



Warfarin loading dose guided by pharmacogenetics is effective and safe in cardioembolic stroke patients – a randomized, prospective study

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Abstract

Warfarin treatment is commonly started with a fixed loading dose that might be associated with an increased risk of bleeding. An individual maintenance dose can then be estimated based on a pharmacogenetic algorithm. Starting treatment with the estimated dose implies a longer time to reach the therapeutic range. Our goal was to compare the safety and efficacy of initiating warfarin treatment with a loading dose guided by pharmacogenetics versus a maintenance dose. The primary endpoint was time in the therapeutic range (TTR) in the first 10 days of treatment. Secondary endpoints were time to the first international normalized ratio (INR) in therapeutic range (2.0–3.0) and occurrence of serious adverse events. Consenting cardioembolic stroke patients were genotyped for *CYP2C9* (cytochrome P450 2C9 gene) and *VKORC1* (vitamin K epoxide reductase complex, subunit 1 gene) polymorphisms and a maintenance warfarin dose was estimated. Patients were randomized into two groups. The loading dose group (LDG) patients received twice the estimated dose in the first 2 days of treatment. The maintenance dose group (MDG) patients received the estimated dose directly from day one. The TTR in the first 10 days was significantly higher in the LDG than in the MDG (50.5% vs. 38.3%, $p = 0.003$). The time to the first INR in this range was significantly shorter in the LDG (5.24 vs. 7.3 days). There were no significant differences in the INR above this range or serious adverse events. Warfarin loading dose guided by pharmacogenetics after recent cardioembolic stroke improved the efficacy of warfarin initiation without increasing the risk of adverse events.

Introduction

Despite the introduction of new oral anticoagulants, warfarin still remains an important anticoagulant drug. Initiation of warfarin treatment is complicated by both the large inter-individual dose variability and warfarin's narrow therapeutic range with a risk of bleeding complications in the case of overcoagulation—especially in patients after

recent ischaemic stroke [1]. Therefore, considerable attention has been given to safe and effective warfarin treatment initiation strategies. Non-genetic initiation strategies have focused on determining the appropriate initiation dose and used loading doses as high as 1.5 mg/kg [2]. However, loading doses of 10 mg and 5 mg are often evaluated and recommended [3–5].

Flexible dosing schemes that estimate the maintenance dose are developed based on the international normalized ratio (INR) response in the first days of therapy [6]. Further attempts to develop a more individualised approach adjust the loading dose by age and comorbid conditions [7]. Apart from non-genetic factors responsible for ~40–50% of the inter-individual dose variability (age, sex, body size, vitamin K intake, state of renal and hepatic metabolism, drug interactions, actual balance of haemostasis, patient compliance, and physicians' adherence to treatment control [8, 9]), genetic factors explain 40–60% of dose variability and are the subject of extensive recent research. The cytochrome P450 2C9 gene

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(*CYP2C9*) and vitamin K epoxide reductase complex, subunit 1 gene (*VKORC1*) were shown to have a conclusive impact and explain ~40% of the warfarin dose variability [9, 10]. This was a crucial step towards pharmacogenetic-guided (PG) dosing.

Numerous pharmacogenetic models were developed to calculate the estimated warfarin maintenance dose before the beginning of warfarin therapy, including both genotypes and defined non-genetic factors for dose prediction [11–17]. In some cases, the INR values were obtained during the warfarin initiation phase [17] or predefined loading doses with respect to genotype [18, 19]. Pharmacogenetic dosing algorithms were tested in a number of trials to determine the benefits of pharmacogenetically guided approaches, but unfortunately these had inconsistent results [11, 19–24]. The current controversy regarding warfarin pharmacogenetics in routine clinical practice can be illustrated in the US example. Indeed, the guidelines of two distinguished scientific societies (American College of Chest Physicians (ACCP) and American Heart Association/American Stroke Association (AHA/ASA)) differ considerably. The ACCP suggests therapy be started with a 10 mg dose daily for the first 2 days rather than starting with the estimated maintenance dose. They even recommend against the routine use of pharmacogenetic testing in the latest ACCP guidelines [25]. In contrast, the AHA/ASA Guidelines for the Primary Stroke Prevention 2014 [26] states that “Pharmacogenetic dosing of vitamin K antagonists may be considered when therapy is initiated”. Finally, the Food and Drug Administration (FDA) in its Coumadin (crystalline warfarin sodium) label favours the pharmacogenetic approach and recommends consideration of both clinical and genetic factors (*CYP2C9* and *VKORC1* genotypes). If the patient’s *CYP2C9* and *VKORC1* genotypes are not known, then a more cautious approach with a lower initial dose (2–5 mg per day) is recommended rather than routine use of higher loading doses because this may increase haemorrhagic and other complications while not offering more rapid protection against clot formation [27].

