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Clinical Application of Genotype-Guided Dosing of Warfarin in Patients with Acute Stroke

Svjetlana Šupe*, Zdravka Poljaković*, Tamara Božina†, Josip Ljevak*, Viola - Macolić-Šarinić‡, Nada Božina§

*Department of Neurology, Intensive Care Unit, University Hospital Center Zagreb, School of Medicine, University of Zagreb, Croatia

†Department of Medical Chemistry, Biochemistry and Clinical Chemistry, School of Medicine, University of Zagreb, Croatia

‡Agency for Medicinal Products and Medical Devices, Zagreb, Croatia

§ Department of Laboratory Diagnostics, Zagreb University Hospital Center, Department of Pharmacology, School of Medicine, University of Zagreb, Croatia

Corresponding author:

Associate Professor Nada Božina, MD, PhD
Department of Laboratory Diagnostics,
University Hospital Center Zagreb
Kišpatićeva 12, 10 000 Zagreb, Croatia
Phone: +38512367249,
Fax: +38512367395
Email: nbozina@kbc-zagreb.hr

Running head: Dosing of Warfarin in Patients with Acute Stroke

Highlights:

Individualized treatment with genotype-guided warfarin dosing instead of fixed dosing reduces the time required for stabilization and improves anticoagulant control with better clinical outcome among acute stroke patients.

Abstract

Background. Patients with certain types of stroke need urgent anticoagulation and it is extremely important for them to achieve fast and stable anticoagulant effect and receive individualized treatment during the initiation of warfarin therapy. **Methods**. We conducted a prospective study among 210 acute stroke patients who had an indication for anticoagulation and compared the impact of *CYP2C9* and *VKORC1* genotype-guided warfarin dosing (PhG) with fixed dosing (NPhG) on anticoagulation control and clinical outcome between groups. **Results**. PhG achieved target INR values earlier, i.e. on average in 4.2 (4.1-4.7, 95%CI) days compared to NPhG [5.2 days (4.7-6.4, 95% CI)] (p=0.0009), spent a higher percentage of time in the therapeutic INR range [76.3% (74.7-78.5, 95% CI) versus 67.1% (64.5-69.6, 95% CI) in NPhG], and spent less time overdosed (INR>3.1) [PhG 0.4 (0.1-0.7, 95% CI), NPhG 1.7 (1.1-2.3, 95% CI) days; p>0.000]. PhG reached stable maintenance dose faster [10 (9.9-10.7, 95% CI) versus 13.9 (13.3-14.7, 95% CI) days in controls; p=0.0049] and had a better clinical outcome in relation to neurological deficit on admission as compared to NPhG. **Conclusion.** We confirmed that warfarin therapy with genotype-guided dosing instead of fixed dosing reduces the time required for stabilization and improves anticoagulant control with better clinical outcome in early stages of warfarin therapy introduction among acute stroke patients, which is essential for clinical practice.

Key words: Stroke, warfarin, *CYP2C9*, *VKORC1,* polymorphisms

INTRODUCTION

Patients with cardioembolic stroke and stroke consequent to a specific condition (cerebral sinus venous thrombosis, dissection of intracranial arteries, hypercoagulable state) require urgent anticoagulation for secondary stroke prevention (1-4). This is important for prevention of arterial and venous thromboembolism, particularly of embolic stroke recurrences, and to reduce a high rate of in-hospital mortality in patients with recurrent embolism (19.6%) (5-6). Despite the emergence of new anticoagulants, warfarin is a widely prescribed first-line anticoagulant for most causes of embolic stoke due to its effectiveness in the management of thromboembolism and to its low cost (7-9).

Warfarin has narrow therapeutic index and at least 20-fold interpatient variations in dose requirements, which can lead to dose-related insufficient or excessive anticoagulation. Most dose-dependent adverse events emerge during introduction of therapy. Risk for hemorrhage or thromboembolism in this initiation period is higher than during later stages (event rates range from 16% to 25%) (10-11). Therefore, stroke patients that require anticoagulation are also at an increased risk of warfarin-dosage side effects and hence of recurrent stroke if they are underdosed or in overdosed conditions due to warfarin-induced brain hemorrhage (12-13). Warfarin pharmacogenetics, association of *CYP2C9* and *VKORC1* gene polymorphisms and dosing algorithms which use this genetic information are well documented (14-18).There are few studies available which have investigated the effectiveness of clinical application of genotype-guided dosing, and such studies conducted among acute stroke patients are specifically scarce (19-22). For these high-risk patients, it is essential to achieve stable anticoagulant effect as early as possible in order to prevent the risk of dosage-related complications by using the initial doses based on individual genotype (17, 23-24). The results of Franchini' s meta-analysis that included nine trials (2812 patients) show that genotype-guided initial warfarin dosing significantly reduced the risk ratio for developing major bleeding events, compared with the control group (RR=0.47; P=0.04), or reduced serious bleeding events by approximately 50% compared to clinically-guided dosing group (25). Data obtained by meta-analysis of randomized clinical trials (Stergiopoulos et al.) indicated that a genotype-guided dosing strategy was not superior to clinical dosing algorithms in terms of INR values, reduction in major bleeding or thromboembolic events (26).

