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Towards improvement of oral anticoagulant therapy

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**Towards Improvement
of
Oral Anticoagulant Therapy**

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Towards Improvement of Oral Anticoagulant Therapy

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ter verkrijging van
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CHAPTER 1

General Introduction

General introduction

Thrombosis, or the formation of a blood clot which hampers blood flow in a bloodvessel, is a very serious disease which can potentially be fatal. Thrombosis can occur in arteries (arterial thrombosis) as well as in veins (venous thrombosis). The most commonly known forms of arterial thrombosis are thrombosis of the coronary arteries and the carotid arteries, which may cause myocardial infarction or ischaemic stroke. Venous thrombosis most commonly presents as an isolated thrombus in vessels of the leg, as a pulmonary embolism (which may be a clot formed in the legs, which has embolised to the pulmonary vasculature), or as a combination of both. An arterial thrombus mainly consists of platelets, whereas in venous thrombosis fibrin is the main component. Treatment and prevention of arterial and venous thrombosis is aimed at inhibiting platelet function or inhibition of coagulation. Three classes of anti-platelet drugs are currently approved for clinical use; cyclooxygenase inhibitors (aspirin), P2Y₁₂ inhibitors (such as clopidogrel), and inhibitors of platelet aggregation (such as Reopro). The most commonly used anticoagulant drugs are heparin and heparin derivatives and vitamin K antagonists.

Vitamin K was discovered in 1929 by the observation of bleeding syndromes among chickens that were fed a fat-free diet [1]. This postulated the existence of a nutritional factor that was essential for normal haemostasis and Dam et al named this factor vitamin K. From clinical observations on patients with liver cirrhosis and obstruction from bile ducts it became clear that there was a direct link between liver function, bile secretion, vitamin K and the synthesis of clotting factors [2].

In the early 1920s a veterinarian in North Dakota, USA described a haemorrhagic diathesis in cattle that was caused by the ingestion of spoiled, sweet clover [3]. In 1929 it was demonstrated that this bleeding disorder was caused by a

deficiency of functional prothrombin [4]. It wasn't until 1940 that chemists of the University of Wisconsin discovered that the anticoagulant substance in the moldy sweet clover was the coumarin derivative 4-hydroxycoumarin [5]. In 1948 the first anticoagulant, warfarin (named for the Wisconsin Alumni Research Foundation), was produced [6]. It was first registered for use as a rodent poison. In 1954 warfarin was approved for medical use in humans. The exact mechanism of action remained unknown until it was demonstrated, in 1978, that warfarin inhibited vitamin K epoxide reductase and hence interfered with vitamin K metabolism [7].

Vitamin K is essential for the function of vitamin K dependent coagulation factors. These specific proteins involved in the coagulation cascade are modified after synthesis by a vitamin K dependent process. Specific amino acid residues in the so-called gla-domain are modified from a glutamic acid to a γ -carboxy glutamic acid by a vitamin K dependent carboxylase. In this process the vitamin K is oxidised to vitamin K epoxide. The γ -carboxy glutamic acid residues have a double negative charge at physiological pH, which is in contrast to the single negative charge of the (non modified) glutamic acid. The introduction of extra negative charge in the gla-domain of the vitamin K-dependent proteins is essential for their Ca^{2+} -mediated interaction with negatively charged cell membranes. Coagulation reactions take place on cellular surfaces – typically an activated platelet or endothelial cell. If the gla-domain of a vitamin K-dependent coagulation factor is not modified, the protein loses its capacity to bind to negatively charged cellular structures and thus is no longer able to participate in coagulation reactions.

Vitamin K antagonists exhibit their effect by interfering with the vitamin K cycle. Because the body is not able to store vitamin K, it is recycled through the vitamin K cycle. When vitamin K is oxidised in the carboxylation process described above it is no longer biologically active, and needs to be reduced by

vitamin K epoxide reductase (VKOR). Vitamin K antagonists have structural similarity to vitamin K, and therefore reduce availability of biologically active, reduced vitamin K.

Vitamin K is a necessary factor to produce the vitamin K-dependent clotting factors II, VII, IX and X, and the anticoagulant proteins C and S. Although both pro- and anticoagulant proteins are no longer post-translationally modified to biological active proteins in the presence of warfarin, the net effect of warfarin is an anticoagulant state. However, when warfarin treatment is initiated, it takes time for already circulating active coagulation factors to be replaced by inactive ones. The half-life of vitamin K-dependent coagulation factors varies from 4-6 hours (FVII) to 42-72 hours for prothrombin, so it will take 4-6 days before levels of activated coagulation factors are reduced to such an extent that sufficient anticoagulation is achieved. In some cases, particularly patients with anticoagulant protein deficiencies, this may lead for a short period to a hypercoagulable state with a risk of thrombosis. In order to bridge the period between initiation of anticoagulant treatment and the moment of sufficient vitamin K antagonist-induced anticoagulation, sometimes patients receive heparin for the first few days after initiation of anticoagulation, as heparin inhibits the coagulation system instantly.

Worldwide there are different types of vitamin K antagonists available. The vitamin K antagonists most frequently used are warfarin, acenocoumarol and phenprocoumon. Warfarin is the vitamin K antagonist of choice in the United States of America, the United Kingdom and many other countries around the world; acenocoumarol and phenprocoumon are frequently used in many European countries. These three vitamin K antagonists mainly differ in their half-life. Acenocoumarol has the shortest half-life of 11 hours, followed by warfarin with 36-42 hours and the longest half-life is seen in phenprocoumon with approximately

140 hours [8-11]. The clearance of these vitamin K antagonists is also different. Acenocoumarol is for its elimination completely dependent on hydroxylation by cytochrome p450 (CYP). Warfarin is also dependent on reduction processes [12]. Phenprocoumon can, in addition to elimination as hydroxylated metabolites, be eliminated as parent compound and is thus less dependent on hydroxylation by CYP.

While vitamin K antagonists decrease the risk of a thrombotic event by inhibiting coagulation, through the same mechanism they increase the risk of severe or even fatal haemorrhage. Prescription of vitamin K antagonists should therefore always be preceded by a careful evaluation whether the benefit will outweigh the bleeding risk. Vitamin K antagonists have a narrow therapeutic window, and frequent monitoring with adjustment of anticoagulant dosage is required to maintain patients within the therapeutic window. The response to vitamin K antagonists in a single patient is highly variable and unpredictable. The intensity of anticoagulation is assessed with a simple laboratory test. In this test, plasma is allowed to clot by addition of a reagent containing tissue factor (the physiological initiator of coagulation), phospholipids and calcium. The time to clot formation is a measure of the functionality of the so-called extrinsic pathway of coagulation, which consists of coagulation factors VII, X, V, II, and fibrinogen. This particular coagulation test is referred to as the prothrombin time (PT). Although the PT is sensitive for anticoagulation with vitamin K antagonists, the tests are poorly standardised between different laboratories. Use of different reagents and equipment results in substantially different PT values from a single blood sample. To overcome this standardisation problem, the INR or international normalised ratio has been developed.

The INR is assessed according to the formula:

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

In this formula ISI is the International Sensitivity Index, which is the calibration factor to correct for the type of thromboplastin and equipment used.

The introduction of the INR system to reflect anticoagulation led to several studies to determine the optimal level of anticoagulation, i.e. the level at which least complications occur [13-16]. The general recommendation is an INR between 2.0 and 3.0. Sometimes, dependent on the indication, a more intense anticoagulation is needed for which the recommendation is an INR between 3.0 and 4.0. In the Netherlands, the Dutch Federation of Anticoagulation Clinics (Federatie Nederlandse Trombosediensten, FNT) proposes target ranges of 2.5 – 3.5 and 3.0 – 4.0.

Patients who are insufficiently anticoagulated (i.e., an INR below the therapeutic window appropriate for their indication) are at increased risk for (re)thrombosis, whereas over-anticoagulated patients show a sharp increase in bleeding risk [16]. In spite of frequent monitoring, the annual risk for experiencing a serious bleeding complication is 1-2% [17,18]. Several studies investigated potential risk factors for haemorrhagic complications, such as increased age, indication for anticoagulant therapy and the use of interacting medication [19-21]. Besides these acquired factors, also genetic factors are shown to be of influence. Several studies have investigated the association between CYP2C9 genotype and warfarin response. Aithal et al. were the first to demonstrate an association between CYP2C9 genotype and warfarin sensitivity. Carriers of a CYP2C9*2 or CYP2C9*3 allele have lower dosage requirement and showed an increased risk for over-

anticoagulation and major bleeding complications in the initial phase of treatment compared to wild-type patients [22]. The presence of polymorphisms in the VKORC1 gene has also been identified to be associated with warfarin response. Carriers of VKORC1 polymorphisms showed a reduced requirement of warfarin dosage [23]. Most studies that investigate the effect of both the genotypes of CYP2C9 and VKORC1 showed that most variation in dosage is explained by polymorphisms in the VKORC1 gene.

Although the quality of oral anticoagulant treatment is already high, improvement is important. The risk for complications rises sharply with INR values below 2.0 and exponentially with INR values above 5.0 [16]. As a result of the large inter- and intra-patient variability in response to a certain dosage, patients may frequently be under- or overanticoagulated, despite frequent monitoring and adjustment of anticoagulant dose. Approximately 30 to 50% of the time, patients' INR is out of range. Improvement can be targeted at several points. First, dosing of vitamin K antagonists can be improved. If physicians are able to predict a patients' required maintenance dosage better, this would result in spending more time within the therapeutic range, and therefore in less complications. Dosing is classically performed by monitoring sequential INR values and the effect of previous dosing adjustments on the INR. Computer algorithms have been introduced to facilitate dosing and to produce a dosing advice based on mathematical processing of previous INR values and anticoagulant dosages. The use of these computer algorithms to assist physicians with their dosing decisions has been shown to lead to equal or improved quality of control of oral anticoagulant treatment compared to unassisted dosing [24-28]. However, a major disadvantage of these algorithms is that they do not generate a dosage proposal in all cases and they do not account for

the sensitivity of the individual patient for the anticoagulant (which may change over time), the half-life of the drug, and the non-linearity of the dose-INR relation.

Second, it is important to identify those patients who are at increased risk for experiencing either a thrombotic or a bleeding complication. Both patients who are unstably anticoagulated (large differences in sequential INRs) and patients who spend much time outside the therapeutic range are at risk. These patients can be more easily recognised when there is a measure to reflect their instability and if risk factors, either environmental or genetic, are identified. If one can recognise these patients actions such as patient education and more frequent monitoring can be taken.

Outline of this thesis

The studies included in this thesis aim to optimise dosing of vitamin K antagonists and control of oral anticoagulant treatment.

In **chapter 2** we describe the results of a double-blind randomised controlled trial in which we compared two computer algorithms for anticoagulant dosing. A newly developed algorithm which incorporated the sensitivity for vitamin K antagonists (ICAD) was compared to an algorithm frequently used in the Netherlands (TRODIS).

The relationships between maintenance dosages between the three most used vitamin K antagonists acenocoumarol, warfarin and phenprocoumon were studied in **chapter 3**. We calculated transition factors for switching from one vitamin K antagonist to another among participants in a randomised controlled trial who were treated with 2 different vitamin K antagonists.

In **chapter 4** the effects of polymorphisms in the CYP2C9 and VKOR genes were investigated in a cohort of patients starting with oral anticoagulant

treatment with acenocoumarol in Italy. We described the effect of these polymorphisms on the dose requirement and the risk of over-anticoagulation.

Instability is considered a risk factor for developing hemorrhagic and thrombotic complications. In **chapter 5** we studied several methods to reflect instability and investigated which method was best associated with hemorrhagic and thrombotic events in patients with mechanical heart valve prosthesis treated with vitamin K antagonists. Determinants of instability were investigated in **chapter 6**.

Finally, in **chapter 7** we present the study design and general results of a trial of which the primary aim was to compare the quality of an oral anticoagulant treatment with warfarin to the quality of treatment with phenprocoumon.

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CHAPTER 2

Improved Control of Oral Anticoagulant Dosing: A Randomized Controlled Trial Comparing Two Computer Algorithms

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J Thromb Haemost 2007; 5: 1644-9

Abstract

Background Efforts to improve dosing quality in oral anticoagulant control include the use of computer algorithms. Since current algorithms are simplistic and give dosage proposals in a small fraction of patients, we developed an algorithm based on principles of system and control engineering that gives proposals in nearly all patients.

Objective We evaluated the new algorithm in clinical practice.

Patients and Methods We conducted a double-blind randomized controlled trial among 712 patients with an indication for long-term anticoagulant treatment at the Leiden Anticoagulation Clinic. We compared oral anticoagulant dosing supported by the new algorithm (ICAD) with the standard algorithm (TRODIS).

Results The percentage of time spent in therapeutic range was similar for the new and standard algorithm group, 79.8% versus 80.2% (Diff 0.4%, 95%CI -1.7% to 2.6%). The new algorithm produced a dosage proposal in 97.5% of visits and the standard algorithm in 60.8% (Diff 36.7%, 95%CI 35.4%-38.0%). 79.3 % of proposals of the new algorithm were accepted by the physician versus 90.9% for the standard algorithm (Diff 11.6%, 95%CI 10.2%-13.0%). This implies that the new algorithm gave an acceptable proposal in 77.4% of all patient visits versus 55.3% for the standard algorithm (Diff 22.1%, 95%CI 20.4%-23.8%).

Conclusions Substantially more dosage proposals were generated and accepted with the new than with the standard algorithm, and the new algorithm will therefore improve the efficiency of anticoagulant monitoring without loss of quality.

Introduction

Management of oral anticoagulant treatment is difficult due to the large variability in the dosage needed to achieve the optimal anticoagulant effect. Sensitivity for vitamin K antagonists not only differs between patients, but also within patients it may vary over time [1].

The use of computer algorithms to assist physicians with their dosing decisions has been shown to lead to equal or improved quality of control of oral anticoagulant treatment compared to unassisted dosing [2-6]. Several algorithms have been developed previously. Poller et al. compared three different computerized systems to assist warfarin control to traditional dosing by experienced doctors. They found roughly similar results for unassisted dosing by physicians and dosing by the three algorithms [7]. In a larger multicenter study Poller et al. evaluated the safety and efficacy of the DAWN AC anticoagulant therapy management system. They found that patients in the computer-dose group spent more time in the target range than patients in the traditional-dose group [8]. An algorithm that is similar to these algorithms is used widely in the Netherlands (TRODIS) [9]. This algorithm generates a dosage proposal in approximately 55% of visits, leaving 45% for unassisted dosing by experienced physicians. In approximately 20% of cases where TRODIS generates a dosage proposal, it is overruled by a physician [10]. All these algorithms are based on an empirical decision-tree that determines whether the same dosage can be maintained, dosage adjustments have to be made or manual intervention by a physician is required. The equations used by the algorithm are based on a simple pharmacodynamic model, which implies a linear function between the INR and the dosage. A major disadvantage of these algorithms is that they do not generate a dosage proposal in all cases and they do not take into account the sensitivity of the patient for

coumarin derivatives (which may change over time), the half-life of the drug, and the non-linearity of the dose-INR relation.

To improve computer assisted dosing, we developed a new dosing algorithm. The Improved Control of Anticoagulant Dosage (ICAD) algorithm is based on a model that comprises pharmacokinetics and pharmacodynamics of the oral anticoagulant drug, pharmacokinetics of the prothrombin complex and the relation between the activity of the prothrombin complex and the measured INR. It consists of two sub models in which the first sub model describes the collective influence of all processes on the effect of the vitamin K antagonist and the second sub model describes the relationship between the dosage and the corresponding INR. The second sub model includes a variable parameter to reflect the sensitivity of the patient that may change over time. In an expert evaluation 194 visits were randomly selected from the anticoagulation clinic database to assess whether the dosage proposal and appointment periods calculated by the algorithm were acceptable. In this evaluation the ICAD algorithm was able to give a good or acceptable proposal in 94.3%. The ICAD algorithm is described in detail elsewhere [11].

In this study we tested the ICAD algorithm in clinical practice in a double-blind randomized controlled trial with the frequently used algorithm 'TRODIS' as reference intervention.

Methods

Study design

The study was conducted at the Leiden Anticoagulation Clinic in the Netherlands. Patients visit the clinic to have their INR measured and they receive their daily dosage prescription the next day by mail [12]. The TRODIS program uses the previous two INRs, the previous dosage schedule, the INR target and range

limiting values of the INR. A dose-response model is used to predict an INR. By comparing this prediction with the measured INR the program computes a new dosage and the time to the next visit. It does not generate a proposal in case of an alert (e.g. when a new drug is prescribed) or a large difference between the actual and the target INR and in that case the physician has to determine the dosage without any help of the algorithm.

The ICAD algorithm also uses the actual measured INR, the target INR and the previous dosage schedule, but differs from TRODIS and all other algorithms by calculating the sensitivity of the patient during the full course of each treatment. This sensitivity is incorporated in the equation to estimate the dosage needed to achieve optimal anticoagulant effect, thus allowing the dose-INR relationship to change over time. As all other algorithms ICAD gives a recommendation for the dosage as well as an appointment period.

A computer program was developed for this study to present dosage proposals of both algorithms in an identical way to keep physicians blinded. This program extracted for every patient the dosage proposal generated by TRODIS along with the INR, previous dosage schedule and all previous INRs from the TRODIS mainframe database. The relevant data were led through the ICAD algorithm, and an ICAD proposal was generated; so for all patients a TRODIS as well as an ICAD proposal was available. Dependent on the group the patient was randomized to, the TRODIS or the ICAD proposal was shown to the physician, on a screen that was identical for both types of proposals. Along with the dosage proposal a recommendation about the appointment period was given. Both ICAD and TRODIS give an indication on how confident the algorithm is about the proposal; for TRODIS, there are two levels of confidence ('high' and 'tentative'), while ICAD expresses confidence in a range of 0-100. These levels of confidence were not shown to the physician to maintain blinding, but are used in the analyzes

presented here. The physician could either accept or overrule the generated proposal. The final dosage was led back to the mainframe database of the anticoagulation clinic and subsequently, as is the routine policy, communicated by mail to the patient.

We obtained approval from the Medical Ethics Review Committee of the Leiden University Medical Center before start of the study and each patient gave written informed consent.

Patients

Enrolment occurred between August 14th and October 16th 2003 at the Leiden Anticoagulation Clinic. Patients were eligible when they were already on anticoagulation with an indication for long-term anticoagulant therapy and were aged between 18 and 80. Patients were excluded when they were on patient self-management, stayed long periods abroad or were in a terminal stage of disease. Randomization was stratified by the indication for oral anticoagulant treatment, age and sex using the minimization method [13]. The study was double blind, e.g., neither patients nor physicians were aware which group the patient belonged to. Follow-up was until September 1st 2004, i.e. maximally about one year.

Analysis

The analysis was performed at two levels, i.e., on the level of the patient and on the level of the proposal. In the first analysis, the primary outcome measure for this comparison was quality of anticoagulant treatment defined as the mean percentage of time spent in the therapeutic range (TTR) calculated with the linear interpolation method [14]. Therapeutic ranges were as they were applied in our routine anticoagulant practice: INR 2.0 to 3.5 for low intensity and INR 2.5 to 4.0 for indications requiring a higher intensity. In case a patient had 2 or less INR

measurements in total, no TTR was calculated. When the time between two INR checks exceeded 9 weeks, no TTR was calculated for this period, and this period was excluded. All periods of hospitalization were excluded.

Secondary outcome measures were the median time between visits, the percentage of time above or below the therapeutic range, the number of dosage proposals generated by the algorithm and clinical events. Bleeding complications were classified as major if they were fatal or necessitated hospitalization. Minor bleeding complications were all other bleeding events, in which ecchymoses were only counted when more than 10 cm diameter and epistaxis only when the duration exceeded 30 minutes.

In the analysis at the level of the proposal, we compared the proposals as they were generated by each algorithm (of which only one was shown to the physician). The primary outcome measure was the quality of the dosage proposals, expressed as the percentage accepted by the physician. In case no proposal was generated we considered this as not accepted. Secondary outcomes at the level of proposal comparisons were the percentage of INRs within the therapeutic range at the next visit, the percentage of accepted appointment periods proposed by the algorithms and whether the dose proposal of the algorithm which was not shown to the physician differed from the given dose.

We knew beforehand that TRODIS is not always capable of generating a dosage proposal. If the algorithm was incapable of generating a proposal, the dosage had to be determined by the physician unassisted by the algorithm. ICAD generated a dosage proposal in nearly all visits, which made it possible to directly compare ICAD with the physician. To avoid bias we selected all INR checks from both randomization groups where TRODIS was not able to generate a proposal. This was possible because for all visits an ICAD as well as a TRODIS proposal was available. In patients randomized to TRODIS the physician determined the

dosage without assistance of the algorithm. In the patients randomized to ICAD, an ICAD proposal was available. We studied the performance of the physician versus ICAD in these two groups by calculating the percentage of INRs within range at the next visit.

Statistical Analysis

We determined the necessary sample size on our primary outcome measure the time in therapeutic range. We felt a difference of 5% in percentage of time spent in therapeutic range to be of clinical relevance. Based on information of several anticoagulation clinics we found a standard deviation of approximately 23% in this outcome measure. With an alpha of 5% and a power of 90% we needed two times 168 patients to detect our clinical relevant difference. To allow us to do subgroup analyzes we included 712 patients.

All outcomes are shown as means or percentages with the corresponding 95% confidence interval of the difference based on T or binomial distributions or medians with the corresponding interquartile range (IQR). All calculations were performed on intention-to-treat basis using the statistical package SPSS version 14.0 (SPSS Inc, Chicago, Ill).

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Seven-hundred-and-twelve patients were randomized, 359 were assigned to ICAD and 353 to TRODIS. Total follow-up time in the ICAD group was 283.1 person-years during which 6007 INR checks were performed. In the TRODIS group follow-up time was 278.7 person-years with 5920 INR checks. Enrolment, randomization, follow-up and analysis of all patients are summarized in figure 1.

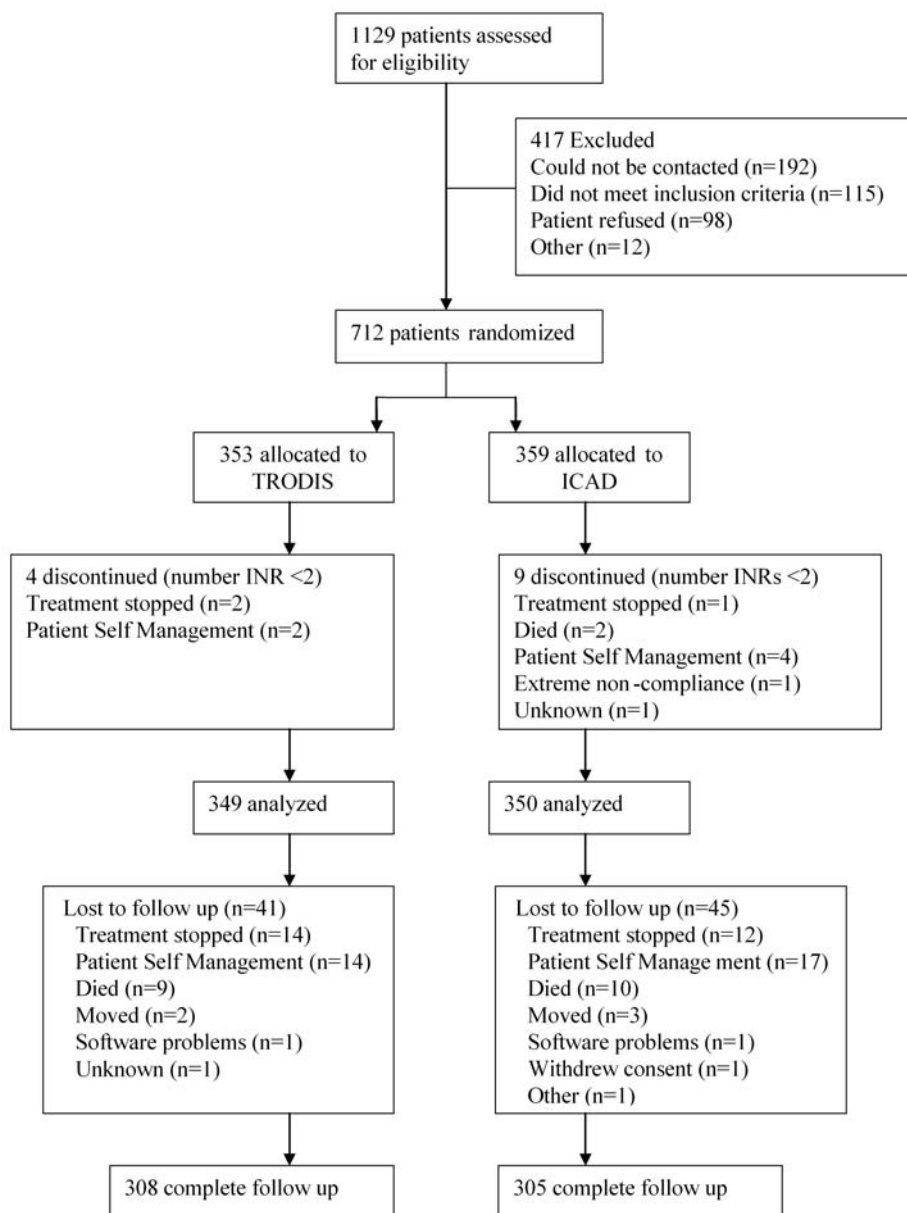


Figure 1. Enrolment, Randomization and Data Analysis

Table 1. Patient characteristics

	TRODIS (n=353)	ICAD (n=359)
Age		
Mean (IQR)	65.7 (59.6-74.8)	64.6 (57.6-74.8)
Sex		
Men (%)	66.6	66.6
Indication		
Atrial Fibrillation (%)	44.5	42.3
Venous thrombosis (%)	11.6	13.3
Heart valve prosthesis (%)	9.9	10.6
Other cardiac indication (%)	20.1	16.7
Peripheral vascular disorder (%)	8.8	10.3
Cerebrovascular (%)	5.1	7.0
Coumarin		
Acenocoumarol (%)	13.6	12.5
Phenprocoumon (%)	85.6	86.1
Switched (%)	0.8	1.4
Intensity (target)		
Low (2.5 – 3.5) (%)	62.9	61.6
High (3.0 – 4.0) (%)	36.3	38.2
Switched (%)	0.8	0.3

Baseline characteristics are shown in table 1.

