EFFECTS OF *CYP2C9* AND *VKORC1* POLYMORPHISMS AND DRUG INTERACTIONS ON COUMARIN ANTICOAGULATION CONTROL

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EFFECTS OF *CYP2C9* AND *VKORC1* POLYMORPHISMS AND DRUG INTERACTIONS ON COUMARIN ANTICOAGULATION CONTROL

EFFECTEN VAN *CYP2C9* EN *VKORC1* POLYMORFISMEN EN GENEESMIDDELINTERACTIES OP DE ANTISTOLLINGS-BEHANDELING MET COUMARINEDERIVATEN

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. W.H. Gispen, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op maandag 7 mei 2007 des middags te 2.30 uur

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Voor Tineke
Paul
Marianne
Frank
Wouter

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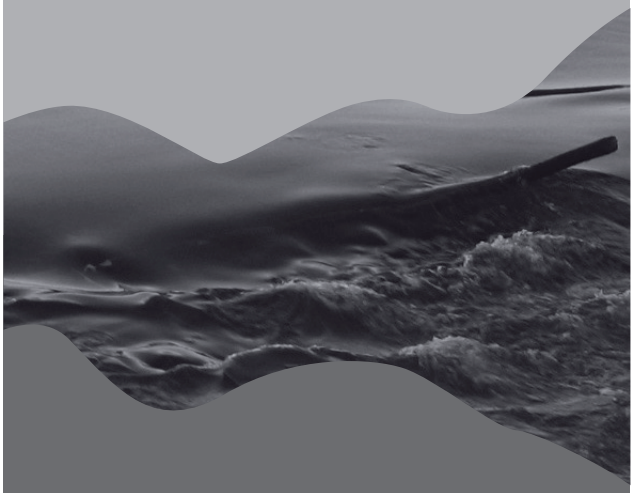
CHAPTER 1



INTRODUCTION

INTRODUCTION

Treatment with oral anticoagulants of the coumarin type (vitamin K antagonists or coumarins) for the prevention of venous and arterial thromboembolism has a history of almost 60 years. Although the effectiveness of coumarins in the prevention of thromboembolism is well established, these drugs are potentially dangerous because of their narrow therapeutic index. The effective dose lies uncomfortably close to the dose at which the risk of disabling or lethal major bleeding, the most feared complication of coumarin therapy, is strongly increased. Therapy with coumarins is further complicated by its unpredictability, dose requirements varying interindividually as well as intraindividually over time. Finding the right balance between the indisputable benefits and risks of coumarin therapy is still a matter of concern,[1,2] and research into factors explaining its variability also has a long history and is still ongoing. This thesis is a contribution to this research area.

Coumarin therapy: clinical applications, mechanism of action, and monitoring

The therapeutic effectiveness of coumarins has been established for the primary and secondary prevention of venous thromboembolism, for the prevention of systemic embolism and stroke in patients with prosthetic heart valves, mitral valve disease or atrial fibrillation, for the prevention of recurrent infarction, stroke or death in patients with acute myocardial infarction, and for the primary prevention of myocardial infarction in high risk men.[3] The most common indication is atrial fibrillation, a cardiac dysrhythmia with a strongly increased risk of stroke, of which coumarins not only reduce the frequency with 60 to 70%,[4,5] but also the severity and mortality,[6] and for which the therapeutic superiority of coumarins over a combination of the potentially safer antiplatelet drugs aspirin and clopidrogrel has been convincingly established.[7]

Coumarins are antagonists of vitamin K, a fat-soluble vitamin that is essential for the formation of clotting factors II (prothrombin), VII, IX, and X of the coagulation cascade. These clotting factors are glycoproteins with glutamic acid residues (Glu), which are transformed by γ-carboxylation into γ-carboxyglutamic (Gla) residues. Calcium binding of the Gla residues leads to the conformational changes that are needed for their effects in the coagulation cascade.[3,8-10] The γ-carboxylation step is catalysed by the vitamin K dependent enzyme γ-carboxylase, the reduced form of vitamin K (vitamin K-hydroquinone) serving as a cofactor. During the carboxylation step vitamin K-hydroquinone is oxidized to

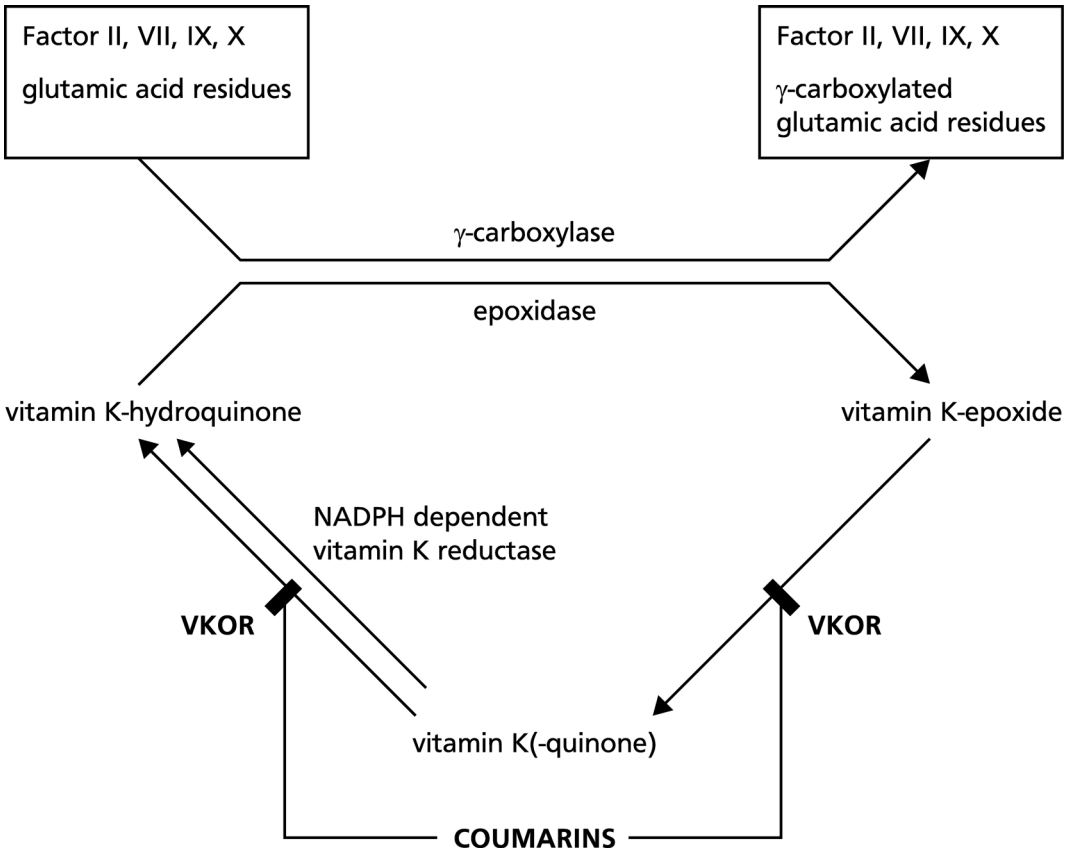
vitamin K 2,3-epoxide. Vitamin K-epoxide has to be rapidly reduced to prevent a vitamin K shortage in tissues. The first step in this reduction process is catalysed by the vitamin K epoxide reductase complex (abbreviated as VKOR) (Figure 1). All coumarins inhibit VKOR, which is the basis for their anticoagulant effect. In contrast to the first step in the reduction process, the second step from vitamin K to vitamin K-hydroquinone is not exclusively catalysed by VKOR, but also by a NADPH dependent reductase. As a consequence the reduction from vitamin K-epoxide to vitamin K is more sensitive to coumarins than the reduction from vitamin K to vitamin K-hydroquinone. But by exclusively inhibiting the first reduction step, the production of vitamin K hydroquinone is reduced to such an extent that γ-carboxylation of vitamin K dependent coagulation factors is effectively decreased. The onset of action of coumarins takes several days, because the decrease of carboxylated clotting factors to sufficiently low levels depends on their half-lives, ranging from 0.25 days for factor VII to 2.5 days for prothrombin (factor II).[11]

Coumarin treatment is usually monitored by the Prothrombin (PT) test, which is indicative for a reduction of the carboxylated factors II, VII, and X following coumarin use.[3] Because the PT test requires thromboplastins which vary in their responsiveness to vitamin K dependent coagulation factors, PT values are not a suitable standard measure for anticoagulation. For a standardized expression of the degree of anticoagulation the International Normalized Ratio (INR) system has been adopted, in which INR is assessed as follows:

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

In this formula ISI is the International Sensitivity Index, a factor correcting for the responsiveness of the used thromboplastin and for the available instrument.[3] The intensity of coumarin treatment depends on the indication. The Dutch federation of anticoagulation clinics (FNT) proposes the following levels: the first intensity group, therapeutic range INR 2.0-3.5, and the second intensity group, therapeutic range 2.5-4.0. Both levels are based on studies in patients with atrial fibrillation[5,6,12] and mechanical heart valves,[13] respectively. Maintaining patients within the therapeutic range is difficult, which is reflected by the fact that coumarins are the only drug group for the monitoring of which specialized institutions have been set up: anticoagulation clinics ('thrombosis services', 'trombosediensten' in Dutch). There is evidence that anticoagulation clinics improve the quality of anticoagulation and are cost saving by preventing major bleeding and thromboembolic events compared with usual medical care.[14-19]

Figure 1: THE VITAMIN K CYCLE AND MECHANISM OF ACTION OF COUMARINS



VKOR = vitamin K-epoxide reductase

The Netherlands have a dense network of 61 regional anticoagulation clinics, monitoring about 300 000 patients. For coumarin dose adjustments on the basis of INR measurements computerized dose-algorithms are available, but the ability of the physicians to manage dose adjustments and good concordance between physician and patient are also essential factors for a safe coumarin therapy.[20] During the year 2005 the frequency of INR monitoring in Dutch anticoagulation clinics varied from 13.3 to 23.8 INR measurements per patient (median value 18.6). In this period, a mean of 78.5% of the patients from the first intensity group and 73.5% of patients from the second intensity group were within the therapeutic range, requirements of the federation of anticoagulation clinics being 70% for the first and 65% for the second group (annual report 2005 of the Dutch federation of anticoagulation clinics, accessible at www.fnt.nl). Although these figures are encouraging, they also accentuate the difficulties in

achieving an adequate treatment in all users of coumarins, despite the increased experience with these drugs and despite the improvement of treatment with the introduction of dosing nomograms.[21] In other words, coumarin therapy is complicated by several factors modifying the dose-effect relationship and resulting in large interindividual and intraindividual differences in dose requirement. Before discussing these factors, several of them being subjects of this thesis, we will give a short survey of the available coumarins and their pharmacokinetics.

Table 1: TERMINAL ELIMINATION HALF-LIVES, MAIN HYDROXYLATION PRODUCTS, AND MAIN METABOLIZING CYP ISOENZYMES OF THE (S)- AND (R)-ENANTIOMERS OF WARFARIN, ACENOCOUMAROL, AND PHENPROCOUMON[a]

Enantiomer	Elimination half-life (hours)[22]	Hydroxylation product	Metabolizing CYP isoenzymes	Ref
(S)-warfarin	24-33	4′-OH 6-OH **7-OH**	2C8, 2C19 2C9 2C9	73
(R)-warfarin	35-58	4′-OH **6-OH** 7-OH **8-OH** 10-OH	2C8, 2C19 1A2, 2C19 1A2, 2C8 1A2, 2C19 3A4	73
(S)-acenocoumarol	1.8[b]	6-OH **7-OH** 8-OH	2C9 2C9 2C9	74
(R)-acenocoumarol	6.6	6-OH 7-OH 8-OH	2C9 2C9 (50%), 1A2, 2C19 2C9, 2C19	74
(S)-phenprocoumon	110-130	4′-OH 6-OH **7-OH**	2C8, 2C9, 3A4 2C9 (60%), 3A4 2C9 (65%), 3A4	75
(R)-phenprocoumon	110-125	4′-OH 6-OH 7-OH	3A4 2C9 (50%), 3A4 2C9 (50%), 3A4	75

Ref = reference, studies of the hydroxylation routes and contributing metabolizing enzymes in vitro; bold print = major metabolic pathway for the enantiomer or major metabolizing enzyme within a hydroxylation route

a) Table is derived from the comparative review of Ufer[22] and modified and supplemented according to the indicated references.

b) Elimination half-life for the *CYP2C9*1/*1* genotype. In carriers of at least one **3* allele, elimination half-life is increased to 9 hours in vivo.[24]

Coumarins: substances and pharmacokinetics

Worldwide three coumarins are used: warfarin, acenocoumarol, and phenprocoumon. This non-alphabetical order is deliberately chosen, because warfarin is by far the most commonly used and best documented of the coumarins. In North America and the United Kingdom warfarin is the principal coumarin, whereas acenocoumarol and phenprocoumon are used on the European continent. In the Netherlands two coumarins are licensed: acenocoumarol and phenprocoumon, of which acenocoumarol is used most. (80.6% according to the annual report 2005 of the Dutch federation of anticoagulation clinics, www.fnt.nl).

All coumarins are 4-hydroxycoumarins with one chiral centre, each coumarin having a (S)- and a (R)-enantiomeric form. For each of the coumarins the (S)-form has a 2- to 5-fold higher anticoagulant potency than the (R)-form.[22] The coumarins are administered as racemic mixtures, consisting of 50% of each of the enantiomers.

The (S)- and (R)-enantiomers of warfarin and phenprocoumon, and (R)-acenocoumarol are rapidly and fully absorbed from the gastrointestinal tract, with a nearly complete bioavailability.[22] (S)-acenocoumarol undergoes extensive first pass metabolism and has a very low bioavailability in most patients (circa 6%), which is much higher in carriers of a *CYP2C9*3* allele (see below).[23] All coumarins are for 98 to 99% bound to plasma albumin. Warfarin and phenprocoumon undergo first-order elimination, acenocoumarol biphasic elimination. Terminal elimination half-lives differ between the coumarins and also between the (S)- and (R)-enantiomers (Table 1). All coumarins undergo extensive, stereoselective metabolism in the liver by hydroxylation reactions into inactive metabolites. These reactions are catalysed by the superfamily of cytochrome P450 enzymes (CYPs). Although there are differences between the metabolic pathways of all enantiomers, there are also notable similarities (Table 1). Differences are most striking for the less active (R)-enantiomers, which are metabolized by a variety of CYP-isoenzymes. However, the more active (S)-enantiomers are mainly metabolized by CYP2C9, although CYP3A4 also participates in the metabolism of (S)-phenprocoumon. Both enantiomers contribute to the pharmacodynamic effect of warfarin and phenprocoumon, but in users of acenocoumarol the anticoagulant effect depends mainly on the (R)-enantiomer, because of the extremely short elimination half-life of its (S)-counterpart (<2 hours). However, in users with a **3* polymorphism for the gene

encoding CYP2C9 (see below), (S)-acenocoumarol does contribute to the anticoagulant effect because the elimination half-life is increased to nine hours.[24]
It is not possible to indicate a first choice coumarin on the basis of available evidence. Because of the difficulties in management of coumarin therapy, a physicians' experience with handling one coumarin can be considered a theoretical advantage. Publicized studies, which have investigated differences between coumarins, are inconclusive. Warfarin has been compared with acenocoumarol in two studies. One study, conducted in the setting of an Italian anticoagulation clinic, showed no difference between warfarin and acenocoumarol,[25] whereas another Italian study showed a more stable anticoagulation with warfarin, without comparing the safety (bleedings!) and efficacy of both drugs.[26] A recent Dutch study claimed an advantage for phenprocoumon over acenocoumarol, because of a more stable anticoagulation and no difference in major bleeding complications, although significantly more minor bleeding complications in phenprocoumon users were reported.[27] The annual report 2005 of the Dutch federation of anticoagulation clinics confirms a higher percentage of phenprocoumon users within the therapeutic range, although a reservation is made because of lack of data on efficacy and major complications (www.fnt.nl).

Variability in the anticoagulation response to coumarins

One of the challenging aspects of drug use in daily practice is the different response of individuals to the same drug. A dosage regimen of a coumarin that protects one subject effectively against thromboembolic events can be insufficiently protective in another subject and can cause a lethal bleeding in a third. It is even possible that a subject is effectively protected against thrombo-embolic events for many years without any adverse effects and that the same coumarin, used to advantage in the same dosage for many years, suddenly gives rise to an invalidating intracranial bleeding. The response to drugs can vary between individuals (interindividual variability) and within individuals (intraindividual variability), which is reflected by differing dose requirements. The interindividual variability results in a 10-fold dosage range for all three coumarins (Table 2). Usually the intraindividual variability is smaller, although some factors like drug interactions or intercurrent diseases can necessitate large dose adjustments.
Part of the interindividual variability in coumarin response can be explained by genetic factors, but there is a considerable contribution of additional factors. One

source of variability is the presence of polymorphisms of the gene encoding CYP2C9, the main metabolizing enzyme of the (S)-enantiomers of coumarins. Quantitatively less important genetic factors are warfarin resistance, attributed to an altered affinity of the warfarin receptor and a mutation in the factor IX propeptide, occurring in <1.5% of the population and increasing the risk of bleeding during coumarin therapy.[3] Theoretically, variations in genes encoding VKOR, γ-carboxylase and the clotting factors II, VII, IX, and X could also affect coumarin response (Figure 1).

Table 2: MAINTENANCE DOSAGES OF COUMARINS[22]

Coumarin	Maintenance dose (mg/day)
warfarin	1.5-12
acenocoumarol	1-9
phenprocoumon	0.7-9

Important additional factors contributing to the variability in coumarin response are age,[28] compliance, variations in vitamin K intake, several disease states, and drug interactions. Vitamin K intake has been identified as a major independent factor interfering with anticoagulation stability,[29] some studies suggesting that a constant intake of low dose vitamin K could contribute to a more stable anticoagulation in unstable patients.[30,31] An obvious effect on coumarin response is established for the following disease states: hepatic disorders, by reducing synthesis of clotting factors;[32] thyroid disorders, probably by changing the catabolism of clotting factors;[32] and heart failure, probably by causing hepatic congestion.[33] Less well established, but suspected disease states are fever, possibly by increasing degradation of clotting factors;[32,34] and malignancies.[32] Of these additional factors drug interactions are of major importance in daily practice.
Drug interactions with coumarins and genetic variations in the gene encoding CYP2C9 will be discussed in more detail below.

Drug interactions with coumarins

Coumarins are highly sensitive to drug interactions, which can affect their pharmacokinetics and pharmacodynamics (Table 3). Because of the narrow therapeutic range of coumarins relatively minor changes in pharmacokinetics or pharmacodynamics have the potency to result in highly relevant adverse outcomes like recurrent thromboembolism or major bleeding. A recent

descriptive study showed that 54% of the patients with atrial fibrillation discharged on warfarin were prescribed at least one other drug that could increase the bleeding risk.[35]

Table 3: INTERACTIONS OF COUMARIN ANTICOAGULANTS: MECHANISMS, EXAMPLES, AND MANAGEMENT

	Examples	Management
Pharmacokinetic		
absorption level	colestyramine	separate dosages
distribution level	—	—
elimination level		
enzyme inhibition	CYP2C9-inhibitors: miconazole, sulphamethoxazole (in co-trimoxazole), phenylbutazone, amiodarone, benzbromarone, gemfibrozil	avoid concurrent use or increase coumarin dosage
enzyme induction	carbamazepine, phenobarbiton, phenytoin, rifampicin	avoid concurrent use or increase coumarin dosage
Pharmacodynamic		
change of blood coagulation	antiplatelet drugs (aspirin, clopidogrel), NSAIDs, heparin	avoid concurrent use or weigh risk against expected benefits
change of thyroid function	thyroid or antithyroid drugs	adjust coumarin dosage

Pharmacokinetic interactions can affect absorption, distribution or elimination of coumarins. The most important absorption interaction occurs with the hardly used bile-acid binding resin colestyramine.[36] The potential reduction of the coumarin effect can be easily avoided by careful separation of the dosages. It has long been thought that protein-binding displacement interactions, which occur in the distribution phase, are of major importance to coumarins. This was probably based on their high degree of binding to plasma albumin, which after displacement by another albumin binding drug would result in a relatively high rise in unbound coumarin concentration. However, the pharmacodynamic effect of such an interaction will be transient because displacement is also accompanied by an increase of elimination, clearance being directly proportional to the free coumarin concentration.[37,38] Whereas pharmacokinetic interactions affecting absorption and distribution have a limited importance in daily practice, interactions at the level of elimination are highly relevant. As we pointed out

earlier, coumarins are extensively hydroxylated by different cytochrome isoenzymes, of which CYP2C9 is the principal metabolizing enzyme for the more active (S)-enantiomers of warfarin and acenocoumarol, and (to a lesser extent) of phenprocoumon (Table 1). As a consequence interactions with strong CYP2C9-inhibiting drugs such as miconazole, phenylbutazone, and sulphamethoxazole (in cotrimoxazole)[39] have a high potency for being clinically relevant and serious interactions with warfarin have been described for all three CYP2C9 inhibitors.[40-47] The potency for serious interactions with strong inhibitors of other CYP isoenzymes is doubtful, because the non-CYP2C9 metabolic pathways are mostly catalysed by multiple enzymes, inhibition of one of them not considerably affecting overall metabolism (see Table 1). However, if the activity of CYP2C9 metabolic pathways is decreased (by genetic causes or by a CYP2C9 inhibitor), it is thinkable that strong CYP3A4 inhibition could severely affect the metabolism of (S)- and (R)-phenprocoumon (see Table 1). Other drugs causing relevant interactions on the elimination level are enzyme-inducing agents like carbamazepine or rifampicin, which can decrease the anticoagulant effects of coumarins by stimulating their metabolism.[48,49]

The most relevant pharmacodynamic interaction effect on coumarins is generated by drugs affecting the coagulation system via other mechanisms like the inhibition of platelet aggregation (aspirin and nonsteroidal anti-inflammatory drugs [NSAIDs]) or by drugs which can damage the gastric mucosa (NSAIDs), increasing the risk and seriousness of upper gastrointestinal bleeding. Other drugs like levothyroxine or antibiotics are indicators of an intercurrent disease (thyroid dysfunction or a feverish infection, respectively), which could affect coumarin pharmacodynamics.

Many interactions with coumarins have been described, giving rise to large contributions to the principal interaction handbooks[50,51] or extensive reviews in medical journals.[52-54] In a recent systematic review, covering the period between 1993 and 2004, Holbrook et al.[53] retrieved 181 articles (out of 642 citations) from the main medical databases containing original reports on interactions and classified the quality of these reports into four categories ranging from poor to excellent. 84% of these reports were of poor quality, 86% of which were single case reports. Not one study could be classified as excellent. This accentuates one of the main problems of drug interactions in general: the poor quantity and quality of evidence, in which case reports still predominate. Other studies providing information about drug interactions are pharmacokinetic studies, randomized clinical trials or epidemiological population based studies.

Pharmacokinetic studies provide valuable insights into changes of plasmaconcentrations and other pharmacokinetic parameters, and into potential problems in clinical practice, but not into the risk of major complications. Randomized clinical trials usually provide valuable insights into the risks and benefits of several pharmacodynamic interactions (for example coumarins and aspirin), but are usually not representative for daily practice leaving ample room for doubts about their applicability in situations deviating from those of the trials.[55] Large epidemiological studies quantifying adverse outcomes of coumarin interactions could provide more information about major bleeding risk in daily practice. However, such studies are surprisingly rare.[56-59]

When a coumarin interaction is identified a decision has to be made about avoidance or acceptance of concurrent use of the coumarin with the interacting drug. For pharmacokinetic interactions affecting the elimination of coumarins, it is theoretically possible to compensate for a decreased or increased metabolism by adjusting dosages. However, because a new adequate coumarin dosage has to be found by trial and error, such adjustments carry the risk of temporary over- or undertreatment in previously stabilized patients. Some authors report difficulties with maintaining a good anticoagulation control when coumarins are used concomitantly with a very strong CYP2C9 inhibitor like miconazole,[46,47] or with a strong inductor like rifampicin.[49] By substituting the interacting drug it is possible to avoid management of an interaction by adjustment. This aspect of interactions of coumarins is underexplored.

Since pharmacodynamically interacting drugs usually do not affect the INR, downward dose adjustments of coumarins carry the risk of undertreatment. Management of such interactions implies a careful weighing of the supposed benefits against the expected increase of the bleeding risk with concurrent use. However, as we have mentioned before, epidemiological studies quantifying bleeding risks are rare. Moreover, not for all drugs affecting anticoagulation the risks of concurrent use with coumarins have been examined.

Finally, to our knowledge no study addressed the simple question whether anticoagulation clinics have all information regarding comedication, without which no adequate management of interactions is possible.

In summary, although many interactions with coumarins have been described, large epidemiological studies aimed at quantifying bleeding risks in daily practice are rare and consequences of the management of drug interactions in daily practice are underexplored.

Genetic factors affecting coumarins

In 1997 the cytochrome P450 isoform CYP2C9 has been identified as the main metabolizing enzyme of the more active (S)-enantiomer of warfarin. The *CYP2C9* gene encoding the homonymous enzyme has been analysed in the same period, the identification of polymorphic alleles giving rise to the assumption that inheritance could play a role in the variability of elimination of CYP2C9 substrates.[60,61]

The *CYP2C9* gene is located on chromosome 10q24.2, spanning about 55-kb encompassing 9 exons and encoding a protein of 490 amino acids.[62,63] The *CYP2C9* gene is highly polymorphic, to date more than 30 non-synonymous variations have been described, their prevalence showing considerable interethnic differences (for updated information see the Human CYP Allele Nomenclature Committee homepage htpp://www.imm.ki.se/CYPalleles/). The most common allele is designated *CYP2C9*1* (wild-type) and predominates in all ethnic groups. The two most important allelic variants in Caucasian populations are *CYP2C9*2* (Arg144Cys) and *CYP2C9*3* (Ile359Leu), encoding enzymes with a decreased activity compared to wild-type alleles. In Caucasian populations allele frequencies for *CYP2C9*2* range from 8 to 19% and for CYP2C9*3 from 4 to 16%, whereas *CYP2C9*2* is completely absent and *CYP2C9*3* exhibits lower frequencies in East-Asian and African or Afro-American populations.[64] Some studies have suggested that other genetic variations in the *CYP2C9* gene could contribute to a decreased CYP2C9 activity in Asian populations, but more concrete information is lacking.[65,66] Because a further discussion of interethnic differences is beyond the scope of this introduction, we will focus on genetic effects in the Caucasian population.

Several studies have examined an association between *CYP2C9* genotype and anticoagulation status in warfarin users. Aithal et al. were the first to demonstrate an association between the *CYP2C9* genotype and warfarin sensitivity in an English case-control study, carriers of a *CYP2C9*2* or *CYP2C9*3* allele having lower warfarin dosage requirements and showing an increased risk of overanticoagulation and major bleeding in the initiation phase of therapy compared to wild-type patients.[67] Several other studies found a decreased warfarin dose requirement in carriers of a variant allele than in wild-type patients.[68-70] Margaglione et al. reported an increased bleeding risk in carriers of a variant allele,[70] whereas Taube et al. did not find a difference between wild-type patients and carriers of a variant allele for overanticoagulation and percentage of time spent within the therapeutic range.[68] Loebstein et al. made a predictive

regression model in which plasma warfarin, age, and *CYP2C9* genotype together explained 48% of the variation in warfarin dose requirement.[69] In this model the *CYP2C9* genotype explained 10% of the variation, suggesting that its contribution to the interindividual variability is small.

Whereas an association between *CYP2C9* genotype and warfarin dose requirement appears to be firmly established, much less is known about the 'European' coumarins acenocoumarol and phenprocoumon. For acenocoumarol a similar association can be expected on the basis of its metabolism (Table 1), CYP2C9 being the major catalyst of the hydroxylation of the (S)-enantiomer. In a small study in 35 outpatients of a Dutch anticoagulation clinic, Thijssen et al. demonstrated an association between possession of the *CYP2C9*3* allele and low acenocoumarol dose requirement,[71] leaving room for more study to clinical consequences of *CYP2C9* polymorphisms in acenocoumarol users.

For phenprocoumon the CYP2C9 sensitivity is less obvious than for the other coumarins, the metabolism of (S)-phenprocoumon depending less on CYP2C9 activity (Table 1). At the other hand, because CYP2C9 participates in the metabolism of both (S)- and (R)-phenprocoumon, clinical consequences of being carrier of *CYP2C9* polymorphisms need to be studied.

Another highly interesting question is whether *CYP2C9* genotyping preceding coumarin therapy could be a useful addition to the already intensive INR monitoring of anticoagulation clinics.

Of course, the identification of new genes encoding proteins in the vitamin K cycle could extend the opportunities of pharmacogenetic research into variability of coumarin response.

OBJECTIVES AND OUTLINE OF THIS THESIS

The objectives of this thesis are to increase our insights into coumarin-drug interactions and into the contribution of genetic factors to the interindividual variability of the coumarins acenocoumarol and phenprocoumon (further designated coumarins).

In Chapter 2, discrepancies between medication records of two anticoagulation clinics and pharmacy records were assessed.

Chapter 3 contains studies in which several drug interactions with coumarins were investigated. Chapter 3.1 describes the management and clinical consequences of the interaction between coumarins and several antibiotics,

particularly the CYP2C9 inhibitor cotrimoxazole, in four anticoagulation clinics. In Chapter 3.2 the effect of selective serotonin reuptake inhibitors (SSRIs) on gastrointestinal and non-gastrointestinal bleeding risk was investigated within a cohort of coumarin users in the PHARMO record linkage system. In Chapter 3.3, the effect of antiplatelet drugs (aspirin, clopidogrel, and dipyridamol) was investigated within the same cohort of coumarin users in the PHARMO record linkage system.

Chapter 4 contains two pharmacogenetic studies in users of acenocoumarol from two anticoagulation clinics. The first study (Chapter 4.1) examined the effects of the *CYP2C9* genotype on time to stable acenocoumarol dosing, severe overanticoagulation, and acenocoumarol dose requirement. In the second study (Chapter 4.2) the effects of the VKORC1 and interactions between the *CYP2C9* and *VKORC1* genotypes on these outcomes were examined.

Chapter 5 contains two similar pharmacogenetic studies in users of phenprocoumon from two anticoagulation clinics. The first study (Chapter 5.1) examined the effects of the *CYP2C9* genotype on phenprocoumon dose requirement and time to stable phenprocoumon dosing and severe overanticoagulation. In the second study (Chapter 5.2) the effects of VKORC1 and interactions between *CYP2C9* and *VKORC1* genotypes on these outcomes were examined.

In Chapter 6, the cost-effectiveness of *CYP2C9* genotyping preceding acenocoumarol therapy was investigated. For this study, which has been extended to a commentary, data from our pharmacogenetic study of Chapter 4.1 and data on major bleeding in users of acenocoumarol from the Rotterdam Study[72] have been used.

Finally, in Chapter 7 the results of this thesis are summarized and put into the broader context of clinical practice and further research.

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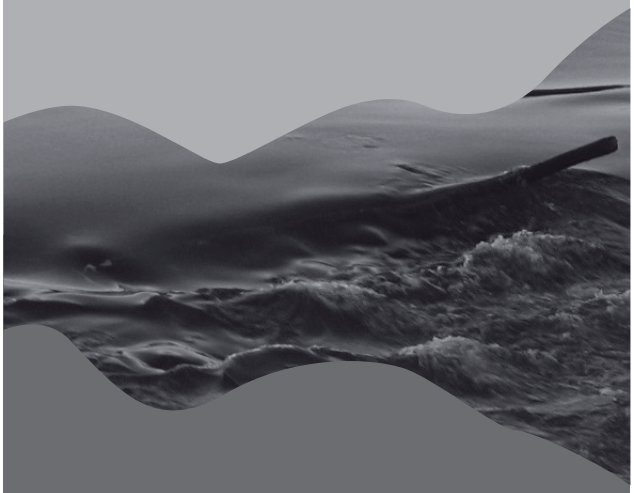
CHAPTER 2



MEDICAL RECORDS OF ANTICOAGULATION CLINICS AND PHARMACY RECORDS

2.1

Discrepancies between medication records of anticoagulation clinics and pharmacy records

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ABSTRACT

Objective

Our objective was to determine whether there were discrepancies between with coumarin anticoagulants interacting medications recorded in medical files of anticoagulation clinics (AC records) and computerized records of community pharmacies (pharmacy records).

Methods

A descriptive study was conducted at two Dutch anticoagulation clinics (ACs). AC records were compared with the pharmacy records. A drug registered in the pharmacy records but not in the AC records was recorded as a discrepancy, while a drug registered in AC records as well as in pharmacy records was recorded as a match.

Results

Of the 117 identified interacting drugs registered in pharmacy records, 32 (27%) were not registered in the AC records. In four out of seven patients for whom the use of a pharmacokinetically interacting drug was not registered in the AC records, several INR (International Normalized Ratio) values exceeded the upper therapeutic range.

Conclusion

This study demonstrates that a substantial percentage of drugs of which an interaction with coumarin anticoagulants can be expected, is not registered in the medical files of anticoagulation clinics.

INTRODUCTION

Oral anticoagulants of the coumarin type are effective in the treatment and prevention of several thromboembolic diseases. However, the therapeutic range of these drugs is very narrow and their dosage has to be frequently adjusted. In the Netherlands monitoring of patients who use coumarin anticoagulants is done by specialized anticoagulation clinics. For a good functioning of anticoagulation clinics it is highly relevant to have all information on factors, which could disturb the anticoagulation status of patients. Some of the factors contributing to the variability of the anticoagulant effects of coumarins are drug interactions, ingestion of varying quantities of vitamin K, infections and fever, severe heart failure and impaired liver function.[1,2] Coumarin anticoagulants are highly sensitive to drug interactions, of which many have been described.[3] For an adequate management of an interaction with a coumarin anticoagulant, the anticoagulation clinic needs all information on interacting comedication of their patients. In all anticoagulation clinics in the Netherlands information on relevant comedication of patients is recorded in the medical files. To assess whether anticoagulation clinics lack important information on comedication, we conducted a descriptive study at two anticoagulation clinics in the Netherlands.

MATERIALS AND METHODS

Design and setting

We conducted a descriptive study at two anticoagulation clinics, AC1 and AC2, in the Netherlands. Anticoagulation clinics monitor patients who use coumarin anticoagulants. In the Netherlands acenocoumarol and phenprocoumon are used. Anticoagulation clinics monitor the International Normalized Ratio (INR) of patients and apply dose adjustments on the basis of the INR measurements (see Table 1). For dose adjustments information about use of potentially interacting drugs is essential. Patients are instructed to inform anticoagulation clinics about all changes in medication, including over the counter medication.

To improve management of interactions with coumarin anticoagulants, the Dutch Standard Management Coumarin Interactions has been edited in 1999.[4] More details of this Standard are provided in Table 1. In both anticoagulation clinics which participated in our study data on medications were electronically registered in the medical patient files. Both anticoagulation clinics registered all

data on relevant comedication which could change effects of coumarin anticoagulants. In addition, AC1 registered all other comedication.

Table 1: ANTICOAGULATION CLINICS, DRUG INTERACTION MECHANISMS, AND STANDARD MANAGEMENT COUMARIN INTERACTIONS

Subject	Description
Anticoagulation clinics	Institutions where patients using warfarin, acenocoumarol or phenprocoumon (coumarins) are being monitored.
What is monitored?	INR (standardized conversion of the prothrombin time; a measure for the degree of anticoagulation). Frequency: a few days to maximally six weeks.
Who monitors?	Specialized physicians. They assess doses on the basis of INR measurements and other medical data, including interacting comedication.
Registering comedication	All potentially interfering comedication is electronically registered in the medical files.
Drug interaction mechanisms:	
Pharmacokinetic	Plasma level of a drug is changed by interference with absorption, distribution or elimination. Usually results in dose adjustment.
Pharmacodynamic	Change of effect or increase of toxicity of a drug without interfering with its pharmacokinetics. Does not always result in dose adjustment.
Standard Management Coumarin Interactions[4]	List of relevant drug interactions with coumarin anticoagulants and guidelines for management by pharmacists and anticoagulation clinics (in the Netherlands). Updated quarterly.
Guidelines for management of coumarin interactions by pharmacists	I. Contact prescribing physician to propose substitution of the interacting drug. Applied to interactions that can be easily avoided by substitution. II. Inform anticoagulation clinic directly as soon as the interacting drug is initiated. Applied to pharmacokinetic interactions which usually result in INR changes and dose adjustments. Designed to increase the probability that information reaches the anticoagulation clinic in time. III. Instruct patient to inform anticoagulation clinic of the initiation of the interacting drug. Applied to frequently occurring interactions which increase risk of bleeding and destabilization but which usually do not result in INR changes.

Previous acenocoumarol – CYP2C9 study

For this study we used the data of our prospective follow-up study on the association between *CYP2C9* genotype and acenocoumarol anticoagulation

status, which ran from November 1998 until September 2002 and was reported elsewhere.[5]

The *CYP2C9* gene encodes for the liver enzyme CYP2C9 which is the main metabolizing enzyme of the coumarin anticoagulants. This study was approved by the Medical Ethical Committee at the University Medical Centre, Utrecht, the Netherlands. All patients were informed on the aims of the study and gave their written consent for participation. Two aspects of the earlier study are important for the present one. First, the informed consent included permission to ask for the community pharmacy records of patients from the first date of acenocoumarol use until maximally six months after the last INR check in the anticoagulation clinic. Second, we excluded patients who used drugs for which a pharmacokinetic interaction with a coumarin anticoagulant has been established. However, because our study was prospective, patients could initiate such interacting drugs during the follow-up period.

Data collection and analysis

The community pharmacies of all patients included in the CYP2C9 study were asked by letter to send the complete computerized pharmacy records of six months, reckoned from the initiation date of acenocoumarol. We defined the computerized pharmacy records of the community pharmacies as 'pharmacy records'. The medication files of the anticoagulation clinics were defined as 'AC records'.

In the Netherlands most patients usually get their prescription drugs in the same community pharmacy. Medication histories of Dutch patients in community pharmacies are usually complete to nearly complete and pharmacy records have been validated as a reliable source of the true drug exposure of patients in the Netherlands.[6] So, we used the pharmacy records as a reference to compare the AC records with.

When a drug in the pharmacy records was not registered at all in the AC records, this was recorded as a discrepancy between the AC records and the pharmacy records. When a drug in the pharmacy records was also registered in the AC records, this was recorded as a match.

We focused our analyses on those drugs for which a relevant interaction with coumarin anticoagulants has been established. In our analyses we differentiated between pharmacokinetically and pharmacodynamically interacting drugs. More details about these mechanisms and their consequences are given in Table 1. Because we excluded patients using pharmacokinetically interacting drugs from

our acenocoumarol CYP2C9 study, we could only identify patients who initiated use of such a drug during the follow-up period, or patients who had been unjustly included because use of an interacting drug was not registered in the medical file of the AC.

Outcomes

The primary outcome of our study was the percentage of discrepancies between AC records and pharmacy records for drugs interacting with coumarin anticoagulants.

The secondary outcome of our study was the number of patients for whom pharamacokinetically interacting drugs were not registered in AC records and whose INR values exceeded the upper therapeutic range.

Table 2: MEDICATION IN RECORDS OF ANTICOAGULATION CLINICS (ACs): MATCHES AND DISCREPANCIES COMPARED TO PHARMACY RECORDS

	AC1 + AC2	AC1	AC2
Number of patients	174	132	42
Number of drugs			
total[a]	1076 (100%)	801 (100%)	275 (100%)
match[b]	628 (58%)	565 (71%)	63 (23%)
discrepancy[c]	448 (42%)	236 (29%)	212 (77%)
Mean number of drugs/patient			
total[a]	6.2	6.1	6.5
match[b]	3.6	4.3	1.5
discrepancy[c]	2.6	1.8	5.0

a) Total number of drugs mentioned in pharmacy records.
b) Match: number of drugs registered in both pharmacy and AC records.
c) Discrepancy: number of drugs registered in pharmacy records but not in AC records.

RESULTS

255 patients of the two anticoagulation clinics gave their informed consent to ask for their pharmacy records over maximally the first six months of treatment with acenocoumarol. We received a print of the pharmacy records of 174 patients (68.2%): 132 (75.9%) were patients of anticoagulation clinic 1 (AC1) and 42 (24.1%) were patients of anticoagulation clinic 2 (AC2). Mean age of all patients was 65.5 years (SD 14.8). The mean period over which the evaluated pharmacy records extended was 4.6 months. The total period of obtained pharmacy records

was 67.1 patient-years: 51.3 years for AC1 and 15.8 years for AC2 (data not shown in table).

The overall percentage of matches between pharmacy records and AC records (drugs that interact and do not interact with the coumarins) was higher for AC1 compared to AC2 (71 and 23%, respectively) reflecting the more selective drug registration of AC2 (Table 2).

Table 3: DRUGS INTERACTING WITH COUMARIN ANTICOAGULANTS IN PHARMACY RECORDS: MATCHES AND DISCREPANCIES COMPARED TO ANTICOAGULATION CLINIC (AC) RECORDS[a]

Interacting drugs	Total[b] (100%)	Match[c] n (%)	Discrepancy[d] n (%)	Management guideline for pharmacists[e]
Pharmacokinetically	**18**	**11 (61)**	**7 (39)**	
allopurinol	3	2 (67)	1 (33)	Inform AC
amiodarone	7	5 (71)	2 (29)	Inform AC
benzbromarone	3	2 (67)	1 (33)	Inform AC
cotrimoxazole	3	1 (33)	2 (67)	a. Propose alternative to prescriber *(preferred)* b. Inform AC *(alternative)*
propafenone	2	1 (50)	1 (50)	Inform AC
Pharmacodynamically	**99**	**74 (75)**	**25 (25)**	
antibiotics	25	18 (72)	7 (28)	Instruct patient to inform AC
acetylsalicylic acid	22	17 (77)	5 (23)	Instruct patient to inform AC
NSAIDs	34	24 (71)	10 (29)	a. Propose alternative to prescriber *(preferred)* b. Instruct patient to inform AC *(alternative)*
SSRIs	6	4 (67)	2 (33)	Instruct patient to inform AC
thyroid drugs	12	11 (92)	1 (8)	Inform AC
Total number	**117**	**85 (73)**	**32 (27)**	
Mean number/patient	**0.67**	**0.49**	**0.18**	

NSAIDs = nonsteroidal anti-inflammatory drugs; SSRIs = selective serotonine reuptake inhibitors

a) Only drugs for which an interaction is described in The Dutch Standard Management Coumarin Interactions[4] were recorded, the total number of patients was 174.
b) The total number of interacting drugs mentioned in pharmacy records.
c) Match: number of drugs registered in both pharmacy and AC records.
d) Discrepancy: number of drugs registered in pharmacy records but not in AC records.
e) According to The Dutch Standard Management Coumarin Interactions.[4]

The biggest mutual difference between anticoagulation clinics was found in Anatomical Therapeutic Chemical group C (drugs for the cardiovascular system):

18% of these drugs were not registered in the medical records of AC1, while this was 90% for AC2 (data not shown in table).
For interacting drugs, the number of discrepancies was 32 out of 117 (27%). This corresponded with 0.18 drug per patient (Table 3). The percentage of discrepancies was higher for pharmacokinetically interacting drugs than for pharmacodynamically interacting drugs (39 and 28%, respectively).
We identified seven patients whose pharmacokinetically interacting drugs were not registered in the medical records of anticoagulation clinics. In four of these patients, several INR values exceeded the upper therapeutic range with at least 0.5 unit (INR>4.0; range 12 to 33% of the totally measured INR values) (data not shown in table).

DISCUSSION

The results of our study, in which we compared registration of interacting drugs in anticoagulation clinics with computerized medication records of community pharmacies, demonstrate that there were considerable discrepancies between AC records and pharmacy records.
Concomitant use of pharmacokinetically interacting drugs nearly always results in considerable dose adjustment of the anticoagulant. Our results show that anticoagulation clinics do not always have this essential information.
A causal relationship between supratherapeutic INR values and lack of information on use of pharmacokinetically interacting drugs can of course not be established in this setting. However, it is reasonable to assume that the ACs could have dosed acenocoumarol more cautiously, if they had been informed about the use of these drugs.
In our study we also identified drugs which interfere in a more indirect way with coumarin anticoagulants. Antibiotics, except cotrimoxazole[7,8] and rifampicin, have no direct interaction with coumarins but are markers of an infection which can in itself interfere with the anticoagulation status. Nonsteroidal anti-inflammatory drugs and low dose acetylsalicylic acid increase the bleeding risk of coumarins,[9,10] and therefore knowledge of their use in ACs is highly desirable.
As far as we know, this study is the first to investigate discrepancies between pharmacy records and medication records of an anticoagulation clinic. Several studies paid attention to discrepancies between medical records in general and pharmacy records. In studies by Leister et al., Christensen et al. and De Maat et

al. discrepancies between pharmacy records and medical records amounted to 36%, 5-14% and 55.1%, respectively.[11-13] The discrepancies for interacting comedication in our study (overall 27%) are reasonably in accordance with the findings in the aforementioned studies.

The percentage of discrepancies we report in our study is the more alarming when we realize that the setting for an adequate exchange of data is favourable for availability of all information on comedication at ACs. The INR of patients is frequently monitored and patients are repeatedly instructed to inform their anticoagulation clinics on their medication. Moreover, Dutch community pharmacies inform anticoagulation clinics in many cases directly on the initiation of interacting drugs by their patients. Therefore, it is reasonable to expect a similar or even higher percentage of discrepancies between medical files and pharmacy records in less favourable settings.

Our study has several limitations. We are aware that the exclusion of users of interacting drugs in the acenocoumarol CYP2C9 study, from which our data were derived, makes the study population different from a randomly selected sample or from a complete population of coumarin users, because pharmacokinetically interacting drugs such as amiodarone are commonly co-prescribed with coumarins. However, this limitation does not apply to pharmacodynamically interacting drugs and does not distract from our main findings. Despite the inevitably limited number of pharmacokinetically interacting drugs in this study, our results convincingly demonstrate a substantial percentage of highly undesired discrepancies. A second limitation is that we did not verify whether the patients of our study always visited the same pharmacy. This could have resulted in an underestimation of the number of discrepancies, because visiting more pharmacies could have increased the number of discrepancies between medical records of the ACs and pharmacy records. A third limitation is that we did not assess the reason for the discrepancies. It could have been possible that AC2 did not register an antibiotic in their files, because the patient indicated that there was no fever, or because an INR check was made on the day the antibiotic was initiated. However, this limitation does not apply to most interacting drugs, so it will not have resulted in a substantial under-estimation of the number of relevant discrepancies.

In conclusion, we think that additional efforts of pharmacists as well as anticoagulation clinics are necessary to improve the undesirable main outcome of our study: lack of essential information on comedication at ACs.

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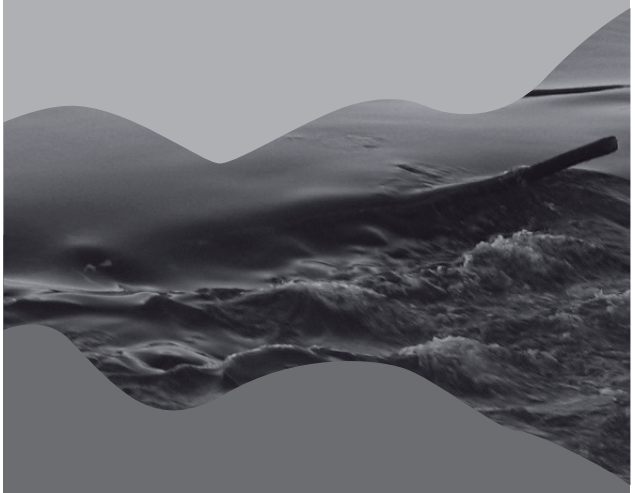
CHAPTER 3



DRUG INTERACTIONS WITH ACENOCOUMAROL AND PHENPROCOUMON

3.1

Coumarin anticoagulants and cotrimoxazole: avoid the combination rather than manage the interaction

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ABSTRACT

Objective

The objective of our study was to examine the management of the interaction between acenocoumarol or phenprocoumon and several antibiotics by anticoagulation clinics, and to compare the consequences of this interaction on users of cotrimoxazole with those for users of other antibiotics.

Methods

A follow-up study was conducted at four anticoagulation clinics in the Netherlands. Data on measurements of the International Normalized Ratio (INR), application of a preventive dose reduction (PDR) of the coumarin anticoagulant, fever, and time within or outside the therapeutic INR range were collected.

Results

The study cohort consisted of 326 subjects. A PDR was given more often to users of cotrimoxazole than to users of other antibiotics. The PDR in cotrimoxazole users resulted in a significantly reduced risk of both moderate overanticoagulation (INR>4.5) and severe overanticoagulation (INR>6.0) compared with no PDR, with odds ratios (ORs) of 0.06 (95% confidence interval [CI] 0.01-0.51), and 0.09 (95% CI 0.01-0.92), respectively. In cotrimoxazole users without PDR, the risk of overanticoagulation was significantly increased compared with users of other antibiotics. All cotrimoxazole users spent significantly more time under the therapeutic INR range during the first six weeks after the course than users of other antibiotics.

Conclusion

PDR is effective in preventing overanticoagulation in cotrimoxazole users, but results in a significantly prolonged period of underanticoagulation after the course. Avoidance of concomitant use of cotrimoxazole with acenocoumarol or phenprocoumon seems to be a safer approach than management of the interaction between these drugs.