Recently, several randomized controlled trials have been published with different conclusions on the clinical utility of genotype-guided dosing of coumarin anticoagulants. A study conducted by Pirmohamed group (EU-PACT trial) found that pharmacogenetic-based dosing was associated with a higher percentage of time in the therapeutic INR range than standard dosing during the initiation of warfarin therapy (27), while Kimmel group (COAG trial) did not confirm these findings for the anticoagulation period of the first 4 weeks of warfarin therapy (28). Also, Verhoef group did not confirm the findings for acenocoumarol or phenprocoumon during 12 weeks after the initiation of therapy in patients with atrial fibrillation or venous thromboembolism (29).

In this study, we compared the impact of *CYP2C9* and *VKORC1* genotype-guided dosing of warfarin with a standardized, fixed dosing regarding anticoagulation control and clinical outcome in acute stroke patients during the first three weeks of therapy.

Patients and methods

During six months, 587 Croatian Caucasian patients (EUCs) with acute ischemic stroke were hospitalized at the Department of Neurology, UHC Zagreb. We conducted a prospective trial among 210 (36% of the total number) patients with an indication for urgent anticoagulation, whose initial brain CT scan was without signs of hemorrhage. Detailed inclusion and exclusion criteria are listed in Table 1.

Power analysis was done before the study. If the statistical significance level is set to $p<0.05$, and power to 0.95, two-tailed Fisher Exact test would need the sample size of n=96 in each group to detect the difference of at least 0.25 in proportion of patients with INR within the therapeutic range (2-3) at fifth day after the introduction of warfarin. Expected proportions were obtained in the pilot study done on the sample of n=20 patients. The proportion of patients with INR within therapeutic range (2-3) was 0.9 in PhG, and 0.4 in NPhG group. For more conservative calculation we set the expected proportions to 0.8 in PhG and 0.55 in NPhG group. Expecting approximately 5-10% of missing data the initial sample size was decided to be n=105 in each group.

Eligible patients (N=210) were centrally registered and stratified according to gender and age, and then assigned to the intervention group [*CYP2C9**2,*3 and *VKORC11173*C>T genotype-guided dosing group, PhG (N=106)] or to the control/fixed dosing group (NPhG, N=104) (Figure 1).

There were no differences between groups in gender (p=0.559), age (p=0.669) or indications for anticoagulation (inclusion criteria; p=0.591). Among the total number of 210 patients, 32% were previously anticoagulated by using warfarin [34.9% (N=36) PhG and 28.8% (N=30) NPhG] and INR on admission was in all of them below the therapeutic range (INR<1.6). Atrial fibrillation was the most common indication for anticoagulation in both groups [56% of the total number; 53 (50.5%) in PhG, 65 (62.5%) in NPhG] (Table 2a).

Since the number of eligible patients, i.e. those with appropriate diagnosis for our study (N=210) was relatively low and inclusion/exclusion criteria practically did not have any impact on patient selection as each patient required equal treatment and was consequently to be allocated in one of two study arms, we used a simple way of grouping (distribution)

patients by alternative allocation of every second patient into one of the two study arms. Moreover, having in mind that we could not influence patient's occurrence and patient's disease status in any manner, we considered that any possible bias was absolutely negligible, by using this method of allocation.

Concomitant therapy and the number of smokers were similar in both groups. Once the ethical committee permission had been obtained, written informed consent form was provided for each PhG patient.

