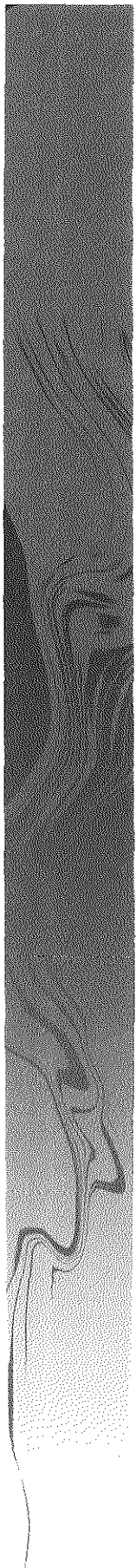
OVERANTICOAGULATION ON COUMARIN ANTICOAGULANTS



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Contents

MANUSCRIPTS BASED ON THE WORK PRESENTED IN THIS THESIS

Chapter 2

Penning-van Beest FJA, Rosendaal FR, Grobbee DE, van Meegen E, Stricker BHCh. Course of the International Normalized Ratio in response to oral vitamin K_1 in patients overanticoagulated with phenprocoumon. Br J Haematol 1999;104: 241-5.

Chapter 3

Penning-van Beest FJA, van Meegen E, Rosendaal FR, Stricker BHCh. Characteristics of anticoagulant therapy and comorbidity related to overanticoagulation. Thromb Haemost 2001;86:569-74.

Penning-van Beest FJA, van Meegen E, Rosendaal FR, Stricker BHCh. Drug interactions as a cause of overanticoagulation on phenprocoumon or acenocoumarol predominantly concern antibacterial drugs. Clin Pharmacol Ther 2001; 69:451-7.

Visser LE, Penning-van Beest FJA, Kasbergen AAH, De Smet PAGM, Vulto AG, Hofman A, Stricker BHCh. Overanticoagulation associated with combined use of antibacterial drugs and coumarin anticoagulants. (submitted)

Penning-van Beest FJA, Geleijnse JM, van Meegen E, Vermeer C, Rosendaal FR, Stricker BHCh. Lifestyle and diet as risk factors for overanticoagulation. J Clin Epidemiol (in press)

Penning-van Beest FJA, Visser LE, Geleijnse JM, Vermeer C, Kasbergen AAH, Hofman A, Stricker BHCh. Deficient dietary intake of vitamin K is associated with an increased risk of overanticoagulation. (submitted)

Chapter 4

Penning-van Beest FJA, Gómez García EB, van der Meer FJM, van Meegen E, Rosendaal FR, Stricker BHCh. Levels of vitamin K-dependent pro- and anticoagulant proteins in overanticoagulated patients. (submitted)

Chapter 5

Penning-van Beest FJA, van Meegen E, Rosendaal FR, Stricker BHCh. Phenprocoumon is superior to acenocoumarol in oral anticoagulant therapy lasting six weeks or more. (submitted)

1

General introduction

Oral anticoagulant therapy

History of development and present use of coumarin anticoagulants

The history of the discovery and development of coumarin anticoagulants started in the 1920s in North Dakota, USA and Alberta, Canada, when cattle fed moldy sweet clover were struck by a new type of serious, hemorrhagic disease. In 1939, Campbell and Link identified the hemorrhagic agent as dicoumarol and in 1941 it was studied as an anticoagulant agent in patients at the Mayo Clinic: its use reduced postoperative thrombosis. However, partly due to fear of unacceptable toxicity, it was not until the 1950s that coumarin derivatives were introduced into medicine (1). The use of coumarins was stimulated by president Eisenhower's treatment with warfarin after a myocardial infarction. At present, oral anticoagulation with coumarin derivatives is an established therapy for a variety of indications (2-8). Well-designed clinical trials have shown that oral anticoagulant therapy is clinically effective in the primary and secondary prevention of venous thromboembolism, in the prevention of systemic arterial embolism in patients with atrial fibrillation or tissue- and mechanical prosthetic heart valves, and in the prevention of stroke, recurrent infarction and death after acute myocardial infarction. Anticoagulants are also recommended in patients with rheumatic heart disease or dilated cardiomyopathy, although for these indications their efficacy has never been demonstrated in randomized trials.

For the acute treatment of thromboembolic disease and for the prevention of postoperative thrombosis, heparin or its derivatives are also used since this drug has an immediate anticoagulant effect. It increases the activity of antithrombin and consequently neutralizes several activated coagulation proteins. However, long-term therapy with heparin is associated with osteoporosis. In addition, daily injections are unpleasant for the patient. Therefore, after the acute phase, therapy with heparin is usually replaced by therapy with coumarins. Other drugs used for prevention or treatment of various thromboembolic disorders are drugs

which inhibit platelet aggregation and thrombolytics which promote the dissolution of thrombi by stimulating the activation of endogenous plasminogen to plasmin (9). Therapy with heparin, platelet inhibiting drugs and thrombolytic drugs are beyond the scope of this thesis and will not be discussed further.

MECHANISM OF ACTION OF COUMARIN ANTICOAGULANTS

Coumarin anticoagulants induce anticoagulation by antagonizing vitamin K. As a cofactor, vitamin K is required for the posttranslational carboxylation of glutamate residues on the N-terminal regions of the vitamin K-dependent coagulation proteins (factor II, VII, IX and X, protein S and protein C) into γ-carboxyglutamate (Gla) residues. The process of γ-carboxylation allows the coagulation proteins to undergo a conformational change in the presence of calcium ions, a necessary requirement for binding to phospholipids on blood platelets and endothelial cells at the site of injury. The carboxylation reaction is catalyzed by a carboxylase that requires the reduced form of vitamin K (vitamin KH_2), molecular oxygen and carbon dioxide. During this reaction, Gla residues are formed and vitamin KH_2 is oxidized to vitamin K epoxide (vitamin KO). Vitamin KO is recycled to vitamin K by a dithiol-dependent vitamin KO reductase and vitamin K, in turn, is reduced to vitamin KH_2 by a dithiol-dependent vitamin K reductase. The vitamin K cycle is depicted in figure 1. Coumarin anticoagulants inhibit vitamin KO reductase and vitamin K reductase, resulting in the accumulation of vitamin KO in the liver and plasma and the depletion of vitamin KH_2. The decrease in vitamin KH_2 impairs the γ-carboxylation and thereby the biological activity of the vitamin K-dependent coagulation proteins (10).

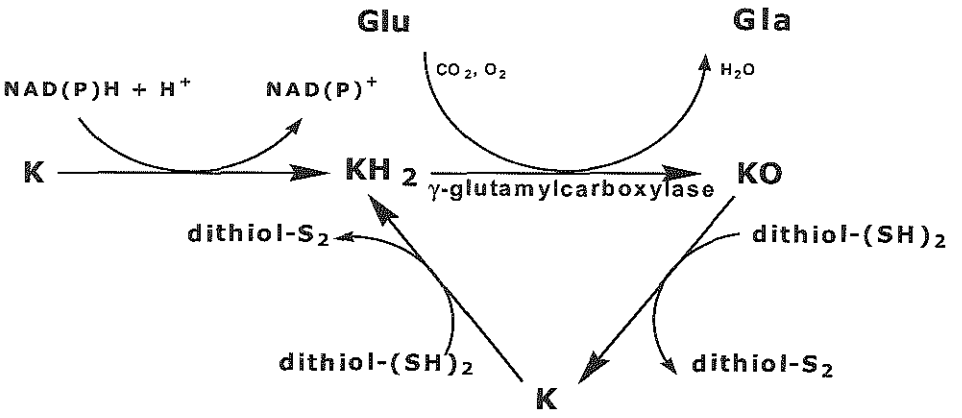

Figure 1. The vitamin K cycle.

Vitamin K can also be reduced to vitamin KH_2 by a NAD(P)H-dependent vitamin K reductase. This enzyme is insensitive to coumarins and is therefore of vital importance in situations requiring reduction of the effect of coumarins. Since NAD(P)H-dependent vitamin K reductase does not reduce vitamin KO, the vitamin K cycle will not be completed and vitamin K can only be used once. Hence, large amounts of vitamin K are required to reduce anticoagulation (10).

The rate of disappearance from the plasma of the vitamin K-dependent coagulation proteins is determined by their half-lives which range from 5 hours for factor VII to 96 hours for factor II (11). In patients on long-term oral anticoagulant therapy at therapeutic levels of anticoagulation, factor X is most strongly, and factor IX is least strongly reduced (12-15). However, there are no reports which focus on the levels of these factors in overanticoagulated patients.

Pharmacokinetics of coumarin anticoagulants

Since the discovery of dicoumarol, several hundred derivatives of coumarin or the related compound indandione have been synthesized. Today, mainly the 4-hydroxycoumarin anticoagulants are in use. Because the toxicity of indandione derivatives is greater than that of the coumarin derivatives, their use is no longer recommended (9). Of the 4-hydroxycoumarin anticoagulants, warfarin is commonly prescribed in the Anglo-Saxon and Scandinavian countries. Acenocoumarol and phenprocoumon are mainly used in Western continental Europe. The structural formulas of warfarin, acenocoumarol and phenprocoumon are shown in figure 2. Whereas the mechanism of action of these three drugs is similar, their pharmacokinetics differ. Absorption from the gastrointestinal tract is rapid and complete. Plasma protein binding, primarily to albumin, is 98% for acenocoumarol and more than 99% for warfarin and phenprocoumon (16). All three drugs are metabolized in the liver by the cytochrome P450 system, especially cytochrome P450 2C9, and the metabolites are predominantly excreted via the kidneys. The relative contribution of cytochrome P450 2C9 to the metabolism differs between the three coumarins as a consequence of the difference in structure. This enzyme plays a more important role in the oxidation of warfarin

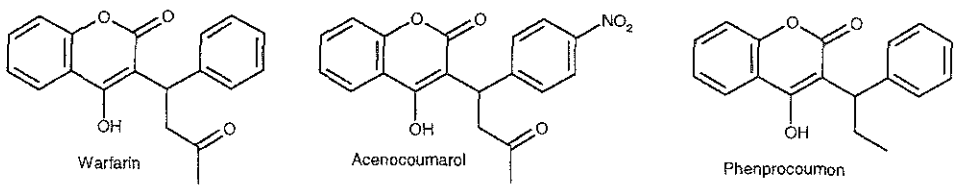

Figure 2. Structural formulas of warfarin, acenocoumarol and phenprocoumon.

and acenocoumarol, than in the oxidation of phenprocoumon. Phenprocoumon is also, for approximately one-third, eliminated as free or conjugated parent drug (16-20). The elimination half-lives of the coumarins vary widely and thereby the duration and onset of effect. Acenocoumarol is a short-acting anticoagulant with a half-life of 8 to 12 hours. Phenprocoumon has a half-life of 65 to 170 hours and so is a long-acting drug and warfarin has an intermediate duration of effect and a half-life of 10 to 45 hours (9, 16). Studies have shown that the use of phenprocoumon or warfarin gives a more stable anticoagulation than the use of acenocoumarol (21-23). This may be explained by the wide fluctuations in the plasma levels of factor VII induced by acenocoumarol because of its short half-life (24, 25). However, it is not known whether the advantage of the long-acting coumarins also applies to the initiation phase of therapy.

Patients may require different doses of coumarins to reach the same level of anticoagulation. The daily maintenance dose of acenocoumarol ranges from 1 to 8 mg, of phenprocoumon from 0.75 to 6 mg and of warfarin from 2 to 10 mg (9). Besides an interindividual variation in dose, the required dose may also vary over time in an individual patient. Since underanticoagulation is ineffective and overanticoagulation may lead to hemorrhage, anticoagulant therapy needs to be monitored and adjusted to steer safely between the Scylla of thrombosis and the Charybdis of bleeding (26).

MONITORING OF ANTICOAGULANT THERAPY

The laboratory test used most often to monitor the intensity of anticoagulant treatment is the one-stage prothrombin time test, introduced by Quick in 1935. This test is sensitive to the reduction of factor II, VII and X. It measures the clotting time of citrated plasma after the addition of thromboplastin, a tissue extract which contains both the tissue factor and the phospholipid necessary to promote the activation of factor X by factor VII. Since the many thromboplastins in current use are manufactured by different methods, they vary in sensitivity to the reduction of vitamin K dependent coagulation proteins. As a result, a similar prothrombin time may reflect very different anticoagulant intensities when different thromboplastins are used. In an attempt to standardize the reporting of prothrombin time in oral anticoagulant control, the International Normalized Ratio (INR) system was introduced in 1983. This system is based on the first primary WHO international reference preparation of thromboplastin, against which every new thromboplastin is calibrated. This way, every prothrombin ratio measured by a calibrated thromboplastin can be converted into an INR, according to the formula 'INR = observed prothrombin time ratio ^{ISI}', where ISI is the International Sensitivity Index, a measure of the responsiveness of the thromboplastin preparation to the reduction of vitamin K dependent coagulation proteins (27). The optimal target range of oral anticoagulant therapy, as recommended by the

Federation of Dutch Thrombosis Centers, lies between an INR of 2.5 and 3.5, or between 3.0 and 4.0 (28, 29), depending on the indication for treatment. The necessary duration of treatment ranges from four weeks to lifelong.

Adequate monitoring of anticoagulant therapy requires experience and specialization. To this purpose, anticoagulation clinics emerged as early as the 1950s in the Netherlands and more recently in Italy, Canada and the United States. The Netherlands have a network of 65 independently operating clinics of which the areas of care cover over 90% of the Dutch population. A specialized system of monitoring has been developed (30, 31), in which nurses who are trained in anticoagulant therapy control play a central role. At the start of therapy, they instruct each patient about anticoagulant therapy. Among other things, the patient is told to inform the clinic about changes in comedication, intercurrent illnesses and bleeding complications. At several outpatient facilities, or when necessary at the patient's home, they collect blood samples. After prothrombin times are assessed at the clinic, dosing of the coumarin is performed by a team of specialized physicians, often with the aid of a computerized dosing program. This program evaluates the stability of the INR and, when possible, proposes a dosing schedule (i.e. in nearly 50% of the patients). In the other patients, dosing is done by the physician according to a standard operating procedure. The dose is printed on a dosage list which the patient receives by mail the next day. If necessary, the patient is telephoned the same day for dose adjustment or vitamin K prescription. The control period depends on the stability of the anticoagulant level and is six weeks at a maximum. Routine audit of the quality of oral anticoagulant therapy, such as the percentage of patients within the target range, is performed at monthly intervals.

Overanticoagulation

Despite monitoring of anticoagulant therapy, overanticoagulation may occur. The response to coumarins may be enhanced by factors influencing the absorption, distribution or elimination of the anticoagulant or vitamin K, or affecting the synthesis, function or clearance of the vitamin K-dependent coagulation proteins (32). Based on case reports and small-scale experiments, a considerable number of drug interactions with coumarin anticoagulants have been reported and summarized (32-35). Critical periods are when a patient stabilized on an anticoagulant commences treatment with an interacting drug or when a patient stabilized on a regimen of an interacting drug and an anticoagulant has the interacting drug withdrawn. In addition, a number of comorbid conditions, among which hepatic dysfunction, hypermetabolic states and congestive heart failure, have been postulated as interfering with oral anticoagulant therapy (33, 36-38). Furthermore, increasing age and female gender have been associated with an enhanced response to coumarins (39). Overanticoagulation after

a dietary modification reducing the intake of vitamin K has been described (40, 41). Large epidemiological studies on the incidence of and risk factors for overanticoagulation in a non-selected population under everyday circumstances, however, are scarce.

In the absence of life-threatening hemorrhagic complications, the usual way to achieve a reduction in anticoagulant effect is to discontinue the drug for two or more days and, depending on the intensity of the INR, administer 1 to 10 mg of vitamin K_1 orally. Anticoagulant therapy is resumed at a lower dose and the INR is measured again within a week (36). Detailed information on changes in the INR in response to vitamin K_1 is not available as INR-values usually are not measured daily. Since reversal of anticoagulation by vitamin K requires synthesis of fully carboxylated coagulation proteins, 24 hours may be needed for a significant reduction of the INR. In case of a serious hemorrhage, the patient should be transfused with prothrombin complex concentrate, supplemented with vitamin K, in order to rapidly reverse the INR (36, 42).

ADVERSE EFFECTS OF COUMARIN ANTICOAGULANTS

Inherent to the mode of action and narrow therapeutic index of coumarin anticoagulants, hemorrhage is the most common adverse reaction and may cause serious morbidity and mortality. It has been reported in virtually every body cavity or organ, the commonest sites being the gastrointestinal tract, the urinary tract, the soft tissues and the oropharynx. The bleeding rate is 7.6 to 16.5 per 100 patient-years of treatment. Major or life-threatening hemorrhage occurs at a rate of 1.4 to 3.6 per 100 patient-years. The duration of anticoagulant therapy and the intensity of anticoagulation are critical determinants of the risk of anticoagulant-associated bleeding. The most important patient-specific risk factors for bleeding are comorbid illness (especially heart-, kidney-, liver- and cerebrovascular disease) and the use of drugs which interfere with hemostasis, e.g. platelet aggregation inhibiting drugs such as the salicylates (29, 43-45). In addition, hepatic injury is a serious, but probably uncommon adverse effect. The first report on coumarin-associated hepatic injury appeared in the German literature in 1963 (46). In the intervening period to date, several case reports have been published, mostly in relation to use of phenprocoumon or warfarin and rarely to acenocoumarol (47-52). Liver damage, mostly hepatocellular damage, manifested two to six months after the start of therapy. This long interval might be compatible with the accumulation of a toxic metabolite in the liver. Other rare but serious complications of coumarins are skin and soft tissue necrosis and the 'purple toes' syndrome (53, 54). Skin and soft tissue necrosis may appear from day three to six after beginning treatment. It occurs in approximately 0.1% of patients, mostly women. The affected site often concerns fatty areas such as breasts, thighs and buttocks. Both heterozygous- and acquired functional protein

C and S deficiency as well as antithrombin III deficiency are risk factors for coumarin-induced skin necrosis (53). The 'purple toes' syndrome has been thought to result from cholesterol embolization (54). Furthermore, since vitamin K is also involved in bone metabolism, the use of coumarin anticoagulants may affect bones (55). A substantial effect of coumarins on young, rapidly growing bone has been proven (56, 57). In adults, the use of coumarin anticoagulants is associated with an increased concentration of undercarboxylated bone proteins (58-61), but the effect on bone mineral density is controversial (62). Use of coumarins during pregnancy poses teratogenic and hemorrhagic risks for the fetus. Warfarin embryopathy, a syndrome characterized by nasal hypoplasia or stippled epiphyses, may result from maternal use of coumarins during the first trimester of pregnancy. Central nervous system abnormalities have been reported following exposure to coumarins at any time during pregnancy (63, 64). In a large cohort study among school-age children the long-term effects of in utero exposure to coumarins were examined. The risk for minor neurological dysfunction was increased and behavioural development was negatively influenced (65, 66). Cognitive functioning and growth in the absence of warfarin embryopathy, were not affected (67, 68). A positive side effect of coumarin anticoagulants which has been postulated is the anti-tumor action. Coumarins appear to reduce the size of small-cell carcinoma of the lung and consequently prolong survival times (69, 70).

Aim and outline of this thesis

Most of the extensive research on oral anticoagulant therapy has focussed on its pharmacological- and biochemical action, prothrombin time calibration, optimal therapeutic intensity and hemorrhagic complications. However, while the risks of overanticoagulation are clear, its treatment and determinants have received little attention. Therefore, the aim of this thesis was to study aspects of overanticoagulation on coumarin anticoagulants among outpatients of an anticoagulation clinic. Overanticoagulation was defined as an INR $\geq$6.0, since at this INR-value the risk of hemorrhage sharply increases (28, 41). Chapter 2 relates to the treatment of overanticoagulation and describes the course of the INR in response to oral vitamin K_1 in overanticoagulated patients. Chapter 3 concerns the incidence of and risk factors for overanticoagulation and includes five studies. Chapter 3.1 focusses on characteristics of anticoagulant therapy and comorbidity associated with overanticoagulation, chapters 3.2 and 3.3 on drug interactions as a cause of overanticoagulation, and chapters 3.4 and 3.5 on lifestyle and diet as risk factors for overanticoagulation. The levels of the vitamin K-dependent pro- and anticoagulant proteins in overanticoagulated patients are evaluated in chapter 4. In chapter 5, several measures of the quality of oral anticoagulant therapy, among which the occurrence of overanticoagulation,

are compared between phenprocoumon and acenocoumarol. Finally, in chapter 6 the main findings are presented, methodological issues and implications for oral anticoagulant therapy are discussed, and recommendations for future research are given.

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2

Treatment of overanticoagulation

Course of the International Normalized Ratio in response to oral vitamin K_1 in patients overanticoagulated with phenprocoumon

ABSTRACT *Oral vitamin K_1 is used for the treatment of excessive anticoagulation. Detailed information on changes in the International Normalized Ratio (INR) in response to vitamin K_1 is not available. We therefore measured the INR on the first seven days following the oral intake of 1 to 5 mg of vitamin K_1 in 24 patients routinely treated with phenprocoumon who had an INR $\geq$6.0 at presentation. The first two days after administration of vitamin K_1 the mean INR decreased by 40% and 23%, respectively. After day 2, the day-to-day proportional change in the mean INR depended on the dose of vitamin K_1 and varied from a decrease of 12% to an increase of 21%. On day 7, the mean INR was higher than on day 2 in three out of five treatment groups. Between day 2 and day 7, in general, 32% of the patients had an INR within the therapeutic zone, 25% had an INR $\geq$6.0 and 8% had an INR <2.0. These findings suggest that our routine treatment of overanticoagulation in patients on phenprocoumon should be intensified to improve its efficacy.*

INTRODUCTION

Coumarin anticoagulants have a narrow therapeutic index (1). The optimal target range of oral anticoagulant therapy, as recommended by the Federation of Dutch Thrombosis Centers, lies between 2.5 and 3.5 International Normalized Ratio (INR) or between 3.0 and 4.0 INR (2, 3), depending on the indication for treatment. When the INR is $\geq$6.0, the risk of bleeding, the most common adverse reaction to coumarin anticoagulants, sharply increases (3). Hence, such an excess anticoagulant effect should be treated promptly and adequately.

In the absence of life-threatening hemorrhagic complications, the usual way to achieve a reduction in anticoagulant effect is to discontinue the drug for two or more days and, depending on the intensity of the INR, administer 1 to 10 mg of vitamin K_1 orally (4). Detailed information on changes in the INR in response to vitamin K_1 is not available since INR-values are usually not measured daily, but only after a week. The literature provides limited information: two studies have been performed on the response of the INR to oral vitamin K_1 (5, 6). In one (5), the INR was measured twice: one or two days after administration of vitamin K_1 and four to seven days after warfarin therapy was resumed. In the other study (6) the INR was determined after one, two and nine days. These studies both focussed on efficacy and expressed the results in the number of patients with an INR below or above 5.0. No data were given on changes in INR over time. Harrell and Kline (7) reported on five patients in whom oral vitamin K_1 was used to treat overanticoagulation. After one or two days the INR had decreased by 58% to 89%.

To obtain detailed insight into the course of the INR after oral administration of vitamin K_1 and to test the efficacy of our routine treatment of overanticoagulation, we determined the INR on the next seven days in patients on phenprocoumon who had an INR $\geq$6.0 at presentation.

METHODS

Patients treated with phenprocoumon (Marcoumar®) by the regional Red Cross anticoagulation clinic The Hague who were prescribed oral vitamin K_1 because of an INR $\geq$6.0 between June 9, 1997 and June 27, 1997, were asked to participate in this prospective study. Patients treated with vitamin K_1 because of a subsequent medical intervention or whose prothrombin time had to be checked within seven days were excluded.

On day 0 patients suspended phenprocoumon therapy and took an oral dose of vitamin K_1 (Konakion® Cremophor EL based drop solution 20 mg/ml). This dose was determined by the INR and the target range of anticoagulation, according to an algorithm employed at the anticoagulation clinic (table 1). Because of individual factors, the algorithm could be deviated from. The required number of

drops were taken with about half a cup of water. The day at which the patient restarted phenprocoumon, as well as the adjustment of the dosage, depended on the patient's individual circumstances: standard practice was to restart phenprocoumon on day 2 and to lower the dosage by about 15%. On the next seven days after taking vitamin K_1, day 1 to day 7, venous blood samples were collected in 3.2% sodium citrate Vacutainer tubes.

The patients were visited at home between 8.00 a.m. and 12.30 a.m. Within five hours after collection the blood samples were centrifuged at 3000 rpm for 10 minutes and plasma was frozen at -20°C. Prothrombin times were measured after the one-week follow-up in order not to affect treatment.

All measurements were done at the same time on an automatic coagulation analyser (Electra 1600C). The thromboplastin used was a human recombinant tissue factor (Ortho® RecombiPlasTin) with an International Sensitivity Index of 1.05 (batch RTF-159).To confirm the assumption of no effect of freezing on the prothrombin time measurement, two blood samples were taken on day 7. Of one of these samples plasma was frozen and the prothrombin time was measured afterwards. Of the other sample the prothrombin time was measured directly. The mean difference in the INR-value between both samples was -0.1 (95%CI -0.5-0.3), indicating that freezing did not affect prothrombin time measurement.

To control for other factors that may affect the INR patients were asked about their health and changes in co-medication and alcohol intake during the study period. Besides, the intake of vitamin K_1 and phenprocoumon were verified with this questionnaire.

The course of the INR is described in terms of the proportional change in the mean INR between two consecutive days. Baseline INR and dose of vitamin K_1 are highly correlated: more vitamin K_1 is prescribed when the INR is higher (table 1). Therefore, we also analysed patients separately according to the dose of vitamin K_1 (1, 2, 3, 4 or 5 mg), starting from the patients being compliant. To test the efficacy of the routine treatment of overanticoagulation, the frequency of INRs $\geq$6.0, INRs within the therapeutic zone (2.0 - 3.5 INR or 2.5 - 4.0 INR) and INRs <2.0 was calculated.

Table 1. Algorithm for the administration of vitamin K_1 in patients on phenprocoumon.

	Target range of anticoagulation	
INR	2.5 - 3.5 INR	3.0 - 4.0 INR
6.0 - 7.9	optional	optional
8.0 - 8.9	2 mg	1 mg
9.0 - 11.9	3 mg	2 mg
12.0 - 14.9	5 mg	4 mg

RESULTS

The number of patients treated with vitamin K_1 and fulfilling the inclusion criteria in the three-week study period was 41. Of the 34 patients we were able to contact, 24 (71%) were willing to participate, 10 men and 14 women. One patient was lost to follow-up after day 2. The mean age of the patients was 70 years (range, 42 to 87 years). They had been using phenprocoumon for 3 days to 19 years, with a median duration of use of 2 years and 4 months. Indications for anticoagulant therapy were: cardiac disease (13 patients), cerebrovascular thromboembolism (4 patients), peripheral arterial disease (3 patients), venous embolism (2 patients) and prophylactic venous treatment (2 patients). The INR at presentation ranged from 7.3 to 14.2, with a mean of 9.3. The mean dose of vitamin K_1 prescribed was 2.7 mg (range, 1 to 5 mg). In 17 out of 24 patients the dose of vitamin K_1 was according to the algorithm; seven patients were prescribed a higher dose.

Figure 1 shows the course of the INR (mean ± SE) during the first week following administration of vitamin K_1 for all patients combined. The first two days after administration of vitamin K_1 the INR decreased. On day 0, the mean INR was 9.3±0.3. On day 1 and day 2, the mean values were 5.6±0.5 and 4.3±0.4, respectively. The proportional decrease in the mean INR was 40% on day 1 and 23% on day 2. After day 2, the mean INR did not decrease further, but slightly

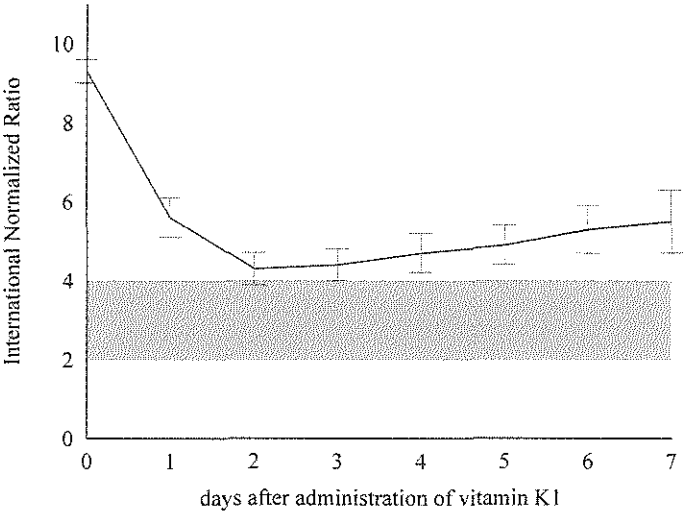

Figure 1. Course of the INR (mean ± SE) during the first week after administration of vitamin K_1 in patients overanticoagulated with phenprocoumon. The grey area indicates the therapeutic zone.

increased instead. The day-to-day proportional increase ranged from 2% to 8%. On day 7, the mean INR was 5.5±0.8, 28% higher than the mean value on day 2. Remarkably, the mean INR did not reach the therapeutic zone at any of the seven days. The pattern of a decrease in the INR during the first two days following the intake of vitamin K_1, was observed for all doses of vitamin K_1 (figure 2, upper panel) and for each of the patients (figure 2, bottom panel). The extent of the response to oral vitamin K_1 varied greatly between patients: the range in INRs on day 0 was much smaller compared to the range on day 1 and day 2. In a few patients, the INR did not decrease immediately but only after one day. The course of the INR after day 2 was not consistent. The day-to-day proportional change in the mean INR depended on the dose of vitamin K_1 and varied from a decrease of 12% to an increase of 21%. On day 7, the mean INR was lower than on day 2 in the 1 mg and the 5 mg group and was higher than on day 2 in the 2, 3 and 4 mg group.

