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**Prevalence of genetic polymorphisms of *CYP2C9* and *VKORC1* - implications for warfarin management and outcome in Croatian patients with acute stroke**

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**Abstract**

**Background:** Data on the prevalence of *CYP2C9* and *VKORC1* genes and their influence on anticoagulant effect and warfarin dose in stroke patients are scarce. The aim of this study was to determine the occurrence and significance of these gene polymorphisms and to establish pharmacogenetic algorithm to estimate the dose of introduction. Also, the goal was to determine tailored safety and intensity of anticoagulation response depending on the allelic variants and their impact on the clinical outcome in acute stroke patients in Croatia.

**Methods**: A total of 106 consented acute stroke patients were tested for *CYP2C9**2,*3 and *VKORC1* 1173C>T gene polymorphisms. We estimated the dose of introduction and monitored anticoagulant effect obtained by INR values, time to reach stable dose, stable maintenance dose, time spent within the therapeutic/supratherapeutic INR range, occurrence of dosage side effects and clinical outcome depending on genotypes.

**Results:** We found that 83% of stroke patients in our study were carriers of multiple allelic variants. The predicted initial dose correlated with the stable warfarin maintenance dose (p=0.0311) and we correctly estimated the dose for 81.5% of 61.3% of study patients who required higher/lower doses than average. Warfarin dosage complications were slightly more frequent among the carriers of *CYP2C9**2,*3 compared to the carriers of *VKORC1* 1173 T alleles (68. 9% versus 62.5%), but their occurrence did not affect the final clinical outcome.

**Conclusion:** Our data indicated rapid and safe anticoagulation achieved by using pharmacogenetically-predicted warfarin dose in high-risk acute stroke patients without increasing the risk of warfarin dosage complications in elderly population.

***Key words:*** stroke, warfarin, genetic polymorphism, *CYP2C9, VKORC1*

## Introduction

Warfarin is the most widely prescribed anticoagulant owing to its effectiveness in primary and secondary prevention and treatment of thromboembolic events coupled with its low cost, in spite of the emergence of newer anticoagulants like dabigatran, rivaroxaban, apixaban (1,2). However, its use is associated with significant morbidity and mortality and makes it the second leading drug-related cause for emergency hospitalization. Most drug-related adverse events emerge during the early initiation of therapy which is a challenging and demanding process. Risks for hemorrhage or thromboembolism as the consequences of under or over-anticoagulation range from 16% to 25% and these events are more frequent during the initiation period than later (3,4,5). These early problems depend on multiple environmental and genetic factors and are related to warfarin narrow therapeutic INR (International Normalized Ratio) range and at least 20-fold interindividual variability in warfarin sensitivity (6).

Common polymorphisms of CYP2C9 and VKORC1 genes have a strong influence on interindividual warfarin sensitivity, variability of anticoagulant effect and dose requirement, therefore accounting for more than one-third of variances associated with a stable therapeutic dose (7,8). An association between the CYP2C9 gene polymorphisms, time to reach first therapeutic and supratherapeutic INR values and increased risk of bleeding during the initiation phase of anticoagulant therapy has been well documented. Two alleles, CYP2C9 *2(Arg144Cys) and CYP2C9*3 (Ile359Leu) out of > 30 CYP2C9 discovered alleles are considered strong risk factors for over-anticoagulation (9). Studies of single nucleotide polymorphisms (SNPs) of VKORC1 -1636G>A and 1173C>T have confirmed the significant influence of VKORC1 gene polymorphisms on warfarin sensitivity and dosing (10-12). CYP2C9 and VKORC1 polymorphisms may explain up to 54 % of warfarin dose variabilities (13-15). Previous studies have been mainly focused on establishing genetic-based warfarin-

dosing algorithms, while only a few studies have investigated the effectiveness of their clinical application (16-18).