Using non-profit website published algorithm *http: //www.WarfarinDosing.org.*, we assessed initial pharmacogenetically-predicted warfarin dose, introducing a loading-dose strategy of warfarin for each patient in PhG during the first two to five days, while subsequently patients were treated on the basis of the INR (28-30). In the control group, warfarin was introduced by a fixed dose of 2 tablets of 3 mg warfarin (6 mg) for the first two to five days, and the doses were subsequently adjusted depending on the measured INR values. INR was measured repeatedly among patients where it was necessary, depending on previous INR values, and for the purposes of this study we recorded INR values on specific dates (INR at admission, on the first day of therapy introduction, at 48 hours, 72 hours, and days 5, 7, 14, and 21.)

Blood samples (10 ml) were taken for INR measurement in both groups and for genotyping in PhG. The following data were recorded and compared between groups: indications for warfarin therapy, respective INR values depending on the day of warfarin introduction (as stated above), predicted dose (only PhG), dose of introduction, time to reach target INR ≥ 2, time/percentage of time spent in the therapeutic (2-3)/supratherapeutic (>3.1) INR values, time to reach a stable maintenance dose, dosage-dependent side effects and neurological deficit. A dose at which the patient was maintained within the therapeutic INR range and which did not change during three consecutive INR measurements for at least five days was defined as the stable maintenance dose.

Warfarin dosage side effects and clinical outcome

Warfarin-dosage side effects were classified into two groups. Group 1 included minor bleeding (asymptomatic microhematuria, slight gingival or vaginal bleeding, and subcutaneous hematoma). Group 2 included major bleeding which was classified into two subgroups: subgroup 2a) bleedings that did not require interruption of therapy, such as mild hemorrhagic transition of infarct zone without worsening of neurological deficit, and

subgroup 2b) bleedings that required interruption of warfarin therapy (large intracerebral hemorrhage with worsening of neurological deficit, extensive urogenital or gastrointestinal bleeding). Patients' neurological deficit on admission was estimated using the NIHSS scale (31). Clinical outcome defined as neurological deficit at the end of the study (follow-up period of three weeks) was assessed using mRS (32).

Endpoints

The primary endpoints to determine by comparing the PhG and NPhG were as follows:

- time to achieve target INR≥2
- proportion of patients who achieved the therapeutic INR 2-3 range depending on the day of measurement
- time/percentage of time spent in the therapeutic/supratherapeutic INR range
- clinical outcome
- association of clinical outcome with the occurrence of dose-dependent side effects.

The secondary endpoints were the following:

- time needed to achieve a stable maintenance dose
- proportion of patients who achieved a stable maintenance dose depending on the day of measurement
- incidence of warfarin dosage-side effects.

Genotyping procedures

DNA was isolated from whole blood by salting out method. 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 (21, 33).

Statistical Analysis

The analysis was carried out by using the statistical software package STATISTICA ver. 6.0 (StatSoft Inc., Tulsa, OK, USA). Quantitative variables were 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).
Descriptive data were presented as frequency and proportion (such as time and percentage of time spent in therapeutic INR range). Student's t-test was used to compare the groups for quantitative variables (time required to achieve the target INR, time needed to achieve stable maintenance dose). χ^2-test was used for comparison of descriptive variables. Analysis of variance (ANOVA) was used for comparison between the groups for quantitative variables depending on distribution. Results were considered statistically significant at the 5% level ($p<0.05$).

Results

Demographic and clinical data, as well as primary and secondary endpoint results are presented in Table 2a and Table 2b.
Newly detected atrial fibrillation was the most frequent indication for warfarin therapy introduction (56.2% of patients). Before stroke, 32% of all included patients were receiving warfarin and on admission to hospital all of them had INR values below the therapeutic INR range (INR<1.6). The most common genotype among 36 underanticoagulated PhG patients, with the prevalence of 19.5% was “wild” homozygous *CYP2C9* wt/wt*, VKORC1* 1173CC genotype.

Primary endpoints

The groups differed significantly in the time needed to reach the target INR≥2 and in the average INR values depending on the day of measurement (p=0.00017) (Table 2b; Figure 2.) PhG achieved INR≥2 earlier, i.e. on average in 4.4 (4.1-4.7, 95% CI) days as compared to NPhG [5.2 (4.7-6.4, 95% CI) days (p=0.0009)].

On day 5, 63.2% of PhG entered the therapeutic INR range compared to 40.4% of NPhG (p=0.0026), on the seventh day 90.6% of PhG patients achieved the target INR compared to 67.3% of NPhG (p=0.0005), and on the 14th day 99.1% of PhG patients achieved the therapeutic INR range compared to 80.8% of NPhG (p=0.0001). On the 21st day (at the end of the study), there were no differences between the groups (p=0.513).