The mean TTR was 79.8% in the ICAD group and 80.2% in the TRODIS group (Diff 0.4%, 95%CI of diff –1.7% to 2.6%). The mean percentage of time spent at sub- or supratherapeutic INRs did not differ: 4.2% of time subtherapeutic in the ICAD group versus 4.4% in the TRODIS group (Diff 0.2% 95%CI of diff – 1.1% to 1.5%), 16.0% of time supratherapeutic in the ICAD group versus 15.4% in the TRODIS group (Diff 0.6%, 95%CI –1.1% to 2.3%). The median time between two visits in the ICAD group was 14 days (interquartile range (IQR) 14-26 days) versus 14 days (IQR 14-22 days) in the TRODIS group (Figure 2).

There were 98 bleeding events (17 major and 81 minor) and 3 thromboembolic complications in 85 patients. Overall incidence of clinical thromboembolic and bleeding events (allowing for more than one event per patient) was 19.4/ 100 py in the ICAD group vs. 16.5/ 100 py in the TRODIS group, yielding a relative risk of 1.2 (95%CI 0.8-1.8).

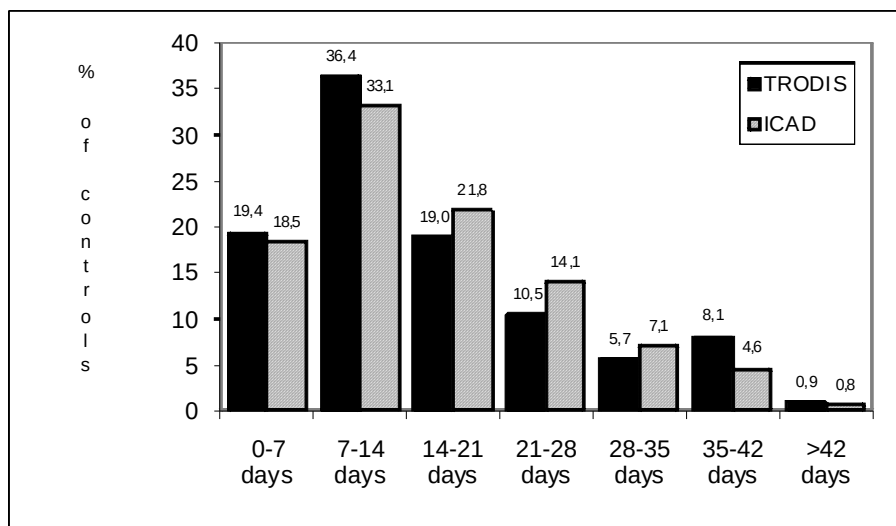


Figure 2. Time between two visits

Twelve of the major bleeding events occurred in the ICAD group (incidence rate 4.2/ 100 person-years), 5 in the TRODIS group (incidence rate 1.8/ 100 person-years). This yielded a relative risk of 2.3 (95%CI 0.8-6.5) of excess bleeding in the ICAD vs. TRODIS group. In the ICAD group 5 major bleeding events were gastrointestinal, 2 haematuria, 1 severe nose bleed and 1 severe skin bleed. In the TRODIS group there were 2 severe nose bleeds, 1 respiratory tract bleed and 1 retroperitoneal bleed. To further investigate the major bleeding events we analyzed in addition to the mean TTR, time spent with an INR <2.0, 2.0 - 3.0, 3.0 - 4.0, 4.0 – 5.0 and above 5.0. Also in this analysis there was no difference between the ICAD group and the TRODIS group. Of six patients there was an INR measurement available within 7 days before the event, of which only one was above range (INR 4.7, ICAD). Of the remaining patients who had their last INR measurement more than 7 days ago three had an INR that was marginally above range (2 ICAD, 1 TRODIS) and two below range (1 ICAD, 1 TRODIS). In none of the patients the dosage was increased at the last INR measurement. In total 21 patients died during

follow up, 4 because of a bleeding complication (3 ICAD, 1 TRODIS). All deaths and complications are listed in table 2.

Table 2. Adverse events and deaths during follow up.

	TRODIS (n=349)	ICAD (n=350)
Bleeding events		
Minor	40	41
Major	4	9
Fatal	1	3
Total	45	53
Thrombo-embolic events	1	2
Deaths		
Bleeds	1	3
Malignancy	1	2
Cardiac	2	0
Respiratory	0	3
Other	1	1
Unknown	4	3
Total	9	12

ICAD was able to generate dosage proposals in 97.5 % of visits, whereas TRODIS generated a proposal in 60.8% (Diff 36.7%, 95%CI: 35.4% to 38.0%). In the ICAD group 79.3% of these dosage proposals was accepted versus 90.9% in the TRODIS group (Diff 11.6%, 95%CI: 10.2% to 13.0%). In total, therefore, 77.4% of patient visits in the ICAD group led to an accepted proposal, which was 55.3% in the TRODIS group (Diff 22.1%, 95%CI 20.4% to 23.8%). In ICAD software problems were the main reason for not generating a proposal, which occurred only rarely. The most important reason for rejecting a proposal was that the dosage change proposed by the algorithm was estimated to be too strong (66.2%). In TRODIS the main reason why a proposal was not generated was an INR change that was too large in relation to the previous INR or the one before that (64.2%). In case of a rejected proposal this was mostly because the dosage change proposed by the algorithm was too strong (66.2%) (Table 3).

Table 3. Reasons for not accepting a dosage proposal.

	ICAD (n=1211) N (%)	TRODIS (n=328) N (%)
Reasons		
Dosage change proposed by the algorithm was too strong	733 (60.5)	217 (66.2)
Dosage change proposed was not strong enough	339 (28.0)	60 (18.3)
Proposal was in the wrong direction	139 (11.5)	51 (15.5)

When the proposals were stratified according to the confidence level the algorithm had given to it, in both groups the proportion of proposals that was accepted rose with the confidence level (Table 4). Along with the dosage proposal the algorithms also provided a proposal for the appointment period. The proposed appointment periods were accepted in 76.5% in the ICAD proposals and 91.4% in the TRODIS proposals (Diff 14.9%, 95%CI of diff: 13.5%-16.3%). When we only considered proposals of which the physician accepted the dosage, 82.1% of the ICAD appointment periods were accepted and 93.4% in the TRODIS group (Diff 11.3%, 95%CI of diff: 9.9-12.7).

Table 4. Percentage of accepted dosage proposals stratified according to the confidence levels.

TRODIS		
	N (%)	% Accepted
TRODIS proposal type		
No proposal	2319 (39.2)	0
Tentative	1465 (24.7)	87.6
Confident	2136 (36.1)	93.1
Total	5920	55.3
ICAD		
	N (%)	% Accepted
ICAD confidence score		
0-20	228 (3.8)	7.9
20-40	367 (6.3)	37.3
40-60	939 (15.6)	64.9
60-80	2001 (33.3)	80.1
80-100	2472 (41.2)	92.4
Total	6007	77.4

In the ICAD group 70.1% of INRs were in range at the next visit, compared to 72.5% of INRs in the TRODIS group (Diff. 2.4%, 95%CI of diff.: 0.7% to 4.1%). When we only consider the accepted proposals, 72.3% of the INRs was in range in the ICAD group, versus 75.7% in the TRODIS group (Diff 3.4%, 95%CI of diff 1.4-5.4). Of the TRODIS proposals that were overruled by the physician, 28.0% had an ICAD proposal which was equal to the dosage that was given by the physician. In the ICAD group, 19.1% of the unaccepted proposals had a TRODIS proposal that was similar to the actual given dosage.

In the comparison between ICAD and the physician, i.e., all patient visits where TRODIS was not capable of giving a proposal, ICAD was able to generate a dosage proposal in 96.9% of cases. Of these, 66.7% was accepted by the physician. In the ICAD group 63.4% of the INRs were within the therapeutic range at the next INR measurement versus 67.4% in the TRODIS group, which were dosed by the physician (Diff 4.0%; 95%CI of diff 1.2–6.8).

Discussion

In this study we compared two computer algorithms for control of anticoagulant dosing. There was no difference in quality of anticoagulant control between the TRODIS and the ICAD algorithm, expressed as mean time in therapeutic range. Also, the time between two visits was similar in both groups, although the interquartile range was broader for the new algorithm. There was a difference in efficiency between the two algorithms. For all visits, TRODIS generated an acceptable proposal in 55.3%, which was 77.4% for ICAD. Finally, in almost all cases where the standard algorithm could not give a proposal, the new algorithm could, and performed equally well as an unassisted physician.

The similar quality of ICAD and TRODIS proposals can be explained in several ways. First, it is possible that an algorithm which uses more information of a patient is not capable of generating dosage proposals better than an algorithm which uses less information. Second, it is possible that there can be no more gain in quality of treatment by improving dosing of anticoagulants. Thirdly, physicians being used to the old algorithm, may have altered unusual but good proposals of the new algorithm. We feel that it is unlikely that an algorithm will ever be capable of incorporating all aspects of patient behavior, such as sudden changes in diet, and that possibly the best that is attainable is an algorithm that does as well as well-trained, dedicated physicians. When one algorithm uses a simple model and only gives proposals for ‘easy’ cases, leaving the more difficult cases to the physicians, while another algorithm gives proposals for virtually all cases and performs as well as the physicians, the result would be as we observed: an increase in efficiency without a concomitant increase in quality of treatment.

Although there was no difference in mean time in therapeutic range between the groups, we did observe a difference in clinical events. Since the study groups were similar in all prognostic variables and the time in therapeutic range was similar for both groups we feel this was due to chance. Also, in the additional analysis we found again no difference between the two groups and most bleeding events were at INR in range, which strengthens our idea that the difference was due to chance.

This study was double blind, so patients in both groups were treated the same way except for the algorithm that was used. Bias resulting from a different attitude towards the two algorithms was therefore prevented. In some cases blinding of the physicians could not be achieved. It was known beforehand that TRODIS often is not able to generate a proposal. Whenever there was no proposal available, the physician knew that in all likelihood this concerned a patient

randomized to the TRODIS group. This was only known for the INR check at that time, for the physician did not see previous proposals. Since in these cases there was obviously no possibility to reject or accept the proposal, it is difficult to imagine that this could have biased the results.

Some patients were lost to follow-up after randomization because of participation in a self management program, end of the prescribed treatment period or other reasons. In both groups the same number of patients was lost to follow-up, and their numbers were small, so that selection due to loss of patients is unlikely.

We have tested the new ICAD algorithm for computer-assisted dosing of oral anticoagulants in a randomized blinded comparison to the algorithm that is currently in use. The ICAD algorithm led to similar quality of anticoagulant control, but proved to perform more efficiently: overall the proportion of proposals that were accepted was 77.4%, versus 55.3% for the old algorithm. Therefore, the newly developed ICAD algorithm is an important gain in the efficiency of the management of oral anticoagulant therapy.

Acknowledgements

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CHAPTER 3

The Relationship between Maintenance Dosages of Three Vitamin K Antagonists: Acenocoumarol, Warfarin and Phenprocoumon

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Abstract

Introduction Vitamin K antagonists of the coumarin type are widely used oral anticoagulants.

Objective We developed a transition algorithm for the maintenance dosages of three frequently used coumarins: warfarin, phenprocoumon and acenocoumarol.

Methods The study was conducted at the Leiden Anticoagulation Clinic. Patients were participants in a trial of which the main objective was to compare the quality of an oral anticoagulant therapy with phenprocoumon to warfarin. We included patients who initiated oral anticoagulant therapy and patients who were already using acenocoumarol. Patients were randomized to a treatment with warfarin or phenprocoumon. Patients who were randomized to warfarin switched to phenprocoumon at end of follow up. We analysed the switch from acenocoumarol to warfarin or phenprocoumon at start of follow up and the switch of warfarin to phenprocoumon at the end of follow up and calculated the transition factors for stable anticoagulation between these three vitamin K antagonists.

Results 58 patients switched from warfarin to phenprocoumon, 39 from acenocoumarol to phenprocoumon and 44 from acenocoumarol to warfarin. The maintenance dose of warfarin was 0.41 (95%CI 0.39- 0.43) times the maintenance dose of phenprocoumon. The transition factor between acenocoumarol and phenprocoumon was 0.84 (95%CI 0.79- 0.89) and between acenocoumarol and warfarin 1.85 (95%CI 1.78- 1.92).

Conclusions We determined the transition factors between warfarin, phenprocoumon and acenocoumarol. With these transition factors physicians are able to estimate the maintenance dose when it is necessary for a patient to switch from one coumarin to the other.

Introduction

Vitamin K antagonists of the coumarin type are widely used oral anticoagulants. They are proven to be effective in the treatment and prevention of arterial and venous thrombosis [1-3]. Worldwide there are different coumarin derivatives available. The coumarins most frequently used are warfarin, acenocoumarol and phenprocoumon. Warfarin is the coumarin of first choice in the United States of America, the United Kingdom and many other countries around the world; acenocoumarol and phenprocoumon are frequently used in many European countries. These three coumarin derivatives mainly differ in their half-life. Acenocoumarol has the shortest half-life of 11 hours, followed by warfarin with 36-42 hours and the longest half-life is seen in phenprocoumon with approximately 140 hours [4-7]. Also the clearance of these coumarins is not similar. Acenocoumarol is for its elimination completely dependent on hydroxylation by cytochrome p450 (CYP). Warfarin is also dependent on reduction processes [8]. Phenprocoumon can, in addition to elimination as hydroxylated metabolites, be eliminated as parent compound and is thus less dependent on hydroxylation by CYP. These differences in dependence on hydroxylation by the CYP enzymes offer an explanation of different responses found in studies investigating the effects of polymorphisms in the CYP2C9 gene [9,10]. Several studies have compared the different coumarins with regard to the quality of treatment, e.g. stability. Most studies have compared the short acting acenocoumarol to the longer acting warfarin or phenprocoumon. The results were mostly in favour of the longer acting coumarins, but not always [11-19].

Sometimes transition from one coumarin to another is required. Reasons to switch can be women trying to get pregnant for whom the use of phenprocoumon is contra-indicated because of its long half-life and acenocoumarol is preferred, the experience of allergic reactions or side effects such as hair loss. Coumarin

sensitivity can be a reason to switch from one coumarin to the other for practical reasons, since a maintenance dose of less than 1 mg of acenocoumarol is difficult to administer (tablets contain 1 mg, and cannot be divided). Finally, patients who are very instable are sometimes thought to benefit from switching to another coumarin derivative with a longer half-life. At present, literature about the transition from one coumarin to another is surprisingly scarce. One study investigated a dosage scheme for transition from phenprocoumon to warfarin in patients treated in an outpatients clinic [20]. The authors found that the dosage for an optimal INR of warfarin is 2.3 times the dosage of phenprocoumon. Applying this transition factor resulted in 75% of patients for whom the right dosage could be determined. No studies are known that included transition to or from acenocoumarol.

We studied the relationship between the maintenance dosages of acenocoumarol, warfarin and phenprocoumon in patients participating in a randomized controlled trial.

Methods

Study design and patient population

Patients participated in a randomized controlled trial conducted at the Leiden Anticoagulation clinic. Inclusion of patients occurred between February 2004 and April 2007. The main objective of the trial was to compare the quality of oral anticoagulant treatment with phenprocoumon versus warfarin. Follow-up was six months. Patients were eligible to participate when they were aged between 18 and 85 years and had an indication for anticoagulant treatment for at least three months. Exclusion criteria were pregnancy or intended pregnancy, renal dialysis, chemotherapy, known allergic reactions for warfarin or phenprocoumon or a contra-indication to oral anticoagulant treatment.

Two patient groups were included in the trial. The first group consisted of patients initiating oral anticoagulant treatment and was recruited in three hospitals, i.e., at the departments of Cardiology and Internal Medicine of the Leiden University Medical Center, Diaconessenhuis Leiden and Rijnland Hospital Leiderdorp and at the department of Orthopedics of the Leiden University Medical Center, all in the Netherlands. Patients were randomized to a treatment with either phenprocoumon or warfarin and were followed until end of treatment or, when the indication required the treatment to continue over 6 months, follow-up ended at this point. Because warfarin is not registered for use in the Netherlands patients who required ongoing treatment and who were randomized to the warfarin group were switched to a treatment with phenprocoumon.

The second group included in this trial consisted of patients already using acenocoumarol and were recruited at the Leiden Anticoagulation clinic. After written informed consent they were randomized and switched to a treatment with either phenprocoumon or warfarin. Follow-up was again 6 months and like patients of the first group, patients randomized to warfarin switched to phenprocoumon at the conclusion of the trial. If they preferred so, patients of this second group could also choose to switch back to acenocoumarol. Figure 1 summarizes the flow of patients through the study.

All patients participating in the trial were part of the routine care in the Anticoagulation clinic. We obtained approval from Medical Ethics Review Committee of the Leiden University Medical Center before start of the study and all patients gave written informed consent before randomization. The trial is registered in the ISRCTN register with identifier ISRCTN60446748 (<http://www.controlled-trials.com>).

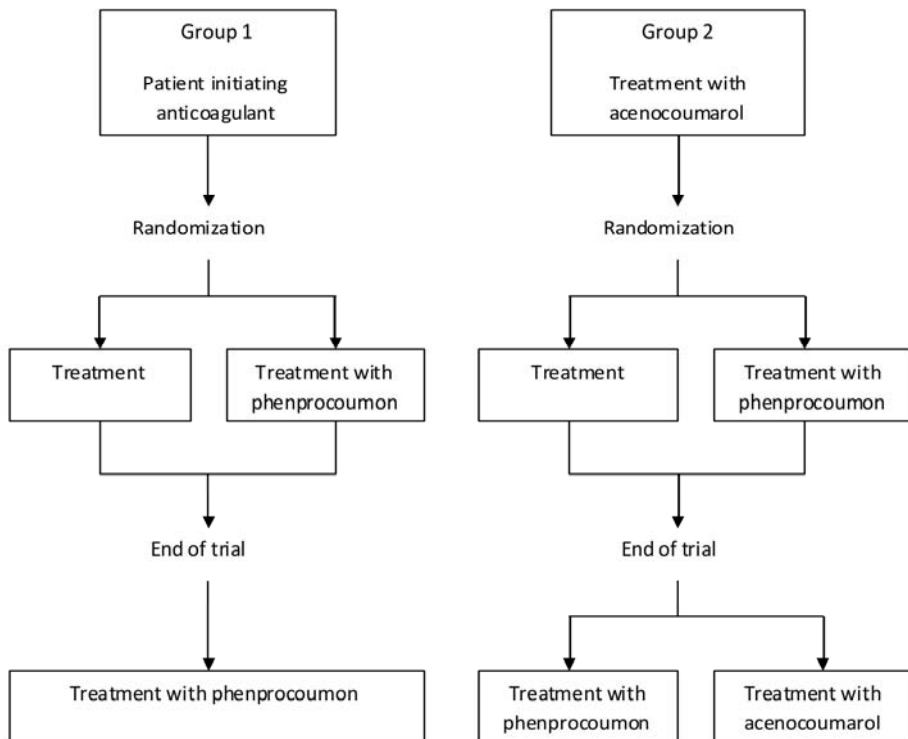


Figure 1. Flowchart of patients in the trial

Analysis

Of the first group of patients, i.e., those who initiated their treatment within the trial, we studied the transition of patients randomized to warfarin who switched to phenprocoumon at end of follow-up. Of the second group of patients, i.e., those already treated with acenocoumarol, we studied the transition from acenocoumarol to phenprocoumon or warfarin at start of follow-up and the transition from warfarin to phenprocoumon at end of follow-up. We did not include the transition from warfarin to acenocoumarol at the end of follow-up because these patients were

already included in the analysis as switchers from acenocoumarol to warfarin when they entered the trial. In a secondary analysis we did look at this transition at the conclusion of the trial separately to investigate whether the transition factor we found was similar to the calculated transition factor at the start of follow-up.

We determined the maintenance dosage by calculating the mean dosage that led to three consecutive INRs within the therapeutic range, with at least 7 days between two INR checks. The maintenance dose was determined over the period closest to the transition date. For the first coumarin used this means the last 3 consecutive INRs in range before switching. After the transition date we considered the first 4 weeks as a wash out period. So, for the second coumarin we searched for the first 3 INRs in range after the wash out period. All determined maintenance dosages were evaluated, and corrected if necessary, by an expert in anticoagulant dosing. Therapeutic ranges were as they are applied in our clinic: INR 2.0-3.5 for indications requiring a low intensity and INR 2.5-4.0 for high intensity.

We performed linear regression analysis. All models were made with starting point at origin. Regression coefficients are given with their 95% confidence intervals. All calculations were performed using the statistical package SPSS version 14.0 (SPSS Inc, Chicago, Ill).

Results

In total 141 transitions were evaluated. Thirty-seven patients initiated their anticoagulant treatment with warfarin and switched to phenprocoumon. Eighty-three patients were already treated with acenocoumarol and of these patients, 39 switched to phenprocoumon and 44 to warfarin. Of these 44 patients randomized to a treatment with warfarin, 21 switched back to phenprocoumon at end of follow-up. General characteristics of all patients are summarized in table 1.

For 13 patients one of the maintenance dosages and for one patient both dosages could not be determined, because they did not have three consecutive INRs in range. The median maintenance dose of phenprocoumon for patients with a target range of 2.0-3.5 (n=68) was 2.09 mg/ day (interquartile range (IQR) 1.50-2.72). For acenocoumarol (n=58) and warfarin (n=81) the maintenance dose was 2.46 mg/ day (IQR 1.79- 3.47) and 4.68 mg/ day (IQR 3.74- 6.60) respectively. Seventy-two patients (51.1%) were stable with their anticoagulant treatment at time of the transition, i.e. the last three INRs were within the therapeutic range. The median interval between the maintenance dose of the first coumarin and the second coumarin was 98 days (IQR 63- 153 days).

