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Association of Cyp2c9 Gene Polymorphism with Bleeding as a Complication of Warfarin Therapy

Marina Samardžija[1], Elizabeta Topić[2], Mario Štefanović[2], Lada Zibar[3], Goran Samardžija[3], Sanja Balen[4], Aleksandar Včev[3], Dragoslav Domanović[5], Jure Mirat[6] and Jerko Barbić[3]

[1] Institute for Transfusion Medicine, University Hospital »Osijek«, Osijek, Croatia
[2] Clinical Institute of Chemistry, School of Medicine, University of Zagreb, University Hospital »Sestre milosrdnice«, Zagreb, Croatia
[3] Internal Medicine Clinic, University Hospital »Osijek«, Osijek, Croatia
[4] Department of Transfusion Medicine, University Hospital Centre »Rijeka«, Rijeka, Croatia
[5] Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia
[6] Cardiology Department, General Hospital »Sveti Duh«, Zagreb, Croatia

A B S T R A C T

*The aim of this study was to determine the association of bleeding as a complication of warfarin therapy with polymorphism of CYP2C9 gene (alleles 1, 2 and 3). The CYP2C9 is the main enzyme for warfarin metabolism. Study included 181 patients receiving warfarin for at least one month. Allele 1 of CYP2C9 gene (in 94.5%) and genotype *1/*1 (57.5%) prevailed. Allele 3 was found in 12.7% patients. Bleeding side-effects occurred in 18 patients (10%). Patients with allele *1 needed significantly higher maintenance warfarin dose (p=0.011). Those with allele *3 had significantly lower maintenance warfarin dose (p=0.005) and higher prothrombin time (PT) at induction (p=0.034). Bleeding occurred significantly more often in those with lower maintenance warfarin dose (p=0.017). Patients with allele *3 had increased risk of bleeding, with marginal significance (p=0.05). Polymorphism of CYP2C9 could determine dose of warfarin therapy and thus it could be related to the risk of bleeding complications. Allele *3 carriers need lower warfarin dose. Therefore, initially reduced warfarin induction dose in allele *3 carriers could avoid more prolonged PT and decrease the risk of bleeding complication.*

Key words: *gene polymorphism, CYP2C9, warfarin, bleeding*

Introduction

The pharmacogenetics represents a molecular ground for drug metabolism on which depend pharmacokinetics and pharmacodinamics of a certain drug[1–3].

The main task of pharmacogenetics is to identify individual genetic variations (gene polymorphism) of several genes which are involved either in drug metabolism or in drug action through drug related molecules (like enzymes, receptors and drug transporters). These genetic variations may cause metabolic differences after administration of the standard dose of a drug and this can lead to more expressed toxicity or treatment failure due to an altered relation between the dose and the concentration of the pharmacologically active substance[4].

A gene is considered polymorphic if its variation was present in more than 1% of normal population. Genes are functionally polymorphic if allelic variants, one or more, are stable in population, and alter the activity of the coded protein in comparison with the wild (normal) type[3,5–7].

In the case when pharmacological activity of a drug or a toxin is associated with catalytic activity of a certain enzyme, factors influencing the activity of the enzyme will determine clinical response to the agent. Enzymes responsible for drugs and activation of other agents and metabolism express wide interindividual variations in proteins expression or catalytic activity, thus resulting in

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various fenotypes. Phenotypes are divided into those of extensive, intermediate, poor and ultraextensive metabolism[1,2,8].

The most important oxidative enzymatic system implicated in drugs metabolism is enzymatic superfamily of cytochrome P450 (CYP). CYP2C9 is one of the four members of human CYP2C subfamily (the others are CYP2C8, CYP2C18, CYP2C19 and CYP2C10 is now considered a variant of CYP2C9), although genetic analyses suggest possible existence of three more CYP2C enzymes. Cytochromes P450 2C8, 2C9, 2C18 and 2C19 show 82% identical aminoacid sequences, but isoforms show minor overlap in substrate specificity. Human gene that codes protein CYP2C9 is located at chromosome 10q24.2 and is greater than 55 kb.

Discovery of 6 different CYP2C9 cDNA sequences encouraged many studies of their metabolic activity and distribution in the population.