In conclusion, there are a wide range of initiation strategies in routine clinical practice due to the disparity in current guidelines. Some physicians hoping for better safety profiles prefer treatment initiation via the predicted maintenance dose (pharmacologically guided in some cases). This implies a longer time to reach the therapeutic range. Others rely on a fixed loading dose that places the patients at an increased risk of overcoagulation, but that can shorten the time needed to reach the therapeutic range and so reduce length of stay in hospital. Thus, we asked ourselves whether individualising the loading dose by pharmacogenetics could lead to a more effective initiation strategy while still retaining the safety profile.

Aim of the study

The aim of the study was to evaluate the safety and efficacy of initiating warfarin treatment via a loading dose guided by pharmacogenetics compared to the maintenance dose. The primary endpoint was time in the therapeutic range (TTR) in the first 10 days of treatment. Secondary endpoints were time to the first INR in therapeutic range (2.0–3.0), time to the first INR above range, TTR in the first 30 days of treatment and occurrence of serious adverse events including INR >4, major bleeding, thromboembolic events, and death.

Methods

This prospective study was conducted in the comprehensive stroke centre affiliated with the Department of Neurology (2nd Faculty of Medicine, Charles University, Motol University Hospital, Prague, Czech Republic) between April 2010 and March 2014 with the Institutional Review Board approval. The study follows the CONSORT statement. Study subjects were recruited from consecutive consenting cardioembolic stroke patients. Informed consent was obtained from all subjects. The patients were genotyped for *CYP2C9* *2,*3 polymorphisms and *VKORC1* AA, AB, and BB haplotypes. Pharmacogenetic testing was done in the Molecular Genetics Laboratory (Hospital Na Homolce, Prague, Czech Republic), which is a fully certified facility with established internal and external genotype quality control procedures. The maintenance warfarin dose was estimated according to the pharmacogenetic algorithm by Sconce et al. [15] corrected for INR range and concomitant drugs [13, 28]. Patients admitted with cardioembolic stroke were randomized using a non-stratified simple randomization procedure into two groups. We used simple mechanism with odd and even number of patient. They started their warfarin treatment on average 7–14 days after stroke depending on the infarct size. The loading dose group (LDG) patients received a loading dose equal to double the estimated dose in the first 2 days of treatment and continued with the estimated dose from day 3. The maintenance dose group (MDG) patients received the estimated dose directly from day 1. Patients were followed for 90 days. The INR was measured on days 0–10 daily as well as day 15, 20, 30, and 90. Although the target INR range was 2–3, we prospectively defined an out-of-range INR value for the purposes of end-point analysis and for clinical dose adjustment. This range was <1.7 or >3.3 and allowed for measurement error to avoid problems inherent in overcorrection [11]. Subsequent warfarin dose corrections were done after day 8 if the INR was below 1.7 and always when the INR was above 3.3 based on a predefined dosing algorithm [11]. Roosendaal’s method was used to calculate the amount of

Table 1 Primers, probes and their concentrations in PCR reaction for HRM analysis

CYP2C9*2	Forward	3'-TTTCGTTTCTCTTCCTGTTAGGAATTGT-5'	10 µM
	Reverse	3'-AGATAGTAGTCCAGTAAGGTCAGTGATATG-5'	0.5 µM
	Probe	3'-GCTTGCTCTTTAACACAGTCCTTAATGCACCTC-P-5'	0.5 µM
CYP2C9*3	Forward	3'-CATGCAAGACAGGAGCCA-5'	0.5 µM
	Reverse	3'-TGAATTTAATGTCACAGGTCAC-5'	10 µM
	Probe	3'-GATACCTTGACCTTCTCCCCACCA-P-5'	2 µM
VKORC1	Forward	3'-CCTCAGCCTCCCAAGTAGTTT-5'	10 µM
	Reverse	3'-AGATAGTAGTCCAGTAAGGTCAGTGATATG-5'	0.5 µM
	Probe	3'-GCTTGCTCTTTAACACAGTCCTTAATGCACCTC-P-5'	0.5 µM

time in the therapeutic range [29]. All patients received the usual clinical care according to the institutional and current therapeutic guidelines [30, 31]. Bleeding episodes were classified according to the International Society on Thrombosis and Haemostasis (ISTH) [32, 33].