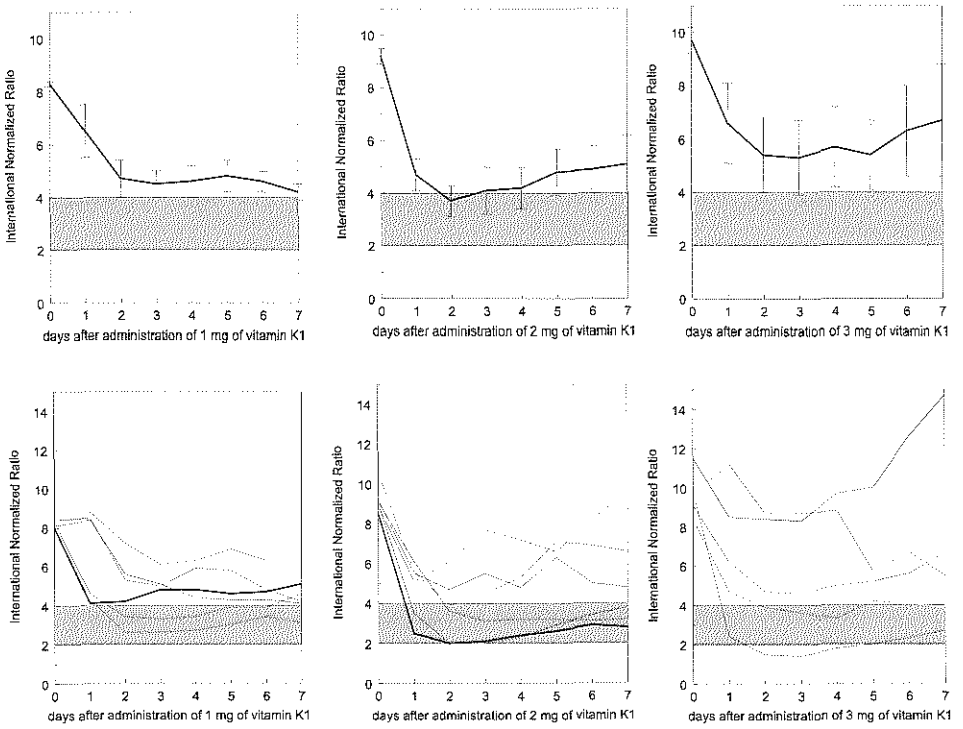

Figure 2. Course of the INR during the first week after administration of 1, 2 and 3 mg of vitamin K_1 in patients overanticoagulated with phenprocoumon (six, six and five patients respectively). The upper panel shows the mean INR ± SE. The bottom panel shows the course of the INR for the individual patients. The grey area indicates the therapeutic zone.

In addition to determining the effect of vitamin K_1 administration on the time-course of the INR, we also looked at its effectiveness in reducing the excess anticoagulant effect to lower and safer levels, preferally in the therapeutic zone. Of 24 patients with an INR $\geq$6.0 on day 0 who received 1 to 5 mg of vitamin K_1, eight (33%) still had an INR $\geq$6.0 on day 1, five (21%) on day 2, 3 and 4, seven (29%) on day 5 and day 6 and six (25%) on day 7. It concerned 12 individual patients, five of whom had an INR $\geq$6.0 on at least five days. All patients had taken less than 5 mg of vitamin K_1. The number of patients with an INR within the therapeutic zone was five (21%) on day 1, eight (33%) on day 2 and day 6, seven (29%) on day 3, day 4 and day 7, and nine (38%) on day 5. Twelve different patients were involved. In three of them the INR was in the therapeutic zone for only one or two days. An INR <2.0 occurred in two patients (8%) on day 2, 3 and 4.

DISCUSSION

To obtain detailed insight into the course of the INR after oral administration of vitamin K_1 in doses of 1 to 5 mg, we measured the INR on the next seven days in patients on phenprocoumon with an INR $\geq$6.0 at presentation. The first two days after administration of vitamin K_1 the mean INR decreased; afterwards it slightly increased again. On all days, the mean INR was above the therapeutic zone. After a week, 25% of the patients were still overanticoagulated (INR $\geq$6.0), only 29% had an INR within the therapeutic zone and none were underanticoagulated.

The routine treatment of overanticoagulation employed at the anticoagulation clinic has not been tested extensively before and is an empirical one. Therefore, one aim of this study was to test its efficacy. Starting from a duration of effect of oral vitamin K_1 of two days, as discussed below, the efficacy of the treatment can be judged on the INR between day 2 and day 7. Overall, just 32% of the patients had an INR within the therapeutic zone, 25% had an INR $\geq$6.0 and consequently were at increased risk of bleeding. An INR <2.0, and so an increased risk of thromboembolism, only occurred on day 2, 3 and 4 in two patients (8%). These figures indicate that the treatment is not sufficiently intense and an adjustment is recommended. Increasing the dosage of vitamin K_1 is one possibility. Another option would be to administer another small dosage of vitamin K_1 on day 1 or day 2. Although this study was only performed at the anticoagulation clinic of The Hague, the conclusion regarding the efficacy of the treatment of overanticoagulation in patients on phenprocoumon most probably also applies to many of the other anticoagulation clinics. Most clinics prescribe comparable doses of vitamin K_1 and some even lower doses.

This study was performed in a setting of routine medical care. As a consequence, in six patients, three of whom had just started oral anticoagulant therapy, the standard practice of restarting phenprocoumon on day 2 and lowering

the dosage by about 15% was deviated from. However, the course of the INR in these patients was comparable with that observed in the patients in whom the standard practice was applied.

Most effect of vitamin K_1 was seen in the first two days. Considering the half-life of vitamin K_1 of approximately 1½ to 3 hours (8, 9), a duration of effect of two days is plausible. The extent of the response to oral vitamin K_1 varied greatly between patients. A difference in the degree of overanticoagulation is a plausible explanation. However, serum levels of phenprocoumon were not determined. The difference between the dose of phenprocoumon at presentation and the dose in a stable condition later on, as a proxy of the degree of overanticoagulation, was available for only a limited number of patients. We did not find a relation between the dose of vitamin K_1 and the proportional decrease in INR between day 0 and day 2. Sex or age also were not related to this decrease. Other factors that may affect the INR were not substantially present and can not have had an appreciable effect on the results.

Our study was restricted to patients on phenprocoumon, since at the anticoagulation clinic of The Hague vitamin K_1 is only incidentally prescribed to patients on acenocoumarol, namely when the INR is $\geq$15.0. Because of a difference in half-life between coumarin anticoagulants, the course of the INR in response to oral vitamin K_1 would be different for other anticoagulants. This aspect should be taken into account when comparing our study with the studies of Weibert et al. (5), Pengo et al. (6) and Harrell and Kline (7), who were performed in patients on warfarin. The former two studies both focussed on efficacy and gave no data on changes in INR over time. In the study of Pengo et al. (6), nearly all patients had an INR below 5.0 on day 1, 2 and 9. The INR at presentation, however, was much lower than in our study. Weibert et al. (5) found that one or two days after administration of vitamin K_1, 73% of the INRs fell within the target zone of 2.0 to 5.0, 10% exceeded 5.0 and 17% was less than 2.0. Four to seven days after warfarin therapy was resumed these percentages were 83%, 1% and 16%, respectively. So, compared to our study, fewer patients had an INR above the target zone, but more patients had an INR below this zone. A higher dosage of vitamin K_1 in proportion to the INR at presentation by Weibert et al. is a plausible explanation. In a report on five patients by Harrell and Kline (7), the INR had decreased by 58% to 89% after one or two days. In our study, the maximum decrease in INR on an individual patient level was 77% on day 1 and 43% on day 2.

In conclusion, in this study among 24 patients on phenprocoumon with an INR $\geq$6.0, the INR decreased during the first two days after administration of 1 to 5 mg of oral vitamin K_1, but slightly increased again afterwards. Only one third of the patients had an INR within the therapeutic zone between day 2 and day 7. To improve its efficacy, the routine treatment of overanticoagulation in patients on phenprocoumon should be intensified.

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3

Risk factors for overanticoagulation

3.1 Characteristics of anticoagulant therapy and comorbidity related to overanticoagulation

ABSTRACT *The risk of hemorrhage when using coumarin anticoagulants sharply increases when the International Normalized Ratio (INR) is ≥6.0. We performed a prospective cohort study with a nested case-control design among 17,056 outpatients of an anticoagulation clinic to determine the incidence of overanticoagulation and to study the association between overanticoagulation and characteristics of anticoagulant therapy and comorbidity. The incidence rate of an INR ≥6.0 was 7.8 per 10,000 treatment days in prevalent users on the starting date and 22.5 per 10,000 treatment days in incident users during the study period. 300 cases with an INR ≥6.0 were compared with 302 randomly selected matched controls with an INR within the therapeutic zone. Information on characteristics of anticoagulant therapy and comorbidity, as well as on potential confounding factors, was collected from the anticoagulant medical record, through the general practitioner, and by interviewing the patient. Patients on acenocoumarol had an increased risk of an INR ≥6.0 compared to patients on phenprocoumon (OR 1.9; 95%CI 1.3-2.7). Chronic diseases associated with overanticoagulation were impaired liver function (OR 2.8; 95%CI 1.1-6.9) and congestive heart failure (OR 1.6; 95%CI 1.04-2.6 in stable condition and OR 3.0; 95%CI 0.8-12.0 in case of a relapse). Acute illnesses associated with overanticoagulation were diarrhea and fever (OR 12.8; 95%CI 1.6-104.9 and OR 2.9; 95%CI 1.1-7.7, respectively). Increased monitoring of INR-values if risk factors are present or avoidance of risk factors could prevent overanticoagulation and potential bleeding complications.*

INTRODUCTION

Coumarin anticoagulants are clinically effective in the prevention of venous and arterial thromboembolism (1). These drugs induce anticoagulation by antagonizing vitamin K, thereby impairing the biological activity of the vitamin K-dependent coagulation factors (factor II, VII, IX and X) (2). Inherent to their mode of action and narrow therapeutic index, hemorrhage is the most common adverse reaction to coumarin anticoagulants. The risk of hemorrhage is strongly associated with the intensity of anticoagulation and sharply increases when the International Normalized Ratio (INR) is $\geq$6.0 (3-6). Such an excess anticoagulant effect should therefore be prevented. This necessitates identification of risk factors for overanticoagulation.

A number of comorbidities are suspected to enhance the response to coumarins (7-10). Hepatic dysfunction may impair the synthesis of coagulation factors. In hypermetabolic states the clearance of coagulation factors is increased. Fat malabsorption and diarrhea impair the absorption of vitamin K. With malignancies, the metabolism of vitamin K and the coumarin anticoagulant may be affected. In congestive heart failure the distribution of the coumarin anticoagulant is altered.

The stability of anticoagulant control depends on the type of anticoagulant used and has been found to be less when using the short-acting acenocoumarol because of fluctuating factor VII levels (11, 12). In addition, the patient's compliance plays a role in stability of control (13, 14). Possibly the risk of overanticoagulation is also related to these factors.

The occurrence of overanticoagulation in a non-selected population under everyday circumstances and the association between overanticoagulation and characteristics of anticoagulant therapy and comorbidity, have not been studied extensively. Therefore, we have conducted a prospective cohort study with a nested case-control design among outpatients of an anticoagulation clinic. We determined the incidence of overanticoagulation (INR $\geq$6.0) and studied the association between overanticoagulation and characteristics of anticoagulant therapy and comorbidity in previously stable patients. This paper is one of a series of three papers on risk factors for overanticoagulation. The other two papers are based on the same study and concern drug interactions and sociodemographic-, lifestyle-, and dietary factors.

METHODS

Setting

In the Netherlands, anticoagulant therapy is monitored by a network of more than 60 independently operating specialized anticoagulation clinics, covering over 90% of the country (15, 16). The study was performed at the regional Red

Cross anticoagulation clinic The Hague, which serves an area of nearly 700,000 inhabitants. All persons in this area with an indication for anticoagulant therapy are referred to this clinic.

Cohort definition

The study cohort consisted of all patients treated with oral anticoagulants at the regional Red Cross anticoagulation clinic The Hague between December 1, 1997 and June 14, 1999. The cohort therefore included prevalent users on the starting date and incident users during the study period. All cohort members were followed until the first occurrence of an INR ≥6.0, the end of their treatment, or the end of the study period (i.e. the day on which the planned number of cases was recruited), whichever came first.

Cases and controls

Subjects for the nested case-control study were identified daily from all patients with an INR measurement on that day. Cases were defined as cohort members with an INR ≥6.0. For each case, one control, matched on target range, was randomly selected from the cohort members with an INR within the therapeutic zone (2.0-3.5 or 2.5-4.0), measured on the same day as the case (index day). Overanticoagulation is often seen during initiation of anticoagulant therapy and in unstable anticoagulation. Since this was not our primary interest, only cases and controls with stable anticoagulation in the three months preceding the index day were eligible. Anticoagulant therapy is considered effective and safe if the patient is kept within the therapeutic zone for more than two-third of the time (17, 18). Therefore, we defined stable anticoagulation as having at least 66% of the INRs within the therapeutic zone and no INRs ≥5.5 in the three months preceding the index day. To judge stability, a minimum of three INRs had to be assessed in the three months preceding the index day. Cases and controls with a hospital admission in this period were excluded, since information on anticoagulant control during admission is often not available at the anticoagulation clinic. As we focussed on sudden overanticoagulation, the INR preceding the assessment on the index day had to be within the therapeutic zone. Patients who were not living independently and those making use of meals on wheels were excluded because these patients may be less able to reliably answer the questions on medication and diet. Since we were primarily interested in overanticoagulation, irrespective of the question whether this was followed by hemorrhage, patients who presented at the index day with a serious bleeding complication were excluded because this may promote recall bias.

Procedure

The study protocol has been approved by the Medical Ethics Committee of the Erasmus University Medical Center Rotterdam. We planned to recruit 300 cases and 300 controls to provide at least 80% power to detect a true odds ratio (OR)

of ≥2.0 for risk factors having a prevalence of 7% among the controls, using a $p<0.05$ to reject the null hypothesis of OR=1.

Information on characteristics of anticoagulant therapy and comorbidity, as well as on potential confounding factors, was collected from the anticoagulant medical record, through the general practitioner (GP), and by interviewing the patient. The interview took place within three weeks after the index day at the private address of the patient, making use of structured questionnaires with mainly closed questions. The interviewers were blinded with respect to the patient's case or control status and the specific research hypotheses. This also applied to the GPs and the pharmacists. Blinding of the patients was not fully feasible, since the INR-value is printed on their dosage list. To obviate this, in the information letter we referred to the problem of overanticoagulation in a general sense.

Characteristics of anticoagulant therapy and comorbidity

The risk period was defined as the four-week period preceding the index day. The following characteristics of anticoagulant therapy were collected from the anticoagulant medical record: indication for anticoagulation, duration of therapy (categorized as ≤1 year, 1 to 5 years and ≥5 years, exclusive of former treatment episodes), type of anticoagulant used, change of type of anticoagulant in the risk period, and the latest dosage of the anticoagulant. The patient was asked about compliance with anticoagulant therapy, i.e. regularity of pill intake and missed or extra pills in the risk period. With respect to comorbidity, chronic comorbidities as well as acute illnesses during the risk period were taken into account. The GP was asked whether the patient had an impaired liver-, biliary- or pancreatic function, an impaired gastro-intestinal absorption, congestive heart failure, hyperthyroidism, or a malignancy. If so, it was asked whether the condition had changed in the risk period. Since anticoagulant therapy likely is titrated to chronic comorbidities and only a relapse or change may be related to overanticoagulation, all chronic comorbidities were categorized as absent, stable in the risk period, and worsened in the risk period. Regarding acute illnesses, the patient was asked about having been ill in the risk period and if so, about his or her complaints and the presence of fever (a temperature ≥38°C). In addition, the GP was asked whether the patient had consulted him in the risk period and if so, with which medical problems.

Cofactors

Acute illnesses and worsened chronic comorbidities may be accompanied by a change in drug use (beside the anticoagulant), weight, physical activity, dietary intake (and thereby intake of vitamin K), and/or alcohol consumption. These factors may also affect the response to oral anticoagulants (19-24) and were thus considered as potential confounders. The associations between these cofactors and overanticoagulation are the main subjects of the two other papers men-

tioned in the introduction.

Statistical analysis

We calculated the cumulative incidence of an INR ≥6.0 using the Kaplan-Meier method, as well as the incidence rate. Both incidence measures were calculated separately for prevalent users on the starting date and incident users during the study period, since overanticoagulation is often seen during initiation of anticoagulant therapy.

Characteristics of anticoagulant therapy and comorbidity related to an INR ≥6.0 were identified using univariate conditional logistic regression analysis at first. Since the unconditional analyses gave comparable results but more statistical power, we finally used unconditional logistic regression to compute unadjusted odds ratios and their 95% confidence intervals (CI). In case a risk factor was absent in either the cases or the controls, a Fisher Exact test was performed instead. To assess characteristics of anticoagulant therapy and stable chronic comorbidities that were independently associated with an INR ≥6.0, all factors of these two categories which were univariately associated at a $p<0.10$, age, sex, and the number of INR determinations in the preceding three months were included in a multiple regression model. A comparable procedure was followed to assess worsened chronic comorbidities and acute illnesses that were independently associated with an INR ≥6.0. Cofactors which were univariately associated with an INR ≥6.0 were included as well if this resulted in a change in one of the odds ratios of 5% or over, starting with the most potent factor.

To determine the importance of the independent risk factors for overanticoagulation in the population, we calculated the population attributable risk percentages (PAR%) according to the following formula (25): PAR% = AR% * (proportion of exposed cases), with AR% = ((OR-1)/OR) * 100.

Results

The prospective cohort consisted of 17,056 patients: 9,508 prevalent users on the starting date, who had on average 380 treatment days (range 1 to 560 days) and 16 INR measurements (range 0 to 72 measurements) and 7,548 incident users during the study period, who had on average 98 treatment days (range 1 to 558 days) and 9 INR measurements (range 1 to 60 measurements). The cumulative incidence of the occurrence of an INR ≥6.0 is represented in figure 1. As expected, overanticoagulation occurred more often in incident users. After six months of follow up, the cumulative incidence was 17% in prevalent users and 29% in incident users. After one year it was 25% and 39%, respectively, and at the end of the study period, i.e. after 560 days of follow up, the cumulative incidence was 34% in prevalent users and 46% in incident users. The number of prevalent users with an INR ≥6.0 was 2,813, which is corresponding to an inci-

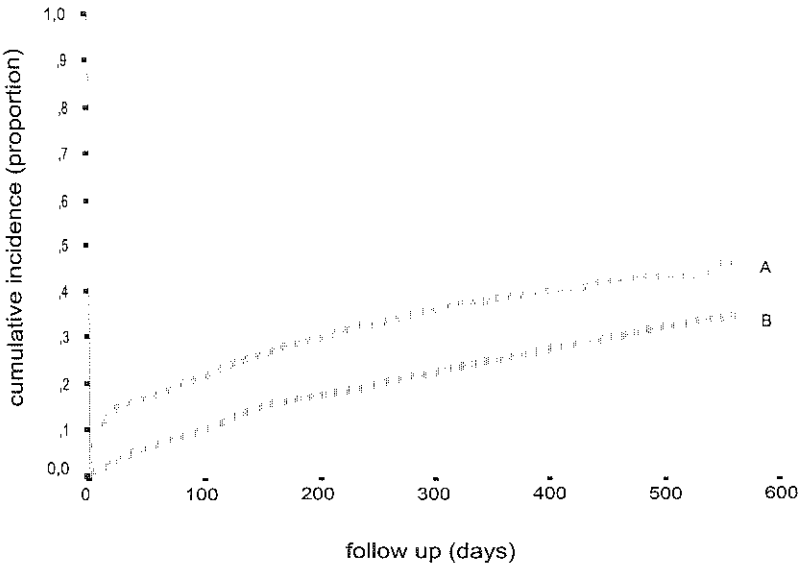

Figure 1. The cumulative incidence of the occurrence of an INR ≥6.0 in incident (A) and prevalent (B) users of acenocoumarol or phenprocoumon.

dence of 18 per 1000 INR measurements and an incidence rate of 7.8 per 10,000 treatment days. 1,663 incident users had an INR ≥6.0, the incidence being 26 per 1000 INR measurements and the incidence rate being 22.5 per 10,000 treatment days.

The nested case-control study included the planned number of 300 cases with a median INR of 6.8 and 302 controls with a median INR of 3.2. The participation among cases and controls was 78% and 85%, respectively. Written informed consent was obtained from every patient. The mean interval between the index day and the interview was fourteen days, for cases as well as for controls. In both case and control groups, the mean age was 68 years, the proportion of men was 58% and 64%, respectively.

The associations between overanticoagulation and characteristics of anticoagulant therapy are shown in table 1. The indication for anticoagulation and the duration of therapy were not related to an INR ≥6.0. Neither was the dosage of the anticoagulant. The type of anticoagulant used, however, was a risk factor for overanticoagulation. Patients on acenocoumarol had an increased risk of 1.9 (95%CI 1.3-2.7) compared to patients on phenprocoumon. The PAR% of overanticoagulation associated with the use of acenocoumarol was 21.3%. A change of type of anticoagulant occurred in only two patients. Regarding compliance, six cases but no controls had taken more pills than prescribed (p=0.02).

With respect to comorbidity (table 2), the only stable chronic comorbidities related to overanticoagulation were impaired liver function and congestive

Table 1. Association between overanticoagulation (INR ≥6.0) and characteristics of anticoagulant therapy[1].

Variable	Cases n=300	Controls n=302	OR (95% CI), univariate	OR (95% CI), multivariate
Age (years, mean±sd)	68.1 ± 12.3	68.2 ± 9.8	1.00 (0.98-1.01)	
Sex				
male	175 (58%)	194 (64%)	1 (reference)	
female	125 (42%)	108 (36%)	1.28 (0.92-1.78)	
Indication for anticoagulation				
atrial fibrillation	37	40	p=0.51	
prosthetic heart valve	31	31		
cardiac disease	110	131		
peripheral arterial disease	76	66		
cerebrovascular thromboembolism	28	18		
venous embolism	16	13		
prophylactic treatment	2	3		
Duration of therapy				
≥ 5 years	122	138	1 (reference)	
1 to 5 years	125	115	1.2 (0.9-1.8)	
≤ 1 year	53	49	1.2 (0.8-1.9)	
Type of anticoagulant				
phenprocoumon	165 (55%)	200 (66%)	1 (reference)	1 (reference)[2]
acenocoumarol	135 (45%)	102 (34%)	1.6 (1.1-2.2)	1.9 (1.3-2.7)[2]
Change of type of anticoagulant	1	1	1.0 (0.1-16.2)	
Dosage of anticoagulant (mg/day, mean±sd)				
phenprocoumon	0.83 ± 0.53	0.76 ± 0.30	1.5 (0.9-2.6)	
acenocoumarol	2.93 ± 1.35	2.73 ± 1.20	1.1 (0.9-1.4)	
Compliance				
no regular intake of anticoagulant	13	9	1.5 (0.6-3.5)	
pills missed	24	23	1.1 (0.6-1.9)	
taken more pills than prescribed	6	0	p=0.02	

[1] Values are numbers unless indicated otherwise.
[2] Type of anticoagulant, stable congestive heart failure, stable impaired liver function, age, sex and the number of INR determinations in the preceding three months were included in the model.

heart failure. The latter condition resulted in an increased risk of an INR ≥6.0 of 1.6 (95%CI 1.04-2.6). The corresponding PAR% was 7.5%. Patients with an impaired liver function had an increased risk of 2.8 (95%CI 1.1-6.9). None of these patients had liver cirrhosis, one case and two controls had chronic active hepatitis. In sixteen cases and in six controls, the liver function was otherwise impaired (including abnormal liver enzymes) and of two cases the kind of impairment was not stated by the GP. The PAR% of overanticoagulation associated with an impaired liver function was 3.9%. A worsening condition was infrequent for most chronic comorbidities. A relapse of congestive heart failure was present in fourteen cases and in three controls and was univariately associated with an increased risk of overanticoagulation of 5.3 (95%CI 1.5-18.8). After adjustment for confounding factors the increased risk was 3.0 (95%CI 0.8-12.0). The corresponding PAR% was 3.1%. Regarding acute illnesses, diarrhea and fever were risk factors for overanticoagulation, with relative risks of 12.8 (95%CI 1.6-104.9) and 2.9 (95%CI 1.1-7.7), respectively. Stratifying for duration of fever (<4 days and ≥4 days) revealed an increased risk of an INR ≥6.0 in both strata (univari-

Table 2. Association between overanticoagulation (INR ≥6.0) and comorbidity[1].

Variable	Cases n=300	Controls n=302	OR (95% CI), univariate	OR (95% CI), multivariate
Chronic comorbidities, stable				
congestive heart failure	60	45	1.5 (0.98-2.3)	1.6 (1.04-2.6)[a]
malignancies	23	21	1.1 (0.6-2.1)	
impaired liver function	18	7	2.7 (1.1-6.5)	2.8 (1.1-6.9)[a]
impaired GI absorption	9	7	1.3 (0.5-3.5)	
hyperthyroidism	4	3	1.3 (0.3-6.0)	
impaired biliary function	1	3	0.3 (0.0-3.2)	
Chronic comorbidities, worsened				
congestive heart failure	14	3	5.3 (1.5-18.8)	3.0 (0.8-12.0)[b]
malignancy	4	2	2.0 (0.4-11.2)	
Acute illnesses				
diarrhea	17	3	6.0 (1.7-20.7)	12.8 (1.6-104.9)[b]
illness of the urinary tract	19	5	4.0 (1.5-10.9)	1.2 (0.4-4.2)[b]
illness of the respiratory tract	93	53	2.1 (1.4-3.1)	1.0 (0.5-1.7)[b]
fever	45	10	5.3 (2.6-10.7)	2.9 (1.1-7.7)[b]

[1] Values are numbers.
[a] Stable congestive heart failure, stable impaired liver function, type of anticoagulant, age, sex and the number of INR determinations in the preceding three months were included in the model.
[b] Relapse of congestive heart failure, diarrhea, illness of the urinary tract, illness of the respiratory tract, fever, age, sex, the number of INR determinations in the preceding three months, use of antibacterial drugs, use of analgesics & NSAIDs, change in weight, change in physical activity and change in frequency of suppers were included in the model.

ate OR 4.4 (95%CI 1.6-12.1) and OR 6.7 (95%CI 2.3-19.7), respectively). The PAR% of overanticoagulation associated with diarrhea and fever were 5.2% and 9.8%, respectively. Illnesses of the urinary or respiratory tract were only univariately associated with an increased risk of an INR ≥6.0 (OR 4.0; 95%CI 1.5-10.9 and OR 2.1; 95%CI 1.4-3.1, respectively).

DISCUSSION

We determined the incidence of overanticoagulation among outpatients of an anticoagulation clinic. Furthermore, we studied the association between overanticoagulation and characteristics of anticoagulant therapy and comorbidity. The incidence rate of an INR ≥6.0 was 7.8 per 10,000 treatment days in prevalent users on the starting date and 22.5 per 10,000 treatment days in incident users during the study period. Since the patients' INRs were not measured daily, the real incidence of overanticoagulation may be higher. Patients with an impaired liver function or congestive heart failure, as well as those using acenocoumarol had an increased risk of an INR ≥6.0. Fever and diarrhea were also risk factors for overanticoagulation. The clinical implication of our findings lies in the possibility of prevention or early detection of excess anticoagulation, and thus of bleeding complications, by paying special attention to these risk factors when

monitoring anticoagulation. For example, patients with an impaired liver function should be monitored carefully and in case of fever, the patient's INR should be measured within seven days. Similarly, the use of phenprocoumon instead of acenocoumarol might be considered. This has been suggested before by others (11, 12), because of the more stable anticoagulant control when using phenprocoumon compared to acenocoumarol.

Diagnostic suspicion bias may play a role in the association of fever and diarrhea with overanticoagulation, since patients are instructed to inform the clinic of acute illnesses. If considered necessary, the patient's INR is measured earlier than the appointed date. Excluding patients whose INRs were measured earlier from the analyses, fever and diarrhea remained risk factors for overanticoagulation.