The most frequent allele Arg144/Ile359 (CYP2C9*1) is considered the wild type. Exchange of cytosine with thymine (C>T) on nucleotide 430 generates Cys144/Ile359 (CYP2C9*2) allelic variant, and exchange of adenine with cytosine (A>C) on nucleotide 1075 results in Arg144/Leu359 (CYP2C9*3) allelic variant. T>C on 1076 codes Arg144/Thr359 (CYP2C9*4) allele, C>G on 1080 codes Arg144/Asp360 (CYP2C9*5) allele, and the last discovered null polymorphism (CYP2C9*6) has adenine deletion on nucleotide 818. Since CYP2C9*1, *2 and *3 were the first discovered alleles, their *in vitro* and *in vivo* activity has been studied the most thoroughly. These alleles make 6 different genotypes: *1/*1 (wild type), *1/*2, *1/*3, *2/*2, *2/*3 and *3/*3. *In vitro* studies showed that CYP2C9*2 and *3 alleles were associated with significantly reduced metabolism of various CYP2C9 substrates in comparison with the wild type. *In vivo* studies report that if there is even only a single allelic variant present, metabolic activity is significantly reduced. Cytochrome P450 CYP2C9 metabolizes clinically important drugs and belongs to the most important enzymes for drugs metabolism in humans. Substrates for CYP2C9 are fluoxetine, losartan, phenitoin, tolbutamid, torsemid, S-warfarin and non-steroidal anti-inflammatory drugs.

The most used oral anticoagulants are derivatives of hydroxycumarines and indandion.

Warfarin (Marivarin, Krka, Slovenia), etilbiscumacetate (Pelentan, Krka, Slovenia), acenocumarol (Sintrom, Novartis Pharma, Switzerland), phenprocumon (Marcoumar, Roche, Switzerland) belong to derivatives of hydroxycumarine. Warfarin is the drug of choice. Two optic isomers of warfarin are in clinical use. Left rotated (S) warfarin is four times more potent than right rotated (R) warfarin. Warfarin half-life (t½) is 36–42 h, it is bound to plasma proteins in circulation, mostly to albumin, and it accumulates in liver microsomes. It is predominantly excreted by kidneys in the form of metabolites. The beginning of action of oral anticoagulants depends on t½ of coagulation factors: the shortest, 4–6 h, for F VII, 3 days for F II, 18–30 h for F IX and 2 days for F X. Therefore, according to some other data, complete anticoagulant action to be achieved needs 48–72 hours, even 5–7 days[9–12]. Warfarin is indicated for treatment and prevention of thromboembolic events. It is used for treatment of venous thrombosis, pulmonary emboli and arterial thrombosis.

Prophylactically it is used for prevention of thrombus formation in patients with artificial heart valves, with intravascular stents, atrial fibrillation, inborn heart valve errors, patients with history of thromboembolic event, those with increased risk of thromboembolic event due to immobilization or surgery, patients with known hypercoagulability (antiphospholipid syndrome, protein C or S deficiency, antithrombin deficiency, factor V Leiden mutation, factor II mutation). There are many contraindications, mostly relative. Therefore, need for anticoagulation therapy and its risk should be considered for every patient.

The most frequent side effect of warfarin therapy is bleeding due to overdosing, interaction with other drugs or certain condition in a patient. Diseases that increase the risk of bleeding are gastrointestinal bleeding, cerebrovascular diseases, inborn heart errors, hypertension, atrial fibrillation, myocardial infarction, renal diseases, liver dysfunction and anemia[13–15]. Peptic ulcer is surprisingly not related to bleeding during warfarin therapy[15]. Nutritional changes (reduced vitamin K intake) and drugs interfering with warfarin metabolism themselves present strong risk for bleeding. Two warfarin isomers are degraded in microsomal liver enzymes by two different pathways. (S)-warfarin is metabolized more rapidly, but significant individual variations are possible since enzymatic activity is influenced by genetic variations for microsomal enzymes (CYP polymorphism) and by external factors. The aim of this study was to determine the association between bleeding as a complication of warfarin therapy and polymorphism of CYP2C9 gene. We hypothesized that genetic variants for CYP2C9 different from the wild type carry increased risk for weak warfarin metabolism and consecutive bleeding complications.

Patients and Methods

The study was approved by the Ethical Committee of University Hospital Osijek.

The inclusion criterion was warfarin therapy for at least one month. The exclusion criteria were the following: treatment with the drugs known to induce or inhibit elimination of the CYP2C9 substrate (barbiturates, ethanol, rifampicin, amiodaron, sulphonamides, fluconasol), mechanical heart valves, repeated myocardial infarctions, antiphospholipid syndrome, history of bleeding preceding warfarin treatment, liver dysfunction.

The study included 181 patients (102 women and 79 men, median age 65 years) who were treated with warfarin for at least a month at the Institute for Transfusion Medicine University Hospital Osijek. All patients were Caucasions and from Eastern Slavonia. The participants signed informed consent.

The methods of the study were the data collection from medical records and CYP2C9 genotyping.

The data regarding the patients' history, warfarin therapy and bleeding complications were taken from the medical records, The historical data included demographic characteristics, underlying illness, check-up prothrombin time (PT) values, warfarin dose and bleeding occurrence.

The genotyping of CYP2C9 gene was done at the Clinical Institute for Chemistry of the University Hospital »Sestre milosrdnice« Zagreb.

Follow-up of anticoagulant therapy was performed by single-step PT test – Dade Innovin Behring. PT was measured every 72 hours following the introduction of the drug, and twice a week for two weeks thereafter (frequency of testing depended on PT stability) and finally once a month in patients with stable PT. The therapeutic range of 2.0–3.0 INR was considered optimal.

