Determinants of hemorrhagic risk during acenocoumarol treatment

[1]Florentina C. Militaru, [2]Sorin Crişan, [3]Ştefan C. Vesa, [4]Adrian Trifa, [2]Valentin Militaru, [1]Anca D. Buzoianu

[1] Department of Pharmacology, Faculty of Medicine, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania;
[2] Fifth Medical Clinic, Department of Internal Medicine, Faculty of Medicine, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; [3] "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; [4] Department of Genetics, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania.

Abstract. Objective: to determine the frequency of haemorrhage occurrence in the analysed batch; to establish the relation between the hemorrhagic risk and the presence of supratherapeutic INR; to assess the concomitant therapy's impact on the haemorrhage risk'; to study the impact of the age and sex on the hemorrhagic risk; to assess the relation between the polymorphisms of the CYP2C9 & VKORC1 genes and the occurrence of hemorrhages. Material and Methods: The study included 223 patients (110 females (49.3%) and 113 males (50.7%)) who were treated with acenocoumarol for one or several of the following clinic situations: 1. deep vein thrombosis (DVT) of the lower limb ± pulmonary thromboembolism; 2. persistent or permanent atrial fibrillation (AF) ± pulmonary thromboembolism. Results: Bleeding complications were registered in 11 patients (4.9%). The presence of a supratherapeutic INR increases the risk of bleeding complications by 3.1 times. Conclusion: our study shows that only the supratherapeutic INR was correlated with the occurrence of haemorrhages.

Key Words: acenocoumarol, hemorrhagic risk, INR, polymorphisms of the CYP2C9 & VKORC1 genes.

Corresponding Author: F. C. Militaru, claudiamilitaru@yahoo.com

Introduction

For 60 years, oral anticoagulants (OAs) have proved their efficacy in treatment or prophylaxis of pulmonary embolism, deep vein thrombosis, stroke and acute myocardial infarction. Although new drugs from the anticoagulant class have been recently launched, at present antivitamins K (AVK) are the benchmark oral anticoagulants.

Oral anticoagulants such as warfarin and acenocoumarol are drugs widely used to prevent and treat thromboembolic diseases, thus being included in the category of the most frequently prescribed drugs. They are characterized by low therapeutic index and intraindividual treatment-response variability. Despite this well-proven efficacy, OAs are associated with an increased hemorrhagic risk which can limit their use, particularly in the case of older people (Becquemont 2008). In France, OAs are associated with 17,000 hospital admissions per year caused by major hemorrhages; in the USA, 20,000,000 patients are treated every year with OAs and 29,000 visits to the doctor are annually registered for bleeding complications. Warfarin is the first cause of the fatal adverse drug reactions in the USA (Wysowski *et al* 2007). The use of AVK is associated with an increased hemorrhagic risk or with thrombotic complications because of

the low therapeutic index and of the numerous drug or food interactions. Several risk factors have been identified for the occurrence of hemorrhages like age, female sex, and drug interactions. Beyond these classical risk factors, in the last decade the pharmacogenetic progress has allowed the identification of genetic factors which can have a major contribution to the occurrence of the hemorrhagic risk.

At present, it is well known that genetic diversity contributes both to the disease susceptibility and to the treatment-response variability. Recent data suggest that most of the drugs are efficient in about 60% of the clinical cases in which they are used and that an important number of patients develop adverse treatment reactions (Hall *et al* 2006).

As regards the OAs, in addition to the demographic and environment factors, researchers also identified genetic polymorphisms which explains a part of the individual OA treatment-response variability – including polymorphisms of the drug-metabolizing genes (CYP2C9) and genetic polymorphisms with an impact on the activity of Vitamin K epoxide reductase, subunit 1 (VKORC1), that is in fact the target of the coumarin anticoagulants. Polymorphisms CYP2C9 were identified about 10 years ago and their frequency in population varies according to the race

(Caucasian, African and Asian). In the Caucasian race, the most common variants are CYP2C9*2 (Arg 144-Cys) present in 8-19% of population and CYP2C9*3 (Ile 359-Leu) present in 6-10% of subjects, which means that about one third of the general population has at least a mutant allele. The mutant enzymes resulted from such polymorphisms are less active than the normal enzymes, which leads to a decrease in the metabolism of the coumarin derivatives: subjects with at least one mutant allele have an increased sensitivity to these derivatives and to the associated hemorrhagic risk, being named `poor metabolizers` (Kirchheiner *et al* 2005).