Table 1. Patient characteristics

	Warfarin to phenprocoumon N=58	Acenocoumarol to phenprocoumon N= 39	Acenocoumarol to warfarin N=44
Age			
Median (IQR*)	69.5 (63.0 – 77.3)	67.0 (61.0-75.0)	66.0 (61.3-73)
Sex			
Men (%)	43 (74.1)	30 (76.9)	37 (84.1)
Intensity of OAC			
Low (2.0-3.5) (%)	51 (87.9)	26 (66.7)	33 (75.0)
High (2.5-4.0) (%)	7 (12.1)	13 (33.3)	11 (25.0)
Indication for OAC			
Atrial fibrillation	45 (77.6)	20 (51.3)	22 (50.0)
Venous thrombosis	5 (8.6)	2 (5.1)	6 (13.6)
Cardiac other	3 (5.2)	7 (17.9)	8 (18.2)
Peripheral arterial	4 (6.9)	7 (17.9)	6 (13.6)
Other	1 (1.7)	3 (7.7)	2 (4.5)

*IQR=Interquartile range

Transition from warfarin to phenprocoumon

The first mean daily dose of phenprocoumon was 0.48 (95%CI 0.46-0.51) times the last mean daily dosage of warfarin. A loading dosage was given on the first day to 89.4% of the patients, and this loading dosage was approximately 1.6 times the mean daily dosage. 83.0% of patients received a loading dosage on the second day after transition and this was 2.3 times the mean daily dosage. A loading dose of 2.3 times the mean daily dosage was given to 63.8% of patients on the third day after

transition. Few patients (27.7%) received a loading dosage on the fourth day after transition and this was 2.1 times the mean daily dosage. In the wash out period of 4 weeks after transition the median percentage time in the therapeutic range was 62.8% (IQR 37.6 – 96.1).

The transition factor between the maintenance dosage of phenprocoumon and warfarin in milligram was 0.41 (95% CI 0.39 – 0.43), indicating that the maintenance dosage of phenprocoumon is 0.41 times the maintenance dosage of warfarin (figure 2).

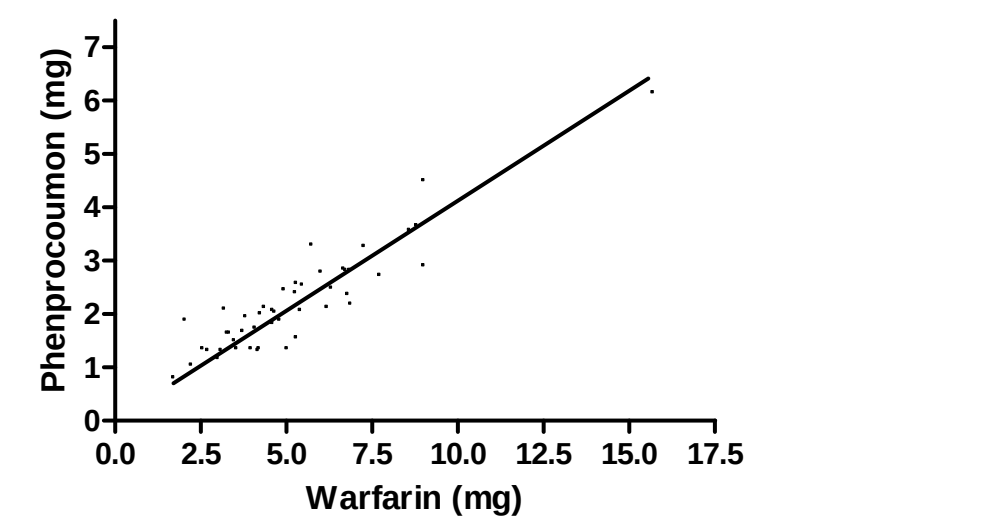


Figure 2. Relation between the maintenance dose of phenprocoumon and warfarin.

Transition from acenocoumarol to phenprocoumon

The first mean daily dose of phenprocoumon was 0.90 (95%CI 0.87-0.94) times the last mean daily dosage of acenocoumarol. A mean loading dosage of 2.5 times the mean daily dosage was given on the first day to 86.8% of the patients. On the

second day 81.6% of the patients received a mean loading dosage of 3.3 times the mean daily dosage, and 63.2% of the patients was prescribed a mean loading dose of 2.5 times the mean daily dosage on the third day after transition. A small fraction of patients (5.3%) received a loading dosage on the fourth day after transition of approximately 2.0 times the mean daily dosage. The median percentage of time in the therapeutic range in the first four weeks was 69.5% (IQR 49.9 – 94.7).

The transition factor between acenocoumarol and phenprocoumon in milligram was 0.84 (95% CI 0.79 – 0.89), meaning that the maintenance dosage of phenprocoumon is 0.84 times the maintenance dosage of acenocoumarol (figure 3).

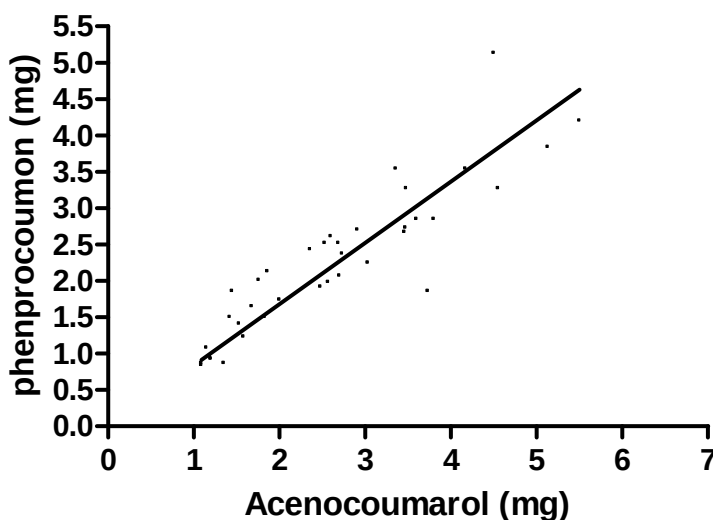


Figure 3. Relation between maintenance dosage of acenocoumarol and phenprocoumon

Transition from acenocoumarol to warfarin

The first mean daily dose of warfarin was 1.59 (95%CI 1.53-1.65) times the last mean daily dosage of acenocoumarol. A loading dosage was given on the first day to 82.9% of the patients, and this loading dosage was on average 1.8 times the

mean daily dosage. 73.2% of the patients also received a loading dosage on the second day after transition and this was 1.8 times the mean daily dosage. A loading dose of 1.6 times the mean daily dosage was given to 36.6% of the patients on the third day after transition. Only one patient received a loading dosage on the fourth day after transition and this was 1.9 times the mean daily dosage. This led to a median percentage of time in therapeutic range in the first four weeks of 66.3% (IQR 48.4 – 95.8).

The transition factor between the maintenance dosage of acenocoumarol and warfarin in milligram was 1.85 (95% CI 1.78 – 1.92), indicating that the maintenance dosage of warfarin is 1.85 times the maintenance dosage of acenocoumarol (figure 4).

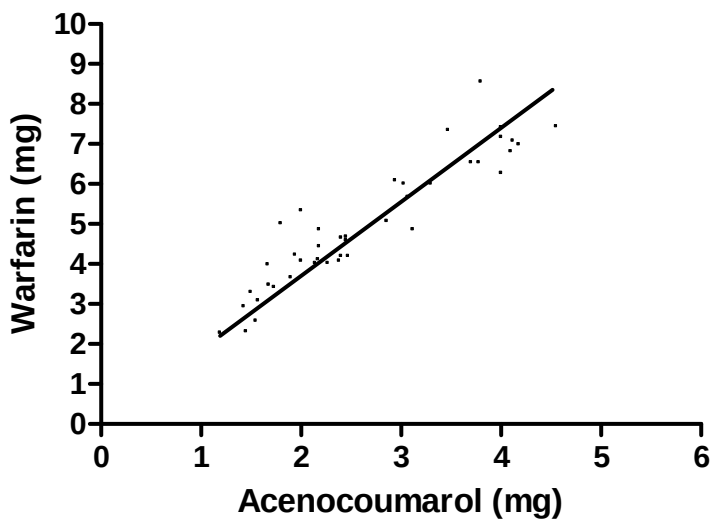


Figure 4. Relation between maintenance dosage of acenocoumarol and warfarin

All transition factors are listed in table 2. Age had minor effects on the transition factors.

Table 2. Transition factors with corresponding 95% confidence intervals

Direction of switch	Transition factor	95% CI
Warfarin to phenprocoumon	0.41	0.39 - 0.43
Phenprocoumon to warfarin	2.36	2.24 - 2.48
Acenocoumarol to phenprocoumon	0.84	0.79 – 0.89
Phenprocoumon to acenocoumarol	1.15	1.08 – 1.22
Acenocoumarol to warfarin	1.85	1.78 – 1.92
Warfarin to acenocoumarol	0.53	0.51 – 0.55

In table 3 the transition factors for different age categories are shown. The transition factors did not differ if we calculated them separately for the two different therapeutic ranges. There were minor differences in the transition factors for stable versus unstable anticoagulated patients which were not clinically relevant.

Table 3. Transition factors with corresponding 95% confidence intervals for different age categories

Direction of switch	Agegroup	N	Transition factor	95%CI
Warfarin to phenprocoumon	<66.7	21	0.40	0.37-0.42
	66.7-74.3	21	0.42	0.38-0.37
	>74.3	16	0.43	0.39-0.48
	Total	58	0.41	0.39-0.43
Acenocoumarol to phenprocoumon	<66.7	17	0.83	0.74-0.93
	66.7-74.3	12	0.83	0.76-0.90
	>74.3	10	0.90	0.83-0.97
	Total	39	0.84	0.79-0.89
Acenocoumarol to warfarin	<66.7	24	1.86	1.74-1.98
	66.7- 74.3	13	1.84	1.73-1.94
	>74.3	7	1.83	1.71-1.96
	Total	44	1.85	1.78-1.92

Of the patients who switched from acenocoumarol to warfarin 21 patients switched back to acenocoumarol at the conclusion of the trial. The transition factor between warfarin and acenocoumarol in these patients in milligram was 0.53 (95%CI 0.50 – 0.56), which is approximately the same as the inverse of the

transition factor we found in patients switching from acenocoumarol to warfarin at start of the trial ($1/1.85 = 0.54$).

Discussion

We investigated the transition factors between the maintenance dosages of the three frequently used coumarins for oral anticoagulant treatment. The maintenance dose of warfarin was 0.41 (95%CI 0.39- 0.43) times the maintenance dose of phenprocoumon. The transition factor between acenocoumarol and phenprocoumon was 0.84 (95%CI 0.79- 0.89) and between acenocoumarol and warfarin 1.85 (95%CI 1.78- 1.92).

Because we had the unique situation of patients switching between warfarin, phenprocoumon and acenocoumarol within a randomized trial, i.e., without a medical reason to switch, we could study the relationship between the maintenance dosages of these three coumarins without bias. Also when we calculate the transition factor between phenprocoumon and warfarin by using the transition factors between acenocoumarol and phenprocoumon and acenocoumarol we find approximately the same result we observed in our patients ($0.84/1.85 = 0.45$ versus 0.41). It should be noted that we investigated the relationship between the maintenance dosages; a transition scheme may also require a loading dose. We gave a description of our management during transition, but we did not investigate whether or not this was the most appropriate way to switch between coumarins.

In some patients the time between the two maintenance doses was very long and we may question whether the dosages were still related to each other since the coumarin sensitivity may vary over time within a patient. However, since these variations are relatively small and random in their direction it is unlikely that this affected our results. This is also supported by the results of our secondary analysis where we found that in patients who switched from acenocoumarol to warfarin and

back to acenocoumarol again the transition factor between acenocoumarol and warfarin is the same as the inverse of the transition factor between warfarin and acenocoumarol.

In our study we found similar results as was found in the study of Kristiansen *et al.* [20]. They reported a transition factor of 2.3 for switching from phenprocoumon to warfarin, where we found a transition factor of 2.4. No literature is known about switching from acenocoumarol to phenprocoumon or from acenocoumarol to warfarin. Several studies showed that the variant alleles CYP2C9*2 or CYP2C9*3 are associated with an increased response to acenocoumarol and warfarin, whereas such an association is unclear for phenprocoumon [10, 21, 22]. These variant alleles could also interfere with the transition factors we presented in this manuscript. Unfortunately it was not possible to investigate this possible effect in our study population since the data were not available. We found minor differences in the transition factors for the different age categories which are not of clinical importance to our opinion.

We calculated the transition factors between the maintenance dosages of the three most frequently used coumarins for oral anticoagulant treatment. With these transition factors physicians can easily determine the maintenance dosage when it is necessary for a patient to switch from one coumarin to another.

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CHAPTER 4

Effects of CYP2C9 and VKORC1 on INR variations and dose requirements during the initial phase of anticoagulant therapy with acenocoumarol

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Abstract

Introduction: Anticoagulants of the coumarin type are effective drugs for the treatment and prevention of thromboembolic diseases. However, they have a narrow therapeutic range and show interindividual and intraindividual variability in dose requirement, largely conditioned by both environmental and genetic factors.

Methods: This prospective study investigated, during the initial phase of acenocoumarol therapy, the effect of *CYP2C9* variant alleles and *VKORC1* haplotypes, single and in combination, in 220 Italians.

Results: *CYP2C9**3 was associated with a 25% dose reduction and an increased risk of over-anticoagulation (INR>6) on day 4. Two copies of the *VKORC1**2 haplotype were associated with a 45% dose reduction and an increased risk of over-anticoagulation. Homozygosity for *VKORC1**3 and *VKORC1**4 was associated with an increased dose requirement and a reduced risk of over-anticoagulation. The *VKORC1**3 or *4 plus *CYP2C9**1 genotype combination was associated with the highest dose requirement and the lowest INR on day 4; *VKORC1**2 plus *CYP2C9**3 was associated with the lowest dose requirement, the highest INR and an increased risk of over-anticoagulation. Even though they spent approximately 50% of the time within the target therapeutic range, *VKORC1**3 or *4 plus *CYP2C9**1 carriers spent a large percentage of the remaining time below and carriers of *VKORC1**2 plus *CYP2C9**3 above the target range.

Discussion: The determination of *VKORC1**3 and *VKORC1**4 haplotypes may be an important addition to *CYP2C9* and *VKORC1**2 genotyping to identify patients at risk of being outside the target range during initial anticoagulation with acenocoumarol.

Introduction

Anticoagulant drugs play an important role in the prophylaxis and treatment of thrombotic events, and are widely used chronically or intermittently in cardiovascular medicine and surgery [1]. For instance, their use has decreased the risk of thrombotic events and death due to acute myocardial infarction by 24% [2] and 80%, respectively [3]. However, the use of vitamin K (VitK) antagonists as oral anticoagulants still poses significant clinical challenges because their therapeutic index is narrow and dose-response relationship unpredictable [4]. The latter situation makes difficult to predict the daily maintenance dose, that for warfarin may range from as little as 0.5 to as much as 60 mg [5] and for acenocoumarol from 1 to 56 mg [6]. These large variations in dose requirements, influenced by pharmacokinetic and pharmacodynamic aspects in turn determined by genetic and environmental factors, demand frequent measurements of the International Normalized Ratio (INR) of the prothrombin time to evaluate the degree of anticoagulation and to assess the need for dosage changing.

Warfarin is the main vit.K antagonist prescribed in the United Kingdom and North-America, while acenocoumarol, the 4'-nitro-analogue of warfarin, is widely used in European countries. Elimination of the two acenocoumarol isomers depends entirely on their hepatic biotransformation by the CYP2C9 enzyme [7, 8]. Carriers of genetic polymorphisms of *CYP2C9*, in particular of the *CYP2C9*2* and *CYP2C9*3* variant alleles [9-10], require smaller doses of acenocoumarol than carriers of the most frequent *2C9*1* wild-type allele and have a higher incidence of minor bleeding episodes [11], even though dose-effect association for the *CYP2C9*2* variant allele was not consistently found [6, 11-17]. The *CYP2C9*3* variant allele is also associated with a reduced likelihood to achieve stability within the target INR range during the first 6 months of acenocoumarol use and with an increased risk of severe anticoagulation ($\text{INR} > 6$) [14].

Oral anticoagulants exert their effect by reducing the regeneration of Vit.K from its epoxide through the inhibition of Vit.K epoxide reductase [18]. This protein is coded by the Vit.K epoxide reductase complex subunit 1 gene (*VKORC1*) [19-20]. Several polymorphisms in the *VKORC1* gene are mainly located in non-coding regions and are in strong linkage disequilibrium (LD), so that a few of them are enough to infer the most common haplotypes that explain the genetic variability of *VKORC1* [21, 22]. Rieder *et al.*, [21] divided the most common haplotypes in two main groups: group A (haplotypes H1 and H2), associated with low warfarin dose requirement and the group B (haplotypes H7-H9), associated with an increased dose requirement. Geisen *et al.*, [22] later described three haplotypes (*VKORC1**2, *VKORC1**3, *VKORC1**4) covering >99% of the genetic variability in Europeans: the *VKORC1**2 haplotype (corresponding to group A) was strongly associated with an increased coumarin sensitivity whereas *VKORC1**3 and *VKORC1**4 (both included into group B) with partial resistance. Even though the combined effect of both *VKORC1* and *CYP2C9* on warfarin [21, 23- 27] was fully investigated, less information is available on acenocoumarol response [15, 28-30]. Thus, this prospective study was designed to investigate the contribution of the common *CYP2C9* variant alleles and *VKORC1* haplotypes in the modulation of acenocoumarol response. We chose to investigate the initial phase of treatment because the first months are particularly problematic, since the safe and effective dose for an individual patient is not known and is determined empirically, with an increased risk of over-anticoagulation and hemorrhagic complications [31]. Our primary purpose was to evaluate the effect of the *CYP2C9* variant alleles and *VKORC1* haplotypes and of their combination on the first INR determination and on mean weekly acenocoumarol dosages. The risk of severe over-anticoagulation (defined as INR>6) at the time of the first INR

determination and the percentage of time spent below, within and above the target INR were also analysed.

Methods

Patients

The study cohort of North-Italian patients was prospectively recruited at two anticoagulant clinics in Milan: the Istituto Clinico Humanitas and San Paolo Hospital. Included were patients aged 18 years or more who started oral anticoagulant therapy with acenocoumarol for arterial or venous thrombosis or non-ischemic heart disease (atrial fibrillation and dilatative cardiopathy). Exclusion criteria were the presence of concomitant severe diseases known to interfere with this treatment (i.e. liver cirrhosis, uremia, malignant diseases). All patients started treatment with the same standard dosage of acenocoumarol during the first 3 days of therapy (4 mg, 4 mg, 2 mg), the first INR being scheduled on day 4 and subsequently once or twice weekly. Acenocoumarol dosing was based on a computerized system (Parma 4.1) which includes an algorithm that recommends the therapeutic dose [32]. Demographic data, indication for anticoagulant therapy, INR target range, concomitant disease, co-medications, diet (vegetarian or not) and bleeding episodes were collected. The daily acenocoumarol doses administered during the initial phase of treatment (arbitrarily set at 52 days, i.e., first 3 days plus 7 weeks) and INR values obtained during control visits at the clinic were also collected. All patients gave written informed consent for analysis of their DNA. The study protocol was approved by the Medical ethics Committee of the participating hospitals

Laboratory methods

Venous blood was collected in sodium citrate 0.105 M. Plasma and cells were separated after centrifugation at 4000 g for 30 min. DNA was isolated from leukocytes using the salting out method [33]. The INRs (International Normalized Ratio) were performed in all centers using the same reagent/instrument combination, namely, human recombinant thromboplastin (RecombiPlasTin, HemosILTM, INstrumentation Laboratory Company, USA), and a Electra 1600 coagulometer (IL, Milan, Italy). All the participating centers performed regular external quality control exercises.

Genotyping

The polymerase chain reactions and endonuclease digestions were used for the detection of the *CYP2C9**2 (c.430 C/T, rs1799853) and *2C9**3 (c.1075 A/T, rs1057910) variant alleles [34]. Three polymorphisms located at the *VKORC1* gene, i.e. c.173+1000 C/T (rs9934438) in intron 1 and c.492+134 G/A (rs7294) in the 3'UTR, both previously identified by D'Andrea *et al.*, [35] and c.173+525 C/T (rs17708472) were analysed. For the analysis of *VKORC1* c.173+1000 C/T a sense (5'-TGACATGGAATCCTGACGTG -3') and antisense (5'-GAGCTGACCAAGGGGAT-3') PCR primers and *HinfI* restriction enzyme (New England BioLabs) were used; for the c.492+134 G/A a sense (5'-ATGGAGTGTTCGGGAGGTG-3') and antisense (5'-ACAGTCCATGGCAGACACAT-3') PCR primers and *AciI* restriction enzyme (New England BioLabs), and for that of the c.173+525 C/T a sense (5'-CGTTAGCATAATGACGGAATACAG-3') and antisense (5'-AACTCCTGACTTCAAGTGATCCAT-3') PCR primers and *BfaI* restriction enzyme (New England BioLabs) were used, respectively.

The analysis of the combination of these polymorphisms allow to establish for each patient the presence of the *VKORC1**1 haplotype, (the putative ancestral

haplotype, characterized by the presence of the normal alleles: c.173+1000 C, c.492+134 G and c.173+525 C polymorphisms [22]), the VKORC1*2 haplotype (characterized by the presence of the variant allele c.173+1000 T and the normal alleles: c.492+134 G and c.173+525 C [22]), the VKORC1*3 haplotype (characterized by the presence of the variant allele c.492+134 A and the normal alleles: c.173+1000 C and c.173+525 C [22]), and the VKORC1*4 haplotype (characterized by the presence of the variant allele c.173+525 T and the normal alleles: c.173+1000 C and c.492+134 G [22]).

Statistical analysis

Continuous variables, expressed as mean (range) were used for age, body surface area (BSA), days of follow-up, INR range, INR values and administered daily doses. BSA was calculated according to the formula: $0.00718 \times \text{height}^{0.725} \times \text{weight}^{0.425}$ [36]. In each patient the doses of acenocoumarol, administered after the first 3 days of standard dosages (4 mg, 4mg, 2 mg), were calculated for weekly periods (day 4-10, day 11-17, day 18-24, day 25-31, day 32-38, day 39-45, day 45-53). In order to analyze the effect of age on dose requirement, age at the start of therapy was divided into seven 10-year categories, the lowest ranging from years 20 to 30 and the highest from years 80 to 90. Dummy variables were used to code for sex, clinical variables (indications for acenocoumarol, presence of concomitant diseases) and concomitant medications. In order to quantify how a single copy or two copies of each *CYP2C9* or *VKORC1* variant alleles affect acenocoumarol dose requirement (according to a additive allelic effect), dummy variable codes were established. As regards *CYP2C9*, 0 was assigned to carriers of two copies of the *CYP2C9**1 wild-type allele, 1 to carriers of one copy of the *CYP2C9**2 variant allele, 2 to carriers of two copies of the *CYP2C9**2 variant allele, 3 to carriers of one copy of the *CYP2C9**3 variant allele, 4 to carriers of both *CYP2C9**2 and

CYP2C9*3 variant alleles and 5 to carriers of two copies of the CYP2C9*3 variant allele. Patients were then divided into groups according to the presence of two wild-type alleles (code: 0), at least one CYP2C9*2 variant allele (heterozygous or homozygous, code: 1) or at least one CYP2C9*3 variant allele (heterozygous or homozygous, code: 2). Heterozygotes for the 2C9*2/*3 genotype were grouped only with patients carrying at least one 2C9*3 variant allele.

As regards *VKORC1*, 0 was assigned to carriers of two copies of the VKORC1*1 ancestral haplotype, 1 to carriers of one copy of the VKORC1*3 haplotype, 2 to carriers of one copy of the VKORC1*4 haplotype, 3 to carriers of two copies of the VKORC1*3 haplotype, 4 to carriers of both VKORC1*3 and VKORC1*4 haplotype, 5 to carriers of two copies of the VKORC1*4 haplotype, 6 to carriers of both VKORC1*2 and VKORC1*3 haplotype, 7 to carriers of both VKORC1*2 and VKORC1*4 haplotype, 8 to carriers of one copy of the VKORC1*2 haplotype and 9 to carriers of two copies of the VKORC1*2 haplotype. The ascending number of the dummy variable codes was assigned to each genotype according to the increased reported effect of the variant alleles on coumarin response [22]. Each group was compared with the wild-type group. Finally, patients were divided according to the possible *CYP2C9* and *VKORC1* genotype combinations.

In order to analyze the effect of *CYP2C9* variant alleles and *VKORC1* haplotypes on dose requirement during the first 7 weeks of therapy, only patients with target INR between 2.0 and 3.0 were considered, while patients with a different INR target were excluded from this analysis. To analyze the effect of *CYP2C9* variant alleles and *VKORC1* haplotypes on the first INR, only patients who had INR measured on day 4 of treatment were considered.