The presence of chronic comorbidities was based on GP diagnoses. Validation of drug use by reference to pharmacy data, revealed that 90% of the patients with a GP diagnosis of congestive heart failure had indeed used drugs for congestive heart failure or ischaemic heart disease. Although postulated as interfering with anticoagulant therapy, malignancies, an impaired gastro-intestinal absorption, hyperthyroidism, and an impaired biliary function were not related to overanticoagulation in our study. Neither in case of a stable condition, nor in case of a relapse in the risk period. This may be explained by the low prevalence of some of these conditions, which requires a larger study population to attain enough statistical power. In addition, the increase in INR by the potentially interfering comorbidity may be of less magnitude than defined in our study.

Compliance may influence the stability of anticoagulant control (13, 14). In our study, as expected, taking more pills than prescribed was associated with overanticoagulation. We were not able to test this association multivariately, but in view of the clear-cut pharmacological pathway this also would have been meaningless. Missing pills and irregularly taking the anticoagulant were not related to overanticoagulation. However, missing pills occurred only occasionally (once or twice in the four-week risk period) and patients who are constantly noncompliant most probably will not become stable and therefore have been excluded a priori.

So far as we are aware of, epidemiological studies on risk factors for overanticoagulation in a non-selected population under everyday circumstances are scarce and were only published for the first time in 1998. Two out of three earlier studies (26, 27) have some limitations. First, in one study (26), the cases were identified during a 12-month period whereas the controls were selected in June only. In the second study (27), cases and controls do not seem to be time-matched either. Second, the number of overanticoagulated patients was small (65 and 31, respectively). Third, only univariate analyses were performed. The third study (28) was well-performed. Diarrhea and taking more warfarin than prescribed were determinants of an INR $\geq$6.0, similar to our study. On the contrary, advanced malignancy was a risk factor for overanticoagulation in their

study, but fever was only univariately associated. Impaired liver function, congestive heart failure, and illnesses of the urinary or respiratory tract were not considered by Hylek et al. An important difference between the study of Hylek et al. and our study is that we only included stable cases and controls. Besides, we used a four-week risk period and they used a one-week risk period. Lastly, the study population of Hylek et al. used warfarin, while our patients used phenprocoumon or acenocoumarol.

Information on the incidence of overanticoagulation under everyday circumstances is even scarcer than information on risk factors for overanticoagulation. In the study of Brigden et al. (27), 0.3% of the INRs were ≥6.0. In the study of Panneerselvam et al. (26), 0.2% of the INRs were >7.0. When expressed in a comparable way, 2.0% of the INRs in our study were ≥6.0 and 0.9% of the INRs were >7.0. The much lower incidence of overanticoagulation reported by Brigden et al. and Panneerselvam et al. may be explained by the lower target range of anticoagulation in their studies.

In conclusion, in this study among previously stable outpatients of an anticoagulation clinic, overanticoagulation was associated with the type of anticoagulant used and with some comorbidities. Increased monitoring of INR-values if risk factors are present or avoidance of risk factors could prevent overanticoagulation and potential bleeding complications.

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3.2 Drug interactions as a cause of over-anticoagulation on phenprocoumon or acenocoumarol predominantly concern antibacterial drugs

ABSTRACT *The risk of hemorrhage when using coumarin anticoagulants sharply increases when the International Normalized Ratio (INR) is ≥6.0. Such overanticoagulation may be caused by drug interactions. We performed a case-control study among previously stable outpatients of an anticoagulation clinic using phenprocoumon or acenocoumarol to identify changes in the use of potentially interacting drugs related to overanticoagulation. 300 cases with an INR ≥6.0 were compared with 302 randomly selected matched controls with an INR within the therapeutic zone. Information on drug use, and potential confounding factors and effect modifiers, was collected from the anticoagulant medical record, through the general practitioner and the pharmacy, and by interviewing the patient. 45 out of 87 potentially interacting drugs were not used in the four weeks preceding the index day and only 15 drugs were used by at least ten patients. A course of sulphamethoxazole+trimethoprim (co-trimoxazole), strongly increased the risk of overanticoagulation (OR 24.2; 95%CI 2.8-209.1), especially in patients on acenocoumarol. Penicillins were associated with a risk of overanticoagulation of 2.4 (95%CI 1.00-5.5). The effect was confined to amoxicillin+clavulanic acid. If possible, the use of co-trimoxazole and amoxicillin+clavulanic acid should be avoided in patients on coumarins. If there is no therapeutic alternative available, increased monitoring of INR-values is warranted to prevent overanticoagulation and potential bleeding complications.*

INTRODUCTION

Coumarin anticoagulants are widely used in the prevention of venous and arterial thromboembolism (1). These drugs induce anticoagulation by antagonizing vitamin K, thereby impairing the biological activity of the vitamin K-dependent coagulation factors (factor II, VII, IX and X) (2). Opposite to their benefit, stands the risk of hemorrhage (3), which is strongly associated with the intensity of anticoagulation and sharply increases when the International Normalized Ratio (INR) is ≥6.0 (4,5). Such overanticoagulation may be caused by drug-drug interactions, to which coumarin anticoagulants are extremely susceptible because of their narrow therapeutic range (6). Critical periods are when a patient stabilized on an anticoagulant starts treatment with an interacting drug or when a patient stabilized on a regimen of an interacting drug and an anticoagulant has the interacting drug withdrawn (7). A considerable number of drug interactions with coumarin anticoagulants, based on case reports and small-scale experiments, have been reported and summarized (6-9). Epidemiological studies quantifying the role of drug interactions in overanticoagulation in a non-selected population on coumarins under everyday circumstances, however, are scarce. Therefore, we have conducted a prospective nested case-control study among outpatients of an anticoagulation clinic. We identified changes in the use of potentially interacting drugs related to an INR ≥6.0 in previously stable patients using phenprocoumon or acenocoumarol and calculated the corresponding odds ratios (OR) and population attributable risk percentages (PAR%). This paper is one of a series of three papers on risk factors for overanticoagulation. The other two papers are based on the same study and concern characteristics of anticoagulant therapy and comorbidity, and sociodemographic-, lifestyle-, and dietary factors.

METHODS

Setting

In the Netherlands, anticoagulant therapy is monitored by a network of more than 60 independently operating specialized anticoagulation clinics, covering over 90% of the country (10, 11). The study was performed at the regional Red Cross anticoagulation clinic The Hague, which serves an area of nearly 700,000 inhabitants. All persons in this area with an indication for anticoagulant therapy are referred to this clinic.

Cohort definition

The study cohort consisted of all patients treated with coumarin anticoagulants at the regional Red Cross anticoagulation clinic The Hague between December 1, 1997 and June 14, 1999. All cohort members were followed until the first occur-

rence of an INR ≥6.0, the end of their treatment, or the end of the study period (i.e. the day on which the planned number of cases was recruited), whichever came first.

Cases and controls

Subjects for the nested case-control study were identified daily from all patients with an INR measurement on that day. Cases were defined as cohort members with an INR ≥6.0. For each case, one control, matched on target range, was randomly selected from the cohort members with an INR within the therapeutic zone (2.0-3.5 or 2.5-4.0), measured on the same day as the case (index day). Overanticoagulation is often seen during initiation of anticoagulant therapy and in unstable anticoagulation. Since this was not our primary interest, only cases and controls with stable anticoagulation in the three months preceding the index day were eligible. Anticoagulant therapy is considered effective and safe if the patient is kept within the therapeutic zone for more than two-third of the time (12, 13). Therefore, we defined stable anticoagulation as having at least 66% of the INRs within the therapeutic zone and no INRs ≥5.5 in the three months preceding the index day. To judge stability, a minimum of three INRs had to be assessed in the three months preceding the index day. Cases and controls with a hospital admission in this period were excluded, since information on anticoagulant control during admission is often not available at the anticoagulation clinic. As we focussed on sudden overanticoagulation, the INR preceding the assessment on the index day had to be within the therapeutic zone. Because of questions about medication and diet, the patients had to be living independently and not making use of meals on wheels. Since we were primarily interested in overanticoagulation, irrespective of the question whether this was followed by hemorrhage, patients who presented at the index day with a serious bleeding complication were excluded because this may promote recall bias.

Procedure

The study protocol has been approved by the Medical Ethics Committee of the Erasmus University Medical Center Rotterdam. We planned to recruit 300 cases and 300 controls to provide at least 80% power to detect a true OR of ≥2.0 for risk factors having a prevalence of 7% among the controls, using a $p<0.05$ to reject the null hypothesis of OR=1.

Information on (changes in) drug use, and potential confounding factors and effect modifiers, was collected from the anticoagulant medical record, through the general practitioner (GP) and the pharmacy, and by interviewing the patient. The interview took place within three weeks after the index day at the private address of the patient, making use of structured questionnaires with mainly closed questions. The interviewers were blinded with respect to the patient's case or control status and the specific research hypotheses. This also applied to the GPs and the pharmacists. Blinding of the patients was not fully

feasible, since the INR-value is printed on their dosage list. To obviate this, in the information letter we referred to the problem of overanticoagulation in a general sense.

(Changes in) drug use

The risk period was defined as the four-week period preceding the index day. The patient was asked to show all currently taken prescription and over-the-counter (OTC) drugs and vitamin supplements. The dosage and frequency of use of these drugs and vitamins were recorded. The patient was asked about dosage changes in the risk period, drugs and vitamins that were started in the risk period, and drugs and vitamins that were discontinued in the risk period. Information in the anticoagulant medical record on dosage changes, start, or discontinuation of a drug, was also considered. Of all patients, the medication history of the preceding six months was obtained from the pharmacy. This history was used to judge the reliability of the patient interview data and anticoagulant medical record data on changes in drug use.

Based on overviews of drugs interacting with anticoagulant therapy (6-8), 69 drugs and 18 drug classes were *a priori* considered as potentially interacting drugs. Since changes in drug use, rather than continuous drug use, pose a risk of overanticoagulation (7), the occurrence of the following situations was defined for every patient, using patient interview data and anticoagulant medical record data: start, dose increase, or irregular and infrequent use (i.e. one or two times a week) of a potentially interacting drug or vitamin enhancing the anticoagulant effect, and discontinuation, dose reduction, or irregular and infrequent use of a potentially interacting drug or vitamin diminishing the anticoagulant effect.

Cofactors

A change in drug use, especially a course of antibacterial drugs and the use of analgesics, most probably occurs in case of an acute illness or a relapse of a chronic comorbidity. These situations may be accompanied by fever and/or result in a change in weight, physical activity, dietary intake (and thereby intake of vitamin K), and/or alcohol consumption. These factors may all affect the response to coumarin anticoagulants (2, 9, 14-18) and were thus considered as potential confounders. The associations between these cofactors and overanticoagulation are the main subjects of the two other papers mentioned in the introduction.

Furthermore, effect modification by the type of anticoagulant may be present. Drugs may interact with coumarin anticoagulants by inducing or inhibiting specific cytochrome P450 enzymes, especially cytochrome P450 2C9 (CYP2C9) (6, 8, 19). The difference in the structure of acenocoumarol and phenprocoumon, although small, may have implications on the relative contribution of cytochrome P450 enzymes to their metabolism (20). The risk of overanticoagulation

when the use of CYP2C9 mediated drugs changes therefore possibly differs with the type of anticoagulant used.

Statistical analysis

Changes in the use of potentially interacting drugs (vitamin supplements included) related to an INR ≥6.0 were identified using univariate conditional logistic regression analysis at first. Since the unconditional analyses gave comparable results but more statistical power, we finally used unconditional logistic regression analysis to compute unadjusted odds ratios and their 95% confidence intervals (CI). In case a risk factor was absent in either the cases or the controls, a Fisher Exact test was performed instead. To assess changes in drug use that were independently associated with an INR ≥6.0, all factors which were univariately associated at a $p<0.10$ were included in a multiple regression model. Beside age, sex, and the number of INR determinations in the preceding three months, cofactors which were univariately associated with an INR ≥6.0 were included if this resulted in a change in one of the odds ratios of 5% or over, starting with the most potent factor. Effect modification by the type of anticoagulant was studied by performing stratified analyses.

To determine the importance of the independent risk factors for overanticoagulation in the population, we calculated the population attributable risk percentages (PAR%) according to the following formula (21): PAR% = AR% * (proportion of exposed cases), with AR% = ((OR-1)/OR) * 100.

Results

The nested case-control study included the planned number of 300 cases with a median INR of 6.8 and 302 controls with a median INR of 3.2. The participation among cases and controls was 78% and 85%, respectively. Written informed consent was obtained from every patient. The mean interval between the index day and the interview was fourteen days, for cases as well as for controls. Characteristics of the study population are presented in table 1. 55% of the cases and 66% of the controls used phenprocoumon, the others acenocoumarol. The mean number of prescription and OTC drugs regularly and frequently used beside the anticoagulant as well as the number of patients using health supplements, was similar for cases and controls.

45 out of 87 potentially interacting drugs or drug classes were not used by the study population in the four-week risk period and 27 were used by less than ten patients. The drugs and drug classes used by at least ten patients are listed in table 2; about one third of the patients had used acetaminophen (paracetamol) in the risk period and a quarter had used HMG-CoA reductase inhibitors, the remaining drugs were used by less than 46 patients. Only for acetaminophen, doxycycline, amoxicillin, amoxicillin+clavulanic acid and

Table 1. Characteristics of the study population[1].

Variable	Cases n=300	Controls n=302	OR (95% CI)
Age (years, mean±sd)	68.1 ± 12.3	68.2 ± 9.8	1.00 (0.98-1.01)
Sex			
male	175 (58%)	194 (64%)	1 (reference)
female	125 (42%)	108 (36%)	1.3 (0.9-1.8)
Indication for anticoagulation			p=0.51
atrial fibrillation	37	40	
prosthetic heart valve	31	31	
cardiac disease	110	131	
peripheral arterial disease	76	66	
cerebrovascular thromboembolism	28	18	
venous embolism	16	13	
prophylactic treatment	2	3	
Type of anticoagulant			
phenprocoumon	165 (55%)	200 (66%)	1 (reference)
acenocoumarol	135 (45%)	102 (34%)	1.6 (1.2-2.2)
Number of drugs[2] (mean±sd)	3.5 ± 2.7	3.7 ± 2.6	1.0 (0.9-1.04)
Use of health supplements			
any supplement	95	111	1.3 (0.9-1.8)
vitamin C	21	18	1.2 (0.6-2.3)
vitamin D	6	6	1.0 (0.3-3.2)
vitamin E	6	6	1.0 (0.3-3.2)
multivitamins	39	33	1.2 (0.7-2.0)

[1] Values are numbers unless indicated otherwise.
[2] Prescribed and OTC drugs regularly and frequently used (i.e. at least three times a week) besides the anticoagulant.

sulphamethoxazole+trimethoprim (co-trimoxazole), a relevant change in use in the risk period occurred in at least ten patients each. Comparing the information on the start of antibacterial drugs given by the patient or mentioned in the anticoagulant medical record with that subtracted from the medication history, revealed no substantial differences, neither in the cases nor in the controls.

The associations between overanticoagulation and changes in drug use are shown in table 3. A course of antibacterial drugs was associated with an INR ≥6.0 (OR 2.8; 95%CI 1.8-4.5). In view of a difference in mechanism of interaction and to be more informative, all antibacterial drugs were also studied individually. Co-trimoxazole most strongly increased the risk of overanticoagulation. After adjustment for confounding factors the increased risk was 24.2 (95%CI 2.8-209.1). The corresponding PAR% was 5.7%. Penicillins were associated with an increased risk of an INR ≥6.0 of 2.4 (95%CI 1.00-5.5). Adjustment for confounders did not change the OR. The PAR% of overanticoagulation associated with the use of penicillins was 3.4%. The effect of penicillins was confined to amoxicillin+clavulanic acid. Doxycycline was only univariately associated with overanticoagulation (OR 2.3; 95%CI 1.1-4.6). Fluoroquinolones and clarithromy-

Table 2. Potentially interacting drugs and drug classes used by at least ten patients.

Drug (class)	number of users	number of users with a relevant[1] change in use
Acetaminophen	179	124
HMG-CoA reductase inhibitors	156	7
simvastatin	93	1
pravastatin	25	2
fluvastatin	13	0
atorvastatin	27	4
cerivastatin	2	0
Omeprazole	45	6
Tetracyclines	43	40
doxycycline	41	38
tetracycline	1	1
minocycline	1	1
Biguanides; metformin	33	0
Penicillins	26	26
amoxicillin	16	16
amoxicillin+clavulanic acid	10	10
Amiodarone	25	3
Ranitidine	23	4
Co-trimoxazole	22	19
Thyroxines	20	0
Fibrates	18	1
clofibrate	1	0
gemfibrozil	13	1
ciprofibrate	4	0
Spironolactone	14	0
Tramadol	12	6
Allopurinol	11	0
Macrolides	11	10
clarithromycin	9	9
azithromycin	2	1

[1] Start, dose increase or irregular and infrequent use (i.e. one or two times a week) of an interacting drug enhancing the anticoagulant effect or discontinuation, dose reduction or irregular and infrequent use of an interacting drug diminishing the anticoagulant effect.

cin were not related to an INR ≥6.0, however, the numbers of patients were small. The stratified analyses revealed that the effect of co-trimoxazole on the risk of overanticoagulation depended on the type of anticoagulant used and was especially present in patients on acenocoumarol.

The analgesic used mainly was acetaminophen. Its use was associated with an increased risk of overanticoagulation of 1.5 (95%CI 0.98-2.2). Adjustment for confounding factors reduced the OR to 1.2.

Table 3. Association between overanticoagulation (INR ≥6.0) and changes in drug use[1].

Variable	Cases n=300	Controls n=302	OR (95% CI), univariate	OR (95% CI), multivariate
antibacterial drugs				
co-trimoxazole	18	1	19.2 (2.6-144.5)	24.2 (2.8-209.1)[a]
amoxicillin	10	6	1.7 (0.6-4.7)	
amoxicillin+clavulanic acid	8	2	4.1 (0.9-19.2)	5.1 (0.6-46.6)[a]
doxycycline	26	12	2.3 (1.1-4.6)	1.4 (0.6-3.6)[a]
ciprofloxacin	3	0	p=0.50	
norfloxacin	1	2	0.5 (0.0-5.6)	
clarithromycin	5	4	1.3 (0.3-4.7)	
analgesics				
acetaminophen	71	53	1.5 (0.98-2.2)	1.2 (0.7-2.0)[a]
salicylates >300mg	2	3	0.7 (0.1-4.0)	
tramadol	5	1	5.0 (0.6-42.3)	
gastro-intestinal drugs				
ranitidine	1	3	0.3 (0.0-3.2)	
omeprazole	2	4	0.5 (0.1-2.8)	
HMG-CoA reductase inhibitors				
atorvastatin	3	1	3.0 (0.3-29.3)	
anti-arrhythmics				
amiodarone	3	0	p=0.12	
vitamin supplements	4	4	1.0 (0.2-4.0)	

[1] Values are numbers. Those drugs for which the number of patients with a relevant change in use was less than three are not included in the table. This concerns tetracycline (1 case / 0 controls), minocycline (0/1), azithromycin (0/1), cefaclor (0/1), ceftibuten (0/1), piroxicam (1/0), cisapride (1/0), simvastatin (0/1), pravastatin (0/2), gemfibrozil (1/0), cholestyramine (1/0), carbamazepine (1/0), phenytoin (1/0), miconazole (1/1), fluoxetine (0/2), chlorthalidone (0/1), metronidazole (1/0).

[a] Co-trimoxazole, amoxicillin+clavulanic acid, doxycycline, acetaminophen, age, sex, the number of INR determinations in the preceding three months, fever, diarrhea, relapse of congestive heart failure, illness of the urinary tract, change in weight and change in alcohol consumption were included in the model.

DISCUSSION

We studied the role of drug interactions in overanticoagulation among outpatients of an anticoagulation clinic. Half of the 87 potentially interacting drugs or drug classes were not used by the study population and only fifteen drugs or drug classes were used by more than ten patients. A relevant change in use in the risk period was infrequent. A course of co-trimoxazole and the use of amoxicillin+clavulanic acid, however, were risk factors for overanticoagulation. The clinical implication of our findings lies in the possibility of prevention or early detection of overanticoagulation, and thus of bleeding complications, by considering the use of antibacterial drugs other than co-trimoxazole and amoxicillin+clavulanic acid. If there is no therapeutic alternative available, increased monitoring of INR-values is warranted, i.e. measuring the INR on the third day after the start of the antibacterial drug and according to this INR three to seven days thereafter. Our study also suggests that acetaminophen is a safe analgesic for patients receiving coumarin anticoagulants.

Many drugs postulated as interacting with anticoagulant therapy on the basis of case reports and small-scale experiments, were not used by our study population or by less than ten patients. This is a reassuring observation, suggesting that these drugs did not play a major role in overanticoagulation under everyday circumstances. In addition, a change in drug use occurred infrequently for many of the 87 drugs. Although we could not judge their association with overanticoagulation because of the small numbers, this finding suggests that these drugs only played a minor role in our study population. Our results regarding the role of drugs in overanticoagulation likely may be generalized since the kind of drugs used in our population is largely the same as used in most other countries.

The types of coumarin used by our study population were phenprocoumon and acenocoumarol. In many countries warfarin is the coumarin of first choice. The results of our study, however, will largely apply to these countries as well. First, interactions of pharmacodynamic nature on receptor level occurring with one anticoagulant may well apply to another anticoagulant (7). Second, the difference in half-life between coumarins will only influence the time of onset and the duration of overanticoagulation (6), but not necessarily affects the baseline risk. Third, drugs that interact by inducing or inhibiting the cytochrome P450 iosenzyme CYP2C9 will affect both acenocoumarol and warfarin (20).

Two mechanisms have been suggested for antibiotic-associated hypoprothrombinemia (22). First, antibacterial drugs affect the vitamin K status by eliminating vitamin K producing micro-organisms from the colon. Second, certain antibacterial drugs directly inhibit the synthesis of the vitamin K-dependent coagulation factors. High-risk antibiotics are cephalosporins containing the N-methylthiotetrazole moiety. In patients receiving anticoagulant therapy, cotrimoxazole may also increase the anticoagulant effect by inhibiting the metabolism of the anticoagulant by trimethoprim (23) or by increasing the plasma concentration of free coumarin by sulphamethoxazole (24). The degree of inhibition of the metabolism may be different for acenocoumarol and phenprocoumon (20). The results of our study are in accordance with this theory. The underlying indication, fever, and other illness-related factors may also be responsible for an increase in the anticoagulant effect when using antibacterial drugs. When adjusting for these potential confounders, doxycycline was no longer associated with overanticoagulation. Amoxicillin+clavulanic acid, however, remained a risk factor. This difference may be caused by a difference in effect on the intestinal microflora.

So far as we are aware of, epidemiological studies on risk factors for overanticoagulation in a non-selected population under everyday circumstances are scarce and were only published for the first time in 1998. Two out of three earlier studies (25, 26) have some limitations. The third study (27) was well-performed, however, changes in drug use were not studied individually. Newly started treatment with potentiating drugs (all combined, half of which antibiotics) and the

use of acetaminophen were independent determinants of an INR ≥6.0. The latter finding is in contrast with our study. This may possibly be explained by the fact that we used a four-week risk period, while they only used the preceding week as potential risk period. Another difference between the study of Hylek et al. and our study is that the study population of Hylek et al. used warfarin, while our patients used phenprocoumon or acenocoumarol. Besides, we only included stable cases and controls. Lastly, potential confounding by a change in weight, physical activity, or alcohol consumption, was not taken into account in the study of Hylek et al.

In conclusion, in this study among previously stable outpatients of an anticoagulation clinic using phenprocoumon or acenocoumarol drug interactions as a cause of overanticoagulation predominantly concerned antibacterial drugs. If possible, the use of co-trimoxazole and amoxicillin+clavulanic acid should be avoided in patients on coumarins. If there is no therapeutic alternative available, increased monitoring of INR-values is warranted to prevent overanticoagulation and potential bleeding complications.

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3.3 Overanticoagulation associated with combined use of antibacterial drugs and coumarin anticoagulants

ABSTRACT *Several case reports associated combined use of coumarins and antibacterial drugs with overanticoagulation. Despite the fact that these drugs are frequently prescribed concurrently, there is little quantitative information on the risks of such complications. To study which antibacterial drugs are associated with overanticoagulation during therapy with coumarins we performed a population-based cohort study in a sample of the Rotterdam Study. The study cohort consisted of all participants who were treated with acenocoumarol or phenprocoumon in the study period from April 1, 1991 through December 31, 1998 and for whom International Normalized Ratio (INR)-data were available. All cohort members were followed until the first occurrence of an INR ≥6.0, the last INR-assessment because of the end of their treatment, death or end of the study period. Data on antibacterial drug use were obtained from regional pharmacies. Of the 1,124 patients in the cohort, 351 developed an INR ≥6.0. Eight antibacterial drugs were multivariately associated with overanticoagulation. Sulfamethoxazole combined with trimethoprim most strongly increased the risk of overanticoagulation (RR 20.1; 95%CI 10.7-37.9). Stratification showed that the induction period of overanticoagulation varied between different antibacterial drugs. Awareness of these drug interactions and more frequent monitoring of INR-values during the initial stages of antibacterial drug therapy are warranted to minimize the risk of bleeding complications.*

INTRODUCTION

Coumarin anticoagulants are extensively used for the treatment and long-term prevention of thromboembolic diseases (1, 2). These drugs induce their anticoagulant effect by antagonizing vitamin K, thereby impairing the biological activity of the vitamin K-dependent coagulation factors (3, 4). The risk of bleeding, the main complication of coumarin anticoagulants, is influenced by the intensity of anticoagulant therapy (5-9), by the patient's underlying clinical disorder (9, 10), and by the concomitant use of other drugs (2, 11, 12). This risk sharply increases when the International Normalized Ratio (INR) is ≥6.0 (13, 14). Growing experience with anticoagulant therapy and increased understanding of drug interactions, have reduced the number of bleeding complications (15). Several drugs can affect the prothrombin time during oral anticoagulant therapy by different mechanisms (11, 12, 16, 17). During the past decades, many case reports associated the use of antibacterial drugs with overanticoagulation (18-24). Prospective studies investigated this association, but these were conducted in young healthy volunteers, were only able to identify interactions that occur relatively frequently and were mostly limited to warfarin (25-31). A recent case-control study suggested that antibacterial drugs are an important cause of overanticoagulation (32). In that study, however, data on drug use came from patient interview and the sample size was too small to study the effect of different antibacterial agents on coumarins. Therefore, we conducted a follow-up study in a large population-based cohort to investigate which antibacterial drugs are associated with overanticoagulation during therapy with coumarins.

METHODS

Setting

Data were obtained from the Rotterdam Study and from the regional outpatient anticoagulation clinic. The Rotterdam Study is a prospective population-based cohort study of neurological-, cardiovascular-, locomotor- and ophthalmologic diseases. All inhabitants of Ommoord, a suburb of the city of Rotterdam in the Netherlands, aged 55 years or over were invited in 1990-1993 to participate in the study. The rationale and design of this study have been described elsewhere (33). The cohort encompasses 7,983 individuals who were all interviewed and investigated at baseline. The anticoagulation clinic monitors all inhabitants of Ommoord with an indication for anticoagulant therapy. The choice of anticoagulant is made by the physician. Prothrombin times are monitored each one to six weeks by reference to the INR, dependent on the stability of the anticoagulant level. Doses are adjusted on the basis of the INR of the patient by computerized dose calculations. For this study data were used from January 1, 1991 through December 31, 1998. More than 99% of participants fill their drugs at

seven regional pharmacies, which are fully computerized. Complete data on drug use were available as of January 1, 1991. The pharmacy data include the Anatomical Therapeutical Chemical (ATC)-code (34), the filling date, the total amount of drug units per prescription, the prescribed daily number of units, and product name of the drugs.

Cohort and outcome definition

The study cohort consisted of all 1,124 participants of the Rotterdam Study, who were treated with acenocoumarol or phenprocoumon in the study period between April 1, 1991 and December 31, 1998 and for whom there were INR-data from the anticoagulation clinic during their treatment. The start date April 1 was chosen to ensure that at least 3 months of medication history from the pharmacy was available for every cohort member. The cohort included prevalent users of coumarins on the starting date and incident users during the study period. All cohort members were followed as of April 1, 1991 for prevalent users and from their first INR assessment for incident users. Both groups were followed until the first occurrence of an INR $\geq$6.0, the last INR-assessment because of the end of their treatment, death or end of the study period, whichever came first. This means that during follow-up, all study members were anticoagulated and regularly assessed for their INR. The date on which an INR $\geq$6.0 was encountered was defined as the index date.

Cofactors

The following baseline patient characteristics were considered as potential determinants for affecting the response of the INR to coumarin anticoagulants: gender, age, hepatic dysfunction (defined as serum aminotransferases > 2x the upper level of normal), hypoalbuminemia ($\leq$ 35 g/l), malignancies, hyperthyroidism, hypertension (systolic blood pressure $\geq$160 mm Hg and/or diastolic blood pressure $\geq$95 mm Hg or use of antihypertensives), congestive heart failure, and low dietary intake of vitamin K (< 1 μg/kg/day). In addition, we considered duration of follow-up and whether the INR measurement on the index date was earlier than according to the INR monitoring scheme. Furthermore, to study the potentially confounding effect of fever or of the indication for treatment we studied the presence or absence of these features in the medical records of the general practitioners. We did this validation for all cases and a random sample from the remainder of the cohort who all had been treated with antibacterial drugs on the index date.