Geisen *et al* described 28 polymorphisms for VKORC1 gene. Three of the haplotypes were found to be responsible for the genetic variability of VKORC1. Most of the oral anticoagulant response variability was attributed to VKORC1*2 haplotype (Geisen *et al* 2005). Researchers found that an elevated response to acenocoumarol in healthy subjects is linked to c.-1639G>A polymorphism of VKORC1*2 (Bodin *et al* 2005). Montes *et al* also showed that allele A of the polymorphism c.-1639G>A of the VKORC1 gene is associated with the necessity of a lower dose of acenocoumarol in patients receiving an anticoagulant therapy (Montes *et al* 2006).

Working Hypothesis

In this study we intended to analyze the impact of various factors on the hemorrhagic risk in patients treated with acenocoumarol. We started from the hypothesis that hemorrhagic risk could be determined both by environmental factors and genetic factors. Consequently, we studied the effect of age and sex on the occurrence of haemorrhage and, in addition, we tried to establish the link between various classes of concomitant drugs and the hemorrhagic risk in patients treated with Acenocoumarol. The novelty is our attempt to determine a relation between the polymorphisms of the CYP2C9 and VKORC1 genes and the occurrence of haemorrhage in these patients.

Objectives

The objectives are: to determine the frequency of haemorrhage occurrence in the analysed batch; to establish the relation between the hemorrhagic risk and the presence of supratherapeutic INR; to assess the concomitant therapy's impact on the haemorrhage risk'; to study the impact of the age and sex on the hemorrhagic risk; to assess the relation between the polymorphisms of the CYP2C9 & VKORC1 genes and the occurrence of hemorrhages.

Material and Methods

The study included 223 patients (110 females (49.3%) and 113 males (50.7%)) who were treated with acenocoumarol and admitted within the 5th Medical Clinic in Cluj-Napoca during the period 2009-2011. It was a transversal analytical observational study.

Patients were included in the study only after they had signed the informed consent form to participate in the study and to undergo genetic determinations.

The protocol of the study was approved by the Ethics Commission of the University.

Criteria of Participation

We accepted patients over 18 years of age, who signed the informed consent form. Patients admitted to the study were treated with acenocoumarol for one or several of the following clinic situations:

1. Deep vein thrombosis (DVT) of the lower limb ± pulmonary thromboembolism;

2. Persistent or permanent atrial fibrillation (AF) ± pulmonary thromboembolism.

Participants in this study had no contraindication to the anticoagulant therapy. The DVT diagnostic was established with the Doppler ultrasound tests. We examined patients with a high degree of clinical suspicion of DVT based on the Wells score for clinical probability (Wells *et al* 2006). We also investigated patients without clinical signs of acute thrombosis but with a high risk of DVT according to the thrombotic risk scale (Caprini *et al* 2006).

For the ultrasound test we used an Aloka SSD 4000 device equipped with a linear transducer of a variable frequency between 7 and 10 MHz and a sector transducer of a variable frequency between 2.5 and 6 MHz.

The AF diagnostic was established against the definitory feature of the ECG tracing, respectively the absence of the P waves and their replacement with the f waves, the irregular rhythm and the amplitude variability of the f waves.

Exclusion Criteria

We did not include persons aged less than 18 years, patients with life expectation shorter than 1 year due to their comorbidities or patients who did not sign the informed consent form. Also we did not included patients who had contraindications to anticoagulant treatment: allergy to heparin or acenocoumarol; current hemorrhage; ulcer in active phase; ischemic stroke upon acceptance or during the last week or hemorrhagic stroke in the last 3 months; bacterial endocarditis in the last 3 months; high blood pressure – systolic over 220 mm Hg or diastolic over 120 mm Hg; thrombocytopenia < 100,000/mmc, antecedents of thrombocytopenia induced by heparin; hepatic insufficiency (INR > 1.8, high ASAT, ALAT).

Considering the hemorrhagic risk, we did not include patients under treatments with non-steroidal anti-inflammatory drugs or with aspirin in doses higher than necessary for thrombocytic anti-aggregation (80-250 mg).

Patients DVT who needed other therapeutic means (thrombolysis, surgical intervention) did not take part in our study. Because of the foetal malformation risk posed by oral anticoagulants, pregnancy was a criterion of exclusion.