Patients with cardioembolic stroke and the stroke consequent to the dissection of extracranial or intracranial arteries or cerebral sinus thrombosis require urgent anticoagulant therapy initiation for secondary stroke prevention. These patients are at a 5-7% risk of embolic stroke recurrence within the first week. At the same time they have an increased risk of bleeding into the infarct zone due to the nature of the ischemic brain damage or to thrombolytic therapy side effects, with subsequent deterioration of neurological deficits (19-23). Therefore, those patients are also at increased risk of warfarin dosage side effects, notably of warfarin-induced brain hemorrhage and it is essential for them to have their initial doses selected on the basis of individual genotype to prevent the additional risk of dosage related complications.

The goals of the current study were to determine the frequencies of SNPs in the CYP2C9 and VKORC1 genes in our group of acute stroke patients, to individualize therapy by using pharmacogenetic-based warfarin-dosing algorithm and to determine tailored security and intensity of anticoagulation response depending on the CYP2C9 and VKORC1 genotypes. We also gauged the impact of these polymorphisms on clinical outcome in studied patients.

## Patients and methods

### *Patients*

In the time period between October 2010 till April 2011, 307 patients were hospitalized with acute ischemic stroke in the Stroke Unit, Department of Neurology, UHC Zagreb. After obtaining ethical permission, the study was conducted in 106 consented patients with acute stroke who had an indication for urgent anticoagulation. Among them, 36 patients were previously anticoagulated with warfarin. Inclusion and exclusion criteria are listed in Table 1.

*Materials and methods*

Upon admission, blood samples (10 ml) were taken for INR measurement and genotyping. Low molecular weight heparin (LMH) had been administered during first five to seven days since admission, before the initial dose of warfarin was introduced. The initial dose of warfarin was calculated by using non-profit website published algorithm *http://www.WarfarinDosing.org.,* which included some demographic data*,* indications for warfarin therapy, concomitant medications (amiodarone, carmabazepine and phenytoin were included) and *CYP2C9* *2*3 and *VKORC1* 1173C>T genotypes (16,23). During hospitalization, the following data were recorded for each patient: gender, age, weight, height, indications for warfarin therapy, several INR values depending on the day of therapy introduction, predicted dose, dose of introduction, stable maintenance dose, dosage-dependent side effects (minor or major bleeding) and clinical outcome at the end of the study reported as mRS (modified Rankin Scale) (23, 24).

After the initial pharmacogenetically predicted warfarin dose had been assessed, we introduced the calculated "mini loading dose"(dose of introduction) of warfarin for first two days (Table 2) (25). Doses were subsequently adjusted depending on the measured INR values. The values of INR were recorded at admission, on the first day of warfarin therapy introduction, after 48 hours, 72 hours, on days 5, 7, 14 and on day 21 of warfarin therapy introduction when the study was terminated. Target INR value was >2, therapeutic range was 2-3 and supratherapeutic values were determined as INR >3.1. Stable maintenance dose was defined as the warfarin dose at which a patient was maintained within the therapeutic INR range and which did not change during three consecutive INR measurements for at least five days.

*Side effects*

Warfarin dosage side effects were defined as minor or major bleeding. Minor bleeding was defined as small subcutaneous hemorrhage, microhematuria or slight gingival or vaginal bleeding. Major hemorrhage was classified into two subgroups: a) bleedings that did not require interruption of therapy, such as mild hemorrhage into the infarct zone without worsening of neurological deficit, b) bleedings that required interruption of warfarin therapy, such as large intracerebral hematoma with deterioration of neurological deficit, extensive gastrointestinal or urogenital bleeding.

*Endpoints*

The primary endpoints to determine were the following:

- frequencies of *CYP2C9* *2,*3 and *VKORC1* C1173T alleles in acute stroke patients
- time to reach the first therapeutic INR
- time to reach stable maintenance dose, and stable maintenance dose according to genotype

The secondary endpoints to determine were as follows:

- time and the percentage of time spent in therapeutic and supratherapeutic INR range
- incidence of warfarin dosage side effects according to genotype
- association of the severity of neurological deficit at discharge reported as mRS with age
- association of final clinical outcome with the occurrence of warfarin dosage complications.