Statistical analysis

Incidence rates were calculated by dividing the number of cases of an INR $\geq$6.0 by the number of days on combined use of a coumarin anticoagulant and an antibacterial drug. To assess antibacterial drugs which were independently associated with an INR $\geq$6.0, all occurring combinations of a coumarin anticoagu-

lant and an antibacterial drug were included separately in a Cox proportional hazards regression model for time-dependent variables to compute relative risks (RR) and their 95% confidence intervals (CI) (35). The model compares exposure to this combination at the index date of each case with that of all subjects in the cohort who are at risk for the outcome of interest. To adjust for potential confounding, cofactors which were associated with an INR ≥6.0 in the univariate analysis were included in the multivariate model, in addition to gender and age, if this caused a change in the point estimate of more than 5 percent. In order to study the time interval between the first treatment day with an antibacterial drug and an INR-value ≥6.0, we distinguished two intervals: 1-3 days and ≥4 days. Stratification by these time-intervals was performed because the induction period differs per mechanism by which antibacterial drugs may cause overanticoagulation. If possible, stratified analyses by the type of anticoagulant were performed. Moreover, separate analyses were performed for prevalent and incident users of coumarin anticoagulants on the starting date.

Table 1. Characteristics of cases and cohort[1].

Variable	Cases n=351	Total Cohort n=1,124	RR (95% CI)[3]
Sex			
female	174 (50%)	590 (52%)	1 (reference)
male	177 (50%)	534 (48%)	1.44 (1.17-1.77)
Age (years, mean (sd))	73 (8)	72 (8)	1.04 (1.03-1.05)
55 - 64 years	49 (14%)	218 (20%)	1 (reference)
65 - 74 years	152 (43%)	496 (44%)	1.28 (0.92-1.76)
75 - 84 years	124 (35%)	40 (30%)	1.85 (1.33-2.58)
≥ 85 years	26 (8%)	70 (6%)	3.17 (1.96-5.14)
Type of anticoagulant			
acenocoumarol	295 (84%)	951 (85%)	1 (reference)
phenprocoumon	56 (16%)	173 (15%)	0.57 (0.43-0.76)
Impaired liver function	3 (1%)	12 (1%)	3.75 (1.18-11.9)
Hypoalbuminemia	1 (0.3%)	2 (0.2%)	1.16 (0.16-8.30)
Malignancies[2]	43 (12%)	94 (8%)	1.67 (1.21-2.30)
Hyperthyroidism	12 (3%)	37 (3%)	1.51 (0.85-2.70)
Hypertension	148 (42%)	434 (39%)	1.08 (0.86-1.35)
Congestive heart failure[2]	83 (24%)	141 (12%)	1.63 (1.27-2.09)
Low intake of vitamin K	7 (2%)	11 (1%)	3.74 (1.76-7.95)

[1] Values are numbers (percentages) unless indicated otherwise.
[2] Assessed by reference to the index date.
[3] Univariate analyses of relative risks were performed with a Cox proportional hazards model.

Results

Of the 1,124 patients in the cohort, 351 developed an INR ≥6.0 after April 1, 1991. The incidence rate was 6.9 per 10,000 treatment days. Baseline characteristics of cases and the total cohort are shown in table 1. Men and old patients had a higher risk of an INR ≥6.0. The risk of overanticoagulation was lowest with phenprocoumon. Hepatic dysfunction, malignancies, congestive heart failure and a low dietary daily intake of vitamin K were associated with an increased risk of an INR ≥6.0 in the univariate analysis. There was no difference in duration of follow-up between cases and the total cohort (mean 559±481 days). The INR

Table 2. Association between overanticoagulation (INR ≥6.0) and use of antibacterial drugs[1].

Antibacterial drug	Cases n=351	Cohort n=1,124	IR[2]	RR (95% CI), crude[3,4]	RR (95% CI), adjusted[5]
Amoxicillin	8	180	32.0	11.1 (5.4-22.9)	10.5 (5.1-21.7)
Amoxicillin & enzyme inhibitor	4	205	18.0	4.6 (1.7-12.4)	5.1 (1.9-13.9)
Amphotericin	0	11		p=0.89	
Azithromycin	0	2		p=0.95	
Cefalexin	0	3		p=0.94	
Cefixime	0	2		p=0.95	
Cefuroxime	0	4		p=0.93	
Ciprofloxacin	0	19		p=0.85	
Clarithromycin	3	69	78.7	11.0 (3.4-35.4)	11.7 (3.6-37.8)
Clindamycin	0	37		p=0.80	
Doxycycline	5	288	32.3	4.2 (1.7-10.2)	4.3 (1.8-10.4)
Erythromycin	0	78		p=0.69	
Flucloxacillin	0	25		p=0.83	
Norfloxacin	3	66	44.6	10.0 (3.1-32.1)	9.8 (3.0-31.6)
Ofloxacin	0	33		p=0.81	
Pheneticillin	1	219	0.2	1.2 (0.2-8.3)	0.9 (0.1-6.1)
Roxithromycin	0	14		p=0.86	
Sulfamethoxazole & trimethoprim	11	125	154.7	20.1 (10.7-37.7)	20.1 (10.7-37.9)
Trimethoprim	2	61	26.8	6.0 (1.4-25.2)	5.6 (1.3-23.1)
Vancomycin	1	10	15.9	17.7 (2.3-138.0)	13.6 (1.7-106.8)

[1] Values are numbers (percentages) unless indicated otherwise.
[2] Expressed as the number of cases per 10,000 days on combined use of a coumarin and an antibacterial drug.
[3] In this time-dependent analysis, exposure in cases and controls is assessed on every index date. As a consequence, the number of assessments in the reference group is much larger than the number of individuals. Hence, crude relative risks can not be calculated with the numbers in this table.
[4] If none of the cases was exposed, p-values are given instead of relative risks.
[5] Adjusted for sex and age.

measurement on the index date was earlier than planned in 8.6% of the cases and in 5.6% of the total cohort (p=0.005).

Twenty different antibacterial drugs were used during the study period, of which eleven were not used in cases. Thirty-eight cases (11%) used antibacterial drugs on the index date. Incidence rates for the individual antibacterial drugs are presented in table 2. Eight antibacterial drugs were univariately as well as multivariately associated with overanticoagulation. The relative risk varied considerably between the different drugs (table 2). Sulfamethoxazole combined with trimethoprim (co-trimoxazole) most strongly increased the risk of overanticoagulation. After adjustment for confounding factors the relative risk was 20.1 (95%CI 10.7-37.9). There was no significant difference in the frequency of fever in cases on antibacterial drugs and individuals in the rest of the cohort who were treated with antibacterial drugs on the index date. Similarly, there was no difference in the indication for antibacterial drugs between cases and the remainder of the cohort (data not shown).

Stratification by time-interval revealed that the adjusted relative risks of overanticoagulation by antibacterial drugs were different for both time intervals (table 3). Relative risks could not be established for both time periods for all antibacterial drugs because overanticoagulation only occurred in one of the time intervals for some drugs. For amoxicillin and co-trimoxazole, relative risks were significantly increased for both time intervals. When comparing the relative risks for both time intervals, the antibacterial drugs with the highest relative risk 1-3 days after start of use were clarithromycin, norfloxacin and trimethoprim. For

Table 3. Associations between overanticoagulation (INR ≥6.0) and use of antibacterial drugs stratified by time interval[1].

Antibacterial drug	RR_{adj} (95% CI)[2], overall	RR_{adj} (95% CI)[2], 1-3 days	RR_{adj} (95% CI)[2], ≥4 days
Amoxicillin	10.5 (5.1-21.7)	7.2 (1.8-29.7)	13.2 (5.6-30.8)
Amoxicillin & enzyme inhibitor	5.1 (1.9-13.9)	-	7.3 (2.7-19.9)
Clarithromycin	11.7 (3.6-37.8)	21.3 (5.0-90.6)	6.3 (0.9-46.3)
Doxycycline	4.3 (1.8-10.4)	2.9 (0.4-20.9)	5.2 (1.9-14.0)
Norfloxacin	9.8 (3.0-31.6)	19.3 (4.5-83.7)	5.0 (0.7-36.6)
Pheneticillin	0.9 (0.1-6.1)	-	0.9 (0.1-6.5)
Sulfamethoxazole & trimethoprim	20.1 (10.7-37.9)	16.6 (5.1-54.4)	23.2 (10.9-49.1)
Trimethoprim	5.6 (1.3-23.1)	9.0 (1.2-67.4)	4.1 (0.6-30.6)
Vancomycin	13.6 (1.7-106.8)	-	15.1 (1.9-120.0)

[1] Time interval is the interval between the first treatment day with an antibacterial drug and an INR ≥6.0.
[2] Adjusted for sex and age.

amoxicillin, doxycycline and co-trimoxazole relative risks of overanticoagulation were most strongly increased ≥4 days after start of the antibacterial drug.

Stratified analyses by the type of anticoagulant could only be performed for amoxicillin and co-trimoxazole. For patients on acenocoumarol the adjusted relative risk of amoxicillin was 8.0 (95%CI 3.5-18.4). For users of phenprocoumon the adjusted relative risk of amoxicillin was 22.4 (95%CI 4.0-126.3). Co-trimoxazole was associated with an adjusted relative risk of overanticoagulation of 17.3 (95%CI 8.6-34.9) in patients on acenocoumarol, and 14.3 (95%CI 2.8-73.8) in patients on phenprocoumon. Numbers appeared to be too small to calculate risks in prevalent users on the starting date. In incident users, risks were largely the same as in the total population (data not shown).

Discussion

The main finding in this population-based cohort study is that several antibacterial drugs were associated with a strongly increased risk of overanticoagulation during oral anticoagulant therapy with acenocoumarol or phenprocoumon. It is likely that these results can be extrapolated to warfarin. Relative risks varied considerably between the different antibacterial drugs, especially after stratification for the time-interval between start of the antibacterial drug therapy and the moment of overanticoagulation. The strongest risk increase was associated with co-trimoxazole. This is in line with an earlier study (32).

Overanticoagulation may be caused by pharmacokinetic- or pharmacodynamic interactions between antibacterial drugs and coumarins. Pharmacokinetic interactions may be caused by plasma protein binding displacement of coumarins or by inhibition of the metabolism of coumarins. Plasma protein binding displacement is a rapid and short-lived effect because the displaced molecules are rapidly metabolized. A small but transient increase in anticoagulant effect can occur (16). Usually this mechanism plays a minor role compared to other mechanisms (16). It may be clinically relevant in the elderly, however, in whom plasma protein binding decreases (36). Unlike enzyme induction, which may take several days or even weeks to develop, inhibition of cytochrome P450 enzymes can occur within two to three days, depending on the elimination half-life of the inhibited drug (16). Pharmacodynamic interactions may result from vitamin K deficiency by elimination of bacterial flora in the gut, and by direct inhibition of the synthesis of the vitamin K dependent coagulation factors (2, 16). Pharmacodynamic mechanisms take at least several days to develop because of already circulating coagulation factors (16). It has been stated that the contribution of bacterial synthesis to vitamin K status in man becomes important only when the dietary intake of the vitamin is markedly decreased (12, 37). Unfortunately, we were not able to investigate this as there were no case patients in our study with a low daily intake of vitamin K who had used antibacterial drugs.

A possible difference in risk of overanticoagulation with the use of acenocoumarol and phenprocoumon, would be caused by their difference in pharmacokinetic properties, as acenocoumarol and phenprocoumon have a similar effect on the elimination of bacterial flora in the gut and the direct inhibition of the synthesis of vitamin K dependent coagulation factors. Especially the variability in elimination kinetics could cause a difference in the contribution of the metabolic component to the pharmacokinetic interactions.

For amoxicillin, relative risks were significantly increased during both time intervals, suggesting that more than one mechanism may be involved. In the literature, nothing was found about possible pharmacokinetic interactions of coumarins with amoxicillin. Hence, the increased risk during the first days after start of amoxicillin is surprising. When amoxicillin was combined with an enzyme inhibitor, however, the relative risk was much lower. For clarithromycin, the relative risk of overanticoagulation was only significantly increased during the first 3 days after start of use. This is in accordance with the literature in which the most frequently suggested mechanism is the inhibition of the hepatic cytochrome P450 mixed-function oxidase system, resulting in a reduced clearance of coumarin anticoagulants and an increase in its effect (16, 18). For doxycycline, several mechanisms have been suggested, but in all published case reports overanticoagulation developed within 7 to 10 days (23, 24). Our data confirm this. For norfloxacin it is clear from our results that the rapid pharmacokinetic interactions are far more important than the delayed pharmacodynamic ones. Mechanisms suggested for norfloxacin to increase prothrombin times are plasma protein binding displacement (16) and inhibition of metabolic clearance (38). For co-trimoxazole the relative risk of overanticoagulation was strongly increased during the first 3 days of antibacterial drug therapy as well as thereafter.

Sulfonamides can strongly displace coumarin anticoagulants from their plasma protein binding sites (16) and it has been suggested that co-trimoxazole, which is metabolized by the CYP2C9 isoenzyme, increases the anticoagulant effect by inhibiting the metabolism of the anticoagulant (39). The degree of inhibition of the metabolism may be different for acenocoumarol and phenprocoumon (40) as acenocoumarol (like warfarin) is predominantly metabolized by CYP2C9 (41).

None of the other antibacterial drugs examined is metabolized by CYP2C9. Especially after ≥4 days, sulfamethoxazole is probably largely responsible for the increased prothrombin times considering the lower relative risk we found for trimethoprim alone. Vancomycin will probably, as a poorly absorbed drug, mainly reduce the bacterial synthesis of vitamin K and thereby increase the INR with a delayed onset (16). This is compatible with our results. However, for vancomycin, as well as for trimethoprim, numbers were too small to draw firm conclusions.

Some potential limitations should be considered in the interpretation of our cohort study. Selection bias was probably negligible as we identified all users

of coumarin anticoagulants in a defined population and because regular INR monitoring makes it unlikely that cases were missed. Also, information bias is not likely as all data on exposure and outcome were recorded similarly for subjects exposed and not exposed to combinations of antibacterial drugs and coumarin anticoagulants without prior knowledge of our study hypothesis. Potential confounding by gender, age, hepatic dysfunction, hypoalbuminemia, malignancies, hyperthyroidism, hypertension, congestive heart failure, low dietary intake of vitamin K, differences in duration of follow-up and INR measurement as scheduled, was dealt with in the multivariate analyses. Whether the INR-assessment on the index date was earlier than according to plan was taken into consideration because patients who are prescribed a known potentially hazardous combination are more likely to be monitored with short intervals. However, neither adjustment for this nor for the average number of assessments during follow-up changed our results. Fever may be a confounding factor as a hypermetabolic state produced by fever might potentiate the response to coumarin anticoagulants, probably by increasing the catabolism of vitamin K-dependent coagulation factors (2). There was, however, no significant difference between the frequency of fever in cases on antibacterial drugs and individuals in the rest of the cohort who were treated with antibacterial drugs on the index date. Furthermore, we excluded confounding by indication as there was no significant difference in the indication for antibacterial agents between cases and the remainder of the cohort. Moreover, confounding by indication would not explain the variation in relative risks between antibacterial agents and would occur similarly for all antibacterial drugs.

In conclusion, in this population-based cohort study among outpatients of an anticoagulation clinic using acenocoumarol or phenprocoumon, several antibacterial drugs were strongly associated with overanticoagulation. The onset of overanticoagulation after start of antibacterial drug therapy varied between drugs. The risk was most strongly increased by a course of amoxicillin, clarithromycin, norfloxacin and co-trimoxazole. Awareness of these drug interactions and more frequent monitoring during the initial stages of antibacterial drug therapy to maintain patients on coumarins within the recommended therapeutic ranges may minimize the risk of bleeding complications.

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3.4 Lifestyle and diet as risk factors for overanticoagulation

ABSTRACT *The risk of hemorrhage when using coumarin anticoagulants sharply increases when the International Normalized Ratio (INR) is ≥6.0. We performed a case-control study among outpatients of an anticoagulation clinic to identify sociodemographic-, lifestyle-, and dietary factors related to overanticoagulation. 300 cases with an INR ≥6.0 were compared with 302 randomly selected matched controls with an INR within the therapeutic zone. Information on sociodemographic factors, lifestyle factors, and diet, as well as on potential confounding factors, was collected by interviewing the patient, from the anticoagulant medical record and through the general practitioner. Age, sex and level of education were not associated with overanticoagulation. Body mass index was negatively related to overanticoagulation (OR 2.37; 95%CI 1.00-5.65, BMI <20 kg/m² vs >25 kg/m²), a beneath average level of physical activity was positively related to overanticoagulation (OR 1.61; 95%CI 1.02-2.53) and never-smokers were more likely to have an INR ≥6.0 compared to smokers (OR 1.70; 95%CI 1.02-2.84). Habitual alcohol consumption, even heavy drinking, was not related to overanticoagulation. However, a recent decrease of alcohol intake increased the risk of an INR ≥6.0 (OR 2.79; 95%CI 1.21-6.43). In addition, weight loss and a vacation were risk factors for overanticoagulation (OR 2.32; 95%CI 1.03-5.22 and OR 10.67; 95%CI 2.48-45.88, respectively). Dietary factors were not associated with overanticoagulation. Increased monitoring of INR-values if risk factors are present or avoidance of risk factors could prevent overanticoagulation and potential bleeding complications.*

INTRODUCTION

Coumarin anticoagulants are widely used in the prevention of venous and arterial thromboembolism (1). These drugs induce anticoagulation by antagonizing vitamin K, thereby impairing the biological activity of the vitamin K-dependent coagulation factors (factor II, VII, IX and X) (2). Opposite to their benefit, stands the risk of hemorrhage (3), which is strongly associated with the intensity of anticoagulation and sharply increases when the International Normalized Ratio (INR) is ≥6.0 (4,5). Such overanticoagulation should therefore be prevented. This necessitates identification of risk factors for overanticoagulation.

Increasing age and female sex have been associated with an enhanced response to coumarins; increased body weight was inversely related to the anticoagulant response (6). In addition, weight loss has been shown to result in decreased factor VII levels (7). A negative association with factor VII has been reported for physical activity, smoking, and intake of alcohol (8,9). Besides, cigarette smoke and alcohol may induce or inhibit cytochrome P450 enzymes (10,11). Cytochrome P450 metabolism is also affected by stress (12). Overanticoagulation after a dietary modification reducing the intake of vitamin K has been described (13,14). Factor VII coagulant activity has been found to be lowered by a diet lower in fat and higher in carbohydrate and fibre (15).

The association between overanticoagulation and sociodemographic-, lifestyle-, and dietary factors in a non-selected population under everyday circumstances, has not been studied extensively. Therefore, we have conducted a prospective nested case-control study among outpatients of an anticoagulation clinic. The aim of the study was to identify sociodemographic-, lifestyle- and dietary factors related to an INR ≥6.0 in previously stable patients. This paper is one of a series of three papers on risk factors for overanticoagulation. The other two papers are based on the same study and concern drug interactions, and comorbidity and characteristics of anticoagulant therapy.

METHODS

Setting

In the Netherlands, anticoagulant therapy is monitored by a network of more than 60 independently operating specialized anticoagulation clinics, covering over 90% of the country (16,17). The study was performed at the regional Red Cross anticoagulation clinic The Hague, which serves an area of nearly 700,000 inhabitants. All persons in this area with an indication for anticoagulant therapy are referred to this clinic.

Cohort definition

The study cohort consisted of all patients treated with coumarin anticoagulants

at the regional Red Cross anticoagulation clinic The Hague between December 1, 1997 and June 14, 1999. All cohort members were followed until the first occurrence of an INR ≥6.0, the end of their treatment or the end of the study period (i.e. the day on which the planned number of cases was recruited), whichever came first.

Cases and controls

Subjects for the nested case-control study were identified daily from all patients with an INR measurement on that day. Cases were defined as cohort members with an INR ≥6.0. For each case, one control, matched on target range, was randomly selected from the cohort members with an INR within the therapeutic zone (2.0-3.5 or 2.5-4.0), measured on the same day as the case (index day). Overanticoagulation is often seen during initiation of anticoagulant therapy and in unstable anticoagulation. Since this was not our primary interest, only cases and controls with stable anticoagulation in the three months preceding the index day were eligible. Anticoagulant therapy is considered effective and safe if the patient is kept within the therapeutic zone for more than two-third of the time (18,19). Therefore, we defined stable anticoagulation as having at least 66% of the INRs within the therapeutic zone and no INRs ≥5.5 in the three months preceding the index day. To judge stability, a minimum of three INRs had to be assessed in the three months preceding the index day. Cases and controls with a hospital admission in this period were excluded, since information on anticoagulant control during admission is often not available at the anticoagulation clinic. As we focussed on sudden overanticoagulation, the INR preceding the assessment on the index day had to be within the therapeutic zone. Patients not living independently and those making use of meals on wheels may be less able to give reliable answers to the questions on diet than persons or couples who prepare their own meals. Therefore, they were excluded. Since we were primarily interested in overanticoagulation, irrespective of the question whether this was followed by hemorrhage, patients who presented at the index day with a serious bleeding complication were excluded because this may promote recall bias.

Procedure

The study protocol has been approved by the Medical Ethics Committee of the Erasmus University Medical Center Rotterdam. We planned to recruit 300 cases and 300 controls to provide at least 80% power to detect a true odds ratio (OR) of ≥2.0 for risk factors having a prevalence of 7% among the controls, using a $p<0.05$ to reject the null hypothesis of OR=1.

Information on sociodemographic factors, lifestyle factors, and diet, as well as on potential confounding factors, was collected by interviewing the patient, from the anticoagulant medical record and through the general practitioner (GP). The interview took place within three weeks after the index day at the private address of the patient, making use of structured questionnaires with mainly

closed questions. In case the partner of the patient prepared the meals, this person assisted in answering the relevant food questions when necessary. The interviewers were blinded with respect to the patient's case or control status and the specific research hypotheses. This also applied to the GPs. Blinding of the patients was not fully feasible, since the INR-value is printed on their dosage list. To obviate this, in the information letter we referred to the problem of overanticoagulation in a general sense.

Sociodemographic factors, lifestyle factors, and diet

The risk period was defined as the four-week period preceding the index day. Especially time-varying changes in factors were expected to pose a risk for overanticoagulation, but steady factors were taken into account as well. Body height and body weight were measured to the nearest centimeter and the nearest 0.5 kg, respectively. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2) and categorized as <20, 20 up to 25, and >25. The patient was asked about his or her level of education (primary, secondary, or higher), extent of physical activity (equal to-, more than-, or less than persons of comparable age and health status) and smoking habits (non-smoker, ex-smoker, or never-smoker and the daily number of cigarettes, cigars, or pipes smoked). In addition, changes in weight, physical activity, and number of smoked units in the risk period were inquired about (no, less, or more and in case of weight the extent of change). Furthermore, it was asked whether the patient had experienced or had been affected by an impressive life event (e.g. removal, divorce, retirement, criminality, death or serious illness of a close friend or relative) in the risk period. Finally, an open question was posed on occurrences in the risk period that had not arisen during the interview.

A semiquantitative food frequency questionnaire including 170 foods and beverages (among which alcohol) was used to assess the patient's habitual diet (i.e. as consumed during the preceding year). The questionnaire has been validated and proven suitable for use in an elderly population (20,21). The patient was also asked whether the number of alcohol units had changed in the risk period (no, less, or more), whether excessive drinking (≥6 drinks/day) had occurred in the two weeks preceding the index day and whether his or her dietary habits had otherwise changed in the risk period and if so, what change occurred. The intake of fat, carbohydrates, fibre, and alcohol was computed using the Dutch food composition table 1993 (22). Intakes were categorized on the basis of tertiles, except for alcohol, the intake of which was categorized as none, <4 drinks/week, 4 drinks/week up to 2 drinks/day, 3 to 6 drinks/day, and ≥6 drinks/day. The vitamin K content of foods, however, is not included in the Dutch food composition table. In order to calculate the intake of vitamin K, we used data on concentrations of vitamin K_1 (phylloquinone) and vitamin K_2 (menaquinones: MK4 through MK10) as had been determined in a large variety of Dutch foods at the Department of Biochemistry and Cardiovascular Research

Institute, Maastricht University. The analytical method used has been described in detail elsewhere (23). For foods consumed in the Netherlands that had not been analysed, concentrations had been derived from data published by others (24-29). This had not been done for vitamin K_2 because of scarcity of data in the literature. The intake of vitamin K was categorized on the basis of tertiles. Since the absorption of vitamin K is strongly dependent on the source from which it is obtained and the length of the aliphatic side chain in the menaquinones (30), the intake was subdivided into vitamin K_1, total vitamin K_2, and all MK-subtypes separately. In the Netherlands, main dietary sources of vitamin K_1 are green leafy vegetables and vegetable oils. Vitamin K_2 is present in meats and eggs (MK4 only), and in fish, sauerkraut, cheese, and other dairy products (MK5 through MK10) (31).

Cofactors

A person's BMI, extent of physical activity, smoking and drinking habits, and habitual diet may be related to the presence of chronic comorbidities. Time-varying changes in lifestyle factors and diet may be the result of an acute illness or a relapse of a chronic comorbidity; situations that may be accompanied by fever and/or a change in drug use. Since acute illnesses, chronic comorbidities, fever, and a change in drug use may also interfere with anticoagulant therapy and enhance the response to coumarins (32-34), these were considered as potential confounders. The associations between these cofactors and overanticoagulation are the main subjects of the two other papers mentioned in the introduction.

Statistical analysis

Sociodemographic-, lifestyle-, and dietary factors related to an INR $\geq$6.0 were identified using univariate conditional logistic regression analysis at first. Since the unconditional analyses gave comparable results but more statistical power, we finally used unconditional logistic regression analysis to compute unadjusted odds ratios and their 95% confidence intervals (CI). In case a risk factor was absent in either the cases or the controls, a Fisher Exact test was performed instead. To assess steady sociodemographic-, lifestyle-, and dietary factors (i.e. age, sex, BMI, level of physical activity, smoking status, and habitual dietary intake and alcohol consumption) that were independently associated with an INR $\geq$6.0, all factors which were univariately associated at a $p<0.10$ were included in a multiple regression model. Beside age, sex, and the number of INR determinations in the preceding three months, comorbidities which were univariately associated with an INR $\geq$6.0 were included if this resulted in a change in one of the odds ratios of 5% or over, starting with the most potent factor. A comparable procedure was followed to assess changes in lifestyle- and dietary factors during the risk period that were independently associated with an INR $\geq$6.0.

To determine the importance of the independent risk factors for overanticoagulation in the population, we calculated the population attributable risk percent-

ages (PAR%) according to the following formula (35): PAR% = AR% * (proportion of exposed cases), with AR% = ((OR-1)/OR) * 100.

RESULTS

The nested case-control study included the planned number of 300 cases with a median INR of 6.8 and 302 controls with a median INR of 3.2. Of the cases, 83% had an INR of 6.0-7.9, 11% had an INR of 8.0-9.9, and 6% had an INR of 10.0-15.0 (the upper measurable INR). The participation among cases and controls was 78% and 85%, respectively. Written informed consent was obtained from every patient. The mean interval between the index day and the interview was fourteen days, for cases as well as for controls. Cardiac disease was the main indication for anticoagulation. 55% of the cases and 66% of the controls used phenprocoumon, the others used acenocoumarol.

Table 1. Association between overanticoagulation (INR ≥6.0) and sociodemographic and lifestyle factors[1].