Quantified Variables

The following data were registered for each patient:

1. General Data: age, sex and environment of origin – urban or rural;

2. Clinical data: indication for which the oral anticoagulant treatment was administrated: deep venous thrombosis, pulmonary embolism, atrial fibrillation; bleeding complications occurred during the oral anticoagulant treatment.

The following bleedings were considered major haemorrhages: fatal haemorrhage; intracranial/intramedullary; intraocular;

retroperitoneal; intra-articular; pericardial; intramuscular with compartment syndrome; hemorrhage leading to a decrease of haemoglobin by at least 2 g%; hemorrhage which requires transfusion of at least two units of blood or erythrocyte mass. The following bleedings have been classified as minor haemorrhages: cutaneous haematoma of at least 100 cm², painful or not painful; epistaxis longer than 5 minutes or repeated at least twice a day or which requires tamponage or electrocoagulation; macroscopic haematuria/urethrorrhagia, spontaneous or which lasts at least 24 hours after catheterism or surgical operation; metrorrhagia; haemoptysis; gastrointestinal bleeding; any other bleeding with clinical consequences for patient.

Ordinary hemorrhages involve: gingivorrhagia, conjunctival bleeding, epistaxis which does not meet the above-mentioned criteria, microscopic haematuria, minor rectorrhage caused by haemorrhoids, minor haematoma/ecchymosis at the injection site. We noted particularly those drugs which can influence the metabolization of the oral anticoagulants by the enzymatic system of the cytochrome P450. We recorded the oral anticoagulant dose per week, expressed in milligrams and INR values considered as: therapeutic INR for values between 2 and 3, supratherapeutic INR for values higher than 3 and subtherapeutic INR for values lower than 2.

3. Genetic data: determination of genotype for gene CYP2C9 and specification of existence of polymorphisms CYP2C9*2 and CYP2C9*3 (heterozygous or homozygous); determination of genotype for gene VKORC1.

4. Data regarding the treatment: patients belonged to two categories: newly diagnosed patients for whom the anticoagulant treatment started during the study and patients who were under chronic treatment with oral anticoagulants before their admission into the study. All patients included in the study underwent the acenocoumarol treatment.

Determination of Genotype

Genotyping for alleles CYP2C9*2 and CYP2C9*3 was based on the method PCR-RFLP (Polymerase chain reaction – restriction fragment length polymorphism) as described in 1999 by Aynacioglu *et al* (1999).

Mainly, an amplicon of 372 base pairs was obtained through PCR to study allele CYP2C9*2; the amplicon was digested during the night with the help of the restriction enzyme Sau96I (Fermentas MBI, Vilnius, Lithuania) which created, in the case of the wild-type allele, 3 fragments of 179, 119 and 74 base pairs. Allele CYP2C9*2 lacks a restriction site for the enzyme Sau96I and creates only two fragments of 253, respectively 119 base pairs. To analyse variant CYP2C9*3, an amplicon of 130 base pairs was obtained through PCR; this amplicon was digested during the night with a restriction enzyme StyI (Fermentas MBI, Vilnius, Lithuania); the wild-type allele was resistant to the StyI digestion. However, allele CYP2C9*3 creates a restriction site for the enzyme StyI and cuts the amplicon of 130 base pairs into 2 fragments, one of 104 base pairs and the other one of 26 base pairs.

Genotyping for polymorphism VKORC1-1693 G>A used the PCR-RFLP method, as described by Wen *et al* (2008). Mainly, an amplicon of 290 base pairs was obtained through PCR. The amplicon was digested during the night with the help of the restriction enzyme MspI (Fermentas MBI, Vilnius, Lithuania). If allele G were present, the amplicon of 290 base pair were cut in 2 fragments of 167 and 123 base pairs; allele A resists to digestion through the restriction enzyme MspI.

Statistical Analysis

Data were electronically recorded with Microsoft Excel XP and statistical analysis was made with SPSS (Statistical Package for the Social Sciences) version 17.

According to their features, data were tagged as nominal, continuous and ordinal variables. As regards nominal and ordinal variables, we calculated frequencies while as regards continuous variables we calculated a central trend (mean or median – depending on the normal distribution checked against the Kolmogorov-Smirnov test).

According to the normal or non-normal distribution of continuous variables, we chose adequate parametric or non-parametric statistical tests. Thus, for the univariate analysis of the continuous variables with asymmetric distribution (non-normal) we used the Mann-Whitney test and the Spearman correlation coefficient. For the verification of the correlation between two variables, with control for the third, we used the partial correlation. For the univariate analysis of the dichotomous variables we used the χ^2 test.