*Genotyping procedures*

DNA was isolated from whole blood by salting out method. In this study, three SNPs were genotyped by TaqMan® Drug Metabolism Genotyping Assays: *CYP2C9**2 (rs1799853) with assay ID C_25625805_10, *CYP2C9**3 (rs1057910) with assay ID C_27104892_10 and

*VKORC1* 1173C>T (*rs* 9934438) with assay ID C_30204875_10. The genotyping was performed by the Applied Biosystems 7500 Real Time PCR System according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). All assays were run in 96 well plates under the same instrument conditions: 2 min at 50°C, 10 min at 95°C, following 50 cycles of 15 sec at 92°C with 90 sec at 60°C extension time. For each SNP, the 25 μl PCR reaction mixture per well consisted of 1.25 μl TaqMan® Drug Metabolism Genotyping Assay Mix (specific for each polymorphism), 12.5 μl TaqMan® Universal PCR Master Mix and 5 -20 ng of genomic DNA diluted in 11.25 μl $dH_20$ (26).

*Statistical Analysis*

Quantitative variables are presented as an arithmetic mean (AM) and standard deviation (SD) when distribution was normal, otherwise as confidence interval (95%CI), median or Interquartile range (IQR).

Qualitative variables are presented as frequency and proportion (such as frequency of alleles or group of alleles, time and percentage of time spent in therapeutic INR values).

Student's t-test or Mann-Whitney U-test were used for comparison among the groups for quantitative variables (time required to achieve the target INR, time to achieve stable maintenance dose). $\chi 2$-test was used for comparison among the groups for qualitative variables. Analysis of variance (ANOVA) or Kruskal-Wallis test, depending on distribution, were used for comparing the groups for quantitative variables. The Spearman rank correlation was used to characterize associations among quantitative variables. Results were deemed statistically significant at the 5% level ($p<0.05$).

The analysis was carried out by using statistical software package STATISTICA ver. 6.0 (StatSoft Inc., Tulsa, OK, USA ).

## Results

*Demographic data and the prevalence of CYP2C9 and VKORC1 gene polymorphisms in stroke patients*

The study included 106 Caucasian patients from Croatia, CEU (56.6% female and 43.4% male), with the mean age of 67.6 years (65.83-70.27, 95%CI). Out of them, 14.15% were younger than 49 years, while 54.76% were older than 71 years.

The most common genotypes were *VKORC1* 1173CC (49.5%) and *CYP2C9* wt/wt (41.5%).Genotypes with reduced enzyme activity, *VKORC1* 1173TT and *CYP2C9* *2,*3 were present in 11.3% and 17.92% of patients respectively, as shown in Table 3.

*Time to reach the first target INR value, proportion of time within the therapeutic INR range and the time spent within supratherapeutic INR values depending on genotype*

Target INR value was ≥ 2, therapeutic INR range was 2-3 and supratherapeutic INR> 3.1. Patients achieved the first target INR ≥ 2 for an average of 4.16 days (4.12-4.66, 95%CI). Significant difference was observed by applying the method of linear interpolation between genotypes in the time needed to achieve the target INR ($p = 0.0257$), assuming that the wild type was the reference group (Table 3). Patients spent 16.09 days (15.69-16.48, 95% CI) within the therapeutic INR range or an average of 79.54% (74.75-78.51, 95%CI) of the time. The carriers of *VKORC1* 1173TT and at least one variant of *CYP2C9* allele (wt/*2,wt/*3,*2/*2,*2/*3,*3/*3) spent the longest proportion of time within the therapeutic INR range (84.19%). "Wild" homozygotes for both genes spent the shortest proportion of time (74.32%) within therapeutic INR range, but without statistically significant difference among genotype groups ($p = 0.370$). The carriers of *CYP2C9* (wt/*2, wt /*3,*2/*2) genotypes

spent the longest time with supratherapeutic INR values (> 3.1), i. e. a period of 7.68 days and they mostly achieved supratherapeutic INR values.