Cytochrome P450 CYP2C9 genotyping (alleles *1, *2 and *3) was performed by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism). Genotypes of CYP2C9 *1/*1, *1/*2, *1/*3, *2/*2, *2/*3 and *3/*3 were identified by determination of polymorphism of length of restriction fragments after digestion of PCR--products by restriction endonucleases (AvaII, NsiI, KpnI).

The data were analyzed using standard statistical methods for descriptive statistics, comparisons and risk determination. Nonparametric tests were applied due to small number of patients in certain subgroups. Statistical analysis was performed by SPSS 10.0 for Windows statistical program package (J.Scientific, San Rafael, CA, USA).

Results

Patients' demographic data, underlying illness and details concerning warfarin therapy are shown in the table 1. Warfarin dose and PT at induction, maintenance dose and optimal PT, time needed to reach optimal PT and duration of warfarin therapy were recorded. Gender was found the confounding factor in respect of warfarin dose. Men needed significantly higher maintenance warfarin dose than women (median 4.5 mg, min. 1.1, max. 9.0 vs 3.5 mg, min. 0.3, max. 9.0; z=–2.571, p=0.010, Mann-Whitney test). The other warfarin therapy parameters were not affected by the gender. Patients older than 65 years were not different in warfarin therapy parameters in comparison with those younger. Smoking or alcohol consumption did not influence the examined warfarin therapy characteristics.

The patients were divided into two subgroups in respect of bleeding complications of the warfarin therapy. One hundred and sixty three of the 181 patients did not have bleeding events (90%), while it occurred in 18 patients (10%). In 14 patients minor bleeding was recorded (1 minor hemoptysis or bloody tinged sputum, 6 epistaxis, 6 hematomas and 1 microhematuria), while 4 patients experienced major bleeding (2 melenas and 2 macrohematurias). Minor bleeding was treated by stopping warfarin only (4 patients), or stopping warfarin was followed by vitamin K administration (10 patients). All patients with major bleeding were hospitalized for median 5 days (min. 3, max. 7) and treated with 20 mg of vitamin K and with fresh frozen plasma (690 mL in average), or with erythrocyte transfusion (1200 mL in average) in the cases of hemoglobin drop of 20 g/L or more. Table 2 presents distribution of CYP2C9 polymorphisms. Genotype *1/*1 prevailed (57.5%). There was only one patient homozygous for *3 (*3/*3). Allele *1 was found in 171 patient, 58 patients had allele *2 and 23 allele *3. (Table 3).

The two groups of patients formed according to the bleeding complication of the therapy were compared. In those older than 65 years of age bleeding was not more frequent. Male gender and smoking did not carry increased risk for bleeding, unlike alcohol consumption (OR 4.371, CI 1.213–15.757). In 14 patients who were alcohol consumers bleeding occurred significantly more often. That finding rendered alcohol consumption the confounding variable. (Table 4) The subgroups of patients divi-

TABLE 1
PATIENTS' CHARACTERISTICS AND WARFARIN THERAPY PARAMETERS (N=181)

Characteristic		value
Gender (male) (n, %)		79 (43.6)
Age (years)		65 (11–88)*
Age older than 65 years (n, %)		82 (45.3)
Diagnosis (n, %)	Deep venous thrombosis	58 (32)
	Atrial fibrillation	33 (18)
	Polytrauma	21 (12)
	Stroke	20 (11)
	Mitral valve stenosis	17 (9)
	Other	32 (18)
Smoking (n, %)		24 (13)
Alcohol consumption (n, %)		14 (8)
Bleeding complication (n, %)		18 (10)
Warfarin dose at induction (mg)		3 (1.5–7.5)*
PT† at induction (INR)§		1.51 (0.97–8.02)*
Maintenance warfarin dose (mg)		4.1±1.8‡
PT† at maintenance warfarin dose – optimal (INR)§		1.94±0.28‡
Time needed to reach optimal PT† (days)		30 (1–450)*
Time of warfarin treatment (months)		5 (1–64)*

*median (minimum-maximum); † prothrombin time; ‡ mean ± SD; §international normalized ratio

TABLE 2
DISTRIBUTION OF CYP2C9 GENOTYPES (N=181)

Genotype	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3
n (%)	104 (57.5)	49 (27.1)	18 (9.9)	5 (2.8)	4 (2.2)	1 (0.6)

TABLE 3
PATIENTS WITH CYP2C9 ALLELE VARIANTS (N=181)

Allele variant	n (%)
*1	171 (94.5) (n=171, homozygous 104, heterozygous 67)
*2	58 (32) (n=58, homozygous 5, heterozygous 53)
*3	23 (12.7) (n=23, homozygous 1, heterozygous 22)