For comparison of the mean INR value on day 4 of therapy and the mean dose requirement during the first 7 weeks of therapy between single and combined

genotypes, one-way ANOVA tests with post-hoc multiple comparison adjustments (Bonferroni for equal variance or Dunnett T3 for non-equal variance) were performed. A multivariate linear regression model was used to assess the independent effect of genetic factors and other environmental variables on the first INR estimate and dose requirement. Relative risks (RR) with 95% confidence interval (CI) were calculated to compare the proportion of patients with INR>6 (severe over-anticoagulation) for single and combined genotypes. Finally, for each genotype combination, the percentage of time (days) spent within, above and below the INR target range during the first 7 weeks of therapy were evaluated using the linear interpolation method described by Rosendaal *et al.* [37]. One-way ANOVA tests with post-hoc multiple comparison adjustments (Bonferroni for equal variance or Dunnett T3 for non-equal variance) were performed to assess the differences in percentages between genotype combinations. 95% confidence intervals (CI) were used to indicate the significance of each test. All statistical analysis were performed using the SPSS software (version 11.5, SPSS Inc, , Chicago, III).

Results

Between 2003 and 2005, 220 Italian patients (all Caucasians) meeting the inclusion criteria were enrolled in the study. Patient characteristics are summarized in Table 1. Two hundred and one (91.4%) completed the scheduled follow-up of 53 days, whereas 19 did not complete follow up or stopped therapy for various reasons. Allele and genotype frequencies for the CYP2C9*2 and CYP2C9*3 variant alleles and for the different VKORC1 haplotypes are summarized in Table 2. None of the patients was homozygous for the reference VKORC1*1 haplotype.

Table 1. Characteristics of patients

Number of patients	220
Age, years [mean,(range)]	65 (23 – 87)
Gender, n (%)	
Male	133 (60.5)
Female	87 (39.5)
BSA, m² [mean, (range)]	1.84 (1.36-2.48)
Follow-up, days [median, (range)]	53 (6 - 53)
Indication for anticoagulant therapy, n (%):	
Arterial thrombosis*	39 (17.7)
Venous thrombosis**	57 (25.9)
Non-ischemic cardiac disease***	124 (56.4)
Target INR, n (%):	
1,8 – 2,2	1 (0.5)
1,8 – 2,5	1 (0.5)
2,0 – 2,5	2 (0.9)
2,0 – 3,0	187 (85.0)
2,5 – 3,5	28 (12.7)
2,5 – 5,0	1 (0.5)
Concomitant diseases, n (%):	
cardiovascular risk factors	73 (33.2)
history of arterial thrombosis	17 (7.7)
history of venous thrombosis	4 (1.8)
benign tumors	7 (3.2)
diseases associated with haemorrhagic risk	2 (0.9)
venous insufficiency of the lower limb	2 (0.9)
Concomitant medications, n (%):	
Drugs known to increase the INR	44 (20.0)
Drugs known to decrease the INR	17 (7.7)
Drugs with unknown effect on INR	109 (49.5)

* Arterial thrombosis includes acute coronary syndromes, stroke, TIA, peripheral arterial emboli

** Venous thrombosis includes deep vein thrombosis and pulmonary embolism

*** Non-ischemic cardiac disease includes atrial fibrillation and dilatative cardiomyopathy

Table 2. Genetic characteristics of patients

Polymorphism	Allele	%	Genotype	N	%
CYP2C9	*1	76.1	*1/*1	132	60.0
	*2	14.8	*1/*2	48	21.8
	*3	9.1	*1/*3	25	11.4
			*2/*2	6	2.7
			*3/*3	4	1.8
			*2/*3	5	2.3
VKORC1	*1	3.35	*1/*1	0	0
	*2	42.63	*1/*3	8	3.6
	*3	36.16	*1/*4	4	1.8
	*4	17.86	*3/*3 ^a	29	13.2
			*3/*4	32	14.5
			*4/*4 ^{d, e}	6	2.7
			*2/*3	60	27.3
			*2/*4	30	13.6
			*1/*2	3	1.4
			*2/*2 ^{b, c}	48	21.8

^a 1 patient was VKORC1 *3/*3 homozygous + *1/*4 heterozygous, ^b 1 patient was VKORC1 *2/*2 homozygous + *1/*3 heterozygous, ^c 1 patient was VKORC1 *2/*2 homozygous + *1/*4 heterozygous, ^d 2 patients were VKORC1 *4/*4 homozygous + *1/*2 heterozygous, ^e 3 patients were VKORC1 *4/*4 homozygous + *1/*3 heterozygous

Effect of single CYP2C9 alleles and VKORC1 haplotypes on the first INR value

This effect was evaluated only in patients (n=164, 74.5%) who had their INR measured on day 4 of treatment. Table 3 shows that carriers of at least one CYP2C9*3 variant allele had a higher mean first INR than CYP2C9*1/*1 wild-type patients, the highest INR being observed for the homozygous CYP2C9*3/*3 genotype [5.6; range: 3.2-10.0; 95%CI of difference vs wild-type patients: -7.68 - 2.14]. The presence of at least one CYP2C9*2 variant allele resulted in a higher INR than that of wild-type patients too.

As the reference VKORC1*1/*1 genotype was not represented, each VKORC1 genotype was compared to VKORC1*2 homozygotes, being the VKORC1*2 haplotype associated with the larger INR variation and the lower warfarin maintenance doses [38]. Homozygotes for the VKORC1*2 haplotype had the highest mean first INR compared to all the other genotypes (Table 3). Homozygotes for the VKORC1*3 haplotype had no difference in mean INR values on day 4 compared to homozygotes for the VKORC1*4 haplotype (2.6; range: 1.4-7.7 vs 2.4, range: 1.4-3.6; 95%CI of diff: -2.14 – 2.32). Heterozygous combinations of the VKORC1*3 or VKORC1*4 haplotypes seem to have at least equal impact on the INR value on day 4 as homozygosity for either VKORC1*3 or VKORC1*4. On the other hand, also VKORC1*1/*2 heterozygotes had higher mean first INR.

The risk of early over-anticoagulation (INR >6 on day 4) associated with the presence of variant alleles was evaluated in comparison to CYP2C9*1/*1 homozygotes and to homozygotes for the VKORC1*2 haplotype. Table 3 shows that carriers of at least one CYP2C9*2 or CYP2C9*3 variant allele had 3.39 and 2.81 times higher risks of over-anticoagulation. The highest risk (6.58 times) was found for CYP2C9*3/*3 homozygotes (95%CI: 0.93 - 45.45). The homozygous VKORC1*2/*2 genotype was characterized by the highest number of INR>6 on day 4 (Table 3).

Table 3. Relative risk (RR) and 95% confidence intervals (CI) of INR>6 on day 4 of acenocoumarol therapy in carriers of variant *CYP2C9* alleles or *VKORC1* haplotypes. References were patients with the *CYP2C9* wild-type genotype and those homozygous for the *VKORC1**2 haplotype, respectively.

	n° of INR at day 4	mean INR on day 4 (range)	p (2-tailed)	[95% CI]	n° INR>6 on day 4	RR [95% CI]
CYP2C9	*1/*1	105	2.9 (1.0 - 6.8)	1.0 (ref)	4	1.0 (ref)
	*2/-	31	3.3 (1.5 - 7.8)	0.42	4	3.39 [0.90 – 12.82]
	*3/-	28	3.7 (1.2 – 10.0)	0.079	3	2.81 [0.67 – 11.90]
VKORC1	*2/*2	32	4.5 (2.2 – 10.0)	1.0 (ref)	7	1.0 (ref)
	*3/*3	21	2.6 (1.4 - 7.7)	0.002	1	0.22 [0.03 – 1.64]
	*4/*4	5	2.3 (1.4 - 3.6)	0.037	0	no risk estimate
	*1/*2	2	3.8 (3.3 - 4.3)	0.97	0	no risk estimate
	*1/*3	7	2.8 (1.1 - 5.9)	0.53	0	no risk estimate
	*1/*4	4	2.2 (1.7 - 3.0)	0.008	0	no risk estimate
	*2/*3	44	2.9 (1.0 - 6.9)	0.003	1	0.10 [0.01 – 0.80]
	*2/*4	26	3.1 (1.0 - 6.7)	0.048	2	0.35 [0.08 – 1.55]
	*3/*4	23	2.4 (1.0 - 4.9)	0.000	0	no risk estimate

Table 4: Mean weekly dose (SD) and 95% CI of acenocoumarol in carriers of variant CYP2C9 or VKORC1.

		Week 1 (days 4-10)	Week 2 (days 11-17)	Week 3 (days 18-24)	Week 4 (days 25-31)	Week 5 (days 32-38)	Week 6 (days 39-45)	Week 7 (days 46-53)
CYP2C9	*1/*1 n=116	mean mg/week (SD) 95% CI	14.3 (6.8) 0 (ref)	14.6 (6.4) 0 (ref)	15.4 (7.2) 0 (ref)	15.7 (7.5) 0 (ref)	15.9 (7.9) 0 (ref)	19.0 (9.1) 0 (ref)
	*2/-	mean mg/week (SD)	13.0 (5.4)	14.1 (6.5)	13.6 (7.6)	13.5 (7.1)	13.8 (7.4)	15.8 (8.6)
	n=45	95% CI	-1.2-3.8	-2.1-3.2	-1.3-4.8	-0.9-5.3	-1.3-5.1	-1.2-5.3
	*3/-	mean mg/week (SD)	11.2 (4.6)	11.0 (5.0)	11.5 (4.3)	11.1 (4.0)	11.4 (4.5)	14.1 (5.3)
	n=26	95% CI	0.4-5.9	0.3-6.9	0.1-7.6	0.8-8.3	0.2-8.2	0.3-8.4
								0.2-9.5
VKORC1	*1/*2 n=3	mean mg/week (SD) 95% CI	10.7 (4.6) -27.9-25.5	11.0 (3.6) -20.2-16.7	17.0 (17.4) -20.4-5.7	11.0 (5.3) -30.9-27.9	10.7 (4.6) -25.7-23.8	12.0 (5.2) -31.1-26.1
	*1/*3 n=7	mean mg/week (SD) 95% CI	11.5 (7.0) -14.8-10.6	13.7 (9.1) -20.9-12.1	15.5 (9.1) -14.8-3.0	15.1 (8.5) -20.9-9.7	15.8 (9.0) -22.3-10.1	16.3 (9.6) -24.3-10.6
	*1/*4 n=3	mean mg/week (SD) 95% CI	14.7 (3.1) -21.6-11.2	18.0 (4.2) -97.6-80.1	19.0 (7.1) -25.2-6.4	18.5 (5.0) -117.8-99.8	16.0 (1.4) -16.7-4.1	15.5 (0.7) 2.2-9.8
	*2/*2 N=41	mean mg/week (SD) 95% CI	9.5 (3.3) 0 (ref)	9.3 (4.7) 0 (ref)	9.6 (4.9) 0 (ref)	9.5 (4.8) 0 (ref)	9.7 (5.0) 0 (ref)	9.5 (4.9) 0 (ref)
	*3/*3 n=26	mean mg/week (SD) 95% CI	16.0 (5.9) 2.2-10.8	16.0 (5.7) 2.2-11.2	16.4 (6.9) 1.32-12.2	17.5 (7.9) 2.0-14.0	18.2 (8.6) 4.1-16.0	22.5 (9.7) 2.1-15.4
	*4/*4 n=6	mean mg/week (SD) 95% CI	15.8 (3.2) -12.9-0.1	17.3 (3.4) 1.1-15.0	15.5 (4.8) -15.4-3.7	15.0 (5.4) -16.6-5.6	16.2 (5.1) -17.6-4.3	18.6 (6.4) -23.2-8.7
	*2/*3 n=48	mean mg/week (SD) 95% CI	14.6 (7.5) 1.2-9.1	15.4 (6.6) 2.2-10.1	15.9 (7.4) 1.6-10.9	16.2 (7.3) 2.4-11.1	15.9 (7.4) 1.8-10.6	16.2 (7.7) 2.1-11.3
	*2/*4 n=26	mean mg/week (SD) 95% CI	14.0 (6.0) 0.6-8.9	14.4 (5.9) 0.4-9.8	14.3 (6.1) -10.2-1.0	14.0 (6.6) -9.8-0.8	14.0 (7.0) -9.9-1.4	14.4 (7.2) -10.7-0.9
	*3/*4 n=27	mean mg/week (SD) 95% CI	15.5 (6.2) 1.6-10.5	15.8 (5.7) 2.1-11.0	16.3 (6.3) 1.2-12.1	16.8 (6.5) 2.2-12.5	17.0 (6.7) 1.9-12.6	17.1 (6.6) 2.3-13.0
								1.8-14.8

Sex, indication for therapy, concomitant diseases, medications and BSA did not affect the first INR. Age at start of therapy was positively associated with the first INR, but was not a confounding factor for the association between genotype and first INR. Multiple linear regression analysis showed that both *CYP2C9* and *VKORC1* genotypes separately influenced the first INR value on day 4 of therapy and that, together with age, they explain 26% of the observed INR variability.

Effect of single CYP2C9 alleles and VKORC1 haplotypes on dose requirement during the first 7 weeks of therapy

This effect was evaluated in 187 patients (85%, only patients with a target INR=2.0-3.0). Table 4 shows that carriership of at least one *CYP2C9**2 allele was associated with a lower mean weekly dose compared to patients carrying only the wild-type allele. The decrease varied from 9% in the first week (1.3 mg) to 17% in the 7th week (3.13 mg). Carriership of at least one *CYP2C9**3 variant allele decreased to a greater degree than *CYP2C9**2 the weekly dose compared to wild-type patients, i.e. from 22% in the first week of therapy (3.1 mg) to 25% in the 7th week (4.8 mg).

Homozygosity for the *VKORC1**2 haplotype was associated with a lower mean weekly dose compared to the other *VKORC1* genotypes, particularly in comparison with carriers of the homozygous *VKORC1**3 haplotype, the decrease varying from 41% in the first week (6.5 mg) to 49% in the 7th week (11.1 mg), and with carriers of the homozygous *VKORC1**4 haplotype, the decrease being around 40% all along the first seven weeks (~ 7 mg). Homozygotes for the *VKORC1**3 and *VKORC1**4 haplotypes showed no differences in the required mean weekly doses [95% CI: -22.59 – 29.92 at week 7]. All the other *VKORC1* genotypes required mean weekly doses in the range between those required by

VKORC1*2/*2 and VKORC1*3/*3.

Concomitant medications and diseases were not associated with dose requirement, whereas BSA, age, sex and indication for therapy were associated factors but not confounders for the association between genotype and dose requirement. Multiple linear regression analysis showed that *CYP2C9* and *VKORC1* genotypes, together with sex, BSA, age at the start of therapy and indication for therapy, accounted for 20% of the observed variability in dose requirement in the first week of therapy. The influence of these factors increased over the following weeks and in the 7th week they accounted for 27% of the variability. The major role on variability was exerted by *VKORC1*, that in the 7th week of therapy accounted for 12% of it, followed by *CYP2C9* genotype (5%).

Effect of CYP2C9 and VKORC1 combinations on the first INR value

Only patients homozygous for the VKORC1*2, VKORC1*3 and VKORC1*4 haplotypes were considered. As VKORC1*3 and VKORC1*4 haplotypes showed no differences in the first INR and dose requirement, homozygous carriers of these haplotypes were combined. In each of the VKORC1*2 and VKORC1*3 or *4 groups, the effect of the *CYP2C9**2 and *3 variant alleles were investigated.

Table 5. Frequency of the combinations between homozygous VKORC1*2 and VKORC1*3 or VKORC1*4 haplotypes and *CYP2C9* alleles in the whole cohort of patients.

GENOTYPE COMBINATION	N of patients from the whole cohort	%	N of patients with target INR of 2.0-3.0	%
VKORC1*2 + CYP2C9*1	31	37.4	27	37
VKORC1*2 + CYP2C9*2	11	13.3	9	12.3
VKORC1*2 + CYP2C9*3	6	7.2	5	6.8
VKORC1*3 or *4 + CYP2C9*1	19	22.9	18	24.7
VKORC1*3 or *4 + CYP2C9*2	9	10.8	7	9.6
VKORC1*3 or *4 + CYP2C9*3	7	8.4	7	9.6
TOTAL	83	100.0	73	100.0

The frequency of each genotype combination in the whole cohort of patients is shown in Table 5.

Figure 1 shows the first INR in patients with different *VKORC1* and *CYP2C9* genotype combinations. Among carriers of *VKORC1**2, associated with the highest mean INR on day 4, the presence of the *CYP2C9**3 variant allele, but not that of the *CYP2C9**2 variant allele, increased the mean INR on day 4 compared to carriers of the *CYP2C9**1 wild-type allele [5.9; range: 3.6–10.0 vs 4.1; range: 2.2–6.6; 95%CI of diff: -0.36 – 3.99]. The *CYP2C9**3 variant allele was also associated with a 5 times increased risk of INR>6.0 [95%CI: 1.07–23.26] compared to the *CYP2C9**1 wild-type allele.

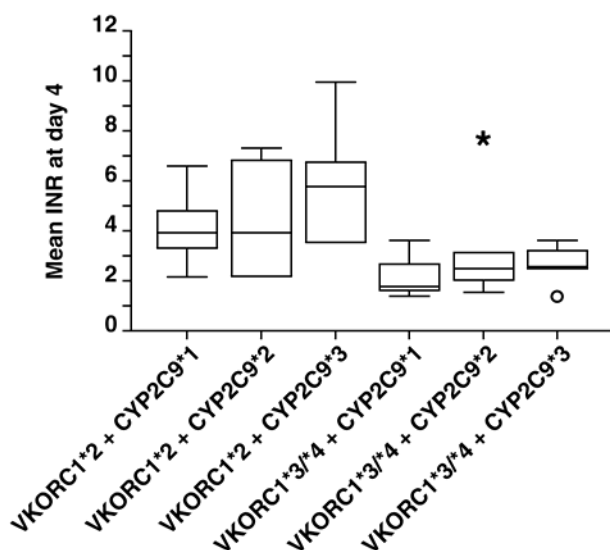


Figure 1. Box plot of first INR values on day 4 of acenocoumarol and therapy in patients with different *CYP2C9* and *VKORC1* genotype combinations.

Among carriers of *VKORC1**3 or *VKORC1**4, the presence of both the *CYP2C9**2 and *CYP2C9**3 variant alleles did not affect the mean INR on day 4 compared to carriers of the *CYP2C9**1 wild-type allele [3.3; range: 1.5–7.8 vs 2.2;

range: 1.4-3.6; 95%CI of diff: -1.18 – 3.34 and 2.7; range: 1.4-3.6 vs 2.2; range: 1.4-3.6; 95%CI of diff: -1.92 – 2.91, respectively].

Effect of CYP2C9 and VKORC1 combinations on weekly dose requirement

Weekly dose requirement was compared only in patients with target INR of 2.0 to 3.0 (n=73). The doses of acenocoumarol required by patients with different *CYP2C9* and *VKORC1* genotype combinations in the first 7 weeks of treatment are shown in Figure 2.

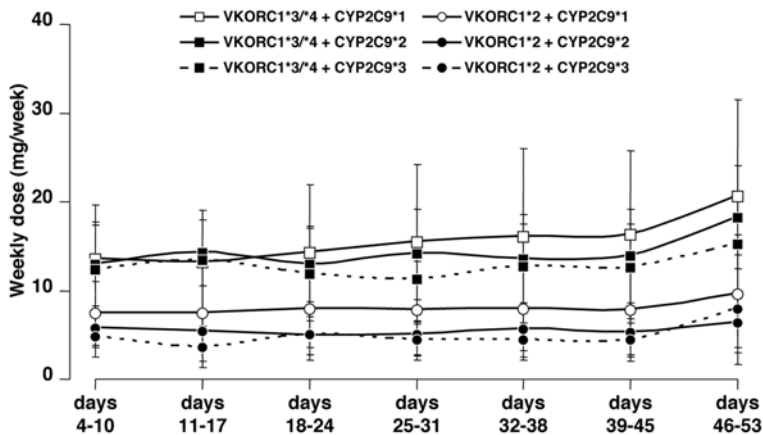


Figure 2. Mean acenocoumarol doses (mg/week, $\pm$ SD) required by patients with different genotype combinations during the first 7 weeks of therapy

Among carriers of *VKORC1**2, associated with the lowest mean weekly dose requirement, the presence of the *CYP2C9**2 variant allele decreased the mean dose required during the first weeks of therapy compared to carriers of *CYP2C9**1. The extent of the reduction ranged from 18% (1.7 mg, 95%CI of diff: -1.48 – 4.89) during the 1st week of therapy to 27% (3.3 mg, 95%CI of diff: -1.95 – 8.51) during the 7th week of therapy. The presence of the *CYP2C9**3 variant allele also decreased the required mean weekly dose compared to *CYP2C9**1. The decrease

ranged from 25% (2.6 mg, 95%CI of diff: -2.25 – 7.35) during the 1st week of therapy to 14% (1.8 mg, 95%CI of diff: -11.2 – 14.78) during the 7th week of therapy.

Among carriers of VKORC1*3 or VKORC1*4, associated with the highest mean weekly dose requirement, the presence of the CYP2C9*2, compared to carriers of CYP2C9*1, decreased the dose requirement from 3.4% (0.6 mg, 95%CI of diff: -7.25 – 8.46) during the 1st week of therapy to 12% (2.4 mg, 95%CI of diff: -10.68 – 15.43) during the 7th week of therapy. The presence of the CYP2C9*3 variant allele also decreased the mean weekly dose required from 7.4% (2.6 mg, 95%CI of diff: -6.98 – 9.33) during the 1st week of therapy to 23% (5.4 mg, 95%CI of diff: -6.48 – 17.2) during the 7th week of therapy.

In all, carriers of both the VKORC1*3 or *4 allele and the CYP2C9*1 wild-type allele had the lowest mean INR on day 4 of therapy and required the highest mean weekly dose during the first weeks of therapy (Figure 1 and 2), while carriers of both VKORC1*2 and CYP2C9*3 alleles had the highest mean INR on day 4 and required the lowest mean weekly dose during the first weeks of therapy. Compared to the former, they had roughly 3 times higher mean INR [5.9 vs 2.2; 95%CI of diff.: 1.48 – 5.99], and required an average 55% lower mean weekly dose (~ 10.5 mg) of acenocoumarol [95%CI of diff.: 2.89 – 14.545 at week 1; -0.83 – 26.38 at week 7].

Effect of CYP2C9 and VKORC1 combinations on the percentage of time spent within, below or above the INR target range

The mean percentages of time spent within, above and below the INR target range were evaluated for each genotype combination. Table 6 shows that carriers of all the combinations spent approximately 50% of the initial phase of treatment in the

target range. However, among carriers of VKORC1*2, carriers of the CYP2C9*3 variant allele spent a higher percentage of time above their target range than carriers of CYP2C9*1 allele and a smaller percentage of time below it. Among carriers of VKORC1*3 or *4, the presence of the CYP2C9*2 or CYP2C9*3 variant alleles had the same effect, increasing the mean percentage of time spent above their target range compared to carriers of the CYP2C9*1 allele and decreasing the percentage of time spent below it.

Table 6. Mean and range of the percentage of time spent in, below or above the INR target range by carriers of different *VKORC1* and *CYP2C9* genotype combinations. The means, the p-value and 95% confidence intervals (CI) were evaluated taking as references the VKORC1*2 + CYP2C9*1 and the VKORC1*3 or *4 + CYP2C9*1 genotype combinations, respectively.