Patient eligibility

To be eligible for the study, patients had to: (1) be indicated for anticoagulant therapy after a recent cardioembolic ischaemic stroke; (2) have a target INR 2.0–3.0; (3) be over 18 years; (4) be of Caucasian origin (self-declared); and finally (5) be compliant with study participation.

Exclusion criteria were refusal to participate, previous treatment with warfarin, active cancer, pregnancy, and concomitant use of other antiplatelet agents or anticoagulants with the exception of bridging low-molecular weight heparin at the time of treatment initiation.

Clinical data

The data were collected from inpatient and outpatient medical records and directly from patients during the hospital stay and regular planned visits with specific emphasis on ischaemic or bleeding complications. Baseline clinical and medical history data included elevated systolic blood pressure, detailed history of vascular disease, renal and hepatic disease, diabetes mellitus, and concomitant medication (prescription and over-the-counter drugs as well as vitamins and herbal products). All patients had an extensive array of examinations including ultrasound of extracranial and intracranial cerebral vessels, CT angiography, brain MRI, as well as transoesophageal echocardiography and ECG monitoring for at least for 72 h. Aetiology of ischaemic stroke was assessed with the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification system [34].

Genotyping

Five millilitres of peripheral blood was obtained during a regularly scheduled blood draw. Genomic DNA was

isolated from whole blood with Puregene Blood Kit (Qiagen Inc., Chatsworth, Calif., USA). Genotyping was performed using the high-resolution DNA melting technique with the LightScanner system (BioFire Inc., Salt Lake City, Utah, USA). The Genotyping Master Mix (Cat. No. HRLS-ASY-0003; BioFire) according to the manufacturer's protocol and primer/probe reagents designed for detection of alleles *2 and *3 of the gene *CYP2C9* and single nucleotide polymorphism 1639-A>G of gene *VKORC1* (Table 1) were used for polymerase chain reaction in a PCR cyclor MultiGene (Labnet International, Inc., Edison, N.J., USA). The *CYP2C9**2 and *VKORC1* 1639-A>G polymorphisms were done in multiplex PCR with thermal profile 94 °C–30 s; 67 °C–30 s; 72 °C–30 s followed by 94 °C–30 s; 70 °C–300 s; 64 °C–300 s cycle to form heteroduplexes. *CYP2C9**3 PCR has the same amplification profile followed by 94 °C–30 s; 25 °C–30 s cycle.

Statistical analysis

The sample size was estimated based on pilot data [35] with the use of two-sided tests with an alpha of 0.05. The TTR in the first 10 days of treatment was predicted to be 0.46 in LDG versus 0.33 in MDG with an assumed standard deviation of 0.3. The sample size was estimated to be 72 (36 per group). Baseline characteristics were compared between the patients in the LDG and MDG using χ^2 -tests for categorical variables and Mann–Whitney *U*-test or independent-samples *t*-test as appropriate for continuous variables.

Predefined subgroups were based on warfarin dose in the first 2 days of treatment. In the LDG group, patients received a loading dose equal to double the estimated dose in the first 2 days of treatment and continued with the estimated dose from day 3. In the MDG group, patients received the estimated dose directly from day 1. Logistic regression was used to assess categorical outcome. The associations between warfarin loading dose and the time to the first INR in therapeutic range were further evaluated using survival analysis techniques. We used the Cox proportional hazards models to compute a hazard ratio (HR) with 95% confidence interval (CI) both unadjusted and

adjusted for the potential confounding covariates. Each clinical variable was tested in the model for statistical significance by a backward stepwise elimination algorithm with a likelihood ratio statistic. Only statistically significant variables with a p -value ≤ 0.1 on univariate testing were included in the final model. Goodness of fit of the model was tested with the Grønnesby and Borgan test for the Cox proportional hazards model [36] with the number of risk groups based on May and Hosmer [37]. Kaplan–Meier survival curves were created and log-rank statistics were calculated. Schoenfeld residuals were calculated for all models to assess a significant departure from the model assumption. We analysed the primary outcome in the modified intention-to-treat population, which included all randomized patients with the exception of patients for whom INR data were not available. Statistical analysis was done using IBM SPSS Statistics 23.0.0 (IBM, USA) and STATA 14 (StataCorp LP, College Station, TX, USA) software.