INTRODUCTION

Coumarin-type anticoagulants have a narrow therapeutic range. One important aspect of their safety is their sensitivity to drug interactions, many of which have been described.[1,2]

There are several reasons why antibiotic use can be considered to be indicative of a change in anticoagulation status in users of coumarin-type anticoagulants. When the antibiotic is used for febrile illness, it may be associated with overanticoagulation.[3,4] In two studies on the interaction between coumarin anticoagulants and antibiotics, the risk of severe overanticoagulation, defined as an International Normalized Ratio (INR) ≥6.0, was increased more in users of sulfamethoxazole-trimethoprim (cotrimoxazole) than in users of other antibiotics.[4,5] Sulfamethoxazole is a strong inhibitor of CYP2C9,[6] the main liver enzyme involved in the metabolism of warfarin,[7] acenocoumarol,[8] and probably phenprocoumon,[9] which could explain this stronger association with overanticoagulation. Current clinical guidelines in the Netherlands for the management of coumarin drug interactions advise healthcare givers to avoid prescribing the concurrent use of cotrimoxazole and coumarins.[10] Nevertheless, in daily practice cotrimoxazole is frequently prescribed to users of coumarins, since physicians in anticoagulation clinics assume that an interaction with cotrimoxazole can be managed in a manner similar to those used to manage interactions that arise with the concurrent use of coumarins with other antibiotics. An anticoagulation clinic will initiate one of the following procedures once it has been notified of the initiation of the use of an antibiotic: (1) measurement of the INR during the antibiotic course and adjustment of the coumarin dose depending on the INR value (a reactive dose-adjustment); (2) a preventive (coumarin) dose reduction (PDR) preceding an INR measurement during or after the antibiotic course, assuming that use of an antibiotic or the intercurrent infection itself increases the risk of overanticoagulation. The PDR approach seems even more relevant to cotrimoxazole than to other antibiotics because the CYP2C9-inhibiting effect of the former might increase the risk of overanticoagulation more than the infectious state alone. However, PDR could also lead to temporary undertreatment, and evidence for the effectiveness of this approach is currently lacking. There are no official guidelines for such dose adjustments, and the application of PDR strongly depends on the personal view of the responsible physician.

The aim of the present study was to examine the management of the interaction between coumarin anticoagulants and antibiotics by anticoagulation clinics and its consequences for users of cotrimoxazole and other antibiotics. To this end, we conducted a prospective follow-up study at four anticoagulation clinics in the Netherlands.

METHODS

Study design

This was a follow-up study conducted at four anticoagulation clinics in the Netherlands. We included patients who were stabilized on one of the coumarin anticoagulants acenocoumarol or phenprocoumon and who had started using one of the following antibiotics between January 2001 and October 2003: cotrimoxazole, amoxicillin, amoxicillin-clavulanic acid, clarithromycin, doxycyclin, nitrofurantoin, norfloxacin, or trimethoprim. In addition to cotrimoxazole, we chose the other antibiotics based on their use for the same kind of infections, mainly those of the urinary and respiratory tract.

The subjects included in our study were prospectively followed during the antibiotic course until the last INR measurement, which occurred within six weeks following the starting date of the antibiotic (follow-up time). We did not intervene in the daily routine of the participating anticoagulation clinics and, in particular, we made no agreements on checking the INR of patients during the antibiotic course, on making additional INR measurements, on the time intervals between INR measurements after the antibiotic courses, or on dose adjustments when antibiotics were prescribed. To assess the consequences of interaction management reliably and to avoid confounding by an unstable anticoagulation status preceding the antibiotic course, we only included stabilized patients in our study. Criteria for the assessment of stability were: (1) use of the coumarin anticoagulant for at least 50 days before the initiation of the antibiotic; (2) availability of at least four INR measurements before the initiation of the antibiotic; (3) the last two INR measurements before initiation of the antibiotic were within the therapeutic range; (4) a maximum of one out of the last four INR measurements or a maximum of 30% of the INR measurements during the 50 days immediately preceding initiation of the antibiotic were outside of the therapeutic range, with no INR being above 5.5. Similar criteria for stability have been used in other studies.[4,5]

We excluded subjects from our analyses in whom the INR was not measured during the course of the antibiotic and who used the antibiotic for a period shorter than three days and longer than 14 days. If the INR was not measured during the course, an interaction effect of the antibiotic could be missed. Antibiotics used for less than three days or more than 14 days are usually prescribed for prophylaxis not for acute infections.
All patients were informed of the aims of the study and were asked for their written consent to participate in the study.

Setting and attitudes of anticoagulation clinics on antibiotic use
All anticoagulation clinics in the Netherlands monitor the INR in outpatients at a frequency varying from a few days to maximally six weeks. The two target therapeutic ranges are the normal therapeutic range (INR 2.0-3.5) and the high therapeutic range (INR 2.5-4.0).
The initiation of the use of an antibiotic is usually reported to the anticoagulation clinics by the patients, their pharmacists and/or the prescribing physicians.
The four anticoagulation clinics participating in this study had different attitudes on the management of the interaction between coumarins and antibiotics. The approach of three of the anticoagulation clinics was to decrease the coumarin dose preventively if cotrimoxazole was prescribed; in the case of cotrimoxazole use, the applied PDR would be in the range of 20-25%. If one of the other antibiotics examined in this study was prescribed, the application of a PDR would depend on the seriousness of the disease and on the occurrence of fever. The fourth anticoagulation clinic had no established protocol for dose reduction but indicated that it would monitor the INR of every user of cotrimoxazole within 3-5 days after initiation of the course.

Data collection
We collected relevant data on the participating patients and recorded these in a database: sex and age of patient; dosage and indication of the coumarin; prescribed antibiotics (indication, dosage, and duration of use); results of INR measurements before, during and after the antibiotic course; comedication; and relevant comorbidities (malignancies, thyroid diseases, heart failure). These data were retrieved from the medical files of the anticoagulation clinics. Patients were asked to indicate on a questionnaire for which infection the antibiotic was prescribed and whether they had suffered from fever during the antibiotic course. We recorded this as fever yes/no in our database. If the coumarin dose was reduced as soon as the antibiotic was started in the absence of an actual INR, we

recorded this as a preventive dose reduction and calculated the percentage of the dose reduction from the data on dosage in the file of the anticoagulation clinic.
In order to assess the anticoagulation status shortly after the antibiotic course, we recorded the time spent within, above, and under the therapeutic range from the starting date of the antibiotic until the last INR measurement within six weeks following the starting date of the antibiotic. Six weeks is the maximal period between two INR measurements if a patient is well stabilized. Furthermore, after a longer follow-up period, differences between patients could be more attributable to other factors than to the infection or antibiotic use. If after the first INR during the antibiotic course no second INR measurement was available within the 6-week period after the starting date of the antibiotic, we recorded no follow-up time and no time spent within, above, or under the therapeutic range.

Outcomes

The end points of our study were chosen to assess the effectiveness of the management of the interaction between coumarin anticoagulants and cotrimoxazole and other antibiotics.
We examined the following parameters in users of cotrimoxazole with and without PDR as well as in users of other antibiotics with and without PDR:

1) occurrence of moderate overanticoagulation (INR>4.5) and severe overanticoagulation (INR>6.0);
2) time spent within, above, and under the therapeutic INR range from the starting date of the antibiotic until the last INR measurement within six weeks following the starting date of the antibiotic.

Calculations and statistical analysis

We assessed the effects of the PDR within the group of users of cotrimoxazole and within the group of users of other antibiotics by comparing the occurrence of overanticoagulation in patients for whom a PDR had been applied with the occurrence of overanticoagulation in patients in whom PDR had not been applied (logistic regression models). We also compared the occurrence of overanticoagulation and time spent within, under, and above the therapeutic range of cotrimoxazole users with users of other antibiotics (reference group). These comparisons were made for patients with PDR and for patients without PDR. Finally, we compared the time spent within, under, and above the therapeutic range in patients for whom a PDR had been applied with those for whom a PDR had not been applied (reference) within the groups of cotrimoxazole users and users of other antibiotics (linear regression models). In all

Table 1: CHARACTERISTICS OF PATIENTS (N=326) USING ANTIBIOTICS, TREATED BY FOUR ANTICOAGULATION CLINICS

Characteristic	Cotrimoxazole (n=43)		Other antibiotics[a] (n=283)	
	PDR +	PDR –	PDR +	PDR –
Age; mean years (SD)	75.4 (10.9)	75.1 (8.2)	72.6 (10.9)	71.4 (11.2)
Follow-up time; mean days (SD)	33.2 (5.6)	28.9 (8.0)	30.4 (7.2)	30.2 (7.2)
INR measurements; mean number (SD)	3.5 (0.9)	3.9 (1.5)	3.5 (1.1)	3.1 (1.1)
Acenocoumarol; mean dose in mg/day (SD)	2.42 (1.26)	2.41 (1.41)	2.61 (1.06)	2.60 (1.12)
Percentage PDR applied in acenocoumarol users; mean (SD)	15.0 (7.6)		10.3 (11.1)	
Phenprocoumon; mean dose in mg/day (SD)	2.81 (0.86)	2.53 (1.02)	2.99 (1.31)	2.36 (1.00)
Percentage PDR applied in phenprocoumon users; mean (SD)	17.9 (15.8)		11.4 (7.0)	
Percentage PDR applied in all coumarin users; mean (SD)	15.4 (8.8)		10.5 (10.6)	
	n=28 (100%)	**n=15 (100%)**	**n=60 (100%)**	**n=223 (100%)**
Men	22 (78.6)	10 (66.7)	30 (50.0)	114 (51.1)
Users of acenocoumarol	24 (85.7)	10 (66.7)	52 (86.7)	169 (75.8)
Fever	18 (64.3)	6 (40.0)	27 (45.0)	120 (53.8)
Normal target therapeutic range[b]	19 (67.9)	5 (33.3)	30 (50.0)	112 (50.2)
Respiratory infections	8 (28.6)	3 (20.0)	33 (55.0)	116 (52.0)
Urinary tract infections	13 (46.4)	8 (53.3)	11 (18.3)	53 (23.8)
Malignancies	1 (3.6)	3 (20.0)	3 (5.0)	9 (4.0)
Thyroid diseases	0	0	1 (1.7)	11 (4.9)
Users of inhibiting drugs	0	1 (6.7)	5 (8.3)	17 (7.6)
Users of inducing drugs	1 (3.6)	0	1 (1.7)	4 (1.8)

PDR + = preventive dose reduction applied; PDR – = preventive dose reduction not applied

a) Other antibiotics: trimethoprim (n=3), doxycyclin (n=104), amoxicillin (n=77), amoxicillin–clavulanic acid (n=36), clarithromycin (n=14), norfloxacin (n=33), nitrofurantoin (n=16).

b) Normal target therapeutic range: INR 2.0-3.5.

models we adjusted for the potential confounding covariates sex, age, target therapeutic range, and fever as indicated by the patient. Covariates were added to the statistical models one at a time. We adjusted for a covariate if it changed the point estimation of the outcome of interest by 5% or more upon inclusion in the model.

Time spent within, above, and under the therapeutic INR range was calculated by the step-up method described by Rosendaal et al.[11]

Although all patients were stable when they were included in our study, we reanalysed our statistically significant outcomes after excluding patients in whom destabilization could be due to factors other than those of infection and/or fever (presence of thyroid disease, malignancy, or use of other enzyme-inhibiting or -inducing drugs).

All statistical analyses were performed with the statistical software package SPSS (version 12.0; SPSS Inc, Ill, USA).

RESULTS

A total of 424 patients who met the inclusion criteria gave their informed consent to participate in our study. Of these patients, 81 did not have assessment of the INR during the antibiotic course, 14 used the antibiotic for less than three days, and 3 used the antibiotic for more than 14 days. This left a study cohort of 326 patients for analysis. All patients were available to follow-up.

A PDR was applied more frequently for users of cotrimoxazole (28/43; 65.1%) than for users of other antibiotics (60/283; 21.2%) (Table 1).

The PDR applied was significantly greater in users of cotrimoxazole than in users of other antibiotics (15.0% and 10.3%, respectively; p-value for difference 0.036; two-sided t-test). The number of INR measurements during follow-up was significantly higher in both users of cotrimoxazole (PDR applied and PDR not applied) and users of other antibiotics (PDR applied) than in users of other antibiotics in whom a PDR was not applied (p-values of 0.028, 0.006, and 0.007, respectively; two-sided t-test). Mean daily dosages for acenocoumarol were lower in users of cotrimoxazole than in users of other antibiotics, but this difference was not statistically significant and even smaller (0.14 mg) after adjustment for differences in age (Table 1).

In cotrimoxazole users, the PDR protected strongly against both moderate and severe overanticoagulation (adjusted odds ratio [OR] 0.06; 95% confidence

interval [CI] 0.01-0.51 for INR>4.5, and adjusted OR 0.09; 95% CI 0.01-0.92 for INR>6). For other antibiotics, the effect of the PDR on overanticoagulation was not as strong and not statistically significant (Tables 2 and 3).

If PDR was applied, the risk of overanticoagulation was not increased in users of cotrimoxazole compared with users of other antibiotics. However, if PDR was not applied, there was a strongly increased risk of moderate as well as severe overanticoagulation in cotrimoxazole users compared with users of other antibiotics (adjusted OR 3.96; 95% CI 1.33-11.8 for INR>4.5, and adjusted OR 3.86; 95% CI 1.03-14.6 for INR>6.0) (Tables 2 and 3).

During the 6-week follow-up, cotrimoxazole users with a PDR spent more time within and less time under the therapeutic range than cotrimoxazole users without a PDR, but these differences were not statistically significant. Users of cotrimoxazole without a PDR spent significantly less time within the therapeutic range than users of other antibiotics with a PDR, whereas significantly more time was spent under the therapeutic range. Moreover, cotrimixazole users with a PDR also spent significantly more time under the therapeutic range than did all users of other antibiotics (adjusted mean difference 6.9%; 95% CI 1.0-12.9) (Tables 2 and 4).

Cotrimoxazole users with more than a 20% PDR spent significantly more time under the therapeutic range than users of other antibiotics (adjusted mean difference 7.4 mg; 95% CI 0.9-14.0; p=0.027). If less than a 20% PDR was applied, the difference between the users of cotrimoxazole and those of other antibiotics shrunk and was no longer significant.

The application of a PDR differed between anticoagulation clinics. Three of the four anticoagulation clinics participating in this study applied PDR as a rule in cotrimoxazole users (83.3-85.7%). In terms of users of other antibiotics, the application of a PDR was more varied: in three of the anticoagulation clinics PDR was sometimes applied (in 17.6-50.8% of all cases), whereas one anticoagulation clinic did not apply the PDR approach at all. The overall percentage of time spent within the therapeutic range during the first six weeks after initiation of an antibiotic ranged from 73.7 to 78.0% at all four anticoagulation clinics. In the anticoagulation clinic that did not apply a PDR, overanticoagulation (INR>4.5) occurred most frequently for the all antibiotics class (26.9% versus 10.8-22.7% in the other clinics), with the difference being most marked for cotrimoxazole (54.4% versus 14.3-16.7% in the other clinics).

We also analysed our data separately for users of acenocoumarol and phenprocoumon. There were no differences in the point estimates of most of

Table 2: OCCURRENCE OF OVERANTICOAGULATION AND TIME SPENT WITHIN, ABOVE, AND UNDER THE THERAPEUTIC RANGE BY PATIENTS USING COTRIMOXAZOLE AND OTHER ANTIBIOTICS[a]

Outcome	Cotrimoxazole (n=43)		Other antibiotics[b] (n=283)	
	PDR +	PDR –	PDR +	PDR –
	n=28 (100%)	n=15 (100%)	n=60 (100%)	n=223 (100%)
INR>4.5	3 (10.7)	25 (89.3)	9 (15.0)	45 (20.2)[c]
INR>6.0	1 (3.6)	4 (26.7)	5 (8.3)	14 (6.3)[d]
	mean (95% CI)	mean (95% CI)	mean (95% CI)	mean (95% CI)
Time within therapeutic range (%)	71.1 (60.4–81.8)	51.8 (34.6–69.0)	76.2 (69.5–82.9)	75.7 (72.2–79.3)[e]
Time above therapeutic range (%)	15.0 (5.7–24.3)	20.3 (10.7–29.8)	12.3 (6.8–17.7)	18.9 (15.5–22.2)[f]
Time under therapeutic range (%)	14.0 (5.6–22.2)	27.9 (7.7–48.1)	11.5 (6.9–16.1)	5.4 (3.7– 7.2)[g]

PDR + = preventive dose reduction applied; PDR – = preventive dose reduction not applied; 95% CI = 95% confidence interval for the reported mean value

a) Calculated for the time from the starting date of the antibiotic until the last INR measurement within 6 weeks following the starting date of the antibiotic. Time within, above, and under therapeutic range was calculated for antibiotic users in whom at least one INR measurement had been performed within 6 weeks after the INR measurement during the antibiotic course. This resulted in exclusion from the analysis of the following number of subjects: cotrimoxazole 2 (1 and 1); other antibiotics 14 (1 and 13); in parentheses the number of subjects with a PDR + and a PDR –, respectively.

b) Other antibiotics: trimethoprim (n=3), doxycyclin (n=104), amoxicillin (n=77), amoxicillin–clavulanic acid (n=36), clarithromycin (n=14), norfloxacin (n=33), nitrofurantoin (n=16).

c) Range: 7.1% for nitrofurantoin to 27.6% for norfloxacin.

d) Range: 0% for nitrofurantoin and trimethoprim to 8.6% for doxycyclin.

e) Range: 71.8% for amoxicillin to 84.8% for norfloxacin.

f) Range: 8.6% for norfloxacin to 22.0% for amoxicillin.

g) Range: 3.3% for norfloxacin to 11.1% for clarithromycin.

Table 3: ODDS RATIOS FOR EFFECT OF PREVENTIVE DOSE REDUCTION AND FOR (SEVERE) OVERANTICOAGULATION IN USERS OF COTRIMOXAZOLE COMPARED WITH USERS OF OTHER ANTIBIOTICS

	OR (95% CI)	P	Adjusted OR[a] (95% CI)	P
Effect of PDR on overanticoagulation				
Cotrimoxazole				
PDR applied, INR>4.5	0.10 (0.02–0.50)	0.005[b]	0.06 (0.01–0.51)	0.010[b]
PDR applied, INR>6.0	0.10 (0.01–1.02)	0.051	0.09 (0.01–0.92)[c]	0.042[b]
PDR not applied	1 (reference)		1 (reference)	
Other antibiotics				
PDR applied, INR>4.5	0.70 (0.32–1.52)	0.37	N.A.	
PDR applied, INR>6.0	1.36 (0.47–3.93)	0.57	N.A.	
PDR not applied	1 (reference)		1 (reference)	
Risk of overanticoagulation				
PDR not applied				
cotrimoxazole, INR>4.5	4.52 (1.56–13.1)	0.006[b]	3.96 (1.33–11.8)	0.013[b]
cotrimoxazole, INR>6.0	5.43 (1.53–19.2)	0.009[b]	3.86 (1.03–14.6)	0.046[b]
other antibiotics	1 (reference)		1 (reference)	
PDR applied				
cotrimoxazole, INR>4.5	0.68 (0.17–2.73)	0.59	N.A.	
cotrimoxazole, INR>6.0	0.41 (0.04–3.66)	0.42	0.30 (0.03–3.05)[d]	0.30
other antibiotics	1 (reference)		1 (reference)	

PDR = preventive dose reduction; OR = odds ratio; N.A. = not applicable (adjustment not applied because including covariates in our model did not result in a change of at least 5% of OR, see text)

a) Adjusted for differences in fever as indicated by patient, age, sex, and target therapeutic range, unless otherwise indicated.
b) Statistically significant difference ($p<0.05$).
c) Adjusted for differences in age and sex.
d) Adjusted for differences in fever as indicated by patient, age, and sex.

Table 4: COMPARISONS OF TIME SPENT WITHIN, UNDER, AND ABOVE THE THERAPEUTIC RANGE (TR) BY USERS OF COTRIMOXAZOLE AND OTHER ANTIBIOTICS[a]

	Mean difference (95% CI)	P	Adjusted mean difference[b] (95% CI)	P
Preventive dose reduction (PDR) applied				
cotrimoxazole, % time within TR	-5.1 (-17.2; 6.9)	0.40	-4.2 (-17.1; 8.6)	0.51
cotrimoxazole, % time above TR	2.7 (- 7.3;12.7)	0.60	1.9 (- 8.9;12.7)	0.73
cotrimoxazole, % time under TR	2.4 (- 6.3;11.1)	0.58	N.A.	
other antibiotics	1 (reference)		1 (reference)	
PDR not applied				
cotrimoxazole, % time within TR	-23.8 (-38.2;- 9.6)	<0.001[c]	-22.3 (-36.6; -8.0)[d]	0.002[c]
cotrimoxazole, % time above TR	1.4 (-11.7;14.5)	0.83	N.A.	
cotrimoxazole, % time under TR	22.5 (14.4;30.6)	<0.001[c]	20.4 (12.4;28.5)[e]	<0.001[c]
other antibiotics	1 (reference)		1 (reference)	
Cotrimoxazole				
PDR applied, % time within TR	19.3 (0.7;37.9)	0.042[c]	14.6 (- 5.8;35.1)[e]	0.16
PDR applied, % time above TR	-5.3 (-19.6; 9.0)	0.46	-4.4 (-19.1;10.3)[f]	0.55
PDR applied, % time under TR	-14.0 (-31.6; 3.6)	0.16	-10.7 (-29.0; 7.5)[f]	0.24
PDR not applied	1 (reference)		1 (reference)	
Other antibiotics				
PDR applied, % time within TR	0.5 (- 7.0; 8.1)	0.89	0.6 (- 6.8; 8.2)	0.87
PDR applied, % time above TR	-6.6 (-13.5; 0.3)	0.061	N.A.	
PDR applied, % time under TR	6.1 (2.0;10.1)	0.003[c]	N.A.	
PDR not applied	1 (reference)		1 (reference)	
Cotrimoxazole, PDR applied				
% time within TR	-4.7 (-15.1; 5.6)	0.37	-3.0 (-13.5; 7.5)[e]	0.58
% time above TR	-2.4 (-11.9; 7.1)	0.61	-3.7 (-13.3; 6.0)[g]	0.46
% time under TR	7.2 (1.2;13.1)	0.018[c]	6.9 (1.0;12.9)[g]	0.022[c]
other antibiotics, PDR applied + PDR not applied	1 (reference)		1 (reference)	

N.A. = not applicable (adjustment not applied because including covariates in our model did not result in a change of at least 5% of mean difference, see text)

a) Calculated for time from starting date of the antibiotic until the last INR measurement within 6 weeks following starting date of the antibiotic.

b) Adjusted for differences in fever as indicated by patient, age, sex, and target therapeutic range, unless otherwise indicated.
c) Statistically significant difference ($p<0.05$).
d) Adjusted for differences in sex.
e) Adjusted for differences in sex and target therapeutic range.
f) Adjusted for differences in age, sex, and target therapeutic range.
g) Adjusted for differences in fever as indicated by patient, sex, and target therapeutic range.

Table 5: MAIN OUTCOMES STRATIFIED FOR USERS OF ACENOCOUMAROL AND PHENPROCOUMON

Outcome	Acenocoumarol (n=254)		Phenprocoumon (n=71)	
	Adjusted OR[a] (95% CI)	**P**	**Adjusted OR[a] (95% CI)**	**P**
Protective effect of preventive dose reduction (PDR)				
Cotrimoxazole				
PDR applied, INR>4.5	0.08 (0.01;0.70)	0.022[b]	0.16 (0.01;4.48)	>0.3[c]
PDR not applied	1 (reference)		1 (reference)	
Risk of overanticoagulation				
PDR not applied				
cotrimoxazole, INR>4.5	4.40 (1.15;16.8)	0.030[b]	3.83 (0.55;26.7)	0.18
other antibiotics	1 (reference)		1 (reference)	
	Mean difference (95% CI)	**P**	**Mean difference (95% CI)**	**P**
% Time within or under therapeutic range (TR)				
cotrimoxazole, % time within TR	-22.1 (-39.1;- 5.0)	0.011[b]	-21.4 (-49.4; 6.6)	0.13
cotrimoxazole, % time under TR	20.3 (10.9;29.7)	<0.001[b]	22.6 (5.7;39.5)	0.010[b]
other antibiotics	1 (reference)		1 (reference)	
cotrimoxazole, PDR applied, % time under TR	9.1 (3.0;15.1)	0.004[b]	- 6.7 (-23.0; 9.6)	0.42
other antibiotics, PDR applied + PDR not applied	1 (reference)		1 (reference)	

a) Adjusted for differences in fever as indicated by patient, age, sex, and target therapeutic range.
b) Statistically significant difference ($p<0.05$).
c) Adjustment not applied because of zero patients with INR>4.5; OR was calculated by increasing the values of each cell of the crosstable with 0.5.

our main outcomes between users of either of these coumarins, with the exception of percentage of time spent under the therapeutic range in phenprocoumon users in whom PDR was applied. However, most of the results that were statistically significant for all coumarin users were also significant for users of acenocoumarol (n=252, 78.2%), whereas they were in most cases not significant for the smaller group of users of phenprocoumon (n=71, 21.8%) (Table 5).

Reanalysis of our results after excluding patients with thyroid diseases and malignancy or those using enzyme-inhibiting or -inducing drugs gave similar point estimates or trends, although there was a loss of significance for severe overanticoagulation in users of cotrimoxazole compared to other antibiotics and for time spent within the therapeutic range for users of cotrimoxazole in whom PDR was not applied (data not shown).

DISCUSSION

The results of the present study, in which we evaluated the management of the interaction between antibiotics and coumarin anticoagulants by anticoagulation clinics, demonstrated that a PDR reduces the risk of overanticoagulation in cotrimoxazole users to the level of other antibiotic users, but also that management of the interaction between coumarins and cotrimoxazole results in a significantly longer period of undertreatment during the first six weeks after initiation of the antibiotic.

In three of the four anticoagulation clinics PDR was applied more frequently and was significantly higher in users of cotrimoxazole than in users of other antibiotics, indicating that anticoagulation clinics are aware of the seriousness of the interaction between coumarins and cotrimoxazole. In the cases and case series that have reported on overanticoagulation and bleeding with the concurrent use of antibiotics and cotrimoxazole[12-17] an effect of the intercurrent infection on the anticoagulation status could not be ruled out. However, Penning-van Beest et al. (case-control study) and Visser et al. (follow-up study) both demonstrated that an increased risk of severe overanticoagulation (INR>6.0) was particularly associated with cotrimoxazole.[4,5] A plausible explanation is the strong inhibition of the main metabolizing enzyme, CYP2C9, of the coumarins by sulfamethoxazole, the sulphonamide component of cotrimoxazole.[6]

Although PDRs as applied in clinical practice are effective in reducing the overanticoagulation risk in cotrimoxazole users, the price that has to be paid for the concurrent use of cotrimoxazole is a significantly prolonged period of underanticoagulation compared with the use of other antibiotics during the first six weeks after the antibiotic course. This difference was more marked in the subgroup of subjects in whom PDR was not applied. Possible explanations for this result are (1) the usually shorter time span between PDR and the first INR measurement (always within the course) compared to the time span between a reactive dose reduction following supratherapeutic INR and subsequent INR measurement (usually after the course), and (2) the higher reactive dose reduction which is applied in the case of severe overanticoagulation (INR>6.0). However, even cotrimoxazole users for whom the PDR had been applied had a significantly prolonged period of underanticoagulation compared with all of the users of other antibiotics (PDR applied and PDR not applied taken together). This last comparison is totally logic because our results strongly suggest that a PDR should always be applied in cotrimoxazole users, whereas this is as a rule not required in users of other antibiotics. The adjusted difference in time spent under the therapeutic range – ranging from 6.9 (PDR applied) to 22.5% (PDR not applied) – corresponds to about 2-7 days of the mean follow-up time of 30 days in otherwise stabilized patients; this time interval is clinically relevant and can be avoided by substituting cotrimoxazole.

It is not difficult to explain the prolonged period of underanticoagulation in cotrimoxazole users. The application of a PDR, which was in this study higher in cotrimoxazole users, might overcompensate for overanticoagulation, whereas the reactive dose reduction following overanticoagulation carries the same risk of overcompensation and undertreatment as PDR. Consequently, the inhibition of CYP2C9 by cotrimoxazole superimposes an additional problem upon the already potentially destabilizing effects of the infection and fever. Because our results for acenocoumarol in the separate analyses were predominantly in agreement with the overall results, our findings primarily apply to acenocoumarol users. It is possible that users of phenprocoumon are less sensitive to interactions with CYP2C9 inhibitors such as cotrimoxazole.[9,18] We do expect that our results also apply to users of warfarin, which seems to be even more CYP2C9 sensitive than acenocoumarol.[19]

Our study has several limitations. Because we retrieved medical data from anticoagulation clinics, it is possible that not all of the relevant data on potentially destabilizing factors, such as malignancies, thyroid diseases, and the use of other

inhibitors of coumarin metabolism, were available. However, by only including patients who were obviously stable at the moment of initiation of the antibiotic, we decreased the chance that such factors changed the anticoagulant status during the antibiotic course. A second limitation is the absence of data on the presence of polymorphisms of the genes encoding the coumarin-metabolizing enzyme CYP2C9 or the pharmacodynamic target of coumarins, VKORC1. The genotypes of both *CYP2C9*[18,20] and *VKORC1* are strongly associated with interindividual variability in coumarin dose requirements.[21-24] Further studies would be needed to assess whether the risk of overanticoagulation in cotrimoxazole users differs between carriers of a *CYP2C9* or *VKORC1* polymorphism and wild-type patients. It should be clear that our results only apply to patients with a stabilized anticoagulation state at the initiation of the antibiotic course.

In conclusion, if cotrimoxazole is prescribed to users of coumarin anticoagulants, the interaction can be managed by applying PDR, which adequately decreases the risk of overanticoagulation, but this successful management comes at the cost of a prolonged period of underanticoagulation after the course. Consequently, rather than managing the interaction it is better to avoid prescribing cotrimoxazole as a therapeutically equivalent alternative is always available.

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3.2

Selective serotonin reuptake inhibitors increase the risk of non-gastrointestinal bleeding in users of acenocoumarol or phenprocoumon

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Submitted

ABSTRACT

Objective

To assess the risk of abnormal gastrointestinal and non-gastrointestinal bleeding associated with the use of selective serotonin reuptake inhibitors (SSRIs) in users of acenocoumarol and phenprocoumon, and to compare this with the relative risk of bleeding due to use of nonsteroidal anti-inflammatory drugs (NSAIDs) and antibiotics.

Methods

We used data from the PHARMO record linkage system including pharmacy and linked hospitalization records of about two million subjects in the Netherlands to conduct a case-control study nested within a cohort of new users of the coumarins acenocoumarol and phenprocoumon. Cases were patients who were hospitalized for a primary diagnosis of abnormal major bleeding while taking a coumarin. Cases were matched with up to four controls for sex, age, coumarin, duration of coumarin use, and dispensing pharmacy. Conditional logistic regression was used to determine odds ratios (OR) and 95% confidence intervals (CI) for the risk of hospitalization for abnormal bleeding associated with concurrent use of SSRIs, NSAIDs, or antibiotics.

Results

We identified 1848 cases of abnormal bleeding (605 gastrointestinal, 1243 non-gastrointestinal). Users of SSRIs had a significantly increased risk of hospitalization for non-gastrointestinal bleeding (OR 1.7; 95% CI 1.1-2.5), but not for gastrointestinal bleeding (OR 0.8; 95% CI 0.4-1.5). Users of NSAIDs had a similar increased risk of non-gastrointestinal bleeding (OR 1.7; 95% CI 1.3-2.2), whereas the relative risk for gastrointestinal bleeding was higher (OR 4.6; 95% CI 3.3-6.5). In users of antibiotics the risk for hospitalization for non-gastrointestinal (OR 4.3; 95% CI 3.1-5.9) as well as gastrointestinal bleeding (OR 2.8; 95% CI 1.7-4.6) was more increased than in users of SSRIs.

Conclusion

In users of acenocoumarol or phenprocoumon, SSRIs increase the risk of hospitalization for non-gastrointestinal bleeding, but not for gastrointestinal bleeding.

INTRODUCTION

Coumarin anticoagulants are effective drugs in the prevention and management of thromboembolic diseases. However, their use is complicated by a narrow therapeutic range and a large inter-individual and intra-individual variability, which necessitates frequent monitoring by specialized anticoagulation clinics. Coumarin anticoagulants are very sensitive to interactions with other drugs of which many have been described.[1,2] Interactions can result in undertreatment as well as overanticoagulation, the latter increasing the risk of major bleeding, the main complication of coumarin anticoagulants.[3-6]

Pharmacodynamic interactions between coumarin anticoagulants and other drugs which are themselves associated with an increased bleeding risk are conceivable. In several population based studies a further increase of the risk of major bleeding in users of coumarin anticoagulants has been convincingly demonstrated for nonsteroidal anti-inflammatory drugs (NSAIDs),[7-9] aspirin,[8,9] and glucocorticoids.[8] Selective serotonin reuptake inhibitors (SSRIs), frequently prescribed as anti-depressants or anxiolytics, have also been associated with an increased risk of upper gastrointestinal bleeding[10-13] as well as abnormal major bleeding in general[14] in several population based studies. These findings and several case reports in which bleedings or increased International Normalized Ratios have been described with concurrent use of coumarin anticoagulants and several SSRIs,[15-20] suggest a pharmacodynamic interaction between SSRIs and coumarin anticoagulants. For the SSRIs fluoxetine and fluvoxamine a pharmacokinetic effect might play a contributing role, since both drugs have been identified as inhibitors of CYP2C9, the main metabolizing enzyme of the more active (S)-enantiomers of warfarin, acenocoumarol, and to a lesser extent phenprocoumon.[21,22] Despite these theoretical considerations a recently conducted population based case-control study did not find an association between the use of SSRIs and an increased risk of hospitalization for upper gastrointestinal bleeding within a cohort of users of warfarin.[23]

To examine an association between concurrent use of SSRIs and coumarin anticoagulants with all possible major bleedings, we conducted a population based case-control study within a cohort of users of the coumarin anticoagulants acenocoumarol or phenprocoumon.

METHODS

Design and setting

We conducted a case-control study, nested within a cohort of new users of acenocoumarol or phenprocoumon, which are the two coumarin anticoagulants licensed in the Netherlands.

The setting of the study was the PHARMO record linkage system (www.pharmo.nl). This system includes the demographic details and complete medication history of more than two million community-dwelling residents of more than 25 population defined areas in the Netherlands from 1985 onwards, further linked to hospital admission records. Since virtually all patients in the Netherlands are registered with a single community pharmacy, independent of prescribing physician, pharmacy records are virtually complete with regard to prescription drugs.

For this study, drug prescribing data and hospitalization data were used. The computerized drug dispensing histories contain information concerning the dispensed drug, dispensing date, prescribing physician, amount dispensed, prescribed dosage regimen, and the estimated duration of use.

Drugs are coded according to the Anatomical Chemical Therapeutic (ATC) classification. The hospital admission and discharge codes are coded according to the International Classification of Diseases, 9th edition (ICD-9-CM).

Cohort and exposure to coumarins

In the cohort of new users of one of the coumarin anticoagulants, all patients of 18 years and older who received a first prescription for acenocoumarol or phenprocoumon between 1991 and 2004 and who did not have a history of hospital admission for major bleeding were included. A patient was defined as a new user of one of these coumarin anticoagulants if none of these drugs had been dispensed before the first coumarin dispensing in the PHARMO database and if a medication history of at least one year before initiation of the coumarin anticoagulant was available. Patients were followed up until either hospital admission for major bleeding, the end of the data collection, death, or discontinuation of acenocoumarol or phenprocoumon, whichever occurred first.

Prescriptions for coumarin anticoagulants do not contain information about the dosage, which is variable and frequently adjusted by anticoagulation clinics. As a consequence duration of coumarin use can not be calculated from the number of dispensed units and the prescribed dosage, necessitating some assumptions. We assumed that treatment was discontinued if more than 180 days passed between

two prescriptions for a coumarin anticoagulant and that the duration of coumarin use ended 180 days after the last recorded dispensing date of acenocoumarol or phenprocoumon. This last assumption has been made to avoid missing of serious major bleeding events which necessitated definite discontinuation of coumarin therapy. The period of 180 days has been estimated on the basis of experience in daily practice. Coumarin anticoagulants are usually dispensed in large quantities (several hundreds of defined daily dosages) and because daily dosages show a large inter-individual variation 180 days could be an under- as well as an overestimation of the duration of use.

Cases and controls

Cases were all patients with a first hospitalization for abnormal bleeding while being treated with acenocoumarol or phenprocoumon. To identify abnormal bleeding we used ICD-9-CM diagnostic codes, which cover the classification of major bleedings described by Fihn et al.[3] for complications of anticoagulant treatment (see appendix). The date of first hospitalization for abnormal bleeding was defined as the index date. For each case up to four non-hospitalized controls were randomly selected from the cohort by risk set sampling. Controls were matched with cases on gender, age (± 5 years), coumarin anticoagulant (acenocoumarol or phenprocoumon), duration of coumarin therapy (± 90 days), and dispensing pharmacy and were assigned the same index date as the corresponding case.

Definition of exposure

We analysed the following a priori chosen SSRIs: citalopram, escitalopram, fluvoxamine, fluoxetine, paroxetine, and sertraline. We also assessed whether nortriptylin and mirtazapin were associated with abnormal bleeding, both being frequently prescribed antidepressants without a significant affinity for the serotonin transporter.[24] In the PHARMO database the duration of use of a dispensed drug is calculated by dividing the number of dispensed units by the prescribed number to be used per day. If the duration of use +10% of an antidepressant ended on or beyond the index date, this was defined as current use of that antidepressant. If the duration of use +10% from a dispensing date ended within 30 days or >30 days before the index date, it was defined as recent use and past use, respectively.

Potential confounders

As confounding comedication we defined current use of NSAIDs (selective COX-2-inhibitors were not included), antiplatelet drugs (low dose aspirin,

clopidogrel, dipyridamole), glucocorticoids, gastroprotective agents (proton pump inhibitors, H2 receptor antagonists, and misoprostol), known inhibitors of coumarin metabolism (amiodarone, allopurinol, benzbromarone, miconazole, fluconazole, and gemfibrozil),[2,25-27] known inducers of coumarin metabolism (carbamazepine, phenytoin, phenobarbitone, and rifampicin),[2,27] and antibiotics (as a proxy for intercurrent infections). For current use of confounding comedication, we used the same definitions as for SSRIs.

Medication history was used as marker for comorbidities. Any use before the index date of thyroid therapy, antidiabetic drugs, antineoplastic agents, and a combination of either ACE inhibitors or angiotensin II antagonists with loop diuretics were proxies for thyroid diseases, diabetes mellitus, malignancies, and heart failure, respectively.

Statistical analysis

We used conditional logistic regression models on the matched sets to estimate the risk of bleeding associated with current use of SSRIs, expressed as odds ratios (OR) with 95% confidence intervals (CI). We also assessed ORs for current use of NSAIDs (selective COX-2-inhibitors not included) and antibiotics as a positive test of the validity of our data set because both drug groups have been strongly associated with an increased risk of major bleeding and severe overanticoagulation in users of coumarins.[7-9,28]

We stratified our analyses by gastrointestinal and non-gastrointestinal bleedings. Moreover, we separately analysed the potentially most invalidating intracranial bleedings. We also stratified our analyses by the CYP2C9 inhibiting SSRIs fluoxetine/fluvoxamine and the other SSRIs.

In sensitivity analyses we reanalysed our results for the assumption that the coumarin use ended maximally 30, 60, or 90 days after the last dispensing date (instead of 180 days) and for users who received more than one prescription for a coumarin anticoagulant. Moreover, we reanalysed our results for bleeding events which occurred after the first 28 days of coumarin therapy, increasing the chance that patients are more or less stabilized because the initiation phase can be attended with problems of dose finding and severe overanticoagulation.

Finally, we also analysed our data for recent past use and past use of the examined drugs.

All statistical analyses were performed using the statistical software package SPSS, version 12 (SPSS Inc, Chicago, Ill, USA).

RESULTS

We identified 70 201 patients who were treated with acenocoumarol or phenprocoumon for a total of 131 707 patient-years. Within this cohort we identified 2403 cases of first bleeding requiring hospitalization (incidence rate 1.82 per 100 patient-years). Of these, 555 cases could not be matched to controls, leaving 1848 cases available for analyses which were matched with 5818 controls. There were 605 gastrointestinal and 1243 non-gastrointestinal bleedings. The most frequently occurring category was upper gastrointestinal bleeding, followed by intracranial bleeding (Table 1). Mean age at the index date was 72.7 years, there were more men than women and almost 90% of the patients used acenocoumarol (Table 2).

Table 1: ADMISSION DIAGNOSIS OF PATIENTS WITHIN A COHORT OF USERS OF ACENOCOUMAROL OR PHENPROCOUMON HOSPITALIZED WITH A FIRST MAJOR BLEEDING EVENT

Bleeding localization	N=1848 (100%)
Gastrointestinal	**605 (32.7%)**
upper gastrointestinal	537 (29.1%)
lower gastrointestinal	68 (3.7%)
Non-gastrointestinal	**1243 (67.3%)**
intracranial	318 (17.2%)
uterus	131 (7.1%)
urinary tract	115 (6.2%)
joint	34 (1.8%)
eye	20 (1.1%)
nose	161 (8.7%)
other[a]	464 (25.1%)

a) Other bleedings: haemoptysis, bleeding complicating a procedure, haemoperitoneum, spontaneous ecchymoses, and bleedings not otherwise specified.

Users of SSRIs had a significantly increased risk of hospitalization for non-gastrointestinal bleeding but not for gastrointestinal bleeding (OR 1.7; 95% CI 1.1-2.5 and OR 0.8; 95% CI 0.4-1.5, respectively). For the non SRIs nortriptylin and mirtazapine no increased risk for both categories of major bleeding was found (Table 3). The relative risk of hospitalization for non-gastrointestinal bleeding associated with use of SSRIs was comparable to the relative risk of bleeding due to the use of NSAIDs, but smaller than the risk in users of antibiotics. As expected, we found that NSAIDs increased the risk of

Table 2: GENERAL CHARACTERISTICS, CURRENT USE OF RELEVANT MEDICATION, AND COMORBIDITIES OF CASES AND CONTROLS (N=7666)

Characteristic	Cases	Controls
Age on index date, mean (SD)	72.7 (10.3)	72.9 (9.7)
	n=1848 (100%)	**n=5818 (100%)**
Male	993 (53.7%)	3173 (54.5%)
Acenocoumarol on index date	1628 (88.1%)	5302 (91.1%)
Selective serotonine reuptake inhibitors	58 (3.1%)	116 (2.0%)
Non serotonergic antidepressants (mirtazapine, nortriptyline)	3 (0.2%)	19 (0.3%)
Nonsteroidal anti-inflammatory drugs	222 (12.0%)	299 (5.1%)
Antiplatelet drugs (aspirine, clopidogrel, dipyridamole)	227 (12.3%)	514 (8.8%)
Glucocorticoids	113 (6.1%)	176 (3.0%)
Gastroprotective agents (proton pump inhibitors, H2-antihistamines, misoprostol)	313 (16.9%)	703 (12.1%)
Inhibitors of coumarin metabolism (amiodarone, allopurinol, benzbromarone, cimetidine, miconazole, fluconazole, gemfibrozil)	153 (8.3%)	385 (6.6%)
Inducers of coumarin metabolism (carbamazepine, phenytoin, phenobarbitone, rifampicin)	21 (1.1%)	66 (1.1%)
Antibiotics	148 (8.0%)	124 (2.1%)
Diabetes mellitus	334 (18.1%)	869 (14.9%)
Thyroid disorders	104 (5.6%)	336 (5.8%)
Heart failure	581 (31.4%)	1507 (25.9%)
Malignancies	38 (2.1%)	91 (1.6%)

Table 3: ASSOCIATION BETWEEN CURRENT USE OF ANTIDEPRESSANTS, NSAIDs, AND ANTIBIOTICS AND HOSPITALIZATION FOR GASTROINTESTINAL AND NON-GASTROINTESTINAL BLEEDING

	Gastrointestinal bleedings				Non-gastrointestinal bleedings			
	Cases n=605 (100%)	Controls n=1914 (100%)	Univariate OR (95% CI)	Multivariate[a] OR (95% CI)	Cases n=1243 (100%)	Controls n=3904 (100%)	Univariate OR (95% CI)	Multivariate[b] OR (95% CI)
SSRIs	15 (2.5%)	42 (2.2%)	1.1 (0.6-2.0)	0.8 (0.4-1.5)	43 (3.5%)	74 (1.9%)	1.8 (1.2-2.6)	1.7 (1.1-2.5)
Non SRIs	0	9 (0.5%)	N.A.	N.A.	3 (0.2%)	10 (0.3%)	1.2 (0.3-4.5)	1.1 (0.3-4.0)
NSAIDs	110 (18.2%)	86 (4.5%)	4.6 (3.3-6.4)	4.6 (3.3-6.5)	112 (9.0%)	213 (5.5%)	1.8 (1.4-2.2)	1.7 (1.3-2.2)
Antibiotics	39 (6.4%)	45 (2.4%)	2.9 (1.8-4.5)	2.8 (1.7-4.6)	109 (8.8%)	79 (2.0%)	4.5 (3.3-6.2)	4.3 (3.1-5.9)

NSAIDs = nonsteroidal anti-inflammatory drugs; (S)SRIs = (selective) serotonin reuptake inhibitors; OR = odds ratio; N.A. = not applicable

a) In the multivariate analysis the following factors were included: current use of SSRIs, NSAIDs, antiplatelet drugs, antibiotics, glucocorticoids, gastroprotective agents (proton pump inhibitors, H2-anthistamines, and misoprostol), inhibitors and inducers of coumarin metabolism, and the comorbities diabetes mellitus, thyroid disorders, heart failure, and malignancies.

b) In the multivariate analysis the same factors were included as for gastrointestinal bleedings except gastroprotective agents.

Table 4: ASSOCIATION BETWEEN CURRENT USE OF SSRIs, NSAIDs, AND ANTIBIOTICS AND HOSPITALIZATION FOR NON-GASTROINTESTINAL BLEEDING FOR VARIOUS ASSUMPTIONS

	Multivariate odds ratios (OR)				
	Last dispensing date of a coumarin – index date: maximally 90 days	Last dispensing date of a coumarin – index date: maximally 60 days	Last dispensing date of a coumarin – index date: maximally 30 days	More than one prescription for a coumarin	Bleedings after the first 28 days of coumarin therapy
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
SSRIs	2.0 (1.2-3.3)	2.0 (1.2-3.5)	1.7 (0.9-3.1)	1.8 (1.1-2.9)	1.8 (1.2-2.9)
NSAIDs	1.8 (1.3-2.3)	1.8 (1.3-2.5)	1.9 (1.3-2.7)	1.8 (1.4-2.4)	1.7 (1.3-2.3)
Antibiotics	4.4 (3.1-6.3)	4.8 (3.2-7.2)	4.0 (2.6-6.2)	4.3 (3.0-6.1)	4.2 (3.0-5.9)

SSRIs = selective serotonin reuptake inhibitors; NSAIDs = nonsteroidal anti-inflammatory drugs; index date = date of first hospitalization for abnormal bleeding

gastrointestinal bleeding more than the risk of non-gastrointestinal bleeding (Table 3).
Separate analysis for intracranial bleeding resulted in not significantly increased risks for users of SSRIs (OR 1.6; 95% CI 0.7-3.4; p=0.26) and NSAIDs (OR 1.6; 95% CI 1.0-2.8; p=0.059), and a just significantly increased risk for antibiotics (OR 2.2; 95% CI 1.0-5.0; p=0.050). Point estimates for SSRIs and NSAIDs were similar to those for all non-gastrointestinal bleedings.
Separate analysis for fluoxetine/fluvoxamine and the other SSRIs did not result in essentially different point estimates for non-gastrointestinal bleedings (OR 1.4; 95% CI 0.7-2.9 and OR 1.8; 95% CI 1.1-3.0, respectively). This also applied to separate analysis for gastrointestinal bleeding (data not shown).
Numbers were too low for a reliable separate analysis for users of SSRIs and phenprocoumon. However, separate analysis for users of acenocoumarol resulted in similar point estimates as for the pooled analysis of users of acenocoumarol and phenprocoumon.
Sensitivity analyses did not change the overall picture of our results. The point estimates for non-gastrointestinal bleeding remained similar if the assumption for the maximal time span between last dispensing date of a coumarin and index date was reduced from 180 days to 90, 60 or 30 days, or if only patients who received more than one prescription for a coumarin anticoagulant were analysed, or if only bleedings after the first 28 days were taken into account (Table 4). Only if the maximal time span between the last dispensing date of the coumarin and index date was reduced to 30 days, significance was lost for the association with SSRIs (p=0.077). Results for gastrointestinal outcomes also did not change with these assumptions (data not shown).
Recent use and past use of SSRIs and NSAIDs showed an immediate attenuation of the effect on non-gastrointestinal bleeding, risk being not increased any more. Recent use of antibiotics showed a still significantly increased risk for non-gastrointestinal major bleeding (univariate OR 2.2; 95% CI 1.8-2.9).

DISCUSSION

The main finding of our study is that SSRIs significantly increase the risk of hospitalization for major non-gastrointestinal, but not for gastrointestinal bleeding, in users of coumarin anticoagulants. Current use of NSAIDs or antibiotics was also independently associated with an increased risk of

gastrointestinal as well as non-gastrointestinal bleeding, the effect of NSAIDs on gastrointestinal bleeding being greatest.

Kurdyak et al. have recently examined a possible association between concurrent use of SSRIs and warfarin and major bleeding.[23] Despite several differences between their study and ours, our findings for gastrointestinal bleedings were similar to Kurdyak's, who reported a multivariate odds ratio of 1.1 (95% CI 0.8-1.7). The consistency of the findings of both studies strongly suggests that SSRIs do not increase the risk of gastrointestinal bleeding in users of coumarins.

At the other hand, we found a substantially increased risk of non-gastrointestinal bleeding in current users of SSRIs. These apparently different effects of SSRIs on gastrointestinal and non-gastrointestinal bleedings in users of coumarins are at first sight unexpected. A first step in the explanation could be our finding that SSRIs appear to increase the non-gastrointestinal bleeding risk to the same extent as NSAIDs (Table 4), whereas there is an obvious difference for gastrointestinal bleedings, NSAIDs markedly increasing the risk where SSRIs show no effect at all.