		% days spent in the INR target range	% days spent below the INR target range	% days spent above the INR target range
VKORC1*2 + CYP2C9*1 n=31	Mean	48.8	19.0	32.3
	Range	20 - 92	0 - 62	0 - 73
	p; [95%CI]	ref	ref	ref
VKORC1*2 + CYP2C9*2 n=11	Mean	47.7	22.6	29.7
	Range	20 - 70	3 - 58	0 - 74
	p; [95%CI]	1.00; [-18.82 - 21.03]	1.0; [-25.79 - 18.48]	1.0 [-21.98 - 27.09]
VKORC1*2 + CYP2C9*3 n=6	Mean	49.1	9.0	41.9
	Range	15 - 85	0 - 25	5 - 85
	p; [95%CI]	1.00 [-41.98 - 41.35]	0.67 [-9.87 - 29.78]	1.00 [-40.83 - 21.54]
VKORC1*3 or *4 + CYP2C9*1 n=19	Mean	50.0	41.5	8.5
	Range	0 - 85	0 - 100	0 - 50
	p; [95%CI]	ref	ref	ref
VKORC1*3 or *4 + CYP2C9*2 n=9	Mean	58.3	21.0	20.8
	Range	4 - 100	0 - 62	0 - 96
	p; [95%CI]	0.99 [-45.74 - 29.31]	0.4 [-10.42 - 51.47]	1.0 [-40.60 - 15.98]
VKORC1*3 or *4 + CYP2C9*3 n=7	Mean (SD)	54.9	24.6	20.4
	Range	38 - 78	3 - 58	0 - 51
	p; [95%CI]	1.0 [-27.72 - 17.90]	0.67 [-15.09 - 48.80]	1.0 [-42.86 - 18.96]

Finally, compared to carriers of the *VKORC1**3 or *4 plus *CYP2C9**1 alleles combination, a lower percentage of time spent below the target range was found in carriers of the *VKORC1**2 plus *CYP2C9**3 combination (9.0%, range: 0-25 vs 41.5, range: 1-100; 95%CI of diff: 8.4 – 56.6). They also spent a higher percentage of time above the target range [41.9%; range:5–85 vs 8.5, range: 0-50; 95%CI of diff: 0.70 – 66.12], in accordance with the correspondingly lower acenocoumarol doses required during the same period and the higher mean INR on day 4.

Exclusion of patients taking concomitant medications known to increase (n= 44) or decrease (n= 17) the INR did not significantly change the observed effect of *VKORC1* and *CYP2C9*. The outcomes of the effect of the *VKORC1* and *CYP2C9* genotype combinations on dose requirement, as well as their effect on the first INR value, the risk of severe over-anticoagulation and the percentage of time spent within, below or above the INR target range remained the same, with no loss of statistical significance. The results of the single *CYP2C9* variant alleles and *VKORC1* haplotypes on the risk of severe over-anticoagulation also remained the same. For the outcomes of the effect of single *CYP2C9* variant alleles on dose requirement, statistical significance for the difference between carriers of at least one *CYP2C9**3 variant allele compared to carriers of only wild-type allele was lost (95%CI: -1.09 – 6.23 at week 1; 95%CI: -0.17 – 1.09 at week 7). For the outcomes of the effect of single *VKORC1* haplotypes on the first INR value, significance for the difference between carriers of the homozygous *VKORC1**4/*4 haplotype compared to carriers of the homozygous *VKORC1**2/*2 haplotype was lost (95%CI: -0.17 – 4.28). The same occurred for carriers of the heterozygous *VKORC1**2/*4 haplotypes (95%CI: -0.60 – 3.33).

Discussion

This study was planned and performed to evaluate the effect of all the common gene variants, previously identified as modifiers of oral anticoagulant therapy with vit.K antagonists, on the initial phase of administration of acenocoumarol, a drug that was studied so far less than warfarin in terms of pharmacogenetics. The initial phase of treatment was chosen as the most critical and unstable, thereby engendering a risk of both over-anticoagulation (and hence of bleeding) and under-coagulation (and of thrombosis).

The first finding of this study is that, pertaining to the effect of the *CYP2C9* gene, only the *CYP2C9**3 variant allele plays an important modifying role during the first 7 weeks of treatment, because its presence significantly reduces the required dose of acenocoumarol and increases the risk of over-anticoagulation (INR>6) on day 4 of treatment, particularly for homozygotes[12-17]. This result is in agreement with Bodin *et al.*, [28] and Schalekamp *et al.*, [29], the latter being the only study that analysed the effect of both *VKORC1* and *CYP2C9* genotypes during the initial phase of acenocoumarol treatment. However, our finding is in contrast with González-Conejero *et al.*, [30], who reported that *CYP2C9* polymorphisms have no effect on the first INR value after 3 days of acenocoumarol therapy. This discrepancy might be due to the fact that González-Conejero *et al.*, performed the analysis combining together *CYP2C9**2 and *CYP2C9**1 carriers.

Pertaining to the *VKORC1* gene, the presence of the ancestral *VKORC1**1 haplotype [22] was found at a low frequency in our Italian population, in contrast with Geisen *et al.*, [22] and Osman *et al.*, [38], who showed that this haplotype is absent in European populations. Interestingly, a rare *VKORC1* mutation, p.D36Y, previously identified in individuals who required an average warfarin dose greater than 10 mg/day [39], was recently reported to tag a unique haplotype found on a *VKORC1**1 background in Ashkenazi and Sephardi Jews [40]. Replication studies

on other Caucasian populations are thus required to establish the real frequency of this allele.

Homozygotes for the VKORC1*2 haplotype required the lowest acenocoumarol doses and had a 4.5 times increased risk of over-anticoagulation (INR>6) on day 4 compared to homozygotes for the VKORC1*3 or VKORC1*4 haplotypes. Both these haplotypes had a similar effect on INR and dose requirements, being associated with higher acenocoumarol doses and lower INR on day 4. This confirms the findings of Osman *et al.*, [38] in warfarin-treated patients. The VKORC1*2 haplotype is characterized by the presence of the c.173+1000 C/T polymorphism, which is in complete linkage disequilibrium with the promoter polymorphism c.-1639G/A, that re-establishes a E-box consensus sequence reducing the promoter activity and thus VKORC1 mRNA expression [41]. The VKORC1*3 haplotype is characterized by the presence of the c.492+134 G/A polymorphism, first identified by D'Andrea *et al.*, [35] in Italian patients on warfarin therapy, and then described by Geisen *et al.* [22] to be associated with a partial resistance to warfarin. As expected from the effect of single alleles, the highest dose requirement and the lowest INR on day 4 were found for the homozygous VKORC1*3 or *4 plus CYP2C9*1 genotype combination, whereas the lowest doses, the highest INR as well as an increased risk of over-anticoagulation were for the VKORC1*2 plus CYP2C9*3 genotype combination. Moreover, in the initial phase of therapy patients carrying all the investigated CYP2C9 and VKORC1 combinations spent the same amount of time in the INR therapeutic range. Carriers of VKORC1*3 or *4 plus CYP2C9*1, associated with a greater resistance to acenocoumarol, spent a large percentage of the remaining time below the target range, whereas carriers of the VKORC1*2 plus CYP2C9*3 combination, associated with a higher sensitivity to the drug, spent a greater amount of the remaining time above the target range. This is in agreement with Schalekamp

et al., [29], who reported that only carriers of a combination of *CYP2C9* and *VKORC1* C1173T polymorphisms have a high risk of over-anticoagulation.

Therefore, based on the obtained results we could conclude that it may be necessary to test not only the *CYP2C9* genotype (particularly the *CYP2C9**3 variant allele) and the *VKORC1* c.173+1000 C/T polymorphism (*VKORC1**2 haplotype), but also the *VKORC1* c.492+134 G/A (*VKORC1**3) and the c.173+525 C/T (*VKORC1**4) to identify patients with the extreme phenotypes: those with a higher sensitivity to acenocoumarol and more exposed to the risk of over-anticoagulation (bleeding); those with a greater resistance to the drug are more exposed to the risk of under-anticoagulation (thrombosis), particularly in the initial phase of treatment.

To our knowledge this is the first work that take into consideration the effect of the *VKORC1**3 and *VKORC1**4 haplotypes other than that of *VKORC1**2 in the initial phase of acenocoumarol therapy.

The *CYP2C9* and the *VKORC1* genotypes, together with age, explain 26% of the observed INR variability. They also account -- together with sex, BSA, age and indications for non-ischemic cardiac disease or venous thrombosis -- for 27% of the variability in dose requirement observed on the 7th week of therapy. In both situations, our results confirm that a major role is exerted by *VKORC1*, followed by *CYP2C9* genotype [23, 28-29]. The variability explained in this study is lower than the variability explained in the warfarin response [42]. These differences might be due to different study design and all variants included in the model, as well as to other unknown genetic and non-genetic variables not considered in this study.

Our study also included also some patients treated with other medications known to interfere with the effect of the anticoagulant drug, with the possibility to affect the results and this seems to be a limit of the study design. However, the regression analysis adjusted for these risk factors did not change the effect of the

CYP2C9 and *VKORC1* genotypes. The same study design was also used in previous reported studies [15, 29, 31, 43-45]. Moreover, the effect of *CYP2C9* and *VKORC1* genotypes was also confirmed after the exclusion of these patients from the analysis.

A limit of the study is that our cohort includes also some patients treated with other medications known to interfere with the effect of the anticoagulant drug, with the possibility to affect the results. However, the same study design was used in other studies [15, 29, 31, 43-45] and the effect of *CYP2C9* and *VKORC1* genotypes was also confirmed after the exclusion of these patients from the analysis.

In conclusion, both the detection of the *VKORC1**2, *3 and *4 haplotypes as well as the *CYP2C9**3 variant allele might be useful to select not only the most sensitive patients, exposed to a higher risk of over-anticoagulation, but also the most resistant ones, exposed to the risk of thrombosis recurrence. On August 2007, the USA Federal Drug Administration updated the label of warfarin to include information on pharmacogenetic testing and to encourage the use of this information in dosing individual patients initiating warfarin therapy [46]. Up to now, several dosing pharmacogenetic algorithms to predict *a priori* the maintenance coumarin dose [23-25, 47-53], as well as a new dose-refinement nomogram to guide clinicians in adjusting warfarin dose [54] have been developed. A randomized study of personalized, pharmacogenetic-guided warfarin dosing using both *CYP2C9* and *VKORC1* variants plus clinical factors in 200 patients was recently published [55], showing an improvement in the accuracy and efficiency of warfarin dose initiation, particularly for wild-type and carriers of multiple variant genotypes. Despite this, a reduction in out-of-range INRs was not achieved.

Future perspective

Pharmacogenetics results can be useful to identify patients more sensitive to coumarin drugs, and thus exposed to a higher risk of over-anticoagulation, as well as those who are more resistant to anticoagulation, and thus exposed to the risk of recurrence of thrombosis. The improvement and validation, by means of prospective clinical trials, of current pharmacogenetic-guided coumarin dosing algorithms, might help to predict the dose requirement of anticoagulant of coumarin type in order to aid clinicians to initiate therapy avoiding adverse events. This issue will be tackled by a prospective double-blind, randomized three-arm trial on 1.965 participants, sponsored by the National Heart, Lung and Blood Institute and meant to compare three possible approaches to guiding warfarin therapy initiation: a strategy based on an algorithm using both clinical and genotypic information on genetic variants in at least CYP2C9 and VKORC1 genes, a strategy based on an algorithm using only clinical information and a standard, guidelinebased strategy [56].

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CHAPTER 5

Prediction of Haemorrhagic and Thrombotic Events in Patients with Mechanical Heart Valve Prostheses Treated with Oral Anticoagulants

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Abstract

Background Variability in the intensity of anticoagulant therapy is considered a risk factor for complications, but it is unclear how best to quantify variability.

Objective We evaluated the association of three methods to measure variability with complications of oral anticoagulant therapy.

Methods We conducted a nested case-control study within a cohort of patients with prosthetic heart valves. 210 patients with a first haemorrhagic or thrombotic event during follow-up were selected with two controls per case, matched on age and sex. We calculated the time spent at an INR below, above and between 2.5 and 4.0; the variance growth rate according to three different methods (A, B1, B2), of which method A combines variability and time in range and methods B1 and B2 purely look at variability.

Results Odds ratios of the variance growth rates for thrombotic events for patients in the 2nd and 3rd tertile varied between 2 and 3, with the highest odds ratio for complications for the method that purely looked at variability. For haemorrhagic complications highest odds ratios were found for measure A which also incorporated time in range, with ORs of 2.6 (CI95 1.3-5.1) and 3.1 (CI95 1.6-6.0) for the 2nd and 3rd tertile as compared to the first. The combination of time spent out of range with the highest tertile of variability increased the risk 2.6-fold (CI95 1.6-4.2) as compared to subjects with stable anticoagulation within the target range.

Conclusion Unstable anticoagulation was associated with haemorrhagic and thrombotic complications. Method A was best associated with complications, but methods B1 and B2, in combination with time spent in range were equally well associated. As we prefer to disentangle variability and intensity of anticoagulation, we propose to use methods B1 or B2 to reflect pure variability of oral anticoagulant therapy.

Introduction

Oral anticoagulants are among the most frequently prescribed drugs worldwide. They are proven to be effective in the treatment of venous thrombosis as well as for prevention of both venous and arterial thrombotic events in patients who are at increased risk[1-3]. While oral anticoagulants decrease the risk for a thrombotic event by inhibiting coagulation, through the same mechanism they increase the risk of severe or even fatal haemorrhage. Approximately 1% of patients will suffer from such a complication of oral anticoagulant therapy per year [4,5].

Given the severity of these adverse events it is of great importance to identify patients who are at highest risk to experience such an event so that precautions can be made. Several studies investigated potential risk factors for haemorrhagic complications, such as increased age, indication for anticoagulant therapy and the use of interacting medication [6-8]. Variability in the intensity of anticoagulant therapy (expressed as the International Normalized Ratio (INR)), as first described by Fihn and colleagues, is considered a risk factor for developing haemorrhagic or thrombotic complications [9]. They investigated whether the variance growth rate was associated with haemorrhagic events in a retrospective cohort of patients using oral anticoagulants for various indications. This variance growth rate is a measure of the time weighted variance of the INR around the target INR and reflects the degree to which a patient's achieved INR deviates from his or her target INR over a prolonged interval. Fihn and colleagues found that patients with a variance growth rate in the highest tertile had a 1.6 times higher risk of experiencing a haemorrhagic event compared to patients in the lowest tertile. Cannegieter radically modified the formula of Fihn and used the degree to which a patient's INR deviates from the previous one, and this variance growth rate does not depend on the target INR [10]. With this formula a patient is defined stable if his or her INRs are around the same value every time, even if this means that, for

example, the INR is constantly above the upper limit of the therapeutic range. Later, Fihn described a third method to calculate the variability in INR [11]. This variance growth rate is approximately the same as the method of Cannegieter, with small differences in the denominator. Variability of the INR, calculated with any of these methods has been shown to be associated with an increased risk of developing a haemorrhagic event with odds ratios varying from 1.6 to 3.0 [9-11], but it is not clear which one is best associated with such events.

Another method to assess the quality of the anticoagulant treatment is the linear interpolation method, which reflects the time spent within the target range, rather than the variability [9,12]. It has been shown that patients who spent less than 45% of the time inside the therapeutic range have a relative risk of 2.8 (95% CI 1.9-4.3) for developing an adverse event compared to patients who spent over 65% in range [13].

In this study we evaluated the three variability measures to assess which one is most associated with the occurrence of a haemorrhagic or a thrombotic event in patients with mechanical heart valve prostheses. We included the time spent in therapeutic range in our analyses since this is the established method in literature to reflect the quality of oral anticoagulant therapy.

Patients and Methods

Patients were participants of the LAVA study, a large cohort study consisting of all patients with mechanical heart valve prosthesis treated in four regional anticoagulation clinics between 1985 and 1993 [14]. The main objective of this study in 1608 patients was to determine the optimal level of anticoagulation for this indication. For all patients the following data were collected: date of birth, sex, all INR measurements with corresponding date, all hospital admissions and deaths. Additional information concerning the hospital admissions were collected from

hospital or general practitioners files. Outcome events were all thrombotic and haemorrhagic events during follow up with cerebral infarction defined as a neurologic deficit of sudden onset documented by CT-scanning (presence of infarction or absence of haemorrhage) or autopsy, peripheral emboli as acute peripheral ischemia proven by angiography, operation or autopsy, valve thrombosis defined as valve impairment by deposition of thrombus on the valve, proven at operation or autopsy, intracranial and spinal haemorrhage defined as neurologic deficit of sudden or subacute onset, confirmed by CT-scanning, surgery or autopsy, and major extracranial haemorrhage as an acute haemorrhagic event that led to death or to hospital admission for treatment of the haemorrhage as the most important reason for hospitalization (haemorrhage that led to hospital admission for diagnostic procedures only was not considered major). Out-hospital haemorrhages of all possible origin, i.e. also traumatic, were included. All strokes that could not be categorized as haemorrhagic or ischemic were designated “unclassified strokes”.

We conducted a nested case-control study within the LAVA cohort. Cases were all patients who experienced a first thrombotic or haemorrhagic event during follow-up. For each case two control persons were selected who were free of a haemorrhagic or thrombotic event at the time of the event of the case, but who were at risk, i.e., they were using oral anticoagulants at that date. For control subjects the index-date was the date of the INR measurement closest to the event date of the case. Control patients were matched on age and sex. A control could be selected more than once or become a case at a later date.

For each patient we calculated the variability of the INR according to three different methods; the variance growth rate by Fihn, the variance growth rate by Cannegieter and the variance growth rate again by Fihn. The formulas used to determine these measures are shown in box 1.

Box 1. Formulas of the variability measures

Variance growth rate Fihn (method A)

$$\sigma^2 = \frac{1}{n} \sum_{i=1}^n \frac{(\text{INR}_i - \text{target}_i)^2}{\tau_i}$$

Variance growth rate Cannegieter (method B1)

$$\sigma^2 = \frac{1}{n} \sum_{i=1}^n \frac{(\text{INR}_{i+1} - \text{INR}_i)^2}{\tau_{i,i+1}}$$

Variance growth rate Fihn (method B2)

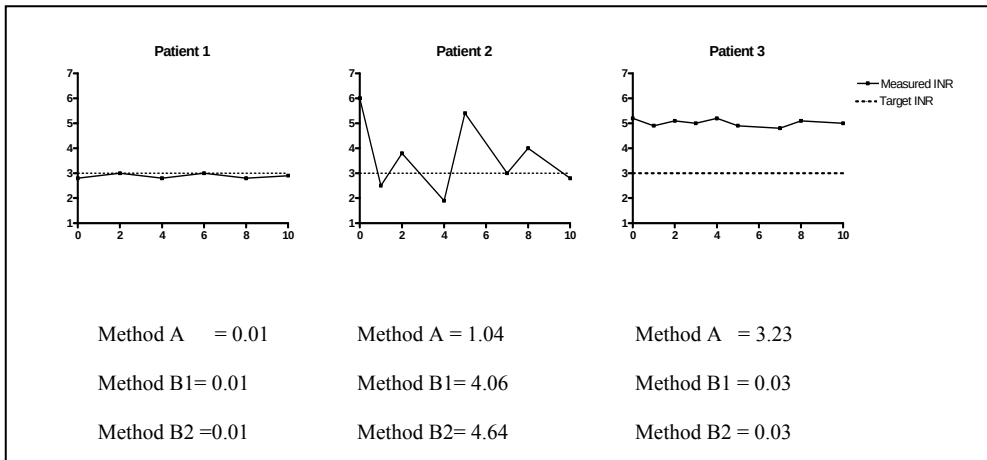
$$\sigma^2 = \frac{1}{n-1} \sum_{i=1}^n \frac{(\text{INR}_{i+1} - \text{INR}_i)^2}{\tau_i}$$

*n is the number of INR measurements, τ is the time in weeks between the present and previous INR measurement.

The variance growth rate by Fihn reflects the degree to which a patient's INR deviates from his or her target INR over a prolonged period. Using this formula for variability, a patient is most stable when his or her INRs are close to the target INR. We will refer to this method as method A from now on. The variance growth rate by Cannegieter reflects the degree to which a patient's INR deviates from the previous one. This formula is a reflection of the true variability not taking into account the intensity of anticoagulation. With this formula a patient is most stable if his or her INRs are around the same level even if this means the INR is constantly above the upper limit of the target range. The 2nd variance growth rate by Fihn is approximately the same as the formula used by Cannegieter, with minor differences in the denominator. Given the minor differences between the formula of Cannegieter and the 2nd formula of Fihn, we will refer to these methods as method B1 and method B2 respectively. Examples of the three measures of variability are given in box 2. Time in, above and below an INR range of 2.5-4.0 was calculated using the linear interpolation method [12]. With this method the

time between two measurements is divided in days and it assumes that the INR value between two measurements will vary linearly from the first, to the value of the second measurement. This way we can calculate how many days were spent at different INR values. Although the target range at the time of the study (1985-1993) was an INR between 3.6 and 4.8 we considered the actual optimal INR range as was determined in 1996; i.e. the level at which least complications occur (INR between 2.5 and 4.0).

Box 2. Example of three patients with target INR 3.0 and the values of three different methods to calculate the variance growth rate.



We calculated all these measures for four different time windows; one year, 6 months, 3 months and 6 weeks prior to the time of the event. For each time window only subjects were included who had INR measurements during the whole period of that time window. The INR measurement at the time of the event was not included in the calculations for the various effect measures, because this was not a routinely measured INR.

The values of the variance growth rates and the percentage of time spent in, above and below INR range 2.5-4.0 were divided into tertiles (good, medium, poor) based on the distribution in the control persons. To assess whether being out of range along with a high variability is predictive we also studied the combination of the time spent at INR 2.5-4.0 and the variability calculated according to method B1 (method A includes the achieved INR and is therefore unsuitable to disentangle variability and time in range). For this combination we divided the time spent at INR 2.5-4.0 into two groups; patients in the good and medium group were considered to be frequently in range and patients in the poor group to be frequently out of range. The variance growth rate was divided into unstable (poor group) or stable (good and medium group). ORs were calculated for patients with high variability who were in INR range 2.5-4.0, patients who were stable but outside the range of INR 2.5-4.0 and those who were both unstable and outside INR range 2.5-4.0, all with stable patients in the INR range as reference category. To investigate whether the effect is specific for the period directly preceding the event, we analysed the different quarters of the year prior to the event. Additionally, we did separate analysis for phenprocoumon and acenocoumarol users.

We performed a matched analysis calculating odds ratios (OR) and 95% confidence intervals (95%CI) using conditional logistic regression models. For all variability measures the lowest tertile is the reference category (which is the low-risk group according to our hypothesis). For the time in therapeutic range the highest tertile is the low-risk group and this was chosen to be the reference category. We performed separate analyses for haemorrhagic events and thrombotic events, as well as an analysis in which the two were combined. For the subgroup analysis for the type of vitamin K antagonist we performed logistic regression analysis adjusting for the matching factors sex and age. The conditional logistic regression models were done with the STATA 8.0 SE software (StataCorp LP, Tx,

USA), logistic regression analysis was performed with the statistical package SPSS version 14.0 (SPSS Inc, Chicago, Ill).

Results

Among the 1608 patients in the LAVA cohort there were 210 first events, consisting of 154 haemorrhagic events and 42 thrombotic events. Fourteen strokes could not be classified as either a haemorrhagic or an ischemic event. Thirty-three patients experienced an intracranial or spinal haemorrhage and 121 an extracranial haemorrhage. The thrombotic and embolic events were almost all cerebral infarctions (n=40) except for two patients who had a peripheral embolism.

Four hundred and twenty control subjects were matched to the 210 cases. One hundred and seventeen patients were selected more than once as a control, of whom 12 patients were selected three times. Thirteen control patients became a case at a later date. There were slightly more women than men, 53.1% versus 46.9%. Median age was 66 years (IQR 58 – 72). General characteristics of cases and controls are shown in table 1.

Table 1. General characteristics of cases and control subjects.

		Cases (n=210)	Controls (n=420)
Age	Median (IQR)	66 (58-72)	66 (58-72)
Sex	Male (%)	99 (46.1)	198 (46.9)
	Female (%)	111 (53.1)	222 (53.1)
Position of heart valve	Aortic (%)	111 (52.9)	233 (55.2)
	Mitral (%)	68 (32.4)	133 (31.7)
	Both (%)	30 (14.3)	52 (12.4)
Coumarin	Phenprocoumon (%)	171 (81.1)	356 (85.2)
	Acenocoumarol (%)	37 (17.2)	62 (14.6)
	Other/ Unknown (%)	2 (1.0)	2 (0.2)

Table 2 shows ORs for haemorrhagic events for all measures (154 cases, 308 controls). The measures were generally most clearly predictive during the time window of three months, while associations became attenuated when the variance growth rates were calculated over longer time intervals. The risk of a haemorrhagic event was highest among patients who spent relatively little time within INR range 2.5-4.0. Method A, which incorporates both time in range and variability was most clearly associated with the risk of haemorrhage. In the time window of three months we found an OR of 2.6 (95%CI 1.3-5.1) for patients in the 2nd tertile and an OR of 3.1 (95% CI 1.6-6.0) for patients in the highest tertile compared to patients in the lowest tertile. The methods that only looked at variability were associated with a 1.6-fold increased risk of haemorrhage in the highest tertile compared to the lowest for a 3-months time window. Odds ratios for all measures for thrombotic events are given in table 3 (42 cases and 84 matched controls).