Variable	Cases n=300	Controls n=302	OR (95% CI), univariate	OR (95% CI), multivariate
Age (years, mean±sd)	68.1 ± 12.3	68.2 ± 9.8	1.00 (0.98-1.01)	
Sex				
male	175 (58%)	194 (64%)	1 (reference)	
female	125 (42%)	108 (36%)	1.28 (0.92-1.78)	
Level of education				
higher	154	144	1 (reference)	
secondary	91	100	0.94 (0.59-1.50)	
primary	55	57	1.11 (0.72-1.71)	
BMI				
> 25 kg/m^2	171	211	1 (reference)	1 (reference)[a]
20 up to 25 kg/m^2	105	80	1.62 (1.14-2.31)	1.74 (1.16-2.61)[a]
< 20 kg/m^2	22	11	2.47 (1.16-5.23)	2.37 (1.00-5.65)[a]
Change in weight ≥2 kg				
no change	222	247	1 (reference)	1 (reference)[b]
weight loss	42	14	3.34 (1.78-6.28)	2.32 (1.03-5.22)[b]
weight gain	8	18	0.49 (0.21-1.16)	0.54 (0.20-1.41)[b]
Level of physical activity				
above average	119	144	1 (reference)	1 (reference)[a]
average	77	85	1.10 (0.74-1.62)	1.01 (0.65-1.57)[a]
beneath average	96	66	1.76 (1.18-2.62)	1.61 (1.02-2.53)[a]
Change in physical activity				
no change	179	219	1 (reference)	1 (reference)[b]
less active	92	54	2.08 (1.41-3.08)	1.13 (0.66-1.95)[b]
more active	28	29	1.18 (0.68-2.06)	0.61 (0.28-1.33)[b]
Smoking status				
smoker	98	100	1 (reference)	1 (reference)[a]
ex-smoker	115	144	0.82 (0.56-1.18)	0.99 (0.64-1.52)[a]
never-smoker	87	58	1.53 (0.99-2.36)	1.70 (1.02-2.84)[a]

- continued -

The associations between overanticoagulation and sociodemographic and lifestyle factors are shown in table 1. Age, sex and level of education were not associated with overanticoagulation. Regarding steady lifestyle factors, BMI, level of physical activity, and smoking status were associated with an INR ≥6.0. Patients with a BMI <20 kg/m^2 had an increased risk of an INR ≥6.0 of 2.37 (95%CI 1.00-5.65) compared to patients with a BMI >25 kg/m^2. The corresponding PAR% was 4.2%. For patients with a BMI of 20 up to 25 kg/m^2 the OR was 1.74 (95%CI 1.16-2.61) and the PAR% was 14.9%. In comparison with patients whose level of physical activity was above average, patients with a beneath average level of physical activity had an increased risk of overanticoagulation (OR 1.61; 95%CI 1.02-2.53, PAR% 12.1%). Never-smokers had an increased risk of overanticoagulation compared to smokers (OR 1.70; 95%CI 1.02-2.84, PAR% 11.9%). Habitual alcohol consumption, even heavy drinking, was not a risk factor for overanticoagulation. Recent occasional excessive drinking neither was associated with overanticoagulation. However, a recent decrease of alcohol intake

Table 1 - continued

Variable	Cases n=300	Controls n=302	OR (95% CI), univariate	OR (95% CI), multivariate
Change in amount smoked				
no change	280	283	1 (reference)	
smoked less	12	8	1.52 (0.61-3.76)	
smoked more	6	11	0.55 (0.20-1.51)	
Alcohol consumption				
none	78	88	1 (reference)	
< 4 drinks/week	72	65	1.25 (0.79-1.97)	
4 drinks/week up to 2 drinks/day	73	58	1.42 (0.90-2.25)	
3 to 6 drinks/day	64	83	0.87 (0.56-1.36)	
≥ 6 drinks/day	9	8	1.27 (0.47-3.45)	
Change in amount drunk				
no change	226	254	1 (reference)	1 (reference)[b]
drunk less	42	13	3.63 (1.90-6.94)	2.79 (1.21-6.43)[b]
drunk more	31	35	1.00 (0.59-1.67)	1.42 (0.74-2.74)[b]
Occasional excessive drinking[2]	8	15	0.53 (0.22-1.26)	
Impressive event	124	106	1.30 (0.94-1.81)	
Vacation	20	2	10.67 (2.48-45.88)	*

[1] Values are numbers unless indicated otherwise.
[2] At least once having drunk ≥6 drinks a day in the two weeks preceding the index day (restricted to patients who habitually drink less than 6 drinks a day).
[a] BMI, level of physical activity, smoking status, intake of fibre, age, sex, the number of INR determinations in the preceding three months, impaired liver function and congestive heart failure were included in the model.
[b] Change in weight ≥2 kg, change in physical activity, change in amount drunk, less often eaten a dinner, eaten less in general, eaten more fat-rich foods, age, sex, the number of INR determinations in the preceding three months, fever, diarrhea, relapse of congestive heart failure, use of antibacterial drugs and use of analgesics&NSAIDs were included in the model.
* Not computed (see discussion).

increased the risk of an INR ≥6.0 (OR 2.79; 95%CI 1.21-6.43, PAR% 9.0%). Another time-varying change in lifestyle factor associated with an increased risk of over-anticoagulation was losing weight. Patients with a recent weight loss of at least two kilograms had an increased risk of 2.32 (95%CI 1.03-5.22). The PAR% of over-anticoagulation associated with a recent weight loss of at least two kilograms was 8.0% Being less active was only univariately related to the risk of an INR ≥6.0 (OR 2.08; 95%CI 1.41-3.08).

With respect to occurrences in the risk period, the experience of an impressive life event was not associated with overanticoagulation. Twenty cases and two controls mentioned having been on vacation. All cases, but neither of the controls had been abroad and only three cases had had their INR checked during

Table 2. Association between overanticoagulation (INR ≥6.0) and dietary factors[1].

Variable	Cases n=300	Controls n=302	OR (95% CI), univariate	OR (95% CI), multivariate
Vitamin K				
> 320 µg/day	102	98	1 (reference)	
225 up to 320 µg/day	92	106	0.83 (0.56-1.24)	
< 225 µg/day	102	98	1.00 (0.68-1.48)	
Fat				
> 38 energy%	100	103	1 (reference)	
33 up to 38 energy%	94	101	0.96 (0.65-1.42)	
< 33 energy%	102	98	1.07 (0.73-1.58)	
Carbohydrates				
< 40 energy%	89	110	1 (reference)	
40 up to 46 energy%	105	94	1.38 (0.93-2.05)	
> 46 energy%	102	98	1.29 (0.87-1.91)	
Fibre				
> 18 gram/day	91	109	1 (reference)	1 (reference)[a]
13.5 up to 18 gram/day	99	106	1.12 (0.76-1.65)	1.04 (0.67-1.62)[a]
< 13.5 gram/day	106	87	1.46 (0.98-2.17)	1.33 (0.84-2.10)[a]
Change in dietary habits				
commencement / discontinuation of a diet	4	4	1.01 (0.25-4.06)	
less often eaten a dinner	45	15	3.40 (1.85-6.25)	1.12 (0.48-2.62)[a]
more often eaten a dinner	4	1	4.06 (0.45-36.29)	
eaten more irregular	2	0	p=0.25	
eaten less vegetables	4	0	p=0.06	
eaten less in general	9	1	9.36 (1.18-74.08)	2.95 (0.31-27.75)[b]
eaten more fat-rich foods	8	2	4.11 (0.87-19.45)	7.67 (1.38-42.67)[b]
other changes	13	6	2.26 (0.85-6.02)	

[1] Values are numbers.
[a] Intake of fibre, BMI, level of physical activity, smoking status, age, sex, the number of INR determinations in the preceding three months, impaired liver function and congestive heart failure were included in the model.
[b] Less often eaten a dinner, eaten less in general, eaten more fat-rich foods, change in weight ≥2 kg, change in physical activity, change in amount drunk, age, sex, the number of INR determinations in the preceding three months, fever, diarrhea, relapse of congestive heart failure, use of antibacterial drugs and use of analgesics&NSAIDs were included in the model.

the vacation. The increased risk of an INR ≥6.0 was 10.67 (95%CI 2.48-45.88). The corresponding PAR% was 6.0%.

The associations between overanticoagulation and dietary factors are represented in table 2. Since vitamin K_1 and all menaquinones gave comparable results, only the total vitamin K intake is included in the table. For none of the dietary factors examined (vitamin K, fat, carbohydrates, and fibre), habitual intake was a risk factor for overanticoagulation. Especially major changes in dietary intake were expected to be related to overanticoagulation, changes resulting in a decreased intake of vitamin K particularly. Of all changes mentioned by the patients, "less often eaten a dinner", nearly always as a consequence of having been ill, occurred most often (in 45 cases and 15 controls). It was, however, only univariately associated with an increased risk of an INR ≥6.0. The same applied to "eaten less in general", which was mentioned by nine cases and one control. On the contrary, "eaten more fat-rich foods" was an independent risk factor for overanticoagulation, with an OR of 7.67 (95%CI 1.38-42.67) and a PAR% of 2.3%.

Discussion

We studied the association between overanticoagulation and sociodemographic-, lifestyle-, and dietary factors. Some lifestyle factors were related to overanticoagulation: BMI was negatively related to overanticoagulation, a beneath average level of physical activity was positively related to overanticoagulation and never-smokers were more likely to have an INR ≥6.0 compared to smokers. Furthermore, a recent decrease of alcohol intake, weight loss, a vacation, and "eaten more fat-rich foods" appeared risk factors for overanticoagulation. The clinical implication of our findings lies in the possibility of prevention or early detection of overanticoagulation, and thus of bleeding complications, by paying special attention to these risk factors when monitoring anticoagulation. For example, fragile and physically inactive patients should be monitored more carefully. Similarly, patients should be advised to have their INR checked when on vacation.

The study population was confined to stably anticoagulated patients because most cases of unstable anticoagulation and overanticoagulation occur during initiation of therapy. Every clinician is aware of this. Would we not have excluded unstable patients, we would have found initiation of therapy as most important risk factor for overanticoagulation, a relatively irrelevant finding. It would have been difficult to release more subtle but also clinically relevant risk factors as the large majority of cases would simply occur within the first weeks of treatment as part of the titration process during the initiation phase of therapy. Clinicians might be more interested in the risk factors they encounter when their patients are on long-term anticoagulant therapy. To gain more insight into such risk fac-

tors, we focussed on patients who were more or less stably anticoagulated but suddenly developed an INR ≥6.0. Potential risk factors were inquired over the four weeks preceding the index day. Due to logistical limitations, the interview took place up to three weeks after the index day. Misclassification of exposure thus may have been present; a patient may have forgotten things or been mistaken regarding the time period in which these occurred. The mean interval between overanticoagulation and interview, however, was similar for cases and controls. Besides, the misclassification is assumed to be nondifferential, resulting in a conservative estimation of the association. Recall bias was prevented by restricting the study population to patients with non-symptomatic overanticoagulation, i.e. by excluding patients who presented at the index day with a serious bleeding complication. In addition, in the information letter we referred to the problem of overanticoagulation in a general sense. The exclusion of patients who presented on the index day with a serious bleeding complication will not have had a substantial effect on our results as this concerned only very few patients: of the 4476 patients with an INR ≥6.0 in the study period, only three patients presented on the index day with a serious bleeding complication. This is explained by the fact that a patient with a serious bleeding complication will visit the hospital and not the anticoagulation clinic.

Ex-smokers had the same risk of overanticoagulation compared to smokers. This unexpected observation argues for the presence of an unknown confounding factor.

The interaction between alcohol consumption and anticoagulant therapy is complex (36). Large intermittent doses cause some enzyme inhibition with resultant increased INR, while chronic heavy use (greater than 60 g/day) causes enzyme induction resulting in a decreased INR. Intermediate use (two to three drinks per day) probably does not alter the anticoagulant metabolism at all. In our study, neither category of habitual alcohol consumption, nor occasional excessive drinking in the two weeks preceding the index day was associated with overanticoagulation.

Having been on vacation greatly increased the risk of overanticoagulation. The concept 'vacation' is a combination of changes in lifestyle factors, dietary habits, and other factors and is, in the context of anticoagulation, difficult to interpret. We did not compute an adjusted OR because this would be meaningless. The effect of vacation on anticoagulation seems to be responsible for the unexpected *increased* risk of an INR ≥6.0 in patients who had "eaten more fat-rich foods". Of the eight cases who mentioned having "eaten more fat-rich foods", seven also mentioned having been on vacation.

Dietary intake did not play a role in overanticoagulation; a reassuring observation. Lack of an effect of habitual dietary intake is plausible, since anticoagulant therapy likely is titrated to a patient's diet. With respect to the absence of an effect of changes in dietary habits, the increase in INR by the dietary modification may have been of less magnitude than defined in our study. Another pos-

sibility is that the dietary changes were too small or lasted too short a time to affect anticoagulant therapy. The case reports on dietary-induced overanticoagulation concerned a drastic dietary modification, viz. giving up consuming 750 to 1000 grams of liver every week (13,14). Regarding the potential interaction between dietary factors and overanticoagulation, it should be kept in mind that not dietary intake, but nutritional status is the real risk factor. The extent to which intake is a good proxy of status depends on the bioavailability of the nutrient in question. For example, green leafy vegetables have a high vitamin K content, but due to tight binding to the thylakoid membranes of the chloroplasts intestinal absorption of vitamin K is poor (30). Furthermore, a decreased dietary intake does not immediately result in a deficient nutritional status.

To our knowledge, only one epidemiological study on risk factors for overanticoagulation in a non-selected population under everyday circumstances including lifestyle- and dietary factors has been published (37). The number of lifestyle- and dietary factors studied, however, was small: alcohol consumption and the intake of vitamin K. Subjects with a higher habitual vitamin K consumption, based on reported weekly intake of twelve vitamin K_1 rich foods, and habitual moderate consumption of alcohol were less likely to have an INR $\geq$6.0. We did not find an association between these factors and overanticoagulation. In addition, decreased oral intake in general was an independent determinant of an INR $\geq$6.0 in the study of Hylek et al., while in our study, the increased risk was no longer significant after adjustment for potential confounders. An important difference between the study of Hylek et al. and our study is that we only included stable cases and controls. Besides, we assessed changes in the preceding four weeks, while they used a one-week risk period. Furthermore, we calculated the intake of vitamin K from the total diet and took vitamin K_2 into account as well. Lastly, the study population of Hylek et al. used warfarin, while our patients used phenprocoumon or acenocoumarol.

In conclusion, in this study among previously stable outpatients of an anticoagulation clinic, some lifestyle factors were related to overanticoagulation. Increased monitoring of INR-values if risk factors are present or avoidance of risk factors, could prevent overanticoagulation and potential bleeding complications.

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3.5 Deficient dietary intake of vitamin K is associated with an increased risk of overanticoagulation

ABSTRACT *A dietary intake of vitamin K of 1 μg/kg body weight per day is required for normal functioning of coagulation factors. Possibly, a deficient intake of vitamin K is associated with overanticoagulation. We performed a population-based cohort study in a sample of the Rotterdam Study to study whether patients with a deficient dietary intake of vitamin K have an increased risk of overanticoagulation (International Normalized Ratio (INR) ≥6.0). The study cohort consisted of all participants of whom dietary intake data have been collected and who were treated with coumarin anticoagulants in the study period from the baseline visit of the Rotterdam Study (1990-1993) through December 31, 1998. All cohort members were followed from their baseline visit of the Rotterdam Study until the first occurrence of an INR ≥6.0, the last INR-assessment during the study period, death or end of the study period. The intake of vitamin K was calculated from the total diet using data on concentrations of vitamin K_1 and vitamin K_2 in foods. An intake of vitamin K below 1 μg/kg body weight per day was considered deficient. Of the 772 patients in the cohort, 227 developed an INR ≥6.0 during the study period. The number of patients in the total cohort with a deficient dietary intake of vitamin K was 12 (1.6%). Of the cases, seven patients (3.1%) had a deficient dietary intake of vitamin K. The adjusted relative risk of overanticoagulation associated with a deficient dietary intake of vitamin K was 9.6 (95%CI 4.0-23.0). To minimize the risk of bleeding complications, patients on oral anticoagulant therapy should be advised to consume vitamin K-rich foods such as green, leafy vegetables.*

INTRODUCTION

Coumarin anticoagulants are widely used in the prevention of venous and arterial thromboembolism (1). These drugs induce anticoagulation by antagonizing vitamin K, thereby impairing the biological activity of the vitamin K-dependent coagulation factors II, VII, IX and X (2). Hemorrhage is the most common adverse reaction to coumarin anticoagulants. Its risk is strongly associated with the intensity of anticoagulation and sharply increases when the International Normalized Ratio (INR) is ≥6.0 (3, 4). For normal functioning of coagulation factors, a habitual dietary intake of vitamin K of 1 μg/kg body weight per day is required (5, 6). Considering the mode of action of coumarin anticoagulants, it is obvious that a low dietary intake of vitamin K will require a low-normal daily dose of coumarins. Because oral anticoagulant therapy is regularly monitored and the dose of coumarins is adjusted in an individual on the basis of the INR, however, there is no reason to assume that a patient's low habitual dietary intake of vitamin K is associated with an increased risk of overanticoagulation.

In the literature there is only limited information on the association between dietary intake of vitamin K and overanticoagulation. Two case reports described overanticoagulation after discontinuation of a weekly consumption of 750 to 1000 grams of liver (7, 8). In a case-control study on risk factors for overanticoagulation, the habitual weekly intake of twelve vitamin K_1-rich foods was inversely associated with the risk of overanticoagulation (9).

We conducted a cohort study in a large population of community-dwelling elderly to study whether patients with a habitual dietary intake of vitamin K below the amount required for normal functioning of coagulation factors have an increased risk of overanticoagulation.

METHODS

Setting

Data were obtained from the Rotterdam Study and from the regional outpatient anticoagulation clinic. The Rotterdam Study is a prospective population-based cohort study of neurological, cardiovascular, locomotor, and ophthalmologic diseases in the elderly. All inhabitants of Ommoord, a suburb of the city of Rotterdam in the Netherlands, aged 55 years or over and living in the district for at least one year were invited in 1990-1993 to participate in the study. The rationale and design of this study have been described elsewhere (10). The cohort comprises 7983 individuals who were all interviewed and investigated at baseline. The regional outpatient anticoagulation clinic monitors all inhabitants of Ommoord with an indication for anticoagulant therapy. The choice of anticoagulant (phenprocoumon or acenocoumarol) is made by the referring physician. The optimal target range of oral anticoagulant therapy, as recommended by the

Federation of Dutch Thrombosis Centers, lies between 2.5 and 3.5 INR or between 3.0 and 4.0 INR, depending on the indication for treatment. INR measurements are performed at a mean interval of two to three weeks, the interval being six weeks at a maximum. Dosing of the coumarin is performed by a team of specialized physicians routinely working at the anticoagulation clinic, with the aid of a computerized dosing program. All laboratory-, clinical- and administrative data as of 1986 are stored in computerized files. For our cohort study, the data until December 31, 1998 were used.

Cohort and outcome definition

The study cohort consisted of all participants of the Rotterdam Study of whom dietary intake data have been collected and who were treated with the coumarins acenocoumarol or phenprocoumon in the study period between the baseline visit of the Rotterdam Study (1990-1993) and December 31, 1998. The cohort included prevalent users at baseline as well as incident users during follow-up. All cohort members were followed from their baseline visit of the Rotterdam Study until the first occurrence of an INR $\geq$6.0, the last INR-assessment during the study period, death or end of the study period, whichever came first. In case a patient had multiple treatment episodes during follow-up, all episodes in the study period were considered. The date on which an INR $\geq$6.0 was encountered was defined as the index date.

Exposure definition

The exposure of interest in this study was habitual dietary intake of vitamin K_1 and K_2. A semiquantitative food frequency questionnaire including 170 foods and beverages was used to assess the habitual diet of each participant as consumed during the preceding year. The questionnaire has been validated and proven suitable for use in an elderly population (11, 12). In order to calculate the intake of vitamin K, we used data on concentrations of vitamin K_1 (phylloquinone) and vitamin K_2 (menaquinones: MK4 through MK10) as have been determined in a large variety of Dutch foods at the Department of Biochemistry and Cardiovascular Research Institute, Maastricht University. The analytical method used has been described in detail elsewhere (13). For foods included in the food frequency questionnaire which have not been analysed, concentrations were derived from data published by others (14-19). This was not done for vitamin K_2 because of scarcity of data in the literature.

The intake of vitamin K was expressed in µg/kg body weight per day. An intake below 1 µg/kg body weight per day was considered deficient (5, 6). If information on weight was missing (n=7), the intake of vitamin K was calculated by reference to the mean weight of male and female cohort members. For women an intake below 72 µg/day and for men an intake below 79 µg/day was considered deficient.

Cofactors

A person's habitual diet is only one aspect of his or her lifestyle and may be related to body mass index (BMI), smoking status and alcohol consumption. In addition, a person's habitual diet may be related to the presence of an impaired liver function, congestive heart failure or malignancies. Since the lifestyle factors and chronic comorbidities mentioned may interfere with anticoagulant therapy and enhance the response to coumarins (20, 21), these were considered as potential confounders. Furthermore, we considered the phase of oral anticoagulant therapy on the index date (initiation phase, i.e. day 1 to day 41, or stabilized phase, i.e. ≥42 days) and, for patients in the stabilized phase, the mean monitoring interval in the three months preceding the index date.

Statistical analysis

The cohort included prevalent users of coumarins at baseline as well as incident users during follow-up. In addition, the moment on which incident users started anticoagulant therapy differed and multiple treatment episodes were considered. Consequently, cohort members were not necessarily receiving anticoagulant therapy during the whole follow-up. Therefore, a time-dependent Cox proportional hazards regression model was used to compute the relative risk (RR) and 95% confidence interval (CI) of overanticoagulation associated with a deficient dietary intake of vitamin K. In this model, the status of a particular determinant at the index date of each case of an INR ≥6.0, is compared to the status of this determinant in all cohort members who are alive and at risk for the outcome. The relative risk was adjusted for age and gender. Furthermore, we adjusted for all cofactors which were univariately associated with an INR ≥6.0 if this caused a change in the point estimate of more than 5 percent. Population attributable risk percentages were calculated with the formula ((RR-1)/RR) * 100 * proportion of exposed cases (22).

RESULTS

The study cohort consisted of 772 patients, with a mean follow-up of 1650±764 days. Nearly 85% of the patients used acenocoumarol and the remainder phenprocoumon. As all vitamin K deficient cases used acenocoumarol, no further distinction is made between the two coumarins. During the study period, 227 patients developed an INR ≥6.0. The mean duration of follow-up in the cases was 1532±627 days. Baseline characteristics of the cases and the total cohort are shown in table 1. Men and women had a more or less similar risk of an INR ≥6.0. Patients of 75 years and older had an increased risk. Furthermore, the risk of overanticoagulation was associated with BMI, current smoking, an impaired liver function, congestive heart failure and malignancies. Patients with an intermediate intake of alcohol tended to have a slightly lower risk of overanticoag-

Table 1. Baseline characteristics of the cases and the total cohort[1].

Variable	Cases n=227	Total cohort n=772
Sex		
male	122 (54%)	386 (50%)
female	105 (46%)	386 (50%)
Age (years, mean (sd))	70.7 (6.8)	69.6 (7.1)
55 - 64 years	44 (19%)	194 (25%)
65 - 74 years	111 (49%)	378 (49%)
≥ 75 years	72 (32%)	200 (26%)
BMI		
> 25 kg/m^2	130 (58%)	499 (65%)
20 - 25 kg/m^2	89 (40%)	255 (33%)
< 20 kg/m^2	5 (2%)	11 (1%)
Smoking status		
never smoker	58 (26%)	232 (30%)
former smoker	103 (45%)	339 (44%)
current smoker	66 (29%)	199 (26%)
Alcohol intake		
none	68 (30%)	225 (29%)
≤ 15 g/day	106 (47%)	366 (47%)
15 - 30 g/day	30 (13%)	107 (14%)
> 30 g/day	23 (10%)	74 (10%)
Impaired liver function[2]	2 (1%)	8 (1%)
Congestive heart failure[3]	56 (25%)	128 (17%)
Malignancies[3,4]	37 (16%)	85 (11%)

[1] Values are numbers (percentages) unless indicated otherwise.
[2] Defined as serum aminotransferases or bilirubine >2x the upper level of normal.
[3] Assessed by reference to the index date.
[4] A diagnosis of a malignancy prior to the index date or within the first year after the index date.

Table 2. Daily dietary intake of vitamin K (median (interquartile range)) in the cases and the total cohort[1].

Variable	Cases	Total cohort
All cohort members	n=227	n=772
Intake of vitamin K (µg)	254 (140)	259 (134)
Intake of vitamin K (µg/kg)	3.6 (1.9)	3.5 (1.8)
Intake of vitamin K_1 (µg)	228 (137)	232 (128)
Intake of vitamin K_2 (µg)	25 (17)	26 (19)
Vitamin K deficient patients	n=7 (3.1%)	n=12 (1.6%)
Intake of vitamin K (µg)	64 (37)	57 (28)
Intake of vitamin K (µg/kg)	0.71 (0.40)	0.73 (0.27)
Intake of vitamin K_1 (µg)	20 (30)	36 (30)
Intake of vitamin K_2 (µg	15 (9)	16 (21)

[1] Because of the use of medians, the intake of vitamin K_1 and K_2 do not add up to the intake of vitamin K.

ulation but the difference with non-use was not significant.

The median daily dietary intake of vitamin K in the total cohort was 259 µg, or 3.5 µg/kg body weight (table 2). Subdivided into vitamin K_1 and vitamin K_2, the median daily intakes were 232 µg and 26 µg, respectively. Case patients had similar median intakes. The number of patients in the total cohort with a deficient dietary intake of vitamin K was 12 (1.6%). In these patients, the median daily intake of vitamin K was 57 µg, that of vitamin K_1 was 36 µg and that of vitamin K_2 was 16 µg. Expressing the intake of vitamin K in µg/kg body weight per day, the intake ranged from 0.20 to 1.0 µg/kg body weight per day, with a median of 0.73. Of the cases, seven patients (3.1%) had a deficient dietary intake of vitamin K, with a median intake of 0.71 µg/kg body weight per day. In the vitamin K deficient cases, the median daily intakes of vitamin K_1 and vitamin

Table 3. The relative risk of overanticoagulation (INR ≥6.0) associated with a deficient dietary intake of vitamin K.

Variable	RR_{crude} (95%CI)[1]	$RR_{adjusted}$ (95%CI)[2]
Deficient dietary intake of vitamin K	9.2 (4.0-20.8)	9.6 (4.0-23.0)
Sex		
male	1.0 (reference)	1.0 (reference)
female	1.2 (0.9-1.5)	0.8 (0.6-1.1)
Age		
55 - 64 years	1.0 (reference)	1.0 (reference)
65 - 74 years	1.0 (0.7-1.5)	1.1 (0.8-1.6)
≥ 75 years	1.5 (1.03-2.2)	1.7 (1.2-2.6)
BMI		
> 25 kg/m^2	1.0 (reference)	1.0 (reference)
20 - 25 kg/m^2	1.6 (1.2-2.1)	1.3 (0.99-1.8)
< 20 kg/m^2	1.2 (0.5-2.9)	1.0 (0.4-2.6)
Smoking status		
never smoker	1.0 (reference)	1.0 (reference)
former smoker	1.0 (0.7-1.4)	1.0 (0.7-1.6)
current smoker	1.5 (1.02-2.1)	1.6 (1.05-2.5)
Alcohol intake		
none	1.0 (reference)	1.0 (reference)
≤ 15 g/day	0.8 (0.6-1.1)	1.2 (0.8-1.7)
15 - 30 g/day	0.6 (0.4-1.01)	1.0 (0.6-1.7)
> 30 g/day	0.8 (0.5-1.3)	1.0 (0.6-1.6)
Impaired liver function	6.6 (1.5-28.7)	9.7 (2.2-43.0)
Congestive heart failure	1.6 (1.1-2.1)	1.5 (1.1-2.1)
Malignancies	2.3 (1.6-3.3)	2.6 (1.7-3.8)

[1] Univariate analyses of relative risks were done with the time-dependent Cox proportional hazards regression model. In this model, the status of a particular determinant at the index date of each case of an INR ≥6.0, is compared to the status of this determinant in all cohort members who are alive and at risk for the outcome. Hence, crude RRs can not be calculated with the numbers in this table.

[2] Adjusted for sex, age, BMI, smoking status, alcohol intake and impaired liver function (missing value indicators included) at baseline, and congestive heart failure and malignancies by reference to the index date.

K_2 were 20 μg and 15 μg, respectively. The crude relative risk of overanticoagulation associated with a deficient dietary intake of vitamin K was 9.2 (95%CI 4.0-20.8) (table 3). Only in one out of the seven patients with a deficient vitamin K intake, overanticoagulation occurred within the first week of treatment. After adjustment for potential confounders the relative risk was 9.6 (95%CI 4.0-23.0). The population attributable risk percentage of overanticoagulation associated with a deficient dietary intake of vitamin K in elderly outpatients of an anticoagulation clinic was 2.8%. Adjustment for the phase of oral anticoagulant therapy on the index date did not substantially change the relative risk. For patients in the stabilized phase, i.e. treated for at least six weeks, the relative risk was also adjusted for the mean monitoring interval in the three months preceding the index date. The adjusted relative risk of overanticoagulation associated with a deficient dietary intake in these patients was 7.1 (95%CI 2.7-18.6). The corresponding population attributable risk percentage was 2.4%.

Discussion

In this population-based cohort study, a deficient intake of vitamin K as calculated from the total diet using data on concentrations of vitamin K_1 and vitamin K_2 in foods, was associated with a considerably increased risk of an INR $\geq$6.0. Apparently, when the habitual dietary intake of vitamin K is below the amount required for normal functioning of coagulation factors, regular monitoring and adjustment of the dose of coumarins does not abolish the risk of overanticoagulation. To minimize the risk of bleeding complications, patients on oral anticoagulant therapy should be advised to consume vitamin K-rich foods such as green, leafy vegetables.

In addition to the fact that the intake of vitamin K is insufficient for normal functioning of coagulation factors, patients on coumarins with a deficient dietary intake of vitamin K are at increased risk of overanticoagulation by antibacterial drugs which may interfere with bacterial synthesis of vitamin K in the colon (23). The contribution of bacterial synthesis of vitamin K in the colon to the vitamin K status becomes important when the dietary intake of the vitamin is markedly decreased (24, 25). As none of our vitamin K deficient cases was exposed to antibacterial drugs on the index date, we were not able to confirm the increased risk of overanticoagulation by antibacterial drugs in patients on coumarins with a deficient dietary intake of vitamin K. If overanticoagulation occurs within the first week of treatment, it may be caused by a relatively too high starting dose of coumarins. In our study, however, this concerned only one vitamin K deficient patient and did not explain the increased risk of overanticoagulation in patients with a deficient dietary intake of vitamin K.