The multivariate analysis consisted in creating several predictive models with the help of the binary logistic regression.

Statistical significance was supplied by p-parameter value lower than 0.05.

Results

Statistic analysis of the patients' age in our study showed a non-normal distribution (the Kolmogorov-Smirnov test). Minimum age was 21, maximum age was 92; the mean was 64 and the median was 66.

Bleeding complications were registered in 11 patients (4.9%). In four cases (1.7%) there were inferior digestive hemorrhages, in two cases (0.8%) there was hemoptysis, in two cases (0.8%) there was hematuria, in two cases (0.8%) multifocal ecchymoses and in one case (0.4%) conjunctival bleeding. None of these hemorrhages involved a lethal danger.

The minimum dose of acenocoumarol administrated to our patients was 2 mg/week, the maximum dose was 49 mg/week, the average dose was 17.2 mg/week, and the median dose was 16 mg. Distribution of dose values for Acenocoumarol was non-normal. Supratherapeutic INR was found in 100 patients during the monitoring period. The maximum INR registered was 9.42. The average of the INR values was 4.41, while the median was 3.11. Distribution of INR values was non-normal.

Patients' characteristics according to the presence of bleeding complications may be seen in Table 1. We can notice that the percentage of patients with bleeding complications and supratherapeutic INR was different from the percentage of the patients without bleeding complications and the difference was statistically significant.

In order to examine the independent impact of each parameter on the risk of bleeding complications, we built a predictive model using the multiple logistic regression (Table 2). We obtained a Nagelkerke R2 value of 0.278 and a Cox & Snell R2 value of 0.09. The model proposed explains 9% of the probability of a

Table 1. Patients' characteristics according to the presence of bleeding complications

Variables	Patients without	Patients with	P
Age (years, median)	66	67	0.95*
Male (no, %)	105 (47%)	5 (2.2%)	1**
Female (no, %)	107 (47.9%)	6 (2.7%)	1**
DVT (no, %)	174 (78%)	8 (3.5%)	0.67**
AF (no, %)	50 (22.4%)	5 (2.2%)	0.18**
Environment of origin: urban (no, %)	101 (45.2%)	4 (1.8%)	0.46**
Environment of origin: rural (no, %)	111 (49.7%)	7 (3.1%)	
Aspirin (no, %)	6 (2.7%)	0	1**
Amiodarone (no, %)	3 (1.3%)	1 (0.4%)	0.48**
Proton-pump inhibitors (no, %)	23 (10.3%)	0	0.51**
Spironolactone (no, %)	19 (8.5%)	3 (1.3%)	0.14**
Statine (no, %)	96 (43%)	4 (1.8%)	0.78**
CYP2C9*2 (no, %)	47 (21%)	2 (0.8%)	1**
CYP2C9*3 (no, %)	37 (9.4%)	3 (1.3%)	0.67**
VKORC1*2 (GG) (no, %)	68 (30.9%)	5 (2.2%)	0.45**
VKORC1*2 (GA) (no, %)	113 (50.6%)	4 (1.8%)	0.45**
VKORC1*2 (AA) (no, %)	73 (32.7%)	4 (1.8%)	0.45**
Dose of acenocoumarol (mg/week, median)	16	19	0.97*
Patients with supratherapeutic INR (no, %)	91 (40.8%)	9 (4%)	0.027**

* Mann-Whitney test

** χ^2 test

patient to present bleeding complications. Among the parameters introduced in the regression, we calculated a statistical significance only for the supratherapeutic INR. The presence of a supratherapeutic INR increases the risk of bleeding complications by 3.1 times.

Discussion

This study aims to analyze the impact of the demographic factors and of the genetic factors on the risk of hemorrhages in patients treated with acenocoumarol.

Outstanding progress has been done over the last years in the field of the anticoagulant therapy, but most of the results in the specialized literature refer to the treatment with warfarin, while acenocoumarol is less studied.

There are relatively few studies which tried to investigate the causes of the oral anticoagulant overdosing and consequently

the hemorrhagic risk, sometimes their results being contradictory (Pindur *et al* 1999; Casais *et al* 2000).