Table 2. Odds ratios for tertiles of the different measures for all haemorrhagic events

		One year		6 Months		3 Months		6 Weeks	
Tertile		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Time at INR 2.5-4.0	Good	1.0	-	1.0		1.0		1.0	
	Medium	1.3	0.7-2.2	1.4	0.8-2.4	1.5	0.8-2.7	1.9	0.9-4.3
	Poor	1.8	1.1-3.2	1.6	0.9-2.7	2.6	1.4-4.8	2.3	1.1-4.9
Time above INR 4.0	Good	1.0		1.0		1.0		1.0	
	Medium	1.0	0.6-1.7	1.0	0.6-1.8	1.1	0.6-1.9	2.5	1.1-5.6
	Poor	1.4	0.8-2.4	1.3	0.8-2.2	1.9	1.1-3.8	2.5	1.2-4.9
Method A	Good	1.0		1.0		1.0		1.0	
	Medium	1.2	0.7-2.1	1.9	1.1-3.4	2.6	1.3-5.1	1.8	0.9-3.7
	Poor	1.4	0.8-2.4	1.9	1.0-3.3	3.1	1.6-6.0	1.4	0.7-2.8
Method B1	Good	1.0		1.0		1.0		1.0	
	Medium	0.9	0.5-1.6	1.1	0.7-2.0	1.2	0.6-2.1	0.8	0.4-1.6
	Poor	0.9	0.6-1.6	1.5	0.9-2.6	1.6	0.9-2.8	1.3	0.7-2.6
Method B2	Good	1.0		1.0		1.0		1.0	
	Medium	1.0	0.6-1.7	1.1	0.6-1.9	1.2	0.7-2.3	0.8	0.4-1.5
	Poor	0.9	0.6-1.6	1.5	0.9-2.6	1.5	0.9-2.7	1.4	0.7-2.7

Table 3. Odds ratios for tertiles of the different measures for all thrombotic events

	Tertile	One year		6 Months		3 Months		6 Weeks	
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Time at INR 2.5-4.0	Good	1.0	-	1.0		1.0		1.0	
	Medium	1.8	0.6-5.3	0.8	0.3-2.2	1.3	0.4-3.8	1.1	0.2-4.8
	Poor	1.4	0.5-4.0	0.7	0.3-1.9	1.5	0.6-4.2	0.9	0.2-4.3
Time below 2.5	Good	1.0		1.0		1.0		1.0	
	Medium	1.3	0.4-4.5	-				-	
	Poor	2.0	0.8-4.8	2.2	0.9-5.6			1.6	0.3-8.0
Method A	Good	1.0		1.0		1.0		1.0	
	Medium	1.5	0.6-4.0	1.3	0.5-3.6	2.1	0.7-6.1	3.2	0.6-16.9
	Poor	1.4	0.5-4.0	1.7	0.5-5.4	1.5	0.5-4.4	1.8	0.4-8.1
Method B1	Good	1.0		1.0		1.0		1.0	
	Medium	0.7	0.3-2.0	3.0	0.9-10.1	3.0	0.8-10.8	0.5	0.1-2.1
	Poor	0.9	0.4-2.5	1.7	0.5-5.6	3.2	1.0-11.1	0.5	0.1-1.8
Method B2	Good	1.0		1.0		1.0		1.0	
	Medium	0.9	0.3-2.3	2.0	0.6-6.4	2.1	0.6-6.8	0.2	0.03-0.9
	Poor	1.0	0.4-2.7	1.3	0.4-4.3	2.5	0.8-7.7	0.4	0.1-1.7

As we found in the analysis with haemorrhagic events, the measures were generally most clearly predictive of an event during the time window of three months and this risk was highest in patients who spent least time in the target zone. Variability method A, that combines variability and time in range showed increased risks with an OR of 1.5 (95% CI 0.5-4.4) for patients in the highest tertile compared to the lowest. The two methods that purely looked at variability now had higher odds ratios, of, for method B1 3.0 (95% CI 0.8-10.8) and 3.2 (95% CI 1-9.8) in the 2nd and highest tertile compared to the lowest tertile and 2.1 (95% CI 0.6-6.8) and 2.5 (95% CI 0.8-7.7) for method B2. Odds ratios for the combination of haemorrhagic and thrombotic events are shown in table 4. Adjustment for the value of the INR at the last measurement before the index date did not affect the outcomes, except in the analysis of thrombotic events where the ORs became more distinct.

Table 4. Odds ratios for tertiles of the different measures for all adverse events

Time window	Tertile	One year		6 Months		3 Months		6 Weeks	
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Time at INR 2.5-4.0	Good	1.0	-	1.0	-	1.0	-	1.0	-
	Medium	1.3	0.8-2.1	1.3	0.8-2.1	1.6	1.0- 2.8	2.0	1.0- 4.0
	Poor	1.5	1.0-2.4	1.4	0.9-2.1	2.5	1.5- 4.3	1.9	1.0- 3.8
Time above 4.0	Good	1.0	-	1.0	-	1.0	-	1.0	-
	Medium	1.1	0.7- 1.7	1.0	0.6- 1.6	1.2	0.7-1.9	2.6	1.3-5.1
	Poor	1.3	0.8- 2.0	1.1	0.7-1.7	1.9	1.2-3.1	1.6	0.9- 2.9
Time below 2.5	Good	1.0	-	1.0	-	1.0	-	1.0	-
	Medium	1.2	0.7-2.2	-	-	-	-	-	-
	Poor	1.6	1.1-2.4	2.0	1.3- 2.9	1.9	1.2- 3.0	1.1	0.6- 2.0
Method A	Good	1.0	-	1.0	-	1.0	-	1.0	-
	Medium	1.3	0.8- 2.0	1.7	1.1-2.8	2.8	1.5- 4.9	1.6	0.9- 2.9
	Poor	1.4	0.9- 2.2	1.8	1.1- 3.0	3.1	1.7-5.4	1.4	0.7- 2.6
Method B1	Good	1.0	-	1.0	-	1.0	-	1.0	-
	Medium	0.9	0.6- 1.5	1.3	0.8-2.0	1.4	0.8- 2.4	1.0	0.5- 1.7
	Poor	1.0	0.6- 1.6	1.5	0.9-2.3	1.8	1.1- 3.0	1.3	0.7- 2.3
Method B2	Good	1.0	-	1.0	-	1.0	-	1.0	-
	Medium	0.9	0.6-1.5	1.2	0.7- 1.9	1.5	0.9- 2.6	1.0	0.5- 1.7
	Poor	1.0	0.6-1.5	1.5	0.9- 2.3	1.8	1.1- 2.9	1.1	0.6- 2.0

Subsequently, we combined the pure variability methods (B1 and B2) with the time spent in and outside the INR target range of 2.5 and 4.0. The risk of a haemorrhagic or thrombotic event was increased 2.6-fold (95%CI 1.6-4.2) for those in the highest tertile of variability and lowest time-in-range tertile as compared to subjects with stable anticoagulation who were usually within the target range (table 5). Variability within an INR range of 2.5 – 4.0 did not affect risk. Again, the effect was only seen in the three months directly preceding the event. In a subgroup analysis of phenprocoumon and acenocoumarol users we found similar results for all measures, but the associations were more pronounced in acenocoumarol users.

Table 5. Odds ratios for the combination of time in therapeutic range and variability calculated with method B1.

Time in therapeutic range*	Variability**	OR _{Haem}	95%CI	OR _{throm}	95%CI	OR _{total}	95%CI
In range	Stable	1.0	-	1.0	-	1.0	-
	Unstable	1.0	0.5-2.0	1.1	0.4-3.5	1.2	0.7-2.1
Outrange	Stable	1.6	0.9-3.1	0.8	0.3-2.6	1.5	0.9-2.6
	Unstable	2.7	1.4 - 4.9	2.5	0.8-7.9	2.6	1.6-4.2

*In range are patients with a time in therapeutic range in the 2nd and highest tertile, outrange are patients in the lowest tertile.

** Stable are patients who have a variance growth rate calculated with method B1 in the lowest or 2nd tertile, unstable are patients in the highest tertile.

Discussion

In this study of 630 subjects with mechanical heart valve prostheses we found that unstable anticoagulation was associated with an increased risk of haemorrhagic and thrombotic complications. Both variability and time spent outside the target range affected risk. The variance growth rate described by Fihn, method A, incorporates both aspects of instability, and was therefore most clearly associated with complications of anticoagulant therapy, especially haemorrhagic episodes. Thrombotic events were most clearly predicted with variability calculated with method B1 and B2, which only concern variability of the INR and not the time within range. The optimal time window to determine these measures was three months.

Periods of time outside the therapeutic range are intrinsically related to the treatment and therefore not avoidable. The problem is to have an instrument to quantify them to obtain a better quality of anticoagulation in order to reduce adverse events. Several studies have been done to investigate potential instruments to quantify this quality, and research concerning this topic is ongoing. Our study

showed similar results as the previous studies have shown [9-11,13]. From these studies we knew that the measures we evaluated could predict which patients were at increased risk, but we did not know which one was best associated since they have never been compared directly to each other. In another study it was shown that patients with episodic overanticoagulation had an increased risk of both haemorrhagic and thrombotic events [15]. The authors suggest that this phenomenon reflects instability of anticoagulation. These results are in line with our findings, since the variability measures increase when patients have episodes of high INR levels.

Overall, the variability according to method A was best associated with complications of oral anticoagulant therapy. However, this method is a composite of time in range and variability. When we combined the methods that only looked at variability with a measure of time in range, this predicted equally well. The importance of the association of risk of the methods that only look at the variability of the INR, is that we show this is a risk factor per se, added to the risk of under- and overanticoagulation. When we adjusted for the value of the last INR before the index date the effect did not disappear, which teaches us that an INR within the therapeutic range in patients with high variability in INRs can not be interpreted the same way as in patients who are very constant in their INRs. Remarkably, the achieved intensity of anticoagulation was less important in predicting thrombotic events than haemorrhagic events.

The lack of association in the time windows of one year and six months may be the result of the dilution of the effect of the variability measures when calculated over a prolonged period. This is also supported by the results of our analysis of quarters of the year prior to an event, where we only found an association in the three months directly preceding the event. Remarkably, a six weeks time window had little association. This may be the result of the limited

number of INRs which are available in such a period. Time between two visits can be as long as six weeks, so in a time window of 6 weeks prior to the event, few patients will have sufficient INR measurements to reliably calculate the various measures. Consequently, three months is the optimal time window to predict thrombotic and haemorrhagic risk by variability.

We have shown that unstable anticoagulation is associated with haemorrhagic and thrombotic complications. The variance growth rate calculated by method A is best associated with complications. However, this method does not inform us on the reason for the increased risk. Since methods B1 and B2 in combination with the time spent at INR 2.5- 4.0 are equally well associated, we prefer to disentangle variability in and intensity of anticoagulation. Disentangling the variability and the time in therapeutic range gives us the opportunity to target more directly either the instability or the inadequate level, hence possibly preventing these events. Therefore we propose to use the variance growth rates by Cannegieter or Fihn, methods B1 and B2 to reflect the variability of oral anticoagulant therapy.

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CHAPTER 6

Determinants of Unstable Anticoagulation in the treatment with Vitamin K antagonists

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Abstract

Background: Patients who are unstably anticoagulated or who spent much time outside their therapeutic range are at an increased risk for complications. Factors associated with unstable anticoagulation are not well known.

Objectives: To investigate the association of patient characteristics, lifestyle factors and treatment-related factors with unstable anticoagulation.

Patients and Methods: Patients were participants in a randomized controlled trial with the main objective to compare two different algorithms for computer-assisted dosing of oral anticoagulants. All patients completed a questionnaire concerning lifestyle factors.

Results: 638 patients (response 91.3%) returned the questionnaire. Unstable anticoagulation was observed more frequently with the use of acenocoumarol versus phenprocoumon (RR 2.3, 95%CI 1.9-2.8), in individuals with daily strenuous physical activity (RR 1.4, 95%CI 0.7-2.7) and less frequently in obese patients (RR 0.7, 95%CI 0.5-1.0). Patients who were underweight (RR 1.5, 95%CI 0.2-12.8), obese (RR 1.8, 95%CI 0.7-4.4), or had daily strenuous physical activity (RR 4.1, 95%CI 0.8-22.1) had an increased risk of spending more than 50% out of range, while regular alcohol use (>1-2-times a week) decreased the risk with 30-50%. Furthermore, patients with high daily anticoagulant dosages were less likely to spend more than 50% out of range than those with low doses.

Conclusion: Unstable anticoagulation is associated with the use of acenocoumarol versus phenprocoumon and strenuous daily physical activity. Obesity decreases the risk for instability. Low anticoagulant dosages, strenuous daily physical activity, being underweight or obese and frequent alcohol use are associated with an increased risk of spending less than 50% in the therapeutic range.

Introduction

Vitamin K antagonists are among the most frequently prescribed drugs worldwide. They are proven to be effective in the treatment and prophylaxis of venous and arterial thrombosis [1-3]. At the same time, vitamin K antagonists are also listed in the top 5 of drugs causing adverse events [4]. In routine clinical practice, vitamin K antagonists result in severe bleeding complications in 1-2% of patients [5-7].

Vitamin K antagonists have a narrow therapeutic window, and frequent monitoring of the anticoagulant effect by measurements of the International Normalised Ratio (INR) with adjustment of anticoagulant dose is required to maintain patients within the therapeutic range. Patients who are insufficiently anticoagulated (i.e., an INR below the therapeutic window appropriate for their indication) are at increased risk for (re)thrombosis, whereas over-anticoagulated patients show a sharp increase in bleeding risk [8]. Keeping patients' INR within the therapeutic window is difficult due to the large variability in the dose needed to achieve the optimal anticoagulant effect within a single patient. Sensitivity for vitamin K antagonists not only differs between patients, but also within patients it may vary over time [9]. The risk for bleeding or thrombosis proportionally increases with the time spent outside the therapeutic window [8]. Bleeding or thrombosis risk is also increased in patients who are unstably anticoagulated, independently from the achieved time within the therapeutic window [10-12]. Thus, low-risk anticoagulation not only requires the patient to be within its therapeutic window for the majority of time, but also requires deviations in INR over time to be small.

In a case-control study, unstable anticoagulation has been shown to have many determinants [13]. Patients who had an INR above 4.5, workers versus pensioners, users of acenocoumarol versus warfarin and patients who had a poor compliance were considered often unstable. Instability in this study was defined

according to the opinion of experts in anticoagulant dosing, because there was a lack of universally accepted criteria for instability. Recently, it has been shown that stability of anticoagulation can be objectively measured by calculating the variance growth rate. This variance growth rate reflects the degree to which a patient's INR deviates from the previous one and is independent from a patient's target INR. Instability defined by this variance growth rate was shown to be associated with an increased risk for bleeding or thrombotic events [10].

In this study our aim was to identify factors that are associated with unstable anticoagulation using the variance growth rate to calculate stability. In addition we studied determinants of the time spent out of range.

Methods

Study design

Patients were participants in a randomized controlled double-blind trial conducted at the Leiden Anticoagulation Clinic in the Netherlands. Main objective of the trial was to evaluate two algorithms (TRODIS and ICAD) for computer-assisted dosing in clinical practice. In total 712 patients participated in the trial. Enrollment occurred between August 14th and October 16th 2003. Patients were eligible when they had an indication for long-term anticoagulant therapy and were between the ages of 18 and 80 years. Patients were excluded when they were participating in the patient self-management program, stayed long periods abroad or were in a terminal stage of disease. Randomization was done stratified by the indication for oral anticoagulant treatment, age and sex using the minimization method [14]. The study was double-blind, e.g., neither patients nor physicians were aware which group the patient was randomized to. Follow-up was until September 1st 2004, i.e. maximally about one year. All patients filled in a questionnaire at the start of the study containing questions about the attitude towards their anticoagulant treatment

and lifestyle factors such as smoking habits, alcohol use, vitamin intake and sporting activities. The study was approved by the Medical Ethics Committee of the Leiden University Medical Center before start of the study and each patient gave written informed consent before randomization. The trial is registered in the ISRCTN trial registration with identifier ISRCTN27801917. The trial is described in more detail elsewhere [15].

Because of our previous finding that the time spent within the therapeutic range was similar for both algorithms [15], and instability was similar (median 0.22, interquartile range (IQR) 0.09-0.57 vs. median 0.26, IQR 0.12-0.61, $p=0.064$) we pooled data of the two groups and considered it as a cohort. We performed analyses for two outcome events. The first outcome event was unstable anticoagulation. Unstable anticoagulation was calculated with the following formula for the variance growth rate:

$$\sigma^2 = \frac{1}{n} \sum_{i=1}^n \frac{(\text{INR}_{i+1} - \text{INR}_i)^2}{\tau_{i,i+1}}$$

In this formula n is the number of INR measurements and τ the time between two visits in weeks. The variance growth rate is a reflection of the amount of deviation of a patient's INR from the previous INR. The σ will be high in patients with highly varying INR values and low if the INR is consequently at the same value every time, even if this means the INR is constantly outside the therapeutic range. The stability was calculated over the first 6 months of follow up. The values of the variance growth rates were divided into tertiles (good, medium, poor), and patients in the third tertile were considered unstably anticoagulated.

In the second analysis we analyzed the time spent outside the therapeutic range. The time in therapeutic range was calculated over the first 6 months after start of the study with the linear interpolation method [16]. We considered patients who spent more than 50% outside their therapeutic range to have experienced the outcome event. Therapeutic ranges were as they were applied in our routine anticoagulant practice: INR 2.0 to 3.5 for low intensity and INR 2.5 to 4.0 for indications requiring a higher intensity. Patients who had less than 3 INR measurements were excluded from the analyses.

We investigated the association of sex, smoking, alcohol use, vitamin use, the use of a specific diet (e.g. low carb, low salt), daily physical activity, sporting activities, BMI, type of vitamin K antagonist and dosage with unstable anticoagulation and time outside range. We developed a questionnaire to measure all factors, except for the type of vitamin K antagonist and dosage used which were derived from the database at the anticoagulation clinic. Smoking was divided in three categories: smokers, former smokers and non-smokers. Former smokers were considered non-smokers if they had quit smoking more than half a year before inclusion in the study. Alcohol use was categorized according to the amount of consumption. Daily physical activity refers to the amount of activity due to work, whereas sporting activities only includes physical activity due to sports. The degree of daily physical activity was categorized by patients themselves as light, moderate, heavy or strenuous. Patients were divided into four categories for Body Mass Index (BMI): underweight (BMI <20), normal weight (BMI 20-25), overweight (BMI 25-30) and obese (BMI>30). The mean daily dosages of vitamin K antagonist were divided into quartiles for phenprocoumon and acenocoumarol separately.

Statistical analyses

We calculated relative risks (RR) for either unstable anticoagulation (variance growth in third tertile) or time outside of range (more than 50% outside range). We report RRs with their corresponding 95% confidence intervals (95% CI). Continuous variables are presented as the median with interquartile range (IQR). All calculations were performed using the statistical package SPSS version 14.0 (SPSS Inc, Chicago, Ill, USA).

Results

Of the 712 patients included in the trial, thirteen patients discontinued treatment before there were 3 INR measurements available. The reasons for discontinuation were: the treatment ended (n=3), participation in the patient self management program (n=6), death (n=2) or other reasons (n=2). Of the 699 patients, 638 (91.3%) filled in the questionnaire and were included in the current analysis. The median age of the participants was 68 years (Interquartile range (IQR) 59-74) and there were slightly more men than women (67.2%). Most patients used phenprocoumon at a low intensity.

Table 1. Patient characteristics (n=638)

Age		
Median (IQR)		68.0 (59-74)
Sex		
Men (%)		67.2
Indication		
Atrial Fibrillation (%)		43.7
Venous thrombosis (%)		12.4
Heart valve prosthesis (%)		10.3
Other cardiac indication (%)		18.2
Peripheral vascular disorder (%)		9.5
Cerebrovascular (%)		6.0
Coumarin		
Acenocoumarol (%)		14.3
Phenprocoumon (%)		85.7
Intensity (target)		
Low (2.0 – 3.5) (%)		62.2
High (2.5 – 4.0) (%)		35.3
Switched (%)		2.5

The main characteristics of the patients are shown in table 1. The patients who did not fill in the questionnaire were not different with respect to age and sex compared to those who filled in the questionnaire. The median value for the instability calculated with variance growth rate was 0.25 (IQR 0.10-0.58). The median time spent in therapeutic range was 82.5% (IQR 69.9 – 94.9). The mean daily dosage of phenprocoumon was 2.27 mg (IQR 1.59-2.72) with a maximum of 9.96 mg. For acenocoumarol users the mean daily dosage was 2.53 mg (IQR 1.67-3.17) with a maximum daily dosage of 6.12 mg.

Determinants of unstable anticoagulation

The 67th percentile of the variance growth rate was 0.45 and patients with a variance growth rate ≥ 0.45 were considered unstably anticoagulated. We investigated the association of the patient characteristics and lifestyle factors sex, BMI, smoking status, daily physical activity, work, sporting activities, alcohol use, vitamin use, the use of a specific diet with unstable anticoagulation (table 2).

Obese patients had a 30% lower risk of unstable anticoagulation than patients with normal weight (RR 0.7, 95% CI 0.5-1.0). Smoking was not associated with unstable anticoagulation. Strenuous physical activity showed a small increased risk for unstable anticoagulation (RR: 1.4, 95% CI 0.7-2.7) compared to patients who had light physical activity. Patients who did not work had a 1.2 times increased risk compared to patients who worked (95% CI 0.9-1.5). We did not observe an association with unstable anticoagulation and sex, vitamin use, sporting activities, the use of a specific diet, or alcohol use.

Table 2. Relative risks for the association between patient characteristics and lifestyle factors and unstable anticoagulation and time out of range.

		Unstable anticoagulation		Time out of range	
		RR	95%CI	RR	95% CI
Sex	Male	1.0 (ref)	-	1.0 (ref)	-
	Female	0.9	0.7-1.2	1.1	0.5-2.1
BMI	Underweight	0.9	0.4-1.9	1.5	0.2-12.8
	Normal weight	1.0 (ref)	-	1.0 (ref)	-
	Overweight	0.9	0.5-1.6	1.1	0.5-2.6
	Obese	0.7	0.5-1.0	1.8	0.7-4.4
Smoking	No	1.0	-	1.0	-
	Former	0.9	0.7-1.2	0.6	0.3-1.3
	Yes	1.0	0.7-1.4	0.6	0.2-1.7
Daily physical activity	Light	1.0 (ref)	-	1.0 (ref)	-
	Moderate	0.7	0.5-1.1	0.9	0.3-2.9
	Heavy	1.0	0.6-1.7	0.6	0.1-5.0
	Strenuous	1.4	0.7-2.7	4.1	0.8-22.1
Work	Workers	1.0 (ref)	-	1.0 (ref)	-
	Non workers	1.2	0.9-1.5	1.3	0.7-2.7
Sporting activities	Yes	1.0 (ref)	-	1.0 (ref)	-
	No	1.1	0.9-1.4	1.0	0.5-1.9
Alcohol use	Never	1.0 (ref)	-	1.0 (ref)	-
	< 1 x per mnd	0.9	0.2-5.0	1.9	0.5-6.3
	< 1 x per week	1.1	0.7-1.6	1.7	0.6-4.7
	1-2 days per week	0.9	0.6-1.3	0.7	0.2-2.3
	3-4 days per week	0.8	0.5-1.3	0.5	0.1-2.6
	5-7 days per week	1.1	0.8-1.5	0.7	0.2-1.7
Vitamin use	No	1.0 (ref)	-	1.0 (ref)	-
	Yes	0.9	0.7-1.2	0.9	0.4-1.9
Specific diet	No	1.0 (ref)	-	1.0 (ref)	-
	Yes	1.2	0.9-1.6	0.6	0.3-1.4

In table 3 RRs are shown of the association between treatment related factors, type of vitamin K antagonist, target INR range, mean daily dosage and unstable anticoagulation. Users of acenocoumarol had a 2.3 fold increased risk for unstable anticoagulation, i.e. a high variance growth rate (95% CI 1.9-2.8). Patients with a high therapeutic range had a slightly increased risk of unstable anticoagulation with a RR of 1.4 (95% CI 1.1-1.7) compared to patients with a low

therapeutic range. Higher anticoagulant dosage tended to give a slightly decreased risk, with relative risks for the 2nd and 3rd quartile of 0.8 (95% CI 0.6-1.1), but this was not seen for patients with the highest dosages (RR 0.9, 95% CI 0.7-1.2). The association between the mean daily dosage and instability was similar for acenocoumarol users and phenprocoumon users.