Among users of coumarins with upper gastrointestinal bleeding underlying known or previously unknown lesions have been identified in up to 70% of patients.[29] This makes a pharmacodynamic interaction between coumarins and NSAIDs, being notorious for their gastrointestinal toxicity, conceivable and not surprisingly a bleeding risk increasing effect of NSAIDs in users of coumarins have been convincingly demonstrated in several population based observational studies.[7,8,30] If SSRIs increase the risk of upper gastrointestinal bleeding in users of coumarins, it is probably not a consequence of gastrointestinal toxicity, but of a reduced platelet aggregation, an effect which has been demonstrated for all SSRIs in patients suffering from depression as well as normal control subjects.[31] Although several population based observational studies demonstrated an association between use of SSRIs as such and upper gastrointestinal bleeding[10,11,13,32] or upper and lower gastrointestinal bleeding,[12] a recent systematic review on this subject concluded that the overall evidence for such an association is weak,[33] suggesting that SSRIs mainly precipitate bleeding in patients with haemostatic defects or in patients who are taking drugs that cause gastrointestinal injury such as NSAIDs. This last assertion is supported by the finding in several studies[10,12,13] of a synergistically increased risk of upper gastrointestinal bleeding with concurrent use of SSRIs and NSAIDs compared to separate use of SSRIs and NSAIDs. A possible explanation of this synergism, apart from the gastrointestinal toxicity of NSAIDs, could be the combination of different

antiplatelet effects of SSRIs and NSAIDs, the first reducing the platelet serotonin, the last reducing the thromboxane synthesis by inhibiting cyclo-oxygenase I. The results of our study and Kurdyak's indicate that such a synergism does not exist for concurrent use of coumarins and SSRIs, the possible effect of SSRIs on pre-existing lesions in the gastrointestinal tract adding nothing to and being offset by the pharmacologically stronger anticoagulant effect of coumarins. However, when we come to discuss non-gastrointestinal bleedings, the mechanism by which NSAIDs increase the bleeding risk is no longer the potential damaging effect on the gastric mucosa, but only the antiplatelet effect. Apparently, for the non-gastrointestinal bleedings in our study inhibition of platelet aggregation does contribute to an increased risk of major bleeding in users of coumarins, making our similar point estimates for the odds ratios of SSRIs and NSAIDs plausible. Our finding that the CYP2C9 inhibiting SSRIs fluoxetine and fluvoxamine did not show an essentially different risk compared to the other SSRIs, strengthens the probability of an underlying pharmacodynamic mechanism. Of course, non-gastrointestinal bleedings do not have similar pathophysiological mechanisms and more study is needed to assess for which bleedings the risk is increased by concurrent use of SSRIs and coumarins. The second most frequently occurring and also most disabling category of bleedings is the intracranial bleeding, for which the stratified analysis suggested a similar increased risk for NSAIDs as well as SSRIs, although statistical significance was not achieved for SSRIs whereas NSAIDs showed a strong trend. This is probably a power problem, the number of users of SSRIs being smaller than the number of users of NSAIDs.

Our results suggest no indication for an increased risk of neither gastrointestinal nor non-gastrointestinal bleedings for nortriptylin and mirtazapine, although numbers were too small for a reliable analysis.

The increase of the major bleeding risk in users of antibiotics is in agreement with several studies which have identified antibiotic therapy as one of the highest risk factors for bleeding[9] or overanticoagulation,[34-36] the most probable explanation being that antibiotic use is a marker for intercurrent infections that can adversely affect anticoagulation control. The risks for gastrointestinal as well as non-gastrointestinal bleedings were markedly higher than in users of SSRIs, suggesting that intercurrent infections are a more important risk factor.

Because of the pharmacodynamic nature of the effect of SSRIs on the bleeding risk in users of the coumarins from our study, we think that our results also apply to warfarin.

Some limitations of our study have to be considered. First, there is the possibility of misclassification of users of coumarins, because we had to make assumptions regarding the duration of coumarin use. There are large inter-individual differences in coumarin dose requirements and the supposed maximal time between the last dispensing date of a coumarin and the index date of 180 days could have been an overestimation, but to the experience of the first author, a community pharmacist, prescriptions of coumarins are being frequently dispensed for an even longer period in the Netherlands. Moreover, a reduction of the maximal time between the last dispensing date of a coumarin and the index date did not result in different outcomes suggesting that our assumptions were valid. A second limitation is that we did not have data on the intensity of anticoagulation (normal or high) or on diseases that we could not derive from drug therapy such as liver- and renal insufficiency, which are also risk factors for major bleeding.[27,29] A third limitation is that we could only evaluate a history of hospitalization for major bleeding from the moment patients were included in the PHARMO record linkage system, implicating that we could have missed information about earlier bleedings in patients. Because of these limitations, more study in a prospective setting of coumarin users is needed to confirm our results and to assess which specific non-gastrointestinal bleedings are most affected by SSRIs.

Because of the pharmacodynamic nature of the effect of SSRIs, correcting for their bleeding risk increase by downward dose adjustments of coumarins could result in values of the International Normalized Ratio, the main measure of the coumarin anticoagulation level, under the therapeutic range. This complicates management of concurrent use of SSRIs and coumarins and the advantages of SSRIs have to be carefully weighed against the drawback of an increased bleeding risk. Given the limitations of our study it goes too far to advise against concurrent use of SSRIs and coumarin anticoagulants, but intensified monitoring of users of SSRIs seems justified.

In conclusion, the results of our study strongly suggest that use of SSRIs increase the risk of hospitalization for non-gastrointestinal bleeding to the same degree as NSAIDs.

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Appendix: LIST OF THE ICD-9-CM CODES OF THE ABNORMAL BLEEDING EVENTS WHICH WERE IDENTIFIED IN CASES

Description	ICD-9 code	N
Gastrointestinal bleedings		
Upper gastrointestinal		
esophageal varices with bleeding	456.0	1
gastric ulcer, acute with haemorrhage	531.0	6
gastric ulcer, acute with haemorrhage and perforation	531.1	1
gastric ulcer, chronic or unspecified with haemorrhage	531.4	80
gastric ulcer, chronic or unspecified with haemorrhage and perforation	531.6	1
duodenal ulcer, acute with haemorrhage	532.0	17
duodenal ulcer, chronic or unspecified with haemorrhage	532.4	54
duodenal ulcer, chronic or unspecified with haemorrhage and perforation	532.6	2
peptic ulcer, acute with haemorrhage without obstruction	533.0	1
gastrojejunal ulcer, chronic or unspecified with haemorrhage and perforation	534.4	1
haematemesis	578.0	26
melaena	578.1	96
haemorrhage of gastrointestinal tract, not otherwise specified	578.9	251
Lower gastrointestinal		
haemorrhage of rectum or anus	569.3	68
Non-gastrointestinal bleedings		
Intracranial		
subarachnoidal haemorrhage	430	22
intracerebral haemorrhage	431	218
nontraumatic extradural haemorrhage	432.0	3
subdural haemorrhage	432.1	65
intracranial haemorrhage, not otherwise specified	432.9	10
Urinary tract		
haemorrhage into bladder wall	596.7	3
haematuria	599.7	102
Uterus		
ovulation bleeding	626.5	1
metrorrhagia	626.6	20
disorder of menstruation or other abnormal bleeding, not otherwise specified	626.9	5
premenopausal haemorrhage	627.0	1
postmenopausal bleeding	627.1	104
Nose		
epistaxis	784.7	161

Description	ICD-9 code	N
Eye		
haemophthalmos except current injury	360.43	1
choroidal haemorrhage, unspecified	363.61	1
hyphaema	364.41	3
conjunctival haemorrhage	372.72	2
vitreous haemorrhage	379.32	13
Joint		
haemarthrosis, site unspecified	719.10	1
haemarthrosis, shoulder	719.11	4
haemarthrosis, upper arm	719.12	1
haemarthrosis, pelvic region and tigh	719.15	3
haemarthrosis, lower leg	719.16	25
Other		
haemoptysis	786.3	115
haemoperitoneum	568.81	6
spontaneous ecchymoses	782.7	1
haemorrhage or haematoma complicating a procedure	998.1	210
haemorrhage, unspecified	459.0	132

ICD-9-CM = International Classification of Diseases, 9th edition

3.3

Effect of antiplatelet drugs on major bleeding in users of acenocoumarol or phenprocoumon

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Submitted

ABSTRACT

Objective

Although oral antiplatelet drugs and coumarins are increasingly combined, the bleeding risk of the combination of clopidogrel or dipyridamole and coumarins is unclear. Therefore, we assessed the risk of major bleeding associated with the use of clopidogrel and dipyridamole next to low dose aspirin in users of acenocoumarol or phenprocoumon.

Methods

We used data from the PHARMO record linkage system including pharmacy and linked hospitalization records of about two million subjects in the Netherlands to conduct a case-control study nested within a cohort of new users of acenocoumarol or phenprocoumon. Cases were patients who were hospitalized for a primary diagnosis of major bleeding while taking a coumarin. Cases were matched with up to four controls for sex, age, coumarin, duration of coumarin use and dispensing pharmacy. Conditional logistic regression was used to determine odds ratios (OR) and 95% confidence intervals (CI) for the risk of hospitalization for major bleeding associated with concurrent use of clopidogrel, dipyridamole, low dose aspirin and combinations of these antiplatelet drugs.

Results

We identified 1848 cases of major bleeding (537 upper gastrointestinal and 1311 other). The risk of major bleeding was significantly increased among users of clopidogrel and aspirin (OR 2.9; 95% CI 1.2-6.9 and OR 1.6; 95% CI 1.3-1.9, respectively), whereas this risk showed a strong trend among users of dipyridamole and combinations of antiplated drugs (OR 1.5; 95% CI 1.0-2.3 and OR 1.8; 95% CI 1.0-3.3. respectively). In all cases, the risks were greater for upper gastrointestinal bleedings than for other bleedings.

Conclusion

Among users of coumarins, all antiplatelet drugs increase the risk of upper gastrointestinal bleeding and the risk of other bleedings to a lesser extent. Concurrent use of dipyridamole or clopidogrel and coumarins is probably not safer than concurrent use of aspirin and coumarins.

INTRODUCTION

Anticoagulants of the coumarin type are highly effective in the prevention of venous and arterial thromboembolism. The most common indication is atrial fibrillation for which the therapeutic superiority over a combination of the antiplatelet drugs aspirin and clopidogrel has been recently established.[1] The principal adverse effect of therapy with anticoagulants of the coumarin type is major bleeding, which can be fatal or invalidating. This increased bleeding risk is an inevitable consequence of the pharmacodynamics of the coumarins, which affect the coagulation cascade by interfering with the activation of clotting factors II, VII, IX, and X, ultimately inhibiting the formation of fibrin.

Antiplatelet drugs interfere with the activation of platelets and are of major importance in the prevention of atherothrombosis in patients suffering from atherosclerosis. There is ample evidence for the effectiveness of antiplatelet therapy in preventing recurrent vascular events in cerebrovascular disease, coronary artery disease, and peripheral artery disease.[2,3]

With the increasing use of antiplatelet drugs, the incidence of concurrent use with coumarin anticoagulants is expected to increase, for example in patients suffering from atrial fibrillation and coronary artery disease. Because of the different pathways along which coumarins and antiplatelet drugs affect haemostasis, an increased major bleeding risk with concurrent use compared with use of coumarins alone is conceivable for all antiplatelet drugs. For concurrent use of aspirin with coumarins such an increased bleeding risk has been convincingly demonstrated in several clinical trials,[4-8] meta-analyses,[9,10] and population based observational studies.[11-14] However, the effect of the newer antiplatelet drugs clopidogrel and dipyridamole on the bleeding risk among users of coumarins is less obvious. For dipyridamole conflicting results have been described,[15,16] whereas hitherto no data have been reported on the risk of concurrent use of clopidogrel, and coumarins. To establish the effect on the bleeding risk of clopidogrel, and dipyridamole among users of coumarins, we conducted a population based case-control study within a cohort of users of the coumarin antagonists acenocoumarol, and phenprocoumon.

METHODS

Design and setting

We conducted a case-control study, nested within a cohort of new users of acenocoumarol or phenprocoumon, which are the two coumarin anticoagulants licensed in the Netherlands. The setting of the study was the PHARMO record linkage system (www.pharmo.nl). This system includes the demographic details, and complete medication history of more than two million community-dwelling residents of more than 25 population defined areas in the Netherlands from 1985 onwards, further linked to hospital admission records. Since virtually all patients in the Netherlands are registered with a single community pharmacy, independent of prescribing physician, pharmacy records are virtually complete with regard to prescription drugs.

For this study, drug prescribing data and hospitalization data were used. The computerized drug dispensing histories contain information concerning the dispensed drug, dispensing date, prescribing physician, amount dispensed, prescribed dosage regimen, and the estimated duration of use.

Drugs are coded according to the Anatomical Chemical Therapeutic (ATC) classification. The Hospital admission and discharge code are coded according to the International Classification of Diseases, 9th edition (ICD-9-CM).

Cohort and exposure to coumarins

In the cohort of new users of one of the coumarin anticoagulants all patients of 18 years and older who received a first prescription for acenocoumarol or phenprocoumon between 1991 and 2004 and who did not have a history of hospital admission for major bleeding were included. A patient was defined as a new user of one of these coumarin anticoagulants if none of these drugs had been dispensed before the first coumarin dispensing in the PHARMO database and if a medication history of at least one year before initiation of the coumarin anticoagulant was available. Patients were followed until either hospital admission for major bleeding, the end of data collection, death, or discontinuation of acenocoumarol or phenprocoumon, whichever occurred first.

Prescriptions for coumarin anticoagulants do not contain information about the dosage, which is variable and frequently adjusted by anticoagulation clinics. As a consequence duration of coumarin use can not be calculated from the number of dispensed units and the prescribed dosage, necessitating some assumptions. We assumed that treatment was discontinued if more than 180 days passed between two prescriptions for a coumarin anticoagulant and that the duration of coumarin

use ended 180 days after the last recorded dispensing date of acenocoumarol or phenprocoumon. This last assumption has been made to avoid missing of serious major bleeding events which necessitated definite discontinuation of coumarin therapy. The period of 180 days has been estimated on the basis of experience in daily practice. Coumarin anticoagulants are usually dispensed in large quantities (several hundreds of defined daily dosages) and because daily dosages show a large interindividual variation 180 days could be an underestimation as well as an overestimation of the duration of use.

Cases and controls

Cases were all patients with a first hospitalization for abnormal bleeding while being treated with acenocoumarol or phenprocoumon. To identify abnormal bleeding we used ICD-9-CM diagnostic codes, which cover the classification of major bleedings described by Fihn et al. for complications of anticoagulant treatment (see Appendix).[17] The date of first hospitalization for abnormal bleeding was defined as the index date. For each case up to four non-hospitalized controls were randomly selected from the cohort by risk set sampling. Controls were matched with cases on gender, age (± 5 years), coumarin anticoagulant (acenocoumarol or phenprocoumon), duration of coumarin therapy (± 90 days), and dispensing pharmacy and were assigned the same index date as the corresponding case.

Definition of exposure

We analysed the following a priori chosen antiplatelet drugs: clopidogrel (ATC code B01AC04), dipyridamole (ATC code B01AC07), and low dose aspirin (30-100 mg) (ATC codes B01AC06 and B01AC08). Since antiplatelet drugs can be used concurrently[18-21] and since such combinations could carry a more increased bleeding risk,[18-20] we separately analysed use of aspirin, clopidogrel, and dipyridamole alone or use of these drugs in combination (including the fixed combination of aspirin and dipyrimole, ATC code B01AC30). In the PHARMO database the duration of use of a dispensed drug is calculated by dividing the number of dispensed units by the prescribed number to be used per day. If the duration of use +10% of an antiplatelet drug ended on or beyond the index date, this was defined as current use of the antiplatelet drug. If the duration of use +10% from a dispensing date ended within 30 days or >30 days before the index date, this was defined as recent use and past use, respectively.

Potential confounders

As potentially confounding comedication we defined current use of nonsteroidal anti-inflammatory drugs (NSAIDs) (selective COX-2-inhibitors were not included), selective serotonin reuptake inhibitors (SSRIs), glucocorticoids, known inhibitors of coumarin metabolism (amiodarone, allopurinol, benzbromarone, miconazole, fluconazole, and gemfibrozil),[22-24] known inducers of coumarin metabolism (carbamazepine, phenytoin, phenobarbitone, and rifampicin),[22,24] and antibiotics (as a proxy for intercurrent infections). Moreover, use of gastroprotective agents (proton pump inhibitors, H2 receptor antagonists, and misoprostol) was defined as confounding comedication for upper gastrointestinal bleeding. For current use of confounding comedication, we used the same definitions as for antiplatelet drugs.

Medication history was used as marker for comorbidities. Any use before the index date of thyroid therapy, antidiabetic drugs, antineoplastic agents, and a combination of ACE inhibitors or angiotensin II antagonists with loop diuretics were proxies for thyroid diseases, diabetes mellitus, malignancies, and heart failure, respectively.

Statistical analysis

We used conditional logistic regression models on the matched sets to estimate the risk of bleeding associated with current use of antiplatelet drugs, expressed as odds ratios (ORs) with 95% confidence intervals. We stratified our analyses by upper gastrointestinal and non upper gastrointestinal bleedings (designated as 'other bleedings'), because these bleedings have different prognostic factors and because we expected that at least use of aspirin would be more associated with upper gastrointestinal bleeding than with other bleedings. For both categories of bleedings we separately analysed clopidogrel, dipyridamole, low dose aspirin, and all combinations of these antiplatelet drugs.

Within the stratum of upper gastrointestinal bleedings we also analysed whether an effect of antiplatelet drugs was modified by gastroprotective drugs.

We separately analysed our results for users of acenocoumarol and for users of phenprocoumon.

In sensitivity analyses we reanalysed our results for the assumption that the coumarin use ended maximally 30, 60, or 90 days after the last dispensing date (instead of 180 days) and for users who received more than one prescription for a coumarin anticoagulant. Moreover, we reanalysed our results for bleeding events which occurred after the first 28 days of coumarin use, increasing the chance that

patients are more or less stabilized because the initiation phase of the coumarin can carry problems such as dose finding and severe overanticoagulation. Finally, we also analysed our data for recent use and past use of the examined drugs.
All statistical analyses were performed using the statistical software package SPSS, version 12 (SPSS Inc, Chicago, Ill, USA).

Table 1: ADMISSION DIAGNOSIS OF PATIENTS WITHIN A COHORT OF USERS OF ACENOCOUMAROL OR PHENPROCOUMON HOSPITALIZED WITH A FIRST MAJOR BLEEDING EVENT

Bleeding localization	N=1848 (100%)
Gastrointestinal	**605 (32.7%)**
upper gastrointestinal	537 (29.1%)
lower gastrointestinal	68 (3.7%)
Non-gastrointestinal	**1243 (67.3%)**
intracranial	318 (17.2%)
uterus	131 (7.1%)
urinary tract	115 (6.2%)
joint	34 (1.8%)
eye	20 (1.1%)
nose	161 (8.7%)
other[a]	464 (25.1%)

a) Other bleedings: haemoptysis, bleeding complicating a procedure, haemoperitoneum, spontaneous ecchymoses, and bleedings not otherwise specified.

RESULTS

We identified 70 201 patients who were treated with acenocoumarol or phenprocoumon for a total of 131 707 patient-years. Within this cohort we identified 2403 cases of first bleeding requiring hospitalization (incidence rate 1.82 per 100 patient-years). 555 of these cases could not be matched to controls, leaving 1848 cases available for analysis which were matched with 5818 controls. There were 537 upper gastrointestinal bleedings, which was the most frequently occurring category, and 1311 other bleedings (Table 1). Mean age at index date was 72.7 years, there were more men than women and almost 90% of the patients used acenocoumarol (Table 2).
Daily dosages of aspirin ranged from 30 to 100 mg for most patients (four out of 536 aspirin users had a daily dosage of 160 mg), daily dosages of dipyridamole ranged from 150 to 450 mg and the daily dosage of clopidogrel was 75 mg.

Table 2: GENERAL CHARACTERISTICS, CURRENT USE OF ANTIPLATELET DRUGS AND RELEVANT COMEDICATION, AND COMORBIDITIES OF CASES AND CONTROLS (N=7666)

Characteristic	Cases	Controls
Age on index date, mean (SD)	72.7 (10.3)	72.9 (9.7)
	n=1848 (100%)	**n=5818 (100%)**
Male	993 (53.7%)	3173 (54.5%)
Acenocoumarol on index date	1628 (88.1%)	5302 (91.1%)
Aspirin alone (30-100 mg)	165 (8.9%)	381 (6.5%)
Clopidogrel alone	11 (0.6%)	11 (0.2%)
Dipyridamole alone	34 (1.8%)	85 (1.5%)
Aspirin + clopidogrel	5 (0.3%)	6 (0.1%)
Aspirin + dipyridamole	12 (0.6%)	30 (0.5%)
Clopidogrel + dipyridamole	0	1 (0.0%)
Nonsteroidal anti-inflammatory drugs	222 (12.0%)	299 (5.1%)
Glucocorticoids	113 (6.1%)	176 (3.0%)
Selective serotonine reuptake inhibitors	58 (3.1%)	116 (2.0%)
Gastroprotective agents (proton pump inhibitors, H2-antihistamines, misoprostol)	313 (16.9%)	703 (12.1%)
Inhibitors of coumarin metabolism (amiodarone, allopurinol, benzbromarone, cimetidine, miconazole, fluconazole, gemfibrozil)	153 (8.3%)	385 (6.6%)
Inducers of coumarin metabolism (carbamazepine, phenytoin, phenobarbitone, rifampicin)	21 (1.1%)	66 (1.1%)
Antibiotics	148 (8.0%)	124 (2.1%)
Diabetes mellitus	334 (18.1%)	869 (14.9%)
Thyroid disorders	104 (5.6%)	336 (5.8%)
Heart failure	581 (31.4%)	1507 (25.9%)
Malignancies	38 (2.1%)	91 (1.6%)

Table 3: ASSOCIATION BETWEEN CURRENT USE OF ASPIRIN (30 AND 80 MG), CLOPIDOGREL, DIPYRIDAMOLE, AND COMBINATIONS OF PLATELET INHIBITORS AND HOSPITALIZATION FOR UPPER GASTROINTESTINAL BLEEDINGS AND OTHER BLEEDINGS

	Cases	Controls	Crude		Adjusted[a]	
Upper gastrointestinal bleedings	**n=537 (100%)**	**n=1684 (100%)**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**
clopidogrel alone	6 (1.1%)	4 (0.2%)	4.7 (1.3-17.0)	0.019[d]	3.6 (0.9-13.5)	0.062
dipyridamole alone	12 (2.2%)	24 (1.4%)	1.9 (0.9-3.9)	0.089	2.2 (1.1-4.6)	0.043[d]
aspirin alone, all dosages	56 (10.4%)	105 (6.2%)	1.9 (1.4-2.8)	<0.001[d]	2.1 (1.5-3.1)	<0.001[d]
concurrent use of antiplatelet drugs[b]	10 (1.9%)	13 (0.8%)	2.7 (1.1-6.3)	0.024[d]	3.4 (1.4-8.4)	0.006[d]
Other bleedings	**n=1311 (100%)**	**n=4134 (100%)**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**
clopidogrel alone	5 (0.4%)	7 (0.2%)	2.6 (0.8-8.3)	0.10	2.4 (0.7-8.0)	0.15
dipyridamole alone	22 (1.7%)	61 (1.5%)	1.0 (0.6-1.8)	0.90	1.3 (0.8-2.1)	0.22
aspirin alone, all dosages	109 (8.3%)	276 (6.7%)	1.4 (1.1-1.8)	0.007[d]	1.4 (1.1-1.7)	0.012[d]
concurrent use of antiplatelet drugs[c]	7 (0.5%)	24 (0.6%)	1.1 (0.5-2.7)	0.78	1.2 (0.5-2.8)	0.71

OR = odds ratio

a) Adjusted for use of NSAIDs, antibiotics, SSRIs, glucocorticoids, inhibitors and inducers of coumarin metabolism, diabetes mellitus, heart failure, thyroid disorders, and malignancies. For upper gastrointestinal, ORs were also adjusted for gastroprotective agents (proton pump inhibitors, H2 antihistamines and misoprostol).

b) Aspirin + clopidogrel, 5 cases/0 controls; aspirin + dipyridamole, 5 cases/12 controls; clopidogrel + dipyridamole, 0 cases/1 control.

c) Aspirin + clopidogrel, 0 cases/6 controls; aspirin + dipyridamole, 7 cases/18 controls.

d) Statistically significant difference ($p<0.05$).

Use of all antiplatelet drugs, including clopidogrel and dipyridamole, increased the risk of upper gastrointestinal bleeding. The effect was marginally not significant for clopidogrel (p-value 0.062), although the point estimate was highest. The point estimate was also higher for dipyridamole than for low dose aspirin (Table 3). Use of gastroprotective drugs did not modify the effect of antiplatelet drugs on the outcome upper gastrointestinal bleeding.

Use of antiplatelet drugs increased the risk of other bleedings to a less extent, only reaching significance for aspirin. Use of dipyridamole resulted in a similar point estimate, whereas the point estimate for clopidogrel was greatest, as it was for upper gastrointestinal bleedings (Table 3).

For the outcome major bleeding (upper gastrointestinal and other taken together) the risks were significantly increased for clopidogrel alone and for aspirin alone, multivariate ORs being 2.9 (95% CI 1.2-6.9) and 1.6 (95% CI 1.3-1.9), respectively. The risks for dipyridamole and combinations of antiplatelet drugs were not significantly increased for all bleedings together although in both cases there was a strong trend towards significance, multivariate ORs being 1.5 (95% CI 1.0-2.3; p-value 0.078) and 1.8 (95% CI 1.0-3.3; p-value 0.051).

Separate analysis for users of acenocoumarol resulted in similar point estimates as for the pooled analyses of users of acenocoumarol and phenprocoumon. Use of aspirin and dipyridamole resulted in somewhat higher point estimates among users of phenprocoumon, both not reaching statistical significance (OR 2.3; p-value 0.097, and OR 2.5; p-value 0.7, respecively). A risk estimate was not possible for clopidogrel (n=1).

Sensitivity analyses did not change the overall picture of our results, risks remaining higher for upper gastrointestinal than for other bleedings. For aspirin the results for upper gastrointestinal and other bleedings are presented in Table 4, for other antiplatelet drugs numbers became lower, resulting in wider confidence intervals without changing the picture of our main findings (data not shown).

Past use of all antiplatelet drugs did not show increased bleeding risks any more (univariate ORs for all bleedings together 1.0, 1.0, and 1.2 for aspirin, clopidogrel, and dipyridamole, respectively, point estimates being similar for upper gastrointestinal bleedings (data not shown). Recent use showed a tendency for an increased bleeding risk in users of aspirin (OR 1.4; p-value 0.083 for all bleedings, and OR 1.5; p-value 0.31 for gastrointestinal bleedings), while the numbers of recent users of clopidogrel (n=5) and dipyridamole (n=10) were too small for analysis.

Table 4: ASSOCIATION BETWEEN CURRENT USE OF ASPIRIN ALONE AND HOSPITALIZATION FOR UPPER GASTROINTESTINAL BLEEDINGS AND OTHER BLEEDINGS FOR VARIOUS ASSUMPTIONS

	Multivariate odds ratios (OR)				
	Last dispensing date of a coumarin – index date: maximally 90 days	**Last dispensing date of a coumarin – index date: maximally 60 days**	**Last dispensing date of a coumarin – index date: maximally 30 days**	**More than one prescription for a coumarin**	**Bleedings after the first 28 days of coumarin therapy**
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Upper GI	2.5 (1.6-4.0)	2.5 (1.5-4.2)	2.2 (1.3-3.9)	2.0 (1.3-3.2)	2.1 (1.4-3.3)
Other	1.5 (1.1-2.0)	1.6 (1.2-2.2)	1.6 (1.1-2.3)	1.3 (1.0-1.8)	1.3 (1.0-1.7)

Index date = date of first hospitalization for abnormal bleeding; GI = gastrointstinal

DISCUSSION

This study demonstrated for the first time that next to aspirin, clopidogrel increases the risk of major bleeding among users of coumarins, whereas we found a strong trend for dipyridamole.

The results of our study regarding aspirin are in agreement with the results of other population based studies[11-14] and with the findings of a recent meta-analysis by Salem et al. which assessed the therapeutic benefits and risks of combined use of aspirin and coumarins compared with use of coumarins alone,[9] their OR for increased major bleeding risk (1.43) being exactly the same as ours. This agreement with other studies is a positive test of the validity of our data set and our research design approach, potentially increasing the validity of our findings for the other antiplatelet drugs.

Although one study showed no effect of clopidogrel on INR among patients receiving long-term warfarin therapy[25] suggesting the absence of a pharmacokinetic interaction, a pharmacodynamic interaction between clopidogrel and coumarins is conceivable because of their differing effects on haemostasis. Clopidogrel is increasingly used as an antiplatelet drug, one trial suggesting that it was more effective and caused significantly less gastrointestinal bleeding than low dose aspirin (325 mg daily).[26] However, in two studies among high risk patients with a history of upper gastrointestinal complications clopidogrel was associated with a high incidence of upper gastrointestinal bleeding,[27,28] one of these studies even demonstrating that combined use of aspirin and a proton pump inhibitor was superior to clopidogrel in the prevention of recurrent ulcer bleeding.[27] In a Danish population based case-control study clopidogrel alone was not associated with an increased risk of upper gastrointestinal bleeding, whereas the combination with aspirin increased the bleeding risk beyond the effect of aspirin alone.[14] The findings of our study similarly suggest that clopidogrel adds to a further increased bleeding risk among users of coumarins and that clopidogrel is not safer than low dose aspirin when used in combination with coumarins, this suggestion being stronger for upper gastrointestinal bleedings than for other bleedings. Although not surprisingly the combination therapy with warfarin, aspirin, and clopidogrel was associated with a significant increased risk of major bleeding compared to therapy with aspirin and clopidogrel,[29] to our knowledge our study is the first to demonstrate an increased bleeding risk for concurrent use of clopidogrel (without aspirin) and coumarins compared with coumarins alone.

The hitherto reported data on the effect of dipyridamole on bleeding risk among users of coumarins are contradictory. Massel et al. reported an increased bleeding risk in a meta-analysis among patients with prosthetic heart valves for combined use of dipyridamole and coumarins compared to coumarins alone,[16] whereas Pouleur et al. did not find an increased risk in another meta-analysis.[15] Our results are in agreement with the findings of Massel et al., who primarily analysed major bleedings as we did. Although dipyridamole unlike aspirin does not inhibit the synthesis of gastroprotecting prostaglandins,[30] a Danish population based observational study found a similarly increased risk of upper gastrointestinal bleedings in users of dipyridamole alone and low dose aspirin alone,[14] which agrees with our finding that dipyridamole increases the risk of upper gastrointestinal bleedings to the same extent as low dose aspirin among users of coumarins.

Our results strongly suggest that all antiplatelet drugs increase the risk of upper gastrointestinal bleedings more than the risk of other bleedings. This was expected for aspirin because of its irreversible and unselective inhibition of cyclo-oxygenase-1 (COX-1), which has a role in the protection of the stomach mucosa.[31] However, in users of coumarins the risk increasing effect of other antiplatelet drugs seems not to be different from the risk increasing effect of low dose aspirin in the dosage range from 30-100 mg.

Our study has several limitations. First, there is the possibility of misclassification of users of coumarins, because we had to make assumptions regarding the duration of coumarin use. However, a reduction of the maximal time between the last dispensing date and the index date did not result in essentially different outcomes, suggesting that our assumptions were valid. A second limitation is that we did not have data on the intensity of anticoagulation (normal or high) or on diseases that we could not derive from drug therapy such as liver and renal insufficiency, which are also risk factors for major bleeding.[24,32] A third limitation is that we could only evaluate a history of hospitalization for major bleeding from the moment patients were included in the PHARMO record linkage system, implicating that we could have missed information about earlier bleedings in patients.

The results of our study give rise to some clinical considerations. Guidelines of the American College of Chest Physicians recommend adding dipyridamole or clopidogrel to warfarin in situations in which a combination of warfarin and an antiplatelet drug is indicated and patients are unable to take aspirin.[33,34] Our findings do not suggest that gastrointestinal safety is better with concurrent use of

coumarins and dipyridamole or clopidogrel than with concurrent use of coumarins and low dose aspirin in the dosage range from 30 to 100 mg.
In summary our results suggest that next to aspirin both clopidogrel and dipyridamole increase the risk of major bleeding among users of coumarins, and that this risk is more increased for upper gastrointestinal bleedings than for other bleedings.

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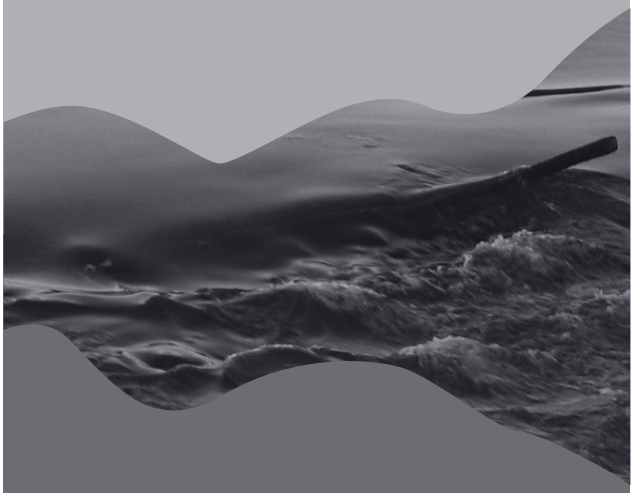
Appendix: LIST OF THE ICD-9-CM CODES OF THE ABNORMAL BLEEDING EVENTS WHICH WERE IDENTIFIED IN CASES

Description	ICD-9 code	N
Gastrointestinal bleedings		
Upper gastrointestinal		
esophageal varices with bleeding	456.0	1
gastric ulcer, acute with haemorrhage	531.0	6
gastric ulcer, acute with haemorrhage and perforation	531.1	1
gastric ulcer, chronic or unspecified with haemorrhage	531.4	80
gastric ulcer, chronic or unspecified with haemorrhage and perforation	531.6	1
duodenal ulcer, acute with haemorrhage	532.0	17
duodenal ulcer, chronic or unspecified with haemorrhage	532.4	54
duodenal ulcer, chronic or unspecified with haemorrhage and perforation	532.6	2
peptic ulcer, acute with haemorrhage without obstruction	533.0	1
gastrojejunal ulcer, chronic or unspecified with haemorrhage and perforation	534.4	1
haematemesis	578.0	26
melaena	578.1	96
haemorrhage of gastrointestinal tract, not otherwise specified	578.9	251
Lower gastrointestinal		
haemorrhage of rectum or anus	569.3	68
Non-gastrointestinal bleedings		
Intracranial		
subarachnoidal haemorrhage	430	22
intracerebral haemorrhage	431	218
nontraumatic extradural haemorrhage	432.0	3
subdural haemorrhage	432.1	65
intracranial haemorrhage, not otherwise specified	432.9	10
Urinary tract		
haemorrhage into bladder wall	596.7	3
haematuria	599.7	102
Uterus		
ovulation bleeding	626.5	1
metrorrhagia	626.6	20
disorder of menstruation or other abnormal bleeding, not otherwise specified	626.9	5
premenopausal haemorrhage	627.0	1
postmenopausal bleeding	627.1	104
Nose		
epistaxis	784.7	161

Description	ICD-9 code	N
Eye		
haemophthalmos except current injury	360.43	1
choroidal haemorrhage, unspecified	363.61	1
hyphaema	364.41	3
conjunctival haemorrhage	372.72	2
vitreous haemorrhage	379.32	13
Joint		
haemarthrosis, site unspecified	719.10	1
haemarthrosis, shoulder	719.11	4
haemarthrosis, upper arm	719.12	1
haemarthrosis, pelvic region and tigh	719.15	3
haemarthrosis, lower leg	719.16	25
Other		
haemoptysis	786.3	115
haemoperitoneum	568.81	6
spontaneous ecchymoses	782.7	1
haemorrhage or haematoma complicating a procedure	998.1	210
haemorrhage, unspecified	459.0	132

ICD-9-CM = International Classification of Diseases, 9th edition

CHAPTER 4



CYP2C9 AND VKORC1 GENOTYPE AND ACENOCOUMAROL

4.1

Acenocoumarol stabilization is delayed in *CYP2C9*3* carriers

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ABSTRACT

Objective

Our objective was to assess whether there is an association between the presence of allelic variants of the gene for cytochrome P450 (CYP) 2C9 and anti-coagulation problems during the initial three to six months of acenocoumarol treatment.

Methods

A prospective follow-up study was performed at two anticoagulation clinics in the Netherlands. Included subjects started with a standard dose regimen as follows: 6 mg on the first day, 4 mg on the second day, and 2 mg on the third day. *CYP2C9* genotypes were assessed, and data on International Normalized Ratio (INR), comedication and comorbidity were collected.

Results

The *CYP2C9* genotype of 231 subjects was assessed. Of these, 147 (63.6%) were wild-type subjects (*CYP2C9*1/*1*), 38 (16.5%) were carriers of *CYP2C9*2*, and 46 (19.9%) were carriers of *CYP2C9*3*. Compared with wild-type subjects, carriers of the *CYP2C9*3* allele had (1) a lower chance to achieve stability in the first six months of therapy (hazard ratio 0.6; 95% confidence interval 0.4-0.9; $p<0.05$), and (2) an increased risk of severe overanticoagulation (INR>6.0) (hazard ratio 3.8; 95% confidence interval 1.5-9.4; $p<0.01$). For both outcomes there was no significant difference between carriers of the *CYP2C9*2* allele and wild-type subjects.

Conclusion

In carriers of the *CYP2C9*3* allele more difficulties in terms of stabilization and overanticoagulation were found as compared with wild-type subjects or *CYP2C9*2* carriers. *CYP2C9* genotyping could be useful to identify potential candidates for more frequent INR controls to minimize problems with acenocoumarol anticoagulation status.

INTRODUCTION

The use of oral anticoagulants of the coumarin type is complicated by considerable problems. The therapeutic range of coumarin derivatives is narrow, and the anticoagulant effectiveness varies strongly both interindividually and intraindividually over time. Several factors can contribute to the variability in the anticoagulant effects of coumarin derivatives, as follows: drug interactions, ingestion of varying quantities of vitamine K, infections, impairment from severe heart failure, and impaired liver function.[1,2] In recent years much attention has been given to a possible association between differences in the cytochrome P450 (CYP) 2C9 genes coding for the coumarin-metabolizing CYP isozyme CYP2C9 and sensitivity for coumarin anticoagulants. Most research in this area has, until now, been focused on warfarin, which is the most frequently used oral anticoagulant worldwide.[3-8]

In all studies on this subject, an association between the possession of the allelic *CYP2C9* variants *CYP2C9*2* and *CYP2C9*3* and a reduced dose need for warfarin has been convincingly demonstrated.[3-8] Some, but not all, of these studies also suggested an increased bleeding risk for warfarin users with one or more of these allelic variants.[3,7] One study demonstrated that the possession of at least one allelic variant of *CYP2C9* was also associated with overanticoagulation and an increased time to achieve stability.[7] In addition, several case reports described commonly encountered difficulties in warfarin therapy, such as bleeding and problems in achieving stability, in patients with two allelic *CYP2C9* variants (*CYP2C9*2/*3* and *CYP2C9*3/*3*).[9,10]

The CYP2C9 subject has been less extensively studied for the anticoagulant acenocoumarol, which is frequently used in European countries. One in vitro study indicated that CYP2C9 plays a role in the metabolism of acenocoumarol.[11] Three studies have convincingly demonstrated an association between the possession of the *CYP2C9*3* allele and a low acenocoumarol dose requirement.[12-14] In one of these studies possession of the *CYP2C9*3* allele, but not the *CYP2C9*2* allele, was also associated with less time spent within the therapeutic range and an increased risk of International Normalized Ratio (INR) values greater than 4.5 in the first days of therapy.[14] In two cases serious early overanticoagulation in patients homozygous for *CYP2C9*3* was described.[15]

None of these studies investigated a possible association between *CYP2C9* genotype and time to achieve stability in acenocoumarol anticoagulant effects. Moreover, the consequences of possession of the *CYP2C9*2* allele on

acenocoumarol effects are less obvious than for warfarin. To elucidate these and other aspects, we conducted a six-months follow-up study in two anticoagulation clinics in the Netherlands. Our objective was to investigate a possible association between *CYP2C9* genotype and acenocoumarol anticoagulant effects in the initial phase of therapy.

METHODS

Study design and patients

The study design was a prospective follow-up study at two anticoagulation clinics in the Netherlands. Both clinics monitor the anticoagulation status of outpatients, and acenocoumarol is the most frequently prescribed oral anticoagulant in both clinics. The INR of patients is regularly monitored, with a frequency varying from a few days to a maximun of six weeks. In the Netherlands two target therapeutic ranges are used: the low therapeutic range (INR of 2.0-3.5) and the high therapeutic range (INR of 2.5-4.0).

We included patients who started therapy at one of the anticoagulation clinics from November 1998 until September 2002 with the following characteristics: use of acenocoumarol, anticoagulant indication for at least three months, 6-4-2 loading dose on the first three days (consisting of 6 mg, 4 mg, and 2 mg consecutively), and INR measurement on the fourth day. The 6-4-2 loading scheme is the most frequently used initial dose for acenocoumarol in the Netherlands. We did not include patients who were taking drugs that pharmacokinetically interact with anticoagulants at the start of acenocoumarol therapy; to identify such drugs, we used the Dutch Standard Management Coumarin Interactions.[16] The pharmacokinetically interacting drugs in this Standard include CYP2C9-inhibiting drugs (e.g. amiodarone, miconazole, and cotrimoxazole) and CYP2C9-inducing agents (e.g. carbamazepine and phenytoin). Recently identified as a CYP2C9 inhibitor but not included in the Dutch Standard is benzbromarone.[17] We did not take into account drugs that are only known as substrates but not inhibitors of CYP2C9 because a substrate of a CYP enzyme does not necessarily decrease the metabolism of another drug via the same enzyme.

During our study, no patients started pharmacodynamically interacting drugs that could confound our outcomes.

The Medical Ethical Committee at the University Medical Centre, Utrecht, the Netherlands, approved our study. Patients who met the aforementioned criteria were informed about the aims of the study and were asked for their written consent. After informed consent was obtained, the remainder of a blood sample from a regular INR control was used for *CYP2C9* genotyping.

Data collection and follow-up time

We collected data on sex, age, genotype, anticoagulant indication, and the corresponding therapeutic INR range, INR measurements, acenocoumarol doses, comorbidity, infections, and comedication as recorded by the anticoagulation clinics in a database. Data on comedication were verified in the patients' pharmacies, with their informed consent.

Patients were followed up from the date of the first acenocoumarol use (the entry date) until one of the end points (described later) was reached or until the end of the observation period, which was set on the last regular anticoagulation clinic visit within 190 days from the entry date.

Genotyping

Deoxyribonucleic acid was extracted from 80 μl whole citrate blood using Generation Capture Column Kit (Gentra Systems, Inc., Minneapolis, Minn, USA) according to the manufacturer's instructions. Polymerase chain reaction (PCR) was performed in a total of 25 μl containing 0.02 μmol/L of each primer, 0.2 μmol/L deoxyribonucleoside triphophates, and 0.6 U r*Taq* polymerase (Amersham Pharmacia Biotech, Amersham Biosciences Corp., Piscataway, NJ, USA), 1x PCR buffer as supplied with the r*Taq*-polymerase, and 10-20 ng of genomic deoxyribonucleic acid. Genotyping for the *CYP2C9*2* allele (Cys144Arg) was done according to Steward et al.[18] with 5'-GGG GAG GAT GGA AAA CAG AGA CTT AC-3' as forward primer, and 5'-TCC TCC ACA AGG CAG CGG GC-3' as reverse primer. PCR conditions were as follows: 5 minutes at 92°C, 35 cycles of 30 seconds at 95°C, 30 seconds at 65°C, and 60 seconds at 72°C, followed by 5 minutes at 72°C. The 263-base pair (bp) PCR product was cleaved with restriction enzyme *Ava*II into 226-bp and 37-bp fragments if it was a product of a wild-type allele and was not cleaved if it was a product of *CYP2C9*2*. Genotyping of the *CYP2C9*3* allele (Ile359Leu) was done according to Sullivan-Klose et al.[19] with two different forward mismatch primers, sul-fw1 5'-AAT AAT AAT ATG CAC GAG GTC CAG AGA TGC-3' and sul-fw2 5'- AAT AAT AAT ATG CAC GAG GTC CAG AGG TAC-3', and primer sul-rv 5'-GAT ACT ATG AAT TTG GGA CTT C-3' as reverse

primer. PCR conditions were as follows: 5 minutes at 94°C, 34 cycles of 45 seconds at 94°C, 45 seconds at 54°C, and 60 seconds at 72°C, followed by 5 minutes at 72°C. The 165-bp PCR product of primers sul-fw2 and sul-rv was not cleaved with *Kpn*I in case of a wild-type allele, whereas the product of other alleles was cleaved with *Kpn*I into 30-bp and 135-bp fragments. Confirmation of the Ile359Leu mutation or any other mutation in codon 359 with primers sul-fw1 and sul-rv gave a 165-bp product that was not cleavable by *Nsi*I, while the 165-bp product of a wild-type allele was cleaved into 31-bp and 134-bp fragments.

Calculation of INR

The INR was calculated as follows:
(prothrombin time of patient / mean prothrombin time of normal subjects)^{ISI}, in which ISI is the international sensitivity index, an adjustment factor for the combination of reagents and coagulometer used.

Outcomes

The end points of this study were chosen to assess the acenocoumarol anticoagulant status. Primary end points were as follows:

1. Time to achieve a first period of stability. This period was calculated as the time (in days from the starting date) until the first of three consecutive INR measurements within the therapeutic range; these INR measurements encompassed a period of at least two weeks with a maximum difference between the mean daily doses of 10%. An analogous definition of stable anticoagulant dosing was used in another study on the association between *CYP2C9* genotype and warfarin anticoagulant status.[7] The mean daily dose of this first period of stability was recorded as the maintenance dose to compare the dose needs for the different genotypes.
2. The hazard ratio (HR) of severe overanticoagulation (defined as INR>6.0) in the observation period. An INR greater than 6.0 is associated with a considerably increased bleeding risk.[20-22] Because we expected the risk of severe overanticoagulation to be more pronounced in the first weeks of acenocoumarol therapy, we also assessed the HR in the first 30 days.

Secondary end points of our study were: initial INR (i.e. INR measured on the fourth day after acenocoumarol was started), and mean dose need during the first period of stability.

Statistical analysis

For comparisons between genotypes, patients were divided into three categories: homozygous patients (*CYP2C9*1/*1*) formed the reference group, and the other two groups consisted of carriers of the *CYP2C9*2* and *CYP2C9*3* alleles. Because of the low prevalence of subjects carrying two allelic variants, heterozygous and homozygous subjects were included in the same genotype category. *CYP2C9*2/*3* subjects were allocated to the *CYP2C9*3* group. We used Cox proportional hazard models to assess the relative risk of achieving a first period of stability in the follow-up period and to assess the risk of severe overanticoagulation. We used linear multiple regression models to assess differences in initial INR and dose requirements between the genotypes. To handle potential confounders, we used adjustment in statistical models and restriction. In the statistical models we adjusted for potential confounders such as sex and age. As confounding comedication, we first considered the use of interacting drugs, such as CYP2C9 inhibitors and inducers, initiated before the end of the follow-up period was reached. Because the number of patients who started such interacting drugs was low, we excluded them from the analyses for which they could confound our results (indicated in the tables).

We adjusted for differences in the use of antibiotics before the end points of overanticoagulation and stability were achieved. Use of antibiotics is indicative for infections, which can delay stabilization of patients. Moreover, in several studies antibiotic use during anticoagulant therapy was associated with overanticoagulation.[23,24]

Another potential confounder is the target INR therapeutic range; it is possible that a higher level of anticoagulation (INR therapeutic range 2.5-4.0) leads to higher mean dose needs, an increased risk of overanticoagulation, and a delay in achieving stability. Thus we adjusted for differences in the target INR therapeutic range in the evaluation of the following end points: time to achieve stability, severe overanticoagulation, and dose need.

We also assessed the use of angiotensin-converting enzyme inhibitors and loop diuretics as a proxy for heart failure, a potential confounder. We analysed the section of our population (60.3%) for whom we had the complete pharmacy records for the follow-up period. The distribution of users of angiotensin-converting enzyme inhibitors and loop diuretics was about equal across genotype groups (13.8% for wild-type subjects, 11.5% for *CYP2C9*2* carriers, and 12.0% for *CYP2C9*3* carriers).

Statistical analyses were performed with the statistical software package SPSS 10 (SPSS Inc., Chicago, Ill, USA).

Table 1: PATIENT CHARACTERISTICS (N=231)

Characteristic	*CYP2C9*1/*1*	*CYP2C9*2* allele[a]	*CYP2C9*3* allele[b]
age; mean years (SD)	65.1 (15.2)	63.3 (16.1)	66.9 (13.5)
maximum follow-up time; days (SD)[c]	166 (35)	175 (38)	175 (46)
Patients	**n=147 (100%)**	**n=38 (100%)**	**n=46 (100%)**
men	85 (57.8%)	22 (57.9%)	26 (56.6%)
women	62 (42.2%)	16 (42.1%)	20 (43.5%)
low INR target therapeutic range (2.0-3.5)	123 (83.7%)	33 (86.8%)	36 (78.3%)
high INR target therapeutic range (2.5-4.0)	24 (16.3%)	5 (13.2%)	10 (21.7%)
Indication for acenocoumarol			
atrial fibrillation	85 (57.8%)	27 (71.1%)	27 (58.7%)
deep vein trombosis or pulmonary embolus	29 (19.7%)	4 (10.5%)	7 (15.2%)
myocardial infarction	8 (5.5%)	4 (10.5%)	5 (10.9%)
postoperative prophylactic	4 (2.7%)		1 (2.2%)
valvular replacement	1 (0.7%)		
vascular prothesis	4 (2.7%)		1 (2.2%)
coronary bypass operation	2 (1.4%)	1 (2.6%)	3 (6.5%)
other indications	14 (9.5%)	2 (5.3%)	2 (4.3%)

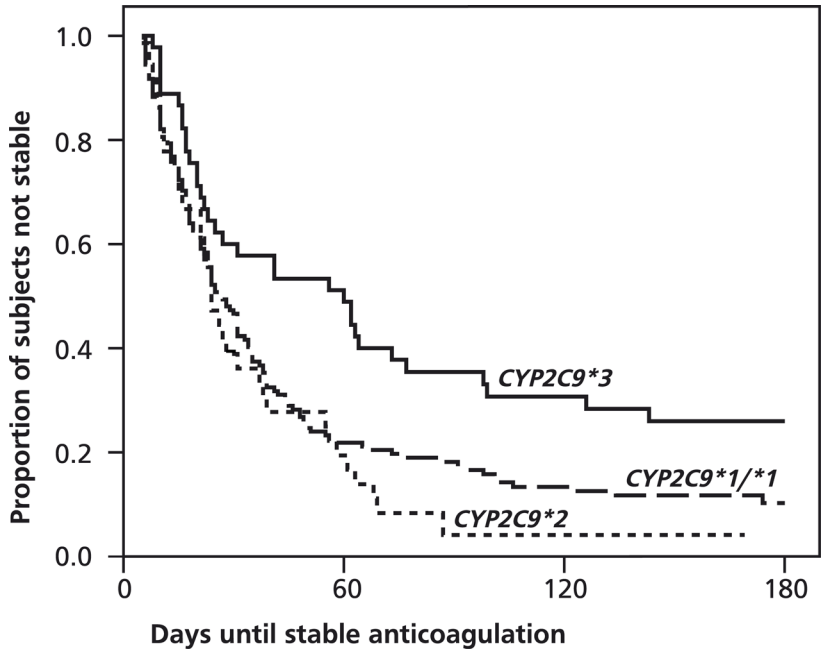
a) Includes 38 *CYP2C9*2* alleles: 34 *CYP2C9*1/*2* and 4 *CYP2C9*2/*2* alleles.
b) Includes 46 *CYP2C9*3* alleles: 42 *CYP2C9*1/*3*, 2 *CYP2C9*2/*3*, and 2 *CYP2C9*3/*3* alleles.
c) Follow-up time until patient stopped anticoagulant therapy or until the last anticoagulation clinic control date within the maximum follow-up period of 190 days was reached.