Hemorrhagic accidents in patients treated with oral anticoagulants are frequent and potentially serious. The frequency of hemorrhages (major and minor) in patients under oral anticoagulant therapy varies from study to study between 3.8% and 16.5% patients per annum (Ameur *et al* 2009).

In our study, the frequency of hemorrhages was 4.9%, all of the bleedings being minor. We cannot compare our results with the literature data because the study design did not provide the registration of hemorrhages in `% per annum`, but we reported the hemorrhages in patients as `% of the total number of patients`. The relation between the old age and an increased risk of hemorrhages was studied by several authors. It is known that OAs are frequently prescribed to older people as this category of population often presents pathologies which require an anticoagulant therapy (Siguret *et al* 2003). The incidence of arterial and venous thromboembolic diseases which require an anticoagulant treatment increases by age.

The results concerning the association between older person and hemorrhagic risk are not convergent. The SPAF-II study showed that patients over 75 years of age treated with OAs for atrial fibrillation have an annual rate of major bleeding significantly more important than the patients under 75 (4.2% versus 1.7%). Van de Meer considered that hemorrhagic risk in patients under an OAs treatment increases 1.5 times for each decade after the age of 40 (1993). However, other authors state that old age has no impact on the risk of haemorrhage in patients treated with oral anticoagulant (Beyth *et al* 1995; Fihn *et al* 1996; Copland *et al* 2001).

Our results do not indicate an increased risk of hemorrhages in older patients treated with acenocoumarol. Like in the age case, there are also controversies regarding the impact of sex on the hemorrhagic risk in persons treated with oral anticoagulant. While the results obtained by Steffensen show that females are more prone to develop bleeding complications, Van der Meer does not find that sex is a risk factor for occurrence of hemorrhages (Van der Meer *et al* 1996; Steffensen *et al* 1997).

With the help of the multiple logistic regression, we did not obtain an important statistical significance in favour of hemorrhages caused by patients' sex.

Although it is known that drugs administrated concomitantly with oral anticoagulants may increase/diminish their effect, in our study concomitant medication did not increase the risk of haemorrhage occurrence. We took into consideration potential interactions between spironolactone and inhibitors of HMG-CoA reductase with oral anticoagulants, because they were the most frequently used concomitant medication for associated diseases presented by patients treated with OAs.

Metabolization of acenocoumarol is made through the enzymatic system of cytochrome P450, CYP2C9 being the most important enzyme involved in its biotransformation (Hermans *et al* 1993). The effects of polymorphisms of the gene CYP2C9 on the Acenocoumarol treatment response variability have been studied in the last period of time. So far the results show that alleles CYP2C9*2 and CYP2C9*3 are key independent factors which influence the patients' sensitivity to the Acenocoumarol's anticoagulant effect. These alleles have been associated with the complications of the oral anticoagulant treatment – over-response

Table 2. Binary logistic regression for bleeding complications

	B	Wald	df	P	OR	95%CI	
						Min	Max
Age	0.347	0.771	1	0.38	1.415	0.652	3.069
Sex	-0.048	0.019	1	0.89	0.953	0.481	1.888
Spironolactone	0.506	1.294	1	0.255	1.659	0.693	3.971
Statine	-0.265	0.524	1	0.469	0.768	0.375	1.571
CYP2C9*2	-0.19	0.184	1	0.668	0.827	0.346	1.974
CYP2C9*3	0.347	0.66	1	0.416	1.415	0.613	3.267
VKORC1*2 (GA)	-1.297	5.501	1	0.179	1.273	0.293	4.808
VKORC1*2 (AA)	0.66	1.403	1	0.236	1.934	0.649	5.761
Dose of acenocoumarol	0.057	1.044	1	0.307	1.059	0.949	1.181
Supratherapeutic INR	1.151	5.797	1	0.016	3.161	1.239	8.066

or hemorrhages (Verstuyft *et al* 2001; Tassies *et al* 2002; Visser *et al* 2004a; Visser *et al* 2004b).

Unlike literature data, our results do not show a statistically significant impact of the alleles CYP2C9*2 and CYP2C9*3 on the occurrence of haemorrhages. Also, according to our study, polymorphisms VKORC1*2 did not increased the hemorrhagic risk either.

It is well known that the intensity of the anticoagulant effect is in relation with the hemorrhagic risk. The results of the ISCOAT (Palareti *et al* 1996) study and also the results reported by Fihn (1996) confirmed that the hemorrhagic risk increases when INR is higher than 4.5.