TABLE 4
RISK FOR BLEEDING COMPLICATIONS IN RESPECT OF POTENTIAL CONFOUNDING FACTORS (N=181)

	OR*	CI†
Male gender (n=79)	1.622	0.581–4.532
Older than 65 years (n=82)	2.036	0.751–5.518
Smoking (n=24)	2.043	0.621–6.821
Alcohol consumption (n=14)	4.371	1.213–15.757

* odds ratio; † confidence interval

TABLE 5
WARFARIN THERAPY IN RESPECT OF BLEEDING COMPLICATIONS (N=181)

	Bleeding*		Statistics†	
	Yes (n=18)	No (n=163)	z	p
Warfarin dose at induction (mg)	3 (1.5–6.0)	3 (1.5–7.5)	–0.022	0.983
PT‡ at induction (INR)§	3.64 (1–6.2)	1.42 (1–8.02)	–3.611	<0.001
Maintenance warfarin dose (mg)	2.13 (0.3–7.5)	4.5 (0.6–9.0)	–2.376	0.017
PT‡ at maintenance warfarin dose – optimal (INR)§	1.9 (1.5–2.5)	1.9 (1.3–2.8)	–0.333	0.739
Time needed to reach optimal PT‡ (days)	45 (10–360)	30 (1–450)	–0.378	0.706
Time of warfarin treatment (months)	4.5 (1–26)	5 (1–64)	–1.033	0.301

* median (minimum-maximum); † Mann-Whitney test; ‡ prothrombin time; §international normalized ratio

ded according to the underlying diseases were not significantly different in respect of bleeding complication.

Warfarin dose at induction of the therapy did not differ between those with and those without bleeding complication. However, the group with bleeding had significantly higher PT at the therapy induction (median INR 3.64, min. 1, max. 6.2 vs median INR 1.42, min. 1, max. 8.02; z=–3.611, p<0.001, Mann-Whitney test). Nevertheless, those with bleeding complication needed significantly lower maintenance dose (median 2.13 mg, min. 0.3, max. 7.5 vs median 4.5 mg, min. 0.6, max. 9.0; z=–2.376, p=0.017, Mann-Whitney test). Optimal PT at maintenance dose did not differ between the groups. The groups did not differ in the time needed to reach the optimal PT and in the duration of the warfarin therapy.(Table 5) The lowest recorded optimal warfarin dose was 0.3 mg, and that was the case of the patient with major bleeding that occurred in the period of induction. The highest optimal warfarin dose was 9.0 mg in two patients with no bleeding event. Significantly different frequency bleeding events (Pearsonχ^2=46.940, p<0.001) was found between the three subgroups according to PT at induc-

TABLE 6
WARFARIN THERAPY IN RESPECT OF CYP2C9 *1 ALLELE VARIANT (N=181)

	Allele *1*		Statistics†	
	Yes (n=171) ‡	No (n=10)	z	p
Warfarin dose at induction (mg)	3 (1.5–7.5)	3 (3–5)	–0.021	0.983
PT§ at induction (INR)¶	1.44 (0.97–8.02)	2.21 (1.02–3.5)	–1.432	0.152
Maintenance warfarin dose (mg)	4.5 (0.3–9)	2.5 (0.6–5.0)	–2.559	0.011
PT§ at maintenance warfarin dose – optimal (INR)¶	1.9 (1.3–2.8)	1.96 (1.6–2.38)	–0.436	0.663
Time needed to reach optimal PT§ (days)	30 (1–450)	60 (3–330)	–0.689	0.491
Time of warfarin treatment (months)	5 (1–64)	10.5 (2–44)	–1.734	0.083

*median (minimum-maximum); † Mann-Whitney test; ‡homozygous 104, heterozygous 67; § prothrombin time; ¶ international normalized ratio

tion of warfarin therapy (INR up to 3.5, between 3.6 and 5.0 and above 5.0). The difference was significant between patients with INR at induction below 3.6 and intermediate INR subgroup (Chi-square=10.242, p=0.017), and between the patients with INR below 3.6 and those above 5.0 (χ^2=46.301, p<0.001). Bleeding occurred in 9 of 163 patients with INR at induction lower than 3.6, in 3 of 9 with intermediate PT and in 6 of 8 patients with PT above 5.0 INR during the induction period. Four patients with PT at induction above 5.0 INR and bleeding complication were homozygous for wild type (*1/*1).