*The average estimated dose, dose of introduction, stable maintenance dose and average time of achieving the stable maintenance dose depending on genotype*

Average estimated dose (De) for all patients was 3.82 mg (3.63- 4.20, 95% CI) and the De differs significantly between genotypes (p=0.000). The calculated "mini loading dose" (dose of introduction, Di) of warfarin for each patient was introduced. The carriers of "wild" *CYP2C9* /*VKORC1* genotypes required the highest Di of 9 mg (8.38-9.62, 95% CI), while the carriers of *VKORC1* 1173 TT and *CYP2C9* (wt/*2,wt/*3,*2/*2,*2/*3,*3/*3) defective alleles required only 4.26 mg (2.70-5.82, 95%CI). The average stable daily maintenance dose (Dm) was up to 3.51 mg (3.44- 4.08, 95% CI) and significantly correlated with the combination of *CYP2C9* and *VKORC1* genotypes (p=0.000), as shown in Table 3. The predicted initial dose correlated with the stable warfarin maintenance dose (Dm) (p=0.0311).

The average time of achieving the Dm amounted to 10.00 (9.96-10.69, 95% CI) days and the stable Dm was most rapidly achieved (in 7 days) by the carriers of homozygous defective alleles for both genes (*CYP2C9* *2*2 or *2*3/ *VKORC1* 1173TT). Since 61.3% (N=65) of 106 study patients required higher (N=15) or lower (N=50) doses of warfarin than usual (<3mg or >6 mg), we correctly estimated the dose for 81.5% of those 65 patients.

*Warfarin dosage side effects and final neurological deficit (mRS) at the discharge depending on genotype*

Warfarin dosage complications were slightly more frequent among the carriers of *CYP2C9* *2*3 alleles (68.9%) when compared to the carriers of *VKORC1* 1173C>T alleles (62.5%). Severe dose dependent side effects were more common in the carriers of *VKORC1* 1173TT

genotype (62.5 %) and the carriers of two defective *CYP2C9* *2 or *3 alleles (37.5%), but without significant difference between genotypes (p=0.0698). Our data indicate no significant correlation between the occurrence of warfarin dosage side effects and the proportion of time spent within the therapeutic INR range (p = 0.5652), although the occurrence of major bleeding was higher in patients (9.59%) who spent more time outside the therapeutic INR range. The age of the patients did not affect the final neurological outcome expressed by mRS (p =0.1562), although all fatal outcomes were observed in patients older than 71 years (42%). The occurrence of side effects dependent on warfarin dosage did not have an impact on final clinical outcome (p=0.1198), as shown in Table 4. We also did not find association of final neurological outcome with the percentage of the time spent within the therapeutic INR range (p=0.5652) or with the time required to achieve a stable therapeutic dose.

**Discussion**

The introduction of warfarin into therapy is a challenging and tedious process that can lead to serious side effects like bleeding, especially among highly vulnerable patients, such as patients with acute ischemic stroke. In this study we analyzed the influence of *CYP2C9* and *VKORC1* 1173 C>T gene polymorphisms on the prediction of the initial dose of warfarin in Caucasian Croatian patients with acute stroke and their impact on the achievement of a fast and stable anticoagulant effect expressed by therapeutic INR range (2-3) and a stable therapeutic dose. Target INR values (≥ 2) were achieved at an average time of 4.16 days. Allelic variants that have contributed to the earlier achievement of target INR values were homozygous "defective" variants of *VKORC1* and *CYP2C9* genes; patients with these variants have attained the target INR already at 1.35 days. The longest time for the anticoagulant effect and achievement of the target INR values was needed by the carriers of "wild" *VKORCl* and *CYP2C9* homozygous alleles (5.05 days).

The shortest proportion of time spent within the therapeutic INR range with slower achievement of target INR was observed in the group of carriers of homozygous "wild" type alleles.