RESULTS

A total of 231 patients who met the selection criteria were included in this study. The characteristics of the cohort are summarized in Table 1. The mean age of all patients at the initiation of therapy was 65.1 years; there were more men than women (57.6 versus 42.4%). The main indication for oral anticoagulant therapy in the cohort was atrial fibrillation. The mean maximum follow-up time (time in days until the last INR control date or until the end of anticoagulant therapy before the maximum follow-up period of 190 days) was 169 days.

In this cohort the *CYP2C9*3* allele was more frequently identified than the *CYP2C9*2* allele, whereas in most studies the last allele is more frequently observed.

Figure 1: KAPLAN-MEIER SURVIVAL CURVES FOR TIME TO ACHIEVE A PERIOD OF STABILITY



Difference between *CYP2C9*3* and *CYP2C9*1/*1* / *CYP2C9*2* was significant (p=0.015); difference between *CYP2C9*2* and *CYP2C9*1/*1* was not significant (p=0.51).

Table 2: TIME TO ACHIEVE FIRST PERIOD OF STABILITY[a]

Genotype	N	Stabilized	Hazard ratios (95% CI) for time to achieve stability			
		n (%)	unadjusted	p	adjusted[b]	p
*CYP2C9*1/*1*	145	126 (86.9)	1		1	
*CYP2C9*2*	37	35 (94.6)	1.23 (0.85–1.80)	0.28	1.14 (0.78–1.69)	0.51
*CYP2C9*3*	45	34 (75.6)	0.60 (0.41–0.87)	0.008[c]	0.62 (0.42–0.91)	0.015[c]

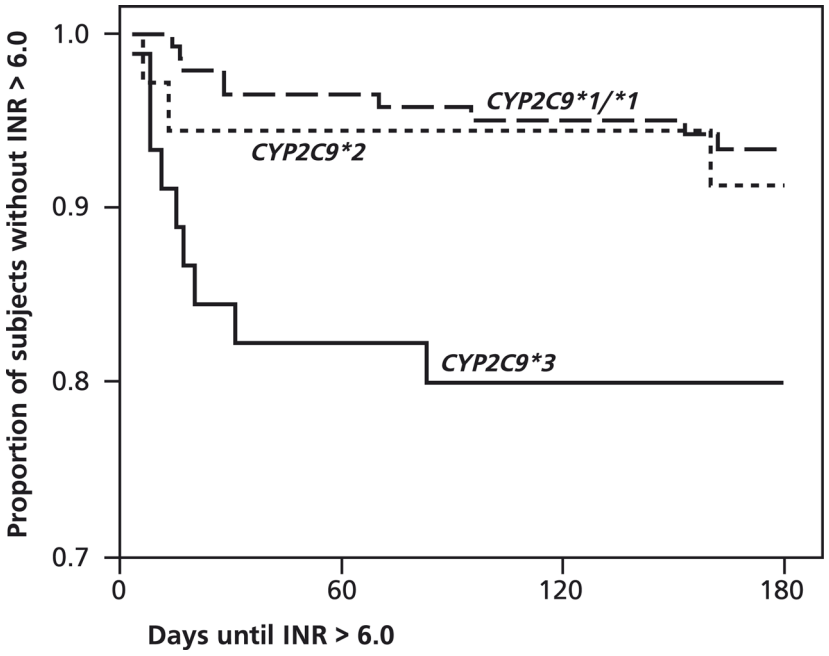
a) We excluded 4 patients from analysis: 2 *CYP2C9*1/*1* carriers who started amiodarone and benzbromarone, 1 *CYP2C9*1/*2* carrier who started amiodarone, and 1 *CYP2C9*1/*3* carrier who started cotrimoxazole. All aforementioned drugs are CYP2C9 inhibitors and were started before stability was achieved.[17,31,32]

b) Adjusted for age, sex, target INR therapeutic range of anticoagulation, and use of antibiotics before stability was achieved.

c) Statistically significant difference (p<0.05).

In Figure 1 the Kaplan-Meier curve is presented for achieving stability. In Table 2 the data regarding achieving stability are presented. The HRs for the *CYP2C9*2* and *CYP2C9*3* carriers are shown. Carriers of the *CYP2C9*3* allele had a significantly lower chance to achieve a period of stability in the first six months of therapy (unadjusted HR 0.6; 95% confidence interval [CI] 0.4-0.9). Use of antibiotics also seemed to be significantly associated with a decreased chance to achieve stability (HR 0.4; 95% CI 0.2-0.8; p=0.011) (data not shown in table). After adjustment, the HR for *CYP2C9*3* carriers remained significantly reduced. The *CYP2C9*3* carriers who did achieve stability in the first 6 months needed significantly more time than wild-type subjects and *CYP2C9*2* carriers (difference 14.9 days; 95% CI 3.2-26.5; p=0.012) (data not shown in table).

Figure 2: KAPLAN-MEIER SURVIVAL CURVES FOR TIME TO SEVERE OVERANTICOAGULATION (FIRST INR MEASUREMENT>6.0)



Difference between *CYP2C9*3* and *CYP2C9*1/*1* / *CYP2C9*2* was significant (p=0.003); difference between *CYP2C9*2* and *CYP2C9*1/*1* was not significant (p=0.57).

In Figure 2 the Kaplan-Meier curve is presented for first assessment of severe overanticoagulation. In Table 3 the data regarding serious overanticoagulation (INR>6.0) are presented. The HRs for the *CYP2C9*2* and *CYP2C9*3* carriers

are shown. Possession of the *CYP2C9*3* allele, but not of the *CYP2C9*2* allele, is associated with a significantly higher risk (adjusted HR 3.8; 95% CI 1.5-9.4) of severe overanticoagulation. This risk is more pronounced in the first 30 days of acenocoumarol therapy (adjusted HR 5.6; 95% CI 1.8-17.1).

Table 3: SEVERE OVERANTICOAGULATION (INR>6.0) OVERALL AND IN FIRST 30 DAYS OF THERAPY

Genotype	N	INR>6.0	Hazard ratios (95% CI) for time to first INR>6.0			
		n (%)	unadjusted	p	adjusted[c]	p
Total study period[a]						
*CYP2C9*1/*1*	146	9 (6.2)	1		1	
*CYP2C9*2*	36	3 (8.3)	1.36 (0.37–5.03)	0.64	1.38 (0.37–5.08)	0.63
*CYP2C9*3*	46	10 (21.7)	3.87 (1.57–9.52)	0.003[d]	3.80 (1.54–9.39)	0.004[d]
First 30 days of treatment[b]						
*CYP2C9*1/*1*	146	5 (3.4)	1		1	
*CYP2C9*2*	38	2 (5.3)	1.59 (0.31–8.18)	0.58	1.61 (0.31–8.32)	0.57
*CYP2C9*3*	46	8 (17.4)	5.54 (1.81–16.9)	0.003[d]	5.59 (1.82–17.1)	0.003[d]

a) We excluded 3 patients from analysis: 1 *CYP2C9*1/*1* carrier who started benzbromarone and 2 *CYP2C9*1/*2* carriers who started amiodarone and cotrimoxazole. These drugs are CYP2C9 inhibitors and were started before first assessment of INR>6.0; cotrimoxazole was in use when the INR >6.0 was assessed.[17,31,32]
b) We excluded 1 patient from analysis: 1 *CYP2C9*1/*1* carrier who started benzbromarone. This drug is a CYP2C9 inhibitor and was started before first assessment of INR>6.0.[17]
c) Adjusted for age, sex, target INR therapeutic range of anticoagulation. No antibiotics were used at the time of overanticoagulation.
d) Statistically significant difference ($p<0.05$).

Table 4: INITIAL INR IN RELATION TO GENOTYPE

Genotype	Initial INR[a] (95% CI)	MD (95% CI)	P	MD_{adj} (95% CI)	P
*CYP2C9*1/*1*	2.7 (2.5–2.8)				
*CYP2C9*2*	2.5 (2.2–2.8)	-0.2 (-0.5–0.2)	0.29	-0.1 (-0.5–0.2)	0.39
*CYP2C9*3*	3.2 (2.9–3.5)	0.5 (0.2–0.8)	0.001[b]	0.5 (0.2–0.8)	0.001[b]

CI = confidence interval; MD = mean difference between *CYP2C9*2*/*CYP2C9*3* alleles and *CYP2C9*1/*1*; MD_{adj} = mean difference adjusted for age and sex
a) Dose scheme of acenocoumarol: 6–4–2 (6 mg on first day, 4 mg on second day, and 2 mg on third day), with first INR measurement on fourth day.
b) Statistically significant difference ($p<0.05$).

In Table 4 the differences in the initial INR (INR measured on the fourth day of therapy) between the genotypes are shown. Possession of the *CYP2C9*3* allele,

but not of the *CYP2C9*2* allele, is significantly associated with a higher initial INR. The initial INR for the *CYP2C9*3* allele is about 0.5 unit higher (3.2 for the *CYP2C9*3* allele versus 2.7 and 2.5 for the wild-type and *CYP2C9*2* allele, respectively).

However, there was no increased risk for initial overanticoagulation in *CYP2C9*3* carriers. On the contrary, the chance on an adequate INR within the therapeutic range was significantly higher for *CYP2C9*3* carriers than for wild-type subjects or *CYP2C9*2* carriers (odds ratio for INR within therapeutic range 3.1; 95% CI 1.4-6.7; p=0.011) (data not shown in table).

Table 5: ACENOCOUMAROL DOSES IN RELATION TO GENOTYPE[a]

Genotype	N	Dose[b] (95% CI)	MD (95% CI)	P	MD_{adj} (95% CI)	P
*CYP2C9*1/*1*	126	2.5 (2.3;2.7)				
*CYP2C9*2*	35	2.5 (2.3;2.7)	-0.0 (-0.4; 0.3)	0.87	-0.1 (-0.4; 0.2)	0.60
*CYP2C9*3*	34	2.0 (1.7;2.3)	-0.5 (-0.8;-0.2)	0.003[c]	-0.5 (-0.8;-0.2)	0.003[c]

CI = confidence interval; MD = mean difference between *CYP2C9*2*/*CYP2C9*3* alleles and *CYP2C9*1/*1*; MD_{adj} = mean difference adjusted for age, sex, and difference in level of anticoagulation

a) We excluded 4 patients for analysis: 2 *CYP2C9*1/*1* carriers who started amiodarone and benzbromarone, 1 *CYP2C9*1/*2* carrier who started amiodarone, and 1 *CYP2C9*1/*3* carrier who started cotrimoxazole. All aforementioned drugs are CYP2C9 inhibitors and were started before stability was achieved.[17,31,32]
b) Mean dose (mg/day) during first period of stability. If stability was not achieved in the maximum follow-up time, no mean dose was computed.
c) Statistically significant difference ($p<0.05$).

In Table 5 the differences in mean daily dose between the genotypes in the first period of stability are shown. The mean daily dose is only computed for persons who achieved a first period of stability in the follow-up period. Possession of the *CYP2C9*3* allele, but not of the *CYP2C9*2* allele, was significantly associated with a lower dose need in the first period of stability in comparison with wild-type subjects. The mean daily dose for *CYP2C9*3* carriers was 0.5 mg lower than for wild-type subjects or carriers of the *CYP2C9*2* allele. This is equivalent to a difference of 3.5 mg in a dose scheme of one week.

The influence of age on the mean daily dose was significant; with 10-year increases in age, there was a decrease in daily acenocoumarol dose need of 0.2 mg (95% CI 0.1-0.3 mg; $p<0.001$). For subjects younger than age 60 years, the mean daily dose was 2.8 mg (95% CI 2.6-3.1 mg); for subjects older than age 80 years, the mean daily dose was 1.7 mg (95% CI 1.5-1.9 mg).

DISCUSSION

Our study demonstrates that the *CYP2C9*3* allele, but not the *CYP2C9*2* allele, is associated with the following: a decreased chance to achieve stability, an increased risk for severe overanticoagulation (INR>6.0), a higher initial fourth-day INR after a standard acenocoumarol starting dose, and a lower acenocoumarol dose need. To our knowledge, our study is the first that has focused on an association between *CYP2C9* genotype and time to achieve a period of stable anticoagulation with acenocoumarol.

Although the benefits of oral anticoagulants for prevention and treatment of venous and arterial thromboembolism are obvious, the use of coumarin derivatives is potentially dangerous and frequent monitoring of the anticoagulant effect is required. Several factors contribute to the difficulties that are frequently encountered in coumarin therapy. One of these factors is the genetically predisposed difference in metabolism of the oral anticoagulants.

The enzyme CYP2C9, like other CYP enzymes located in the endoplasmatic reticular membrane of the liver, plays an important role in phase I metabolism of several frequently prescribed drugs, such as warfarin, phenytoin, losartan, and tolbutamide.[25-27]

The *CYP2C9* gene has several allelic variants, which code for enzymes with reduced catalytic properties. In addition to the wild-type allele (called *CYP2C9*1*), up to now, the following five officially numbered allelic variants have been identified: *CYP2C9*2* (Arg144Cys), *CYP2C9*3* (Ile359Leu), *CYP2C9*4* (Ile359Thr), *CYP2C9*5* (Asp360Glu), and *CYP2C9*6* (null allele). The *CYP2C9*4*, **5* and **6* alleles are very rare.[27,28] We investigated only the frequently occurring *CYP2C9*2* and *CYP2C9*3* alleles. Although our study was not designed to investigate an association between possession of polymorph *CYP2C9* alleles and actual bleeding, the occurring of INR values greater than 6.0 is potentially dangerous and associated with a strongly increased bleeding risk.[21,22]

Our results also suggest that the search for a stable dose regimen with acenocoumarol in carriers of *CYP2C9*3* is more difficult and takes longer than in wild-type subjects or carriers of *CYP2C9*2*. This is a potentially important finding, because it reflects problems for anticoagulation clinics in assessing an acenocoumarol dose that provides more than two consecutive INR values within the therapeutic range. The finding that almost 25% of *CYP2C9*3* carriers did not reach such a stable dose period even after 6 months of acenocoumarol

therapy indicates that, in the long term, more frequent INR controls are probably needed for *CYP2C9*3* carriers. A possible pharmacological explanation could be the presence of detectable quantities of (S)-acenocoumarol in *CYP2C9*3* carriers. Acenocoumarol is a racemic mixture of two enantiomers, (S)-acenocoumarol and (R)-acenocoumarol, which are both pharmacologically active.[29] As a matter of fact, (S)-acenocoumarol is intrinsically more active than (R)-acenocoumarol, but because of the rapid metabolic clearance of the (S)-enantiomer, the pharmacological activity lies mainly with (R)-acenocoumarol.[30] (S)-acenocoumarol is almost exclusively metabolized to inactive 6- en 7-hydroxylation products by CYP2C9, whereas (R)-acenocoumarol is partly metabolized by CYP2C9.[11] Thijssen et al.[12] demonstrated that only in *CYP2C9*3* carriers (S)-acenocoumarol was detectable 16 to 18 hours after intake, whereas (S)-acenocoumarol was not detectable in wild-type subjects and *CYP2C9*2* carriers. Thus it seems that in *CYP2C9*3* carriers two active enantiomers contribute to the anticoagulant effect, in contrast with non-*CYP2C9*3* carriers, in whom only the (R)-enantiomer is responsible for the effect. Apart from different pharmacodynamic properties, the two enantiomers seem to have different pharmacokinetic profiles, with the area under the time-concentration curve, maximum concentrations, and elimination half-life being higher for (R)-acenocoumarol.[30] Both enantiomers can potentially contribute to 24 hours of biological activity of racemic acenocoumarol. It is imaginable that intraindividual pharmacokinetic variations of two enantiomers over time might disturb a stable anticoagulant status more than variations over time in one enantiomer, certainly if such variations differ between the enantiomers.

Our study corroborates the conclusions from other authors that the *CYP2C9*3* allele is associated with a reduced dose need. This difference with the wild-type and *CYP2C9*2* subjects amounts to 0.5 mg daily (20%) for stabilized patients, which is equivalent to 3.5 mg in a 1-week dose scheme. We found no difference in dose need between carriers of the *CYP2C9*2* allele and wild-type subjects.

Our results do not indicate that *CYP2C9*3* carriers have an increased risk of overanticoagulation at the initial INR control, so it does not seem necessary to decrease the most frequently used loading dose (6-4-2) for *CYP2C9*3* carriers.

Although we cannot conclude from our study that *CYP2C9* genotyping is cost-effective, our results at least suggest the possibility that *CYP2C9* genotyping could contribute to a safer acenocoumarol anticoagulant status. It would be interesting to investigate whether another dose algorithm or more frequent INR controls of *CYP2C9*3* carriers could reduce the risk of severe

overanticoagulation and increase the chance of achieving stability within a reasonable period. Our results suggest that the algorithms used for acenocoumarol dosing might not be adequate for patients with the *CYP2C9*3* gene.

In accordance with other acenocoumarol studies, our results indicate that possession of the *CYP2C9*2* allele has less impact for acenocoumarol than for warfarin. This could indicate that for known *CYP2C9*2* carriers acenocoumarol is possibly a better choice as a coumarine anticoagulant than warfarin.

We believe that this study may also have implications for the management of drug interactions of acenocoumarol with moderate to strong inhibitors of CYP2C9. If a genetically reduced CYP2C9 activity is associated with an increased risk for overanticoagulation and with a decreased chance to achieve stability, it is imaginable that imposing a reduced CYP2C9 activity by an interacting drug has analogous consequences.

Our study has several limitations. We had only medical data from anticoagulation clinics. As a consequence, we were not certain that we had all relevant data about destabilizing malignancies, liver diseases, seriousness of heart failure, and vitamin K use, because these are not routinely mentioned in the anticoagulation clinic records.

Furthermore, the follow-up period of our study was relatively short, so we could not assess differences in stability between *CYP2C9* genotypes over a longer period.

Apart from these limitations, the associations found in our study at least indicate a significant trend toward a different anticoagulant status for *CYP2C9*3* carriers.

In conclusion, the results of our study suggest that possession of at least one *CYP2C9*3* allele is associated with a higher initial INR, a lower dose need, an increased risk for severe overanticoagulation, and a longer time needed to achieve stability. Further studies are required to assess whether identification of *CYP2C9*3* carriers will result in safer acenocoumarol handling in these patients and also whether this identification is cost-effective.

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4.2

VKORC1 and *CYP2C9* genotypes and acenocoumarol anticoagulation status: interaction between both genotypes affects overanticoagulation

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ABSTRACT

Objective

Our objective was to assess the effects of *VKORC1* and *CYP2C9* genotypes on severe overanticoagulation and time to achieve stability and their contributions to dose requirement during the initial phase of acenocoumarol treatment.

Methods

A prospective follow-up study was conducted at two anticoagulation clinics in the Netherlands. We assessed the *CYP2C9* genotype (*CYP2C9*2* and *CYP2C9*3* polymorphisms) and the *VKORC1 C1173T* genotype of the subjects and collected data on international normalized ratio, dose, comedication, and comorbidity.

Results

Of the 231 patients in the cohort, 150 (64.9%) had a *VKORC1 C1173T* polymorphism and 84 (36.4%) had a *CYP2C9*2* or *CYP2C9*3* allele. Only carriers of a combination of a *CYP2C9* polymorphism and a *VKORC1* polymorphism had an increased risk of severe overanticoagulation compared with subjects with no polymorphism or only one polymorphism (hazard ratio 3.83; 95% confidence interval [CI] 1.62-9.05). The time to achieve stability was associated with the possession of the *CYP2C9* genotype, not with the *VKORC1* genotype (hazard ratio for *CYP2C9*3* allele compared with *CYP2C9* wild-type 0.59; 95% CI 0.40-0.87). Patients with a *VKORC1* polymorphism required significantly lower doses than *VKORC1 CC* wild-type patients. A larger part of the variability in dose requirement was explained by the *VKORC1* genotype than by the *CYP2C9* genotype (21.4% and 4.9%, respectively).

Conclusion

Being a carrier of a combination of polymorphisms of *VKORC1* and *CYP2C9*, rather than of one of these polymorphisms, is associated with severe overanticoagulation. The time to achieve stability is mainly associated with the *CYP2C9* genotype.

INTRODUCTION

Anticoagulants of the coumarin type are effective drugs for the treatment and prevention of thromboembolic diseases. However, the use of these drugs is accompanied by considerable problems as a consequence of the high interindividual variability, as well as intraindividual variability, in dose requirement. This variability can partly be explained by age, drug-drug and drug-food interactions, infections, ingestion of varying quantities of vitamin K, heart failure, impairment of liver function,[1-5] and *CYP2C9* genotype.[6-23]

Polymorphisms of the *CYP2C9* gene, which encodes the main metabolizing enzyme of coumarins, have been extensively studied. An association between possession of at least one *CYP2C9*2* or *CYP2C9*3* allele and reduced dose requirement, severe overanticoagulation, major bleeding risk, and retarded stabilization has been convincingly demonstrated for warfarin,[6,8,9,11,16,19,24-27] acenocoumarol,[12,15,18,22] and phenprocoumon.[14,28]

Recently, the presence of polymorphisms in the *VKORC1* gene has drawn attention as another source of variability in the response to coumarins. The enzyme vitamin K epoxide reductase (VKOR) reduces Vitamin K 2,3-epoxide to the biologically active vitamin K hydroquinone, which catalyses the production of the carboxylated blood-clotting proteins II, VII, IX en X. Coumarins act by inhibiting VKOR activity, their target having been identified as the protein vitamin K reductase complex subunit 1 (VKORC1) encoded by the homonymous gene *VKORC1*.[29,30] In several studies an association between the presence of polymorphisms of the *VKORC1* gene and a reduced dose need of warfarin[31-35] and acenocoumarol[36] has been demonstrated. In most of these studies the *VKORC1* genotype explained a larger part of the variation in dose requirement than did the *CYP2C9* genotype.

Because the aforementioned studies mainly focused on dose requirements as an outcome of coumarin sensitivity, we conducted a study in acenocoumarol using outpatients to assess the effects of *VKORC1* and *CYP2C9* genotypes on severe overanticoagulation and time to achieve stability.

METHODS

Study design and patients

This study was conducted in the same cohort of acenocoumarol-using outpatients in whom we earlier examined the association between *CYP2C9*

genotype and anticoagulation status. For full details not described in this article, we refer to the report of our study previously published in this Journal.[15]

In brief, the original study was a prospective follow-up study at two anticoagulation clinics in the Netherlands. We included patients who started therapy from November 1998 until September 2002 with the following characteristics: use of acenocoumarol, anticoagulant indication for at least three months, and loading dose of 6, 4, and 2 mg on the first three days of therapy, respectively, with the first international normalized ratio (INR) measurement being taken on the fourth day. We did not include patients who were taking drugs that pharmacokinetically interact with coumarins at the start of acenocoumarol therapy. We excluded from analysis patients who started pharmacokinetically interacting drugs during the follow-up period of the study. These drugs were identified by the Dutch Standard Management Coumarin Interactions.[37]

The Medical Ethical Committee at the University Medical Centre, Utrecht, the Netherlands, approved our earlier study,[15] in which we used a blood sample from a regular INR measurement for *CYP2C9* genotyping. All patients who met the aforementioned criteria were informed about the aims of the study and were asked for their written consent, which included a statement that the samples would be preserved for ten years maximally for examination of analogue research questions.

Data collection and follow-up time

We collected data on sex, age, anticoagulant indication and the corresponding therapeutic INR range, INR measurements, acenocoumarol doses, comorbidity, infections, and comedication as recorded by the anticoagulation clinics. For *CYP2C9* genotyping, we used blood samples from regular INR measurements at the anticoagulation clinics. We assessed only the presence of the *CYP2C9*2* and *CYP2C9*3* alleles, which are the most frequently occurring variant alleles in white populations. We reanalysed the deoxyribonuclic acid samples from the *CYP2C9* genotyping to assess the *VKORC1 C1173T* genotype. The single-nucleotide polymorphism *C1173T* in intron 1 of the *VKORC1* gene appears to be as informative about coumarin sensitivity as five *VKORC1* haplotypes, which are predictive for the warfarin dose requirement and which together account for 96% to 99% of the total haplotypes in European-American white populations.[35]

Patients were followed up from the date of the first acenocoumarol use (entry date) until the end of the observation period, which was set on the last regular anticoagulation clinic visit within 190 days from the entry date.

Genotyping

For *CYP2C9* genotyping, we refer to our earlier reported study.[15]

For the detection of the *VKORC1 C1173T* polymorphism a LightCycler (Roche Diagnostics, Mannheim, Germany) assay was performed (Roijers JFM, unpublished data, 2006). During the melting-curve analysis, the hybridization probes dissociate from the target deoxyribonuclic acid at specific melting temperatures. The presence of a C allele introduces a destabilizing mismatch, which results in a decreased melting temperature. Comparison between LightCycler genotyping and digestion with a restriction enzyme showed completely concordant results.

Assessment of presence or absence of single-nucleotide polymorphism *C1173T* results in three different genotypes, as follows: *VKORC1 CC*, *VKORC1 CT*, and *VKORC1 TT*.

Outcomes

The primary end points of our study were:

1. Severe overanticoagulation (defined as INR>6.0) during the observation period. An INR greater than 6.0 is associated with a considerably increased bleeding risk.[38,39]
2. Time to achieve the first period of stability. This period was calculated as the time (in days from the entry date) until the first of three consecutive INR measurements within the therapeutic range, with these INR measurements encompassing a period of at least two weeks, with a maximum difference between the mean daily dosages of 10%.

Secondary end points of our study were mean daily dosage during the first period of stability and the percentage of variability in dose need that could be explained by *VKORC1* and *CYP2C9* genotypes.

Statistical analysis

For assessment of deviations of allelic frequencies from Hardy-Weinberg equilibrium, we used the chi-square test. To assess hazard ratios of severe overanticoagulation and time to achieve stability, we used Cox proportional hazard models. To assess differences in mean acenocoumarol dosage during the first period of stability and percentage of variability explained by *VKORC1* and *CYP2C9* genotypes, we used linear regression models. We examined effect

Table 1: CHARACTERISTICS OF PATIENTS TAKING ACENOCOUMAROL (N=231)

Characteristic	*CYP2C9* genotype[a]			*VKORC1* genotype[b]		
	*CYP2C9*1/*1*	*CYP2C9*2*	*CYP2C9*3*	*VKORC1 CC*	*VKORC1 CT*	*VKORC1 TT*
age; mean years (SD)	65.1 (15.2)	63.3 (16.1)	66.9 (13.5)	67.4 (13.8)	64.4 (14.6)	62.3 (17.9)
maximum follow-up time[c]; mean days (SD)	166 (35)	175 (22)	175 (22)	172 (28)	170 (32)	162 (33)
INR measurements; mean number (SD)	14.4 (3.8)	14.3 (2.9)	15.4 (3.8)	14.9 (3.5)	14.6 (3.8)	13.8 (3.7)
Patients	**n=147 (100%)**	**n=38 (100%)**	**n=46 (100%)**	**n=81 (100%)**	**n=111 (100%)**	**n=39 (100%)**
men	85 (57.8%)	22 (57.9%)	26 (57.6%)	46 (56.8%)	66 (59.5%)	21 (53.8%)
low INR target therapeutic range: INR 2.0-3.5	123 (83.7%)	33 (86.8%)	36 (78.3%)	67 (82.7%)	89 (80.2%)	36 (92.3%)
Indication for acenocoumarol treatment						
atrial fibrillation	85 (57.8%)	27 (71.1%)	27 (58.7%)	52 (64.2%)	65 (58.6%)	22 (56.4%)
deep vein thrombosis or pulmonary embolus	29 (19.7%)	4 (10.5%)	7 (15.2%)	11 (13.6%)	18 (16.2%)	11 (28.2%)
other indications	33 (22.4%)	7 (18.4%)	12 (26.1%)	18 (22.2%)	28 (25.2%)	6 (15.4%)
Relevant comedication during follow-up						
CYP2C9-inhibiting drugs	2 (1.4%)	2 (5.3%)	1 (2.2%)	2 (2.5%)	2 (1.8%)	1 (2.6%)
NSAIDs	20 (13.6%)	3 (7.9%)	8 (25.8%)	7 (8.6%)	20 (18.0%)	4 (10.3%)
antibiotics	28 (19.0%)	5 (13.2%)	10 (21.7%)	20 (24.7%)	20 (18.0%)	3 (7.7%)

a) Full details regarding CYP2C9 genotype are as follows: *CYP2C9*1/*1*, n=147(63.6%); *CYP2C9*1/*2*, n=34 (14.7%); *CYP2C9*1/*3*, n=42 (18.2%); *CYP2C9*2/*2*, n=4 (1.7%); *CYP2C9*2/*3*, n=2 (0.1%); *CYP2C9*3/*3*, n=2 (0.9%); percent is percentage of total patients. Allele frequencies are as follows: *1, 80.1%; **2*, 9.5%; and **3*, 10.4%.

b) Allele frequencies are as follows: *C-1173* allele, 59.1%; *T-1173* allele, 40.9% (Hardy-Weinberg χ^2=0.01, p=0.92).

c) Follow-up until patient stopped acenocoumarol or until last anticoagulation clinic control date within maximum follow-up of 190 days was reached.

d) Two patients started amiodarone, two patients started cotrimoxazole, and one started benzbromarone during follow-up.

Table 2: INITIAL INR, ACENOCOUMAROL MEAN DAILY DOSES, OVERANTICOAGULATION, AND STABILITY FOR COMBINATIONS OF *VKORC1* AND *CYP2C9* GENOTYPES

Combined genotype *VKORC1—CYP2C9*	N (100%)	INR>6.0 n (%)[b]	Patients stabilized n (%)[b]	Days until stabilization mean (SD)	Mean daily dose[a] (mg/day) mean (95% CI)	Initial INR mean (95% CI)
*CC—*1/*1*	47	5 (10.6)	42 (89.4)	38 (29)	3.0 (2.6-3.3)	2.4 (2.1-2.8)
*CC—*2*	18	1 (5.6)	18 (100)	34 (23)	2.8 (2.5-3.1)	2.4 (2.0-2.8)
**1/*2*	16	0	16 (100)	37 (22)	2.8 (2.4-3.1)	2.4 (2.0-2.9)
**2/*2*	2	1 (50.0)	2 (100)	8	2.9	2.1
*CC—*3*	14	1 (7.1)	11 (78.6)	30 (26)	2.2 (1.7-2.6)	3.2 (2.6-3.8)
**1/*3*	11	0	8 (72.7)	27 (28)	2.3 (1.8-2.7)	3.1 (2.5-3.8)
**2/*3*	2	0	2 (100)	24	2.5	2.4
**3/*3*	1	1 (100)	1 (100)	62	0.9	5.5
*CT—*1/*1*	67	2 (3.0)	60 (89.6)	31 (12)	2.5 (2.3-2.7)	2.5 (2.3-2.7)
*CT—*2*	15	1 (6.7)	13 (86.7)	24 (20)	2.2 (1.9-2.6)	2.6 (2.0-3.1)
**1/*2*	13	1 (7.7)	12 (92.3)	22 (19)	2.3 (2.0-2.6)	2.3 (1.9-2.7)
**2/*2*	2	0	1 (50.0)	56	1.4	4.1
*CT—*3*	27	6 (22.2)	20 (74.1)	56 (49)	2.0 (1.6-2.4)	3.2 (2.8-3.6)
**1/*3*	26	5 (19.2)	19 (73.1)	55 (50)	2.1 (1.7-2.4)	3.2 (2.8-3.6)
**3/*3*	1	1 (100)	1 (100)	64	0.4	3.2
*TT—*1/*1*	31	2 (6.5)	24 (77.4)	22 (12)	1.7 (1.6-1.9)	3.5 (3.2-3.8)
*TT—*2*						
**1/*2*	3	1 (33.3)	3 (100)	37 (21)	2.1 (0.8-3.5)	2.5 (1.4-3.6)
*TT—*3*						
**1/*3*	4	2 (50.0)	3 (75.0)	48 (23)	1.4 (-0.3- 3.1)	3.4 (3.0-3.9)

a) Doses in first period of stability (only assessed for patients in whom stability was achieved).
b) Percent refers to the percentage of patients with the corresponding combined genotype.

modification by introducing product terms in our models between the *VKORC1* and *CYP2C9* genotypes and between each of these genotypes and other factors, such as sex and age. In all models we adjusted for the potential confounders age, sex, and target therapeutic range. To adjust for confounding comedication, the best strategy would have been to include potential interacting drugs as time-varying covariates in our models. However, files from anticoagulation clinics from which we retrieved our data did not provide reliable information on using time of comedication. Therefore, we excluded all subjects who initiated use of cytochrome P450 (CYP) 2C9-inhibiting drugs from our analyses. Moreover, we tested the robustness of our findings by reanalysing our outcomes after exclusion of those patients who started to use antibiotics or nonsteroidal anti-inflammatory drugs (NSAIDs), which can both contribute to overanticoagulation.[40,41]

RESULTS

Of the 231 patients who met the criteria of our study, five started to use a pharmacokinetically interacting drug during the follow-up period and were excluded from further analysis (Table 1).

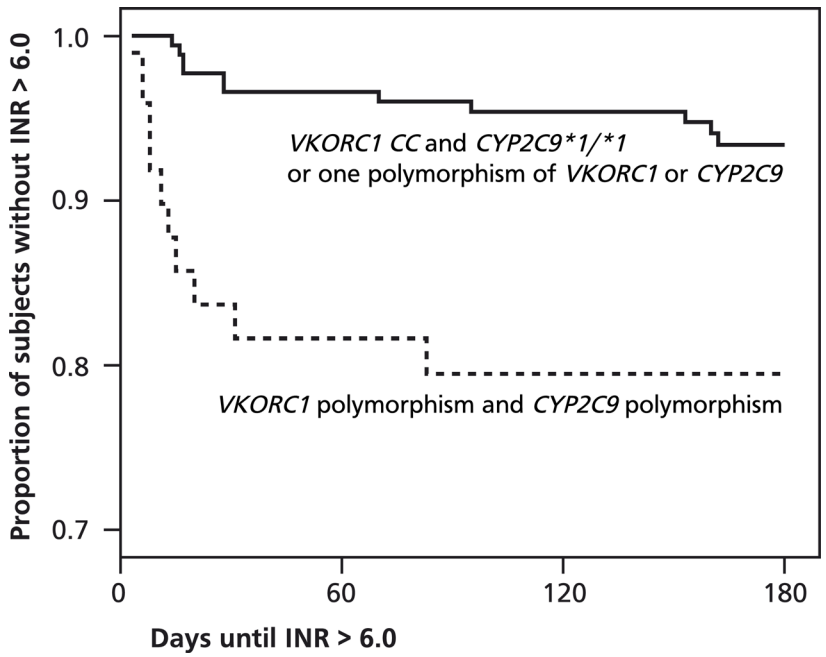
The frequencies of the *VKORC1 CC*, *VKORC1 CT*, and *VKORC1 TT* genotypes were 35.1%, 48.1%, and 16.9%, respectively. The frequencies of the *CYP2C9*1*, *CYP2C9*2*, and *CYP2C9*3* genotypes were 63.6%, 16.5%, and 19.9%, respectively. Allelic frequencies of both genotypes were in Hardy-Weinberg equilibrium. There were more men than women included in our study (57.8% versus 42.2%). The mean follow-up period was 172 days and the mean number of INR measurements during the follow-up was 14.7 (Table 1).

For combined *VKORC1* and *CYP2C9* genotypes, numbers of patients, numbers of patients with severe overanticoagulation, numbers of patients in whom stabilization was achieved, days until the first period of stability was achieved, mean dose requirements in the first period of stability, and initial INR values are summarized in Table 2.

For the association between severe overanticoagulation (INR>6.0) and *CYP2C9* and *VKORC1* genotypes, effect modification was found between both genotypes (p=0.020 for product term in Cox model). Carriers of a polymorphism of CYP2C9 and *VKORC1* had a significantly increased risk of severe overanticoagulation compared with wild-type subjects or carriers of only one

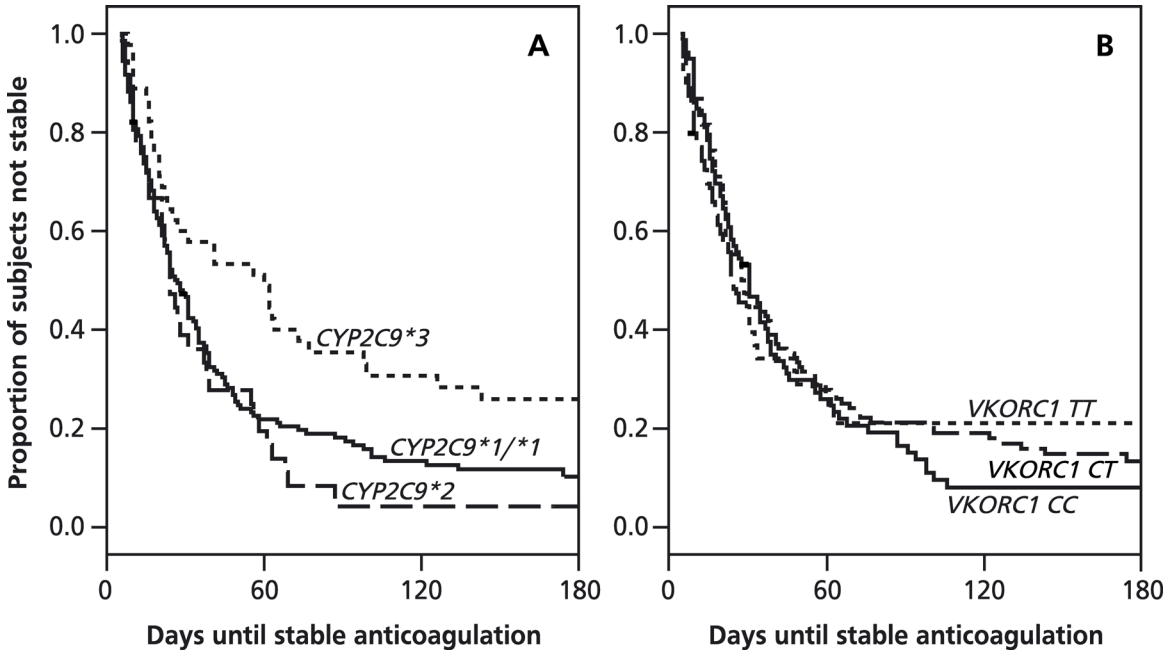
polymorphism of *CYP2C9* or *VKORC1* (adjusted hazard ratio 3.85; 95% confidence interval [CI] 1.62-9.11) (Table 3 and Figure 1). Stratified analysis revealed that within subjects with at least one *VKORC1* polymorphism, the risk of severe overanticoagulation was increased in carriers of a *CYP2C9* polymorphism compared with *CYP2C9* wild-type subjects (adjusted hazard ratio 5.83; 95% CI 1.82-18.6). However, within subjects with the *VKORC1* wild-type, the risk of severe overanticoagulation was not increased in carriers of a *CYP2C9* polymorphism compared with *CYP2C9* wild-type subjects (Table 3). Similarly, within carriers of a *CYP2C9* polymorphism, there was a strong trend toward an increased risk in subjects with at least one *VKORC1* polymorphism compared with *VKORC1* wild-type subjects (adjusted hazard ratio 4.16; 95% CI 0.88-9.15); in contrast, within subjects with the *CYP2C9* wild-type, the overanticoagulation risk was not increased in carriers of a *VKORC1* polymorphism. There was even a weak trend toward a decreased risk compared with subjects with the *VKORC1* wild-type (adjusted hazard ratio 0.37; 95% CI 0.10-1.40) (Table 3).

Figure 1: KAPLAN-MEIER SURVIVAL CURVE FOR TIME TO SEVERE OVERANTICOAGULATION



The difference between *VKORC1* polymorphism plus *CYP2C9* polymorphism (*dashed line*) and no polymorphism or only one polymorphism (*solid line*) was significant (log rank test: p=0.001).

Figure 2: KAPLAN-MEIER SURVIVAL CURVES FOR TIME TO ACHIEVE PERIOD OF STABILITY, PLOTTED FOR *CYP2C9* GENOTYPE (**A**) AND THE *VKORC1* GENOTYPE (**B**)



A: The difference between *CYP2C9*3* (*dashed line*) and *CYP2C9*1/*1* (*solid line*) was significant (log rank test: p=0.008).
B: There were no significant differences between the *VKORC1* genotypes (log rank test for comparison of *VKORC1 TT* with *VKORC1 CC*: p=0.54).

The probability to achieve the first period of stability within the first six months of therapy was decreased in carriers of at least one *CYP2C9*3* allele compared with carriers of the *CYP2C9* wild-type (hazard ratio 0.59; 95% CI 0.40-0.87; p=0.007 [adjusted for differences in age, sex, *VKORC1* genotype, and target therapeutic range]). Moreover, if patients achieved stability within the follow-up time, it took significantly more time for carriers of at least one *CYP2C9**3 allele compared with *CYP2C9* wild-type subjects (mean difference 13.7 days; 95% CI 1.9-25.6; p=0.023 [adjusted for differences in age, sex, target therapeutic range, and *VKORC1* genotype]). There was no significant difference between carriers of at least one *CYP2C9*2* allele and the *CYP2C9* wild-type (adjusted hazard ratio 1.16; 95% CI 0.78-1.70; p=0.47) (Figure 2A). For subjects with a *VKORC1* polymorphism, the chance to achieve stability was not significantly different from that in subjects with the *VKORC1* wild-type: Hazard ratios for *VKORC1 CT* and *VKORC1 TT* genotypes compared with the *VKORC1*

wild-type were 1.06 (95% CI 0.77-1.46; p=0.71) and 0.82 (95% CI 0.53-1.28; p=0.38), respectively (adjusted for differences in age, sex, *CYP2C9* genotype, and target therapeutic range) (Figure 2B) (data not shown in table). We found no effect modification in our Cox model between the *VKORC1* and *CYP2C9* genotypes as we did for severe overanticoagulation.

For subjects in whom stability was achieved compared with subjects with the *VKORC1* wild-type, the presence of a *VKORC1* polymorphism was associated with a significantly lower mean daily acenocoumarol dosage. The adjusted mean difference was 0.56 mg (95% CI 0.34-0.78 mg) for *VKORC1 CT* subjects and 1.34 mg (95% CI 1.03-1.56 mg) for *VKORC1 TT* subjects (Table 4). The mean acenocoumarol dose was decreased in carriers of at least one *CYP2C9*3* allele compared with *CYP2C9* wild-type subjects. After adjustment, we also found a decreased dose requirement in carriers of at least one *CYP2C9*2* allele compared with *CYP2C9* wild-type subjects (adjusted mean difference 0.29 mg; 95% CI 0.01-0.56 mg). For all combinations of at least one *VKORC1* and *CYP2C9* polymorphism, except *VKORC1 CC* plus *CYP2C9*2*, the mean daily dose requirement was significantly decreased compared with subjects with the *VKORC1* and *CYP2C9* wild-type (Table 4).

The combination of age, *VKORC1* genotype, and *CYP2C9* genotype explained 39.1% of the variation in mean daily dosage, with adjusted R^2 values of 12.8% for age, 21.4% for *VKORC1* genotype, and 4.9% for *CYP2C9* genotype (data not shown in table).

We identified 38 subjects (16.4%) with a supratherapeutic initial INR, assessed on the fourth day of acenocoumarol use after the fixed starting dose of 6-4-2 mg. The risk of a supratherapeutic initial INR was only increased in users of the *VKORC1 TT* genotype compared with both *VKORC1 CT* and *VKORC1 CC* subjects (odds ratio 5.07; 95% CI 2.00-12.8 [adjusted for differences in age, sex, and *CYP2C9* genotype]). On the other hand, there was no difference between carriers of a *CYP2C9*2* or *CYP2C9*3* allele and CYP2C9 wild-type subjects or between *VKORC1 CT* subjects and *VKORC1 CC* subjects (data not shown in table).

Exclusion of all users of NSAIDs and antibiotics did not change our results considerably, even without a loss of statistical significance of our main findings (data not shown).

Table 3: ASSOCIATION BETWEEN SEVERE OVERANTICOAGULATION (INR>6.0) AND COMBINED *VKORC1* AND *CYP2C9* GENOTYPES, STRATIFIED BY *VKORC1* AND *CYP2C9* GENOTYPE[a]

	Hazard ratio			
	unadjusted (95% CI)	**p**	**adjusted (95% CI)[b]**	**p**
Total study population				
VKORC1 CC and *CYP2C9*1/*1*, or *VKORC1* polymorphism and *CYP2C9*1/*1*, or *VKORC1 CC* and *CYP2C9* polymorphism	1 (reference)		1 (reference)	
VKORC1 polymorphism and *CYP2C9* polymorphism	3.68 (1.56-8.67)	0.003[c]	3.85 (1.62-9.11)	0.002[c]
Population with *CYP2C9*1/*1*				
VKORC1 CC	1 (reference)		1 (reference)	
VKORC1 polymorphism	0.38 (0.10-1.40)	0.14	0.37 (0.10-1.40)[d]	0.14
Population with *CYP2C9* polymorphism				
VKORC1 CC	1 (reference)		1 (reference)	
VKORC1 polymorphism	3.66 (0.80-16.7)	0.094	4.16 (0.88-19.5)	0.071
Population with *VKORC1 CC*				
*CYP2C9*1/*1*	1 (reference)		1 (reference)	
CYP2C9 polymorphism	0.55 (0.11-2.82)	0.47	0.46 (0.10-2.76)	0.54
Population with VKORC1 polymorphism				
*CYP2C9*1/*1*	1 (reference)		1 (reference)	
CYP2C9 polymorphism	5.53 (1.73-17.6)	0.004[c]	5.83 (1.82-18.6)	0.003[c]

a) For product term CYP2C9 × VKOR in Cox model: p=0.020.
b) Adjusted for differences in age, sex and target therapeutic range.
c) Statistically significant difference (p<0.05).
d) Adjusted for differences in age and sex, with all patients with the VKORC1 CC genotype being within the low INR target therapeutic range.

Table 4: DOSE DIFFERENCES OF ACENOCOUMAROL ACCORDING TO *VKORC1* GENOTYPE, *CYP2C9* GENOTYPE AND COMBINED *VKORC1* AND *CYP2C9* GENOTYPE[a]

Genotype	N	Mean difference			
		unadjusted (95% CI)	p	adjusted (95% CI)	p
Separate genotypes					
VKORC1 CC	71	1 (reference)		1 (reference)	
VKORC1 CT	93	–0.44 (–0.70;–0.18)	0.001[b]	–0.56 (–0.78; 0.34)[c]	<0.001[b]
VKORC1 TT	30	–1.05 (–1.41;–0.71)	<0.001[b]	–1.34 (–1.56;–1.03)[c]	<0.001[b]
*CYP2C9*1/*1*	126	1 (reference)		1 (reference)	
*CYP2C9*2*	34	–0.01 (–0.34; 0.32)	0.96	–0.29 (–0.56;–0.01)[d]	0.039[b]
*CYP2C9*3*	34	–0.51 (–0.84;–0.18)	0.003[b]	–0.55 (–0.82;–0.28)[d]	<0.001[b]
Combined genotypes					
*VKORC1 CC—CYP2C9*1/*1*	42	1 (reference)		1 (reference)	
*VKORC1 CC—CYP2C9*2*	18	–0.20 (–0.64; 0.24)	0.38	–0.32 (–0.71; 0.07)[e]	0.11
*VKORC1 CC—CYP2C9*3*	11	–0.78 (–1.32;–0.24)	0.004[b]	–0.81 (–1.28;–0.34)[e]	0.001[b]
*VKORC1 CT—CYP2C9*1/*1*	60	–0.46 (0.78;–0.14)	0.005[b]	–0.62 (–0.91;–0.34)[e]	<0.001[b]
*VKORC1 CT—CYP2C9*2*	13	–0.75 (–1.25;–0.24)	0.004[b]	–0.92 (–1.36;–0.48)[e]	<0.001[b]
*VKORC1 CT—CYP2C9*3*	20	–0.98 (–1.40;–0.55)	<0.001[b]	–1.12 (–1.50;–0.74)[e]	<0.001[b]
*VKORC1 TT—CYP2C9*1/*1*	24	–1.24 (–1.64;–0.83)	<0.001[b]	–1.45 (–1.81;–1.09)[e]	<0.001[b]
*VKORC1 TT—CYP2C9*2*	3	–0.83 (–1.72; 0.12)	0.086	–1.72 (–2.58;–0.86)[e]	0.001[b]
*VKORC1 TT—CYP2C9*3*	3	–1.60 (–2.54;–0.66)	0.001[b]	–1.45 (–2.28;–0.62)[e]	<0.001[b]

a) Patients in whom a stable dose was not reached during follow-up were not included in the analysis.
b) Statistically significant difference ($p<0.05$).
c) Adjusted for differences in CYP2C9 genotype, age, sex, and target therapeutic range.
d) Adjusted for differences in VKORC1 genotype, age, sex, and target therapeutic range.
e) Adjusted for differences in age, sex, and target therapeutic range.

