publishing entirely with Creative Commons licenses. In parallel, he joined the Thromboembolic Disease Unit as an undergraduate student (head: dr. Franco Piovella).

He graduated with honours in 2009 with a thesis on fondaparinux for heparin-induced thrombocytopenia and started to work as a resident at the department of Internal Medicine (head: Prof. Carlo Balduini) of the Pavia University Hospital. In 2011, together with his girlfriend Alice, he helped settling a Primary Health Center in Mahuninga, Tanzania, with NGO YouAid. In 2012-13, he attended the department of Vascular Medicine of the Academic Medical Center, University of Amsterdam, where the present thesis was conceived, under the supervision of Prof. dr. Saskia Middeldorp and dr. Michiel Coppens, and the auspices of Prof. dr. Harry Büller. He qualified as an Internist in summer 2015.

He currently lives with Alice and their daughter Agata, now 2 years old, in Mainz (Germany) and works as a research associate in the field of pulmonary embolism at the Center for Thrombosis and Hemostasis (head: Prof. dr. Stavros Konstantinides) of the Johannes Gutenberg University Hospital.

Agata and he are taking in German with varying results.

De paranimfen

Paulien G. de Jong and G. Jacopo Nicoletti