The details concerning warfarin therapy were compared in respect of possession of the certain CYP2C9 allele variant. Warfarin dose and PT at induction were not different between the patients with and without allele *1. However, those with allele *1 needed significantly higher maintenance dose in comparison with the patients without the wild type allele (median 4.5 mg, min. 0.3, max. 9.0 vs median 2.5, min. 0.6, max. 5; z=–2.559, p=0.011, Mann-Whitney test). They did not differ in optimal PT, in the time needed to reach it, and in the duration of warfarin therapy. (Table 6) The patients with allele *2 did not differ from those without allele *2 in the examined details concerning warfarin therapy. (Table 7) Subgroup of patients with allele *3 did not differ in induction warfarin dose from those without allele *3, but the therapy

TABLE 7
WARFARIN THERAPY IN RESPECT OF CYP2C9 *2 ALLELE VARIANT (N=181)

	Allele *2*		Statistics†	
	Yes (n=58) ‡	No (n=123)	z	p
Warfarin dose at induction (mg)	3 (2–6)	3 (1.5–7.5)	–0.147	0.883
PT§ at induction (INR) ¶	1.44 (0.97–4.6)	1.52 (0.97–8.02)	–0.290	0.771
Maintenance warfarin dose (mg)	4 (0.6–7.5)	4.5 (0.3–9)	–1.059	0.289
PT§ at maintenance warfarin dose – optimal (INR) ¶	1.9 (1.5–2.8)	1.9 (1.3–2.7)	–0.490	0.624
Time needed to reach optimal PT§ (days)	30 (1–360)	40 (1–450)	–0.857	0.391
Time of warfarin treatment (months)	5.5 (1–49)	5 (1–64)	–0.139	0.889

* median (minimum-maximum); † Mann-Whitney test; ‡homozygous 5, heterozygous 53; §prothrombin time; ¶ international normalized ratio

TABLE 8
WARFARIN THERAPY IN RESPECT OF CYP2C9 *3 ALLELE VARIANT (N=181)

	Allele *3*		Statistics†	
	Yes (n=23)‡	No (n=158)	z	p
Warfarin dose at induction (mg)	3 (2–6)	3 (1.5–7.5)	–1.165	0.244
PT§ at induction (INR) ¶	2 (1.02–7.3)	1.44 (0.97–8.02)	–2.116	0.034
Maintenance warfarin dose (mg)	3 (0.3–6.5)	4.5 (0.6–9)	–2.790	0.005
PT§ at maintenance warfarin dose – optimal (INR) ¶	1.83 (1.5–2.5)	1.9 (1.3–2.8)	–0.607	0.544
Time needed to reach optimal PT§ (days)	30 (1–450)	30 (3–360)	–1.265	0.206
Time of warfarin treatment (months)	8 (1–64)	5 (1–49)	–0.379	0.705

* median (minimum-maximum); † Mann-Whitney test; ‡homozygous 1, heterozygous 22; §prothrombin time; ¶ international normalized ratio

TABLE 9
WARFARIN THERAPY IN RESPECT OF CYP2C9 GENOTYPE (N=181)

	CYP2C9 genotype‡					
	*1/*1§ (n=104)	*1/*2§ (n=49)	*1/*3§ (n=18)	*2/*2§ (n=5)	*2/*3§ (n=4)	*3/*3 (n=1)
Warfarin dose at induction (mg)	3 (1.5–7.5)	3 (2–6)	3 (2–6)	3 (3–4.5)	3 (3–5)	4
PT* at induction (INR) †	1.48 (0.97–8.02)	1.41 (0.97–4.6)	1.75 (1.06–7.3)	2.21 (1.02–2.9)	1.7 (1.03–3.5)	2.8
Maintenance warfarin dose (mg)†	4.5 (1.1–9)	4.5 (0.75–7.5)	3 (0.3–6.5)	2.5 (1.5–4.5)	3 (0.6–5)	1.5
PT* at maintenance warfarin dose – optimal (INR)†	1.9 (1.3–2.7)	1.9 (1.5–2.8)	1.9 (1.5–2.5)	1.74 (1.6–1.91)	2.26 (2–2.38)	2.8
Time needed to reach optimal PT* (days)	45 (1–450)	30 (1–360)	27 (4–360)	45 (3–330)	60 (30–180)	60
Time of warfarin treatment (months)	5 (1–40)	5 (1–49)	11 (1–64)	3.5 (2–13)	16 (7–44)	8

*prothrombin time; †international normalized ratio; ‡χ^2=12.854, p=0.025, Kruskal-Wallis H test; post hoc *1/*1 vs *1/*3 z=–2.309, p=0.021, Mann-Whitney test; §median (minimum-maximum)

produced significantly more prolonged PT at induction in those having the allele in question (median INR 2, min. 1.02, max. 7.3 vs median 1.44, min. 0.97, max. 8.02; z=–2.116, p=0.034, Mann-Whitney test). Patients with allele *3 also needed lower warfarin maintenance dose than the patients without the allele (median 3 mg, min. 0.3, max. 6.5 vs median 4.5 mg, min. 0.6, max. 9; z= –2.790, p=0.005). The patients with allele *3 did not differ in optimal PT, time needed to reach it and in the duration of the warfarin therapy from those without that allele. (Table 8)