DISCUSSION

The results of our study, in which we evaluated the role of *CYP2C9* and *VKORC1* genotypes on the anticoagulation status of patients taking acenocoumarol, strongly suggest that the association between the possession of the variant allele *CYP2C9*2* or *CYP2C9*3* and severe overanticoagulation that we found in our earlier study[15] is modified by the *VKORC1 1173CT* genotype, with only carriers of a combination of *CYP2C9* and *VKORC1* polymorphisms having an increased risk. However, the probability of achieving stability within the follow-up period appears to be mainly associated with the *CYP2C9* genotype, although the *VKORC1* genotype explains a considerably larger part of the variation in mean daily dosage than the *CYP2C9* genotype.

The allele frequencies we found for the C-1173 allele (59.1%) and the *T-1173* allele (40.9%) were in accordance with other studies in several white populations, with *T-1173* allele frequencies varying from 39.1% to 45.8%.[33,36,42]

Our results regarding the association of the *VKORC1* genotype with coumarin dose requirements are in agreement with the findings of other studies in users of acenocoumarol, as well as warfarin.[32-35,43]

Sconce et al.[34] and Wadelius et al.[32] made predictive regression models for dose requirements in warfarin-using patients in English and Swedish anticoagulation clinics, respectively. Both models with *CYP2C9* genotype, *VKORC1* genotype, age, and several other factors predicted 54% to 56% of the variation in dose requirement. Our model with *CYP2C9* genotype, *VKORC1* genotype, and age explained a smaller part of the variation in dose requirement (39.1%), but we had a smaller number of variables at our disposal. Our finding that the *VKORC1* genotype contributed more than the *CYP2C9* genotype to the variability in dose requirement was in agreement with studies in warfarin users of Rieder et al.,[35] Wadelius et al.,[32] and Bodin et al.[36] In contrast, in the studies of D'Andrea et al.[33] and Sconce et al.,[34] a larger contribution of the *CYP2C9* genotype than of the *VKORC1* genotype to the dose requirement has been reported. This could be because of the higher proportion of *CYP2C9* variant alleles in their studies compared with our study: for *CYP2C9*2* and *CYP2C9*3*, D'Andrea et al.[33] had allelic frequencies of 17.0% and 8.8%, respectively; Sconce et al.[34] had frequencies of 14.2% and 9.4%, respectively; and we had frequencies of 9.5% and 10.4%, respectively. Furthermore, differences in the percentage of explained variance and relative contribution of different genotypes can also be a consequence of differences in inclusion and exclusion criteria between studies.

Of the aforementioned authors, only Bodin et al.[36] studied the effects of the *CYP2C9* and *VKORC1* genotypes as determinants of acenocoumarol sensitivity, whereas the other studies were aimed at warfarin. However, Bodin et al.[36] conducted their study in healthy volunteers. As far as we know, our study is the first to examine the effects of the combined *VKORC1* and *CYP2C9* genotypes in acenocoumarol-using patients.

In a case-control study Reitsma et al.[44] found an increased bleeding risk in phenprocoumon-using carriers of a *VKORC1* polymorphism compared with *VKORC1* wild-type subjects, whereas such an increased risk was not found in acenocoumarol users. Although this study failed to show an effect for acenocoumarol, the finding for phenprocoumon underlines the increased sensitivity of carriers of a *VKORC1* polymorphism compared with *VKORC1* wild-type subjects. A limitation of this otherwise interesting study was the lack of data on the *CYP2C9* genotype, which makes a comparison with our finding of an interaction between both genotypes affecting overanticoagulaton impossible.

Our study adds two interesting novel findings to all other hitherto performed studies. First, the risk of severe overanticoagulation is significantly increased in patients with a combination of polymorphisms of *VKORC1* and *CYP2C9*, as compared with patients with no polymorphism or maximally one polymorphism of either gene. This necessitates a reconsideration of the findings of earlier studies that demonstrated an increased risk of overanticoagulation in carriers of a *CYP2C9* polymorphism[9,15,18,22] and underlines the importance of the knowledge of both *VKORC1* and *CYP2C9* polymorphisms to identify patients at risk of severe overanticoagulation. Our finding that within *CYP2C9* subjects there was a weak trend toward a decreased risk of overanticoagulation in carriers of a *VKORC1* polymorphism compared with *VKORC1* wild-type subjects was not expected and is probably a chance finding. Other studies are needed to elucidate this subject further.

Our second novel, and at first sight, unexpected finding is that the search for a stable acenocoumarol dose regimen appears to be associated more with the *CYP2C9* genotype than with the *VKORC1* genotype. Only carriers of at least one *CYP2C9*3* allele had a decreased chance to achieve stability compared with patients with the *CYP2C9* wild-type or carriers of a *CYP2C9*2* allele. This seems to contrast with our finding that *VKORC1* polymorphisms account for a larger part of the variability in dose need. However, especially because the possession of a *VKORC1* polymorphism is more associated with a lower dose need, dose adjustments in carriers of such polymorphisms will point faster to a

lower dose need compared with dose adjustments in carriers of only a *CYP2C9* polymorphism. This might result in faster stabilization with a lower dose. On the other hand, possession of the 'pharmacokinetic' *CYP2C9*3* allele results in detectable quantities of the more active (S)-enantiomer of acenocoumarol in contrast to patients with only *CYP2C9*1* or **2* alleles (or both of these alleles), in whom the coumarin effect is exclusively based on the less active (R)-enantiomer.[45] So, without *CYP2C9* genotyping, anticoagulation clinics unknowingly manage two anticoagulants with different pharmacokinetic profiles in carriers of a *CYP2C9*3* allele,[15] independent of the *VKORC1* genotype. This indicates that knowledge of the *CYP2C9* genotype could be more important for the guidance to a stable acenocoumarol dose in the long term than knowledge of the *VKORC1* genotype.

Our study indicates that using data of both the *VKORC1* and *CYP2C9* genotypes might contribute to the development of new and safer dosing and monitoring algorithms in acenocoumarol users. Of course, this issue has to be further examined in prospective studies. Some limitations of our study have to be considered. Because we only had medical data of anticoagulation clinics at our disposal, we could have missed relevant data about comorbidities and comedication. Furthermore, because our study was originally designed to examine the effects of two *CYP2C9* alleles on acenocoumarol anticoagulation status, the numbers of patients with overanticoagulation and nonstabilization in the combined strata of *CYP2C9* and *VKORC1* polymorphisms had become too low for examination of risks in specific combinations of genotypes such as *CYP2C9*3* and *VKORC1 TT*. However, our results proved to be robust after exclusion of users of NSAIDs and antibiotics from our cohort.

In conclusion, our study shows that in acenocoumarol users the possession of a combination of polymorphisms of *VKORC1* and *CYP2C9*, rather than the possession of one of these polymorphisms, is associated with severe overanticoagulation and that the time to achieve a period of stability seems to be associated only with the *CYP2C9* genotype. Being a carrier of a *VKORC1* polymorphism or a *CYP2C9* polymorphism is associated with lower acenocoumarol dose requirements compared with those in wild-type subjects, with the *VKORC1* genotype explaining a larger part of the variability in dose requirement.

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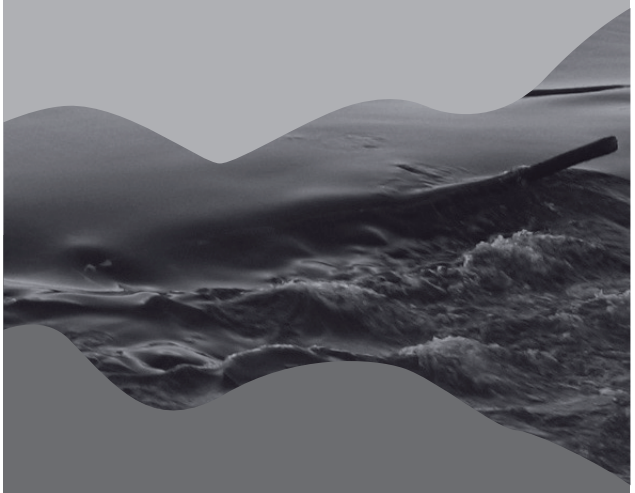
CHAPTER 5



CYP2C9 AND VKORC1 GENOTYPE AND PHENPROCOUMON

5.1

Effects of cytochrome P450 2C9 polymorphisms on phenprocoumon anticoagulation status

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ABSTRACT

Objective

Our objective was to assess whether there is an association between the presence of allelic variants of the gene for cytochrome P450 (CYP) 2C9 and anticoagulation problems during the initial phase of phenprocoumon treatment.

Methods

A prospective follow-up study was performed at two anticoagulation clinics in the Netherlands. Included subjects started phenprocoumon during the study period, had their first check of the International Normalized Ratio (INR) on the third or fourth day of therapy and had an indication for the low therapeutic range (INR 2.0-3.5). *CYP2C9* genotypes (*CYP2C9*1*, *CYP2C9*2*, and *CYP2C9*3*) were assessed and data on indication, INR checks, comedication, and comorbidity were collected.

Results

After genotyping, 284 subjects were available for analysis. Of these, 186 (65.5%) were homozygous carriers of the *CYP2C9* wild-type allele (*CYP2C9*1/*1*), 61 (21.5%) were carriers of the *CYP2C9*2* allele and 37 (13.0%) were carriers of the *CYP2C9*3* allele. Compared with homozygous *CYP2C9*1/*1* subjects, carriers of *CYP2C9*2* or **3* had an increased risk of severe overanticoagulation (INR>6.0). The hazard ratio (HR) for *CYP2C9*2* versus *CYP2C9*1/*1* was 3.09 (95% confidence interval (CI) 1.56-6.13; p=0.001), and HR for *CYP2C9*3* versus *CYP2C9*1/*1* was 2.40 (95% CI 1.03-5.57; p=0.042). Carriers of *CYP2C9*2* also had a lower chance to achieve stability in the follow-up period. HR for *CYP2C9*2* versus *CYP2C9*1/*1* was 0.61 (95% CI 0.43-0.85; p=0.003). Carriers of the *CYP2C9*2* or **3* allele needed a significantly lower phenprocoumon dosage compared with homozygous *CYP2C9*1/*1* subjects.

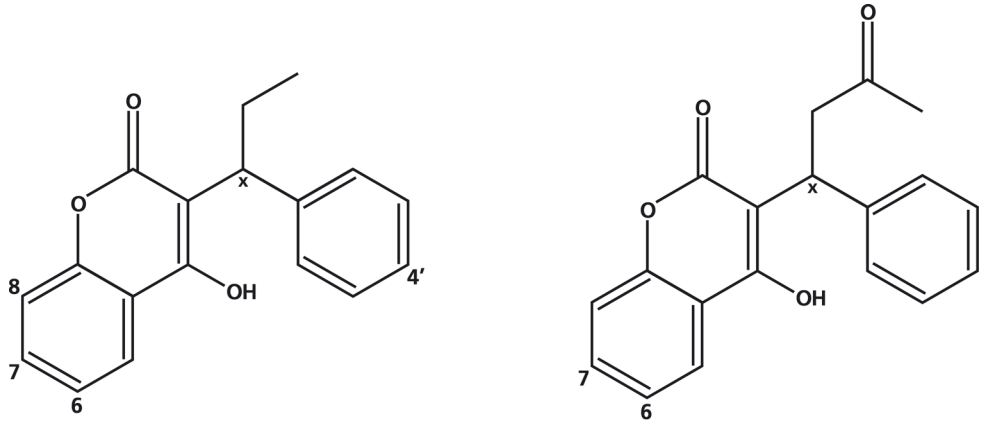
Conclusion

The presence of at least one *CYP2C9*2* or **3* allele in phenprocoumon users is associated with an increased risk of severe overanticoagulation. Similar to warfarin and acenocoumarol, phenprocoumon had a lower dosage requirement in carriers of *CYP2C9*2* or **3* compared with that in *CYP2C9* wild-type subjects.

INTRODUCTION

Oral anticoagulants of the coumarin type are used for the prevention and treatment of several thromboembolic disorders, such as atrial fibrillation, deep vein thrombosis, and pulmonary embolus.[1] Coumarin anticoagulants have a narrow therapeutic range and show a large interindividual and intraindividual variation in dose need, which requires frequent control of the anticoagulant effect and dosage adjustment. Well-known factors that contribute to instability in anticoagulant therapy are drug interactions, variable vitamine K intake, infections, and diseases, such as heart failure and impaired liver function.[1] Furthermore, the presence of genetic polymorphisms in the gene for cytochrome P450 (CYP) 2C9 has been recently identified as a cause of problems with coumarin therapy. For warfarin, the most frequently used coumarin anticoagulant worldwide, it has been demonstrated that the presence of one of the *CYP2C9* polymorphic alleles, *CYP2C9*2* or *CYP2C9*3*, results in a reduced dose need and more bleeding complications and overanticoagulation.[2-9]

Figure 1: STRUCTURES OF PHENPROCOUMON AND WARFARIN



The (S)-enantiomers of both coumarins have a higher anticoagulant potency compared with that of the (R)-enantiomers. CYP2C9 is involved in the 4'- and 7-hydroxylation and in the quantitatively less important 6- and 8-hydroxylation of (S)- and (R)-phenprocoumon. CYP2C9 is involved in the 6- and 7-hydroxylation of (S)-warfarin, with (S)-7-hydroxywarfarin being the major metabolite in vivo.

For acenocoumarol, several studies indicate that the presence of the *CYP2C9*3* allele, but not the *CYP2C9*2* allele, is associated with a reduced maintenance dose and an increased risk of severe overanticoagulation.[10-16] Surprisingly little is

known regarding the role of CYP2C9 in the metabolism of phenprocoumon, a coumarin anticoagulant that is frequently used in European countries. Phenprocoumon is structurally very similar to warfarin: phenprocoumon has an ethyl side chain, where warfarin has an acetonyl side chain (Figure 1). As for warfarin, the (S)-enantiomer of phenprocoumon has a stronger anticoagulant effect than the (R)-enantiomer (relative potency of 1.6-2.6 for (S)- versus (R)-phenprocoumon).[17] An in vitro study showed that both (S)-phenprocoumon and (R)-phenprocoumon are metabolized at least partly via the CYP isozyme CYP2C9 to 4'-, 6-, 7-, and 8-hydroxymetabolites, with 4'-hydroxylation and 7-hydroxyation being the major pathways for (S)-phenprocoumon. However, the same study demonstrated that both stereoisomeric forms of phenprocoumon are less effectively metabolized via CYP2C9 compared with warfarin.[18] To date, three studies have been conducted that addressed a possible association between *CYP2C9* genotype and phenprocoumon.[16,19,20] These studies have not led to an unequivocal conclusion regarding an association between the *CYP2C9* genotype and phenprocoumon anticoagulant status. The aim of our study was to elucidate further the influence of the presence of *CYP2C9* polymorphisms on phenprocoumon anticoagulation status.

METHODS

Study design and patients

We conducted a prospective follow-up study on outpatients at the anti-coagulation clinics of Leiden and The Hague, the Netherlands. In both clinics phenprocoumon is the most frequently used coumarin anticoagulant. The International Normalized Ratio (INR), an indicator for the extent of anticoagulation, is regularly monitored with a frequency ranging from a few days to a maximum of 6 weeks. This frequency mainly depends on the results of INR measurements and the stability of the anticoagulant status. After each INR check, a dose scheme is determined with the aid of a computerized dosing program. In the Netherlands two target therapeutic ranges are being used: the low therapeutic range (INR 2.0-3.5) and the high therapeutic range (INR 2.5-4.0).

We included patients who started phenprocoumon therapy between October 2002 and July 2003 and met the following characteristics: indication for anticoagulant treatment at least three months, INR check on the third or fourth day, and low target therapeutic range (INR 2.0-3.5). Loading doses were not the

same for all subjects and depended mainly on the physician who initiated phenprocoumon therapy. However, all patients were supervised by the anticoagulation clinic, starting on the third or fourth day of phenprocoumon use, during the entire follow-up period. We included only patients with the low therapeutic range to increase the homogeneity of our cohort.

Exclusion criteria were liver failure, thyroid disease, and use of pharmacokinetically interacting drugs at the start of phenprocoumon therapy. All drugs for which an interaction with anticoagulants is established are listed in the Dutch Standard Management Coumarin Interactions, which is used as a reference by the Dutch anticoagulation clinics.[21] These clinically important drug interactions can also be found in the review by Harder and Thurmann.[22] Examples of interacting drugs are CYP2C9 inhibitors (e.g. amiodarone, benzbromarone, and cotrimoxazole) or CYP2C9 inducers (e.g. carbamazepine). Case patients who began pharmacokinetically interacting drugs during follow-up were excluded from our analyses (see Statistical analysis section).

The Medical Ethical Committee of the Leiden University Medical Centre approved the study. Patients who met the aforementioned criteria were informed about the aims of our study and gave written consent.

Data collection and follow-up period

We collected data on sex, age, genotype, indication for anticoagulant therapy, INR, phenprocoumon doses, comorbidity, infections, and comedication as recorded by the anticoagulation clinics in a database. Although our study was not designed to detect differences in bleeding risks, we also collected data on bleeding events. In addition, we recorded the frequency of INR monitoring in the patients (see Table 1). The weekly dosage for each patient was assessed from the dose schemes. Dose schemes are indicated as mean daily dosages in the patients' records, which we converted into mean weekly dosages. The remainder of a regular blood sample was used for *CYP2C9* genotyping. No additional blood or other body material was needed for this study.

Patients were followed up from the first date of phenprocoumon use (entry date) until the end of the observation period, which was set on the last regular anticoagulation clinic visit within 180 days from the entry date.

Genotyping

The *CYP2C9* genotypes were assessed in two different laboratories. The main reason for switching from one laboratory to another was the possibility offered during the course of our study to perform genotypings more cheaply and quickly

within our own institution (Utrecht University, Utrecht, the Netherlands). Both laboratories routinely genotype *CYP2C9*1*, **2*, and **3* alleles by use of validated methods.

The *CYP2C9* genotypes of the first 231 subjects were assessed according to a method described in our previous study, which was recently published in this Journal,[15] by use of primers for *CYP2C9*2* and CYP2C9*3, as described by Steward et al.[23] and Sullivan-Klose et al.,[24] respectively.

The *CYP2C9* genotypes of the other 71 subjects were assessed as follows: Genomic deoxyribonucleic acid was isolated from peripheral blood according to the method of Boom et al.[25] Genotyping of *CYP2C9*2* was performed with polymerase chain reaction (PCR) followed by restriction enzyme analyses as described by Sullivan-Klose et al.,[24] with a modification of the PCR protocol. For the identification of the *CYP2C9*3* polymorphism, specific primers were developed to amplify a 208-base pair fragment, which contains the A1075C polymorphism located in exon 7, forward primer 5'-GAA CGT GTG ATT GGC AGA AA-3', and reverse primer 5'-TCG AAA ACA TGG AGT TGC AG-3' (Invitrogen Life Technologies, Paisley, United Kingdom). PCR amplification was performed by use of a 1x PCR buffer containing 1.5 mmol/L magnesium chloride (Opti-Prime, buffer No.5; Stratagene, Cedar Creek, Tex, USA), 200 nmol/L of each primer, and a PCR protocol consisting of an initial denaturation step at 94°C for 7 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 57°C for 1 minute, and extension at 72°C for 1 minute, with a final extension step at 72°C for 7 minutes. The PCR product was purified for sequencing by use of ExoSAP-IT (Amersham Biosciences, Uppsala, Sweden) and sequenced in an ABI Prism 3100-Avant Genetic Analyser (Applied Biosystems Inc., Foster City, Calif, USA). The ABI Prism SeqScape v2.0 program was used to analyse the PCR fragment for the single-nucleotide polymorphism (Applied Biosystems Inc.).

Outcomes

The primary end points of our study were as follows:

1. Severe overanticoagulation (defined as INR>6.0) during the observation period. An INR greater than 6.0 is associated with a considerably increased bleeding risk.[26-28]
2. Time to achieve a first period of stability. This period was calculated as the time (in days from the entry date) until the first of three consecutive INR checks within the therapeutic range. These INR values must have

encompassed a period of at least two weeks with a maximum difference between the mean weekly dosages of phenprocoumon of 10%. An analogous definition of stable anticoagulant control was used in a study on the association between *CYP2C9* genotype and warfarin.[5]

The secondary endpoint of our study was mean weekly dosage of phenprocoumon during the first period of stability. This dosage was calculated as the mean of the three weekly dosages that resulted in the first three consecutive INR values within the therapeutic range. As a consequence these dosages were only calculated for subjects in whom stability was achieved during the follow-up period.

Statistical analysis

For assessment of deviations of allelic frequencies from Hardy-Weinberg equilibrium, we used the chi-square test. For comparisons between genotypes, patients were divided into three categories; homozygous carriers of the *CYP2C9* wild-type allele (*CYP2C9*1/*1*) formed the reference group, and the other two groups consisted of carriers of the *CYP2C9*2* and *CYP2C9*3* alleles. Because of the low prevalence of subjects carrying two allelic variants, heterozygous and homozygous subjects were included in the same genotype category. *CYP2C9*2/*3* subjects were allocated to the *CYP2C9*3* group. Detailed information on the separate genotypes is presented in the tables. To assess hazard ratios (HRs) of severe overanticoagulation and time to achieve stability, we used Cox proportional hazards models. To assess differences in mean phenprocoumon dosage during the first period of stability, we used linear regression models. In all models we adjusted for the potential confounders age, sex and time between INR measurements. We excluded subjects who started to use a CYP2C9-inhibiting or CYP2C9-inducing drug during the follow-up period from our analyses. Statistical analyses were performed with the statistical software package SPSS 10 (SPSS Inc., Chicago, Ill, USA).

RESULTS

There were 302 subjects who met the criteria of our study and were genotyped. Of these subjects, 18 started to use a pharmacokinetically interacting drug during the follow-up period and were excluded (amiodarone in 9, cotrimoxazole in 3, benzbromarone in 2, allopurinol in 2, carbamazepine in 1, and fluconazole in 1).[22,29,30]

Table 1: CHARACTERISTICS OF PATIENTS ON PHENPROCOUMON TREATED BY TWO ANTICOAGULATION CLINICS (N=284)

Characteristic	CYP2C9*1/*1	CYP2C9*2	CYP2C9*3
Age; mean years (SD)	64.3 (15.8)	65.6 (16.1)	66.0 (15.0)
Maximum follow-up period; mean days (SD)[a]	152 (35)	149 (39)	155 (34)
INR measurements; mean number (SD)	13.8 (3.8)	14.1 (3.9)	13.8 (3.7)
Patients	**n=186 (100%)**	**n=61 (100%)**	**n=37 (100%)**
Men	102 (54.8%)	36 (59.0%)	19 (51.4%)
Women	84 (45.2%)	25 (41.0%)	18 (48.6%)
Indication for phenprocoumon treatment			
atrial fibrillation	94 (50.5%)	37 (60.7%)	21 (56.8%)
deep vein thrombosis	43 (23.1%)	11 (18.0%)	4 (10.8%)
pulmonary embolus	39 (21.0%)	11 (18.0%)	8 (21.6%)
other indications	10 (5.4%)	2 (3.3%)	4 (10.8%)
Users of antibiotics	36 (19.4%)	12 (19.7%)	11 (29.7%)
Users of oral contraceptives or postmenopausal hormonal treatment	5 (2.7%)	2 (3.3%)	1 (2.7%)
Patients with congestive heart failure[b]	18 (9.7%)	4 (6.6%)	4 (10.8%)

Full details of genotypes are as follows: *CYP2C9*1/*1*, n=186 (65.5%); *CYP2C9*1/*2*, n=56 (19.7%); *CYP2C9*1/*3*, n=29 (10.2%); *CYP2C9*2/*2*, n=5 (1.8%); *CYP2C9*2/*3*, n=6 (2.1%); *CYP2C9*3/*3*, n=2 (0.7%); percent is percentage of total patients.
Allelic frequencies are as follows: **1*, 80.4%; **2*, 12.7%; and **3*, 6.9%.
Hardy Weinberg χ^2=0.861, p=0.83.
a) Time in days until the end of phenprocoumon therapy or until the last INR check within the maximum follow-up period of 180 days.
b) No subjects had other relevant comorbidities, such as liver dysfunction or thyroid dysfunction during the follow-up period.

Table 2: HAZARD RATIO (HR) OF VARIANT ALLELES OF *CYP2C9* VERSUS WILD-TYPE WITH REGARD TO SEVERE OVERANTICOAGULATION (INR>6.0)

Genotype		Patients with INR>6.0	Events with INR>6.0	Hazard ratio (95% CI)			
		n (%)[a]	n (%)[b]	Unadjusted	p	Adjusted[c]	p
CYP2C9*1/*1	(n=186; 2573 INR controls)	**17 (9.1)**	**20 (0.8)**	**1 (reference)**		**1 (reference)**	
CYP2C9*2	(n= 61; 859 INR controls)	**16 (26.2)**	**23 (2.7)**	**3.19 (1.61-6.32)**	**0.001[d]**	**3.09 (1.56-6.13)**	**0.001[d]**
*CYP2C9*1/*2*	(n= 56; 771 INR controls)	15 (26.8)	20 (2.6)				
*CYP2C9*2/*2*	(n= 5; 88 INR controls)	1 (20.0)	3 (3.4)				
CYP2C9*3	(n= 37; 511 INR controls)	**8 (21.6)**	**11 (2.2)**	**2.40 (1.03-5.56)**	**0.041[d]**	**2.40 (1.03-5.57)**	**0.042[d]**
*CYP2C9*1/*3*	(n= 29; 411 INR controls)	7 (24.1)	10 (2.4)				
*CYP2C9*2/*3*	(n= 6; 73 INR controls)	1 (16.7)	1 (1.4)				
*CYP2C9*3/*3*	(n= 2; 27 INR controls)	0	0				

a) Percent refers to the percentage of patients with the corresponding genotype.
b) Percent refers to the percentage of total INR measurements within the corresponding genotype.
c) Hazard ratio adjusted for age, sex and time between INR measurements.
d) Statistically significant difference ($p<0.05$).

The frequencies of the *CYP2C9*1*, *CYP2C9*2*, and *CYP2C9*3* alleles were 80.4%, 12.7%, and 6.9%, respectively. The allelic frequencies were in Hardy-Weinberg equilibrium (p=0.82). There were more men than women included in our study (55.3% versus 44.7% for all genotypes). The mean age of all patients was 64.8 years. The mean follow-up period was 152 days, and the mean number of INR checks during the follow-up period was 13.9 (Table 1).

Carriers of at least one *CYP2C9*2* or *CYP2C9*3* polymorphic allele had a significantly increased risk of severe overanticoagulation compared with the wild-type subjects (for *CYP2C9*2* and *CYP2C9*3*, adjusted HRs were 3.09 [95% confidence interval (CI) 1.56-6.13], and 2.40 [95% CI 1.03-5.57], respectively) (Table 2).

Overanticoagulation occurred in four patients during a bleeding event. Of these, one patient (*CYP2C9*1/*2*) had gastro-intestinal bleeding leading to hospital admission, and the other three patients (two with *CYP2C9*1/*1* and one with *CYP2C9*1/*2*) had minor bleeding.

Overall, 29 patients (10.2%) in our cohort had bleeding during follow-up period. We found no significant differences in bleeding risk for patients with at least one variant allele compared with patients with the *CYP2C9*1/*1* genotype (HR 1.57; p=0.23) (data not shown in table).

The hazard ratios for overanticoagulation, as well as the statistical significance of the results, did not essentially change when the cohort was analysed after exclusion of those subjects who used at least one antibiotic during the follow-up period; for *CYP2C9*2* and *CYP2C9*3*, adjusted HRs were 2.56 (95% CI 1.09-6.02; p=0.031) and 2.92 (95% CI 1.12-7.57; p=0.028), respectively (data not shown in table). Exclusion of users of oral contraceptives and hormonal replacement therapy also did not result in an essential shift in the results; for *CYP2C9*2* and *CYP2C9*3*, adjusted HRs were 3.35 (95% CI 1.67-6.71; p=0.001) and 2.47 (95% CI 1.06-5.80; p=0.037), respectively (data not shown in table).

In the first 45 days of therapy, the association between genotype and overanticoagulation was less strong and only statistically significant for *CYP2C9*2* carriers. HRs for *CYP2C9*2* and *CYP2C9*3* were 2.56 (95% CI 1.16-5.67; p=0.020) and 1.80 (95% CI 0.65-5.01; p=0.26), respectively (data not shown in table). This indicates that the risk appears to increase further after the first six weeks of therapy.

Carriers of the *CYP2C9*2* allele had a significantly lower chance of achievement of a period of stability during the first six months of therapy (adjusted HR 0.61;

95% CI 0.43-0.85), and more days were also required to achieve stability in *CYP2C9*2* carriers. For carriers of the *CYP2C9*3* allele, the chance to achieve stability was not significantly different from that in wild-type subjects (Table 3). Exclusion of those subjects who used at least one antibiotic during the follow-up period did not essentially change the hazard ratios for achieving stability or the statistical significance of the results; for *CYP2C9*2* and *CYP2C9*3*, adjusted HRs were 0.63 (95% CI 0.43-0.92; p=0.016) and 0.91 (95% CI 0.57-1.45; p=0.68), respectively (data not shown in table).
Exclusion of the users of oral contraceptives and postmenopausal hormonal substitution also did not change the results with regard to overanticoagulation and time to achieve stability; for *CYP2C9*2* and *CYP2C9*3*, adjusted HRs were 0.61 (95% CI 0.43-0.86; p=0.005) and 0.94 (95% CI 0.63-1.39; p=0.74), respectively (data not shown in table).
For subjects in whom stability was achieved, the presence of the *CYP2C9*2* or the *CYP2C9*3* allele was associated with a significantly lower mean weekly dosage of phenprocoumon compared with that in homozygous *CYP2C9*1/*1* subjects. The adjusted mean difference of the weekly dosage was 3.7 mg (95% CI 1.9-5.5 mg) for *CYP2C9*2* carriers and 4.4 mg (95% CI 2.4-6.5 mg) for *CYP2C9*3* carriers (Table 4). Age had a significant influence on the mean weekly dosage of phenprocoumon; with 10 years' increase in age, the mean weekly dosage decreased by 1.6 mg (95% CI 1.1-2.0 mg; p<0.001 (data not shown in table). The factor age explained a greater percentage of the variability in dosage than the factor genotype (16.8% versus 10.3%).

DISCUSSION

The results of our study, in which we evaluated the role of *CYP2C9* genotypes on the anticoagulation status of patients using phenprocoumon, strongly suggest that the presence of at least one polymorphic *CYP2C9* allele is associated with an increased risk of severe overanticoagulation and a lower maintenance dosage in the first six months of therapy. Moreover, carriers of the *CYP2C9*2* allele had a decreased chance to achieve stability.
The allelic frequencies of the variant alleles were well in accordance with most studies in which *CYP2C9* genotyping in a white population was performed. For the *CYP2C9*2* allele (frequency of 12.7%) in British, German and Dutch CYP2C9 studies a range of 10.6% to 19.1% was reported.[31,32] For the

Table 3: HAZARD RATIO OF VARIANT ALLELES OF *CYP2C9* VERSUS WILD-TYPE WITH REGARD TO TIME TO ACHIEVE FIRST PERIODE OF STABILITY

Genotype	Patients total	Patients stabilized n (%)[a]	Time to achieve stability mean days (SD)	Hazard ratio (95% CI) Unadjusted	p	Adjusted[b]	p
CYP2C9*1/*1	**186**	**160 (86.0)**	**32 (26)**	**1 (reference)**		**1 (reference)**	
CYP2C9*2	61	**44 (72.1)**	**52 (36)**	**0.58 (0.41-0.80)**	**0.001[c]**	**0.61 (0.43-0.85)**	**0.003[c]**
*CYP2C9*1/*2*	56	40 (71.4)	51 (35)				
*CYP2C9*2/*2*	5	4 (80.0)	60 (43)				
CYP2C9*3	**37**	**30 (81.1)**	**31 (35)**	**0.94 (0.64-1.39)**	**0.75**	**0.93 (0.63-1.38)**	**0.71**
*CYP2C9*1/*3*	29	23 (79.3)	29 (25)				
*CYP2C9*2/*3*	6	5 (83.3)	50 (67)				
*CYP2C9*3/*3*	2	2 (100)	14 (6)				

a) Percent refers to the percentage of patients with the corresponding genotype.
b) Hazard ratio adjusted for age, sex and time between INR measurements.
c) Statistically significant difference ($p<0.05$).

Table 4: DOSE DIFFERENCES OF PHENPROCOUMON IN STABILIZED PATIENTS ACCORDING TO *CYP2C9* GENOTYPE

Genotype	Number of patients	Mean dose[a] mg/wk (95% CI)	Mean difference[b] (95% CI)			
			Unadjusted	p	Adjusted[c]	p
CYP2C9*1/*1	**160**	**17.4 (16.4;18.4)**	**1 (reference)**		**1 (reference)**	
CYP2C9*2	**44**	**13.2 (11.7;14.8)**	**–4.2 (–6.1;–2.2)**	**<0.001[d]**	**–3.7 (–5.5;–1.9)**	**<0.001[d]**
*CYP2C9*1/*2*	40	13.2 (11.7;14.8)				
*CYP2C9*2/*2*	4	13.1 (0.3;25.9)				
CYP2C9*3	**30**	**12.6 (11.0;14.3)**	**–4.8 (–6.6;–2.4)**	**<0.001[d]**	**–4.4 (–6.5;–2.4)**	**<0.001[d]**
*CYP2C9*1/*3*	23	12.7 (10.6;14.8)				
*CYP2C9*2/*3*	5	12.9 (10.0;15.8)				
*CYP2C9*3/*3*	2	11.4				

a) Mean dose during first period of stability. If stability was not achieved in the follow-up period, no mean dose was computed.
b) Mean difference between *CYP2C9*2 / CYP2C9*3* alleles *and CYP2C9*1/*1* allele.
c) Mean difference adjusted for sex and age.
d) Statistically significant difference ($p<0.05$).

CYP2C9*3 allele (frequency of 6.9%), the range in these studies was 5.3% to 10.5%.

Our results are in agreement with the findings of several studies with warfarin but differ from the findings of several studies with acenocoumarol in regard to the *CYP2C9*2* genotype. For warfarin, an increased risk of overanticoagulation and a decreased maintenance dosage were demonstrated in *CYP2C9*2* and *CYP2C9*3* carriers, as in our study.[5] For the coumarin anticoagulant acenocoumarol, a decreased maintenance dosage and an increased risk for overanticoagulation were demonstrated in *CYP2C9*3* carriers but not in *CYP2C9*2* carriers,[10,11,15,16] although some studies also point to a significant effect of the *CYP2C9*2* allele on acenocoumarol clearance and steady-state concentrations.[13,33]

For phenprocoumon, a few pharmacogenetic studies have been conducted, which did not result in a consistent insight into the influence of the *CYP2C9* genotype on phenprocoumon anticoagulation status.[16,19,20] The study by Kirchheiner et al.[20] examined pharmacokinetics in healthy volunteers, and demonstrated differences in metabolic capacity as a result of both the *CYP2C9*2* and **3* alleles. Although these differences were smaller compared with those for warfarin and acenocoumarol, the results of this study are in agreement with our finding that the phenprocoumon maintenance dosage is decreased in both *CYP2C9*2* carriers and *CYP2C9*3* carriers.

Hummers-Pradier et al.[19] and Visser et al.[16] conducted patient studies. Hummers-Pradier et al. found an increased bleeding risk in *CYP2C9*3* carriers but no differences in dosage need between *CYP2C9* genotypes. The increased bleeding risk in *CYP2C9*3* carriers points to differences in sensitivity with regard to the pharmacodynamic effects of phenprocoumon between *CYP2C9* genotypes, which is in agreement with our finding of an increased risk of overanticoagulation in carriers of a variant allele. The fact that, in contrast to our study, no differences in dosage were found, could be because of the smaller number of patients included and the use of another method to calculate the phenprocoumon dosage need. As mentioned before, our study was not designed to assess bleeding risks, so it is difficult to compare our findings on bleeding with those of Hummer-Pradiers et al.

Visser et al.[16] found no differences in dosage need and risk for overanticoagulation between carriers of a *CYP2C9* polymorphism and homozygous *CYP2C9*1/*1* carriers. Part of this apparent discrepancy could be a result of differences in sample size; we investigated 284 phenprocoumon users, as

compared with 204 in their study, in which, moreover, the percentage of carriers of the *CYP29*3* allele was relatively low (8.3% versus 13% in our study). Another substantial difference compared with our study is the method of assessment of dosage need; we restricted the calculation of dosage to periods of stable anticoagulation, which results in less variability in the calculated dosages. Although the study of Visser et al. did show a trend toward a lower dosage need in carriers of a *CYP2C9* polymorphism, the combination of a lower sample size, a lower frequency of *CYP2C9*3* carriers, and the use of another method to assess dosage needs could explain the failure to demonstrate a significant effect. Their finding that there were no significant differences in overanticoagulation risks between genotypes could be because of the aforementioned power problem and the fact that overanticoagulation was only assessed during the first six weeks after the start of phenprocoumon in their study, whereas our study showed that the relationship with overanticoagulation is stronger after this first six-week period. The difference we found between *CYP2C9*2* and *CYP2C9*3* carriers in the time to achieve a first period of stability is interesting and unexpected. Although the dosage need does not seem to be different in *CYP2C9*2* and *CYP2C9*3* carriers (Table 4), the process of finding this dosage appears to be more difficult in *CYP2C9*2* carriers. The outcome parameter 'time to achieve a first period of stability' could at first sight seem of limited value, because of possible variations in the frequency of INR monitoring. However, we did not find substantial differences in the number of controls and the follow-up period between the genotypes (Table 1), and, moreover, we adjusted the calculated hazard ratios for differences in time between INR measurements. Because these adjustments did not substantially change the outcomes, 'time to achieve stability' appears to be a useful parameter for assessing differences in anticoagulation status in our study.

Our study has several limitations. Because we obtained medical data from anticoagulation clinics, it is possible that we did not have all relevant data on potentially destabilizing malignancies, heart failure, seriousness of infections, and vitamine K use. However, we could exclude subjects with potentially destabilizing liver insufficiency and thyroid dysfunctions from entry in our study. Comedication could be a confounder for severe overanticoagulation or time to achieve stability. We were able to eliminate the influence of inhibitors or inducers of CYP2C9, which we excluded from analysis. Another confounder might be the use of antibiotics. The three users of the antibiotic sulfamethoxazole-trimethoprim (cotrimoxazole), a strong CYP2C9 inhibitor,

were excluded from analysis.[34] Although some studies suggest an association between the use of antibiotics and severe overanticagulation,[35,36] such an association is not firmly established for all antibiotics. Besides, if an association exists, it will also depend on the seriousness of the infection and the time during which the antibiotic is used. Because details about the seriousness of infections and time of antibiotic use were lacking, we reanalysed our data for overanticoagulation and time to achieve stability after exclusion of all patients who used an antibiotic during the follow-up period. This exclusion did not result in essentially other outcomes with regard to overanticoagulation and time to achieve stability, which is probably a good indication that lack of information on infections did not influence our findings.

Recently, a possible influence of oral contraceptives on the CYP2C9 phenotype has been suggested, indicating that the use of oral contraceptives or postmenopausal hormonal treatment could have confounded our results.[37] After restriction of the users of these therapies, our results did not change, so this potential factor did not interfere as a confounder in our study.

In conclusion, our study shows that in phenprocoumon users the presence of at least one *CYP2C9*2* or *CYP2C9*3* allele is associated with an increased risk of severe overanticoagulation and a lower maintenance dosage. *CYP2C9*2* carriers have a lower chance to achieve a first period of stability within a period of six months after the start of phenprocoumon therapy.

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5.2

VKORC1 and *CYP2C9* genotypes and phenprocoumon anticoagulation status: interaction between both genotypes affects dose requirement

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ABSTRACT

Objective

Our objective was to assess the effects of *VKORC1* and *CYP2C9* genotypes on the anticoagulation status of patients during the initial six months of phenprocoumon treatment.

Methods

In a prospective follow-up study, we assessed the *CYP2C9* and the *VKORC1 C1173T* genotypes of patients initiating phenprocoumon. We used linear regression models and Cox proportional hazard models to determine the effects of the *VKORC1* and *CYP2C9* genotypes on phenprocoumon dose requirements, overanticoagulation, and time to achieve stability.

Results

Allele frequencies of interest within the cohort (n=281) were 40.8% *VKORC1* T-1173, 12.8% *CYP2C9*2*, and 6.9% *CYP2C9*3*. In patients with the *VKORC1 CC* genotype carriers of a *CYP2C9* polymorphism needed dosages that were nearly 30% lower than those for *CYP2C9*1/*1* patients ($p<0.001$). In patients with a *VKORC1* polymorphism, differences between carriers of a *CYP2C9* polymorphism and *CYP2C9*1/*1* were far smaller and largely not statistically significant. A larger part of the variability in dose requirement was explained by the *VKORC1* genotype than by the *CYP2C9* genotype (28.7% and 7.2%, respectively). Carriers of a combination of a *CYP2C9* polymorphism and a *VKORC1* polymorphism had a strongly increased risk of severe overanticoagulation (hazard ratio [HR] 7.20; $p=0.002$). Only carriers of a *CYP2C9*2* allele had a decreased chance to achieve stability compared to *CYP2C9*1/*1* patients (HR 0.61; $p=0.004$).

Conclusion

The *VKORC1* genotype modifies the effect of the *CYP2C9* genotype on phenprocoumon dose requirements. A combination of polymorphisms of both genotypes is associated with a strongly increased risk of overanticoagulation, whereas delayed stabilization is mainly associated with the *CYP2C9* genotype.

INTRODUCTION

Anticoagulants of the coumarin type are effective drugs for the treatment and prevention of thromboembolic diseases. However, these drugs have a narrow therapeutic range and show a large interindividual and intraindividual variability in dose requirement, which necessitates frequent monitoring of the anticoagulant effect and dosage adjustments. Known factors contributing to this variability are age, drug-drug interactions, ingestion of varying quantities of vitamin K, heart failure, infections, impairment of liver function,[1-5] and polymorphisms of the *CYP2C9* gene, which encodes for the main metabolizing enzyme of the coumarins.[6-10]

The presence of polymorphisms in the *VKORC1* gene has been recently identified as another source of variability in the response to coumarins. The enzyme vitamin K epoxide reductase (VKOR) reduces vitamin K 2,3-epoxide to the biologically active vitamin K hydroquinone, which catalyses the production of the blood-clotting proteins II, VII, IX, and X by carboxylation of glutamic acid residues. Coumarins interfere with this carboxylation by inhibiting VKOR through their recently identified target protein vitamin K reductase complex subunit 1 (VKORC1), which is encoded by the homonymous gene *VKORC1*.[11,12] In several studies, an association between the presence of polymorphisms of the *VKORC1* gene and a reduced dose requirement of warfarin,[10,13-20] acenocoumarol,[21-23] and phenprocoumon[23] has been demonstrated. Most of the studies that examined the effects of both the *VKORC1* and the *CYP2C9* genotypes showed that a larger part of the variation in dose requirement was explained by the *VKORC1* than by the *CYP2C9* genotype,[14,17,18,20-22] suggesting that the *VKORC1* genotype has a larger impact on the anticoagulation status. However, in a recent study in acenocoumarol users we demonstrated that being a carrier of a combination of variant alleles of *CYP2C9* and *VKORC1*, rather than of one variant allele, is associated with severe overanticoagulation, underlining the importance of both genotypes for the anticoagulation status.[21]

Associations between the *CYP2C9* genotype and the anticoagulation status have been convincingly demonstrated for warfarin and acenocoumarol,[6-10] carriers of a *CYP2C9*2* or *CYP2C9*3* allele requiring lower coumarin dosages and having an increased tendency to severe overanticoagulation and retarded stabilization. However, this *CYP2C9* sensitivity is less clear for phenprocoumon, a frequently used coumarin in European countries. Recently, we found an association

between possession of *CYP2C9*2* or **3* polymorphisms and a lower dose requirement, severe overanticoagulation, and delayed stabilization (for the **2* allele) in a Dutch population of phenprocoumon users,[24] whereas another Dutch study did not find such associations.[25] The clinical relevance of the findings of our study has been questioned[26] because only a minor impact of the *CYP2C9* genotype on phenprocoumon metabolism has been found in pharmacokinetic studies.[27-29] Of course, it might be possible that the results of our earlier phenprocoumon study have been confounded by the at-that-time unknown *VKORC1* genotype. In order to examine the effects of both the *VKORC1* and *CYP2C9* genotypes on phenprocoumon sensitivity and their relative contributions to dose requirements, we also assessed the *VKORC1 C1173T* polymorphisms in the same cohort of outpatients of two Dutch anticoagulation clinics.

METHODS

Study design and patients

This study was conducted in the same cohort of phenprocoumon-using outpatients in whom we earlier examined the association between *CYP2C9* genotype and anticoagulation status. For full details not described in this article, we refer to the report of our study previously published in this journal.[24]

In brief, the original study was a prospective follow-up study at two Dutch anticoagulation clinics in patients who started phenprocoumon therapy between October 2002 and July 2003. Exclusion criteria were hepatic dysfunction, thyroid disease, and use of pharmacokinetically interacting drugs at the start of phenprocoumon therapy. These data were retrieved from the medical files of the anticoagulation clinics. Pharmacokinetically interacting drugs were inhibitors of CYP2C9 like gemfibrozile, strong inhibitors of CYP3A4 like itraconazole, and inducers of liver enzymes like carbamazepine. They were identified by means of the Dutch Standard Management Coumarin Interactions, which is used as a reference by all Dutch anticoagulation clinics and pharmacies[30] and can also be found in the review by Harder and Thurmann.[31] The Medical Ethical Committee of the Leiden University Medical Centre approved our study.[24]

Data collection and follow-up period

We collected patients' characteristics and clinical data as recorded by the anticoagulation clinics in a database. The weekly dosage for each patient was

assessed from the dose schemes. For *VKORC1* genotyping, we used the samples in which we assessed the *CYP2C9* genotypes in our earlier study. The *VKORC1 C1173T* genotype was assessed in intron 1 of the *VKORC1* gene. This polymorphism appears to be as informative about warfarin sensitivity as five *VKORC1* haplotypes, which are predictive for the warfarin dose requirement and which together account for 96-99% of the total haplotypes in European-American Caucasian populations.[17]
Patients were followed up from the first date of phenprocoumon use (entry date) until the end of the observation period of maximally 180 days.

Genotyping

For *CYP2C9* genotyping, we refer to our previous study.[24] A LightCycler (Roche Diagnostics, Mannheim, Germany) assay was used for the detection of the *VKORC1 C1173T* polymorphism. During the melting curve analysis, the hybridization probes dissociate from the target DNA at specific melting temperatures. The presence of a C-allele introduces a destabilizing mismatch with the fluorescenting probes, which results in a decreased melting temperature. During setup, all LightCycler analyses (n=25; nine *CC*, eleven *CT*, and five *TT*) were compared with restriction fragment length polymorphism. Both methods of analysis were checked by testing externally obtained patient samples of known *VKORC1 C1173T* (*CC*, *CT*, and *TT*) genotype (courtesy of Wadelius et al., Uppsala, Sweden). The genotypes of the provided samples were established with minisequencing based on primer oligo base extension and matrix-assisted laser desorption/ionization-time of flight mass spectrometry. Comparison between LightCycler genotyping and restriction fragment length polymorphism samples showed completely concordant results.[21]
Assessment of presence or absence of the single-nucleotide polymorphism *C1173T* results in three different genotypes: *VKORC1 CC* (wild-type), *VKORC1 CT*, and *VKORC1 TT*.

Outcomes

The outcomes that were chosen to establish the effects of both *CYP2C9* and *VKORC1* genotypes on three representative parameters of coumarin sensitivity were:

1. Mean weekly phenprocoumon dosage during the first period of stability;
2. Severe overanticoagulation (defined as INR>6.0) during the observation period. An INR>6.0 is associated with a considerably increased major bleeding risk;[32,33]

3. The time to achieve a first period of stability, which was calculated as the time (in days from the entry date) until the first of three consecutive INR measurements within the therapeutic range, with a maximum difference between the mean daily dosages of 10%.

Calculations and statistical analysis

For assessment of deviations of allele frequencies from Hardy-Weinberg equilibrium, we used the χ^2 test.

For comparisons between genotypes, patients were divided into three categories for both *CYP2C9* and *VKORC1*. For the *CYP2C9* genotype, homozygous carriers of the *CYP2C9* wild-type allele (*CYP2C9*1/*1*) formed the reference group, the other two groups consisted of carriers of the *CYP2C9*2* and *CYP2C9*3* alleles. Because of the low prevalence of subjects carrying two allelic variants, heterozygous and homozygous subjects were included in the same genotype category. *CYP2C9*2/*3* subjects were allocated to the *CYP2C9*3* group, but we also analysed our outcomes after allocating *CYP2C9*2/*3* subjects to the *CYP2C9*2* group. Moreover, we reanalysed all our outcomes after having made a separate category of homozygous carriers of *CYP2C9* allelic variants (*CYP2C9*2/*2*, *CYP2C9*2/*3*, and *CYP2C9*3/*3*). For the *VKORC1 C1173T* genotype, homozygous carriers of the *VKORC1* wild-type allele (*VKORC1 CC*) formed the reference group, the other two groups consisted of patients with the *VKORC1 CT* and the *VKORC1 TT* genotype.

When we found an interaction between the *CYP2C9* and *VKORC1* genotypes, we compared all combined *CYP2C9–VKORC1* genotypes with patients who were homozygous carriers of the wild-type allele of both genotypes (*VKORC1 CC–CYP2C9*1/*1*) as a reference.

Mean dose requirements were calculated for patients who achieved stability during the follow-up period, because dose requirements in non stabilized patients are, by definition, less certain and possibly not representative for the definitive dose requirements after stabilization. To assess whether our results changed considerably if we included all patients, we compared the dose requirements in stabilized patients with the mean dose requirement in all patients from the 30th day after the entry date until the end of the follow-up period.

To assess differences in mean weekly phenprocoumon dosages during the first period of stability and percentage of variability explained by *VKORC1* and *CYP2C9* genotypes, we used linear regression modelling.

To compare our phenprocoumon dosages with the study that did not take the *CYP2C9* genotype into account, we recalculated a weighted mean for patients with the *VKORC1 CC*, *VKORC1 CT*, and *VKORC1 TT* genotypes by means of the following equation:

$$\text{Dose } VKORC1\ XX = (D_{VKORC1\ XX\text{ - CYP2C9*1/*1}} \times N_{CYP2C9*1/*1} / N_{VKORC1\ XX}) + (D_{VKORC1\ XX - CYP2C9*2} \times N_{CYP2C9*2} / N_{VKORC1\ XX}) + (D_{VKORC1\ XX - CYP2C9*3} \times N_{CYP2C9*3} / N_{VKORC1\ XX})$$

in which XX is *CC*, *CT* or *TT*; D is the dose; and N is the number of patients within the *VKORC1 XX* stratum.