Explanation for this could be the observation that "wild" type *VKORC1* allele carriers are often also carriers of rare mutations and require significantly higher doses or can even develop resistance to warfarin (11). However, these mutations are not analyzed in routine clinical practice and we do not have these data. The longest proportion of time spent within the therapeutic INR range was observed for the group of patients carriers of defective alleles. Such patients require lower doses of warfarin and they are less likely carriers of *VKORC1* rare mutations, all of which could contribute to more accurate dosing (27,28).

Our data suggest the dominant effect of *CYP2C9* gene polymorphisms on early achievement of target INR and supratherapeutic INR> 3.1 values, which is consistent with some published data (12-15,29,30). However, opposite data were also obtained, pointing to the greater role of *VKORC* 11173TT in achieving the target INR, although *CYP2C9* *2 and *3 allele variants were responsible for the earlier achievement of supratherapeutic INR values (31,32).

Estimated initial doses and stable maintenance doses did not differ among the group of genotypes, indicating that our pharmacogenetically based warfarin-dosing algorithm could effectively predict the actual warfarin maintenance dose, providing clinicians with a reliable tool for managing successful anticoagulation (33-35). Homozygous carriers of *VKORC1/CYP2C9* defective alleles required the lowest daily maintenance dose of 1 mg, while the highest dose of 6 mg per day was needed by homozygous "wild" type carriers.

Relatively high proportion (61.3%) of our patients required doses that are higher or lower than the average, which is a significantly higher proportion compared to data from Anderson's study where 46% of patients required lower or higher doses than average (14). This difference could be explained by possible different frequencies of tested polymorphisms in general

population. Patients with embolic stroke, particularly cardioembolic stroke caused by atrial fibrillation as was the case in 77% of our respondents, have a 5-7% risk for embolic stroke recurrences within 7-12 days after the initial stroke, with immediate risk for intracerebral hemorrhage as the result of damage related to the nature of the disease (20,21). For this vulnerable group of patients (who are under- or over anticoagulated), it is crucial to achieve anticoagulant effect and stable therapeutic dose as soon as possible, while pharmacogenetic testing seems valuable and promising.

In this study, the average time of achieving stable maintenance dose was up to 10 days. An interesting finding is in one of our patients, the carrier of *VKORC1CT/ CYP2C9* wt/wt genotype, whose estimated initial dose was 4.5 mg according to pharmacogenetically based algorithm. He reached the target INR very slowly and only after 19 days achieved the stable therapeutic dose of 12 mg per day, which was more than twice the estimated value, although he did not receive any concomitant therapy. This might be explained in several ways: he is a very fast metabolizer for *CYP2C9* due to possibly different transcriptional regulation of *CYP2C9* (34); he is the carrier of rare *VKORC1* mutations predisposing resistance or has a molecular mechanism for warfarin resistance independent of the regulation of the *CYP2C9* and *VKORC1* genotypes (36,37).

We confirmed the relatively high prevalence of 83% of *VKORC1* and *CYP2C9* multiple allele carriers among our stroke patients with one (27.3%), two (46.2%) or more defective alleles that require significantly lower doses compared to the "wild" type carriers. This was also demonstrated by the research of Caldwell and Ruan in whose study 73.2 % of patients were carriers of one or more *CYP2C9* or *VKORC1* polymorphisms (38,39). Stroke patients who stand to benefit most from genotyping are precisely those who have the greatest number of deficient polymorphisms.

The presented data are consistent with previous studies that have pointed to the dominant impact of *VKORC1* gene polymorphisms on stable maintenance dose.

Studies of inter-ethnic differences in sensitivity to warfarin confirmed that the *VKORC1* polymorphism had significantly greater influence on the variability of dose in Asians and Caucasians than in Afro-Americans as the result of race-specific differences in the frequency of *VKORC1* polymorphisms (40-43). A studies conducted confirmed that this is due to differences in MAFs (minor allele frequency) across racial groups. As the MAF increases, the percentage of variation in dose explained by minor allele increases, with the highest variance explained at MAF of 60% to 70%. Minor allele frequencies for *VKORC1* -1639G>A in Asian, white, and black population are, 0.91, 0.39, and 0.11 respectively. The frequencies are calculated by using genotype information from subjects in International Warfarin Pharmacogenetics Consortium (44).