Subgroups of patients according to the CYP2C9 genotype were compared in respect of warfarin therapy. The 6 subgroups did not differ in warfarin dose at induction and in the PT reached by that initial therapy. However, they did differ significantly in the warfarin dose needed to maintain optimal PT (χ^2 =12.854, p=0.025, Kruskal-Wallis test). Genotype *3/*3 was excused from *post hoc* analysis, while there was only one patient in the subgroup. *Post hoc* Mann-Whitney test found the significant difference between genotypes *1/*1 and *1/*3 (4.5 mg, min. 1.1, max. 9.0 vs 3 mg, min. 0.3, max. 6.5; z=–2.309, p=0.021). The 6 subgroups did not differ in the optimal PT, time needed to reach it and in the duration of warfarin therapy. (Table 9)

Table 10 shows the distribution of bleeding events in the subgroups formed according to the CYP2C9 genotype.

The study examined the risk for bleeding complication of warfarin therapy in the presence of certain CYP2C9 allele. Only marginal significance was found for the increased risk carrying by allele *3 (χ^2=4.511, p=0.05). (Table 11).

TABLE 10
BLEEDING COMPLICATION IN RESPECT OF CYP2C9 GENOTYPE (N=181)

Genotype	*1/*1 (n=104)	*1/*2 (n=49)	*1/*3 (n=18)	*2/*2 (n=5)	*2/*3 (n=4)	*3/*3 (n=1)
n (%)	12	1	4	0	1	0

TABLE 11
RISK FOR BLEEDING COMPLICATION IN RESPECT OF CYP2C9 ALLELE VARIANTS (N=181)

	OR*	CI†
*1 (n=171, homozygous 104, heterozygous 67)	0.994	0.119–8.326
*2 (n=58, homozygous 5, heterozygous 53)	0.239	0.053–1.076
*3‡ (n=23, homozygous 1, heterozygous 22)	3.098	0.989–9.705

*odds ratio; † confidence interval; ‡χ^2=4.511, p=0.05

The three subgroups of patients, as divided according to the bleeding occurrence, were not different considering the underlying diagnosis (Pearson χ^2= 25.581, p=0.375).

Discussion

Genetic variability in response to drugs between individuals is currently of a great scientific interest considering the important therapeutically consequences.

Enzymatic family P450 (CYP) is the most important system implicated in biotransformation of drugs and other xenobiotics in humans. Enzymes CYP2C9, CYP2C19, CYP2D6 and CYP3A4 are responsible for oxidative metabolism of more than 90% currently used drugs. Warfarin is the most frequently prescribed anticoagulant in Europe and North America[16,17,18]. CYP2C9 is the main enzyme responsible for warfarin metabolism. Beside the wild type of the allele (CYP2C9*1), there are two another allelic variants (CYP2C9*2 and *3) identified, which resulted from point mutations of CYP2C9 gene[19]. Higashi[20] reports that the alleles *2 and *3 cause 30% and 80% reduction in enzymatic activity, respectively, as compared with the wild type. According to Aithal[21] the allele *2 has 12% of the activity of the wild type, and the allele *3 less than 5% of the same. Meyer[3] reports data on 0.2–1.0% of Caucasians being CYP2C9 weak metabolizers (homozygous or heterozygous for alleles *2 and *3), and on 14.0–37.0% Caucasians heterozygotes for CYP2C9 *2 and *3, the feature responsible for reduced enzymatic activity. In comparison with the cited paper our patients (all Caucasions from Eastern Slavonia) were homozygous for allele *1 in quiet less proportion (57.5%), whereas so-called weak metabolizers were more frequent (42.5% homozygotes or heterozygotes for alleles *2 and *3). All pharmacogenetic studies point out that ethnic origin should be considered by investigating pharmacogenetic distinctions and pharmacotherapy. Identification of CYP2C9 genotype prior to warfarin therapy initiation is an example of benefit of the applied pharmacogenetics in routine daily practice.

Taube and al.[22], Scordo and al.[23,] Wittkovsky[24], Freeman[25], Haining[26], Takashi[27], Ingelman-Sundberg[7], Ogg[28], Aithal[21] and other authors report on genetic polymorphism of CYP2C9 resulting in variants *2 and *3, which have reduced catalytic activity in S-warfarin metabolism in comparison with the wild type.

Aithal and al.[21] studied polymorphism of CYP2C9 in regard with warfarin dose and risk for bleeding complication of the treatment in Great Britain in 36 patients receiving ≤1.5 mg of warfarin, 52 patients receiving standard dose and in 100 healthy controls. They report on the lack of significant difference in CYP2C9 genotype distribution between healthy controls and patients on standard dose.

While in patients with *1/*2 genotype optimal warfarin dose was by 20% reduced in comparison with the wild type carriers needs, CYP2C9*3 mutation, even in heterozygotes, showed an excellent relation of *in vitro* and *in vivo* results in the sense of reduced warfarin metabolism and reduced optimal dose needed. Wild type allele *1 carriers in our study needed higher warfarin dose to maintain optimal PT, whereas carriers of allele *3 required lower maintenance dose. Standard induction dose in allele *3 carriers led to more prolonged PT. These findings are in concordance with presumably reduced warfarin metabolism with CYP2C9 allele variants other than wild type.