We also determined the contributions of the *CYP2C9* and *VKORC1* genotypes, age, and sex to the phenprocoumon dose requirements, for which we used a linear regression model with these factors and with product terms between the *VKORC1* and *CYP2C9* genotypes. The adjusted mean R^2 value of the model explained the variability of the mean weekly dosage. Partial R^2 values for each of the contributing factors were assessed by a backward selection procedure.

To assess hazard ratios (HRs) of severe overanticoagulation and time to achieve stability, we used Cox proportional hazard modelling.

We examined effect modification by introducing product terms in our models between the *VKORC1* and *CYP2C9* genotypes and between each of these genotypes and other factors such as sex and age. In all models, we adjusted for the potential confounders age and sex and the comorbidity heart failure, which has been identified as an independent risk factor for severe overanticoagulation.[34]

To adjust for confounding comedication, the best strategy would have been to include potential interacting drugs as time-varying covariates in our models. However, files from anticoagulation clinics from which we retrieved our data did not provide reliable information on duration of comedication use. Therefore, we excluded all subjects who started using CYP2C9 inhibiting drugs during the follow-up time from our analyses.[30,31]

Moreover, we tested the robustness of our findings by reanalysing our outcomes after exclusion of those patients who used nonsteroidal anti-inflammatory drugs which are known CYP2C9 substrates and antibiotics, which can both contribute to overanticoagulation and instability.[3,35]

Statistical analyses were performed with the statistical software package SPSS 12 (version 12.0; SPSS Inc, Chicago, Ill, USA).

Table 1: CHARACTERISTICS OF PATIENTS TAKING PHENPROCOUMON TREATED BY TWO ANTICOAGULATION CLINICS (N=281)

Characteristic	*CYP2C9* genotype[a]			*VKORC1* genotype[b]		
	*CYP2C9*1/*1*	*CYP2C9*2*	*CYP2C9*3*	*VKORC1 CC*	*VKORC1 CT*	*VKORC1 TT*
Age; mean years (SD)	64.3 (15.8)	65.6 (16.1)	66.0 (15.0)	63.9 (15.0)	64.3 (15.8)	67.9 (16.7)
Maximum follow-up time; mean days (SD)	151 (35)	148 (40)	155 (34)	146 (41)	151 (35)	161 (24)
INR measurements; mean number (SD)	13.8 (3.8)	14.1 (3.9)	13.8 (3.7)	13.7 (3.7)	13.6 (3.8)	14.8 (3.9)
	n=183 (100%)	**n=61 (100%)**	**n=37 (100%)**	**n=106 (100%)**	**n=121 (100%)**	**n=54 (100%)**
Men	101 (55.2%)	36 (59.0)	19 (51.4)	56 (52.8)	70 (57.9)	30 (55.6)
Indication for phenprocoumon treatment						
atrial fibrillation	93 (50.8%)	37 (60.7%)	21 (56.8%)	52 (49.1%)	66 (54.5%)	33 (61.1%)
deep vein thrombosis	43 (23.5%)	11 (18.0%)	4 (10.8%)	21 (19.1%)	27 (22.3%)	10 (18.5%)
pulmonary embolus	37 (20.2%)	11 (18.0%)	8 (21.6%)	26 (24.5%)	21 (17.4%)	9 (16.7%)
other indications	10 (5.5%)	2 (3.3%)	4 (10.8%)	7 (6.6%)	7 (5.8%)	2 (3.7%)
Relevant comedication during follow-up						
NSAIDs[c]	12 (6.6%)	4 (6.6%)	8 (21.6%)	8 (7.5%)	11 (9.1%)	5 (9.3%)
antibiotics	34 (18.6%)	12 (19.7%)	11 (29.7%)	20 (18.9%)	27 (22.3%)	10 (18.5%)
Patients with congestive heart failure	18 (9.8%)	4 (6.6%)	4 (10.8%)	9 (8.5%)	12 (9.9%)	5 (9.3%)

NSAIDs = nonsteroidal anti-inflammatory drugs

a) Full details regarding *CYP2C9* genotype are as follows: *CYP2C9*1/*1*, n=183(65.1%); *CYP2C9*1/*2*, n=56 (19.9%); *CYP2C9*1/*3*, n=29 (10.3%); *CYP2C9*2/*2*, n=5 (1.8%); *CYP2C9*2/*3*, n=6 (2.1%); *CYP2C9*3/*3*, n=2 (0.7%); percent is percentage of total patients. Allele frequencies are as follows: **1*, 80.3%; **2*, 12.8%; and **3*, 16.9% (Hardy Weinberg χ^2= 0.93, p=0.82).

b) Allele frequencies are as follows: *C-1173* allele, 59.2%; *T-1173* allele, 40.8% (Hardy-Weinberg χ^2=3.29, p=0.070).

c) Only known CYP2C9 substrates have been included (in this cohort celecoxib, diclofenac, ibuprofen, meloxicam, and naproxen).[35]

Table 2: PHENPROCOUMON MEAN WEEKLY DOSES, OVERANTICOAGULATION (INR>6.0), DAYS UNTIL STABILIZATION AND STABILITY FOR COMBINATIONS OF *VKORC1* AND *CYP2C9* GENOTYPES

Combined genotype *VKORC1—CYP2C9*	N (100%)	INR>6.0 n (%)[a]	Patients stabilized n (%)[a]	Days until stabilization mean (SD)	Mean weekly dose (mg/week) mean (95% CI)
*CC—*1/*1*	63	3 (4.8)	55 (87.3)	71 (39)	22.4 (20.4–24.3)
*CC—*2*	27	4 (14.8)	19 (70.4)	81 (43)	15.4 (13.5–17.4)
**1/*2*	26	4 (15.4)	18 (69.2)	83 (44)	15.3 (13.3–17.3)
**2/*2*	1	0	1 (100)	52	17.0
*CC—*3*	16	4 (25.0)	11 (68.8)	51 (31)	15.6 (13.2–17.9)
**1/*3*	11	3 (27.3)	7 (63.6)	45 (26)	16.8 (13.6–20.0)
**2/*3*	4	1 (25.0)	3 (75.0)	69 (45)	12.7 (6.2–19.1)
**3/*3*	1	0	1 (100)	42	15.8
*CT—*1/*1*	95	12 (12.6)	83 (87.4)	62 (33)	16.4 (15.4–17.3)
*CT—*2*	16	5 (31.3)	9 (56.3)	78 (39)	15.0 (11.3–18.8)
**1/*2*	14	5 (35.7)	8 (57.1)	79 (41)	14.1 (10.6–17.6)
**2/*2*	2	0	1 (50.0)	74	22.5
*CT—*3*	10	0	8 (80.0)	49 (11)	14.4 (12.4–16.6)
**1/*3*	9	0	7 (77.8)	48 (12)	14.3 (11.6–17.0)
**2/*3*	1	0	1 (100)	56	14.7
*TT—*1/*1*	25	2 (8.0)	22 (88.0)	68 (40)	10.6 (9.1–12.2)
*TT—*2*	18	7 (38.9)	16 (88.9)	82 (41)	9.4 (7.0–11.8)
**1/*2*	16	6 (37.5)	14 (87.5)	78 (38)	9.8 (7.2–12.5)
**2/*2*	2	1 (50.0)	2 (100)	104	6.5
*TT—*3*	11	4 (36.4)	11 (100)	63 (34)	8.5 (6.4–10.6)
**1/*3*	9	4 (44.4)	9 (100)	69 (35)	8.1 (5.8–10.5)
**2/*3*	1	0	1 (100)	31	13.2
**3/*3*	1	0	1 (100)	43	7.4

a) Percent refers to the percentage of patients with the corresponding combined genotype.

RESULTS

Of the 284 patients we analysed in our earlier study,[24] three had no blood or DNA samples left for analysis. The remaining 281 patients were all available for analysis (Table 1).

Allele frequencies of *VKORC1 C-1173* and *VKORC1 T-1173* were 59.2% and 40.8%, respectively. Allele frequencies of *CYP2C9*1*, *CYP2C9*2*, and *CYP2C9*3* were 80.3%, 12.8%, and 6.9%, respectively. Allele frequencies of both genotypes were in Hardy-Weinberg equilibrium (Table 1).

For combined *VKORC1* and *CYP2C9* genotypes, numbers of patients within each combination of genotypes, mean weekly doses, numbers of patients with severe overanticoagulation, numbers of patients in whom stability was achieved, and number of days until stability was achieved are summarized in Table 2.

The *CYP2C9* and *VKORC1* genotypes modified each other's effects on dose requirements. In our regression models, we found a statistical interaction between the *CYP2C9* and *VKORC1* genotypes, p-values for two of the product terms in our regression model being lower than 0.05. In carriers of a *CYP2C9*2* or *CYP2C9*3* allele with the *VKORC1 CC* genotype, the weekly dosages were considerably and significantly lower than in *VKORC1 CC–CYP2C9*1/*1* patients (point estimates of the percentages of dose reduction compared to *CYP2C9*1/*1*: 27.7% for *CYP2C9*2* and 28.1% for *CYP2C9*3*). However, if patients had also the *VKORC1 CT* or *VKORC1 TT* genotype, the differences between carriers of a *CYP2C9*2* or *CYP2C9*3* allele and *CYP2C9*1/*1* patients were far smaller and generally not statistically significant. Only in patients with the *VKORC1 TT* genotype, the difference between *CYP2C9*1/*1* and *CYP2C9*3* patients was marginally significant (Table 3). The combination of *VKORC1* genotype, *CYP2C9* genotype, interaction between both genotypes, age, and sex explained 54.7% of the variation in mean weekly dosage, adjusted mean R^2 being 28.7% for *VKORC1* genotype, 14.1% for age, 7.2% for *CYP2C9* genotype, 1.6% for interaction between *VKORC1* and *CYP2C9* genotypes, and 0.8% for sex.

Analysis of the mean dose during the entire follow-up period from the 30th day after entry as a measure for dose requirement did not result in other insights. The explained percentage of variation in mean weekly dosage as well as the adjusted dosage differences between combined genotypes were fully comparable with the differences we found for stabilized patients and statistical significance was

maintained for all found differences and for the product terms between the *CYP2C9* and *VKORC1* genotypes (data not shown).

Table 3: DOSE DIFFERENCES IN PHENPROCOUMON FOR COMBINED *VKORC1* AND *CYP2C9* GENOTYPES[a,b,c]

Genotype[d]	N	Adjusted difference (mg/week) mean (95% CI)[e]	Adjusted difference (%)
*VKOR CC – CYP2C9*1/*1*	55	reference, 22.4 mg/week	reference
*VKOR CC – CYP2C9*2*	19	– 6.2 (– 8.6;– 3.9)	–27.7
*VKOR CC – CYP2C9*3*	11	– 6.3 (– 9.1;– 3.4)	–28.1
*VKOR CT – CYP2C9*1/*1*	83	– 6.0 (– 7.6;– 4.5)	–26.8
*VKOR CT – CYP2C9*2*	9	– 7.4 (–10.6;– 4.3)	–33.0
*VKOR CT – CYP2C9*3*	8	– 7.7 (–11.0;– 4.4)	–34.3
*VKOR TT – CYP2C9*1/*1*	22	–11.1 (–13.3;– 8.9)	–49.6
*VKOR TT – CYP2C9*2*	16	–12.4 (–15.0;–10.0)	–55.4
*VKOR TT – CYP2C9*3*	11	–13.6 (–16.5;–10.8)	–60.7

a) Mean dose (in mg/week) of stabilized patients (if stability was not achieved, no mean dose was computed).
b) P-values for interaction between genotypes: *VKORC1 CT* x *CYP2C9*2*, 0.012; *VKORC1 CT* x *CYP2C9*3*, 0.059; *VKORC1 TT* x *CYP2C9*2*, 0.008; and *VKORC1 TT* x *CYP2C9*3*, 0.062, in which *VKORC1 CT*, *VKORC1 TT*, *CYP2C9*2* and *CYP2C9*3* are dummies in the linear regression equation: mean phenprocoumon dose=a + b.*VKORC1 CT* + c.*VKORC1 TT* + d.*CYP2C9*2* + e.*CYP2C9*3* which was used to assess an interaction between the *VKORC1* and *CYP2C9* genotypes, and in which *a* is the intercept and *b-e* are coefficients of the separate factors in the equation.
c) Raw differences are not shown, because they were very similar to the adjusted differences.
d) P-values for other comparisons: within *CYP2C9*2* stratum difference between *VKORC1 CC* and *VKORC1 CT*, p=0.41; and difference between *VKORC1 CC* and *VKORC1 TT*, p<0.001. Within *CYP2C9*3* stratum difference between *VKORC1 CC* and *VKORC1 CT*, p=0.21; and difference between *VKORC1 CC* and *VKORC1 TT*, p<0.001. Within *VKORC1 CT* stratum there were no significant differences between *CYP2C9*1/*1* and *CYP2C9*2*, p=0.42 or *CYP2C9*3*, p=0.22. Within *VKORC1 TT* stratum difference between *CYP2C9*1/*1* and *CYP2C9*2*, p=0.53; and difference between *CYP2C9*1/*1* and *CYP2C9*3*, p=0.036.
e) All found differences were statistically significant (p<0.001). Dose requirements were adjusted for differences in heart failure, sex and age.

For the outcome severe overanticoagulation, we found no statistical interaction between the *VKORC1* and *CYP2C9* genotypes. After adjustment for potential confounders, including *VKORC1* genotype, carriers of a *CYP2C9*2* or **3* allele had a significantly increased risk of severe overanticoagulation compared to subjects with the *CYP2C9*1/*1* wild-type. If both alleles were considered separately, the risk was only significantly increased in *CYP2C9*2* carriers (p=0.001), whereas there was a strong trend in *CYP2C9*3* carriers (p=0.060). Patients with the *VKORC1 TT* genotype had a significantly increased risk of

severe overanticoagulation compared to *VKORC1 CC* patients, whereas this risk was not significantly increased in patients with the *VKORC1 CT* genotype (Table 4). If combined *VKORC1–CYP2C9* genotypes were considered, the risk for severe overanticoagulation was most strongly increased in patients with a combination of *CYP2C9*2* or *CYP2C9*3* with either *VKORC1 CT* or *VKORC1 TT* compared to patients with no polymorphism of *VKORC1* or *CYP2C9* (*VKORC1 CC–CYP2C9*1/*1*) (Table 4, Figure 1).

Table 4: ASSOCIATION BETWEEN SEVERE OVERANTICOAGULATION (INR>6.0) AND *CYP2C9* AND *VKORC1* GENOTYPES AND COMBINED *CYP2C9* AND *VKORC1* GENOTYPE[a]

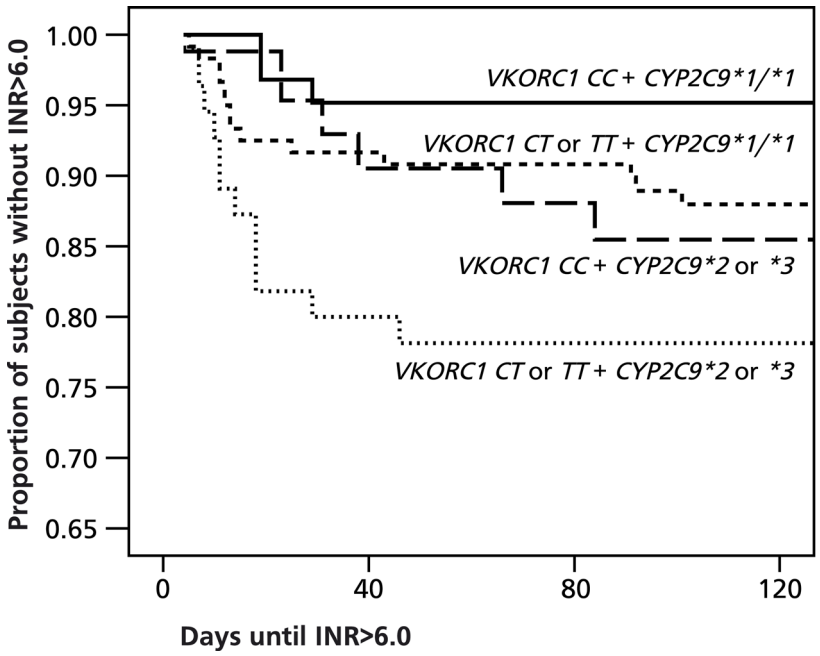
Genotype	Adjusted HR (95% CI)	P-value
Separate genotypes		
*CYP2C9*1/*1*	1 (reference)	
*CYP2C9*2* or **3*	3.02 (1.62–5.65)[b]	0.001[e]
*CYP2C9*2*	3.37 (1.68–6.75)[b]	0.001[e]
*CYP2C9*3*	2.26 (0.96–5.30)[b]	0.060
VKORC1 CC	1 (reference)	
VKORC1 CT or *TT*	1.92 (0.96–3.83)[c]	0.067
VKORC1 CT	1.69 (0.79–3.64)[c]	0.18
VKORC1 TT	2.28 (1.02–5.10)[c]	0.045[e]
Combined genotypes		
*VKORC1 CC – CYP2C9*1/*1*	1 (reference)	
*VKORC1 CC – CYP2C9*2* or **3*	4.56 (1.20–17.3)[d]	0.026[e]
*VKORC1 CT or TT – CYP2C9*1/*1*	2.72 (0.78–9.49)[d]	0.12
*VKORC1 CT or TT – CYP2C9*2* or **3*	7.20 (2.10–24.7)[d]	0.002[e]

HR = hazard ratio
a) Raw differences are not shown, because they were very similar to the adjusted differences.
b) Adjusted for differences in *VKORC1* genotype, heart failure, sex, and age.
c) Adjusted for differences in *CYP2C9* genotype, heart failure, sex, and age.
d) Adjusted for differences in heart failure, sex, and age.
e) Statistically significant difference ($p<0.05$).

We also found no statistical interaction between the *VKORC1* and *CYP2C9* genotypes for time to achieve stability. Within the *CYP2C9* genotype, we found differences in time to achieve stability; within the *VKORC1* genotype, we did not find any differences (Figure 2). In patients with a *CYP2C9*2* allele, the chance to achieve stability within the follow-up period was significantly decreased compared to *CYP2C9*1/*1* patients (adjusted HR 0.61; 95% CI 0.43-0.86; p=0.004), whereas we found no changed risk for *CYP2C9*3* patients

(adjusted HR 1.01; 95% CI 0.68-1.49; p=0.98) (Figure 2A). In patients with the *VKORC1 CT* or *VKORC1 TT* genotype, the chance to achieve stability was not significantly different from *VKORC1 CC* patients (adjusted HR 1.01; 95% 0.75-1.36; p=0.95 for *VKORC1 CT* and 1.16; 95% CI 0.81-1.68; p=0.42 for *VKORC1 TT*) (Figure 2B).

Figure 1: KAPLAN-MEIER SURVIVAL CURVES FOR TIME TO SEVERE OVER-ANTICOAGULATION

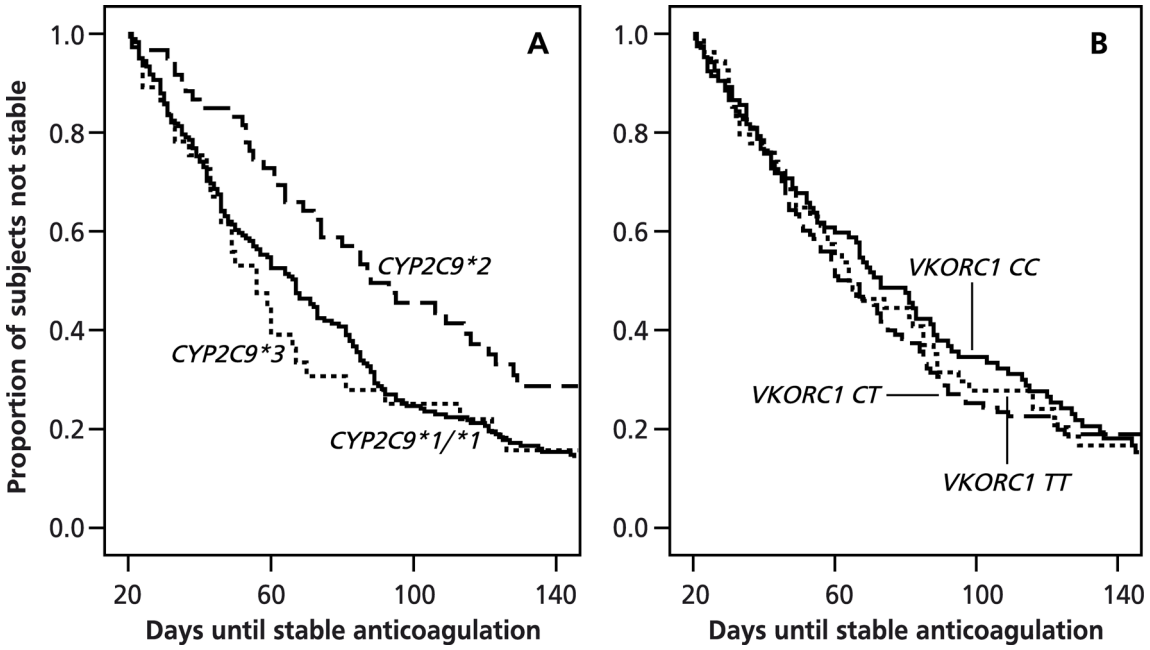


Hazard Ratio (adjusted for differences in age, sex and heart failure) of *VKORC1 CT* or *VKORC1 TT* + *CYP2C9*2* or **3* versus *VKORC1 CC* + *CYP2C9*1/*1* was 7.20, p=0.002.

Analysis of our data after allocating patients with the *CYP2C9*2/*3* genotype to the *CYP2C9*2* group (instead of the *CYP2C9*3* group) did not change our results (data not shown).

Analysis of our data with a separate group of homozygous carriers of two *CYP2C9* variant alleles (*CYP2C9*2/2*, *CYP2C9*2/*3*, and *CYP2C9*3/*3*) also did not change our results. Point estimates for homozygous carriers were generally similar to those for the heterozygous carriers of a wild-type and a variant allele, in most cases with loss of clinical significance as a consequence of low numbers in this group.

Figure 2: KAPLAN-MEIER SURVIVAL CURVES FOR TIME TO ACHIEVE FIRST PERIOD OF STABILITY, PLOTTED FOR THE *CYP2C9* GENOTYPE (**A**) AND THE *VKORC1* GENOTYPE (**B**)



A) Hazard Ratio (HR), adjusted for differences in age, sex, heart failure, and *VKORC1* genotype, of *CYP2C9*2* versus *CYP2C9*1/*1* was 0.61, p=0.004.

B) There were no significant differences between *VKORC1 CC*, *CT* and *TT:* HR of *VKORC1 CT* versus *VKORC1 CC* was 1.01, p=0.95; and of *VKORC1 TT* versus *VKORC1 CC* was 1.16, p=0.42.

Exclusion of all users of nonsteroidal anti-inflammatory drugs and antibiotics did not change our results considerably. The outcomes for dosage and time to achieve stability remained the same, without any loss of significance. For the outcome severe overanticoagulation, significance for the risk in carriers of the *CYP2C9*2* allele compared to *CYP2C9*1/*1* wild-type patients was lost (HR 2.36; p=0.072), whereas significance was just achieved for the risk in carriers of a *CYP2C9*3* allele (HR 3.46; p=0.016). For the combined *VKORC1–CYP2C9* genotypes, significance for the increased risk in *VKORC1 CC–CYP2C9*2* or *3 patients compared to *VKORC1 CC–CYP2C9*1/*1* patients was lost (HR 2.70; p=0.20), but the risk in patients with a *VKORC1* and a *CYP2C9* polymorphism was even more strongly increased than in our main cohort (HR 8.68; p=0.005) (further data not shown).

DISCUSSION

The results of our study, in which we evaluated the effects of *VKORC1* as well as *CYP2C9* genotypes on the anticoagulation status of patients taking phenprocoumon, strongly suggest that the association between possession of a variant *CYP2C9* allele and a decreased mean weekly dose requirement we found in our earlier study within this cohort[24] is modified by the *VKORC1* genotype.

The allele frequencies we found for the C-1173 allele (59.3%) and the T-1173 allele (40.8%) were in accordance with other studies in several populations, T-1173 allele frequencies varying from 39.1% to 45.8%.[15,21,22,36]

Concerning the lower maintenance dose in carriers of a *VKORC1* polymorphism, our findings are in good agreement with the study of Reitsma et al.,[23] the only other study that examined the association between *VKORC1 C1173T* genotype and phenprocoumon anticoagulation. Their main findings were an increased risk of bleeding and decreased dose requirement in carriers of at least one *T* allele compared to *VKORC1 CC* patients. In contrast to our study, Reitsma et al.[23] did not take the *CYP2C9* genotype into account. However, it is possible to compare our results with Reitsma et al.[23] if we calculate the weighted mean of the daily phenprocoumon dosages of patients with the *VKORC1 CC*, *VKORC1 CT*, and *VKORC1 TT* genotypes, adjusting for the frequencies of the *CYP2C9* genotypes. These recalculations result in the following dose requirements in stabilized patients: *VKORC1 CC* 2.9 mg/day (Reitsma et al.[23] 2.9 mg/day), *VKORC1 CT* 2.3 mg/day (Reitsma et al.[23] 2.6 mg/day), *VKORC1 TT* 1.5 mg/day (Reitsma et al.[23] 1.4 mg/day). Our recalculated mean dose requirements for the different *VKORC1* genotypes are in remarkable agreement with those of Reitsma et al.,[23] corroborating a decreased phenprocoumon dose requirement in patients with a *VKORC1* polymorphism compared to *VKORC1* wild-type patients.

Compared to *CYP2C9* wild-type patients, carriers of a *CYP2C9*2* or **3* allele needed considerably lower doses if they also had the *VKORC1 CC* genotype. However, if patients had the *VKORC1 CT* or *TT* genotype, differences in dose requirement between *CYP2C9* wild-type patients and carriers of a *CYP2C9*2* or **3* allele were much smaller and not in all cases statistically significant. In fact the *CYP2C9*1/*1* → *CYP2C9*2* or **3* shift within the *VKORC1 CC* stratum has about the same impact as the *VKORC1 CC* → *VKORC1 CT* shift in general (Table 3). The finding that the impact of being a carrier of a variant allele of *CYP2C9* on phenprocoumon dose requirement changes with the *VKORC1*

genotype has not been reported in similar studies with the other coumarins warfarin[13-20] and acenocoumarol.[21,22] A possible explanation for this remarkable effect modification could be that the sensitivity to the (S)- and (R)-enantiomers of phenprocoumon changes with the *VKORC1* genotype. Like the other coumarins, phenprocoumon is a racemic mixture of a (S)- and a (R)-enantiomer, (S)-phenprocoumon being biologically more active (1.6 to 2.6 ×) than (R)-phenprocoumon.[37] The impact of the *CYP2C9* genotype on clearance is far greater for (S)-phenprocoumon than for (R)-phenprocoumon. Although *CYP2C9* polymorphisms reduce the metabolism of (S)-phenprocoumon considerably, the overall metabolism of (both (S)- and (R)-) phenprocoumon depends less on the *CYP2C9* genotype than the overall metabolism of acenocoumarol or warfarin.[27,29] So, the more (S)-phenprocoumon contributes to the overall anticoagulant activity, the greater the role of the *CYP2C9* genotype. It is possible that (S)- and (R)-phenprocoumon display larger differences in anticoagulant activity for the VKORC1 target protein produced by *VKORC1* wild-type patients than for the VKORC1 target protein produced by *VKORC1 CT* or *TT* patients. We are aware that this explanation is speculative and has to be tested in pharmacodynamic studies. Of course, we also have to consider the possibility that the effect modification we found was a chance finding, because our results are derived from a reanalysis of a data set in which we did not define *a priori* that we wanted to test for an interaction between genotypes. So, confirmation of our findings in an independent data set is warranted.

Few studies have examined the association between the phenprocoumon anticoagulation status and the *CYP2C9* genotype. The pharmacokinetic studies by Kirchheiner et al.[29] and Ufer et al.[27] were conducted in healthy volunteers and demonstrated a more limited role for the enzyme CYP2C9 in the overall elimination of (S)- and (R)-phenprocoumon than in the elimination of warfarin and acenocoumarol. However, their finding that the elimination of (S)-phenprocoumon is considerably reduced in carriers of *CYP2C9* polymorphism supports the significant dose differences we found between carriers of a *CYP2C9*2* or **3* allele and *CYP2C9*1/*1* patients with the *VKORC1 CC* genotype. In two studies of Hummers-Pradier et al.[38] and Visser et al.,[25] no significant differences in dose requirements between patients with different *CYP2C9* genotypes were found. In contrast, we found significant differences in dose requirements between carriers of a *CYP2C9*2* or **3* allele and *CYP2C9* wild-type patients in our earlier study, which was conducted within the same population as this one.[24] Our results strongly suggest that the contribution to

differences between *CYP2C9* genotypes is mainly provided by patients with the *VKORC1 CC* genotype. In Caucasian populations, the percentage of patients who have the *VKORC1 CC* genotype is smaller than 40% (in our study 37.7%), which means that overall differences between *CYP2C9* genotypes will only be found in large populations if the *VKORC1* genotype is not taken into account. As a consequence, the larger number of patients in our study compared to the studies of Visser et al.[25] and Hummers-Pradier et al.[38] seems a plausible explanation for the apparent discrepancies between our study and theirs. The explained variability in dose requirement by the combination of *VKORC1*, *CYP2C9* genotype, age, and several other factors is in accordance with other studies in users of warfarin,[16,18,22] as is our finding that the *VKORC1* genotype explains a larger part of the dose variability than the *CYP2C9* genotype.[17,18,21,22] In contrast with a study we recently conducted in acenocoumarol users,[21] we found no modification of the association between the *CYP2C9* genotype and severe overanticoagulation by the *VKORC1* genotype. The risks of having *CYP2C9* and *VKORC1* variant alleles seem to be additive rather than multiplicative. This indicates that coumarins differ in their sensitivities to combinations of *VKORC1* and *CYP2C9* genotypes, which is in itself not surprising in view of the earlier noticed differences in *CYP2C9* sensitivities.

In this study, we only found an association between being a carrier of the *CYP2C9*2* allele and a decreased chance to achieve stability compared with *CYP2C9*1/*1* subjects. This indicates that the process of finding the right dose requirement is most difficult in *CYP2C9*2* carriers. That the search for a stable phenprocoumon dose regimen is more associated with the *CYP2C9* genotype than with the *VKORC1* genotype is in agreement with our findings in another study we recently conducted in acenocoumarol users.[21]

Some limitations of our study have to be considered. Because we only had medical data from anticoagulation clinics, we could have missed relevant data about comorbidities and comedication. However, we were able to exclude subjects with potentially destabilizing hepatic dysfunction and thyroid disease from entry in our study, which has enhanced the homogeneity of our cohort. A second limitation is the lack of knowledge about the duration of potentially confounding comedication use. However, we were able to eliminate users of CYP2C9 inhibiting drugs from analysis. Moreover, reanalysis of all outcomes after exclusion of all incident users of antibiotics and NSAIDs during follow-up did not result in essentially other outcomes.

In conclusion, our study shows that in phenprocoumon users the differences in dose requirements between patients with different *CYP2C9* genotypes are modified by the *VKORC1* genotype, that differences between carriers of a *CYP2C9*2* or **3* allele and patients with the *CYP2C9*1/*1* genotype are mainly relevant in *VKORC1 CC* patients and that the *VKORC1* genotype explains a larger part of the dose variability than the *CYP2C9* genotype. Overanticoagulation is most strongly associated with possession of polymorphisms of *VKORC1* and *CYP2C9*, whereas time to achieve stability is only associated with the *CYP2C9* genotype. These results suggest that preceding knowledge of both *VKORC1* and *CYP2C9* genotypes could contribute to a safer treatment with phenprocoumon.

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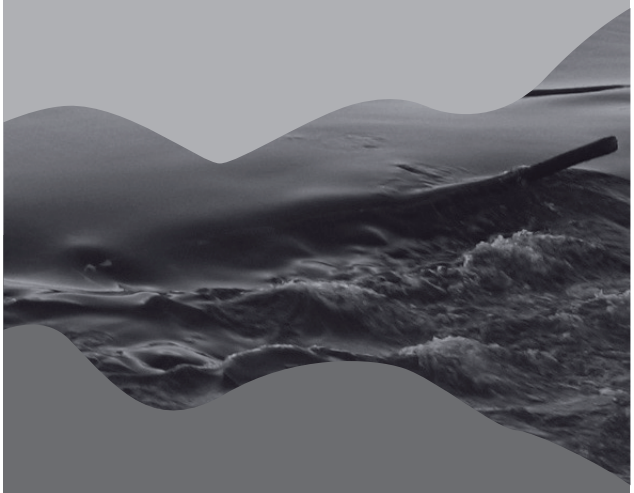
CHAPTER 6



COST-EFFECTIVENESS OF GENOTYPING

6.1

CYP2C9 genotyping in acenocoumarol treatment: is it a cost-effective addition to international normalized ratio monitoring?

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ABSTRACT

Objective

Our objective was to analyse the cost of preventing major bleeding episodes by *CYP2C9* genotyping in acenocoumarol-using outpatients, monitored at Dutch anticoagulation clinics.

Methods

We designed a decision analytic model in which a hypothetical cohort of acenocoumarol-starting outpatients of 55 years or older was followed during 12 months. We evaluated two possible outcomes (bleeding and no bleeding) and two strategies: (1) no genotyping, and (2) *CYP2C9* genotyping prior to or shortly after initiating acenocoumarol therapy. We used a third party payer perspective. The probabilities of bleeding, prevalence of bleeding, and data on *CYP2C9* polymorphisms were based on results of a number of studies in Dutch anticoagulation clinics. Costs of genotyping, treatment of major bleedings, and monitoring at anticoagulation clinics were derived from Dutch economic studies and organizations. We performed sensitivity analyses for incidence rate of major bleedings, possible reduction of major bleeding rate, difference in bleeding rate between wild-type subjects and carriers of a polymorphism, and additional INR-measurements. Our main outcome measure was the cost to prevent one major bleeding by *CYP2C9* genotyping, assuming that *CYP2C9* genotyping could result in a reduction of the bleeding risk in carriers of a *CYP2C9* polymorphism.

Results

The marginal cost-effectiveness of genotyping (cost to avoid one major bleeding) would vary from dominance (cheaper compared to no genotyping) to Euro 4233 if *CYP2C9* genotyping costs Euro 30 to Euro 55 and if prior knowledge of the *CYP2C9* genotype results in a 20% reduction of the incidence rate of major bleeding. Sensitivity analysis revealed that our model was sensitive to the extent of reduction of the major bleeding rate in carriers of an identified *CYP2C9* polymorphism. Our model was also sensitive to the incidence rate of major bleedings, to the relative risk of major bleeding in carriers of a *CYP2C9* polymorphism compared to wild-type patients, and to the cost of treatment of major bleeding. Selection of candidates for *CYP2C9* genotyping on the basis of their initial INR (>2.5) could further reduce the cost to prevent one major bleeding (to Euro 2210 for the base case).

Conclusion

The cost-effectiveness of *CYP2C9* genotyping in acenocoumarol users depends on several factors. In some circumstances *CYP2C9* genotyping could be cost-effective. However, for a definitive assessment of the cost-effectiveness of *CYP2C9* genotyping, prospective studies to assess the effect of genotyping on the reduction of major bleeding are urgently needed.

We conducted a cost-effectiveness study of which the results has been worked up into a commentary, in which the separate factors affecting cost-effectiveness could be discussed more extensively.

INTRODUCTION

Oral anticoagulants of the coumarin type are effective for the treatment and prevention of thromboembolic events. However, these drugs have a very narrow therapeutic window, and their pharmacodynamic effect can vary inter-individually, as well as intraindividually, over time. There are several factors that can contribute to the variability in dose requirement: drug interactions; comorbidities, such as deteriorating heart failure, hepatic insufficiency, or thyroid dysfunction; infections; and variable vitamin K intake.[1-4] During recent years, the *CYP2C9* genotype has been recognized as an important source of variability.

The *CYP2C9* gene encodes the cytochrome P450 (CYP) isozyme CYP2C9, which is the main enzyme catalyzing the metabolism of the more active (S)-enantiomer of the therapeutically used coumarins warfarin, acenocoumarol, and phenprocoumon.[5,6] Several studies convincingly demonstrated that being carrier of at least one *CYP2C9*2* or *CYP2C9*3* allele is associated with an increased risk of overanticoagulation and a decreased coumarin dose requirement. Most of these studies have been conducted with warfarin,[7-10] but similar effects have been demonstrated in users of phenprocoumon[11] and acenocoumarol.[12-14] Moreover, in several studies among coumarin users possession of at least one variant allele has been associated with an increased bleeding risk.[8,9,15,16] In most of these studies *CYP2C9* genotyping preceding coumarin treatment is suggested as a means to identify patients with an increased risk of overanticoagulation or bleeding, suggesting that genotyping can be useful in reducing this major complication of coumarin therapy. However, coumarin therapy is usually frequently monitored by assessing International Normalized Ratios (INRs), in many countries by specialized anticoagulation clinics. As a consequence, the cost-effectiveness of relatively expensive *CYP2C9* genotyping preceding frequent INR monitoring cannot be taken for granted. Recently, You et al.[17] studied the economic consequences of *CYP2C9* genotyping preceding initiation of warfarin therapy. This study offered interesting insights into the potential cost-effectiveness of *CYP2C9* genotyping in warfarin users.

Since the study of You et al.,[17] new data have been provided about the increased bleeding risk in acenocoumarol-using carriers of *CYP2C9*2* or *CYP2C9*3* alleles by Visser et al.[16] Moreover, in an earlier study we have demonstrated that, in acenocoumarol users, the first INR, assessed on the fourth day after a starting dose of 6, 4, and 2 mg on the first three days, was increased in carriers of at least one *CYP2C9*3* allele.[14] Selection of patients based on their first INR could

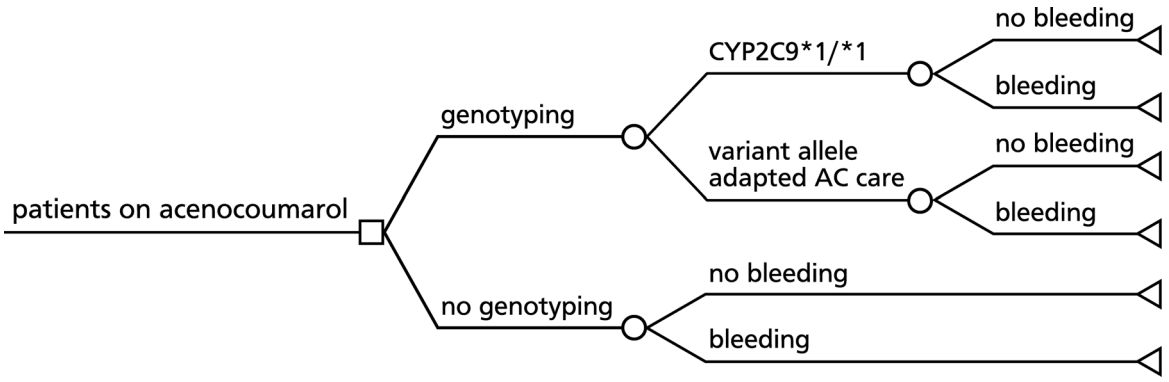
therefore increase the percentage of carriers of a *CYP2C9* polymorphism. So it is possible that a selection of suitable candidates for *CYP2C9* genotyping could improve cost-effectiveness.

With these recent data in mind, it is interesting to speculate further on the cost-effectiveness of *CYP2C9* genotyping in acenocoumarol therapy and to consider whether there are scenarios in which *CYP2C9* genotyping could be a useful addition to INR monitoring.

ANALYSING COST-EFFECTIVENESS OF CYP2C9 GENOTYPING IN COUMARIN USERS

The cost-effectiveness of *CYP2C9* genotyping in coumarin users should be focused on the possibility to prevent major bleeding, because an increased risk of major bleeding is the only clinically important outcome that has hitherto been described in coumarin using carriers of a *CYP2C9*2* or *CYP2C9*3* allele.[8,9,15,16]

Figure 1: COST-EFFECTIVENESS MODEL



AC = anticoagulation clinic
Results presented in tables 2, 3, and 4 were calculated with this decision analytic model.

To evaluate the economic outcomes of *CYP2C9* genotyping, a decision analytic model as depicted in Figure 1 may be used. Two strategies are represented in this model: (1) no genotyping (nongenotyped group) and (2) *CYP2C9* genotyping preceding or shortly after initiation of acenocoumarol (genotyped group). For both strategies, the two possible outcomes are bleeding and no bleeding. With this model, the marginal cost-effectiveness can be calculated by dividing the

difference of the costs of the genotyping strategy (Cg) and the no genotyping strategy (Cng) by the difference of the number of bleedings in genotyped (Eg) and nongenotyped (Eng) patients, according to the following formula:

$$\text{Marginal cost-effectiveness} = (Cg - Cng) / |Eg - Eng|$$

This marginal cost-effectiveness is the cost to avoid one major bleeding episode by *CYP2C9* genotyping and is the main outcome of interest. It consists of the cost of *CYP2C9* genotyping, the cost of INR monitoring by anticoagulation clinics, the additional number of INR measurements needed for adapting care in carriers of a *CYP2C9* allele, the cost of acenocoumarol tablets for one year, and the cost of treatment of major bleeding. If the marginal cost-effectiveness of genotyping is indicated as dominant, the genotyping strategy is cheaper than the no genotyping strategy.

Because no association has been reported between possession of *CYP2C9* polymorphisms and thromboembolic risk, it is not necessary to include thromboembolic events in the decision analytic model as You et al.[17] did.

Base case and sensitivity analysis

In assessing cost-effectiveness of a strategy – in our case *CYP2C9* genotyping – it is customary to analyse a base case and to perform sensitivity analyses. A 'base case' is defined as the scenario with the best possible estimations of the parameters in the model, derived from the literature or from other sources, such as anticoagulation clinics in our case. Because models can be very sensitive to relatively small changes in parameters and because it might be difficult to make a best possible estimation of parameters from the literature, sensitivity analyses are a regular part of cost-effectiveness studies. Sensitivity analyses are performed by varying the values of parameters in the model over certain ranges, with determination of the limits of such ranges being based on literature data or logical reasoning.

Base case example: a Dutch setting of acenocoumarol-using outpatients

As a base case example, we have worked out our model for a setting of Dutch anticoagulation clinics. Since the 1950s, a dense network of anticoagulation clinics ('thrombosis services') has provided care for coumarin using outpatients. These anticoagulation clinics are mostly supervised by physicians who have developed experience in coumarin dosing on the basis of INR measurements that are being performed with a frequency of a few days until, maximally, six weeks, depending on the achieved stability and on whether an INR measurement is within or outside the target therapeutic range. For dose

adjustments, computerized programs are available at all anticoagulation clinics in which individual histories of earlier coumarin doses, INR measurements, and clinical events such as bleedings are recorded.
In this setting coumarin users are being intensively monitored on the basis of INR values.

Parameters in the decision analytic model

In Table 1 we have summarized the parameters that we have used in our base case example as well as the ranges for sensitivity analysis. Later in the text, we give a short description of each of these parameters; their consequences for cost-effectiveness of *CYP2C9* genotyping will be discussed in depth in the next section of this article.
All costs are expressed in Euros, reindexed to the year 2004. All analyses were performed with DATA 3.5 software (Treeage Software, Williamstown, Massachusetts, USA).

Prevalence of the CYP2C9 polymorphisms CYP2C9*2 and CYP2C9*3. The *CYP2C9* polymorphisms that have been studied thus far in coumarin users are *CYP2C9*2* and *CYP2C9*3*. For our base case example, we derived the prevalence of these polymorphisms from three Dutch studies in users of acenocoumarol and phenprocoumon, which included 1591 subjects[11,13,14]: the mean prevalence of carriers of at least one *CYP2C9*2* or *CYP2C9*3* allele was 36%. Moreover, we worked out a scenario in which the percentage of carriers of a *CYP2C9* polymorphism was increased by selection based on their first INR. We demonstrated that, in acenocoumarol users, the first INR, assessed on the fourth day after initiation with a starting dose of 6, 4, and 2 mg on the first three days, was increased in carriers of at least one *CYP2C9*3* allele.[14] In this study 51.5% of the subjects had an initial INR greater than 2.5, with the prevalence of carriers of a polymorphism being 0.44.

Incidence rate of major bleeding. For our base case example, we used the incidence rate of major bleeding events in acenocoumarol users reported by Visser et al.[16] Bleedings were defined as major if these resulted in death, hospitalization, blood transfusion, or surgery. Intracranial, intra-articular, and intramuscular bleeding events were also classified as major. The incidence rate of major bleeding in acenocoumarol users with the *CYP2C9* wild-type found in this study was 4.16 per 100 patient-years, and the incidence rate of major bleeding in carriers of a *CYP2C9* polymorphism was 6.86 per 100 patient-years.

Table 1: SURVEY OF PARAMETERS IN DECISION ANALYTIC MODEL AND COSTS

Factor	Base case	Range for sensitivity analysis	Reference
Prevalence of *CYP2C9* polymorphisms **2* and **3*	0.36	—	11,13,14 [a]
Prevalence of *CYP2C9* polymorphisms **2* and **3* after selection of genotyping candidates based on first INR on day 4	0.44	—	14 [b]
Incidence rate of major bleeding in *CYP2C9*1/*1* patients (number / 100 patient-years)	4.16[c]	2.00-6.00 [d]	16
Incidence rate of major bleeding in carriers of a *CYP2C9* polymorphism (number / 100 patient-years)	6.8 [e]	—	16
Relative risk of major bleeding in carriers of *CYP2C9* polymorphism compared to wild-type patients	1.649	1.05-2.2 [f]	—
Reduction of incidence rate of major bleedings in carriers of *CYP2C9* polymorphism after genotyping	0.8	0.6 -1.0 [g]	assumed value
Cost of *CYP2C9* genotyping (Euro) [h]	55	20-30-55	—
Cost of INR monitoring at anticoagulation clinics (Euro)	204.60	—	data TSN [i]
Additional number of INR measurements needed for adapted care in carriers of *CYP2C9* polymorphism	5	0-10	assumed value
Cost of acenocoumarol tablets for one year (Euro)	28.20	—	FK [j]
Cost of treatment of major bleeding (Euro)	10 622	8000-15 000	18,19 [k]

TSN = Dutch Thrombosis Foundation; FK = Farmacotherapeutisch Kompas

a) The total number of patients in these three studies was 1591.
b) In the referred study in acenocoumarol users the first INR (assessed on the fourth day after initiation of acenoumarol therapy) was significantly increased in *CYP2C9*3* carriers.
c) There were 29 major bleeding episodes in 696.5 patient-years, as follows: 14 digestive tract, 5 intracranial, 2 fatal, and 8 other (data provided by author of reference 16).
d) Range for sensitivity analysis: the 95% confidence interval of bleeding risk in *CYP2C9*1/*1* subjects.
e) There were 20 major bleedings in 291.6 patient-years, as follows: 11 digestive tract, 3 intracranial, 1 fatal, and 5 other (data provided by author of reference 16).
f) Range for sensitivity analysis is 95% confidence interval.
g) Range for sensitivity analysis: 0.61 is the factor with which the bleeding rate in carriers of a *CYP2C9* polymorphism would be fully reduced to the bleeding rate in wild-type subjects.
h) The cost of *CYP2C9* genotyping in the base case was the cost we paid in our last study[11]; the range for the sensitivity analysis was the lowest and highest amount paid per genotyping in the Netherlands (information from different laboratories).
i) The TSN collects data on costs of all Dutch anticoagulation clinics. Costs are charged for each INR assessment (mean cost Euro 10.23), with assessments at patients' homes being more expensive compared with assessments in anticoagulation clinics (Euro 10.83 versus Euro 9.63). Mean number of INR measurements was 20 per year.
j) The FK was edited by the Dutch College of Health Care Insurances, providing prescription guidelines and information of costs for prescribers and pharmacists in the Netherlands.
k) Weighted mean of costs of treatment of aneurysmal subarachnoid bleeding[18] and of bleeding peptic ulcer.[19]

Relative risk of major bleeding in carriers of CYP2C9 polymorphism compared with CYP2C9 wild-type subjects. From the aforementioned data of Visser et al.,[16] it can be calculated that the relative risk of major bleeding (RRb) is 1.649 (6.86/4.16) for our base case example.

Reduction of incidence rate of major bleeding in carriers of polymorphism after CYP2C9 genotyping. Because no studies on bleeding rate reduction after *CYP2C9* genotyping are available, we have to assume a value. For our base case example, we assumed that this risk reduction is 20% (relative risk reduction [RRR]=0.8), halfway between 1 (no risk reduction after genotyping) and 0.61 (a complete reduction toward the major bleeding risk in *CYP2C9* wild-types).

Cost of CYP2C9 genotyping. For our base case example, we used the amount of Euro 55, which we paid in the two studies we have conducted in users of acenocoumarol[14] and phenprocoumon in 2001-2003.[11]

Cost of INR monitoring. For our base case example we calculated the mean cost of care of anticoagulation clinics from the yearly data of the Dutch Thrombosis Foundation, which keeps statistics on costs and frequency of INR measurements. We used the data from the year 2004. In the first year of treatment the number of INR measurements was, on average, 20. For adapting care after *CYP2C9* genotyping, we assumed five additional INR measurements in genotyped carriers of a *CYP2C9* polymorphism (25% additional INR measurements).

Cost of major bleeding. For our base case example, we derived the cost of major bleeding from two Dutch studies. Roos et al.[18] calculated the cost of aneurysmal subarachnoid bleeding in the first year after diagnosis, and De Leest et al.[19] calculated the cost of treatment of bleeding peptic ulcers. We considered the cost of treatment of subarachnoidal bleeding to be representative of the cost of intracranial bleeding and reindexed the results of the study of Roos et al. to the year 2004. In the study of De Leest et al., the costs depended on the localization and seriousness of the peptic ulcer, ranging from Euro 10 000 to Euro 26 000. We estimated the costs of treatment of a bleeding ulcer conservatively at Euro 11 900, which is a weighted mean of the costs of bleeding without perforation in the duodenum and in the stomach. For the total costs of major bleeding, we calculated a weighted mean using only the data of gastrointestinal and intracranial bleeding, because costs of other major bleeding sites have not been studied. This could have resulted in an underestimation of the cost of major bleeding in our example, but in the sensitivity analyses we varied the cost of major bleeding.