Although the variability in dose explained by *VKORC1* differed by racial groups at the population level, possession of the minor allele (-1639A or 1173T) at an individual level was associated with the similar decrease in warfarin dose requirement irrespective of race.

*CYP2C9* allele frequencies also vary considerably among racial groups. Minor allele *CYP2C9**2 frequencies in the white, Asian and black population are estimated to be 0.13, 0, and 0.03 respectively, and for *CYP2C9**3 frequencies they are 0.07, 0.04 and 0.02, respectively, according to IWPC (International Warfarin Pharmacogenetics Consortium). Thus, aggregate minor allele frequencies for *CYP2C9* range from 4% in Asians and 5% in blacks to 20% in whites, and these differences may account for some of the population-level differences in the use of warfarin-dosing algorithms in racial groups. Smaller range of the *CYP2C9* allele frequencies across racial groups compared to *VKORC1* is the reason why relative contribution to racial differences in dosing is larger for *VKORC1* than for *CYP2C9*.

In Croatian population, the prevalence of variant *CYP2C9**2 and *3 alleles is estimated to be 35% (Božina), which is similar to some other data published for Caucasians (41,43). Additional *CYP2C9* variant alleles with reduced activity (*CYP2C9**5,*6,*8, and *11) contribute to dose variability among African Americans. Other genes that could predict warfarin dose, but with much lower impact, are *CYP4F2* (primary liver vitamin K1 oxidase that catalyzes the metabolism of vitamin K1) and *CALU* (coding for Calumenin, Ca2+-binding protein) in some but not all populations (44,45).

Most of the side effects related to warfarin therapy occur at the very beginning of the drug introduction, in our study mostly between 6-12 days. The incidence of warfarin dosage complications did not exceed the incidence stated results of published research where it ranged from 5-17%, or even up to 25% (46-48). We found no significant association of the development of warfarin dosage complications with the proportion of the time spent within the target therapeutic INR range, although the incidence of major bleeding occurred in those subjects who spent more time outside the therapeutic INR range (9.59% of respondents). We found that warfarin dosage complications were slightly more frequent in the carriers of defective *CYP2C9* alleles compared to the carriers of defective *VKORC1* alleles (68.9% versus 62.5%). Serious dosage-related complications were more frequent among the carriers of both *CYP2C9* defective alleles that commonly contribute to over-anticoagulation. Similar observations of an increased risk of bleeding among the carriers of *CYP2C9* *2 and *3 alleles have been reported in some previous studies, with almost 3-fold increase in risk in the carriers of *3 alleles (10,49-50). Among patients with major hemorrhage, three had discrete hemorrhagic transition into the infarct zone confirmed by brain CT findings without any signs of clinical deterioration. We did not interrupt warfarin therapy in these patients, but adjusted the dose with careful monitoring of INR, clinical parameters and brain CT. We also continued warfarin therapy in four patients with the occurrence of microhematuria and in one with

vaginal bleeding, because we estimated that they had higher risk of serious thromboembolic events consequential to the interruption of therapy compared to the symptoms caused by the warfarin dosage side effects. In four patients, INR values were within therapeutic range, while three patients with defective *CYP2C9* alleles had INR>3.1. Three days after completing the follow-up study, one patient with *VKORC1* 1173TT and *CYP2C9* *2/*3 genotypes developed an extensive gastrointestinal bleeding despite the fact that INR values were within the therapeutic range. We applied 20 mg vitamin K followed by intravenous infusion of 690 ml of fresh frozen plasma without the need for blood transfusion and the patient has good clinical recovery.