Statistical analysis of our study indicates that a supratherapeutic INR is a risk factor for the occurrence of hemorrhages and there is a difference between the percentage of patients with bleeding complications and supratherapeutic INR and patients without bleeding complications (p=0.027); the difference is statistically significant.

Conclusion

Although literature data show that there are several factors which may increase the hemorrhagic risk in patients treated with acenocoumarol, our study shows that only the supratherapeutic INR was correlated with the occurrence of haemorrhages.

References

Ameur, Y. B., Chaabane, O., Zairi, I., Longo, S., Battikh, K., Slimane, M. L., 2009. Les accidents hemorragiques graves sous antivitamines K. Etude descriptive et pronostique. La Tunisie Medicale 87(11):763-769.

Aynacioglu, A. S., Brockmöller, J., Bauer, S., Sachse, C., Güzelbey, P., Ongen, Z., Nacak, M., Roots, I., 1999. Frequency of cytochrome P450 CYP2C9 variants in a Turkish population and functional relevance for phenytoin. Br J Clin Pharmacol 48(3):409-415.

Becquemont, L., 2008. Evidence for a pharmacogenetic adapted dose of oral anticoagulant in routine medical practice. Eur J Clin Pharmacol 64:953–960.

Beyth, R. J., Landefeld, C. S., 1995. Anticoagulants in older patients: a safety perspective. Drugs Aging 6:45-54.

Bodin, L., Verstuyft, C., Tregouet, D. A., Robert, A., Dubert, L., Funck-Brentano, C., *et al*, 2005. Cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase (VKORC1) genotypes as determinants of acenocoumarol sensitivity. Blood 106(1):135-140.

Caprini, J., 2006. Venous Thromboembolism Risk Factor Assessment. http://www.crmhealthcare.net/docs/67450a_CapriniRiskAssesemntTool.pdf

Casais, P., Sanchez Luceros, A., Meschengieser, S., Fondevila, C., Santarelli, M., Lazzari, M., 2000. Bleeding risk factors in chronic oral anticoagulation with acenocoumarol. Am J Hematol 63:192–196.

Copland, M., Walker, I. D., Tait, R. C., 2001. Oral anticoagulation and bleeding complications in an elderly population with atrial fibrillation. Arch Intern Med 161(17):2125-2128.

Fihn, S. D., Callhan, C. M., Martin, D. C., McDonnell, M. B., Henikoff, J. G., White, R. H., 1996. The risk for and severity of bleeding complications in elderly patients treated with warfarin. Ann Intern Med 124:970-979.

Geisen, C., Watzka, M., Sittinger, K., Steffens, M., Daugela, L., Seifried, E., 2005. VKORC1 haplotypes and their impact on the inter individual and inter-ethnical variability of oral anticoagulation. Thrombosis and haemostasis 94:773-779.

Hall, I. P., Pirmohamed, P., 2006. Pharmacogenetics. 1st ed, Taylor & Francis Group, New York.

Hermans, J. J., Thijssen, H. H., 1993. Human liver microsomal metabolism of the enantiomers of warfarin and acenocoumarol: P450 isozyme diversity determines the differences in their pharmacokinetics. Br J Pharmacot 110:482-490.

Kirchheiner, J., Brockmöller, J., 2005. Clinical consequences of cytochrome P4502C9 polymorphisms. Clin Pharmacol Ther 77:1-16.

Montes, R., Ruiz de Gaona, E., Martinez-Gonzales, M. A., Alberca, I., Hermida, J., 2006. The c.-1639G>A polymorphism of the VKORC1 gene is a major determinant of the response to acenocoumarol in anticoagulated patients. Br J Haematol 133:183-187.

Palareti, G., Leali, N., Coccheri, S., Poggi, M., Manotti, C., D'Angelo, A., *et al*, on behalf of the Italian Study on Complications of Oral Anticoagulant Therapy, 1996. Bleeding complications of oral anticoagulant treatment: an inception-cohort, prospective collaborative study (ISCOAT). Lancet 348:423–428.

Pindur, G., Mörsdorf, S., Schenk, J., Krischek, B., Heinrich, W., Wenzel, E., 1999. The overdosed patient and bleedings with oral anticoagulation. Semin Thromb Hemost 25:85-88.

Siguret, V., Esquirol, C., Debray, M., Gouin, I., Andreux, J. P., Pautas, E., 2003 Surdosage en antivitamine K dans une population de patients hospitalisés âgés de plus de 70ans. Presse Med 32:972-977.