Results

We enrolled 103 patients after recent cardioembolic stroke who met the inclusion criteria. These were randomized and genotyped; 88 patients were included in the final analysis - 41 in the LDG and 47 in the MDG. Detailed reasons for exclusion or discontinuation are given in Fig. 1. The mean age of study population was 68.5 years (SD 13.6) with 40 men and 48 women included. Gender, weight, and height were not statistically different between the groups. There were no statistically significant differences in comorbid conditions (ischaemic heart disease, hypertension, hyperlipidaemia, diabetes mellitus, hepatic, or renal disease) or concomitant medications (amiodarone, other antithrombotic medication, use of statins, proton pump inhibitors, antidepressants, number of antihypertensive agents) between the compared groups. The only significant difference was a higher frequency of smokers in the LDG (Table 2). The genetic profile of the study population showed no

Fig. 1 Patient disposition and workflow

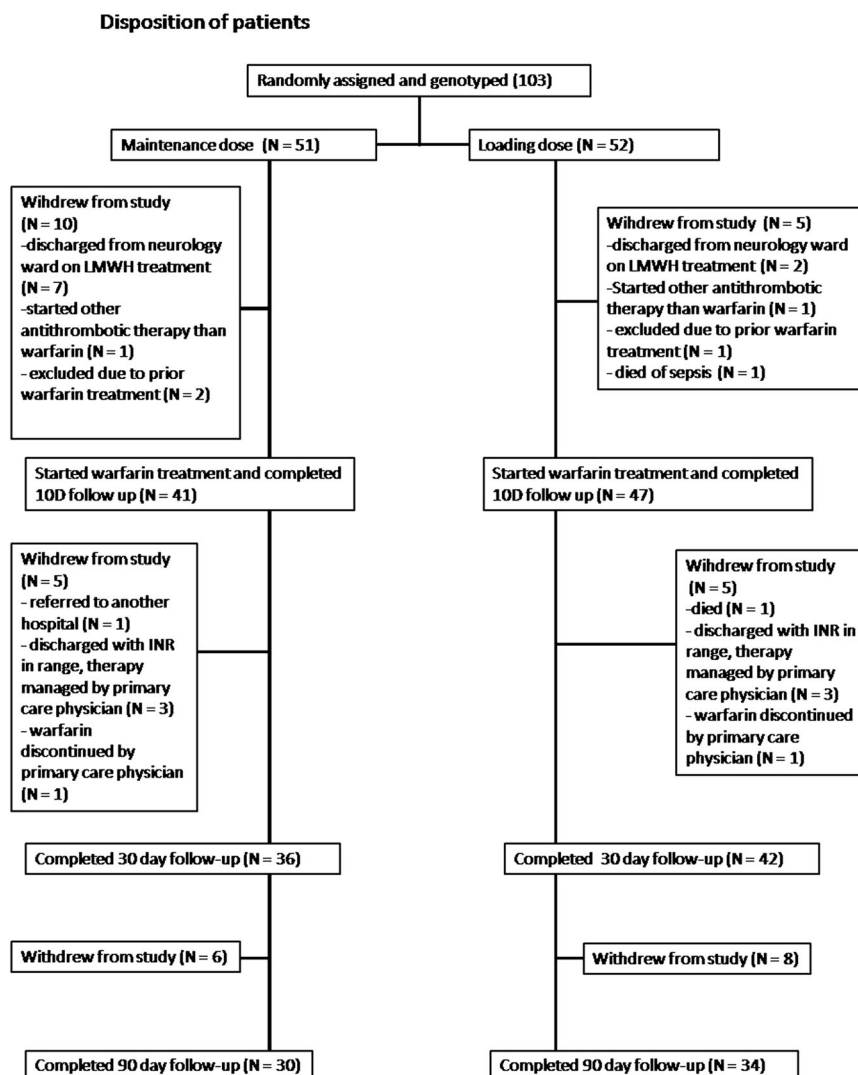


Table 2 Demographics and clinical characteristics

Variable	LDG	MDG	All patients	Groups differed significantly ($p < 0.05$)*
Subjects, No. (%)	41 (46.6%)	47 (53.4%)	88	
Men, No. (%)	20 (48.8%)	21 (44.7%)	41 (46.6%)	0.701
Age, mean (SD), years	67.6 (13.7)	69.2 (13.6)	68.5 (13.6)	0.580
Age >80 years, No. (%)	9 (22.0%)	8 (47.1%)	17 (19.3%)	0.559
Height, mean (SD), cm	170.0 (2.1)	170.0 (9.9)	170.0 (10.0)	0.998
Weight, mean (SD), kg	83.6 (19.3)	84.3 (18.2)	84.0 (18.6)	0.864
<i>Comorbid conditions, No. (%)</i>				
Hypertension	29 (70.7%)	33 (70.2%)	62 (70.5%)	0.958
Ischaemic heart disease	27 (65.9%)	29 (61.7%)	56 (63.6%)	0.686
Smoking	14 (34.1%)	4 (8.5%)	18 (20.5%)	0.003*