Results of large studies confirmed that the incidence and prevalence of atrial fibrillation, which is the most common cause of cardioembolic stroke, increase with age (51). In light of these findings, clinical significance of our study lies in the fact that we pointed to a safe therapeutic intervention using pharmacogenetically-predicted warfarin dose, particularly in elderly acute stroke patients (54.76% were older than 71 years). We found no association of warfarin dosage complications with age or with the final clinical outcome reported as mRS, although all fatal outcomes were observed in patients aged over 71 years. We primarily related such outcomes to the severity of the initial brain damage caused by stroke (49,52-53).

**Conclusions**

Data from our study confirmed the relatively high prevalence of VKORC1 and CYP2C9 multiple allele carriers among Croatian patients with ischemic stroke (83%), which has a significant impact on required therapeutic dose. By using the pharmacogenetically-based warfarin-dosing algorithm that includes CYP2C9 and VKORC1 genotypes, we correctly estimated the required dose for 81.5% of 61.3 % of patients who needed higher or lower than average doses, which is crucial for rapid and safe anticoagulation. Bearing in mind that over

33% (in some studies even 42%, in Croatian population about 35%) of patients with acute stroke require immediate application of anticoagulant therapy, the clinical value of this study lies in the fact that we pointed to the safe therapeutic intervention by using pharmacogenetically-predicted warfarin dose in this high-risk group of patients without increasing the risk of warfarin dosage complications in elderly population (54,55).

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**Tables**

**Table 1.** Inclusion and exclusion criteria for the patients with ischemic stroke that required anticoagulant therapy

| Inclusion criteria | Exclusion criteria |
|---|---|
| 1. Previously taking warfarin due to:<br>• atrial fibrillation, mechanical heart valves, deep vein thrombosis or pulmonary embolism<br>2. Newly detected atrial fibrillation confirmed by HOLTER ECG<br>3. Acute dissection of extracranial or intracranial arteries<br>4. Foramen ovale apertum (FOA) with septal aneurysm<br>5. Cerebral venous sinus thrombosis | 1. Bleeding detected by brain CT scan except in patients with cerebral venous sinus thrombosis<br>2. Malignancy<br>3. Pregnancy<br>4. Hepatic and renal insufficiency |

**Table 2.** Demographic data, average doses of warfarin (estimated, introduction and maintenance doses), anticoagulant effect of warfarin (expressed by the time to reach target INR values, time and proportion of time spent within the therapeutic/supratherapeutic INR range) and time to achieve a stable daily maintenance dose.

| **Patients** | **N** | **-/+ 95% CI** | **Median** |
|---|---|---|---|
| Age | 106 | 65.1-70.3 | 72.0 |
| Weight | 106 | 73.1-77.2 | 75.0 |
| De | 106 | 3.6-4.2 | 3.8 |
| Di | 106 | 5.8-6.6 | 6.0 |
| Dm | 106 | 3.4-4.0 | 3.5 |
| T-Dm | 106 | 9.9-10.7 | 10.0 |
| Tm | 106 | 15.7-16.8 | 16.7 |
| To | 106 | 0.07-0.7 | 0.0 |
| T % | 106 | 74.7-79.5 | 78.5 |
| T tg | 106 | 4.1-4.6 | 4.2 |

N (number of patients); Age (year, median), Weight (Kg, median), 95% CI (+/-95% confidence interval); De (estimated dose, mg); Di (dose of introduction, mg); Dm (stable daily maintenance dose, mg); T-Dm (time to achieve a stable daily maintenance dose, day); Tm (time spent within the therapeutic INR range, day); T% (proportion of time spent within the therapeutic INR range); To (time spent within the supratherapeutic INR>3.1, day); Ttg (time to reach target INR values, day)