Some potential limitations should be considered in the interpretation of our findings. Selection bias was probably negligible as we identified all users of cou-

marin anticoagulants in a defined population and because regular INR monitoring makes it unlikely that cases were missed. Information bias is also unlikely as all data on exposure and outcome were gathered prospectively and recorded similarly for all cohort members without prior knowledge of our study hypothesis. Misclassification of exposure may be present since the habitual dietary intake of vitamin K was assessed at baseline and may have changed during follow-up. However, misclassification of exposure usually leads to a conservative estimate of the relative risk. This suggests that the actual risk of overanticoagulation in patients with a deficient dietary intake of vitamin K may be higher. Potential confounding by sex, age, BMI, smoking status, alcohol consumption, impaired liver function, congestive heart failure, and malignancies was dealt with in the multivariate analyses. Similarly, the phase of therapy on the index date and, for patients in the stabilized phase, the mean monitoring interval in the three months preceding the index date were considered. Although our study pertained to the coumarins acenocoumarol and phenprocoumon, it is likely that the results can be extrapolated to warfarin because here, vitamin K plays a similar role.

In our study population, and in the Netherlands in general, the dietary intake of vitamin K is high and vitamin K deficiency is rare. Therefore, the public health impact of a deficient dietary intake of vitamin K on overanticoagulation is probably modest considering the population attributable risk percentage in elderly outpatients of an anticoagulation clinic of 2.8%. However, in the USA, the mean dietary intake of vitamin K_1 is much lower and only 80 μg/day in young adults and 150 μg/day in older adults (26). In these populations, the occurrence of an INR ≥6.0 associated with a deficient dietary intake of vitamin K, may concern many more patients.

In conclusion, in this population-based cohort study, outpatients of an anticoagulation clinic with a deficient dietary intake of vitamin K had an increased risk of overanticoagulation. Since overanticoagulation is associated with an increased risk of hemorrhages, patients on oral anticoagulant therapy should be advised to consume vitamin K-rich foods such as green, leafy vegetables.

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4

Coagulant levels in overanticoagulation

Levels of vitamin K-dependent pro- and anticoagulant proteins in overanticoagulated patients

ABSTRACT *Coumarin anticoagulants impair the biological activity of the vitamin K-dependent pro- and anticoagulant proteins. There are no reports which focus on the levels of these proteins in overanticoagulated patients. Therefore, we determined the levels of factor II, VII, IX and X, protein C and S in 25 randomly selected overanticoagulated patients (International Normalized Ratio (INR) ≥6.0) and in 25 matched controls with an INR within the therapeutic zone. Furthermore, to study a possible effect of the cause of overanticoagulation, coagulant levels were compared between 16 overanticoagulated patients with fever in the preceding two weeks and 24 overanticoagulated patients with stable congestive heart failure. The pattern of procoagulant level reductions in the three groups of overanticoagulated patients was largely the same as in controls: factor X was the lowest and factor IX the highest. The difference was that in overanticoagulated patients factor VII was relatively low among the procoagulant factors compared to controls. Protein C was lower than protein S in overanticoagulated patients with congestive heart failure, but was similar to protein S in the other study groups. In overanticoagulated patients with fever, the vitamin K-dependent coagulation proteins except factor X were significantly lower than in overanticoagulated patients with congestive heart failure, especially factor VII and protein S.*

INTRODUCTION

Coumarin anticoagulants induce anticoagulation by antagonizing vitamin K, thereby impairing the γ-carboxylation and consequently the biological activity of the vitamin K-dependent pro- and anticoagulant proteins (factor II, VII, IX and X, protein C and S) (1). The rate of disappearance from the plasma of these proteins is determined by their half-lives, which range from 5 hours for factor VII to 96 hours for factor II (2).

A number of studies has been done on the extent to which the different vitamin K-dependent procoagulant factors are depressed in patients on long-term oral anticoagulant therapy at therapeutic levels of anticoagulation (3-6): factor X is most strongly, and factor IX is least strongly reduced. However, to our knowledge, there are no reports which focus on the levels of these factors in overanticoagulated patients. Therefore, we determined the levels of factor II, VII, IX and X, protein C and S in a subset of patients who participated in a prospective nested case-control study on the association between overanticoagulation and characteristics of anticoagulant therapy and comorbidity. Coagulant levels were compared between a random sample of overanticoagulated patients (International Normalized Ratio (INR) ≥6.0) and matched controls with an INR within the therapeutic zone (2.0-3.5 or 2.5-4.0). To study a possible effect of the cause of overanticoagulation on coagulant levels, overanticoagulated patients with fever were compared to those with congestive heart failure.

METHODS

Setting

The present study is part of a prospective nested case-control study on the association between overanticoagulation and characteristics of anticoagulant therapy and comorbidity performed at the regional Red Cross anticoagulation clinic The Hague (7). In short, cases with an INR ≥6.0 were compared with randomly selected controls with an INR within the therapeutic zone (2.0-3.5 or 2.5-4.0, depending on the indication), matched on index day and target range. Cases and controls had to be stably anticoagulated in the three months preceding the index day and the INR preceding the assessment on the index day had to be within the therapeutic zone. Information on characteristics of anticoagulant therapy, comorbidity and potential confounders in the four weeks preceding the index day was collected from the anticoagulant medical record, through the general practitioner and the pharmacy, and by interviewing the patient. Patients on acenocoumarol appeared to have an increased risk of an INR ≥6.0 compared to patients on phenprocoumon. Regarding comorbidity, impaired liver function, congestive heart failure, diarrhea and fever turned out to be risk factors for overanticoagulation (7).

Patients

For the present study four groups were selected from the larger nested case-control study: group 1, 25 randomly selected overanticoagulated patients; group 2, 25 matched controls; group 3, 16 overanticoagulated patients with fever in the two weeks preceding the index day, but with none of the following chronic comorbidities that are suspected to interfere with anticoagulant therapy (8-11): impaired liver-, biliary- or pancreatic function, impaired gastro-intestinal absorption, congestive heart failure, hyperthyroidism, or a malignancy; group 4, 24 overanticoagulated patients suffering from congestive heart failure, but with none of the following acute risk factors for overanticoagulation (7, 12, 13) in the four weeks preceding the index day: fever, diarrhea, a change in the use of potentially interacting drugs, a change in the number of alcohol units and a vacation. The patients with congestive heart failure had been in a stable condition in the four weeks preceding the index day, i.e. no relapses had occurred in this period. Fever and congestive heart failure were studied as causes of overanticoagulation because for these risk factors a sufficient number of patients fulfilled the above mentioned inclusion criteria. Furthermore, the mechanism by which overanticoagulation is caused is different for these risk factors. The random sample of overanticoagulated patients (group 1) included five patients with fever and three patients with congestive heart failure.

Laboratory methods

For the nested case-control study, subjects were identified daily from all patients with an INR measurement on that day. For this measurement, venous blood was collected in 3.2% sodium citrate tubes. The prothrombin time was measured in plasma, obtained by centrifugation at 3000 rpm for 10 minutes, on an Elektra 1800 C automated coagulometer with a human recombinant tissue factor thromboplastin (RecombiPlastin, Instrumentation Laboratory, Breda, The Netherlands) with an International Sensitivity Index between 0.93 and 1.00 during the study period, according to the different batches used. After determining the INR, the plasma was stored at -80°C until use for the present study. The pro- and anticoagulant proteins were determined at the department of Hematology, Erasmus University Medical Center Rotterdam on a Sysmex CA-1500 automated coagulometer (Dade-Behring, Brussels, Belgium). The levels were measured by functional one-stage clotting assay using immuno-depleted plasma (Biopool, Kordia, Leiden, The Netherlands) with Thromborel®S (Dade-Behring, Brussels, Belgium) for factor II, VII and X, and Platelin®LS (bioMérieux, 's-Hertogenbosch, The Netherlands) for factor VIII and IX. The levels of protein S and protein C were determined according to the instructions of the manufacturers, i.e. Roche Diagnostics, Almere, The Netherlands and Instrumentation Laboratory, Breda, The Netherlands, respectively. Fibrinogen was assessed according to Clauss (Dade-Behring, Brussels, Belgium). The interassay (intra-assay) coefficients of variation of the assays of factor II, VII, VIII, IX and X, protein C and S, and fibrinogen are 3.3%

(4.8%), 2.1% (8.0%), 2.8% (6.5%), 1.3% (6.2%), 3.0% (4.5%), 1.7% (4.2%), 4.7% (9.5%) and 1.9% (4.3%), respectively. Factor VIII and fibrinogen were only determined in the latter two study groups to differentiate between acute fever and chronic congestive heart failure as causes of overanticoagulation. Normal values of factor VIII and fibrinogen are 0.60-1.40 U/ml and 1.5-3.6 g/l, respectively.

To compare the balance between coagulation and anticoagulation between study groups, the ratio of the mean of the procoagulant factors II, VII, IX and X to the mean of the anticoagulant proteins C and S was calculated for each patient in addition to the individual coagulant levels.

Statistical analysis

Comparisons between study groups were conducted by Student's t-test or by Mann-Whitney U test in case of non-normally distributed variables. Paired Student's t-test or Wilcoxon matched pairs signed rank sum test were used for comparisons within study groups. The comparisons within and between randomly selected overanticoagulated patients and matched controls were also performed separately for users of acenocoumarol and users of phenprocoumon. We did not perform stratified analyses in overanticoagulated patients with fever and overanticoagulated patients with congestive heart failure, because of small numbers in either of the strata. Instead, comparisons between groups were adjusted for the type of anticoagulant in a multivariate linear regression model. All tests were two-sided with rejection of the null hypothesis at a p-value <0.05.

RESULTS

We compared the coagulant levels between randomly selected overanticoagulated patients, who had a median INR of 7.0, and matched controls, who had a median INR of 2.9. The characteristics of these two study groups are listed in table 1; age, sex and the type of anticoagulant did not differ significantly. The levels of factor II, VII, IX and X, protein C and S in the randomly selected overanticoagulated patients and matched controls are shown in table 2. Since the median and the mean of the majority of variables differed only slightly, for ease of interpretation mean values are given in the table. As expected, all vitamin K-dependent coagulation proteins were much lower in the overanticoagulated patients compared to their controls. This was observed with the use of both acenocoumarol and phenprocoumon (data not shown). The absolute difference in mean level ranged from 0.04 for factor X to 0.17 for factor IX; the relative difference ranged from 36% for factor X to 58% for factor VII. The ratio of the mean of the procoagulant factors II, VII, IX and X to the mean of the anticoagulant proteins C and S was the same in overanticoagulated patients and controls (median (interquartile range) 0.72 (0.28) and 0.72 (0.12), respectively). So, on average, the procoagulant factors and the anticoagulant proteins were lowered by the

same percentage in the overanticoagulated patients and their controls.

In both study groups, of the procoagulant factors, factor X was the lowest and factor IX the highest; the anticoagulant proteins C and S were similar. The difference between the two groups was that in controls factor VII was significantly higher than factor II, whereas in overanticoagulated patients factor VII was relatively low among the procoagulant factors compared to controls and similar to factor II. When stratifying for the type of anticoagulant, in controls using phenprocoumon all procoagulant factors differed significantly from each other (factor X<II<VII<IX), but in controls using acenocoumarol factor II and VII were similar. In overanticoagulated patients, with the use of phenprocoumon factor II and VII tended to be similar (p=0.08) and when using acenocoumarol factor II and VII and factor VII and X were similar (data not shown).

Table 1. Characteristics of the study groups[1].

Variable	Controls (n=25)	Random cases (n=25)	Cases with fever (n=16)	Cases with heart failure (n=24)
INR	2.9 (0.7)	7.0 (1.3)	7.0 (1.9)	6.8 (1.6)
Age (years)	71.0 (11.5)	73.0 (10.0)	72.5 (19.5)	76.5 (11.5)
Sex				
male	18 (72%)	14 (56%)	7 (44%)	15 (62%)
female	7 (28%)	11 (44%)	9 (56%)	9 (38%)
Type of anticoagulant				
phenprocoumon	14 (56%)	15 (60%)	5 (31%)	16 (67%)
acenocoumarol	11 (44%)	10 (40%)	11 (69%)	8 (33%)

[1] Values are medians (interquartile ranges) or numbers (%).

Table 2. Levels of factor II, VII, IX and X, protein C and S (U/ml) in randomly selected overanticoagulated patients and matched controls[1].

Variable	Controls (n=25)	Random cases (n=25)
Factor II	0.18 (0.04)[a]	0.10 (0.05)[d]
Factor VII	0.24 (0.07)[a]	0.10 (0.06)[b,d]
Factor IX	0.38 (0.09)	0.21 (0.09)[d]
Factor X	0.11 (0.03)[a]	0.07 (0.03)[b,c,d]
Protein C	0.32 (0.08)	0.18 (0.12)[d]
Protein S	0.30 (0.08)	0.14 (0.10)[d]

[1] Values are means (SD).
[a] p<0.001 X versus II, II versus VII, and VII versus IX within controls.
[b] p<0.001 X versus II and VII versus IX within random cases.
[c] p<0.05 X versus VII within random cases.
[d] p<0.001 random cases versus controls.

The pattern of level reductions was also studied in overanticoagulated patients with fever and in those with congestive heart failure. The INR, age and sex were similar in these two study groups, but the type of anticoagulant was different (table 1). As shown in table 3, in both groups of overanticoagulated patients, factor X was the lowest, factor IX the highest, and factor VII and II were similar, as was found in the randomly selected overanticoagulated patients. Protein C was lower than protein S in overanticoagulated patients with congestive heart failure, whereas protein S was relatively low and similar to protein C in overanticoagulated patients with fever.

When comparing coagulant levels between overanticoagulated patients with fever and those with congestive heart failure, factor II, VII, and IX, protein C and S were significantly lower in overanticoagulated patients with fever (table 3, p-values adjusted for the type of anticoagulant). The difference was most pronounced for factor VII and protein S. Factor X, as well as factor VIII and fibrinogen, was similar in overanticoagulated patients with fever and overanticoagulated patients with congestive heart failure. The ratio of the mean of the procoagulant factors II, VII, IX and X to the mean of the anticoagulant proteins C and S tended to be smaller in overanticoagulated patients with congestive heart failure (median (interquartile range) 0.55 (0.40) versus 0.68 (0.48) in overanticoagulated patients with fever; p=0.07, after adjustment for type of anticoagulant).

Table 3. Levels of factor II, VII, VIII, IX and X, protein C and S (U/ml) and fibrinogen (g/l) in overanticoagulated patients with fever and overanticoagulated patients with congestive heart failure[1].

Variable	Cases with fever (n=16)	Cases with heart failure (n=24)
Factor II	0.10 (0.05)	0.14 (0.07)[e]
Factor VII	0.09 (0.03)[c]	0.15 (0.07)[d,f]
Factor IX	0.15 (0.05)	0.20 (0.08)[e]
Factor X	0.06 (0.02)[a,b]	0.07 (0.03)[d]
Protein C[2]	0.12 (0.09)	0.16 (0.05)[d,e]
Protein S	0.14 (0.07)	0.26 (0.09)[g]
Factor VIII[3]	1.31 (0.33)	1.22 (0.38)
Fibrinogen[3]	4.09 (0.94)	4.08 (0.67)

[1] Values are means (SD).
[2] Missing in 3 cases with heart failure.
[3] Determined in 13 and 22 cases respectively.
[a] p<0.01 X versus II within cases with fever.
[b] p<0.05 X versus VII within cases with fever.
[c] p<0.01 VII versus IX within cases with fever.
[d] p<0.001 X versus II, X versus VII, VII versus IX, and C versus S within cases with heart failure.
[e] p<0.05 cases with heart failure versus cases with fever, adjusted for type of anticoagulant.
[f] p<0.01 cases with heart failure versus cases with fever, adjusted for type of anticoagulant.
[g] p<0.001 cases with heart failure versus cases with fever, adjusted for type of anticoagulant.

Discussion

We compared the levels of factor II, VII, IX and X, protein C and S between randomly selected overanticoagulated patients (INR ≥6.0) and matched controls with an INR within the therapeutic zone. Except for factor VII which was relatively low among the procoagulant factors in overanticoagulated patients, the pattern of level reductions was the same: factor X was the lowest, factor IX the highest and protein C and S were similar. To study a possible effect of the cause of overanticoagulation on coagulant levels, overanticoagulated patients with fever were compared to those with congestive heart failure. The pattern of level reductions in these two groups of overanticoagulated patients was largely similar and the same as that observed in the randomly selected overanticoagulated patients. The only difference was that protein C was lower than protein S in overanticoagulated patients with congestive heart failure. In overanticoagulated patients with fever, the vitamin K-dependent coagulation proteins except factor X were lower than in overanticoagulated patients with congestive heart failure, especially factor VII and protein S.

The pattern of procoagulant level reductions found in our controls is in accordance with other studies (5, 6, 14). The absence of a difference in factor II and VII in controls on acenocoumarol may be explained by the fact that blood was collected 12-18 hours after drug intake. With the use of acenocoumarol, due to its short half-life, factor VII fluctuates and about 16 hours after drug intake the level is lowest (15). Besides, the stratified analysis was based on relatively small numbers.

All overanticoagulated patients were stably anticoagulated in the three months preceding the index day. Furthermore, the INR preceding the assessment on the index day was within the therapeutic zone. So, in all patients the overanticoagulated state was more or less acute, irrespective of its cause. Consequently, of the procoagulant factors, factor VII was expected to be most depressed because of its shortest half-life. This is confirmed by our observation that factor VII was relatively low and similar to factor II in all overanticoagulated patients. Analogously, one would expect protein C to be lower than protein S in acute overanticoagulation. However, in overanticoagulated patients with fever and in the randomly selected overanticoagulated patients, two-third of whom had an acute risk factor for overanticoagulation, protein S was as low as protein C. This may be explained by an additional decrease in the percentage of free and functionally active protein S as a consequence of an increased plasma level of C4b-binding protein, an acute phase protein to which protein S binds (16, 17).

To our knowledge, no studies have been reported yet which focus on the pattern of coagulant level reductions in overanticoagulated patients. In an article of Makris et al. on oral anticoagulant reversal (14), levels of factor II, VII, IX and X in warfarin-treated patients (including 11 overanticoagulated patients with a median INR of 14.0 (interquartile range 7.4)) are described. Although not explic-

itly stated, in their overanticoagulated patients factor IX was the highest and factor II and VII were similar, as we found in our patients. On the contrary, in their patients the level of factor X did not differ from the levels of factor II and VII. Possibly, the lower procoagulant levels in the overanticoagulated patients of Makris et al. as compared to ours or the different type of anticoagulant are responsible for this difference in results.

Factor VIII and fibrinogen were included as acute phase response markers to differentiate between acute fever and chronic congestive heart failure as causes of overanticoagulation. However, they did not differ between the two study groups and were also high-normal in overanticoagulated patients with congestive heart failure. An elevated plasma level of fibrinogen in patients with congestive heart failure compared to controls has also been reported by Cooke at al. (18). High factor VIII levels are compatible with congestive heart failure due to ischemic heart disease (19, 20). The vitamin K-dependent coagulation proteins were lower in overanticoagulated patients with fever than in overanticoagulated patients with congestive heart failure. This may have to do with the different mechanisms by which overanticoagulation is caused. Fever increases the clearance of coagulation factors (10). In congestive heart failure unbound coumarin accumulates at the site of hepatic receptors resulting from redistribution of body water (11). Besides, the synthesis of coagulation factors may be impaired due to liver congestion (10). Because of already circulating coagulation proteins, interference with their elimination by fever will reduce coagulation levels faster than interference with the synthesis of coagulation proteins in congestive heart failure. The large difference in protein S may additionally be explained by the effect of fever or inflammation on the plasma level of C4b-binding, as mentioned earlier. The ratio of the mean of the procoagulant factors to the mean of the anticoagulant proteins, in other words the balance between coagulation and anticoagulation, was similar in controls, randomly selected overanticoagulated patients and overanticoagulated patients with fever. The smaller ratio, i.e. disbalance towards anticoagulation, in overanticoagulated patients with congestive heart failure is the result of the relatively high level of protein S in these patients. As a consequence of the disbalance towards anticoagulation, overanticoagulated patients with congestive heart failure might be at increased risk of bleeding.

In conclusion, in overanticoagulated patients (INR $\geq$6.0) the pattern of coagulant level reductions was largely the same as in controls with an INR within the therapeutic zone, except for factor VII which was relatively low. In overanticoagulated patients with fever, the vitamin K-dependent coagulation proteins were lower than in overanticoagulated patients with congestive heart failure, especially factor VII and protein S.

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5

QUALITY OF ORAL ANTICOAGULANT THERAPY; PHENPROCOUMON VERSUS ACENOCOUMAROL

Phenprocoumon is superior to acenocoumarol in oral anticoagulant therapy lasting six weeks or more

ABSTRACT *Long-acting coumarins have advantages over acenocoumarol. However, it is not known whether this also applies to the initiation phase of therapy. We performed a cohort study among 7740 newly enrolled patients of an anticoagulation clinic and compared the quality of oral anticoagulant therapy between phenprocoumon and acenocoumarol in three phases of therapy: the initiation phase (day 1 up to day 31), the short-term phase (day 32 up to day 122) and the long-term phase (day 123 up to day 365). In the short-term phase and especially the long-term phase, patients on phenprocoumon were more likely to spend at least 70% of the time within the therapeutic zone, to have no International Normalized Ratios (INR) ≥6.0 and to have no INRs <2.0. Furthermore, their INRs were closer to the target INR and their interval of monitoring was longer. In the initiation phase, however, the quality of oral anticoagulant therapy was slightly better with acenocoumarol. The percentage of patients with a major hemorrhage did not differ substantially between the two types of coumarin. In conclusion, for outpatients of an anticoagulation clinic phenprocoumon seems to be preferred. Only in case a patient needs oral anticoagulant therapy for a short time (<6 weeks), acenocoumarol might be considered.*

INTRODUCTION

Coumarin anticoagulants are widely used in the prevention of venous and arterial thromboembolism (1). These drugs induce anticoagulation by antagonizing vitamin K, thereby impairing the biological activity of the vitamin K-dependent coagulation factors II, VII, IX and X (2). In contrast to this benefit, they carry a risk of hemorrhage (3), which is strongly associated with the intensity of anticoagulation and sharply increases when the International Normalized Ratio (INR) is ≥6.0 (4, 5). An INR <2.0 is associated with a higher thromboembolic risk (5). Anticoagulant therapy is considered effective and safe if the patient's INR is kept within the therapeutic zone (2.0-3.5 or 2.5-4.0, depending on the indication (5, 6, 7)) for as much of the time as possible (8, 9). Hence, adequate monitoring of anticoagulant therapy is important. To this purpose, the Netherlands have a national network of experienced anticoagulation clinics, using a specialized system of monitoring (10, 11). Two types of coumarin are used: phenprocoumon, which has a half-life of 65 to 170 hours, and acenocoumarol, which has a half-life of 8 to 12 hours. Warfarin, which has a half-life of 10 to 45 hours and is the coumarin of first choice in many countries, is not licensed in the Netherlands (12).

Three studies have suggested that the use of phenprocoumon or warfarin gives a more stable anticoagulation than the use of acenocoumarol (13-15). This may be explained by the large fluctuations in plasma levels of factor VII induced by acenocoumarol because of its short half-life (16, 17). In the study of Pattacini et al. (13), the higher quality of oral anticoagulant therapy with warfarin was absent in the subgroup with venous thromboembolism. An explanation was sought in the shorter treatment period, since in the beginning of therapy instability of the INR occurs with all coumarins. In this study, however, the one-year follow-up started on average 100 days after the beginning of therapy, while the initiation phase is usually defined as the first month of treatment.

In view of these contradictory results, we performed a cohort study among newly enrolled patients of an anticoagulation clinic in which we compared the quality of oral anticoagulant therapy between phenprocoumon and acenocoumarol in three phases of therapy. By including a large number of newly enrolled patients treated in one clinic, experienced in monitoring oral anticoagulant therapy with both drugs, we aimed at arriving at highly reliable estimates.

METHODS

Setting

The study was performed at the regional Red Cross anticoagulation clinic The Hague, which serves an area of nearly 700,000 inhabitants. All persons in this area with an indication for anticoagulant therapy are referred to this clinic. Each year, approximately 15,000 patients are monitored. INR measurements are per-

formed at a mean interval of two to three weeks. About 60% of the patients are treated with phenprocoumon and about 40% with acenocoumarol, the choice of coumarin being determined by the preference of the prescriber (i.e. the general practitioner or physician). All laboratory-, clinical- and administrative data as of 1984 are stored in computerized files. For our cohort study, the data until September 1, 1999 were used.

Cohort definition

The study cohort consisted of patients who were newly referred to the regional Red Cross anticoagulation clinic The Hague between January 1, 1997 and September 1, 1999 because of the following indications: prosthetic heart valve, atrial fibrillation, myocardial infarction, coronary bypass, vascular surgery, pulmonary embolism, deep venous thrombosis and short-term prophylactic treatment. Hereby, we excluded indications for which there were less than 50 users of either phenprocoumon or acenocoumarol. Furthermore, patients were excluded if their target zone differed from the usual ones and in case monitoring of the INR started more than two weeks after referral. In the latter situation, outpatient therapy was often initiated and monitored elsewhere, for example at the rehabilitation clinic. Cohort members were followed for 365 days, starting from their first INR measurement. Follow-up ended earlier in the following situations: end of study period, end of therapy, change of coumarin, change of target zone and change of indication. Since the quality of oral anticoagulant therapy can only be judged reliably in case of regular monitoring, follow-up also ended when the interval between INR assessments exceeded 42 days. Similarly, patients with less than three INR measurements during follow-up were excluded.

Monitoring of oral anticoagulant therapy

Venous blood was collected in 3.2% sodium citrate tubes. The prothrombin time was measured in plasma, obtained by centrifugation at 3000 rpm for 10 minutes, on an Elektra 1800 C automated coagulometer. From January 1997 until October 1997, an Elektra 1600 C was used. The thromboplastin used was a human recombinant tissue factor (Ortho® RecombiPlastin) with an International Sensitivity Index between 0.93 and 1.00 during the study period, according to the different batches of thromboplastin used. Prothrombin times were expressed as INRs.

Dosing of the coumarin was performed by a team of specialized physicians routinely working at the regional Red Cross anticoagulation clinic The Hague, with the aid of a computerized dosing program (TRODIS, Hiscom, Leiden). This program evaluates the stability of the INR and, when possible, proposes a dosing schedule (i.e. in nearly 50% of the patients). In the other patients, dosing was done by the physician according to a standard operating procedure. Details of the computerized dosing program have been published previously (18).

Quality of oral anticoagulant therapy

For each individual patient several measures of the quality of oral anticoagulant therapy were determined. First, we calculated the percentage of INRs within the therapeutic zone. This measure, however, is likely to underestimate the quality of oral anticoagulant therapy because unstable patients are checked more frequently than stable patients (10). Second, we therefore also counted the number of days the patient spent within the therapeutic zone, expressed as a percentage of the total follow-up time. To this end we computed the INR on a daily basis, assuming that the INR linearly increases or decreases over the interval between two consecutive measurements. This approximation method has been extensively described elsewhere (18). Since anticoagulant therapy is considered of high quality if the patient's INR is kept within the therapeutic zone for at least 70% of the time (8, 9), a cut-off point of 70% was used to dichotomize the percentage of INRs and the percentage of time within the therapeutic zone. Preferably, the INR is as closest to the target INR as possible (3.0 or 3.5, depending on the indication). We therefore also calculated the mean deviation of the INRs from the target INR. Because of the increased risk of complications (4, 5), the occurrence of INRs $\geq$6.0 as well as the occurrence of INRs <2.0 were assessed. Besides, in view of the burden on the patient of regular assessments, the mean interval of monitoring was calculated. In addition to these measures of therapeutic quality, the occurrence of hemorrhages, the most common adverse reaction to coumarins, and the occurrence of thromboembolisms during oral anticoagulant therapy were assessed. Hemorrhages were classified as major if these led to death, necessitated hospitalization, blood transfusion or surgery, or if these concerned intracranial-, intra-articular- or intra-muscular bleeding. Minor hemorrhages were defined as all other overt non-fatal bleeding, including skin bleeds of more than 10 cm in diameter and nose bleeds lasting for at least 30 minutes.

The quality of oral anticoagulant therapy was determined over the total follow-up and over three different phases of therapy, i.e. the initiation phase (day 1 up to day 31), the short-term phase (day 32 up to day 122) and the long-term phase (day 123 up to day 365). The latter analyses were restricted to patients in whom oral anticoagulant therapy was built up in the usual way, i.e. at least three INR measurements during the initiation phase.