Genetic polymorphism of CYP2C9, allele *3 and allele *2, is associated with an increased risk of an extreme anticoagulant response by the time of the induction of the therapy and bleeding complication[3,21,27,29,30].

Landefeld and Beyth reported on annual incidence of bleeding based on the data gathered from literature: fatal 0.6%, severe 3.0% and weak 9.6%. They cite the four independent risk factors for severe bleeding: age over 65, history of stroke and gastrointestinal bleeding, and 1 or more concomitant diseases beside the main disease[14].

Age over 65 was not associated with the risk of bleeding in our patients, even though they were in significant proportion over 65 years of age (45,3%). History of stroke did not increase the risk for bleeding in our patients. However, we found increased risk for bleeding in alcohol consumers.

Aithal[21] reports on the association of bleeding incidence with warfarin therapy with the intensity of anticoagulant therapy and prothrombin time deviation. In our patients bleeding occurred more often in those with more prolonged PT at induction and in patients requiring lower maintenance dose. Thus, our finding confirms the association found in the cited report. The lowest maintenance warfarin dose in our study was 0.30 mg, and it was in the case of a patient who experienced major bleeding during the induction of the therapy. The risk of bleeding is higher at the beginning of the treatment. Several studies showed that the relative risk for bleeding rose with the duration of the drug therapy, while the other authors reported on a higher risk for bleeding during the first 90 days after the therapy was stabilized once[9]. When divided in respect of bleeding complication, our patients did not differ in the duration of warfarin therapy. They were not different concerning the time needed to achieve optimal PT. These findings suggest that warfarin dose and effect on PT have more important role for bleeding complications than the treatment time variables.

Ogg and al.[28] describe bleeding complications in two patients homozygous for allele *3 during introduction of warfarin therapy. In our study only one patient was homozygote for allele *3, with optimal daily warfarin dose of 1.5 mg, and did not experience bleeding complication. This could be explained by frequent and careful prothrombin time control and precise warfarin dose adjustment, but having only one patient certainly limits the conclusions.

The subgroups of patients divided according to INR at induction differed significantly regarding the bleeding complication of warfarin therapy (Pearson Chi-square = 46.940, $p<0.001$). Bleeding was more common in those with higher INR values. Palareti reports on higher risk of bleeding with INR above 4.5, and the exponential increment of that risk with INR above 5.0[14]. Our data suggest that INR above 3.5 significantly increase the likelihood for bleeding side-effect.

The patients divided according to the underlying illness did not differ significantly in the frequency of bleeding complications.

Our survey is the first study of CYP2C9 gene polymorphism in the population of Eastern Croatia. The ultimate goal of pharmacogenetic polymorphism investigations is to create pharmacogenetic identification card for each patient. CYP2C9 alleles 2* and 3* are known as weak metabolizers. Our findings support the presumable hypothesis. Identifying allele *3 carriers could enable reducing warfarin dose at induction, thus avoiding extreme PT prolongation with standard dose and decreasing the risk of bleeding complication. However, bleeding occurs in the wild type CYP2C9 carriers, too. Actually, if one excludes relative proportions most of the patients with bleeding carried CYP2C9*1/*1 genotype, which indicated the possibility of the existence of another CYP2C9 variants in the same patients that were not identified by the available laboratory methods.

Therefore, careful titration of warfarin dose and regular PT check-ups are priorities in warfarin therapy monitoring. CYP2C9 genotype seems to be not the only determinant of the clinical response to warfarin therapy and bleeding side-effects. According to our findings gender and alcohol consumption deserve some attention.