**Table 3.** Frequencies of the carriers of *CYP2C9**2,*3 and *VKORC1* 1173C>T gene polymorphisms in the group of acute stroke patients, time do achieve target INR**≥2** and the average stable daily maintenance dose depending on the genotype

| genotype | N (%) | T(days) | T 95%CI. | Dm (mg) | Dm 95%CI |
|---|---|---|---|---|---|
| | 106(100%) | 4.39 | 4.12-4.66 | 3.76 | 3.4-4.08 |
| 1.CC,wt/wt | 18(17%) | 5.05 | 4.47-5.63 | 5.7 | 5.1-6.2 |
| 2.CC,wt/*2 | 14(13.2%) | 3.88 | 3.28-4.49 | 4.1 | 3.4-4.8 |
| 3.CC,wt/*3 | 6(5.6%) | 4.55 | 3.59-5.51 | 3.8 | 2.5-5.2 |
| 4.CC,*2/*2 | 9(8.5%) | 4.62 | 3.56-5.68 | 3.7 | 2.7-4.7 |
| 5.CC,*2/*3 | 5(4.7%) | 3.58 | 2.38-4.78 | 2.4 | 1.7-3.0 |
| 6.CT,wt/wt | 19(17.9%) | 4.93 | 4.13-5.73 | 4.9 | 3.6-5.3 |
| 7.CT,wt/*2 | 8(7.6%) | 4.47 | 3.69-5.25 | 2.9 | 2.3-3.7 |
| 8.CT,wt/*3 | 13(12.3%) | 4.47 | 3.29-5.65 | 2.7 | 2.1-3.2 |
| 10.CT,*2/*3 | 2(1.9%) | 2.45 | 0.91-3.99 | 1.3 | -1.9-4.4 |
| 11.TT,wt/wt | 7(6.6%) | 3.89 | 3.53-4.25 | 2.3 | 1.5-2.9 |
| 12.TT,wt/*2 | 1(0.9%) | 3.84 | | 2.5 | |
| 13.TTwt/*3 | 1(0.9%) | 2.18 | | 2.0 | |
| 14.TT,*2/*3 | 2(1.9%) | 3.210 | 2.83-3.45 | 1.5 | 1.5-1.5 |
| 15.TT,*3/*3 | 1(0.9%) | 1.350 | | 1.0 | |

N (number and percentage of the carriers); 95% CI (+/-95% confidence interval) ;
T - Average time to achieve target INR ≥ 2 depending on the *CYP2C9* and *VKORC1* genotype (day); Dm - Average stable daily maintenance dose (mg), according to the *CYP2C9* and *VKORC1* genotype

**Table 4.** Frequencies of the patients according to the severity of neurological deficit at the discharge expressed by mRS, depending on warfarin dosage side effects
($\chi 2 = 17.87$, df = 12, p = 0.1198)

| mRS | N(%)Without side effect | N(%)Side effect- 1 | N(%)Side effect- 2 | Row - Totals |
|---|---|---|---|---|
| **0** | 13 | 1 | 1 | 15 |
| **%** | 14,44% | 14,29% | 11,11% | |
| **Row %** | 86,67% | 6,67% | 6,67% | |
| **1** | 23 | 1 | 1 | 25 |
| **%** | 25,56% | 14,29% | 11,11% | |
| **Row %** | 92,00% | 4,00% | 4,00% | |
| **2** | 17 | 1 | 2 | 20 |
| **%** | 18,89% | 14,29% | 22,22% | |
| **Row %** | 85,00% | 5,00% | 10,00% | |
| **3** | 23 | 1 | 0 | 24 |
| **%** | 25,56% | 14,29% | 0,00% | |
| **Row %** | 95,83% | 4,17% | 0,00% | |
| **4** | 9 | 2 | 2 | 13 |
| **%** | 10,00% | 28,57% | 22,22% | |
| **Row %** | 69,23% | 15,38% | 15,38% | |
| **5** | 3 | 1 | 1 | 5 |
| **%** | 3,33% | 14,29% | 11,11% | |
| **Row %** | 60,00% | 20,00% | 20,00% | |
| **6** | 2 | 0 | 2 | 4 |
| **%** | 2,22% | 0,00% | 22,22% | |
| **Row %** | 50,00% | 0,00% | 50,00% | |

| | | | | |
|---|---|---|---|---|
| **Total N** | 90 | 7 | 9 | 106 |

N ( number and frequencies of patients); mRS( modified Rankin Scale); Side effect 1 (minor bleeding); Side effect 2 (major bleeding)