Statistical analysis

As we were primarily interested in the percentage of patients spending at least 70% of their INRs/of the time within the therapeutic zone, a multivariate risk-ratio model was used to calculate adjusted relative risks (RR) with 95% confidence intervals (CI). This model was also used for comparing the percentage of patients: [1] without INRs $\geq$6.0, [2] without INRs <2.0, [3] with hemorrhages and [4] with thromboembolisms. For comparisons of continuous variables (i.e. the mean deviation of the INRs from the target INR and the mean interval of monitoring) adjusted differences and their 95%CIs were computed using multivari-

ate linear regression analysis after taking the natural logarithm. Because of the use of log-transformed variables, the model predicts geometric rather than arithmetic means and regression coefficients approximate relative differences rather than absolute differences. Relative differences were calculated as the anti-log of the regression coefficient minus one (20). All multiple regression models included age, sex and the indication for therapy (treatment or prophylaxis of arterial thrombosis, treatment of venous thrombosis, prophylaxis of venous thrombosis) and, when relevant, the length of follow-up.

Results

The study cohort included 3,158 patients using phenprocoumon, with a median follow-up of 116 days and 4,582 patients using acenocoumarol, with a median follow-up of 56 days. The general characteristics of the study population are presented in table 1.

The quality of oral anticoagulant therapy, as determined over the total follow-up (table 2), was in favour of phenprocoumon. First, a higher percentage of patients spent at least 70% of the time within the therapeutic zone (RR 1.09; 95%CI 1.05-1.13). When expressed as INRs within the therapeutic zone, similar results were obtained. Second, the INRs for patients on phenprocoumon were somewhat closer to the target INR. Third, the percentage of patients without INRs ≥6.0 tended to be higher with phenprocoumon, but the difference was not significant. Fourth, a higher percentage of patients on phenprocoumon had no INRs

Table 1. General characteristics of the study cohort[1].

	Phenprocoumon n=3,158	Acenocoumarol n=4,582
Age (years, mean (sd))	64.9 (17.0)	62.3 (18.0)
0 - 20 years	37 (1%)	117 (2%)
21 - 40 years	291 (9%)	542 (12%)
41 - 60 years	768 (24%)	1,102 (24%)
61 - 80 years	1,511 (48%)	2,227 (49%)
81 -100 years	551 (18%)	594 (13%)
Sex		
male	1,526 (48%)	1,917 (42%)
female	1,632 (52%)	2,665 (58%)
Indication		
treatment or prophylaxis of arterial thrombosis	1,876 (59%)	1,232 (27%)
treatment of venous thrombosis	790 (25%)	480 (10%)
prophylaxis of venous thrombosis	492 (16%)	2,870 (63%)
Follow-up (days, median (IQR[2]))	116 (195)	56 (74)

[1] Values are numbers (percentages) unless indicated otherwise.
[2] Interquartile range.

<2.0 (RR 1.07; 95%CI 1.05-1.10). Lastly, the interval of monitoring was slightly longer in users of phenprocoumon.

The advantages of phenprocoumon over acenocoumarol, as were present over the total follow-up, did not apply to all phases of therapy (table 3). During the initiation phase, only the percentage of patients without INRs <2.0 was higher in users of phenprocoumon (RR 1.16; 95%CI 1.10-1.22). The percentage of patients spending at least 70% of the INRs within the therapeutic zone, the deviation of the INRs from the target INR and the interval of monitoring did not differ between the two types of coumarin. The percentage of patients spending at least 70% of the time within the therapeutic zone tended to be higher with acenocoumarol, but the difference was not significant. The percentage of patients without INRs ≥6.0 was in favour of acenocoumarol (RR 0.78; 95%CI 0.68-0.89). During both other phases, all measures of the quality of oral anticoagulant therapy were in favour of phenprocoumon, but the differences were most pronounced during the long-term phase. Patients on phenprocoumon were 1.54 times as likely to spend at least 70% of the time within the therapeutic zone, were 2.39 times as likely to have no INRs ≥6.0 and were 1.53 times as likely to have no INRs <2.0. Furthermore, their interval of monitoring was 25% longer and their INRs were 25% closer to the target INR.

The study populations concerning the different phases of therapy differed considerably in indication for therapy. Of the patients included in the long-term analysis 94% used coumarins for treatment or prophylaxis of arterial thrombosis. For the patients included in the short-term analysis this concerned 73% while 24% were treated because of venous thrombosis. In the study population concerning the initiation phase, 46% of patients were treated because of an arterial indication and 34% for prophylaxis of venous thrombosis. Restricting the analyses

Table 2. The quality of oral anticoagulant therapy over the total follow-up[1].

Measure of quality	Phenprocoumon n=3,158	Acenocoumarol n=4,582	adj[3]. RR / *rel. dif.*[4] (95%CI)
≥70% of INRs within ther. zone	1,123 (36%)	1,368 (30%)	1.09 (1.05-1.13)
≥70% of time within ther. zone	1,700 (54%)	2,131 (46%)	1.09 (1.04-1.15)
deviation INRs from target (median (IQR[2]))	1.06 (0.58)	1.12 (0.53)	*-0.05 (-0.07- -0.03)*
no INRs ≥6.0	2,338 (74%)	3,786 (83%)	1.09 (0.99-1.19)
no INRs <2.0	1,179 (37%)	1,034 (23%)	1.07 (1.05-1.10)
interval of monitoring (days, median (IQR))	10.2 (6.8)	7.0 (4.1)	*0.05 (0.04-0.07)*

[1] Values are numbers (percentages) unless indicated otherwise.
[2] Interquartile range.
[3] Adjusted for age, sex, indication and length of follow-up.
[4] The relative differences are to be interpreted as the differences expressed in percentages between the two drugs. For example, the interval of monitoring is 5% longer in patients on phenprocoumon.

to patients using coumarins for treatment or prophylaxis of arterial thrombosis revealed similar results and did not change the interpretation of the observed differences in therapeutic quality between phenprocoumon and acenocoumarol over the three phases of therapy. For example, during the initiation phase 35% of the patients on phenprocoumon and 36% of the users of acenocoumarol spent at least 70% of the time within the therapeutic zone (RR 0.98; 95%CI 0.93-1.04). The percentage of patients without INRs ≥6.0 was 80% and 85%, respectively (RR 0.81; 95%CI 0.68-0.95).

In addition to the therapeutic quality of oral anticoagulant therapy, the occurrence of hemorrhages and thromboembolic complications during follow-up were

Table 3. The quality of oral anticoagulant therapy over different phases of therapy[1].

Measure of quality	Phenprocoumon	Acenocoumarol	adj[3]. RR / *rel. dif* (95%CI)
Initiation phase	n=2,674	n=3,325	
≥70% of INRs within ther. zone	782 (29%)	995 (30%)	1.00 (0.96-1.03)
≥70% of time within ther. zone	1,004 (38%)	1,334 (40%)	0.96 (0.92-1.01)
deviation INRs from target (median (IQR[2]))	1.28 (0.85)	1.23 (0.63)	*0.00 (-0.03-0.03)*
no INRs ≥6.0	2,154 (81%)	2,928 (88%)	0.78 (0.68-0.89)
no INRs <2.0	1,348 (50%)	1,105 (33%)	1.16 (1.10-1.22)
interval of monitoring (days, median (IQR))	6.2 (1.6)	6.2 (1.6)	*0.01 (0.00-0.02)*
Short-term phase	n=1,519	n=995	
≥70% of INRs within ther. zone	847 (56%)	462 (46%)	1.20 (1.11-1.30)
≥70% of time within ther. zone	984 (65%)	588 (59%)	1.14 (1.03-1.27)
deviation INRs from target (median (IQR))	0.78 (0.47)	0.96 (0.47)	*-0.18 (-0.21- -0.15)*
no INRs ≥6.0	1,386 (91%)	859 (86%)	1.54 (1.23-1.94)
no INRs <2.0	1,065 (70%)	523 (53%)	1.55 (1.40-1.72)
interval of monitoring (days, median (IQR))	15.2 (6.8)	13.0 (5.1)	*0.14 (0.11-0.17)*
Long-term phase	n=500	n=303	
≥70% of INRs within ther. zone	310 (62%)	109 (36%)	1.61 (1.39-1.85)
≥70% of time within ther. zone	357 (71%)	163 (54%)	1.54 (1.28-1.86)
deviation INRs from target (median (IQR))	0.74 (0.38)	1.00 (0.50)	*-0.25 (-0.29- -0.20)*
no INRs ≥6.0	430 (86%)	204 (67%)	2.39 (1.82-3.15)
no INRs <2.0	326 (65%)	137 (45%)	1.53 (1.31-1.79)
interval of monitoring (days, median (IQR))	22.1 (13.0)	17.4 (8.6)	*0.25 (0.19-0.32)*

[1] Values are numbers (percentages) unless indicated otherwise.[2] Interquartile range.[3] Adjusted for age, sex and indication.

Table 4. The percentage of patients with hemorrhages or thromboembolic complications during follow-up[1].

	Phenprocoumon n=3,158	Acenocoumarol n=4,582	adj[2]. RR (95%CI)
minor hemorrhages	137 (4%)	75 (2%)	1.81 (1.34-2.48)
major hemorrhages	30 (0.9%)	23 (0.5%)	1.10 (0.62-1.99)
thromboembolisms	19 (0.6%)	7 (0.2%)	1.77 (0.74-4.20)

[1] Values are numbers (percentages).
[2] Adjusted for age, sex, indication and length of follow-up.

considered. A total of 277 hemorrhages occurred in 262 patients, corresponding to 11.0 hemorrhages per 100 patient-years. It concerned 224 minor hemorrhages, i.e. 8.9 per 100 patient-years and 53 major hemorrhages, i.e. 2.1 per 100 patient years. As shown in table 4, the percentage of patients with a minor hemorrhage was higher in users of phenprocoumon (RR 1.81; 95%CI 1.34-2.48). The percentage of patients with a major hemorrhage did not differ between the two types of coumarin. Twenty-six patients had a thromboembolism during follow-up, which corresponds to an incidence rate of 1.0 per 100 patient-years. Nine patients had a myocardial infarction, seven patients had a cardiovascular accident, six patient had a deep venous thrombosis and four patients had a pulmonary embolism; none of the events was fatal. There was no difference in the percentage of patients with a thromboembolic complication between phenprocoumon and acenocoumarol.

DISCUSSION

We performed a cohort study among a large number of newly enrolled patients of an anticoagulation clinic and compared the quality of oral anticoagulant therapy between phenprocoumon and acenocoumarol in three phases of therapy. The results clearly show that the use of phenprocoumon has advantages over the use of acenocoumarol in the short-term and especially the long-term phase of therapy. Patients on phenprocoumon were more likely to spend at least 70% of the time within the therapeutic zone, to have no INRs $\geq$6.0 and to have no INRs <2.0. Additionally, they had INRs closer to the target INR and a longer interval of monitoring. In the initiation phase, the quality of oral anticoagulant therapy seems to be slightly better with acenocoumarol. Our results suggest that newly referred patients should preferably be prescribed phenprocoumon. Only in case a patient needs oral anticoagulant therapy for a short time (i.e. up to about six weeks), acenocoumarol might be considered.

The regional Red Cross anticoagulation clinic The Hague has an extensive

experience in monitoring oral anticoagulant therapy, both on phenprocoumon and acenocoumarol: each year approximately 15,000 patients are monitored, of whom about 60% are treated with phenprocoumon and about 40% with acenocoumarol. Dosing of the coumarins is performed with the aid of a computerized dosing program. A difference in monitoring experience with the two coumarins, therefore, is not a likely explanation for the observed differences in quality of oral anticoagulant therapy. The choice of coumarin is determined by the preference of the prescriber and is also hospital-related. However, the monitoring of anticoagulant therapy by the anticoagulation clinic is not influenced by the preference of the prescriber or hospital. The choice of coumarin may also be determined by the patient profile, e.g. compliance or bleeding risk. This plays probably only a limited role. Since the study cohort only consisted of new patients, selection bias by the anticoagulation history of a patient was excluded. Furthermore, the major precribers prescribed the same coumarin to more than 95% of their patients. As anticoagulant therapy may be ended because of non-compliance, depletion of susceptibles may have occurred, i.e. noncompliant patients were included in the initiation phase analysis but not in the long-term phase analysis. This concerned only four patients using acenocoumarol and three patients using phenprocoumon, and would not explain the consistent advantages of phenprocoumon over acenocoumarol in the short-term and long-term phase of therapy. The measures of the quality of oral anticoagulant therapy determined in this study, are related to the length of follow-up. For example, the longer the follow-up, the more INR assessments and the higer the chance to have an INR $\geq$6.0 or the longer the follow-up, the more stably anticoagulated and the longer the monitoring interval. The length of follow-up was either constant or adjusted for in the multivariate analyses beside age, sex and the indication for therapy. Age and length of follow-up were included in the model as continuous variables. Optimal control of counfounding by including age and length of follow-up as categorical variables with dummies (20-years and 4-weeks classes, respectively) did not substantially change the RRs. Similarly, restricting the analyses to patients using coumarins for treatment or prophylaxis of arterial thrombosis revealed roughly similar results and did not change its interpretation. A cut-off point of 70% was used to dichotomize the percentage of INRs and the percentage of time within the therapeutic zone. When using 80% or 90% as cut-off point, the results still were in favour of phenprocoumon, but the differences between the two coumarins were smaller.

The advantages of phenprocoumon over acenocoumarol concerning the quality of oral anticoagulant therapy may be explained by the difference in half-life. Because of its short half-life, the use of acenocoumarol induces large fluctuations in plasma levels of factor VII (16, 17). Hence, irregular compliance may have more consequences with the use of acenocoumarol than with the use of phenprocoumon. Moreover, acenocoumarol may be more sensitive to interference by the use of co-prescribed drugs inducing or inhibiting cytochrome P450

enzymes (21).

The short half-life of acenocoumarol is not only disadvantageous. An important advantage is the more direct reaction to dosing changes. This may be helpful in finding stability, as is reflected by the result that the quality of oral anticoagulant therapy was slightly better with acenocoumarol during the initiation of therapy.

Opposite to the better therapeutic quality of oral anticoagulant therapy with phenprocoumon, stands an increased risk of minor hemorrhages. However, this should not prevent the use of phenprocoumon since the risk of major hemorrhages and thromboembolic complications were not increased.

In conclusion, for outpatients of an anticoagulation clinic phenprocoumon seems to be preferred since this coumarin gave a better quality of oral anticoagulant therapy than acenocoumarol in the short-term and especially the long-term phase of therapy. Only in case a patient needs oral anticoagulant therapy for a short time (<6 weeks), acenocoumarol might be considered.

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6 General discussion

Background

Oral anticoagulation with coumarin derivatives is an established, widely used therapy for the prevention of venous and arterial thromboembolism (1). Coumarin anticoagulants induce anticoagulation by antagonizing vitamin K, thereby impairing the γ-carboxylation and consequently the biological activity of the vitamin K-dependent coagulation proteins (factor II, VII, IX and X, protein C and S) (2). In patients on long-term oral anticoagulant therapy at therapeutic levels of anticoagulation, factor X is most strongly, and factor IX is least strongly reduced (3-6). However, there are no reports which focus on the levels of these factors in overanticoagulated patients.

Three types of coumarin with a similar mechanism of action but different pharmacokinetics are used. In Western continental Europe acenocoumarol and phenprocoumon are mainly used. Acenocoumarol is a short-acting anticoagulant and phenprocoumon is a long-acting drug. Warfarin, which has an intermediate duration of effect, is commonly prescribed in the Anglo-Saxon and Scandinavian countries (7, 8). Studies have shown that the use of phenprocoumon or warfarin gives a more stable anticoagulation than the use of acenocoumarol (9-11). However, it is not known whether the advantage of the long-acting coumarins also applies to the initiation phase of therapy.

Coumarin anticoagulants have a narrow therapeutic index (12). The optimal target range of oral anticoagulant therapy, as recommended by the Federation of Dutch Thrombosis Centers, lies between an International Normalized Ratio (INR) of 2.5 and 3.5, or between 3.0 and 4.0 (13, 14), depending on the indication for treatment. As patients may require different doses of coumarins to reach the same level of anticoagulation and because the required dose may vary over time in an individual patient, anticoagulant therapy needs to be monitored. Despite monitoring of anticoagulant therapy, overanticoagulation may occur. When the INR is $\geq$6.0, the risk of hemorrhage, the most common adverse

reaction to coumarin anticoagulants, sharply increases (14, 15). Hence, such an excess anticoagulant effect should preferably be prevented and should be treated promptly and adequately if present. Based on case reports and small-scale experiments, a considerable number of drug interactions with coumarin anticoagulants as a cause of overanticoagulation have been reported and summarized (16-19). In addition, a number of comorbid conditions have been postulated as interfering with oral anticoagulant therapy (17, 20-22). Furthermore, increasing age and female gender have been associated with an enhanced response to coumarins (23) and overanticoagulation after a dietary modification reducing the intake of vitamin K has been described (24, 25). However, large epidemiological studies on the incidence of and risk factors for overanticoagulation in a non-selected population under everyday circumstances are scarce. In the absence of life-threatening hemorrhagic complications, orally administered vitamin K_1 is used to reduce the excess anticoagulant effect (20). In case of a serious hemorrhage, the patient should be transfused with prothrombin complex concentrate, supplemented with vitamin K, in order to rapidly reverse the INR (20, 26). Detailed information on changes in the INR in response to oral vitamin K_1, however, is not available.

Although the risks of overanticoagulation are clear, its treatment and determinants have received little attention. Most of the extensive research on oral anticoagulant therapy has focussed on its pharmacological- and biochemical action, prothrombin time calibration, optimal therapeutic intensity and hemorrhagic complications. Therefore, the aim of this thesis was to study aspects of overanticoagulation on coumarin anticoagulants among outpatients of an anticoagulation clinic. In this chapter, the main findings will be summarized and the general methodological issues inherent to the study of risk factors for overanticoagulation will be discussed. In addition, the implications for oral anticoagulant therapy and recommendations for future research will be given.

MAIN FINDINGS

Treatment of overanticoagulation

In chapter 2, we describe a prospective study in which we provide detailed insight into the course of the INR after oral administration of vitamin K_1 in patients overanticoagulated with phenprocoumon. The study was restricted to patients on phenprocoumon, since overanticoagulated patients on acenocoumarol are only prescribed vitamin K_1 when the INR is >15.0. The first two days after administration of vitamin K_1 the mean INR decreased by 40% and 23%, respectively. After day 2, the day-to-day proportional change in the mean INR depended on the dose of vitamin K_1 and varied from a decrease of 12% to an increase of 21%. On day 7, the mean INR was lower than on day 2 in the 1 mg and the 5 mg group and was higher than on day 2 in the 2, 3 and 4 mg group. In addition to

determining the effect of vitamin K_1 administration on the time-course of the INR, we also looked at its effectiveness in reducing the excess anticoagulant effect to lower and safer levels, preferally in the therapeutic zone. Between day 2 and day 7, in general, 32% of the patients had an INR within the therapeutic zone, 25% had an INR ≥6.0 and 8% had an INR <2.0.

Risk factors for overanticoagulation

We performed a prospective cohort study with a nested case-control design among previously stable outpatients of an anticoagulation clinic to determine the incidence of overanticoagulation. Furthermore, we studied the association between overanticoagulation and characteristics of anticoagulant therapy and comorbidity, drug interactions, and sociodemographic-, lifestyle-, and dietary factors. The incidence rate of an INR ≥6.0 was 7.8 per 10,000 treatment days in prevalent users on the starting date and 22.5 per 10,000 treatment days in incident users during the study period (chapter 3.1). Patients on acenocoumarol had an increased risk of an INR ≥6.0 compared to patients on phenprocoumon. Regarding comorbidity, impaired liver function, congestive heart failure, diarrhea and fever were independent risk factors for overanticoagulation (chapter 3.1). About the role of drug interactions in overanticoagulation, half of the 87 potentially interacting drugs or drug classes which have been reported in the medical literature were not used by the study population. Only fifteen drugs or drug classes were used by more than ten patients, and a relevant change in use in the risk period was infrequent. A course of co-trimoxazole and the use of amoxicillin+clavulanic acid were independent risk factors for overanticoagulation (chapter 3.2). Sociodemographic- as well as dietary factors were not associated with overanticoagulation. Body mass index was negatively related to overanticoagulation, a beneath average level of physical activity was positively related to overanticoagulation, and never-smokers were more likely to have an INR ≥6.0 compared to smokers. Habitual alcohol consumption, even heavy drinking, was not related to overanticoagulation. However, a recent decrease of alcohol intake increased the risk of an INR ≥6.0. In addition, weight loss and a vacation were independent risk factors for overanticoagulation (chapter 3.4).

In the case-control analysis, drug interactions as a cause of overanticoagulation predominantly concerned antibacterial drugs. However, the sample size of this study was too small to judge the association with overanticoagulation for several antibacterial drugs. Therefore, we conducted a cohort study in a large population of community-dwelling elderly to investigate which antibacterial drugs are associated with overanticoagulation (chapter 3.3). Eight antibacterial drugs were multivariately associated with overanticoagulation. Co-trimoxazole most strongly increased the risk of overanticoagulation. The onset of overanticoagulation after start of antibacterial drug therapy varied between different antibacterial drugs, probably due to different causal mechanisms. The second cohort study in this large population of community-dwelling elderly described in chap-

ter 3.5 showed that patients with a habitual dietary intake of vitamin K below the amount required for normal functioning of coagulation factors, i.e. 1 μg/kg body weight per day, had an increased risk of overanticoagulation.

Coagulant levels in overanticoagulation

The levels of the vitamin K-dependent pro- and anticoagulant proteins in randomly selected overanticoagulated patients, in overanticoagulated patients with fever in the preceding two weeks and in overanticoagulated patients with stable congestive heart failure are described in chapter 4. This study was part of the prospective nested case-control study on the association between overanticoagulation and characteristics of anticoagulant therapy and comorbidity (chapter 3.1). The pattern of procoagulant level reductions in the three groups of overanticoagulated patients was largely the same as in controls with an INR within the therapeutic zone: factor X was the lowest and factor IX the highest. The difference was that in overanticoagulated patients factor VII was relatively low among the procoagulant factors compared to controls. Protein C was lower than protein S in overanticoagulated patients with congestive heart failure, but was similar to protein S in the other study groups. In overanticoagulated patients with fever, the vitamin K-dependent coagulation proteins except factor X were significantly lower than in overanticoagulated patients with congestive heart failure, especially factor VII and protein S.

Quality of oral anticoagulant therapy; phenprocoumon versus acenocoumarol

The prospective nested case-control study on the association between overanticoagulation and characteristics of anticoagulant therapy and comorbidity described in chapter 3.1 showed that patients on acenocoumarol had an increased risk of an INR $\geq$6.0 compared to patients on phenprocoumon. In chapter 5, several measures of the quality of oral anticoagulant therapy, among which the occurrence of overanticoagulation, are compared between phenprocoumon and acenocoumarol in three phases of therapy. In the short-term phase and especially the long-term phase, patients on phenprocoumon were more likely to spend at least 70% of the time within the therapeutic zone, to have no INRs $\geq$6.0 and to have no INRs <2.0. Furthermore, their INRs were closer to the target INR and their interval of monitoring was longer. In the initiation phase, however, the quality of oral anticoagulant therapy was slightly better with acenocoumarol. The percentage of patients with a major hemorrhage did not differ substantially between the two types of coumarin.

METHODOLOGICAL CONSIDERATIONS

Study design

In epidemiology, several study designs are used to estimate frequency of occur-

rence and measures of association. To determine the incidence of and risk factors for overanticoagulation we performed a prospective cohort study with a nested case-control design. This study design is an efficient approach to examine multiple risk factors. However, due to the often retrospective assessment of exposure in a case-control study the internal validity may be more easily threatened by selection- and information bias than other analytic study designs. Furthermore, a case-control study is inefficient for the evaluation of rare exposures, unless the sample size is very large or the attributable risk percentage is high (27, 28). Because of the latter limitation of case-control studies, we also performed prospective cohort analyses in the Rotterdam Study to investigate which antibacterial drugs are associated with overanticoagulation and to study whether patients with a deficient dietary intake of vitamin K have an increased risk of overanticoagulation. An important disadvantage of cohort studies is that the validity can be seriously affected by 'loss to follow-up' (27, 28). However, this was not an issue as patients in chapters 3.3 and 3.5 were selected on the basis of presence of INR-data at the anticoagulation clinic, and because in the Rotterdam Study follow-up information is collected for every individual.

Validity

The validity of case-control- and prospective cohort studies on risk factors for overanticoagulation may be threatened by three types of bias: selection bias, information bias and confounding. These potential biases will be discussed in the next paragraphs.

- *Selection bias* – Selection bias occurs when the association between the determinant and the outcome is different for those who participated and those who would be eligible for study. This type of bias is a particular problem in case-control studies, since both the exposure and outcome have occurred at the time subjects are selected for study. An important potential cause of selection bias in case-control studies is non-response. Non-responders may differ from responders with respect to risk factor status. In terms of relative risks, the association between exposure and outcome will especially be distorted only if the non-response is non-random with respect to both exposure and outcome (27, 28). In our case-control study, the participation rate was slightly lower among cases. However, the status of the examined risk factors in non-responders is unknown. Would non-response be related to exposure, it is most likely that patients with e.g. chronic comorbidities or acute illnesses are less likely to participate. Consequently, risk estimates would have been biased towards a relative risk of one.

- *Information bias* – Information bias results from systematic errors in the way exposure or outcome are assessed. There are several specific types of information bias, depending on the source of noncomparability (27, 28). In a case-con-

trol study, recall bias arises when individuals with a particular adverse health outcome remember and report their previous exposure differently from those who are not affected. In our study, recall bias was prevented by restricting the study population to patients with non-symptomatic overanticoagulation, i.e. by excluding patients who presented on the index day with a serious bleeding complication. In addition, in the information letter we referred to the problem of overanticoagulation in a general sense. In the case-control study on drug interactions, comparing the information on the start of antibacterial drugs given by the patient or mentioned in the anticoagulant medical record with that prospectively recorded by the pharmacy, revealed that recall bias was not a problem. Interviewer bias in a case-control study refers to any systematic difference in the gathering, recording or interpretation of information on exposure from study participants. To prevent this type of bias, structured questionnaires with mainly closed questions were used and the interviewers, general practitioners and pharmacists were blinded with respect to the patient's case or control status and the specific research hypotheses. Diagnostic suspicion bias occurs when the chance of diagnosing the outcome is different for exposed and unexposed individuals. It may play a role in the associations of fever, diarrhea and several drug interactions with overanticoagulation, since patients are instructed to inform the clinic of these occurrences. If considered necessary, the patient's INR is measured earlier than the appointed date. We dealt with diagnostic suspicion bias by excluding patients whose INRs were measured earlier from the case-control analyses and by adjustment for earlier INR-assessment in the prospective cohort analysis. Misclassification occurs when subjects are erroneously categorized with respect to either exposure or outcome status. In our case-control study, potential risk factors were inquired over the four weeks preceding the index day. Due to logistical limitations, the interview took place up to three weeks after the index day. Misclassification of exposure thus may have been present; a patient may have forgotten things or been mistaken regarding the time period in which these occurred. The mean interval between overanticoagulation and interview, however, was similar for cases and controls. Besides, the misclassification is assumed to be random, resulting in a conservative estimation of the association. In the prospective cohort analysis in the Rotterdam Study concerning deficient dietary intake of vitamin K, misclassification of exposure may also have been present since the habitual dietary intake of vitamin K was assessed at baseline and may have changed during follow-up. However, also this would have led to a conservative estimate of the relative risk rather than to an overestimation.

- *Confounding* – Confounding is a distortion of the estimated exposure effect that results from the fact that the effect of an extraneous variable is mixed with or mistaken for the actual exposure effect. Before a variable is considered a confounder it should fulfil three criteria. First, it must be an independent risk factor

for the outcome among the unexposed. Second, it must be associated with the exposure variable in the source population from which the cases are derived. Third, it must not be affected by the exposure or the outcome; in particular, it cannot be an intermediate step in the causal pathway between exposure and the outcome. Confounding can lead to an over- or underestimation of the true association between exposure and outcome, depending on the direction of the associations which the confounding factor has with exposure and outcome. Confounding can even change the apparent direction of an effect (27, 28). In our case-control- and prospective cohort studies, potential confounders were taken into account and, when necessary, adjusted for in the statistical analyses.

Generalizability

The ability to generalize study results is determined by the representativeness of the study subjects. As validity is a prerequisite for generalizability, the primary concern in the design of any study must be validity, not generalizability (27, 28). Therefore, patients not living independently and those making use of meals on wheels, as well as patients who presented on the index day with a serious bleeding complication were excluded from our case-control study. Furthermore, the study population was confined to stably anticoagulated patients because most cases of unstable anticoagulation and overanticoagulation occur during initiation of therapy. Every clinician is aware of this and more interested in the risk factors they encounter when their patients are on long-term anticoagulant therapy. Would we not have excluded unstable patients, it would have been difficult to release these more subtle but also clinically relevant risk factors. We would have found initiation of therapy as most important risk factor for overanticoagulation, a relatively irrelevant finding. However, there is no reason to assume that the observed risk factors can not be generalized.

Clinical implications

Overanticoagulation increases the risk of hemorrhage and should therefore be prevented and treated promptly and adequately if present. Although the risks of overanticoagulation are clear, its treatment and determinants have received little attention. The studies presented in this thesis especially released several risk factors for overanticoagulation, but also showed that the routine treatment of overanticoagulation employed at the anticoagulation clinic was not sufficient.