Hyperlipidaemia	21 (51.2%)	24 (51.1%)	45 (51.1%)	0.998
Type 2 diabetes mellitus	11 (26.8%)	10 (21.3%)	21 (23.9%)	0.542
Peptic ulcer disease	2 (4.9%)	2 (4.3%)	4 (4.5%)	>0.999
Hepatic disease	4 (9.8%)	3 (6.4%)	7 (8%)	0.700
Renal insufficiency	4 (9.8%)	5 (10.6%)	9 (10.2%)	>0.999
<i>Concomitant medication</i>				
Amiodarone, No. (%)	4 (9.8%)	2 (4.3%)	6 (6.8%)	0.411
Statin, No. (%)	29 (70.7%)	36 (76.6%)	65 (73.9%)	0.532
PPI, No. (%)	11 (26.8%)	10 (21.3%)	21 (23.9%)	0.542
SSRI No. (%)	6 (14.2%)	7 (14.9%)	13 (14.8%)	0.973
Antiplatelet drugs (acetylsalicylic acid), No. (%)	6 (14.6%)	3 (6.4%)	9 (10.2%)	0.294
Diuretics, No. (%)	11 (26.8%)	12 (25.5%)	23 (26.1%)	0.890
OAD, No. (%)	6 (14.6%)	8 (17.0%)	14 (15.9%)	0.760
Number of antihypertensive agents, mean (SD)	1.44 (1.58)	1.45 (1.40)	1.44 (1.47)	0.980
<i>CYP2C9 genotype, No. (%)</i>				
*1/*1	27 (65.9%)	31 (66%)	58 (65.9%)	0.992
*1/*2	6 (14.6%)	6 (12.8%)	12 (13.6%)	0.799
*1/*3	6 (14.6%)	10 (21.3%)	16 (18.2%)	0.420
*2/*2	1 (2.4%)	0 (0.0%)	1 (1.1%)	0.466
*2/*3	1 (2.4%)	0 (0.0%)	1 (1.1%)	0.466
*3/*3	0	0	0	
<i>VKORC1 genotype, No. (%)</i>				
BB haplotype (high dose)	18 (43.9%)	23 (48.9%)	41 (46.6%)	0.637
AB haplotype	19 (46.3%)	20 (42.6%)	39 (44.3%)	0.721
AA haplotype (low dose)	4 (9.8%)	4 (8.5%)	8 (9.1%)	>0.999
<i>Total number of variant alleles CYP2C9 and VKORC1, No. (%)</i>				
No variant allele	13 (31.7%)	16 (34.0%)	29 (33.0%)	0.816
1 variant allele	17 (41.5%)	22 (46.8%)	39 (44.3%)	0.615
2 or more variant alleles	11 (26.8%)	9 (19.1%)	20 (22.7%)	0.391
<i>Follow-up, No. (%)</i>				
Follow-up 30 days	36 (87.8%)	43 (91.5%)	79 (89.8%)	0.728
Follow-up 90 days	30 (73.2%)	34 (72.3%)	64 (72.7%)	0.930
Dose adjustments day 1–10, mean (SD)	0.88 (1.36)	0.87 (1.12)	0.88 (1.23)	0.983