REFERENCES

1. TOPIĆ E, Laboratorijsko istraživanje bolesti jetre (Medicinski fakultet Sveučilišta u Zagrebu, Zagreb, 2001). — 2. ŠERMAN D, STAVLJENIĆ-RUKAVINA A, SERTIĆ J, BULIĆ-JAKUŠ F, Metode molekularne biologije u medicini (Medicinska naklada, Zagreb, 2001). — 3. MEYER UA, Lancet, 356 (2000) 1667. — 4. WOLF RC, SMITH G, SMITH RL, BMJ, 320 (2000) 987. — 5. MEYER UA, Annu Rev Pharmacol Toxicol, 37 (1997) 269. — 6. EVANS, WE, RELLING MV, Science, 286 (1999) 487. — 7. INGELMAN-SUNDBERG M, OSCARSON M, McLELLAN RA, Trends Pharmacol Sci, 20 (1999) 342. — 8. LINDER MW, PROUGH RA, VALDES RJR, Clin Chem, 43 (1997) 254. — 9. BARBARA MA, Blood Components and Pharmacologic Agents in the Treatment of Congenital and Acquired Bleeding Disorders (AABB Press, Bethesda, 2000). — 10. HIRSH J, DALEN JE, ANDERSON DR, POLLER L, BUSSEY H, ANSELL J, Chest, 114 (1998) 445. — 11. VRHOVAC B, Interna medicina (Naprijed, Zagreb, 1997). — 12. BULAT M, GEBER J, LACKOVIĆ Z, Medicinska farmakologija (Medicinska naklada, Zagreb, 1999). — 13. FONNE-PFISTER R, MEYER UA, Biochem Pharmacol, 37 (1988) 3829. — 14. VRHOVAC B, REINER Ž, Farmakoterapijski priručnik (Med-Ekon, Zagreb, 2000). — 15. HARRISON TR, Principles of Internal Medicine. (McGraw-Hill, 1994). — 16. IYER L, RATAIN MJ, Eur J Cancer, 34 (1998) 1493. — 17. WILLIAMS PA, OSME J, WARD A, ANGOVE HC, MATAK VINKOVIC D, Nature, 424 (2003) 464. — 18. GONZALES FJ, NEBERT DW, TIG, 6 (1990) 182. — 19. LEE CR, GOLDSTEIN JA, PIEPER JA, Pharmacogenetics, 12 (2002) 251. — 20. HIGASHI MK, VEENSTRA DL, KONDO LM, WITTKOVSKY AK, SRINOUANPRACHANH SL, FARIN FM, RETTIE AE, JAMA, 287 (2002) 1690. — 21. AITHAL GP, DAY CP, KESTEVEN PJL, DALY AK, Lancet, 353 (1999) 717. — 22. TAUBE J, HALSALL D, BAGLIN T, Blood, 96 (2000) 1816. — 23. SCORDO MG, PENGO V, SPINA E, DAHL ML, GUSELLA M, PADRINI R, Clin Pharmacol Ther, 72 (2002) 702. — 24. WITTKOWSKY AK, J Thromb Thrombolysis, 12 (2001) 67. — 25. FREEMAN BD, ZEHNBAUER BA, McGRATH S, BORECKI I, BUCHMAN TG, Surgery, 128 (2000) 281. — 26. HAINING RL, HUNTER AP, VERONESE ME, TRAGER WF, RETTIE AE, Arch Biochem Biophys, 333 (1996) 447. — 27. TAKAHASHI H, ECHIZEN H, Clin Pharmacokinet, 40 (2001) 587. — 28. OGGS MS, BRENNAN P, MEADE T, HUMPHRIES SE, Lancet, 354 (1999) 1124. — 29. WADELIUS M, SORLIN K, WALLRMAN O, KARLSSON J, YUE QY, MAGNUSSO PK, WADELIUS C, MELHUS H, Pharmacogenomics J, 4 (2004) 40. — 30. MARGAGLIONE M, COLAIZZO D, D'ANDREA G, BRANACACCIO V, CIAMPA A, GRANDONE E, Thromb Haemost, 84 (2000) 775.

M. Samardžija

Department of Transfusion medicine, University Hospital »Osijek«, Osijek, Croatia
e-mail: samrdzija.marina@kbo.h

POVEZANOST POLIMORFIZMA GENA ZA CYP2C9 I KRVARENJA NASTALOG KAO KOMPLIKACIJA TERAPIJE VARFARINOM

SAŽETAK

Cilj istraživanja bio je odrediti povezanost krvarenja kao komplikacije liječenja varfarinom i polimorfizma gena za CYP2C9 (aleli 1, 2 i 3). CYP2C9 je glavni enzim u metabolizmu varfarina. U istraživanje je uključen 181 bolesnik liječen varfarinom najmanje 1 mjesec. Nađena je najveća učestalost alela 1 gena za CYP2C9 (u 94,5%) i genotipa *1/*1 (57,5%). Alel 3 je nađen u 12,7% bolesnika. Krvarenje se pojavilo u 18 bolesnika (10%). Ispitanicima koji su imali alel *1 trebala značajno je veća doza održavanja varfarina (p=0,011). Nositelji alela *3 su trebali značajno manju dozu održavanja varfarina (p=0,005) a dulje protombinsko vrijeme (PT) uz indukcijsku terapiju (p=0,034). Krvarenje se javilo češće u osoba s manjom potrebnom dozom održavanja varfarina (p=0,017). Nositelji alela *3 imali su povišen rizik krvarenja, s graničnom statističkom značajnošću (p=0,05). Polimorfizam gena za CYP2C9 povezan je s potrebnom dozom varfarina i posljedičnim rizikom krvarenja kao komplikacije te terapije. Posjedovanje alela *3 zahtijeva manju dozu varfarina. Stoga bi se smanjenjem induktivne doze varfarina u nositelja alela *3 moglo izbjeći prekomjerno produljenje PT i smanjiti rizik komplikacija krvarenja.