On the seventh day after warfarin introduction, supratherapeutic INR>3.1 was reached only by 6 (5.7%) PhG patients compared to 15.4% of NPhG patients, and on the 14th day by 1 (0.9%) PhG patient compared to 16 (17.2%) NPhG patients who had INR>3.1. Finally, only 9.3% of PhG patients achieved INR>3.1 in the monitored period, as opposed to 37.5% in NPhG [difference (control group minus genotype-guided group) 28.2 percentage points, p>0.000] (Table 3).

PhG spent more time in the therapeutic INR range [16.1 (15.7-16.5, 95% CI) days or 76.6% (74.7-78.5, 95% CI) of time] as compared to NPhG [14.1 (13.6-14.6, 95% CI) days or 67.1% (64.5-69.6, 95% CI) of time] (p=0.0049) (Table 2b).

PhG spent less time in supratherapeutic INR>3.1 range [0.4 (0.1-0.7, 95% CI) day] as compared to NPhG [1.7 (1.1-2.3, 95%CI) days] (p=0.000).

Using the method of linear interpolation and Student's t-test, we confirmed differences between the PhG and NPhG in the time of entry into the target INR range (p=0.000), in the percentage of time spent in the therapeutic INR range (p=0.000), in the total time spent in the therapeutic INR range (p=0.000), as well as in the time spent outside the therapeutic INR range (p=0.000). We adjusted the doses and monitored clinical parameters, INR levels and brain CT findings in both groups if it was necessary, and found no differences between groups in the occurrence of warfarin-dosage side effects (χ^2=0.688; df=2; p=0.7089). Side effects were observed in 16 (14.8%) of PhG patients, 7 (6.6%) had minor and 9 (8.3%) had major bleeding (Table 2a). Three patients in subgroup 2a had a discrete hemorrhagic transition of infarct zone without clinical deterioration. Warfarin side effects were observed in 18 (17.5%) NPhG patients, 10 (9.7%) patients had minor and 8 (7.8%) had major bleeding. In subgroup 2a, three patients developed less severe hemorrhagisation of infarct zone without clinical worsening and three developed microhematuria, without discontinuation of the therapy. On the 19th day of therapy, one patient had extensive intracerebral hemorrhage with clinical deterioration and subsequent death (subgroup 2). The groups did not differ in the occurrence of warfarin-dosage side effects depending on the proportion of time in the therapeutic INR

range (p=0.560), although major bleeding in both groups occurred in those subjects who spent more time outside the therapeutic INR range.

Neurological deficit on admission expressed by the NIHSS was 10.5 (SD5.00) for PhG and 9.7 (SD 3.69) for NPhG. At the end of the study (a follow-up period of three weeks), residual neurological deficit (final clinical outcome) presented by mRS was 2.1 (SD 1.58) for PhG and 2.4 (SD 1.36) for NPhG. By comparing the neurological deficit on admission and at the end of the study (using analysis of variance for repeated measurement), we found difference with better recovery in PhG (p=0.043) (Table 2a; Figure 3).

We found no association of the final clinical outcome with the appearance of the dosage-dependent side effects in PhG (p=0.1198), in contrast to the positive correlation in NPhG (p=0.0006). The association of clinical outcome with the appearance of complications was also confirmed by using Spearman correlation coefficients (rho=0.2617, p=0.0075).

Secondary endpoint

PhG achieved stable dose earlier, i. e. in 10 (9.9-10.7, 95% CI) days as compared to NPhG [13.9 (13.3-14.7, 95% CI) days] (p=0.0049).

The groups differed in the proportion of patients who achieved stable maintenance dose depending on the time (p=0.000) (Figure 4).

Within seven days of the therapy introduction, 9.4% of PhG subjects achieved stable dose but none from the NPhG. On the 10^{th} day, cumulatively 59.5% of PhG subjects achieved stable dose as compared to 11.5% in NPhG. By the 12^{th} day, a total of 94.3% of PhG subjects had stable maintenance dose as opposed to 39.4% of NPhG subjects. After two weeks, 97.1% of PhG and 60.5% of NPhG subjects achieved stable dose.