Using the χ^2 -test, Fisher's exact test or Mann–Whitney U -test as appropriate

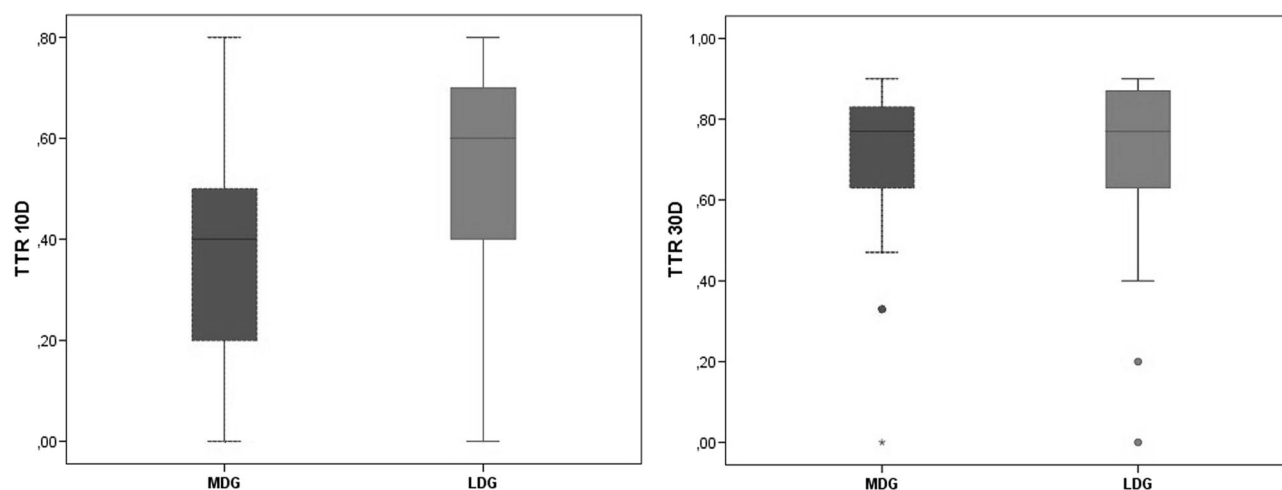


Fig. 2 Comparison of time in therapeutic range in the first 10 days (left) and the first 30 days (right) in loading dose (LDG) and maintenance dose (MDG) groups. This is illustrated by box plots where the

horizontal line inside each box indicates the median, and the top and bottom of the box indicates the interquartile range. The I bars indicate the 5th and 95th percentiles

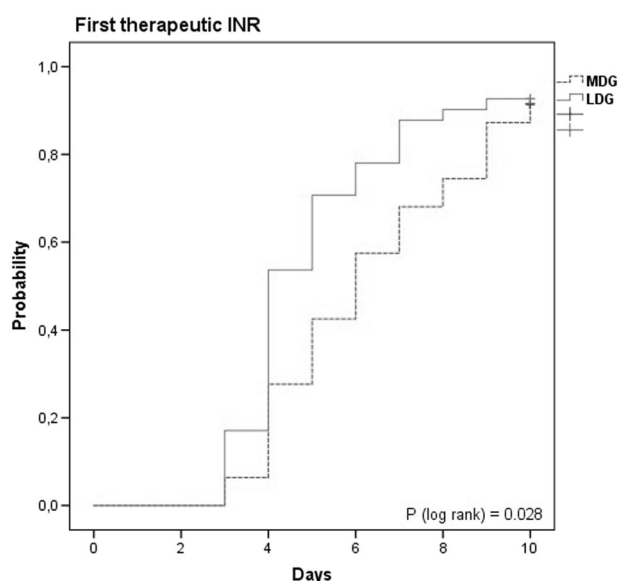


Fig. 3 Cumulative Kaplan–Meier estimates of the time to the first INR in therapeutic range in the loading dose (LDG) and maintenance dose (MDG) groups

statistically significant difference either in *CYP2C9* or *VKORC1* genotype frequencies between the groups. The mean loading dose of warfarin was 8.91 mg (4–16 mg) in the LDG, and the mean maintenance dose of warfarin was 4.64 mg (2–9 mg) in the MDG. The number of warfarin dose changes in the first 10 days did not differ between groups.