Table 2: COST-EFFECTIVENESS OF *CYP2C9* GENOTYPING IN ACENOCOUMAROL USERS (BASE CASES, AS DISCUSSED IN TABLE 1)

CYP2C9 polymorphisms	Genotyping		No genotyping		Marginal		
Prevalence	Effect: bleeding episodes/ 100 p-yrs	Cost: Euro/ 100 p-yrs	Effect: bleeding episodes/ 100 p-yrs	Cost: Euro/ 100 p-yrs	Effect: bleeding episodes/ 100 p-yrs	Cost: Euro/ 100 p-yrs	C/E
0.36	4.6392	79 899	5.1334	77 807	0.4942	2092	4233
0.44	4.7457	81 440	5.3497	80 105	0.6040	1335	2210

C/E = marginal cost-effectiveness (cost to avoid one bleeding by *CYP2C9* genotyping); p-yrs = patient-years
Results reported in this table were calculated with the decision analytic model, represented in Figure 1.

Table 3: RESULTS OF SENSITIVITY ANALYSES REGARDING MARGINAL COST-EFFECTIVENESS FOR PARAMETERS IN DECISION ANALYTIC MODEL

	All patients genotyped [a]			Selection of patients genotyped [b]		
	Euro 20	Euro 30	Euro 55	Euro 20	Euro 30	Euro 55
Incidence rate major bleeding episodes in wild-type (per 100 p-yrs)						
2.00	5 546	9 754	20 276	4 015	7 459	16 067
2.40	2 851	6 358	15 126	1 576	4 445	11 619
3.00	156	2 962	9 977	D	1 432	7 171
3.60	D	698	6 544	D	D	4 205
4.16	D	D	4 233	D	D	2 210
4.60	D	D	2 812	D	D	982
5.60	D	D	413	D	D	D

RRR						
0.95	20 469	28 563	48 798	17 526	24 148	40 704
0.90	4 924	8 971	19 088	3 452	6 763	15 041
0.85	D	2 440	9 185	D	968	6 487
0.80	D	D	4 233	D	D	2 210
0.70	D	D	D	D	D	D
RRb						
1.05	1 592	4 772	12 721	436	3 038	9 542
1.30	D	1 812	8 232	D	411	5 664
1.50	D	154	5 718	D	D	3 493
1.649	D	D	4 233	D	D	2 210
1.90	D	D	2 278	D	D	521
Additional INR measurements [c]						
10	877	2900	7 959	141	1 797	5 935
7	D	665	5 723	D	D	3 700
5	D	D	4 233	D	D	2 210
3	D	D	2 742	D	D	719
0	D	D	507	D	D	D
Cost of major bleeding						
Euro 8 000	D	1 796	17 343	D	693	4 831
Euro 9 000	D	796	12 343	D	D	3 831
Euro 10 000	D	D	7 343	D	D	2 831
Euro 11 000	D	D	2 343	D	D	1 831
Euro 12 000	D	D	D	D	D	831

p-yrs = patient-years; D = dominant strategy, more effective and cheaper than not genotyping; RRR = relative risk reduction (factor with which the incidence rate of major bleeding in carriers of a polymorphism can be reduced when the CYP2C9 genotype is known); RRb = relative risk of major bleeding in carriers of a polymorphism compared to wild-type patients

a) The prevalence of polymorphisms was 0.36.

b) The prevalence of polymorphisms was 0.44.

c) Estimated number of additional INR measurements in carriers of a polymorphism after genotyping.

Cost-effectiveness of *CYP2C9* genotyping: which parameters are important?

In our base case example the marginal cost-effectiveness (cost to avoid one bleeding) was Euro 4233 if all patients were genotyped. If patients in whom an initial INR greater than 2.5 was assessed on the fourth day of therapy were selected, the marginal cost-effectiveness was Euro 2210 (Table 2). In the next subsections we consider the significance of these parameters with regard to the cost-effectiveness based on sensitivity analyses we have performed by varying the values of our base case example (Table 3).

Prevalence of *CYP2C9*2* and *CYP2C9*3* polymorphisms

The results of our base case calculations show that the selection of suitable candidates for *CYP2C9* genotyping based on their first INR improves the cost-effectiveness of *CYP2C9* genotyping. In our base case example this selection increased the prevalence of *CYP2C9* polymorphisms from 36% to 44% (an increment of 22%), resulting in a reduction of the cost to avoid one major bleeding of nearly 50% for our base case example (Table 2). We think that the selection we propose is justified because the fixed acenocoumarol starting dose of 6, 4, and 2 mg on the first three days did not result in severe overanticoagulation in carriers of the *CYP2C9*3* allele and only resulted in a significantly increased initial INR on comparison with wild-type subjects (mean initial INR 3.2 versus 2.5; $p<0.01$; more *CYP2C9*3* subjects having a therapeutic INR[14]).

On the other hand, a low prevalence of *CYP2C9*2* and *CYP2C9*3* polymorphisms in a population will decrease the chance that *CYP2C9* genotyping is a useful, cost-effective addition to INR monitoring.

Cost of *CYP2C9* genotyping

The cost of *CYP2C9* genotyping is of course an important issue in determining cost-effectiveness. The cost of genotyping is decreasing rapidly. Whereas we paid Euro 55 for *CYP2C9* genotyping in our studies (the amount we used in our base case example), we have been informed by several Dutch laboratories that *CYP2C9* genotyping will cost between Euro 20 and Euro 30 in the near future. Therefore we performed all sensitivity analyses of our base case example for three possible values of cost of *CYP2C9* genotyping: Euro 20, Euro 30 and Euro 55. Decrease in cost of genotyping from Euro 55 to Euro 20 strongly increases the cost-effectiveness for all possible scenarios. If all patients are genotyped, for most scenarios, *CYP2C9* genotyping becomes the dominant strategy if *CYP2C9* genotyping costs Euro 20, whereas dominance for *CYP2C9* genotyping is almost

never reached if it costs Euro 55. If a selection of patients is genotyped, dominance of *CYP2C9* genotyping is achieved for many scenarios, even if it costs Euro 30 (Table 3).

Incidence rate of major bleeding

A low incidence rate of major bleeding in wild-type patients involves a high cost per avoided bleeding episode. If the bleeding rate in subjects with the *CYP2C9* wild-type is 2.00 per 100 patient-years, the marginal cost of the genotyping strategy varies from Euro 4000 to Euro 16 000 in patients with an initial INR greater than 2.5 and from Euro 5500 to Euro 20 000 for all patients (Table 3). In patients with an initial INR greater than 2.5, *CYP2C9* genotyping becomes the dominant strategy if the major bleeding rate exceeds 3.00 per 100 patient-years and if genotyping costs Euro 20 to Euro 30, whereas for all patients, genotyping becomes dominant if the major bleeding rate exceeds 4.00 per 100 patient-years. If *CYP2C9* genotyping costs Euro 55, as in our base case, *CYP2C9* genotyping becomes the dominant strategy only if the major bleeding rate exceeds 5.60 per 100 patient-years in all patients.

These examples show that the cost-effectiveness of *CYP2C9* genotyping depends strongly on the incidence rate of major bleedings. Unfortunately, data on the incidence rate of major bleedings in users of coumarin anticoagulants differ widely, even within the same country. In the base case example we used, the major bleeding risk in wild-type acenocoumarol users was 4.16 per 100 patient-years, derived from Visser et al.[16] However, van der Meer et al.[20] reported lower major bleeding rates of 2.7 and 2.1 per 100 patient-years for two different years in the setting of a Dutch anticoagulation clinic. In a recent study in the same setting, the incidence rate of major bleedings was 2.64 per 100 patient-years in patients of 60 years or older.[21] Although more phenprocoumon users were included in these latter studies, another study found no difference in the major bleeding rate between phenprocoumon users and acenocoumarol users.[22] If we assume an incidence rate of only 2.4 per 100 patient-years in wild-type subjects, the cost to avoid one major bleeding episode would be Euro 15 126 for our base case.

In studies with warfarin as an anticoagulant, higher major bleeding rates have been reported.[8,9,23] Higashi et al.,[8] who studied the association between possession of *CYP2C9*2* or *CYP2C9*3* alleles and anticoagulation status in warfarin users, found major bleeding rates of 5.6 and 12.5 per 100 patient-years for *CYP2C9* wild-type subjects and carriers of *CYP2C9*2* or *CYP2C9*3* allele, respectively.

If we apply the incidence rate of 5.6 per 100 patient-years for wild-type subjects to our model, it would cost only Euro 413 to avoid one major bleeding episode with our base case price of Euro 55 for genotyping (Table 3).
It is conceivable that incidence rates of major bleeding differ between subgroups within a population, which could result in differences in cost-effectiveness as a consequence. Van der Meer et al.[20] demonstrated that age was an important risk factor for major bleeding. Therefore, *CYP2C9* genotyping probably will be more cost-effective in older patients. To assess the cost-effectiveness of *CYP2C9* genotyping in a certain setting of coumarin users, knowledge of the major bleeding risk incidence in that setting is pivotal.

Relative risk of major bleeding

An important parameter for the assessment of cost-effectiveness to avoid one major bleeding episode by *CYP2C9* genotyping is the relative risk of major bleeding in carriers of a *CYP2C9* polymorphism compared with *CYP2C9* wild-type subjects (RRb). As Table 3 shows, a low RRb is unfavourable for cost-effectiveness of *CYP2C9* genotyping. In the two studies that examined major bleeding differences between *CYP2C9* wild-type subjects and carriers of a *CYP2C9* polymorphism, RRb varied roughly from 1.6 to 2.2. Visser et al.[16] found a value of 1.65 (6.86/4.16) in acenocoumarol users, whereas Higashi et al.[8] found a value of 2.23 (12.5/5.6) in warfarin users. This difference might be partly explained by the finding in some studies that the sensitivity for acenocoumarol is mainly increased in carriers of a *CYP2C9*3* allele,[12,14] whereas the sensitivity for warfarin is increased in carriers of a *CYP2C9*2*, as well as a *CYP2C9*3* allele.[8,9,24] All the same, it seems to be reasonable to assume that RRb is higher than 1.5 in users of warfarin, as well as acenocoumarol. In that case *CYP2C9* genotyping would become the dominant strategy if it would cost Euro 30 or less (Table 3).

Relative risk reduction of major bleeding

The most important parameter in our model for which no value can be derived from studies is the factor with which the major bleeding rate can be reduced in carriers of a *CYP2C9* polymorphism toward the rate in wild-type subjects (RRR). If RRR equals 0.95 (5% risk reduction), the cost of the genotyping strategy varies from Euro 20 000 to Euro 49 000 for all patients and from Euro 17 500 to Euro 40 700 for selected patients with an initial INR greater than 2.5. If the bleeding rate can be reduced by 30% (RRR=0.7), genotyping becomes the dominant strategy for all considered costs of genotyping (Table 3).

Table 4: THRESHOLD ANALYSIS OF COSTS OF GENOTYPING NEEDED TO ACHIEVE DOMINANCE OR MARGINAL COST-EFFECTIVENESS LESS THAN 4000 EURO

	Incidence rate of major bleeding episodes in wild-type patients (per 100 p-yrs)	RRR	Cost of genotyping to achieve dominant strategy (Euro)	Cost of genotyping to achieve marginal C/E <4000 Euro (Euro)
All patients (prevalence of polymorphism = 0.36)	2.0	0.9	12-13	16-17
		0.8	24-25	33-34
		0.7	36-37	50-51
	3.0	0.9	18-19	25-26
		0.8	36-37	50-51
		0.7	55-56	80-81
	4.0	0.9	24-25	35-36
		0.8	48-49	67-68
		0.7	73-74	101-102
Selection of patients with initial INR>2.5 (prevalence of polymorphism = 0.44)	2.0	0.9	14-15	20-21
		0.8	29-30	43-44
		0.7	44-45	61-62
	3.0	0.9	22-23	30-31
		0.8	44-45	61-62
		0.7	67-68	92-93
	4.0	0.9	30-31	41-42
		0.8	59-60	82-83
		0.7	89-90	123-124

RRR = relative risk reduction; p-yrs = patient-years

The relative risk of bleeding in carriers of a polymorphism compared with wild-type patients was 1.6, and the number of additional INR measurements needed in carriers of a polymorphism was 5.

Voora et al.[25] demonstrated that prospective dosing of warfarin, based on the *CYP2C9* genotype, resulted in a similar time to achieve a stable warfarin dose in carriers of a polymorphism and in wild-type subjects. However, in this study the risk of overanticoagulation remained increased in carriers of a polymorphism, suggesting that an association between more adequate dosing and a decrease in overanticoagulation cannot be taken for granted. Prospective studies over longer periods are needed to assess whether *CYP2C9* genotyping can contribute to a decreased bleeding rate and to what extent. Without such studies, reliable statements about the cost-effectiveness of *CYP2C9* genotyping cannot be made.

Cost of (additional) INR monitoring

It is unknown whether preceding knowledge of the *CYP2C9* genotype should result in additional INR measurements in carriers of a *CYP2C9* polymorphism. If it is possible to steer computerized dosing programs toward lower doses basd on a *CYP2C9* polymorphism, additional INR measurements might not be needed. For our base case example, we assumed 5 additional INR measurements for the first year of acenocoumarol treatment. If *CYP2C9* genotyping would cost Euro 20 to Euro 30, it would become the dominant strategy even if up to seven additional INR measurements per year would be needed. However, if *CYP2C9* genotyping would cost Euro 55, the number of additional INR measurements would markedly affect the cost-effectiveness of *CYP2C9* genotyping (Table 3).
Of course, it is theoretically possible that *CYP2C9*-genotyped dose guiding could result in less INR monitoring, which would make *CYP2C9* genotyping even more cost-effective. For our analyses, we have not assumed savings in the number of INR measurements after *CYP2C9* genotyping.

Cost of major bleeding

Because prevention of major bleeding appears to be the only clinical important outcome that can be achieved by *CYP2C9* genotyping, the cost of major bleeding is an essential issue. The cost-effectiveness of *CYP2C9* genotyping increases with the cost of treatment of major bleeding, and *CYP2C9* genotyping would become the dominant strategy if major bleeding would cost Euro 8000 to Euro 12 000 and if genotyping would cost Euro 20 (Table 3). If genotyping would cost Euro 30, it would only cost Euro 1796 to avoid one major bleeding episode if the cost of treatment were Euro 8000. Finally, if genotyping is relatively expensive (Euro 55), there is a sharp decrease in the cost to avoid one major bleeding episode if the cost of treatment of major bleeding increases from Euro 8000 to Euro 12 000 (Table 3). So, if *CYP2C9* genotyping is relatively

expensive, the cost of major bleeding is an important parameter in the assessment of cost-effectiveness.

ACCEPTABLE COST TO PREVENT ONE MAJOR BLEEDING EPISODE AND THRESHOLD ANALYSES

Obviously, a clear agreement about the acceptable cost to avoid one major bleeding episode is lacking. In the Netherlands the accepted cost for gaining a life-year is Euro 20 000.[26] It is possible to derive an acceptable cost for avoiding one major bleeding episode from the bleeding data we used in our base case example. On the basis of our data, 7 of 29 bleeding episodes (24.1%) in wild-type subjects were intracranial or fatal, whereas this figure was 4 of 20 bleeding episodes (20.0%) in carriers of a polymorphism. Because these bleeding episodes have a direct impact on the duration and quality of life, it seems reasonable to agree that an amount of 20% to 24% of Euro 20 000 would be acceptable for a health care payer; this could be rounded to Euro 4000 as the acceptable cost to avoid one major bleeding episode.

Using our base case example, we performed a threshold analysis in which we assessed at which cost of genotyping dominance or a marginal cost-effectiveness of less than Euro 4000 per avoided bleeding episode of the genotyping strategy would be achieved. For this threshold analysis, RRb was fixed at 1.6 and the number of additional INR measurements per year in carriers of a *CYP2C9* polymorphism was fixed at 5 per year. This threshold analysis revealed that if genotyping is relatively cheap (Euro 25-Euro 30), only scenarios with a low bleeding rate reduction (RRb=0.9) fail to achieve dominance, but a marginal cost-effectiveness of Euro 4000 is possible if the bleeding rate incidence in wild-type subjects is greater than 3.0 per 100 patient-years. Even if genotyping is relatively expensive (>Euro 50), dominance or a marginal cost-effectiveness of Euro 4000 is achieved for a number of scenarios (Table 4).

SUMMARY AND PERSPECTIVE

The marginal cost to avoid one major bleeding episode by *CYP2C9* genotyping appears to be sensitive to a number of parameters. Some of these parameters are virtually unknown (reduction of major bleeding rate in carriers of a *CYP2C9* polymorphism), vary between populations (major bleeding rate in wild-type

subjects and prevalence of *CYP2C9* polymorphisms), or changing in time (cost of genotyping). These uncertainties, especially the ability to reduce the major bleeding rate by *CYP2C9* genotyping, prevent us from concluding unequivocally that *CYP2C9* genotyping is valuable in addition to INR monitoring of anticoagulation clinics. However, our base case example, our sensitivity analyses, and our threshold analysis all show that, even in a setting characterized by intensive INR monitoring, *CYP2C9* genotyping could be a cost-effective strategy under certain circumstances and a potentially useful addition to INR monitoring.

Although we worked out an example with acenocoumarol in a Dutch setting, the data provided in Tables 3 and 4 are perfectly applicable to warfarin and other settings. As we pointed out in this commentary, it is possible that in warfarin users the risk of major bleeding in carriers of a *CYP2C9* polymorphism compared with *CYP2C9* wild-type subjects (RRb) is higher than in acenocoumarol users. So, this higher RRb could result in a more easily attainable cost-effectiveness for warfarin users compared with acenocoumarol users. For settings in which the major bleeding risk is higher or lower or in which the cost of treatment of major bleeding is different, the cost-effectiveness of *CYP2C9* genotyping can be observed in Tables 3 and 4.

An important and challenging recently discovered source of variability in coumarin users is the *VKORC1* genotype. In two hitherto conducted studies the *VKORC1* genotype contributed more to the variability in warfarin dose requirement than the *CYP2C9* genotype,[27,28] whereas one study found an equal contribution for both genotypes[29] and one study found a larger contribution of the *CYP2C9* genotype.[30] A study in healthy volunteers suggested that the *VKORC1* genotype contributed more to acenocoumarol sensitivity than the *CYP2C9* genotype.[31] However, none of these studies addressed the question of whether there was an association between the *VKORC1* genotype and major bleeding. The only study that found an increased bleeding risk in phenprocoumon using carriers of a *VKOR C1173T* polymorphism compared with *VKORC1* wild-type subjects did not take the *CYP2C9* genotype into account.[32]

There is a strong need for studies examining whether the *VKORC1* genotype, either alone or in combination with the *CYP2C9* genotype, is associated with major bleeding in coumarin users. As long as this information is lacking, it is justified to discuss the cost-effectiveness of *CYP2C9* genotyping as we have

done, because the *CYP2C9* genotype appears to be clearly associated with major bleeding, whereas this is less obvious for the *VKORC1* genotype thus far.
All the same, it is interesting to consider how cost-effectiveness would change if the *VKORC1* genotype would appear to have a larger impact on major bleeding than the *CYP2C9* genotype. Suppose that the possession of a *VKORC1* polymorphism, for example the *VKORC1 C1173T* polymorphism, is more strongly associated with an increased major bleeding risk than the possession of a *CYP2C9* polymorphism. In that case we can replace the *CYP2C9* genotype in our model with the *VKORC1* genotype. The percentage of carriers of a polymorphism would increase to more than 50%, which is what most previously conducted studies have found. With a stronger association between the *VKORC1* genotype and major bleeding risk, RRb would be higher than 1.6, which was the lowest value found in studies with CYP2C9. If, finally, VKORC1 genotyping would cost as much as *CYP2C9* genotyping, it is obvious that the cost-effectiveness of *VKORC1* genotyping would be more easily attained than by *CYP2C9* genotyping.
In conclusion, this commentary indicates several scenarios in which cost-effective *CYP2C9*-guided (or even *VKORC1*-guided) coumarin therapy is plausible. Therefore a randomized clinical trial examining the impact of knowledge of the *CYP2C9* and the *VKORC1* genotypes on preventing major bleeding is warranted.

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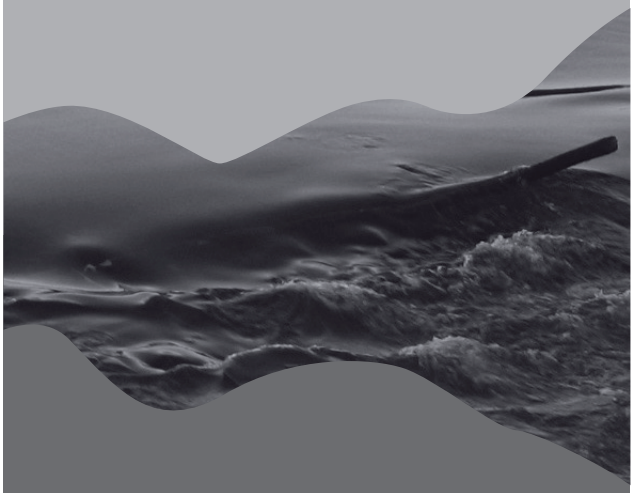
CHAPTER 7



GENERAL DISCUSSION

INTRODUCTION

The narrow therapeutic range and interindividual as well as intraindividual variability of vitamin K antagonists (coumarins) are still a matter of concern. Despite the fact that coumarins have been applied successfully for more than half a century and despite the useful contribution of anticoagulation clinics to a more efficacious and safer coumarin treatment, they still cause considerable problems in daily practice.[1] In the recently published results of the Dutch Hospital Admission Related to Medication (HARM) study coumarins have been identified as one of the main causes of avoidable hospital admissions, taking a second place between the far more frequently prescribed drug groups of antiplatelet drugs and non-steroidal anti-inflammatory drugs (NSAIDs) (report accessible at www.knmp.nl).

Because of the obvious difficulties in achieving an effective and safe anticoagulation with coumarins, their replacement by the recently developed and possibly equally effective direct thrombin inhibitor ximelagatran, for which no monitoring is needed, seemed a matter of time.[2] However, the potential hepatic toxicity and possible association with an increase of coronary events have prevented the approval of the latter drug by registration authorities.[3] As a consequence the apparently aged coumarins remain clear first choice drugs for several common diseases such as atrial fibrillation which are associated with an increased risk of thromboembolism, making research into the variability in their response still useful and challenging. This research already started in 1943, two years after the introduction of warfarin, when Richards et al. published a study in which fever was identified as an environmental factor increasing its anticoagulant effect.[4]

Factors contributing to the variability in coumarin response are coumarin-drug and coumarin-food interactions, comorbidities, age, bodyweight, patient adherence, and genetic variation.

Nowadays it is well recognized that of the environmental factors affecting coumarin anticoagulation use of interacting drugs can contribute considerably to the intraindividual variability in coumarin response.[5] It is also known that the quality of the evidence for drug-drug interactions is often doubtful.[6] Several studies in this thesis were aimed at increasing our insights into coumarin interactions and at providing more evidence on the clinically relevant end point major bleeding (Chapter 3).

Genetic factors can play an important role in the individual variation in drug response. The pharmacokinetics as well as pharmacodynamics of a drug can be

affected by genetic factors, potentially contributing to changes in drug response with clinical consequences.[7-9] It is conceivable that the large ranges in dose requirements for coumarins could at least partly be due to genetic variations. During the last decade, many studies investigated genetic factors affecting the warfarin response.[10,11] Several studies in this thesis were aimed at gaining more insight into the role of gene variations in the response of acenocoumarol (Chapter 4) and phenprocoumon (Chapter 5), and also into the possible economic consequences of genotyping preceding coumarin therapy (Chapter 6).
In this chapter we will discuss our main findings and put them into the broader context of potential clinical implications and further research. For a more detailed discussion of the separate studies and their findings and limitations we refer to the previous chapters.

MAIN FINDINGS

Medication records of anticoagulation clinics

Several studies have reported considerable discrepancies between medical records and pharmacy records,[12-14] suggesting that medical treatment is not always based on complete information. For anticoagulation clinics which are specialized institutions improving the quality of anticoagulation compared with usual medical care,[15-17] information about concomitant use of interacting drugs is pivotal. To assess the completeness of medication records of anticoagulation clinics (AC records), we compared them with pharmacy records (Chapter 2.1). We found considerable discrepancies between medical records and pharmacy files: of 117 interacting drugs registered in pharmacy records, 27 (32%) were not registered in AC records, indicating that essential information which is needed for adequate adjustments of coumarin therapy is lacking. Since interacting drugs are an important source of intraindividual variability, this is highly undesirable and potentially dangerous and it accentuates the need for central electronic patient files, in which complete care processes are registered and which can be consulted by all health care providers. A short-term solution is promotion and extension of direct information flows from pharmacies to anticoagulation clinics, which could circumvent the potentially less reliable information flow from patient to anticoagulation clinic. Of course, instructions for an adequate registration of relevant concurrently used drugs and comorbidities in medical files should be an important issue in quality guidelines of anticoagulation clinics.

Drug interactions: evidence

One of the problems of evaluating drug interactions is the generally poor quality of evidence. A recent systematic review concluded that 86% of the analysed articles on coumarin interactions were case reports.[6] In 2003 Juurlink et al. published a pioneering study which used population based data to evaluate hospital admissions for drug toxicity following the co-prescription of drugs with known interactions.[18] Although the contribution of interaction effects to the feared risk of major bleeding in users of coumarins is well recognized, few studies have systematically investigated an association between hospitalization for major bleeding and co-prescribed potentially interacting drugs.[19-23] For several drugs which are themselves associated with an increased bleeding risk, such as selective serotonin reuptake inhibitors (SSRIs) and the antiplatelet drug clopidogrel, the consequences of concomitant use with coumarins have not been established. We studied the effect of serotonin reuptake inhibitors (SSRIs) in a case-control study nested within a cohort of users of acenocoumarol and phenprocoumon (Chapter 3.2). We found that SSRIs increased the risk of major non-gastrointestinal bleeding to the same extent as NSAIDs, possibly because of their antiplatelet effects. We did not find an increased risk of upper gastrointestinal bleedings, which agreed with the results of another study.[23] A point of interest is that more attention is needed for non-gastrointestinal bleedings, such as the potentially disabling intracranial bleeding, as an end point for interaction studies, since interaction effects on non-gastrointestinal bleedings could differ from the effects on the more extensively studied upper gastrointestinal bleedings.

Because of their different effects on haemostasis an interaction between antiplatelet drugs and coumarins is conceivable. However, only the bleeding risk increasing effect of aspirin among users of coumarins is well established,[24] whereas data are scarce for the antiplatelet drugs clopidogrel and dipyridamole. We demonstrated that the effect on bleeding risk of clopidogrel and dipyridamole among users of coumarins is probably similar to the effect of aspirin and possibly even greater for clopidogrel (Chapter 3.3).

Despite the fact that we conducted these studies in the PHARMO record linkage system, which includes complete medication histories of more than 2 million community dwelling residents, we had relatively few cases and controls exposed to SSRIs and the newer antiplatelet drugs available. Although SSRIs, dipyridamole and clopidogrel are frequently prescribed in daily practice, concurrent use with coumarins is apparently rare. This underlines the value of epidemiological studies in large populations to quantify serious interaction effects

between not commonly co-prescribed drugs and the conduction of interaction studies in such settings should be encouraged.

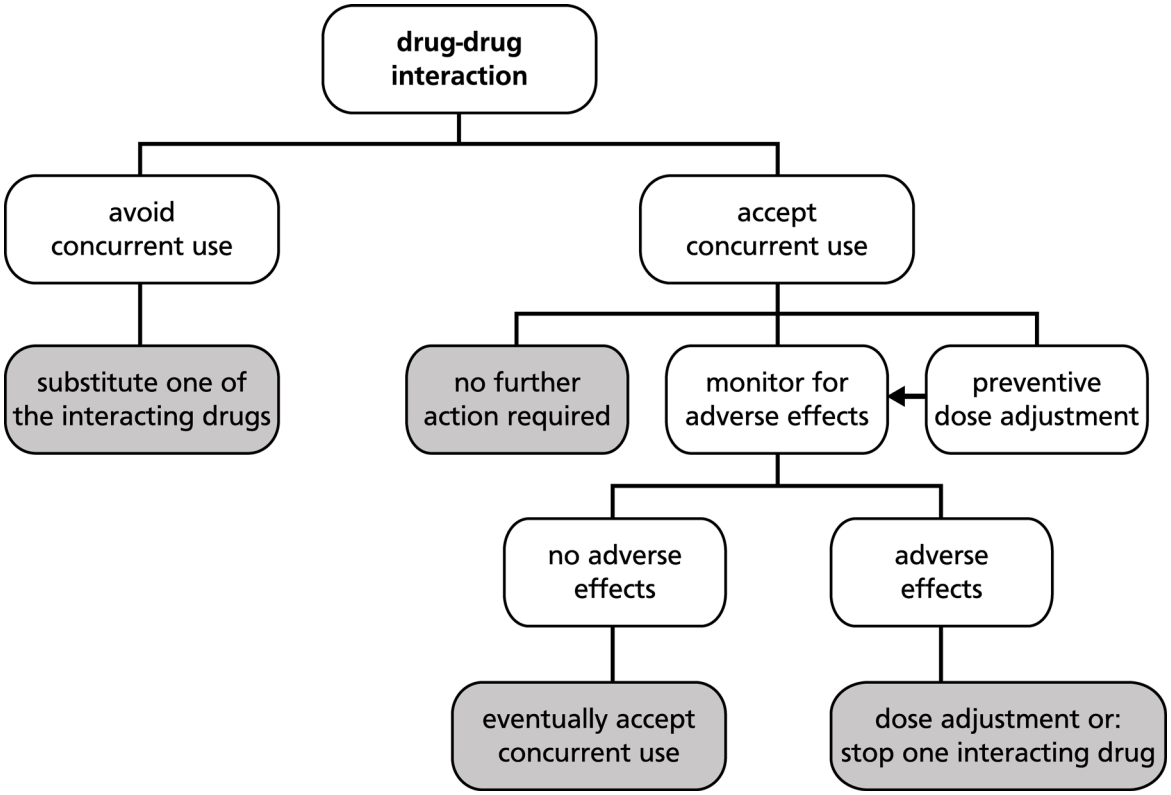
Drug-drug interactions: management

When a drug interaction is detected, the main management options are avoidance or acceptance of concurrent use of the interacting drugs. Concurrent use can be accepted without any special precautions or under condition of preventive dose adjustments (mainly applicable to pharmacokinetic interactions) or monitoring for clinical consequences (Figure 1). Acceptance of an interacting drug combination usually results in a period of monitoring and dose adjustments during which a patient can become destabilized, especially if the target drug of the interaction has a narrow therapeutic range. This risk can be avoided by substituting an interacting drug for a non-interacting therapeutic equivalent. In this section we will discuss interaction management from the viewpoint of the pharmacist and his potential role in enhancing drug safety. Adequate management of drug-drug interactions is one of the pharmacists' contributions to the reduction of drug related problems and not only requires thorough knowledge of the pharmacology of the interaction, but an equally thorough knowledge of the pros and cons of alternative therapies. The interactions which have been studied in this thesis illustrate some of the problems of interaction management.

We assessed the consequences of management of the interaction between coumarins and sulphamethoxazole-trimethoprim, of which the sulphamethoxazole is a strong CYP2C9 inhibitor[25] retarding the metabolism of coumarins (Chapter 3.1). Although several studies have found an increased risk of severe overanticoagulation[26,27] and major bleedings[19] in users of cotrimoxazole, and although the guidelines for management of coumarin interactions advise against concurrent use, in daily practice this interaction is accepted and managed by dose adjustments. We demonstrated that concurrent use of coumarins and cotrimoxazole resulted in a significantly increased period of anticoagulant undertreatment compared to concurrent use of coumarins and other antibiotics, probably because downward dose adjustments have to be applied more frequently in users of cotrimoxazole than in users of not pharmacokinetically interacting antibiotics. Because an equivalent alternative to cotrimoxazole is always available, the logical consequence of this finding is that concurrent use of cotrimoxazole and coumarins should be avoided and should not be managed by monitoring. The broader implication is that short-term concurrent use of drugs

with a narrow therapeutic range (for example coumarins, lithium, digoxin) and inhibitors or inducers of their elimination can be better managed by avoidance than by monitoring.

Figure 1: BASIC MANAGEMENT OF DRUG-DRUG INTERACTIONS



The approach of the interaction between coumarins and SSRIs (Chapter 3.2) is more complex. Although SSRIs were shown to increase the risk of major non-gastrointestinal bleeding, these drugs have no effect on the International Normalized Ratio (INR), neither directly nor by affecting the pharmacokinetics of coumarins. As a consequence management by a downward dose adjustment of the coumarin carries the risk of undertreatment. However, a number of studies convincingly demonstrated that use of SSRIs is safer than use of tricyclic antidepressants in patients with several cardiovascular diseases.[28-31] Moreover, SSRIs have less anticholinergic effects which are otherwise problematic in elderly patients,[32] whereas another alternative like venlafaxine can cause undesirable cardiovascular effects.[33,34] A more extensive discussion of the pharmacotherapy for

depression and anxiety being beyond the scope of this thesis, these limited considerations indicate some of the potential drawbacks of substituting SSRIs in cardiovascular compromised and elderly patients. As a consequence management of this interaction requires a careful weighing of the advantages of SSRIs as first choice drugs against the drawback of an increased bleeding risk.

Similar considerations apply to the interaction between coumarins and antiplatelet drugs (Chapter 3.3). Because of their therapeutic superiority over antiplatelet drugs in atrial fibrillation, concomitant use of coumarins with antiplatelet drugs can be expected among patients who suffer from atrial fibrillation as well as ischaemic heart disease. However, since the increased bleeding risk for such combinations has been firmly established, concurrent use of coumarins and antiplatelet drugs is only justified in situations for which the therapeutic benefit is also established, which is only the case among patients with mechanical heart valves.[24] As a consequence management of this interaction also requires a careful weighing of the risks and benefits.

In summary, the cotrimoxazole interaction has to be managed by a simple intervention to convince the prescriber of the necessity of substitution for which pharmacological arguments prevail, whereas the interactions with SSRIs or antiplatelet drugs require consultation with the prescriber, in which the pharmacological arguments have to be weighed against medical benefits of treatment and in the case of SSRIs also against potential drawbacks of second choice alternatives.

From the viewpoint of pharmacists both examples underline the need for well-designed pharmacotherapy courses in pharmacy curricula and in post-academic education aimed at educating pharmacists for their responsibilities in optimizing drug safety. Several studies have demonstrated that pharmacists' interventions positively contribute to the safety of drug therapy.[35-37] However our cotrimoxazole study suggests that pharmacists apparently accept concomitant use of cotrimoxazole with coumarins, indicating that simple interventions are not always applied and offering opportunities to enhance the pharmacists' contribution to drug safety.

Genetic variability: *CYP2C9*

Genetic variance of genes encoding metabolizing enzymes, transporters, receptors, and ion channels can modify the effectiveness and safety of drugs. The increased knowledge of pharmacokinetics and pharmacodynamics of drugs and the completion of the Human Genome Project, in which human DNA has been

mapped and sequenced, offer great opportunities to improve our insights into the variability in drug response. Research into genetic factors which could explain the well recognized variability in coumarin response has led to the identification of CYP2C9 as the main metabolizing enzyme of (S)-warfarin in 1997,[38] followed by the identification of VKORC1 as its target protein on vitamin K epoxidase in 2004.[39,40]

The most common *CYP2C9* variants in Caucasian populations, designated as *CYP2C9*2* and *CYP2C9*3*, have been identified in the coding region, giving rise to changes in the primary amino acid sequence of CYP2C9 with the potency of modifying its enzyme function.[41,42] Up to 2005 research on genetic factors affecting coumarins was almost exclusively focused on the *CYP2C9* gene. A great number of studies in different Caucasian populations reported an association between decreased warfarin dose requirements and possession of *CYP2C9*2* or *CYP2C9*3* variant alleles[43-57] and several also found an increased risk of overanticoagulation[43,44,46,52,58] or bleeding[43,44,59] in carriers of a variant allele compared to wild-type subjects.

Because less was known about the clinical consequences of being carrier of *CYP2C9* variant alleles in users of the other coumarins, we conducted two separate prospective follow-up studies in users of acenocoumarol and in users of phenprocoumon. By performing each of these studies in two anticoagulation clinics in which the coumarin of interest predominated, we aimed to rule out bias because of lack of experience with the other coumarin.

Our results for acenocoumarol (Chapter 4.1) showed that carriers of a *CYP2C9*3* variant allele had lower acenocoumarol dose requirements, an increased risk of severe overanticoagulation (INR>6), and a lower chance to achieve stability during the follow-up period of 6 months compared to wild-type subjects, which agreed with the results of simultaneously conducted studies in users of acenocoumarol.[60-63] Effect of being carrier of the *CYP2C9*2* allele was small[62] or even absent,[60,61,63] in contrast to the findings for warfarin.

However, the association we found between being carrier of a *CYP2C9*2* or **3* allele and increased phenprocoumon sensitivity (with equal effects for the *CYP2C9*2* and *CYP2C9*3* alleles) (Chapter 5.1) was unexpected and contrasted with the findings of several other studies.[62,64,65]

Only a small part of the intraindividual variability appears to be explained by the *CYP2C9* genotype. In multiple regression models from several studies the *CYP2C9* genotype accounted for 5 to 27% of the variation in warfarin dose

requirements,[45,48,66] for 14% in acenocoumarol dose requirements,[63] and for 10.3% in phenprocoumon dose requirements (Chapter 5.1).

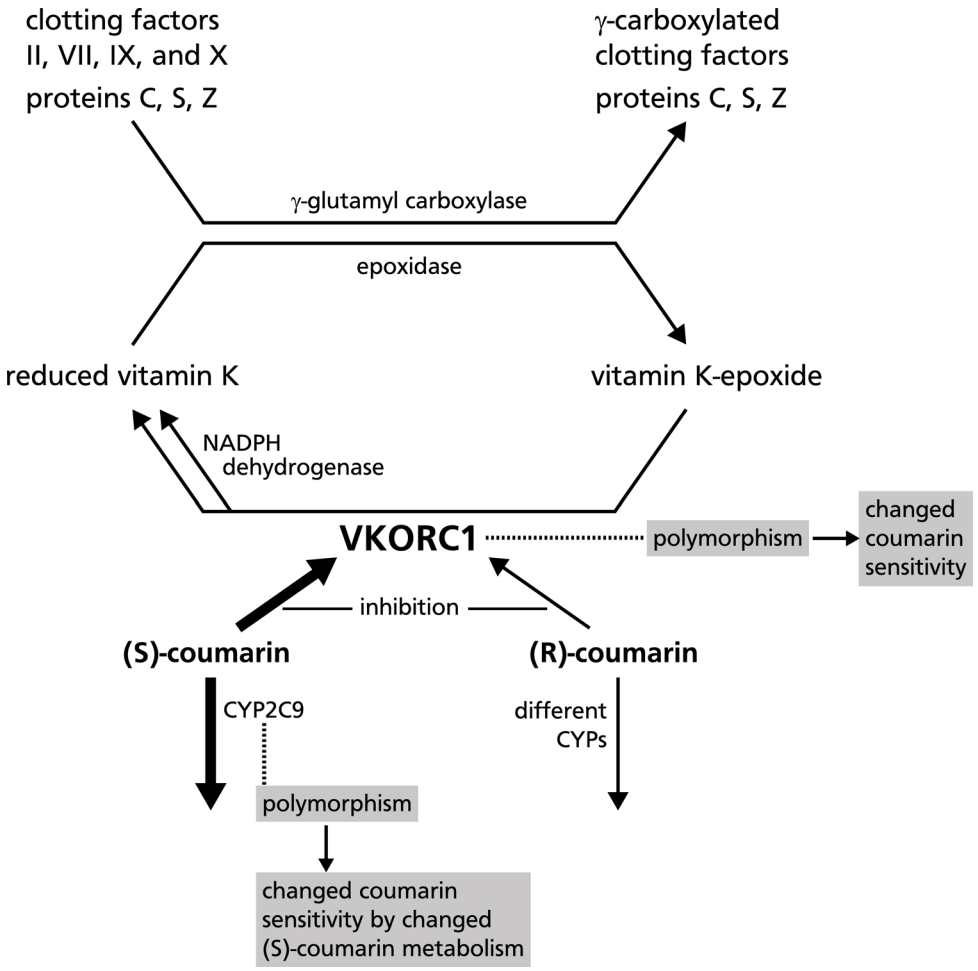
Despite this apparently small contribution of the *CYP2C9* genotype to coumarin sensitivity, its obvious association with an increased major bleeding risk in users of warfarin,[43,44,59] acenocoumarol,[67] and even phenprocoumon[64] raised the expectation that knowledge of the *CYP2C9* genotype preceding coumarin therapy could decrease the incidence of major bleeding. Several of these studies have been conducted in anticoagulation clinics,[44,64,67] suggesting that the usual INR monitoring in anticoagulation clinics do not prevent additional bleedings in genetically predisposed patients. We investigated the potential economic consequences of *CYP2C9* genotyping preceding acenocoumarol use (Chapter 6.1), showing that genotyping could be cost-effective in many scenarios, even if we assumed a modest reduction of the incidence rate of major bleeding in carriers of a *CYP2C9* variant allele. Of course, the missing link in all models evaluating cost-effectiveness in *CYP2C9* genotyping is the lack of any evidence that preceding knowledge of the genotype will improve coumarin dosing to such an extent that a reduction of the incidence rate of major bleeding is actually achieved without increasing thromboembolic risk. While two trials tested the feasibility of model-based warfarin dose initiation using *CYP2C9* genotype[68] and prospective *CYP2C9* based dosing of warfarin,[69] the significance of the *CYP2C9* genotype was put into another perspective with the identification of the *VKORC1* gene.

Genetic variability: *VKORC1* and *CYP2C9* genotypes

In 2004 a protein of 163 amino acids of the vitamine K epoxidase (VKOR) enzyme complex, designated as VKORC1, has been identified as the target for warfarin.[39,40] The homonymous gene encoding this protein is *VKORC1*, which is located on chromosome 16p11.2, spanning about 5-kb, and encompassing 3 exons and 2 introns. The *VKORC1* gene is polymorphic, but in contrast to the *CYP2C9* variants the common *VKORC1* variants are noncoding, indicating that they alter the mRNA expression and level of protein synthesis rather than modifying the amino acid sequence of VKORC1.[70,71]

VKORC1 can be considered a pharmacodynamic gene affecting the sensitivity to coumarins at the level of their physiological target, whereas *CYP2C9* can be considered a pharmacokinetic gene affecting this sensitivity at the level of the hepatic elimination (Figure 2). Theoretically, interaction effects between both genes are thinkable.

Figure 2: THE ROLE OF *VKORC1* AND *CYP2C9* IN THE ACTIVITY OF COUMARINS



A number of studies has examined the effects of the *CYP2C9* and *VKORC1* genotypes on anticoagulation status in users of warfarin[70,72-80] and acenocoumarol.[81] All found clear associations between being carrier of *VKORC1* variant alleles and decreased coumarin dose requirements, most studies reporting that the *VKORC1* genotype contributed more to the variability in dose requirements than the *CYP2C9* genotype.[70,73,75,77-81] Only one study examined a clinical outcome and reported an increased risk of major bleeding in phenprocoumon-using carriers of the *VKORC1 C1173T* polymorphism compared to patients without this polymorphism, but unfortunately this study did not take the *CYP2C9* genotype into account.[82]

In both studies we conducted on the effects of the *VKORC1 C1173T* polymorphism and the *CYP2C9*2* and **3* alleles in users of acenocoumarol

(Chapter 4.2) and phenprocoumon (Chapter 5.2), we found interaction effects between the *VKORC1* and *CYP2C9* genotypes.
In users of acenocoumarol only carriers of a combination of a *VKORC1 C1173T* polymorphism and a *CYP2C9*2* or **3* allele had an increased risk of severe overanticoagulation compared to subjects with no polymorphism or only one polymorphism, indicating a synergistic effect of both genotypes on the risk of overanticoagulation.
In users of phenprocoumon the *CYP2C9* genotype mainly affected differences in dose requirements in patients without a *VKORC1 C1173T* polymorphism. Interestingly, in both studies the time to achieve stable anticoagulation was only affected by the *CYP2C9* genotype, not by the *VKORC1* genotype, whereas in both studies the *VKORC1* genotype contributed more to the differences in coumarin dose requirements than the *CYP2C9* genotype (Table 1).
Although in a majority of studies the *VKORC1* genotype explains a greater part of the variation in coumarin dose requirements, our findings clearly indicate that research on either *CYP2C9* or *VKORC1* as single genetic factors will lead to important gaps in information about the effects of gene variations on coumarin sensitivity and such studies should be discouraged and avoided.
The most important potential clinical consequences of our findings regarding the effects of the *CYP2C9* and *VKORC1* genotypes are the following:
Assessment of *CYP2C9* as well as *VKORC1* genotypes will more accurately predict acenocoumarol-using patients at risk for overanticoagulation and bleeding, since only possession of variant alleles of both genes seems to be associated with overanticoagulation,
The *VKORC1* genotype probably predicts the sensitivity for a pharmacokinetic effect of CYP2C9 inhibiting drugs in phenprocoumon users, since the *CYP2C9* genotype mainly affects dose requirements in the *VKORC1 CC* stratum,
The combination of *CYP2C9* genotype (**2* and **3* alleles), *VKORC1 C1173T* genotype, and age predict 40-55% of the dose requirements of acenocoumarol and phenprocoumon, increasing the potency of a significant contribution of both genotypes to more accurate dose algorithms.
However, two points need to be accentuated. First, despite the fact that combined *VKORC1* and *CYP2C9* genotypes together with other factors can explain more than 50% of the variability in dose requirements, there is still no study which has examined whether the effect of knowledge of these genotypes preceding coumarin therapy actually improves the anticoagulation control. Second, despite the remarkable increase of studies examining the effects of the

VKORC1 genotype, there are still more studies which examined and found an association between the *CYP2C9* genotype and clinical outcomes such as bleeding or overanticoagulation (Table 2).
Nonetheless, if studies would demonstrate that the *VKORC1* genotype contributes more to the bleeding risk than the *CYP2C9* genotype, the decision analytic model of our cost-effectiveness study to *CYP2C9* genotyping would be perfectly applicable to *VKORC1* genotyping, because a greater part of the Caucasian population carries a variant allele for the *VKORC1* gene than for the *CYP2C9* gene, making *VKORC1* genotyping potentially more cost-effective than *CYP2C9* genotyping (Chapter 6.1).

OTHER ASPECTS OF GENETIC VARIABILITY IN COUMARIN THERAPY

Some other aspects of genetic factors need to be discussed. First the role of ethnicity in coumarin response, second the potential role of other genetic variants of the *CYP2C9* and *VKORC1* genes and third the role of other genes which could contribute to the until now unexplained part of the variation in coumarin dose requirements.

Ethnicity

Differences in coumarin sensitivity between ethnic groups already have been described before the effect of genetic factors became subject of study in coumarin users, several studies reporting that Chinese populations need lower warfarin dosages than Caucasian populations.[83,84] Recent studies examining different ethnic populations reported up to 40% lower mean warfarin dose requirements in Chinese and Japanese than in Caucasian populations.[79,80,85] This can be partly explained by differences in the occurrence of genetic variations of the *CYP2C9* and *VKORC1* genes. Of the most common variant alleles in Caucasian populations, *CYP2C9*2* (8-34%) and **3* (8-23%), the **2* allele is completely absent in Asians and less common in Afro Americans (2-8%), whereas the **3* allele exhibits lower frequencies in Asians (3-8%) and Afro Americans (1-4%).[86,87]
Recently resequencing of *CYP2C9* in Japanese patients revealed a haplotype distribution which differed from an earlier defined Caucasian one,[77] and discovered a number of novel non-synonymous SNPs (of which one null allele and three defective alleles) suggesting that other *CYP2C9* genetic variants than *CYP2C9*2* and **3* could potentially account for decreased warfarin dose requirements.[88]

Table 1: CONTRIBUTIONS OF THE *VKORC1* AND *CYP2C9* GENOTYPES AND OTHER FACTORS TO THE VARIABILITY IN COUMARIN DOSE REQUIREMENTS (EXPRESSED AS PERCENTAGE EXPLAINED VARIATION [R^2] IN MULTIPLE REGRESSION MODELS)

Coumarin	Population	Total model	*VKORC1*	*CYP2C9*	Other factors in the regression model	Ref.
Warfarin	Italian		13.8	21.5		(72)
Warfarin	Swedish	56	28.5	11.2	age 9.5, bodyweight 4.9, interacting drugs 3.7, target INR 1.5	(73)
Warfarin	Italian	54.2	5.0	17.5	age 16.7, height 16.0	(74)
Warfarin	USA[a]		21	6		(70)
Warfarin	USA[b]	51.4	28.8	10.2	age 3.7, bodyweight 11.4, CYP2C9 inhibiting drugs 3.7, CYP2C9 inducing drugs 2.3, smoking status 3.3, target INR 2.0, vitamin K intake 1.2, genotypes clotting factors: X I/D 2.0, VII D/D 1.3, factor C I/I 1.3	(75)
Warfarin	USA[b]	45	15.1	18.3	age, sex, and weight 12	(76)
Warfarin	Hong Kong Chinese	60.8	31	7.9	age 21.5, sex 0.4	(103)
Warfarin	Japanese	50	16.5	13.4	*CYP2C19* 4.5, interacting drugs 18.3, diagnosis 7.3	(78)
Warfarin	Chinese	46	18	7	age 16, sex 6	(79)
	Malay	46	37		age 4, sex 5	
	Indian	43	15	29	age-, sex -	
Warfarin	Japanese	33.3	5.9	5.2	genotype γ-glutamylcarboxylase 4.6, age 1.7, sex 8.1, weight 7.8	(91)
Warfarin	Japanese, Indian	60.2	34.9	13.0	age 17.4	(104)
Warfarin	Swedish	73	31.7	15.9	age 9.2, bodyweight 5.7, other genotypes: protein C 9 + epoxide hydrolase 1 + γ-glutamylcarboxylase + orosomucoid 1 gene 10	(92)
Warfarin	Israelian	63	21	20	age 9.6, weight 12.1	(105)
Acenocoumarol	French[c]	54	37[c]	13[c]	bodyweight 4	(81)
Acenocoumarol	Dutch	39	21.4	4.9	age 12.8	Ch. 4.2
Phenprocoumon	Dutch	54.7	28.7	7.2	age 14.1	Ch. 5.2

a) Study in different ethnic groups, percentages apply to Caucasian patients.
b) Ethnic groups not specified.
c) Reduction of factor VII in healthy volunteers.