Discussion

Our investigation is the only study conducted exclusively among patients with acute stroke that need urgent anticoagulation, except for individual case reports and some studies of warfarin dosing formula among stroke patients in the available literature (21,23). Goals of our study were to determine whether pharmacogenetically-guided warfarin dosing could improve anticoagulation control and safety of warfarin treatment in comparison with standard, fixed dosing among stroke patients with indications for anticoagulation. The presented results confirm the value of implementing a pharmacogenetic algorithm that includes *CYP2C9* *2,*3

and *VKORC1* 1173C> T gene polymorphisms in the prediction of the initial dose of warfarin. We found significant between-group differences, with earlier achievement of therapeutic INR levels and confirmed better anticoagulation control in PhG reported by the longer time spent in the therapeutic INR 2-3 range. Differences became apparent between the 5^{th} and 14^{th} day, with higher proportion of time spent in the therapeutic INR range and lower proportion of time spent in the supratherapeutic INR range, which is consistent with previously published data (27, 34-36) and highlights the importance of initiating warfarin therapy with a genotype-guided dose in the early stage of the therapy introduction. When considering the impact of different parameters in warfarin dose prediction, it is important to be aware of MAFs (minor allele frequency) for VKORC1 and CYP2C9. Actually, 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. MAFs for *VKORC1* -1639G>A in Asian, white, and black population were 0.91, 0.39, and 0.11, respectively. *CYP2C9* variant allele frequencies are also rather variable among racial groups (4% in Asians, 5% in blacks, 20% in whites), which may explain some of the population-level differences in application of warfarin-dosing algorithms in racial groups (37-41).

Our data differ from a study conducted among orthopedic patients in which authors did not find any differences in the anticoagulation control or in the incidence of complications between PhG and NPhG (19). In their study, 13% of patients had only *CYP2C9* allelic variants, 44% of patients had *VKORC1* polymorphisms, and 19% of patients had polymorphism for both genes, in contrast to our PhG group where polymorphism for only *CYP2C9* gene was present in 32% of patients, 24.5% of patients had only the *VKORC1* gene polymorphism, and 28.3% had polymorphism for both genes. This may contribute to differences of our results. In our study, serious complications were more frequent among carriers of *VKORC1* 1173C>T alleles (62.5%) and carriers of both defective *CYP2C9**2 and *3 alleles (37.5%), but without significant difference between the genotypes.

Secondary endpoints (stable anticoagulant effects) were also achieved in our study. Data confirmed that genotype-guided introduction reduced the time required for stabilization, i.e. PhG achieved a stable maintenance dose earlier, with greater proportion of stable anticoagulated patients depending on time as compared to NPhG. Our data demonstrated the difference between clinical outcome and the occurrence of complications between the two groups. We found no relations between the occurrence of warfarin-dosage side effects and the clinical outcome in PhG in contrast to positive correlation found in NPhG. This fact is

probably associated with the occurrence of more severe bleeding in the fixed-dosing group of patients. There is still no sufficient evidence to support the use of pharmacogenetics to prevent major complications and improve clinical outcome (39). The already mentioned different results obtained in several randomized controlled trials could be due to different approaches to dosing. EU-PACT trial applied fixed-dosing approach while clinical dosing algorithm was used in the COAG trial (27,28). Further, differences in the trial populations could have influence on study results, since additional variants discovered in African Americans could contribute to dose requirements (40,41).The strength of our study lies in investigation of a homogenous population of acute ischemic stroke patients among whom atrial fibrillation was the most frequent indication for warfarin therapy. In addition, the study was conducted in hospital settings where experienced cerebrovascular neurologists comprehensively monitored patients with ischemic stroke (42). Our data are consistent with the most recently published findings and remarks by Schwarz group (43). They found genotype-based dosing to be superior in patients who had atrial fibrillation as compared to patients who had venous thromboembolism, with the clear benefit when the participants harbored two or more variant alleles. This was the case in our study where homozygosity for "defective" variants of *VKORC1* and *CYP2C9* genes contributed to the earlier achievement of target INR and to the longest proportion of time spent within the therapeutic INR range in comparison to carriers of wild type alleles. This finding could be at least partly explained by the observation that “wild” type *VKORC1* allele carriers can also be carriers of rare mutations and they require significantly higher doses, or can even develop resistance to warfarin. However, these mutations are not analyzed in routine clinical practice (44).