Based on our findings, the dose of vitamin K_1 has been increased and the coumarin is discontinued for one more day to improve the treatment of the excess anticoagulant effect. Besides, vitamin K_1 is now administered at a lower INR than before. Paying special attention to risk factors for overanticoagulation when monitoring oral anticoagulant therapy, offers the possibility to prevent

overanticoagulation. If possible, risk factors should be avoided. For example, the use of phenprocoumon instead of acenocoumarol might be considered. A better quality of oral anticoagulant therapy on phenprocoumon than on acenocoumarol in the short-term and especially the long-term phase of therapy supports a preference of phenprocoumon to acenocoumarol in patients who need oral anticoagulant therapy for six weeks or more. Similarly, patients on oral anticoagulant therapy should be advised to consume vitamin K-rich foods such as green, leafy vegetables to prevent a deficient dietary intake of vitamin K. Furthermore, the use of certain antibacterial drugs, especially co-trimoxazole, should be restricted. If there is no therapeutic alternative available, more frequent monitoring of INR-values during antibacterial drug therapy is warranted. Then, it should be taken into account, however, that some antibacterial drugs induce overanticoagulation already within two to three days after start of antibacterial drug therapy, while for other drugs this takes more than four days. Increased monitoring of INR-values may also be advisable for patients with non-avoidable risk factors such as fever, diarrhea or weight loss. Patients with an impaired liver function or congestive heart failure should be monitored more carefully. Also, it is advised that patients have their INR checked when on vacation.

To be able to prevent overanticoagulation by paying special attention to risk factors for overanticoagulation when monitoring oral anticoagulant therapy, physicians should be aware of these risk factors and their presence. Therefore, it is as important that patients are aware of the necessity to inform the anticoagulation clinic about occurrences such as changes in comedication, intercurrent illnesses or weight loss. This should be emphasized at the start of therapy and should be brought to the attention of patients at regular intervals thereafter.

RECOMMENDATIONS FOR FUTURE RESEARCH

To determine risk factors for overanticoagulation on coumarin anticoagulants we performed a nested case-control study among outpatients of an anticoagulation clinic. However, this design is inefficient for the evaluation of rare exposures, as we encountered when studying the role of drug interactions and chronic comorbidities in overanticoagulation. For several drugs and some comorbidities numbers were too small to judge their association with overanticoagulation. A large cohort study - for instance within automated data bases such as the PHARMO Record Linkage System (29) or the Integrated Primary Care Information (IPCI) project (30) - might be required to study these potential risk factors for overanticoagulation, as we already did with respect to antibacterial drugs.

The results presented in chapter 5 show that the use of phenprocoumon has advantages over the use of acenocoumarol in the short-term and especially the long-term phase of therapy. However, opposite to the better therapeutic quality

of oral anticoagulant therapy with phenprocoumon, stands an increased risk of minor hemorrhages. The use of warfarin also gives a more stable anticoagulation than the use of acenocoumarol (9). The advantages of phenprocoumon and warfarin over acenocoumarol may be explained by the difference in half-life. Because of its short half-life, the use of acenocoumarol induces wide fluctuations in the plasma levels of factor VII (31, 32). It would be interesting to compare the quality of oral anticoagulant therapy and the risk of hemorrhages between phenprocoumon and warfarin. Possibly, warfarin, which has an intermediate half-life, both has a high quality of oral anticoagulant therapy and a low risk of hemorrhages.

In oral anticoagulant therapy not only overanticoagulation poses a risk to patients, but also the opposite situation, underanticoagulation. While overanticoagulation increases the risk of hemorrhages, underanticoagulation is associated with a higher thromboembolic risk (14). Noncompliance is an obvious risk factor for underanticoagulation. A number of other factors, among which drug interactions and comorbid conditions, have been postulated to decrease the response to coumarins (7). However, as for overanticoagulation, these potential risk factors for underanticoagulation should be further investigated in a large epidemiological study in a non-selected population under everyday circumstances.

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7

Summary
Samenvatting

7.1 Summary

Oral anticoagulation with coumarin derivatives is an established, widely used therapy for the prevention of venous and arterial thromboembolism. These drugs induce anticoagulation by antagonizing vitamin K, thereby impairing the γ-carboxylation and consequently the biological activity of the vitamin K-dependent coagulation proteins (factor II, VII, IX and X, protein C and S). Coumarin anticoagulants have a narrow therapeutic index. As patients may require different doses of coumarins to reach the same level of anticoagulation and because the required dose may vary over time in an individual patient, anticoagulant therapy needs to be monitored. Despite monitoring, overanticoagulation may occur. When the International Normalized Ratio (INR) is ≥6.0, the risk of hemorrhage, the most common adverse reaction to coumarin anticoagulants, sharply increases. Hence, such an excess anticoagulant effect should preferably be prevented and should be treated promptly and adequately if present. Although the risks of overanticoagulation are clear, its treatment and determinants have received little attention. Most of the extensive research on oral anticoagulant therapy has focussed on its pharmacological- and biochemical action, prothrombin time calibration, optimal therapeutic intensity and hemorrhagic complications. Therefore, the aim of this thesis was to study aspects of overanticoagulation on coumarin anticoagulants among outpatients of an anticoagulation clinic (**chapter 1**).

Chapter 2 relates to the treatment of overanticoagulation and describes the course of the INR in response to oral vitamin K_1 in overanticoagulated patients. Oral vitamin K_1 is used for the treatment of excessive anticoagulation. Detailed information on changes in the INR in response to vitamin K_1 is not available. We therefore measured the INR on the first seven days following the oral intake of 1 to 5 mg of vitamin K_1 in 24 patients routinely treated with phenprocoumon who had an INR ≥6.0 at presentation. The first two days after administration of vitamin K_1 the mean INR decreased by 40% and 23%, respectively. After day 2, the day-to-day proportional change in the mean INR depended on the dose of

vitamin K_1 and varied from a decrease of 12% to an increase of 21%. On day 7, the mean INR was higher than on day 2 in three out of five treatment groups. Between day 2 and day 7, in general, 32% of the patients had an INR within the therapeutic zone, 25% had an INR ≥6.0 and 8% had an INR <2.0. These findings suggest that the routine treatment of overanticoagulation in patients on phenprocoumon should be intensified to improve its efficacy.

Chapter 3 concerns the incidence of and risk factors for overanticoagulation. **Chapters 3.1, 3.2** and **3.4** are based on the same prospective cohort study with a nested case-control design. The study cohort consisted of all patients treated with coumarin anticoagulants at the regional Red Cross anticoagulation clinic The Hague between December 1, 1997 and June 14, 1999 (n=17,056). All cohort members were followed until the first occurrence of an INR ≥6.0, the end of their treatment, or the end of the study period. The nested case-control study included 300 prospectively gathered cases with an INR ≥6.0 and 302 randomly selected controls with an INR within the therapeutic zone, matched on index day and target range. Cases and controls had to be stably anticoagulated in the three months preceding the index day. Information on risk factors in the four weeks preceding the index day was collected from the anticoagulant medical record, through the general practitioner and the pharmacy, and by interviewing the patient. In **chapter 3.1**, we describe the incidence of overanticoagulation and the association between overanticoagulation and characteristics of anticoagulant therapy and comorbidity. The incidence rate of an INR ≥6.0 was 7.8 per 10,000 treatment days in prevalent users on the starting date and 22.5 per 10,000 treatment days in incident users during the study period. Patients on acenocoumarol had an increased risk of an INR ≥6.0 compared to patients on phenprocoumon (OR 1.9; 95%CI 1.3-2.7). Chronic diseases associated with overanticoagulation were impaired liver function (OR 2.8; 95%CI 1.1-6.9) and congestive heart failure (OR 1.6; 95%CI 1.04-2.6 in stable condition and OR 3.0; 95%CI 0.8-12.0 in case of a relapse). Acute illnesses associated with overanticoagulation were diarrhea and fever (OR 12.8; 95%CI 1.6-104.9 and OR 2.9; 95%CI 1.1-7.7, respectively). **Chapter 3.2** addresses the role of drug interactions in overanticoagulation. Forty-five out of 87 potentially interacting drugs were not used in the four weeks preceding the index day and only 15 drugs were used by at least ten patients. A course of sulphamethoxazole+trimethoprim (co-trimoxazole), strongly increased the risk of overanticoagulation (OR 24.2; 95%CI 2.8-209.1), especially in patients on acenocoumarol. Penicillins were associated with a risk of overanticoagulation of 2.4 (95%CI 1.00-5.5). The effect was confined to amoxicillin+clavulanic acid. **Chapter 3.4** relates to sociodemographic-, lifestyle-, and dietary factors as risk factors for overanticoagulation. Age, sex and level of education were not associated with overanticoagulation. Body mass index was negatively related to overanticoagulation (OR 2.37; 95%CI 1.00-5.65, BMI <20 kg/m^2 vs >25 kg/m^2), a beneath average level of physical activity was

positively related to overanticoagulation (OR 1.61; 95%CI 1.02-2.53) and never-smokers were more likely to have an INR ≥6.0 compared to smokers (OR 1.70; 95%CI 1.02-2.84). Habitual alcohol consumption, even heavy drinking, was not related to overanticoagulation. However, a recent decrease of alcohol intake increased the risk of an INR ≥6.0 (OR 2.79; 95%CI 1.21-6.43). In addition, weight loss and a vacation were risk factors for overanticoagulation (OR 2.32; 95%CI 1.03-5.22 and OR 10.67; 95%CI 2.48-45.88, respectively). Dietary factors were not associated with overanticoagulation. In conclusion, in previously stable outpatients of an anticoagulation clinic using phenprocoumon or acenocoumarol, overanticoagulation was associated with the type of anticoagulant used and with some comorbidities and lifestyle factors. Drug interactions as a cause of overanticoagulation predominantly concerned antibacterial drugs. If risk factors are present, increased monitoring of INR-values might prevent overanticoagulation and potential bleeding complications. Similarly, if possible, the use of co-trimoxazole and amoxicillin+clavulanic acid should be avoided in patients on coumarins.

The sample size of the case-control study was too small to judge the association with overanticoagulation for several antibacterial drugs. Therefore, we conducted a population-based cohort study in a sample of the Rotterdam Study to investigate which antibacterial drugs are associated with overanticoagulation, as described in **chapter 3.3**. The study cohort consisted of all participants who were treated with acenocoumarol or phenprocoumon in the study period from April 1, 1991 through December 31, 1998 and for whom INR-data were available. All cohort members were followed until the first occurrence of an INR ≥6.0, the last INR-assessment because of the end of their treatment, death or end of the study period. Data on antibacterial drug use were obtained from regional pharmacies. Of the 1,124 patients in the cohort, 351 developed an INR ≥6.0. Eight antibacterial drugs were multivariately associated with overanticoagulation. Sulfamethoxazole combined with trimethoprim most strongly increased the risk of overanticoagulation (RR 20.1; 95%CI 10.7-37.9). Stratification showed that the induction period of overanticoagulation varied between different antibacterial drugs. Awareness of these drug interactions and more frequent monitoring of INR-values during the initial stages of antibacterial drug therapy are warranted to minimize the risk of bleeding complications.

In **chapter 3.5**, we examined whether patients with a deficient dietary intake of vitamin K have an increased risk of overanticoagulation. We performed a population-based cohort study in a sample of the Rotterdam Study. The study cohort consisted of all participants of whom dietary intake data have been collected and who were treated with coumarin anticoagulants in the study period from the baseline visit of the Rotterdam Study (1990-1993) through December 31, 1998. All cohort members were followed until the first occurrence of an INR ≥6.0, the last INR-assessment during the study period, death or end of the study period. The intake of vitamin K was calculated from the total diet using data on con-

centrations of vitamin K_1 and vitamin K_2 in foods. An intake of vitamin K below 1 µg/kg body weight per day was considered deficient. Of the 772 patients in the cohort, 227 developed an INR ≥6.0 during the study period. The number of patients in the total cohort with a deficient dietary intake of vitamin K was 12 (1.6%). Of the cases, seven patients (3.1%) had a deficient dietary intake of vitamin K. The adjusted relative risk of overanticoagulation associated with a deficient dietary intake of vitamin K was 9.6 (95%CI 4.0-23.0). To minimize the risk of bleeding complications, patients on oral anticoagulant therapy may be advised to consume vitamin K-rich foods such as green, leafy vegetables.

Chapter 4 presents the levels of the vitamin K-dependent pro- and anticoagulant proteins in overanticoagulated patients. The levels of factor II, VII, IX and X, protein C and S were determined in 25 randomly selected overanticoagulated patients (INR ≥6.0) and in 25 matched controls with an INR within the therapeutic zone. Furthermore, to study a possible effect of the cause of overanticoagulation, the coagulant levels were compared between 16 overanticoagulated patients with fever in the preceding two weeks and 24 overanticoagulated patients with stable congestive heart failure. The pattern of procoagulant level reductions in the three groups of overanticoagulated patients was largely the same as in controls: factor X was the lowest and factor IX the highest. The difference was that in overanticoagulated patients factor VII was relatively low among the procoagulant factors compared to controls. Protein C was lower than protein S in overanticoagulated patients with congestive heart failure, but was similar to protein S in the other study groups. In overanticoagulated patients with fever, the vitamin K-dependent coagulant levels except factor X were lower than in overanticoagulated patients with congestive heart failure, especially factor VII and protein S.

Chapter 5 describes a cohort study among 7740 newly enrolled patients of an anticoagulation clinic which was performed to compare the quality of oral anticoagulant therapy between phenprocoumon and acenocoumarol in three phases of therapy: the initiation phase (day 1 up to day 31), the short-term phase (day 32 up to day 122) and the long-term phase (day 123 up to day 365). In the short-term phase and especially the long-term phase, patients on phenprocoumon were more likely to spend at least 70% of the time within the therapeutic zone, to have no INRs ≥6.0 and to have no INRs <2.0. Furthermore, their INRs were closer to the target INR and their interval of monitoring was longer. In the initiation phase, however, the quality of oral anticoagulant therapy was slightly better with acenocoumarol. The percentage of patients with a major hemorrhage did not differ substantially between the two types of coumarin. In conclusion, for outpatients of an anticoagulation clinic phenprocoumon seems to be preferred. Only in case a patient needs oral anticoagulant therapy for a short time (<6 weeks), acenocoumarol might be considered.

Finally, in **chapter 6** the main findings are summarized and the general methodological issues inherent to the study of risk factors for overanticoagulation are discussed. In addition, the implications for oral anticoagulant therapy and recommendations for future research are given.

7.2 Samenvatting

Orale antistolling met coumarinederivaten wordt veel gebruikt ter voorkoming van veneuze en arteriële trombo-embolieën. Deze geneesmiddelen remmen de bloedstolling door als antagonist van vitamine K de γ-carboxylering en daarmee de biologische activiteit van de vitamine K-afhankelijke stollingsfactoren (factor II, VII, IX and X, proteïne C and S) te blokkeren. Coumarine anticoagulantia hebben een smalle therapeutische breedte. Patiënten kunnen een verschillende coumarinedosering nodig hebben voor hetzelfde niveau van antistolling. Daarnaast kan voor een individuele patiënt de benodigde dosering variëren over de tijd. Antistollingsbehandeling moet daarom regelmatig worden gecontroleerd. Ondanks deze controle kan de stolling te sterk worden geremd (het zogenoemde 'doorschieten'). Wanneer de International Normalized Ratio (INR, maat voor de intensiteit van antistolling) ≥6.0 is, neemt het risico op een bloeding, de meest voorkomende bijwerking van coumarine anticoagulantia, sterk toe. Excessieve antistolling moet dus bij voorkeur worden voorkomen en dient prompt en adequaat te worden behandeld indien aanwezig. Alhoewel de risico's van een doorgeschoten antistolling duidelijk zijn, hebben de behandeling ervan en de risicofactoren ervoor weinig aandacht gekregen. Het meerendeel van de vele onderzoeken op het gebied van orale antistollingsbehandeling concentreerden zich op de farmacologische- en biochemische werking van coumarines, het calibreren van de protrombinetijd, de optimale therapeutische intensiteit en bloedingscomplicaties. Het doel van dit proefschrift was daarom om aspecten van het doorschieten van de antistolling op coumarine anticoagulantia te bestuderen bij patiënten van een trombosedienst (**hoofdstuk 1**).

Hoofdstuk 2 betreft de behandeling van doorgeschoten antistolling en beschrijft het verloop van de INR na orale toediening van vitamine K_1. Oraal toegediend vitamine K_1 wordt gebruikt ter behandeling van excessieve antistolling. Er is geen gedetailleerde informatie over de veranderingen in de INR na inneming van vitamine K_1. Daarom hebben wij de INR gemeten op de eerste zeven dagen na inneming van 1 tot 5 mg vitamine K_1 bij 24 patiënten die werden behandeld

met fenprocoumon en een INR ≥6.0 hadden. De eerste twee dagen na orale toediening van vitamine K_1 nam de gemiddelde INR met 40% respectievelijk 23% af. Na dag 2 was de proportionele verandering in de gemiddelde INR tussen twee opeenvolgende dagen afhankelijk van de dosis vitamine K_1 en varieerde van een afname van 12% tot een toename van 21%. Op dag 7 was de gemiddelde INR hoger dan op dag 2 in drie van de vijf behandelingsgroepen. Tussen dag 2 en dag 7 had, in het algemeen, 32% van de patiënten een INR binnen het therapeutische gebied, 25% had een INR ≥6.0 en 8% had een INR <2.0. Deze bevindingen suggereren dat de behandeling van doorgeschoten antistolling op phenprocoumon moet worden geïntensiveerd teneinde de effectiviteit ervan de verbeteren.

Hoofdstuk 3 gaat over de incidentie van en risicofactoren voor het doorschieten van de antistolling. De **hoofdstukken 3.1, 3.2** en **3.4** zijn gebaseerd op dezelfde prospectieve cohortstudie met daarbinnen een genest patiënt-controle-onderzoek. Het studiecohort bestond uit alle patiënten die werden behandeld met coumarine anticoagulantia bij de Stichting Rode Kruis Trombosedienst 's-Gravenhage en omstreken tussen 1 december 1997 en 14 juni 1999 (n=17056). Alle patiënten werden gevolgd tot het eerste optreden van een INR ≥6.0, het einde van hun behandeling of het einde van de studieperiode. Het geneste patiënt-controle-onderzoek omvatte 300 patiënten met een INR ≥6.0 en 302 willekeurig geselecteerde controles met een INR binnen het therapeutische gebied, gematcht op indexdag en streefgebied. Patiënten en controles moesten stabiel zijn ontstold in de drie maanden voorafgaande aan de indexdag. Informatie over risicofactoren in de vier weken voorafgaande aan de indexdag werd verzameld uit het dossier van de trombosedienst, via de huisarts en de apotheek en door middel van een interview met de patiënt. In **hoofdstuk 3.1** beschrijven we de incidentie van doorgeschoten antistolling en de associatie tussen doorgeschoten antistolling, karakteristieken van antistollingsbehandeling en comorbiditeit. De incidentiedichtheid van een INR ≥6.0 was 7.8 per 10000 behandeldagen voor prevalente gebruikers op de startdatum en 22.5 per 10000 behandeldagen voor incidente gebruikers tijdens de studieperiode. Patiënten op acenocoumarol hadden een toegenomen risico op een INR ≥6.0 vergeleken met patiënten op fenprocoumon (odds ratio (OR) 1.9; 95% betrouwbaarheidsinterval (95%BI) 1.3-2.7). Chronische ziekten die waren geassocieerd met het doorschieten van de antistolling waren een gestoorde leverfunctie (OR 2.8; 95%BI 1.1-6.9) en hartfalen (OR 1.6; 95%BI 1.04-2.6 in stabiele conditie en OR 3.0; 95%BI 0.8-12.0 in geval van een recidief). Acute ziekten die waren geassocieerd met doorgeschoten antistolling waren diarree en koorts (OR 12.8; 95%BI 1.6-104.9 respectievelijk OR 2.9; 95%BI 1.1-7.7). In **hoofdstuk 3.2** komt de rol van geneesmiddelinteracties bij het doorschieten van de antistolling aan de orde. Vijfenveertig van de 87 potentieel interacterende geneesmiddelen werden niet gebruikt in de vier weken voorafgaande aan de indexdag en slechts 15 geneesmiddelen werden gebruikt

door ten minste tien patiënten. Een kuur met sulfamethoxazol+trimethoprim (co-trimoxazol) verhoogde het risico op doorschieten sterk (OR 24.2; 95%BI 2.8-209.1), met name bij patiënten op acenocoumarol. Penicillines waren geassocieerd met een risico op doorgeschoten antistolling van 2.4 (95%BI 1.00-5.5). Dit effect was beperkt tot amoxicilline+clavulaanzuur. **Hoofdstuk 3.4** betreft sociaaldemografische-, leefstijl- en voedingsfactoren als risicofactoren voor het doorschieten van de antistolling. Leeftijd, geslacht en opleidingsniveau waren niet geassocieerd met doorschieten. Body mass index (BMI) - een maat voor overgewicht - was negatief geassocieerd met het doorschieten van de antistolling (OR 2.37; 95%BI 1.00-5.65, BMI <20 kg/m^2 vs >25 kg/m^2), een lager dan gemiddeld niveau van lichamelijke activiteit was positief geassocieerd met doorschieten (OR 1.61; 95%BI 1.02-2.53) en patiënten die nooit hadden gerookt hadden een grotere kans op een INR ≥6.0 dan rokers (OR 1.70; 95%BI 1.02-2.84). Gebruikelijke alcoholconsumptie, zelfs overmatig drinken, was niet geassocieerd met doorgeschoten antistolling. Echter, een recente vermindering van de alcoholinneming verhoogde het risico op een INR ≥6.0 (OR 2.79; 95%BI 1.21-6.43). Daarnaast waren gewichtsverlies en vakantie risicofactoren voor het doorschieten van de antistolling (OR 2.32; 95%BI 1.03-5.22 respectievelijk OR 10.67; 95%BI 2.48-45.88). Voedingsfactoren waren niet geassocieerd met doorschieten. Concluderend kan worden gesteld dat in voorheen stabiele patiënten van een trombosedienst die fenprocoumon of acenocoumarol gebruiken, het doorschieten van de antistolling was geassocieerd met het type anticoagulans en met sommige onderliggende ziekten en leefstijlfactoren. Geneesmiddelinteracties als oorzaak van het doorschieten van de antistolling betrof met name antibacteriële middelen. In aanwezigheid van risicofactoren kan het frequenter controleren van de INR het doorschieten van de antistolling en potentiële bloedingscomplicaties mogelijk voorkomen. Eveneens zou, indien mogelijk, het gebruik van co-trimoxazol en amoxicilline+clavulaanzuur moeten worden vermeden bij patiënten die coumarines gebruiken.

Het aantal patiënten in het patiënt-controle-onderzoek was te klein om de associatie met doorschieten te beoordelen voor verscheidene antibacteriële middelen. Daarom voerden we een analyse uit binnen het Rotterdamse prospectieve cohortonderzoek (ERGO), teneinde te onderzoeken welke antibacteriële geneesmiddelen zijn geassocieerd met het doorschieten van de antistolling (**hoofdstuk 3.3**). Het studiecohort bestond uit alle deelnemers die werden behandeld met acenocoumarol of fenprocoumon in de studieperiode van 1 april 1991 tot 31 december 1998, en van wie INR-gegevens beschikbaar waren. Alle cohortleden werden gevolgd tot het eerste optreden van een INR ≥6.0, de laatste INR-bepaling vanwege het einde van hun behandeling, overlijden of einde van de studieperiode. Gegevens over het gebruik van antibacteriële middelen werden verkregen via regionale apotheken. Van de 1124 patiënten in het cohort ontwikkelden er 351 een INR ≥6.0. Acht antibacteriële middelen waren geassocieerd met doorgeschoten antistolling. Sulfamethoxazol in combinatie met tri-

methoprim verhoogde het risico op doorschieten het sterkst (relatief risico (RR) 20.1; 95%BI 10.7-37.9), een bevinding die sterk overeenkomt met het onderzoek uit hoofdstuk 3.2. Stratificatie liet zien dat de periode tussen het starten van de behandeling met het antibacteriële geneesmiddel en het doorschieten varieerde tussen verschillende antibacteriële middelen. Het zich bewust zijn van deze geneesmiddelinteracties en het frequenter controleren van de INR in de beginfase van een antibacteriële kuur wordt aangeraden om het risico op bloedingscomplicaties te beperken.

In **hoofdstuk 3.5** onderzochten we of patiënten met een deficiënte voedingsinneming van vitamine K een verhoogd risico op het doorschieten van de antistolling hadden. We voerden hiertoe een cohortanalyse uit bij een deel van de ERGO-deelnemers. Het studiecohort bestond uit alle deelnemers van wie voedingsgegevens waren verzameld en die werden behandeld met coumarine anticoagulantia in de studieperiode tussen de aanvang van de ERGO-studie (1990-1993) en 31 december 1998. Alle cohortleden werden gevolgd tot het eerste optreden van een INR $\geq$6.0, de laatste INR-bepaling tijdens de studieperiode, overlijden of einde van de studieperiode. De inneming van vitamine K werd berekend over de totale voeding op basis van de vitamine K_1- en K_2-gehalten van voedingsmiddelen. Een vitamine K-inneming beneden 1 µg/kg lichaamsgewicht per dag werd als deficiënt beschouwd. Van de 772 patiënten in het cohort ontwikkelden er 227 een INR $\geq$6.0. Het aantal patiënten in het totale cohort met een deficiënte voedingsinneming van vitamine K was 12 (1.6%). Van de patiënten met een doorgeschoten antistolling hadden er zeven (3.1%) een deficiënte inneming van vitamine K. Het gecorrigeerde relatieve risico op doorgeschoten antistolling geassocieerd met een deficiënte voedingsinneming van vitamine K was 9.6 (95%BI 4.0-23.0). Teneinde het risico op bloedingscomplicaties zo klein mogelijk te houden zou patiënten op orale antistollingsbehandeling kunnen worden geadviseerd om vitamine K-rijke voedingsmiddelen te eten, zoals groene bladgroenten.

Hoofdstuk 4 geeft de niveaus weer van de vitamine K-afhankelijke pro- en anticoagulante eiwitten bij patiënten met een doorgeschoten antistolling. De niveaus van factor II, VII, IX en X, proteïne C en S in het plasma werden bepaald bij 25 willekeurig geselecteerde patiënten met een doorgeschoten antistolling (INR $\geq$6.0) en bij 25 op dag gematchte controles met een INR binnen het therapeutische gebied. Verder werden, om een mogelijk effect van de oorzaak van doorschieten te bestuderen, de niveaus van de stollingseiwitten vergeleken tussen 16 doorgeschoten patiënten met koorts in de voorafgaande twee weken en 24 doorgeschoten patiënten met stabiel hartfalen. Het patroon van de reductie van stollingsfactoren bij de drie groepen patiënten met een doorgeschoten antistolling was grotendeels hetzelfde als voor de controlegroep: factor X was het laagst en factor IX het hoogst. Het verschil was dat bij patiënten met een doorgeschoten antistolling factor VII relatief laag was vergeleken met de con-

troles. Proteïne C was lager dan proteïne S bij doorgeschoten patiënten met hartfalen, maar was gelijk aan proteïne S bij de andere studiegroepen. Bij doorgeschoten patiënten met koorts waren de niveaus van de vitamine K-afhankelijke stollingseiwitten, behalve factor X, lager dan bij dergelijke patiënten met hartfalen, met name factor VII en proteïne S.

Hoofdstuk 5 beschrijft een cohortonderzoek dat werd uitgevoerd bij 7740 nieuw geregistreerde patiënten van een trombosedienst teneinde de kwaliteit van orale antistollingsbehandeling te vergelijken tussen fenprocoumon en acenocoumarol over drie fasen van behandeling: de initiatiefase (dag 1 tot en met dag 31), de korte-termijn-fase (dag 32 tot en met dag 122) en de lange-termijn-fase (dag 123 tot en met dag 365). In de korte-termijn-fase en met name in de lange-termijn-fase hadden patiënten op fenprocoumon een grotere kans om tenminste 70% van de tijd INRs binnen het therapeutische gebied te hebben, om geen INRs $\geq$6.0 te hebben en om geen INRs <2.0 te hebben. Daarnaast lagen hun INRs dichter bij de streefwaarde en was hun controleinterval langer. In de initiatiefase was de kwaliteit van orale antistollingsbehandeling echter iets beter met acenocoumarol. Het percentage patiënten met een ernstige bloedingscomplicatie verschilde nauwelijks tussen de twee types coumarine. Wij concluderen dat voor trombosedienstpatiënten fenprocoumon de voorkeur heeft. Alleen indien een patiënt voor een korte periode (<6 weken) orale antistollingsbehandeling behoeft, kan acenocoumarol worden overwogen.

Ten slotte worden in **hoofdstuk 6** de belangrijkste resultaten samengevat en bespreken we de algemene methodologische aspecten die inherent zijn aan het bestuderen van risicofactoren voor het doorschieten van de antistolling. Vervolgens worden de consequenties voor orale antistollingsbehandeling besproken en aanbevelingen gedaan voor verder onderzoek.

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