Table 2: EFFECTS OF POLYMORPHISMS ON THE CLINICAL OUTCOMES BLEEDING AND OVERANTICOAGULATION IN USERS OF COUMARINS

Coumarin	Studied genotypes	Description of effect on clinical outcome	Reference
Warfarin	*CYP2C9*	increased risk of major bleeding and first INR>4 in low dose warfarin groups	(43)
Warfarin	*CYP2C9*	increased risk of overanticoagulation and bleeding in carriers of **2* or **3* allele	(44)
Warfarin	*CYP2C9*	increased bleeding risk in carriers of a **2* or **3* allele	(59)
Warfarin	*CYP2C9*	increased risk of overanticoagulation (INR>3) in carriers of **2* or **3* allele	(52)
Acenocoumarol	*CYP2C9*	increased risk of overanticoagulation (INR>6) in carriers of a **2* or **3* allele	(62)
Acenocoumarol	*CYP2C9*	increased risk of major bleeding in carriers of a **2* or **3* allele	(67)
Acenocoumarol	*CYP2C9*	increased risk of overanticoagulation (INR>4.5) in carriers of a **2* or **3* allele and less time spent within therapeutic range in carriers of a *3 allele	(61)
Phenprocoumon	*CYP2C9*	increased risk of bleeding in carriers of a *CYP2C9*3* allele	(64)
Phenprocoumon	*VKORC1*	increased risk of bleeding in carriers of the *VKORC1 C1173T* polymorphism	(82)
Acenocoumarol	*CYP2C9* + *VKORC1*	increased risk of overanticoagulation (INR>6) in carriers of a *CYP2C9*2* or **3* allele and a *VKORC1 C1173T* polymorphism	Chapter 4.2
Phenprocoumon	*CYP2C9* + *VKORC1*	increased risk of overanticoagulation (INR>6) in carriers of a *CYP2C9*2* or **3* allele and in homozygous carriers of a *VKORC1 C1173T* polymorphism	Chapter 5.2

Table 3: SELECTION OF OTHER GENETIC FACTORS THAN *CYP2C9* AND *VKORC1* WHICH COULD AFFECT COUMARIN RESPONSE [11]

Factor	Gene	Physiologic function
α-1-acid glycoprotein 1, orosomucoid 1	*ORM1*	carrier of warfarin in blood
α-1-acid glycoprotein 2, orosomucoid 2	*ORM2*	carrier of warfarin in blood
Apolipoprotein E	*APOE*	receptor binding ligand facilitating vitamin K uptake in liver
Epoxide hydrolase, microsomal	*EPHX1*	subunit of VKOR complex containing a vitamin K 2,3 epoxide binding site
NADPH dehydrogenase quinone 1	*NADPH*	contributes to the reduction of vitamin K-quinone to vitamin K-hydroquinone (Figure 2)
Calumenin	*CALU*	interferes with the transfer of reduced vitamin K-hydroquinone from VKOR to γ-glutamyl-carboxylase
γ-glutamyl carboxylase	*GGCX*	catalyses the carboxylation of vitamin K-dependent clotting factors
Clotting factor II (prothrombin)	*F2*	
Clotting factor VII	*F7*	
Clotting factor IX	*F9*	
Clotting factor X	*F10*	
Protein C	*PROC*	inactivates clotting factors Va and VIIIa together with protein S
Protein S	*PROS1*	inactivates clotting factors Va and VIIIa together with protein C
Protein Z	*PROZ*	contributes to the inactivation of clotting factor Xa

Variations in the *VKORC1* gene also show interethnic differences. Rieder et al. inferred five *VKORC1* haplotypes from 10 common SNPs and divided these into a low dose haplotype group A and a high dose haplotype group B. The frequency of haplogroup A was 89% in Asian Americans and 37% in European Americans.[70] Correspondingly, another study found that the *VKORC1 C1173T* polymorphism had a frequency of 89% in Japanese and 42% in Caucasian patients.[80] Because the *VKORC1 C1173T* or *G1639A* are more common in Asian subjects than *CYP2C9*2* or *3 alleles, the *VKORC1* genotype is probably more important for predicting lower or higher dose requirements than the *CYP2C9* genotype.[78]

A more detailed discussion of this subject going beyond the scope of this thesis, the main conclusion is that there are considerable interethnic differences in coumarin dose requirements and in distribution of *CYP2C9* and *VKORC1* genetic variants. The greater coumarin sensitivity, which in Asians could be partly explained by the high incidence of the *VKORC1 C1173T* polymorphism, should be kept in mind when Asian or African patients visit anticoagulation clinics.

Other variants of *CYP2C9* and *VKORC1*

Although the *CYP2C9*2* and **3* alleles are by far the most commonly studied variants of *CYP2C9*, many other variations of this gene have been identified (for updated information see the Human CYP Allele Nomenclature Committee homepage htpp://www.imm.ki.se/CYPalleles/). Veenstra et al. defined the CYP2C9 haplotype structure in European Americans.[77] From 60 common SNPs, 23 haplotypes were inferred which were divided into 6 major haplotype groups. Further analysis showed that only haplotypes bearing the **2* and **3* allele showed evidence for an increased warfarin sensitivity. These results suggest that other *CYP2C9* variants besides the **2* and **3* alleles will not add useful information on warfarin sensitivity in Caucasian populations.

As described in the former section, Rieder et al. defined five common *VKORC1* haplotype groups, which were predictive for warfarin dose requirements and which accounted for 96% of the total haplotypes in a Caucasian population. One *VKORC1* polymorphism such as *C1173T* is as informative as these five haplotype groups.[70]

In summary, the findings of these studies suggest that in research in Caucasian populations no more relevant clinical information will be obtained by assessing

other genetic variants of *CYP2C9* and *VKORC1* than *CYP2C9*2* or **3*, or *VKORC1 C1173T*, respectively.

Other genetic factors

Variants of genes encoding enzymes or transporters which affect the pharmacokinetics of coumarins or variants of genes encoding proteins in the vitamin K cycle could be of potential interest to coumarin sensitivity (Figure 2, Table 3). Several studies found small, but significant contributions of polymorphisms of the *GGCX* gene.[73,89-92] One study showed a large contribution of one polymorphism in the F7 gene, but it did not take the *VKORC1* genotype into account.[89] The contribution of the *APOE* genotype appears to be small.[93,94] Recently, Wadelius et al. developed a predictive regression model of *VKORC1*, *CYP2C9*2/*3*, *PROC*, age, bodyweight, and interacting drugs which explained 62% of the variability of warfarin dose requirements. Adding the genetic factors *EPHX1*, *GGCX*, and *ORM1-2* to the model increased the total part of the explained variability to 73%.[92]

Wadelius' study provided the best available model, predicting almost three quarter of warfarin dose requirements. However, the explained percentages of variance of several models with only the *VKORC1* and *CYP2C9*2/*3* genotypes and additional factors like age and bodyweight were not far behind (Table 1), suggesting that *VKORC1* and *CYP2C9* are the major predictive genetic factors involved.

For research into the impact of other genetic factors than *VKORC1* and *CYP2C9*, the candidate gene approach seems to be most appropriate. Since the contribution of coumarin transporters to coumarin sensitivity is relatively unknown, genes encoding such transporters are suitable candidate genes for further research. Other suitable and underexplored candidate genes are genes encoding the clotting factors II, VII, IX and X (Table 3).

DRUG-DRUG INTERACTIONS: FURTHER EVIDENCE AND RESEARCH

Setting

Our study in which we investigated the effects of SSRIs on the bleeding risk in users of acenocoumarol and phenprocoumon (Chapter 3.2) clearly showed that large data sets are required to quantify the risk of serious outcomes following the use of potential harmful and rarely occurring combinations of drugs. We used the PHARMO record linkage system, including complete medication histories of

more than 2 million community dwelling residents. However, apart from the merit of providing large numbers of patients and complete medication histories, the PHARMO record linkage system has several major limitations. Focusing on coumarin interactions, the main problem is the lack of data on INR measurements and target therapeutic ranges. Because major bleeding risks are associated with the anticoagulation intensity and with INR variability, both factors can be considered important confounders in epidemiological studies on major bleedings.[95-98] A second drawback is the lack of data on patient adherence and on comorbidities such as liver insufficiency, malignancies, and feverish diseases, also limiting our information on confouders. And finally, duration of coumarin use cannot be assessed in the PHARMO record linkage system because data on coumarin dosage are not available, increasing the risk of misclassification of coumarin users.

Anticoagulation clinics provide the best setting to conduct prospective studies on coumarin interactions, the medical records containing all INR measurements, target therapeutic range, bleeding events, recurrent thromboembolic events, comorbities, and relevant comedication, although improvement of information on the latter factor is a matter of concern (Chapter 2.1). Recording medical files from all Dutch anticoagulation clinics in a central database would provide marvellous opportunities for further research on many aspects of coumarin anticoagulation control, including the effect of interacting drugs. Efforts to realize centralization of medical data of anticoagulation clinics for the purpose of research should be strongly encouraged.

Genetic modification of drug-drug interaction effects

There are interindividual differences in the pharmacokinetic and pharmacodynamic effects of interacting drugs. It is possible that the consequences of taking an interacting drug are serious in one patient and hardly recognizable in another one. A recent pioneering study, evaluating the effect of nonsteroidal anti-inflammatory drugs (NSAIDs) on the anticoagulation status of acenocoumarol and phenprocoumon, revealed that NSAIDs which are also CYP2C9 substrates increased the risk of severe overanticoagulation in carriers of a *CYP2C9*2* or **3* allele, whereas wild-type patients were unaffected.[99] Since *CYP2C9*2* and **3* alleles occur in 20-35% of a Caucasian population, an effect on INR or (S)-coumarin plasma concentrations would probably have remained unnoticed in a regular pharmacokinetic study, the number of subjects being

usually between 10 to 20. This study clearly shows that the sensitivity for an interacting drug can be modified by the *CYP2C9* genotype.
Evaluating interaction effects in populations which are genotyped for *VKORC1* and *CYP2C9* could provide valuable information on the magnitude of interaction risks in different patient groups. It is conceivable that other CYP2C9 substrates also cause interaction effects in users of coumarins with *CYP2C9* variant alleles. The sulphonylureas are obvious candidates for further study, since they are CYP2C9 substrates[100] and several case reports described serious increased hypoglycaemic as well as anticoagulant effects when concurrently used with warfarin, whereas some studies showed no mutual interaction effects.[101]
It is also possible that effects of pharmacodynamically interacting, bleeding risk increasing drugs differ between patients with different *VKORC1* genotypes. One study demonstrated an increased bleeding risk in users of phenprocoumon with the *VKORC1 C1173T* polymorphism.[70] It would be interesting to examine whether the effect on the bleeding risk of drugs such as aspirin is modified by the *VKORC1* genotype.

SELF-MONITORING OF ORAL ANTICOAGULATION

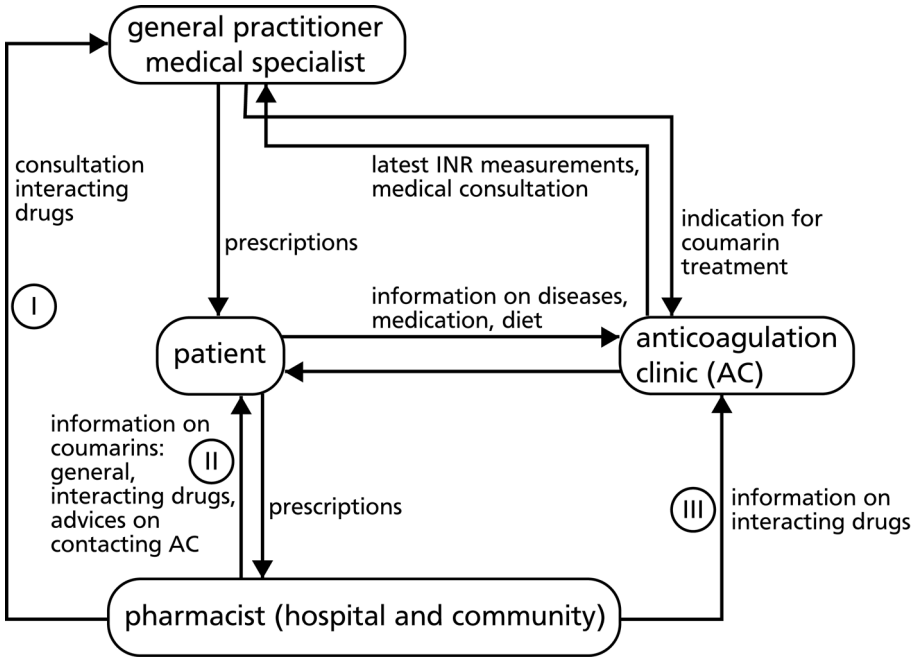
The role of self-monitoring of oral anticoagulation is increasing. A recent systematic review and meta-analysis demonstrated that self-management of coumarins can improve the quality of oral anticoagulation, decreasing the number of thromboembolic events and mortality.[102] Several of our studies demonstrated difficulties with coumarin management in carriers of variant alleles of *CYP2C9* and *VKORC1*. It is conceivable that the benefit of self-monitoring is greater in these patients at risk than in patients in whom regular management of coumarins is less problematic. So, it would be interesting to investigate whether knowledge of *VKORC1* and *CYP2C9* genotype preceding coumarin therapy could be helpful in selecting suitable candidates for self-monitoring.

ROLE OF THE PHARMACIST

Despite the major care-providing function of anticoagulation clinics in coumarin anticoagulation control, community and hospital pharmacists can play an important role as well. In 1999 the Dutch Committee for Coumarin-Drug Interactions finished its first edition of the Guidelines for Management of

Coumarin Interactions (accessible at www.fnt.nl). The Committee has been established in 1996 and consists of physicians from anticoagulation clinics and pharmacists. The main objective is to evaluate evidence for and to issue management guidelines on coumarin-drug interactions for prescribers as well as dispensing pharmacists. From 1999 these guidelines have been implemented in Dutch community pharmacies.

Figure 3: MUTUAL RELATIONSHIPS IN COUMARIN ANTICOAGULATION CONTROL



I) relationship pharmacist–physician;
II) relationship pharmacist–patient;
III) relationship pharmacist–anticoagulation clinic.

In Figure 3 the lines I, II, and III indicate the relationships between pharmacists and other care providers in coumarin anticoagulation control. Contacts with prescribing physicians (line I) mainly concern coumarin-drug interactions, in which pharmacists have full opportunities to display their competence as drug experts by advising equivalent alternatives for interacting drugs, guided by the above mentioned guidelines. Pharmacists should be aware of their responsibility to estimate the seriousness of interaction effects and to accentuate the need for avoidance of concurrent use of drugs such as miconazole and cotrimoxazole. Of course, contact with patients (line II) is one of the mainstays of daily practice in

community pharmacies. Although anticoagulation clinics provide excellent information, offering clear-written instructions to their patients (accessible at www.fnt.nl) and taking care to educate patients about coumarins, pharmacists can have an important additive role by educating patients as well, by stimulating therapy adherence, by advising about safe Over The Counter products and by being aware of potential causes of destabilization. For example, if a coumarin user asks for an antidiarrhoeal agent there is no drug-drug interaction, but there is potentially destabilizing diarrhoea for which a patient has to be referred to the anticoagulation clinic. The results of our study on discrepancies (Chapter 2) strongly suggest that direct contacts between pharmacies and anticoagulation clinics (line III) could contribute to the completeness of information on drug use in the latter's medical files.

Additionally, pharmacists could contribute to further insights into coumarin safety by reporting drug interaction effects to the Dutch Pharmacovigilance Foundation Lareb, but also to the Committee for Coumarin Drug Interactions (interacties@fnt.nl).

And finally, anticoagulant therapy should be on the agenda of pharmacotherapeutical consultation groups in which both general practitioners and pharmacists participate.

FUTURE RESEARCH

This thesis raised several questions, the most important being the following: is knowledge of *CYP2C9* and *VKORC1* genotypes a useful addition to INR monitoring? Although the effects of the *CYP2C9* and *VKORC1* genes on coumarin anticoagulation level are well established, prospective studies into the effects of knowledge of these genotypes preceding coumarin therapy are desperately missing. Designing and conducting such studies will be the major challenge for the coming years, perhaps more than conducting new studies aimed at elucidating effects of other genetic factors affecting the pharmacokinetics or pharmacodynamics of coumarins.

Even if we succeed in demonstrating that this knowledge contributes to safer drug therapy, coumarin-drug interactions will always affect intraindividual variability in coumarin response. As a consequence more studies on the effects of interacting drugs on the coumarin anticoagulation level remain to be necessary

and should be extended to research to genetic determined modification of drug interaction effects.

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SUMMARY

Since coumarins are drugs with a narrow therapeutic range, research into the many factors affecting their wide interindividual and intraindividual variability in dose requirements and anticoagulation response could contribute to a safer management of these therapeutically very effective drugs. The aim of this thesis was to increase our insights into the effects of drug interactions on coumarin anticoagulation control and into the effects of variations of the *CYP2C9* and *VKORC1* genes on the response of the coumarins acenocoumarol and phenprocoumon, which have been less extensively studied than the worldwide most applied coumarin warfarin. The studies of this thesis have been performed in several Dutch anticoagulation clinics, and in the PHARMO record linkage system, which includes the demographic details, complete medication history and hospital diagnoses of more than two million community dwelling residents in the Netherlands.

Chapter 1 gives an overview of the characteristics of the coumarins and of several aspects of coumarin-drug interactions. Moreover, we discuss the *CYP2C9* genotype, its potential contribution to coumarin sensitivity and the findings of other authors before we started our own studies. Finally, an outline of this thesis is given.

In **Chapter 2** a study is described in which we examined discrepancies between medication records of two anticoagulation clinics and pharmacy records. Because pharmacy records in the Netherlands are complete to nearly complete, they are a valid reference to compare medical records of anticoagulation clinics with. Of 117 interacting drugs registered in pharmacy records, 32 (27%) were not registered in the records of anticoagulation clinics. Among several patients of whom pharmacokinetically interacting drugs were not registered, the International Normalized Ratio (INR) exceeded the upper therapeutic range.

In **Chapter 3** studies are described in which we examined several coumarin-drug interactions. In **Chapter 3.1** we compared the effect of cotrimoxazole on coumarin anticoagulation control with the effect of several other antibiotics in stabilized patients of four anticoagulation clinics. We found that a preventive dose reduction (PDR) was more frequently applied in users of cotrimoxazole and that the applied PDR resulted in a significantly reduced risk of overanticoagulation. Among patients without PDR cotrimoxazole increased the risk of overanticoagulation more than other antibiotics. However, we also found

that all cotrimoxazole users spent significantly more time (range 2 to 7 days) under the therapeutic INR range during the first six weeks after the antibiotic course compared with other antibiotics. This is probably the consequence of the preventive as well as reactive coumarin dose reductions which are applied when cotrimoxazole is prescribed. We concluded that it is better to avoid cotrimoxazole in users of coumarins than to manage the interaction between coumarins and cotrimoxazole by preventive or reactive dose reductions. In **Chapter 3.2** we examined the risk of major bleeding associated with the use of serotonin reuptake inhibitors (SSRIs) among users of acenocoumarol or phenprocoumon and we compared this with the relative risk of bleeding due to use of non-steroidal anti-inflammatory drugs (NSAIDs) and antibiotics for which interactions with coumarins have been established in other studies. In this case-control study, nested within a cohort of users of acenocoumarol or phenprocoumon within the PHARMO record linkage system, we found that users of SSRIs had a significantly increased risk of hospitalization for non-gastrointestinal bleeding, odds ratio (OR) being 1.7, 95% confidence interval (CI) 1.1-2.5. This risk was comparable to the risk among users of NSAIDs (OR 1.7, 95% CI 1.3-2.2) and lower than the risk among users of antibiotics (OR 4.3, 95% CI 3.1-5.9). However, users of SSRIs showed no increased risk of gastrointestinal bleeding (OR 0.8, 95% CI 0.4-1.5), in contrast to users of NSAIDs (OR 4.3, 95% CI 3.1-5.9) and antibiotics (OR 2.8, 95% CI 1.7-4.6). In **Chapter 3.3** we described a nested case-control study which has been conducted within the same cohort of coumarin users. In this study we assessed the relative risk of bleeding due to use of the antiplatelet drugs clopidogrel and dipyridamole and compared this with the risk due to low dose aspirin, for which several studies have firmly established an increased risk of major bleeding among users of coumarins. We found that all antiplatelet drugs increased the risk of major bleeding, suggesting that clopidogrel and dipyridamole are not safer compared to low dose aspirin when concurrently used with coumarins. The risk of major bleeding was significantly increased among users of clopidogrel and aspirin (OR 2.9; 95% CI 1.2-6.9 and OR 1.6; 95% CI 1.3-1.9, respectively), whereas this risk showed a strong trend among users of dipyridamole and combinations of antiplatelet drugs (OR 1.5; 95% CI 1.0-2.3 and OR 1.8; 95% CI 1.0-3.3, respectively). The effects on the risk of upper gastrointestinal bleeding were higher than the effects on the risk of other bleedings.

In **Chapter 4** studies are described in which we examined the effects of *CYP2C9* and *VKORC1* polymorphisms on the anticoagulation status among users of acenocoumarol. The studies in this chapter have been conducted at two anticoagulation clinics in which acenocoumarol was the most frequently used coumarin. In **Chapter 4.1** we examined the effects of being carrier of the *CYP2C9* variant alleles *CYP2C9*2* or *CYP2C9*3* on time to achieve stable anticoagulation, severe overanticoagulation (INR>6), first INR on the 4th day of acenocoumarol therapy and acenocoumarol dose requirements. During the follow-up period of six months carriers of a *CYP2C9*3* allele had a lower chance to achieve stability (hazard ratio [HR] 0.6, 95% CI 0.4-0.9) and showed an increased risk of severe overanticoagulation (HR 3.8, 95% CI 1.5-9.4) compared with *CYP2C9*1/*1* patients. Moreover, carriers of a *CYP2C9*3* allele needed significantly lower dosages (20%) and had a significantly higher first INR. Within the period of this research project the *VKORC1* gene encoding the target protein of coumarins on the enzyme vitamin K epoxidase had been identified. In **Chapter 4.2** we examined the effects of the *VKORC1 C1173T* genotype and the *CYP2C9* genotype within the same cohort of acenocoumarol users. For the association between severe overanticoagulation and *CYP2C9* and *VKORC1* genotypes, we found effect modification between both genotypes. Only carriers of a combination of a *CYP2C9* polymorphism (**2* or **3* allele) and a *VKORC1* polymorphism had an increased risk of severe overanticoagulation compared with subjects with no polymorphism or only one polymorphism (HR 3.8, 95% CI 1.6-9.1). Patients with a *VKORC1* polymorphism needed significantly lower acenocoumarol dosages than *VKORC1 CC* wild-type patients, a larger part of the variability in dose requirement being explained by the *VKORC1* than by the *CYP2C9* genotype (21.4% and 4.9%, respectively). The combination of *VKORC1* and *CYP2C9* genotypes and age together explained almost 40% of the variation in acenocoumarol dose requirements. However, time to achieve stability was only associated with possession of the *CYP2C9*3* allele.

In **Chapter 5** two similar studies on the effects of *CYP2C9* and *VKORC1* polymorphisms on the anticoagulation status among users of phenprocoumon are described. In **Chapter 5.1** we found that being carrier of the *CYP2C9*2* or the *CYP2C9*3* variant allele was associated with a significantly increased risk of severe overanticoagulation (for **2* HR 3.1, 95% CI 1.6-6.1; and for **3* HR 2.4, 95% CI 1.0-5.6) compared to *CYP2C9*1/*1* patients. Carriers of a *CYP2C9*2*

or **3* allele needed significantly lower phenprocoumon dosages than *CYP2C9* wild-type subjects (difference 25-28%). Unexpectedly, only carriers of a *CYP2C9*2* allele had a decreased chance to achieve stability within the follow-up period (HR 0.6, 95% CI 0.4-0.9). In **Chapter 5.2** we examined the effects of the *VKORC1 C1173T* genotype and the *CYP2C9* genotype within the same cohort of phenprocoumon users. Again, we found effect modification between the *CYP2C9* and *VKORC1* genotypes, this time on the outcome phenprocoumon dose requirement. The effects of being carrier of a *CYP2C9*2* or **3* allele which we found in Chapter 5.1, appeared to apply mainly to patients with the *VKORC1 CC* wild-type. Among patients with the *VKORC1 CC* genotype, carriers of a *CYP2C9*2* or **3* allele needed nearly 30% lower dosages than *CYP2C9*1/*1* patients, whereas among patients with a *VKORC1* polymorphism differences between carriers of a *CYP2C9*2* or **3* allele and *CYP2C9*1/*1* were far smaller and largely not statistically significant. Carriers of a combination of a *VKORC1* polymorphism and a *CYP2C9* polymorphism had a strongly increased risk of severe overanticoagulation (HR 7.2, 95% CI 2.1-24.7), but, similar to what we saw in users of acenocoumarol, the chance to achieve stability within the follow-up period was only associated with the *CYP2C9* genotype. The *VKORC1* and *CYP2C9* genotypes and age together explained 55% of the variation in phenprocoumon dose requirements, the *VKORC1* genotype explaining a larger part of the variation than the *CYP2C9* genotype (28.7% and 7.2%, respectively).

In **Chapter 6** the economic consequences of *CYP2C9* genotyping in patients preceding acenocoumarol therapy are evaluated. For this analysis we designed a decision analytic model in which a hypothetical cohort of acenocoumarol using outpatients was followed during 12 months. We used a third party payer perspective and assessed under which conditions prevention of serious bleeding by *CYP2C9* genotyping could be cost-effective. Important factors within our model were: (1) incidence of serious bleeding in users of acenocoumarol; (2) relative risk of serious bleeding in carriers of a *CYP2C9*2* or **3* allele compared to patients with the *CYP2C9*1/*1* genotype; (3) cost of medical treatment of serious bleeding; (4) prevalence of *CYP2C9*2* or **3* alleles in the Dutch population; (5) costs of *CYP2C9* genotyping. Our assumptions for each of these factors was based on literature data and partly on data from Dutch laboratories (cost of genotyping). Our analyses showed that *CYP2C9* genotyping could be cost-effective, even when we assumed that genotyping would result in a

relatively modest 20% reduction of the incidence of major bleeding in carriers of a *CYP2C9* polymorphism. Our model appeared to be sensitive to all above mentioned factors. If *CYP2C9* genotyping would cost 20 to 30 Euro, there are many scenarios for which genotyping becomes the dominant strategy (cheaper and more effective than not genotyping). Our model could also be used for *VKORC1* genotyping. Since in Caucasian populations the percentage of carriers of a *VKORC1 C1773T* allele is higher than the percentage of carriers of a *CYP2C9*2* or **3* allele, cost-effectiveness would be more easily attained by *VKORC1* genotyping than by *CYP2C9* genotyping, provided that being carrier of a *VKORC1* polymorphism is similarly associated with an increased risk of major bleeding as being carrier of a *CYP2C9* polymorphism, which has hitherto not been demonstrated in studies.

In **Chapter 7** we summarized the results of our studies and placed them into the broader perspective of clinical implications and further research.

SAMENVATTING

Coumarinederivaten (coumarines) zijn geneesmiddelen die worden toegepast bij een verhoogde neiging tot bloedstolling, zoals bij trombose, sommige hartziekten (bijvoorbeeld boezemfibrilleren) en na implantatie van hartkleppen. In Nederland worden acenocoumarol en fenprocoumon gebruikt, wereldwijd wordt warfarine het meest toegepast. Hoewel deze middelen uitermate effectief zijn, is de therapeutische breedte klein: dat wil zeggen dat de dosering waarbij een therapeutisch gewenst antistollingseffect wordt bereikt, dicht ligt bij de dosering waarbij het effect te sterk of juist te zwak is. Bij een te sterke antistolling is de kans op ernstige bloedingen verhoogd, bij een te zwakke antistolling zijn coumarines niet effectief en blijft een verhoogd risico van trombose bestaan. De gevoeligheid voor coumarinederivaten verschilt sterk tussen individuen onderling (interindividuele variatie), maar kan bovendien ook binnen hetzelfde individu in de tijd variëren (intraindividuale variatie). Daarom moeten gebruikers van coumarines regelmatig worden gecontroleerd door trombosediensten, die de dosering bijstellen aan de hand van bepalingen van de mate van antistolling die wordt uitgedrukt in de International Normalized Ratio (INR). Er zijn twee instellingsgebieden: de eerste intensiteitsgroep (therapeutische INR 2,0-3,5) en de tweede intensiteitsgroep (therapeutische INR 2,5-4,0). Zelfs bij een goede instelling is het risico van bloedingen verhoogd, bij een INR hoger dan 6 is het bloedingsrisico sterk verhoogd.
Het doel van dit proefschrift was om het inzicht te vergroten in de effecten van andere geneesmiddelen (interacties) en in de effecten van variaties in twee genen, *CYP2C9* en *VKORC1*, op de antistollingsbehandeling met acenocoumarol en fenprocoumon.
De studies in dit proefschrift zijn deels uitgevoerd in samenwerking met diverse Nederlandse trombosediensten en deels in de Nederlandse PHARMO RLS database die demografische gegevens, volledige medicatiehistories en ziekenhuisdiagnoses bevat van meer dan twee miljoen Nederlanders.

In **Hoofdstuk 1** is een overzicht gegeven van de belangrijkste kenmerken van de coumarines en van algemene aspecten van interacties tussen coumarines en andere geneesmiddelen. Bovendien wordt in dit hoofdstuk het *CYP2C9* gen besproken, de mogelijke consequenties van variaties in dit gen voor de antistollingsbehandeling en bevindingen van andere onderzoekers naar de effecten van het *CYP2C9* genotype op antistollingsbehandeling met warfarine. Tenslotte wordt een algemeen overzicht van het proefschrift gegeven.

Omdat coumarines zeer gevoelig zijn voor interacties met andere geneesmiddelen, is het van groot belang dat trombosediensten goed op de hoogte zijn van het medicijngebruik van hun patiënten. In **Hoofdstuk 2** hebben wij de verschillen onderzocht tussen de medicatiegegevens zoals bekend bij de trombosediensten en die bij openbare apotheken. Omdat medicatiehistories in Nederlandse openbare apotheken nagenoeg compleet zijn, zijn zij bruikbaar als standaard om de medicatiegegevens van trombosediensten mee te vergelijken. Van de 117 geneesmiddelen met een belangrijke wisselwerking met coumarines die wij terugvonden in de medicatiehistories van apotheken, ontbraken er 32 (27%) in de medicatiegegevens van de trombosediensten. Bij verschillende patiënten van wie interagerende geneesmiddelen niet waren geregistreerd bij trombosediensten, werden te hoge INR waarden gevonden.

In **Hoofdstuk 3** worden studies beschreven waarin wij diverse geneesmiddelinteracties met coumarines nader hebben onderzocht. In **Hoofdstuk 3.1** vergeleken wij het effect van het antibioticum cotrimoxazol op de kwaliteit van de antistollingsbehandeling met het effect van enkele andere antibiotica bij patiënten die stabiel waren ingesteld op één van de coumarines acenocoumarol of fenprocoumon. Antibiotica worden voorgeschreven bij infecties die gepaard kunnen gaan met koorts. Koorts kan een antistollingsbehandeling ontregelen. Daarnaast kan het antibioticum cotrimoxazol de afbraak van coumarines via de lever remmen. Wij vonden dat een preventieve dosisreductie (PDR) vaker werd toegepast bij cotrimoxazolgebruikers en dat de toegepaste PDR resulteerde in een significant verminderd risico van te sterke antistolling (INR>6). Bij patiënten zonder PDR verhoogde cotrimoxazolgebruik het risico van te sterke antistolling meer dan gebruik van andere antibiotica. Echter, wij vonden eveneens dat cotrimoxazolgebruikers gedurende de eerste zes weken na de antibioticumkuur gedurende een significant langere tijd (2-7 dagen) onderbehandeld (te zwakke antistolling) waren in vergelijking met gebruikers van andere antibiotica. Dit is waarschijnlijk het gevolg van de relatief grote preventieve en reactieve verlagingen van de coumarinedoseringen die worden toegepast als cotrimoxazol wordt voorgeschreven. Onze conclusie is dat cotrimoxazol beter geheel kan worden vermeden bij coumarinegebruikers in plaats van te trachten de interactie tussen cotrimoxazol en coumarines af te handelen door dosisaanpassingen van het coumarine.
In **Hoofdstuk 3.2** hebben wij het risico van ernstige bloedingen onderzocht als gevolg van gelijktijdig gebruik van de selectieve serotonine heropnameremmers

(SSRIs) met coumarines. SSRIs zijn veel gebruikte geneesmiddelen bij depressie en angststoornissen, waarvoor in eerder onderzoek een verhoogde kans op maagbloedingen is gevonden. Als het gebruik van middelen op zichzelf gepaard gaat met een verhoogde kans op bloedingen, is het nuttig na te gaan of dit bij gelijktijdig gebruik met coumarines niet leidt tot een extra verhoogd bloedingsrisico. Wij onderzochten in deze studie eveneens het risico van ernstige bloedingen bij gelijktijdig gebruik van niet-steroïde anti-inflammatoire pijnstillers (NSAIDs) of antibiotica met coumarines. In deze studie in een groep van coumarinegebruikers binnen de PHARMO RLS database vonden wij dat gebruikers van SSRIs een 1,7× verhoogde kans hadden op ziekenhuisopname als gevolg van ernstige bloedingen buiten het maagdarmkanaal. Dit risico bleek vergelijkbaar met het risico voor gebruikers van NSAIDs (risico ook 1,7× verhoogd) en was lager dan het risico voor gebruikers van antibiotica (risico 4,3× verhoogd). Hoewel een verhoging van 1,7× niet veel lijkt, is dit voor coumarinegebruikers wel degelijk relevant, omdat zij door hun behandeling al een verhoogd bloedingsrisico hebben. Het risico van een bloeding in het maagdarmkanaal was bij gebruikers van SSRIs echter niet verhoogd, terwijl dit risico juist sterk verhoogd was bij gebruikers van NSAIDs (4,3×) en antibiotica (2,8×). In **Hoofdstuk 3.3** hebben wij het risico van ernstige bloedingen onderzocht als gevolg van gebruik van middelen die de samenklontering van bloedplaatjes remmen (plaatjesaggregatieremmers) en daardoor de kans op bloedingen bij gebruikers van coumarines theoretisch extra kunnen verhogen. In Nederland worden als plaatjesaggregatieremmers clopidogrel, dipyridamol en laaggedoseerd acetylsalicylzuur (ook bekend als Aspirine®) gebruikt. Van acetylsalicylzuur is al uit ander onderzoek bekend dat het bij gelijktijdig gebruik met coumarines het bloedingsrisico verder kan verhogen. Het doel van ons onderzoek was om ook het risico van gebruik van clopidogrel en dipyridamol vast te stellen, omdat deze middelen – met name clopidogrel – soms worden aanbevolen als alternatief voor acetylsalicylzuur als dit niet verdragen wordt. Wij vonden dat gebruik van alle plaatjesaggregatieremmers het bloedingsrisico bij coumarinegebruikers verhoogde. Bij gebruikers van clopidogrel, dipyridamol en acetylsalicylzuur was het risico van ernstige bloedingen respectievelijk 2,9×, 1,5× en 1,6× verhoogd. Bij gebruikers van een combinatie van plaatjesaggregatieremmers was het risico 1,8× verhoogd. Deze risicoverhoging was statistisch significant voor gebruikers van acetylsalicylzuur of clopidogrel en bereikte net geen statistische significantie voor gebruikers van dipyridamol en combinaties van plaatjesaggregatieremmers. De effecten op het risico van

bloedingen in het bovenste deel van het maagdarmkanaal waren voor alle plaatjesaggregatieremmers hoger dan de effecten op het risico van andere bloedingen.

In **Hoofdstuk 4** zijn twee studies beschreven waarin wij de effecten van polymorfismen van de genen *CYP2C9* en *VKORC1* op antistollingsbehandeling bij gebruikers van acenocoumarol hebben onderzocht. Het gen *CYP2C9* is een stukje erfelijk materiaal dat codeert voor het enzym CYP2C9. Coumarines worden in de lever 'afgebroken' door CYP2C9 en omgezet in onwerkzame afbraakproducten. Bij mensen met afwijkingen in dit gen ('polymorfismen') wordt een minder actief CYP2C9 geproduceerd, waardoor coumarines langer werken en theoretisch lagere doseringen van deze middelen nodig zijn. Het 'normale' gen wordt aangeduid als *CYP2C9*1*, de meest voorkomende polymorfismen bij mensen van het Kaukasische ('blanke') ras worden aangeduid als *CYP2C9*2* en *CYP2C9*3*; deze polymorfismen komen voor bij 8-19%, respectievelijk 4-16% van de Kaukasische populatie. Het gen *VKORC1* codeert voor het eiwit VKORC1 waarop coumarines aangrijpen om hun antistollingsactiviteit te kunnen ontplooien. Als er een polymorfisme van *VKORC1* aanwezig is, is er naar alle waarschijnlijkheid minder van het 'aangrijpingspunt' beschikbaar voor coumarines en zijn er dus theoretisch lagere doseringen nodig. Het meest informatieve polymorfisme wordt aangeduid als *VKORC1 C1173T*, kortweg *VKORC1 T*, het 'normale' gen wordt dan aangeduid als *VKORC1 C*. Het *VKORC1 T* polymorfisme komt voor bij 55-65% van de Kaukasische populatie. Ieder mens heeft voor elke eigenschap twee genen, zulke bij elkaar horende genen worden ook 'allelen' genoemd. Een genotype wordt weergegeven door beide allelen, bijvoorbeeld *CYP2C9*1/*1* (normaal genotype) of *CYP2C9*1/*3*. De drie mogelijke genotypen van *VKORC1* zijn: *VKORC1 CC* (normaal), *VKORC1 CT* en *VKORC1 TT*.
Omdat het *VKORC1* gen kort geleden is ontdekt nadat onze studies naar de effecten van *CYP2C9* op de antistollingsbehandeling al waren gepubliceerd, hebben wij in de hoofdstukken 4 en 5 eerst de studies naar de effecten van alleen het *CYP2C9* genotype opgenomen, en vervolgens de studies bij dezelfde patiëntengroepen naar de effecten van zowel het *CYP2C9* als het *VKORC1* genotype.
De studies in dit hoofdstuk zijn uitgevoerd bij twee trombosediensten waarin acenocoumarol het frequentst gebruikte coumarine was. In **Hoofdstuk 4.1** hebben wij de effecten onderzocht van het bezit van één of meer van de

CYP2C9 allelen *CYP2C9*2* of *CYP2C9*3* op de tijd om een stabiele antistolling te bereiken, op het optreden van zogenaamde 'doorgeschoten' antistolling (gedefinieerd als INR>6), op de eerste INR op de vierde dag van de acenocoumarolbehandeling en op de acenocoumaroldosering. De patiënten werden maximaal zes maanden gevolgd. Patiënten met een *CYP2C9*3* allel hadden een circa 40% kleinere kans om een stabiele antistolling te bereiken en een 3,8× hoger risico van doorgeschoten antistolling (INR>6) vergeleken met patiënten met het *CYP2C9*1/*1* genotype. Bovendien hadden dragers van een *CYP2C9*3* allel 20% lagere acenocoumaroldoseringen nodig en hadden zij een significant hogere eerste INR waarde op de vierde dag van de acenocoumaroltherapie.
In **Hoofdstuk 4.2** hebben wij de effecten van het *VKORC1 C1173T* genotype en het *CYP2C9* genotype onderzocht binnen hetzelfde cohort van acenocoumarolgebruikers. In dit onderzoek vonden wij dat beide genen elkaars effect beïnvloeden op een doorgeschoten antistolling (INR>6), dit verschijnsel wordt ook effectmodificatie genoemd. Alleen dragers van een combinatie van polymorfismen van *CYP2C9* (**2* of **3* allel) en *VKORC1* (21,2% van de studiepopulatie) hadden een verhoogd risico van doorgeschoten antistolling (3,8×) vergeleken met patiënten zonder polymorfisme of met slechts één polymorfisme van één van beide genen. Patiënten met een *VKORC1* polymorfisme hadden lagere acenocoumaroldoseringen nodig dan patiënten met het *VKORC1 CC* genotype; een groter deel van de variatie in dosisbehoefte werd verklaard door het *VKORC1* genotype dan door het *CYP2C9* genotype (respectievelijk 21,4% en 4,9%). De combinatie van *VKORC1* en *CYP2C9* genotype en leeftijd verklaarde bijna 40% van de variatie in dosisbehoefte van acenocoumarol. Echter, de tijd om een stabiele antistolling te bereiken was uitsluitend geassocieerd met het bezit van een *CYP2C9*3* allel, niet met het *VKORC1* genotype.

In **Hoofdstuk 5** zijn twee soortgelijke studies beschreven naar de effecten van *CYP2C9* en *VKORC1* polymorfismen op de antistollingsbehandeling bij gebruikers van fenprocoumon. In **Hoofdstuk 5.1** toonden wij aan dat het bezit van een *CYP2C9*2* of *CYP2C9*3* allel samenhangt met een verhoogd risico van doorgeschoten antistolling (INR>6) vergeleken met patiënten met het *CYP2C9*1/*1* genotype (voor **2* was dit risico 3,1× verhoogd, voor **3* 2,4×). Dragers van een *CYP2C9*2* of **3* allel hadden een 25-28% lagere fenprocoumon dosering nodig dan patiënten met het *CYP2C9*1/*1* genotype.

Enigszins onverwacht bleken alleen patiënten met een *CYP2C9*2* allel een circa 40% verlaagde kans op stabiele antistolling te hebben binnen de periode waarin ze werden gevolgd.
In **Hoofdstuk 5.2** onderzochten wij de effecten van het *VKORC1 C1173T* genotype en het *CYP2C9* genotype binnen hetzelfde cohort van fenprocoumongebruikers. Ook in deze studie vonden wij dat de *CYP2C9* en *VKORC1* genotypen elkaars effect beïnvloedden, nu op de dosering van fenprocoumon. Het effect van het bezit van een *CYP2C9*2* of **3* allel op de fenprocoumondosering dat wij hadden gevonden in hoofdstuk 5.1 bleek voornamelijk te gelden voor patiënten met het *VKORC1 CC* genotype (37,7% van de studiepopulatie). Binnen de groep patiënten met het *VKORC1 CC* genotype hadden dragers van een *CYP2C9*2* of **3* allel ruim 30% lagere fenprocoumondoseringen nodig dan patiënten met het *CYP2C9*1/*1* genotype. Bij patiënten met een *VKORC1* polymorfisme waren de verschillen in fenprocoumondosering tussen dragers van een *CYP2C9*2* of **3* allel en patiënten met het *CYP2C9*1/*1* genotype veel kleiner en in de meeste gevallen niet statistisch significant. Dragers van een combinatie van een *VKORC1* polymorfisme en een *CYP2C9* polymorfisme hadden een 7,2× verhoogd risico op ernstige overontstolling; de kans om binnen de periode waarover patiënten werden gevolgd (maximaal zes maanden) een stabiele antistolling te bereiken was alleen geassocieerd met het *CYP2C9* genotype, evenals bij acenocoumarolgebruikers. De combinatie van *VKORC1* en *CYP2C9* genotypen en leeftijd verklaarde ruim 55% van de variatie in fenprocoumondosering; hierbij verklaarde het *VKORC1* genotype een groter deel van de variatie dan het *CYP2C9* genotype (respectievelijk 28,7% en 7,2%).

In enkele studies is gevonden dat bij coumarinegebruik het risico van ernstige bloedingen bij dragers van een *CYP2C9*2* of **3* allel ruim 60% hoger ligt dan bij patiënten met het *CYP2C9*1/*1* genotype. Het is denkbaar dat kennis van het *CYP2C9* genotype voorafgaand aan een behandeling met een coumarine het bloedingsrisico bij dragers van een *CYP2C9*2* of **3* allel zou kunnen verlagen tot het niveau van patiënten met het *CYP2C9*1/*1* genotype.
Daarom hebben wij in **Hoofdstuk 6** de mogelijke economische consequenties onderzocht van het vaststellen van het *CYP2C9* genotype voorafgaand aan behandeling met acenocoumarol. Voor deze analyse werd een beslismodel ontwikkeld waarin een hypothetische groep van acenocoumarolgebruikers gedurende 12 maanden werd gevolgd. We gingen in dit model uit van het

perspectief van de (zorg)verzekeraar, waarbij we probeerden vast te stellen of het voorkómen van ernstige bloedingen door voorafgaande kennis van het *CYP2C9* genotype kosteneffectief was. Belangrijke factoren in ons model waren: (1) de incidentie van ernstige bloedingen bij acenocoumarolgebruikers; (2) het relatief risico van ernstige bloedingen bij dragers van een *CYP2C9*2* of **3* allel ten opzichte van patiënten met het *CYP2C9*1/*1* genotype; (3) de kosten van medische behandeling van een ernstige bloeding; (4) voorkomen van *CYP2C9*2* of **3* allelen in de Nederlandse populatie; (5) kosten van *CYP2C9* genotypering. De invulling van deze factoren hebben wij gebaseerd op literatuuronderzoek en voor een deel op gegevens van verschillende laboratoria, met name de kosten van genotypering van 55 Euro. Voor één belangrijke factor moest een aanname worden gedaan: de mogelijke reductie van het risico van ernstige bloedingen bij dragers van een *CYP2C9*2* of **3* allel. Wij stelden deze risicoreductie in eerste instantie op 20%. In ons zogenaamde economische basismodel kwamen wij uit op een bedrag van 4233 Euro om één ernstige bloeding te voorkomen. Omdat wij aannamen dat circa 4000 Euro een aanvaardbaar bedrag zou zijn voor het voorkomen van een ernstige bloeding, leverde ons basismodel net geen kosteneffectiviteit op. Echter, ons model bleek gevoelig voor alle bovengenoemde factoren en met name bij lagere kosten van genotypering (20 tot 30 Euro, wat volgens de huidige inzichten haalbaar is) zijn er veel scenario's denkbaar waarin *CYP2C9* genotypering kosteneffectief is. Een groot aantal van deze scenario's hebben wij weergegeven in hoofdstuk 6. Zo zou in ons basismodel genotypering al de dominante strategie worden als het 30 Euro (in plaats van 55 Euro) zou kosten (dominant: genotyperen is even effectief en uiteindelijk goedkoper dan niet genotyperen, met andere woorden genotyperen kost de verzekeraar minder dan het behandelen van de bloedingen die zouden worden 'gemist' zonder genotypering!). Als de prijs van genotypering 20 Euro zou zijn, zou genotyperen zelfs al dominant worden bij een reductie van het bloedingsrisico bij dragers van een *CYP2C9*2* of **3* allel met slechts 15%.
Ons model zou ook gebruikt kunnen worden voor de analyse van *VKORC1* genotypering voorafgaand aan acenocoumaroltherapie. Omdat in Kaukasische populaties het percentage dragers van een *VKORC1 C1173T* allel hoger is dan het percentage dragers van een *CYP2C9*2* of **3* allel, wordt kosteneffectiviteit wellicht gemakkelijker bereikt met *VKORC1* genotypering dan met *CYP2C9* genotypering. Voorwaarde voor deze aanname is wel dat het bezit van een *VKORC1* polymorfisme in ongeveer dezelfde mate is geassocieerd met

bloedingen als het bezit van een *CYP2C9* polymorfisme. Dit laatste wordt wel aangenomen, maar is nog niet uit onderzoek gebleken.

In **Hoofdstuk 7** tenslotte hebben wij de resultaten van onze studies samengevat en in een breder perspectief besproken. Wij hebben aangegeven dat met name onderzoek nodig is naar de mogelijkheid om met kennis van het *CYP2C9* en *VKORC1* genotype de effectiviteit en veiligheid van behandeling met coumarines te verbeteren. Tevens hebben wij aangegeven dat meer onderzoek naar de gevolgen van interacties met coumarines in grote patiëntenpopulaties gewenst is.

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Voor dit onderzoek heb ik veel samengewerkt met trombosediensten in Nederland. Ik ben erg onder de indruk geraakt van de professionele wijze waarop deze instellingen zorg verlenen aan patiënten. Het vele dat ik heb geleerd over de dagelijkse praktijk van de begeleiding van coumarinegebruik is één van de leukste aspecten van dit onderzoekstraject geweest. De eerste die ik in dit verband graag wil bedanken is Hanneke de Vries-Goldschmeding, internist en hematoloog en directeur van de trombosedienst in Utrecht. Beste Hanneke, jij bent mijn mentrix geworden op het gebied van coumarinebehandeling. Onze eerste contacten dateren al van 1994 toen ik voor een klein onderzoek bij patiënten van onze apotheek met jou heb samengewerkt. Later heb jij ondersteund dat ik lid werd van de Commissie Interacterende Medicatie Coumarines, waarvan je een zeer inspirerende voorzitter was. Het is vooral aan jouw inzet te danken dat dit geleid heeft tot een landelijke standaard die nu door alle Nederlandse trombosediensten en apotheken wordt gebruikt. De geduldige, deskundige en vriendelijke wijze waarop je mijn vele vragen hebt beantwoord, heb ik altijd zeer op prijs gesteld. Ik ben er trots op dat je medeauteur bent van drie artikelen in dit proefschrift en dat ik een bijdrage mocht leveren aan je afscheidssymposium als directeur van de trombosedienst Utrecht.

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LIST OF PUBLICATIONS
related to the thesis

PUBLICATIONS RELATED TO THE THESIS

Schalekamp T, Smit C, van Geest-Daalderop JHH, de Vries-Goldschmeding H, de Boer A. Discrepancies between medication records of anticoagulation clinics and pharmacy records. Pharmacoepidemiol Drug Saf 2006;15:823-8.

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Tom Schalekamp was born on 7 March 1954 in The Hague, the Netherlands.
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Tom lives in Odijk together with his wife Tineke. They have one daughter, Marianne, and three sons, Paul, Frank, and Wouter.