We are aware that a limitation of this study is a relatively small number of enrolled patients and that our results need to be confirmed by further studies on larger populations of patients. Nevertheless, from the clinical point of view, we were able to detect clinically relevant differences. Our group of patients differed from respondents in other studies since they were in life-threatening condition due to the acute brain infarction, with an increased risk of bleeding and poor outcome due to the nature of the disease itself or because of warfarin-dose-dependent over/under-anticoagulation. The risk of early embolic stroke recurrence range between 5-7% within the first week, with simultaneously increased risk of hemorrhagic transformation of an ischemic infarct related to the nature of the ischemic brain damage and the risk of warfarin-induced brain hemorrhage due to overanticoagulation (45,46). Data from our study confirmed faster anticoagulant control and, bearing in mind that acute stroke

patients are at increased risk for recurrent embolism or warfarin-induced brain hemorrhage, we also confirmed safer anticoagulant control among them, with better clinical outcome and minor neurological deficit using genotype-guided warfarin dosing. This is important for the prevention of arterial and venous thromboembolism among acute stroke patients, particularly of embolic stroke recurrences.

Conclusion

Our study confirmed that initiating warfarin therapy with genotype-guided dosing has the greatest impact on anticoagulation control during the early stages of therapy introduction. This individualized treatment reduces the time required for stabilization, which is important for fast and stable anticoagulation in clinical practice, particularly among stroke patients who are at increased risk of brain hemorrhage caused by the nature of the disease itself. At the same time, they undergo a high risk of warfarin-dose dependent events, risk of stroke recurrence due to embolisms in underdosed conditions, or warfarin-induced brain hemorrhage in overdosed state. We confirmed better clinical outcome with minor neurological deficit using *CYP2C9* and *VKORC1* genotype-guided dosing compared to fixed warfarin dosing.

Study Limitations

A limitation of the study might be the size of observed patient groups. Randomisation and concealment of allocation are limitations, and could have biased the results. The lack of knowledge on control subjects' genotype could have had impact on the results.
However, the obtained data indicated significant findings which need to be confirmed in replication studies and in other populations.

Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1. Inclusion and exclusion criteria for the study

Inclusion criteria	1. Previously taking warfarin due to atrial fibrillation, mechanical heart valves, deep vein thrombosis, pulmonary embolism 2. Newly discovered atrial fibrillation confirmed by HOLTER-ECG 3. Acute dissection of intracranial arteries 4. Patent foramen ovale with septal aneurysm 5. Cerebral venous sinus thrombosis
Exclusion criteria	1. Age<18 year 2. Hemorrhage in the brain, detected by CT scan, except in patients with cerebral venous thrombosis 3. Malignancy, pregnancy 4. Hepatic/renal insufficiency

Table 2a. Demographic/ clinical data and some endpoint results among PhG/NPhG patients

Patients	**PhG (N=106)**	**NPhG (N=104)**
Gender	Female 60 (56%)	Female 62 (60%)
Age (year)	67.6(SD 13.5)	69.1(SD 12.2)
Weight (kg)	75.6(SD 10.3)	74.5(SD 10.6)
Height (cm)	171(SD 12.5)	172(SD 13.5)
P A*	36(34.9%)	30(28.8%)
nAF*	53(50.5%)	65(62.5%)
ADA*	10(9.5%)	5(4.8%)
PFO*	3(2.9%)	2(1.9%)
VST*	3(2.9%)	2(1.9%)
ND-a NIHSS*	10.5(SD 5.00)	9.7(SD 3.69)
ND-d mRS*	2.1(SD 1.58)	2.4(SD 1.36)
SE*	16(15.1%)	18(17.5%)
SE mi	7(6.6%)	10(9.7%)
SE ma	9(8.5%)	8(7.8%)

N (number), PhG (pharmacogenetic-dosing group of patients), NPhG (fixed-dosing group of patients), SD (standard deviation); PA* **(**Previously anticoagulated**)**; nAF* (Newly discovered atrial fibrillation confirmed by HOLTER-ECG); ADA* (Acute dissection of intracranial arteries); PFO* (Patent foramen ovale with septal aneurysm); VST* (Cerebral venous sinus thrombosis); ND-a NIHSS* (neurologic deficit at the admission expressed by NIHSS); ND-d mRS* (neurologic deficit at the end of the study, expressed by mRS); SE* (warfarin-dosage side effects); SE mi (warfarin-dosage side effects – minor bleeding); SE ma (warfarin-dosage side effects- major bleeding)