Table 3. Relative risks for the association between several treatment related factors and unstable anticoagulation and time out of range.

	Unstable anticoagulation		Time out of range	
	RR	95%CI	RR	95% CI
Vitamin K antagonist				
Phenprocoumon	1.0 (ref)	-	1.0 (ref)	-
Acenocoumarol	2.3	1.9-2.8	0.7	0.2-2.0
Target zone				
Low (2.0-3.5)	1.0 (ref)		1.0 (ref)	
High (2.5-4.0)	1.4	1.1-1.7	1.0	0.5-2.0
Switched	2.3	1.1-3.0	2.3	0.5-10.8
Dosage				
Lowest quartile	1.0 (ref)		1.0 (ref)	
Second quartile	0.8	0.6-1.1	0.3	0.1-0.7
Third quartile	0.8	0.6-1.1	0.4	0.2-1.0
Highest quartile	0.9	0.7-1.2	0.2	0.1-0.6

Determinants for time spent outside the therapeutic range

Thirty eight (6.0%) patients spent more than 50% of the time outside the therapeutic range. Patients being underweight or obese patients tended to be at increased risk for spending 50% or more outside the therapeutic range with RRs of 1.5 (95% CI 0.2-12.8) and 1.8 (95% CI 0.7-4.4) compared to patients with normal weight, although their confidence intervals contained 1.0. Former smokers (smoking terminated less than half a year before inclusion into the study) and smokers had a 40% lower risk compared to patients who did not smoke or stopped smoking more than half a year before inclusion. Patient regularly using alcohol (more than 1-2 time a week) had a 30-50% decreased risk to be out of range compared to patients who never use alcohol. We did not observe an association

between time out of range and sex, the use of vitamin supplements and sporting activities.

In contrast to the results for unstable anticoagulation there was a decreased risk for acenocoumarol users to be out of range compared to phenprocoumon users (RR 0.7, 95% CI 0.2-2.0) (table 3). There was a strong association between the anticoagulant dosage and spending more than 50% out of range. Patients in the highest quartile of the anticoagulant dosage had an 80% reduced risk compared to patients in the lowest quartile (RR: 0.20, 95% CI 0.1-0.6).

Discussion

In this study we have shown that unstable anticoagulation is observed more frequently with the use of acenocoumarol versus phenprocoumon, in individuals with daily strenuous physical activity and less frequent in obese patients. Patients who are obese, have daily strenuous physical activity or are frequent alcohol users have a higher risk of spending more time outside the therapeutic range. Furthermore, increased anticoagulant dosage substantially reduced the risk of time spent outside range.

Our study showed similar results as the study of Palareti et al. [13]. They showed that users of the short acting acenocoumarol had an increased risk for unstable anticoagulation compared to users of the longer acting warfarin. We found that also compared to the longest acting vitamin K antagonist phenprocoumon, acenocoumarol users were at an increased risk for instability. It is remarkable that the use of acenocoumarol was associated with unstable anticoagulation but not with spending 50% or more time outside the therapeutic range. Overall, acenocoumarol users did have a lower mean time spent within range, but the proportion of patients with a time outside the therapeutic range of more than 50% was smaller compared to phenprocoumon. We can explain this by the fact that

phenprocoumon users have a wider range in time in therapeutic range compared to acenocoumarol users with a large amount of patients spending more than 80% of time within the therapeutic range. This leads to an overall better quality of treatment for phenprocoumon compared to acenocoumarol, although the number of patients who spent less than 50% in range is larger. Instability is associated with the time spent in range, but the association is stronger with higher time in range. Several studies have shown that vitamin K antagonists with a shorter half-life are associated with poorer quality (less time within the therapeutic range) of anticoagulant treatment, although some studies did not confirm these findings [17-19]. Our study indicates that this poorer quality might be caused by an increased instability rather than spending more time out of range.

Patients requiring higher anticoagulant dosages had a decreased risk for spending more than 50% of the time outside the therapeutic range. Patients who require low anticoagulant dosages may be more sensitive to small changes in the dosage, since these dose changes are relatively larger for small dosages than for high dosages. In earlier studies it has been shown that carriers of the CYP2C9 polymorphisms CYP2C9*2 and CYP2C9*3 need lower anticoagulant dosages and have a higher risk for adverse events [20-22]. Also the VKORC1 haplotype has been shown to be of influence on the anticoagulant dosages. The decreased risk for spending more time out of range we found for patients with high anticoagulant dosages might be explained by CYP2C9 genotype and VKORC1 haplotype. Unfortunately we could not investigate this in our study since DNA samples were not available.

Of the lifestyle factors we investigated, only daily physical activity was positively associated with unstable anticoagulation and spending more than 50% of the time out of range. In contrast to the results of the study by Palareti et al. we observed only a small increased risk for patients who did not work compared to

workers. Obesity was shown to decrease the risk for unstable anticoagulation and increase the risk for spending more than 50% outside the therapeutic range. This contradictory finding was caused by several obese patients who were stably anticoagulated with INR values around the upper or lower values of the therapeutic range.

We used a questionnaire to obtain information about lifestyle factors so it is possible that patients filled in favourable answers for these factors since it is well known that some lifestyle factors such as smoking and drinking large amounts of alcohol have negative effects on health. If this is true we would have found risks that are underestimated.

In conclusion, unstable anticoagulation defined by the variance growth rate occurs more frequently with the use of acenocoumarol versus phenprocoumon, in patients with strenuous daily physical activity and is reduced in obese patients. Spending more than 50% outside the therapeutic range was associated with obesity, strenuous daily physical activity, frequent alcohol use and lower anticoagulant dosages.

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CHAPTER 7

A Randomised Comparison of the Quality of anticoagulant therapy with Two Vitamin K antagonists: Warfarin versus Phenprocoumon

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Abstract

Context Several studies have compared different vitamin K antagonists with regard to the quality of treatment, e.g. stability. The results were mostly, but not always, in favour of the longer acting vitamin K antagonists. Only one randomised study compared warfarin and phenprocoumon.

Objective The aim of our study was to investigate whether oral anticoagulant treatment with warfarin leads to a more or less stable anticoagulant status than is achieved with phenprocoumon.

Methods We conducted a randomised controlled trial among patients with an indication for anticoagulant treatment at the Leiden Anticoagulation Clinic. We included patients who initiated oral anticoagulant therapy and patients who were already using a different vitamin K antagonist (acenocoumarol), and who were switched. We compared an oral anticoagulant treatment with warfarin with a treatment with phenprocoumon. Follow-up was 6 months. The primary outcome measure was the percentage of time spent in therapeutic range.

Results From March 1st 2004 to January 2008, 312 patients finished the follow period and were included in this analysis. One patient died before the first INR measurement. The median time spent in the therapeutic range was 74.7% (IQR 58.8-87.0). Thirty eight percent of patients spent more than 80% of the time in therapeutic range. There was no difference in time spent in the therapeutic range between men and women, and between patients who initiated oral anticoagulant treatment and patients who switched from acenocoumarol. The median time between two visits was 11.7 days (IQR 10.0 – 13.9).

Trial registration The trial is registered in the ISRCTN register with identifier ISRCTN60446748. (www.controlled-trial.org)

Introduction

Coumarins are among the most frequently used drugs worldwide and the number of indications for which they are prescribed is still growing. Worldwide there are different vitamin K antagonists available. There are three different types most frequently used: acenocoumarol, warfarin and phenprocoumon. These vitamin K antagonists mainly differ in half-life: Acenocoumarol has the shortest half-life of 11 hours, followed by warfarin with 36-42 hours and the longest half-life is seen in phenprocoumon with approximately 140 hours [1-4]. The clearance of these coumarins also is dissimilar. Acenocoumarol is for its elimination completely dependent on hydroxylation by cytochrome p450 (CYP). Warfarin is also dependent on reduction processes [5]. Phenprocoumon can, in addition to elimination as hydroxylated metabolites, be eliminated as parent compound and is thus less dependent on hydroxylation by CYP. These differences in dependence on hydroxylation by the CYP enzymes offer an explanation of different responses found in studies investigating the effects of polymorphisms in the CYP2C9 gene on clearance of specific coumarin classes [6,7]. Several studies have compared these different vitamin K antagonists with regard to the quality of treatment, e.g. stability. Most studies have compared the short acting acenocoumarol to the longer acting warfarin or phenprocoumon. The results were mostly, but not always, in favour of the longer acting vitamin K antagonists. [8-16]. Only one randomised comparison between warfarin and phenprocoumon was made by Rodman *et al.* in 1964 [15]. In their comparative study they evaluated 4 different anticoagulants: phenprocoumon, bishydroxycoumarin, diphenadione and warfarin. They concluded that phenprocoumon appeared to be superior to the other anticoagulants in terms of ease of maintenance therapy, although all others were also satisfactory for clinical use.

The aim of our study was to investigate whether oral anticoagulant treatment with warfarin leads to a more stable oral anticoagulant treatment than is achieved with phenprocoumon. In this report we will discuss the design of the study and give general results regarding the quality of oral anticoagulant treatment of the participating patients.

Methods

Study design

We performed a randomised controlled trial comparing two groups: patients receiving oral anticoagulant treatment with phenprocoumon and patients using warfarin. We used block randomisation stratified for the centre a patient was referred from to assure an equal number of patients on both anticoagulants among patients from each centre. The study was not blinded since dosing of warfarin and phenprocoumon has to be done in different ways due to the difference in half life. For safety reasons, it is also necessary that patients and physicians are aware of which vitamin K antagonists they are taking.

The study was conducted at the Leiden Anticoagulation Clinic in the Netherlands. At this anticoagulation clinic most patients are treated with phenprocoumon (approximately 80%), all other patients are treated with acenocoumarol. Warfarin is not registered for use in the Netherlands.

Patients

Recruitment of patients occurred from March 1st 2004 and is ongoing. Two patient groups were included in the trial. The first group consisted of patients initiating oral anticoagulant treatment and was recruited from three hospitals, all in the greater Leiden area, i.e., the departments of Cardiology and Internal Medicine of the Leiden University Medical Center, Diaconessenhuis Leiden, and Rijnland

Hospital Leiderdorp and at the department of Orthopedics of the Leiden University Medical Center. Patients were eligible to participate when they were aged between 18 and 85 years and had an indication for anticoagulant treatment which required treatment for at least three months. Exclusion criteria were pregnancy or intended pregnancy, renal dialysis, chemotherapy, known allergic reactions to warfarin or phenprocoumon or a contra-indication to oral anticoagulant treatment. Only patients who lived in the working area of the Leiden Anticoagulation clinic could participate in the study. Patients were randomised to a treatment with either phenprocoumon or warfarin and were followed until end of treatment or, when the indication required the treatment to continue over 6 months, follow-up ended at 6 months.

The second group included in this trial consisted of patients already using acenocoumarol and who were recruited at the Anticoagulation clinics of Leiden and The Hague. We chose patients using acenocoumarol so that for the study participants always had to switch to a treatment with another vitamin K antagonist, and there was no possibility of attrition of susceptibles specific to phenprocoumon. Patients were eligible to participate when they were aged between 18 and 85 years and had an indication for long term anticoagulant treatment. For this group the same exclusion criteria were applied as was for the first group. After written informed consent they were randomized and switched to a treatment with either phenprocoumon or warfarin and follow-up was again 6 months.

All patients participating in the trial were part of the routine care in the Anticoagulation clinic. We obtained approval from Medical Ethics Review Committee of the Leiden University Medical Center before start of the study and all patients gave written informed consent before randomization. The trial is registered in the ISRCTN register with identifier ISRCTN60446748 (<http://www.controlled-trials.com>).

Analyses

We analysed all patients treated at the Leiden Anticoagulation clinic who finished the follow-up period at January 1st 2008. The primary outcome measure was quality of anticoagulant therapy, which was defined as the mean percentage of time spent in the therapeutic range. We used the linear interpolation method to determine this quantitative measure of quality of anticoagulant therapy [17]. Therapeutic ranges were as they were applied in our routine anticoagulant practice: an INR between 2.0 and 3.5 for most indications (i.e. venous thrombosis, atrial fibrillation) and between 2.5 and 4.0 for patients needing a high intensity of anticoagulation (e.g. patients with prosthetic heart valves). In case a patient had less than 2 INR measurements in total, no time in therapeutic range was calculated, and the patient was excluded. When the time between two INR measurements exceeded 9 weeks, no time in therapeutic range was calculated for this period, and this period was excluded.

Secondary outcome measures were the median time between visits, the time above and below the therapeutic range and the time needed to achieve an INR within range after start of treatment. Bleeding complications were classified as major when they were fatal or necessitated hospitalisation. Minor bleeding complications were all other bleeding events, in which ecchymoses were only counted when more than 10 cm in diameter and epistaxis only when the duration exceeded 30 minutes.

Statistical Analyses

All outcomes are shown as means or percentages with the corresponding 95% confidence interval of the difference based on t or binomial distributions or

medians with the corresponding interquartile range (IQR). All calculations were performed using the statistical package SPSS version 14.0 (SPSS Inc, Chicago, Ill).

Results

From March 1st 2004 to January 2008, 405 patients were included of whom 312 patients finished the study. One patient dropped out, while 75 had not completed the 6 months study duration. Of the 312 patients, 162 were randomised to the warfarin group and 150 to the phenprocoumon group. The study population consisted of 218 patients who initiated an oral anticoagulant therapy (112 warfarin group, 106 phenprocoumon group) and 94 patients who were already treated with oral anticoagulants (of whom 50 were switched to warfarin, and 44 to phenprocoumon).

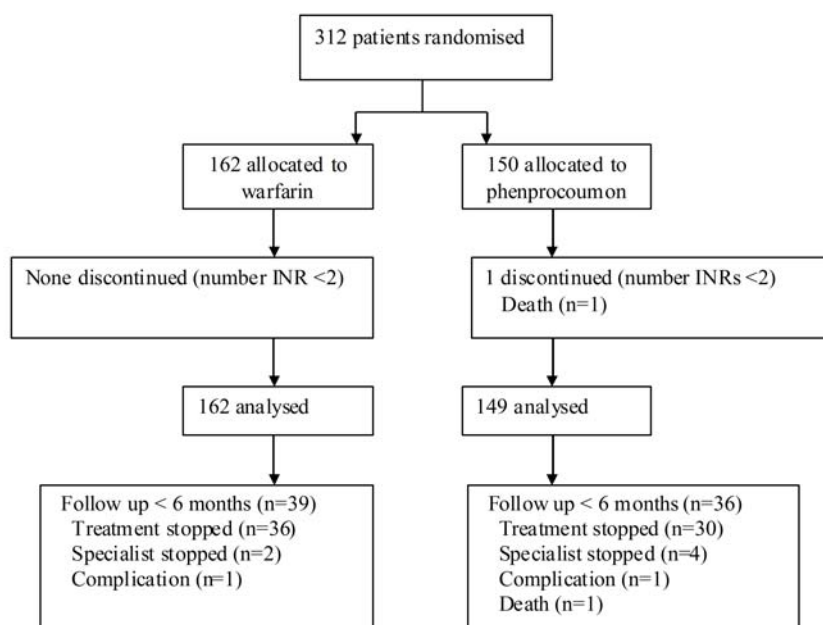


Figure 1. Enrollment, Randomisation and Data Analysis

One patient discontinued the treatment before the first INR measurement because of a bleeding complication (phenprocoumon group). The total follow up time was 119.3 person years, 63.2 person years in the warfarin group and 56.1 person years in the phenprocoumon group. Seventy five patients had a follow up shorter than 6 months for various reasons. Two patients stopped the treatment because of treatment-related complications (1 warfarin group, 1 phenprocoumon group) and 1 patient died of unknown cause (phenprocoumon group). Figure 1 summarises enrolment, randomisation, follow up and analysis of all patients.

Baseline characteristics are shown in table 1. There were more men than women (201 (62.2%) women versus 111 men (34.4%)), equally divided over the two treatment groups. Median age was 66 years (IQR 58-73). The majority of patients started with oral anticoagulants because of atrial fibrillation or venous thrombosis. Almost all patients had a target intensity of INR 2.5 – 3.5. Demographic characteristics, indication for oral anticoagulant treatment, and target intensity of anticoagulation did not differ between the two groups.

Table 1. Patient characteristics

	Patients treated with warfarin (n=162)	Patients treated with phenprocoumon (n=149)
Age		
Mean (IQR)	64.1 (59-73)	63.3 (56-74)
Sex		
Men (n, %)	110 (67.9)	91 (61.1)
Indication		
Atrial Fibrillation (n, %)	79 (48.8)	70 (47.0)
Venous thrombosis (n, %)	31 (19.1)	25 (16.8)
Heart valve prosthesis (n, %)	9 (5.6)	9 (6.0)
Other cardiac indication (n, %)	9 (5.6)	9 (6.0)
Prophylactic (n, %)	32 (19.8)	32 (21.5)
other (n, %)	2 (1.2)	4 (2.7)
Intensity (target)		
Low (2.5 – 3.5) (n, %)	144 (88.9)	129 (86.6)
High (3.0 – 4.0) (n, %)	15 (9.3)	20 (13.4)
Switched (n, %)	3 (1.8)	0 (0.0)
Group		
Initiating oral anticoagulant treatment	112 (69.1)	105 (70.5)
Switched from acenocoumarol	50 (30.9)	44 (29.5)

The median time spent within the therapeutic range was 74.7% (interquartile range (IQR) 58.8-87.0). Patients who switched from acenocoumarol to warfarin or phenprocoumon spent an equal amount of time in the therapeutic range as patients initiating treatment (75.8% versus 73.4%). There was no difference in time in therapeutic range between men and women (74.5% versus 75.1%). One hundred and nineteen patients (38.1%) spent more than 80% of the time within their therapeutic range, 228 (73.1%) spent more than 60% of time in range (figure 2).

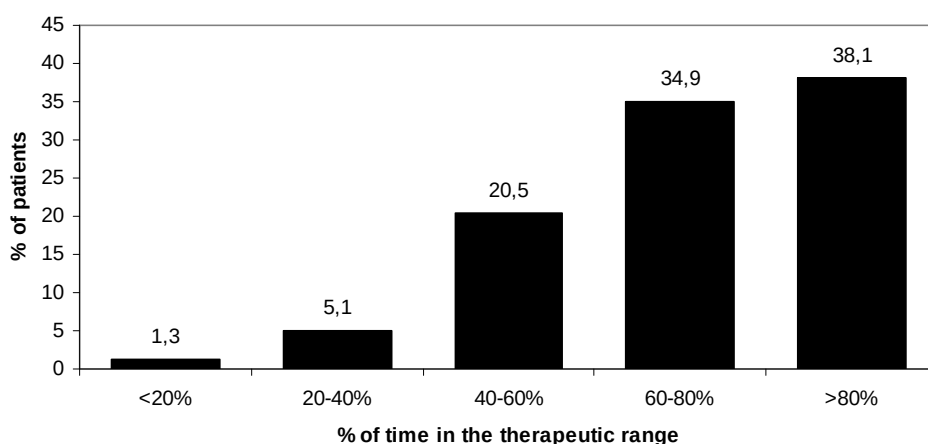


Figure 2. Percentage of time spent within therapeutic range.

Time between two visits is shown in figure 3. Overall, the median time between two visits was 11.7 days (IQR 10.0 – 13.9), with 59.2% of patients with a median time between two visits between 10 and 15 days.

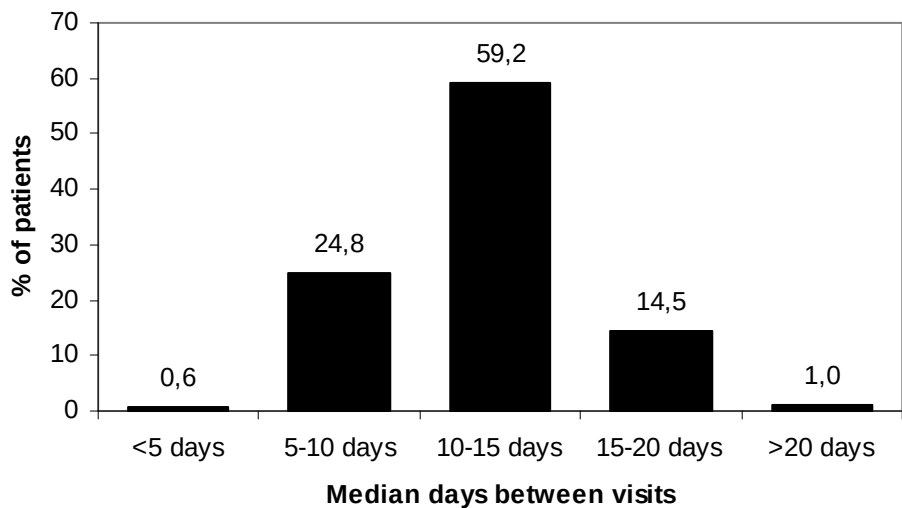


Figure 3. Median time between two visits

Discussion

In this study we described the study design of our trial of which the primary aim is to compare the quality of a treatment with warfarin to the quality of a treatment with phenprocoumon. In addition we gave an overview of the overall quality of anticoagulant treatment, without giving the results for the two treatment groups separately since the trial is ongoing. The overall quality of anticoagulant treatment was high, with a median time spent within the therapeutic range of 74.7% (IQR 58.8-87.0).

This study has several potential limitations. Firstly, in our anticoagulation clinic the majority of patients is treated with phenprocoumon. This may potentially bias the present comparison between anticoagulant treatment with phenprocoumon versus warfarin, since the physicians dosing the patients are experienced in dosing phenprocoumon, but inexperienced in dosing warfarin. We will investigate this phenomenon with an analysis in which we compare patients randomised to a

treatment with warfarin in the beginning of the trial to patients randomised to warfarin at the end of the trial to see whether there was a large learning effect in warfarin dosing among the physicians.

Secondly, this study was not blinded. Blinding of trials was introduced to ensure random allocation, to avoid selective drop-outs, to avoid differential co-treatment, and to avoid bias in the assessment of the outcome measures. It is believed that randomised controlled trials are optimally valid when patients as well as physicians or investigators are blinded for which treatment a patient is allocated to. In trials comparing vitamin K antagonists however, it is not possible without extended sham procedures to blind patients and physicians since the half life of the vitamin K antagonist is needed to determine the dosage optimally. We believe that at the level of the patient we did not introduce any bias as result of the non blindness of the trial. The outcome measure of this trial was the time spent in therapeutic range, i.e. the achieved INR. It is not likely that the patients' knowledge about the vitamin K antagonist he or she is taking is of any influence on the measured INR. It could in theory affect a patients' compliance to the treatment, but we do not have any reason to believe that the compliance will be different among the two groups. The knowledge of the physician to which treatment a patient is allocated to can be of influence on the time spent in therapeutic range. For example, a physician may be more cautious in one of the two treatment groups which would result in a shorter time between two INR measurements and less rigorous dosage changes. This would be evident in the data as a shorter time between two INR measurements in the group with the highest proportion of time spent in the therapeutic range.

The patients included in our trial are all out-patients, and therefore represent a selection of all patients treated with vitamin K antagonists with a relatively high health status, since a relevant proportion of patients treated with

vitamin K antagonists will be in-patients. Because this is a randomized trial this will not affect the validity of the results. In-patients are more likely to receive multiple and interacting medications and are more likely to have co morbidities. One may question therefore whether the results can be generalised to the total population of patients using vitamin K antagonists though there is no reason to assume this would not be the case

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CHAPTER 8

General Discussion & Summary

General Discussion & Summary

Although the quality of oral anticoagulant treatment has improved over time, there still is considerable risk to experience a thrombotic and haemorrhagic complication. The risk for complications increases when patients are either under- or overanticoagulated. Improvement in dosing strategies is warranted. The studies included in this thesis aim to optimise dosing of vitamin K antagonists and control of oral anticoagulant treatment.

Strategies for improvement of dosing of oral anticoagulants.