Steffensen, F. H., Kristensen, K., Ejlersen, E., Dahlerup, J. F., Sorensen, H. T., 1997. Major bleeding complications during oral anticoagulant therapy in a Danish population-based cohort. J Intern Med 242:497–503.

Tassies, D., Freire, C., Pijoan, J., Maragall, S., Monteagudo, J., Ordinas, A., Reverter, J. C., 2002. Pharmacogenetics of acenocumarol: cytochrome P450 CYP2C9 polymorphisms influence dose requirements and stability of anticoagulation. Haematologica 87:185-191.

Van de Meer, F. J. M., Rosendaal, F. R., Vandenbroucke, J. P., Briët, E., 1993. Bleeding complications in oral anticoagulant therapy. Arch Intern Med 153:1557-1562.

Van der Meer, F. J. M., Rosendaal, F. R., Vandenbroucke, J. P., Briët, E., 1996. Assessment of a bleeding risk index in two cohorts of patients treated with oral anticoagulants. Thromb Haemost 76:12–16.

Verstuyft, C., Morin, S., Robert, A., Loriot, M. A., Beaune, P., Jaillon, P., Becquemont, L., 2001. Early acenocoumarol overanticoagulation among cytochrome P4502C9 poor metabolizers. Pharmacogenetics 11:735-737.

Visser, L. E., van Vliet, M., van Schaik, R. H., Kasbergen, A. A., De Smet, P. A., Vulto, A. G., *et al*, 2004. The risk of overanticoagulation in patients with cytochrome P450 CYP2C9*2 or CYP2C9*3 alleles on acenocoumarol or phenprocoumon. Pharmacogenetics 14:27-33.

Visser, L. E., van Schaik, R. H., van Vliet, M., Trienekens, P. H., De Smet, P. A., Vulto, A. G., *et al*, 2004. The risk of bleeding complications in patients with cytochrome P450 CYP2C9*2 or CYP2C9*3 alleles on acenocoumarol or phenprocoumon. Thrombosis and Haemostasis 92:61-66.

Wells, P., Scarvelis, D., 2006. Diagnosis and treatment of deep-vein thrombosis. CMAJ 175(9):1087–1092.

Wen, M. S., Lee, M., Chen, J. J., Chuang, H. P., Lu, L. S., Chen, C. H., *et al*, 2008. Prospective study of warfarin dosage requirements based on CYP2C9 and VKORC1 genotypes. Clin Pharmacol Ther 84(1):83-89.

Wysowski, D., Nourjah, P., Swartz, L., 2007. Bleeding complications with warfarin use. Arch Intern Med 167:1414–1419.

Authors

•Florentina C. Militaru, Department of Clinical Pharmacology,"Iuliu Haţieganu" University of Medicine and Pharmacy, 6th Pasteur Street, 400349, Cluj-Napoca, Cluj, Romania, EU, email: claudiamilitaru@yahoo.com

•Sorin Crişan, Department of Internal Medicine, 5th Medical Clinic, Municipal Hospital,"Iuliu Haţieganu" University of Medicine and Pharmacy, 11th Tăbăcarilor Street, 400139, Cluj-Napoca, Cluj, Romania, EU, email: crisan.sorin@gmail.com

•Ştefan C. Vesa, "Iuliu Haţieganu" University of Medicine and Pharmacy, 43rd Victor Babeş Street, 400012, Cluj-Napoca, Cluj, Romania, EU, email:stefanvesa@gmail.com

•Adrian Trifa, Department of Medical Genetics,"Iuliu Haţieganu" University of Medicine and Pharmacy, 6th Pasteur Street, 400349, Cluj-Napoca, Cluj, Romania, EU, email: adi_trifa@yahoo.co.uk

•Valentin Militaru, Department of Internal Medicine, 5th Medical Clinic, Municipal Hospital,"Iuliu Haţieganu" University of Medicine and Pharmacy, 11th Tăbăcarilor Street, 400139, Cluj-Napoca, Cluj, Romania, EU, email: Valentin.Militaru@umfcluj.ro

•Anca D. Buzoianu, Department of Clinical Pharmacology,"Iuliu Haţieganu" University of Medicine and Pharmacy, 6th Pasteur Street, 400349, Cluj-Napoca, Cluj, Romania, EU, email: abuzoianu@umfcluj.ro