Table 2b. Primary and secondary endpoints results among PhG/NPhG patients

Patients	**PhG (N=106)** 95%CI	**NPhG (N=104)** 95%CI
De	3.6-4.2	0
Di	5.8-6.6	6.0
Dm	3.4-4.0	3.6-4.5
T-Dm*	10(9.9-10.7)	13.9(13.3-14.7)
Tm*	16.1(15.7-16.4)	14.1(13.5-14.6)
To*	0.0(0.07-0.7)	1.6(1.0-2.3)
T %*	76.6(74.7-78.5)	67.0(64.5-69.5)
T tg*	4.2(4.1-4.6)	5.2(4.7-6.3)

N (number of PhG/NPhG patients); 95% CI (+/-95% confidence interval); De (estimated dose, mg); Di (dose of introduction, mg); Dm (stable maintenance dose, mg); T-Dm* (time needed to achieve stable maintenance dose, days); Tm* (time spent within the therapeutic INR range, days); To* (time spent within the INR>3.1, days); T %* (proportion of time within the therapeutic INR range); T tg* (time required to reach target INR values, days)

Table 3. Frequencies of PhG and NPhG patients who achieved target INR ≥ 2, therapeutic INR 2-3, supratherapeutic INR>3.1 or INR>4, depending on the day of INR measurement

Day of INR measur.	N(%) PhG < targetINR	N(%) PhG in target INR 2-3	N(%) PhG INR>3.1	N(%) PhG INR>4	N(%) NPhG <target INR	N(%) NPhG in target INR 2-3	N(%) NPhG INR>3.1	N(%) NPhG INR>4
INR(3)	88(83.2)	18(16.98)	0	0	97(93.27)	7(6.73)	0	0
INR(5)	36(33.96)	67(63.21)	0	3(2.83)	56(53.85)	42(40.38)	4(3.85)	2(1.92)
INR(7)	4(3.77)	96(90.57)	5(4.72)	1(0.94)	18(17.31)	70(67.31)	14(13.46)	2(1.92)
INR(14)	0	105(99.06)	0	1(0.94)	4(3.85)	84(80.77)	14(13.46)	2(1.92)
INR(21)	2(1.89)	104(98.11)	0	0	1(0.96)	102(98.08)	1(0.96)	0

N(%) PhG (number and percentage of pharmacogenetic-dosing group of patients); N(%) NPhG (number and percentage of fixed-dosing group of patients)

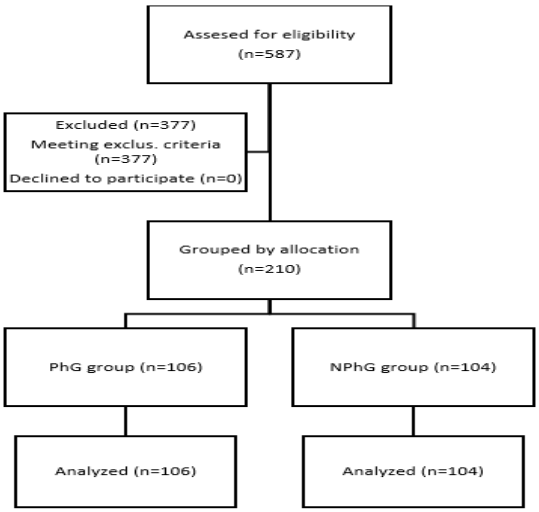

Figure 1. Consort diagram

Assessed for eligibility (587 Caucasian patients with acute ischemic stroke), grouped by allocation [eligible patients with an indication for urgent anticoagulation with warfarin that meets inclusion criteria, 36% (N=210) of total], PhG group (patients in pharmacogenetically-guided group, N=106); NPhG group (patients without pharmacogenotypisation, fixed-dosing group, N=104)

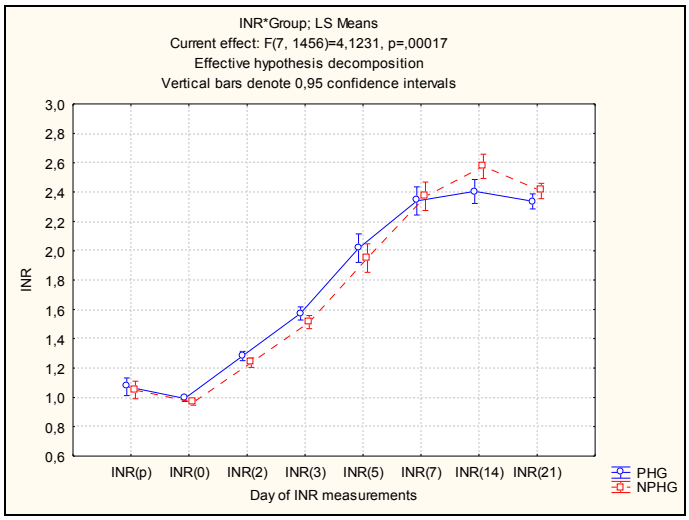

Figure 2. Time required to achieve the target International Normalized Ratio (INR)≥2 and the average INR values depending on the day of INR measurements and monitoring in PhG (pharmacogenetically-guided) and NPhG (fixed dosing) patients (p=0.0017)