In our double-blind controlled trial comparing a simple dosing algorithm to an algorithm which incorporated the patients' sensitivity for vitamin K antagonists, we showed that there was no increase in quality of treatment, expressed as time spent within the therapeutic range (**chapter 2**). Also, the time between two visits was similar in both groups, although the interquartile range was broader for the new algorithm. However, the new algorithm was more efficient, as more dosage proposals were generated and accepted. For all visits combined, the standard algorithm generated an acceptable proposal in 55.3% of cases, which was 77.4% for the new algorithm. Finally, in almost all cases in which the standard algorithm did not result in a dosage proposal, a proposal was generated by the new algorithm. In these cases, the new algorithm performed as well as an unassisted physician.

In **chapter 3** we investigated the 'transition factors' between the maintenance dosages of three frequently used vitamin K antagonists for oral anticoagulant treatment. We observed that the maintenance dose of warfarin was 0.41 (95%CI 0.39- 0.43) times the maintenance dose of phenprocoumon. The transition factor between acenocoumarol and phenprocoumon was 0.84 (95%CI 0.79- 0.89) and between acenocoumarol and warfarin 1.85 (95%CI 1.78- 1.92).

In the prospective cohort study consisting of 220 Italian patients initiating oral anticoagulant treatment with acenocoumarol described in **chapter 4**, we showed that CYP2C9*3 was associated with a 25% dose reduction and an increased risk of over-anticoagulation (INR>6) on day 4. Two copies of the VKORC1*2 alleles were associated with a 45% dose reduction and an increased risk of over-anticoagulation. Both VKORC1*3 and VKORC1*4 homozygosity was associated with an increased dose requirement and a reduced risk of over-anticoagulation. The VKORC1*3 or *4 + CYP2C9*1 genotype combination was associated with the highest dose requirement and the lowest INR on day 4; VKORC1*2 + CYP2C9*3 with the lowest dose requirement, the highest INR and an increased risk of over-anticoagulation. Even though they spent approximately 50% of the time within the target therapeutic range, VKORC1*3 or *4 + CYP2C9*1 carriers spent a large percentage of the remaining time above and carriers of VKORC1*2 + CYP2C9*3 below the target range.

Relevance of improved dosing strategies for clinical practice

In recent years, interest in individualised dosing of pharmaceuticals based on a genetic profile has increased. For some drugs, genetic factors can be responsible for the majority of patient variability. Variability in response to vitamin K antagonists is in part mediated by genetic variability. In accordance with the results presented in chapter 4, several studies have reported variants in CYP2C9 and VKORC1 genes to be important for patients using warfarin or phenprocoumon, although there are some conflicting results [1-6]. Individualised dosing which incorporates the specific genotype of a particular patient could result in more appropriate dosages and less frequent over-anticoagulation in the initial phase of treatment. As we showed in chapter 4, patients who have a genotype which makes them more sensitive to vitamin K antagonists need lower maintenance dosages and

are at increased risk for overanticoagulation in the initial phase of treatment. It is likely that reducing the starting dosages of these patients results in a reduction of overanticoagulation, and thus a decreased risk for bleeding complications. However, knowledge of the specific genotype of a patient in the maintenance phase of treatment is less likely to result in an improvement of therapy as reflected by the time spent within the therapeutic range. As demonstrated in chapter 2, dosing during the maintenance phase assisted by a computerised algorithm which incorporated the sensitivity of a patient for the anticoagulant did not result in an increased quality of treatment. The sensitivity factor used by the algorithm was based on the patients' response to previous dosages. This response partly depends on the genotype of a particular patient, and therefore the effect of the genotype is already reflected in the calculated sensitivity factor.

Using one single algorithm for generation of dosage proposals is most efficient for daily clinical practice. An algorithm which incorporates the genotype of the patient may be beneficial compared to an algorithm without this information, due to a possible advantage in the initial phase of treatment. But, the response to vitamin K antagonists is not entirely dependent from a patient's genetic profile. Removing the variability due to the genetic profile still leaves a substantial variability in response to vitamin K antagonists and the effect on clinical endpoints may not outweigh the costs for genetic testing. A prerequisite for genotype-based dosing is the availability of bedside genotype tests, since the genotype is already required for the first dosing proposal. To investigate the potential benefit of genotype-based dosing a randomised controlled trial should be performed.

Using the calculated transition factors reported in chapter 3 physicians are able to estimate the new maintenance dosage in case a patient needs to switch from one vitamin K antagonist to another. However, two important limitations for the use of

these transition factors require attention. First, the genotype may also influence these transition factors, as from other studies it is known that sensitivity for different vitamin K antagonists varies per genotype. In theory, in a patient who has a genotype which results in an extreme sensitivity for warfarin but not for phenprocoumon, switching may result in either under- or over-coagulation depending on the direction of the switch. In our study described in chapter 3, we could not investigate this since DNA was not available. Second, we only determined the transition factor between maintenance dosages. Since switching from one vitamin K antagonist to another also requires a transition scheme, it remains unknown what dosages are needed in the initial days after switching for optimal safety. Only one other study investigated a dosage scheme for transition from phenprocoumon to warfarin in patients treated in an outpatient clinic [7]. Because of these two important limitations, patients who switch from one vitamin K antagonist to another require frequent monitoring during the initial phase after transition.

Future studies that investigate switching between different types of vitamin K antagonists should address the potential influence of genotype on the transition factors, as well as the optimal dosing scheme for transition.

Stability of oral anticoagulant treatment.

Besides improving dosing of oral anticoagulants, monitoring of patients is also important. If one could identify patients at high risk for complications, precautions can be taken to avoid these complications.

In a study of 630 subjects with mechanical heart valve prostheses we found that unstable anticoagulation was associated with an increased risk of haemorrhagic and thrombotic complications (**chapter 5**). Both variability and time spent outside the target range affected risk. The variance growth rate described by Fihn, method

A, incorporates both aspects of instability, and was therefore most clearly associated with complications of anticoagulant therapy, especially haemorrhagic episodes. Thrombotic events were most clearly predicted with variability calculated with method B1 and B2, which only concerns variability of the INR and not the time within range. The optimal time window to determine these measures was three months. In **chapter 6** we showed that unstable anticoagulation is observed more frequently with the use of acenocoumarol versus phenprocoumon, in individuals with daily strenuous physical activity, and less frequently in obese patients. Patients who are obese, have daily strenuous physical activity, or are frequent alcohol users have a higher risk of being more frequently out of range. Furthermore, increased anticoagulant dosage substantially reduced the risk of being frequently out of range.

Finally, in **chapter 7** we described the study design of our trial of which the primary aim is to compare the quality of a treatment with warfarin to the quality of a treatment with phenprocoumon. In addition we gave an overview of the overall quality of anticoagulant treatment, without giving the results for the two treatment groups separately since the trial is ongoing. The overall quality of anticoagulant treatment was high, with a median time spent within the therapeutic range of 74.7% (IQR 58.8-87.0).

The quality of oral anticoagulant treatment depends on a combination of the percentage of time spent within the therapeutic range and instability of anticoagulation. Both factors have their own risk factors and may require their own management.

The achieved intensity of anticoagulation was less important in predicting thrombotic events than haemorrhagic events. Variability, however, seems to be most predictive for thrombotic complications. This may be because time spent

outside the therapeutic range for the greater part consists of overanticoagulation rather than underanticoagulation. Highly variable INRs result in a disturbed balance between procoagulant and anticoagulant factors which may lead to a procoagulant state, although there is no clear explanatory mechanism. Initiation of oral anticoagulant treatment is associated with a temporary hypercoagulable state due to the difference in half life between pro- and anticoagulant factors. Each time the INR rises again in a patient who is unstably anticoagulated, a hypercoagulable state may be temporarily present as a result of a similar mechanism.

Other studies have also shown that variability is a risk factor for complications of anticoagulant treatment [8-10]. For daily clinical practice this means that physicians should not interpret an INR within the therapeutic range for a patient with highly variable INRs the same as an INR within range of a patient whose INRs are always within range.

Several studies have compared the quality of treatment with acenocoumarol to a treatment with warfarin or phenprocoumon. The results with respect to stability are mostly, but not always in favour of the longer acting vitamin K antagonists [11-19]. In our study described in chapter 6 acenocoumarol was shown to be a risk factor for unstable anticoagulation, which is also in accordance with an earlier study investigating risk factors for unstable anticoagulation [20]. Acenocoumarol should therefore only be used in patients in whom short acting drugs are preferred, such as women trying to get pregnant. In all other patients a longer acting vitamin K antagonist should be used, either warfarin or phenprocoumon. Which one of these two vitamin K antagonists results in the highest quality of treatment can be answered with the results of the trial described in chapter 7.

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CHAPTER 9

Samenvatting

Samenvatting

Ondanks dat de kwaliteit van de antistollingsbehandeling met vitamine K antagonisten door de jaren heen is verbeterd, bestaat er nog steeds het risico op het krijgen van een trombotisch- dan wel bloedingscomplicatie. Het risico op een complicatie neemt toe wanneer patiënten te sterk, dan wel niet sterk genoeg ontstold zijn. Verbeteringen in doseerstrategieën zijn daarom wenselijk. In dit proefschrift worden diverse onderzoeken beschreven die als doel hebben het doseren of controleren van de orale antistollingsbehandeling te optimaliseren.

Strategieën voor het verbeteren van doseren van orale anticoagulantia

In een dubbelblind gerandomiseerde gecontroleerde trial hebben we het doseren met behulp van een eenvoudig computer algoritme hebben vergeleken met doseren met behulp van een computer algoritme waarin de gevoeligheid van de patiënt voor vitamine K antagonisten geïncorporeerd was. In deze studie hebben we laten zien dat de kwaliteit van de behandeling niet toeneemt bij gebruik van het nieuwe algoritme, gemeten aan de hand van het percentage tijd dat in de therapeutische zone is doorgebracht (**hoofdstuk 2**). Ook de tijd tussen twee INR controles was gelijk in beide groepen, hoewel de interquartile range (IQR) wijder was bij het nieuwe algoritme. Aan de andere kant was het nieuwe algoritme wel efficiënter omdat deze meer doseervoorstellen genereerde en de voorstellen ook vaker door de arts geaccepteerd werden. Het standaard algoritme genereerde een acceptabel voorstel in 55.3% van de gevallen, tegen 77.4% door het nieuwe algoritme. Tot slot, in bijna alle gevallen waarbij het standaard algoritme niet in staat was een doseervoorstel te genereren, was dit wel het geval bij het nieuwe algoritme. In deze gevallen was het nieuwe algoritme net zo goed als een arts zonder de hulp van een computer algoritme.

Soms moet een patient switchen van vitamine K antagonist vanwege een overgevoeligheidsreactie of een zwangerschapswens, waarbij een langwerkend middel niet geprefereerd wordt. In **hoofdstuk 3** hebben we omrekeningsfactoren onderzocht tussen de onderhoudsdoseringen van de 3 meest gebruikte vitamine K antagonisten. We observeerden dat de onderhoudsdosering van warfarine 0.41 (95%BI 0.39-0.43) keer de onderhoudsdosering van fenprocoumon was. De omrekeningsfactor tussen acenocoumarol en fenprocoumon was 0.84 (95%BI 0.79-0.89) en tussen acenocoumarol en warfarine 1.85 (95%BI 1.78- 1.92).

In een prospectief cohort onderzoek met 220 Italiaanse patiënten die startten met een orale antistollingsbehandeling met acenocoumarol beschreven in **hoofdstuk 4**, laten we zien dat CYP2C9*3 geassocieerd was met een 25% dosis reductie en een verhoogd risico op een doorgeschoten antistolling (INR>6) op dag 4. Twee kopieën van de VKORC1*2 allelen waren geassocieerd met een 45% dosis reductie en een verhoogd risico een doorgeschoten antistolling. Zowel VKORC1*3 als VKORC1*4 homozygotie was geassocieerd met een verhoogde dosis behoefte en een verlaagd risico op doorgeschoten antistolling. Het VKORC1*3 of *4 en CYP2C9*1 genotype gecombineerd was geassocieerd met de hoogste dosis behoefte en de laagste INR op dag 4; VKORC1*2 en CYP2C9*3 met de laagste dosis behoefte, de hoogste INR en een verhoogd risico op een doorgeschoten antistolling. Hoewel ze beiden ongeveer 50% van de tijd binnen de therapeutische zone zijn, zijn dragers van VKORC1*3 of *4 + CYP2C9*1 een hoger percentage van de overgebleven tijd boven de therapeutische zone, terwijl dragers van VKORC1*2 + CYP2C9*3 een hoger percentage van de tijd beneden de therapeutische zone zijn.

Relevantie van verbeterde doseerstrategieën voor de klinische praktijk

In de afgelopen jaren is interesse in het doseren van medicijnen op individuele basis gebaseerd op het genetische profiel toegenomen. Voor sommige medicijnen kunnen genetische factoren voor een belangrijk deel verantwoordelijk zijn voor van de variabiliteit bij patiënten. Ook de variabiliteit in de respons op vitamine K antagonisten kan gedeeltelijk worden verklaard door genetische variabiliteit. In overeenstemming met de resultaten gepresenteerd in hoofdstuk 4 hebben verschillende andere studies gerapporteerd dat variaties van CYP2C9 en VKORC1 belangrijk zijn voor patiënten die warfarine of fenprocoumon gebruiken, alhoewel dit niet in alle studies gevonden is. Een patiënt-specifiek doserings advies dat rekening houdt met het specifieke genotype van de patiënt leidt mogelijk tot betere doseeradviezen en minder frequent doorschieten van antistolling in de startfase van de behandeling. Zoals we hebben laten zien in hoofdstuk 4, hebben patiënten met een genotype dat hen gevoeliger maakt voor vitamine K antagonisten een lagere onderhoudsdosering en hebben zij een verhoogd risico op het doorschieten van de antistolling in de beginfase van behandeling. Het is waarschijnlijk dat het reduceren van de startdoseringen van deze patiënten resulteert in het minder frequent doorschieten van de antistolling, en dus mogelijk een verlaagd risico op bloedingscomplicaties. Echter, kennis van het specifieke genotype van de patiënt is waarschijnlijk minder van belang in de onderhoudsfase van de behandeling. Zoals beschreven in hoofdstuk 2 resulteerde het doseren met behulp van een computer algoritme waarin de gevoeligheid voor het antistollingsmiddel geïncorporeerd was niet tot een verhoogde kwaliteit van de behandeling in de onderhoudsfase. De gevoeligheidsfactor die gebruikt werd door het algoritme was gebaseerd op de respons van de patiënt op eerdere doses. Deze respons hangt gedeeltelijk af van het genotype van de specifieke patiënt waardoor het effect van het genotype al opgenomen is in de berekende gevoeligheidsfactor.

Het gebruiken van één algoritme voor het generen van doseervoorstellen is het meest efficiënt voor de dagelijkse klinische praktijk. Een algoritme dat rekening houdt met het genotype van de specifieke patiënt is mogelijk geschikter dan een algoritme zonder deze informatie, door het mogelijke voordeel in de startfase van behandeling. Echter, de respons op vitamine K antagonisten is niet alleen maar afhankelijk van het genetisch profiel van de patiënt. Het verwijderen van de variabiliteit in de respons veroorzaakt door het genetisch profiel, laat nog een substantieel gedeelte van de variabiliteit in de respons op vitamine K antagonisten over en het effect op klinische eindpunten weegt mogelijk niet op tegen de kosten van het genotyperen. Een voorwaarde voor het genotype gemedieerde doseren is het beschikbaar zijn van sneltesten, aangezien het genotype al voorhanden moet zijn voor het eerste doseeradvies. Om dit mogelijke voordeel van genotype gemedieerd doseren te onderzoeken zou er een gerandomiseerde gecontroleerde trial uitgevoerd moeten worden.

Met behulp van de berekende vermenigvuldigingsfactoren gerapporteerd in hoofdstuk 3 kunnen artsen de nieuwe onderhoudsdosering schatten wanneer een patiënt van één vitamine K antagonist naar een andere moet wisselen. Echter, er zijn twee belangrijke beperkingen voor gebruik van deze vermenigvuldigingsfactoren. Allereerst, het genotype van de patiënt kan van invloed zijn op de vermenigvuldigingsfactor, omdat uit andere onderzoeken bekend is dat de gevoeligheid voor verschillende vitamine K antagonisten per genotype verschilt. In theorie kan wisselen bij een patiënt met een genotype dat resulteert in een extreme gevoeligheid voor warfarine maar niet voor fenprocoumon, resulteren in een te lage dan wel een doorgeschoten antistolling, afhankelijk van de richting van het wisselen. In onze studie beschreven in hoofdstuk 3 konden we dit helaas niet onderzoeken omdat van deze patiënten geen DNA beschikbaar was. Ten

tweede hebben we alleen vermenigvuldigingsfactoren berekend voor de onderhoudsdosering. Aangezien voor het wisselen van de ene vitamine K antagonist naar de andere ook een overgangsschema nodig is, blijft onbekend welke doses in de eerste dagen na het wisselen nodig zijn voor een optimale veiligheid. Slechts één andere studie heeft een overgangsschema voor het wisselen van fenprocoumon naar warfarine onderzocht in poliklinische patiënten. Vanwege deze twee belangrijke beperkingen, zal in de eerste dagen na het wisselen toch frequente INR controle nodig zijn.

Toekomstige studies die het wisselen tussen verschillende types vitamine K antagonisten onderzoeken moeten aandacht besteden aan de invloed van het genotype op de vermenigvuldigingsfactor, alsmede aan het optimale doseringsschema voor de periode vlak na het wisselen.

Stabiliteit van orale antistollingsbehandeling.

Naast het verbeteren van het doseren van antistollingsmiddelen, is het monitoren van patiënten ook belangrijk. Wanneer men in staat is patiënten te identificeren die een verhoogd risico hebben op complicaties, kunnen er voorzorgsmaatregelen worden getroffen teneinde deze complicaties te voorkomen.

In een onderzoek met 630 deelnemers met mechanische hartklep prothesen vonden we dat instabiele antistolling geassocieerd was met een verhoogd risico op bloedings- en trombotische complicaties (**hoofdstuk 5**). Zowel instabiliteit als de tijd buiten de therapeutische zone hadden invloed op dit risico. Er bestaan diverse methoden om instabiliteit weer te geven. De variance growth rate beschreven door Fihn, methode A, houdt rekening met beide aspecten van instabiliteit, en was waarschijnlijk daardoor het meest geassocieerd met complicaties van antistollingsbehandeling, vooral bleedingcomplicaties. Trombotische complicaties werden het best voorspeld met de variabiliteit berekend met methode B1 en B2,

een methode die alleen de variabiliteit weergeeft van de INR en niet de tijd doorgebracht in de therapeutische zone. Het optimale tijdwindow om deze maten te berekenen was 3 maanden. In **hoofdstuk 6** hebben we laten zien dat instabiele antistolling vaker gezien wordt bij patiënten die acenocoumarol gebruiken ten opzichte van fenprocoumon gebruikers, bij patiënten die dagelijks zware lichamelijke activiteit hadden en minder vaak in obese patiënten. Patiënten met obesitas, dagelijkse zware lichamelijke activiteit of patiënten die frequent alcohol gebruiken hadden een verhoogd risico meer tijd buiten de therapeutische zone door te brengen. Verder hadden patiënten met een hoge dosis van hun antistollingsmiddel een substantieel lager risico om vaak buiten de therapeutische zone te zijn.

Tenslotte beschrijven we in **hoofdstuk 7** het studie design van onze trial waarin het hoofddoel is de kwaliteit te vergelijken van een antistollingsbehandeling met warfarine ten opzichte van fenprocoumon. Tevens geven we hier een overzicht van overall kwaliteit van de antistollingsbehandeling, zonder de resultaten van beide groepen weer te geven omdat de trial nog niet beëindigd is. De kwaliteit was hoog, met een mediane tijd doorgebracht in de therapeutisch zone van 74.7% (IQR 58.8-87.0).

De kwaliteit van een orale antistollingsbehandeling hangt af van een combinatie van het percentage tijd doorgebracht in de therapeutische zone en de instabiliteit van de antistolling. Beide aspecten van de kwaliteit van de behandeling hebben hun eigen risicofactoren en beide aspecten hebben wellicht een geïndividualiseerde aanpak nodig.

De bereikte intensiteit van de antistolling was minder belangrijk in het voorspellen van trombotische complicaties dan van bloedingscomplicaties. De variabiliteit lijkt echter het meest voorspellend te zijn voor trombotische

complicaties. Dit kan veroorzaakt worden omdat de tijd doorgebracht buiten de therapeutische zone voor het grootste gedeelte bestaat uit doorgeschoten antistolling in plaats van antistolling beneden de therapeutische zone. Hoge variabele INRen resulteren in een verstoorde balans van procoagulante en anticoagulante factoren en dit kan leiden tot een tijdelijke procoagulante status, alhoewel daar nog geen duidelijk verklarend mechanisme voor is. Het starten met een orale antistollingsbehandeling is geassocieerd met een tijdelijke hypercoagulabele status, vanwege de verschillen in halfwaarde tijd van de pro- en anticoagulante factoren. Iedere keer wanneer de INR stijgt in een instabiel ontstolde patiënt kan deze hypercoagulabele staat aanwezig zijn als gevolg van eenzelfde mechanisme.

Andere studies hebben ook laten zien dat variabiliteit een risico factor is voor complicaties van orale antistollingsmedicijnen. Voor de dagelijkse klinische praktijk betekent dit dat artsen een INR binnen de therapeutische zone van een patiënt met zeer variabele INRen niet hetzelfde moeten interpreteren dan een INR in de therapeutische zone van een patiënt waarvan de INR altijd binnen de zone zijn.

Verskillende onderzoeken hebben de kwaliteit van een antistollingbehandeling met acenocoumarol vergeleken met een behandeling met warfarine of fenprocoumon. De resultaten zijn meestal, maar niet altijd in het voordeel van de langer werkende vitamine K antagonisten. In onze studie beschreven in hoofdstuk 6 was het gebruik van acenocoumarol een risico factor voor instabiele antistolling, wat overeen komt met de resultaten van een eerdere studie die risico factoren voor instabiele antistolling onderzocht heeft. Acenocoumarol zou daarom alleen gebruikt moeten worden in patiënten bij wie kortwerkende medicijnen de voorkeur verdienen, zoals vrouwen met een zwangerschapswens. Bij alle andere patiënten

heeft een langer werkende vitamine K antagonist de voorkeur, zowel warfarine of fenprocoumon. Welke van deze 2 vitamine K antagonisten resulteert in de hoogste kwaliteit van de behandeling kan beantwoord worden met de resultaten van de trial beschreven in hoofdstuk 7.

Dankwoord

Curriculum Vitae

Dankwoord

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Curriculum Vitae

Yvonne van Leeuwen is op 03 oktober 1979 geboren te Delft. In 1998 werd het gymnasium diploma behaald aan het Christelijk Lyceum in Delft. Aansluitend begon zij de studie Biomedische Gezondheidswetenschappen aan de Radboud Universiteit Nijmegen. Met als hoofdvak ‘Epidemiologie’ en bijvakken ‘Evaluatie in de gezondheidszorg’ en ‘Infectieziekten’ liep zij onder andere stage bij de afdeling Klinische Epidemiologie van het Leids Universitair Medisch Centrum, het Kwaliteitsinstituut voor de gezondheidszorg CBO en de Inspectie van Infectieziekten, Landelijke coördinatiecentrum Infectieziektebestrijding. In december 2002 behaalde zij het doctoraalexamen. Hierna is zij als promovenda aangesteld op de afdeling Klinische Epidemiologie van het Leids Universitair Medisch Centrum onder leiding van prof. dr F.R. Rosendaal en dr F.J.M. van der Meer. Het onderzoek waarvan de resultaten staan beschreven in dit proefschrift werd in samenwerking met de Trombosedienst Leiden uitgevoerd. Een gedeelte is tot stand gekomen door een samenwerking met het Bianchi Bonomi Hemophilia and Thrombosis Center, Universiteit van Milaan, Italië. Per augustus 2007 is de auteur in dienst als postdoc onderzoeker op de afdeling Huisartsgeneeskunde van het Erasmus Medisch Centrum onder leiding van dr. M.Y. Berger. Hier verricht zij onderzoek binnen de onderzoekslijn “Aandoeningen bij kinderen in de huisartsenpraktijk” met een focus op gastrointestinale klachten.