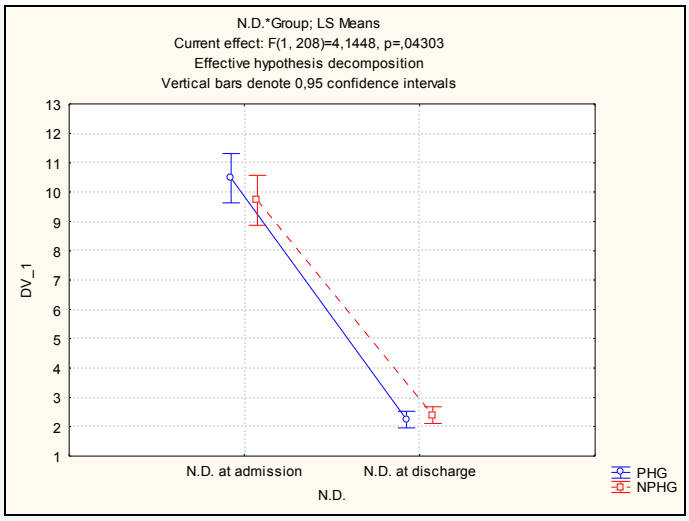

Figure 3. Differences between the neurological deficit at admission (ND at admission, expressed by NIHSS) and neurological deficit at the end of the study (ND at discharge, expressed by mRS) between PHG (pharmacogenetic-dosing group) and NPHG (fixed-dosing group) (p=0.043)

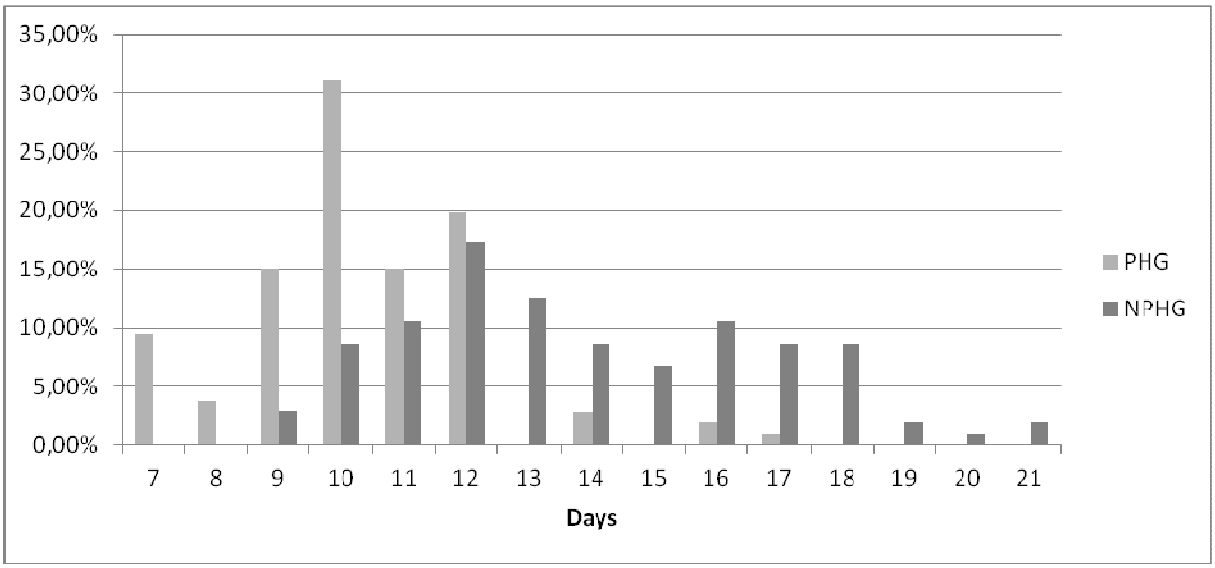

Figure 4. Frequencies of patients in pharmacogenetic-dosing group (PHG) and fixed-dosing group (NPHG), depending on the day of achieving stable maintenance dose (p=0.000)