The primary endpoint of our study the time in the therapeutic range (TTR) in the first 10 days of the treatment was significantly higher in the LDG ($50.5\% \pm 3.6\%$) than in the MDG ($38.3\% \pm 4.1\%$, $p = 0.003$) (Fig. 2).

Patients in the LDG reached therapeutic range faster than the patients in the MDG—the mean time to the first

INR in therapeutic range was 5.24 days (± 0.37) in the LDG and 7.3 days (± 1.09) in the MDG ($p = 0.008$). The Kaplan–Meier curves for the time required to reach the first therapeutic INR varied significantly between the groups (log-rank test, $p = 0.028$) (Fig. 3). The difference between groups in terms of effective anticoagulation was significant by day 5 (61.0% vs. 31.9%), but was not longer statistically significant on day 10 (92.7% vs. 91.5%). The odds ratio for having therapeutic INR by day 5 for the LDG compared to the MDG was 3.33 (95% CI, 1.39–8.02, $p = 0.007$). More detailed results are given in Table 3, median INRs in the first 10 days of treatment are presented in Fig. 4.

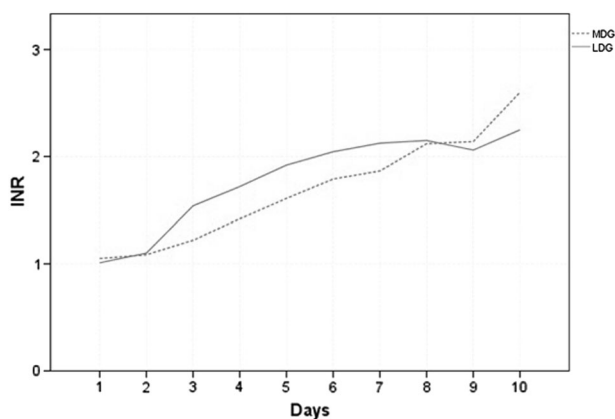
In contrast to the significantly higher TTR in the first days of treatment in the LDG, the TTR in the first 30 days of treatment was similar in both groups: 71.5% ($\pm 4.6\%$) in the LDG and 70.0% ($\pm 3.7\%$) in the MDG, $p = 0.275$, (Fig. 2).

Univariate analysis results are given in Table 4. Patients over 80 are less likely to have INR in the range with a HR of 0.51 (95% CI 0.28–0.93, $p = 0.028$). In the final adjusted Cox model, only age above 80 years and loading dose were included based on predefined parameters. The remaining factors did not reach significance. The adjusted hazard ratio for INR in therapeutic range in the LDG compared to the MDG patients was HR_{adj} 1.66 (95% CI 1.06–2.60, $p = 0.026$). The Grønnesby and Borgan goodness-of-fit test results failed to reach significance ($p = 0.59$) indicating a good overall fit of the final model.

There was no statistically significant difference in cumulative occurrence of INRs above therapeutic range in the first 10 days of treatment—neither in INRs above 4 nor above 5 (Table 3). The mean time spent above therapeutic range in the first 10 days was 5.1% ($\pm 1.8\%$) in the LDG and

Table 3 Secondary endpoints. Odds ratio for LDG compared to MDG

Variable	LDG	MDG	All patients	Odds ratio (95% confidence interval), statistical significance
INR day 5 in therapeutic range, No. (%)	25 (61.0%)	15 (31.9%)	40 (45.5%)	3.33 (1.39–8.02), $p = 0.007$
INR day 10 in therapeutic range, No. (%)	38 (92.7%)	43 (91.5%)	81 (92.0%)	1.18 (0.25–5.60), $p = 0.837$
INR >3.3 during completed follow-up, No. (%)	9 (22.0%)	15 (32.6%)	24 (27.2%)	0.58 (0.22–1.52), $p = 0.269$
INR >3.3 in the first 10 days of treatment, No. (%)	8 (20.0%)	13 (28.3%)	21 (24.4%)	0.64 (0.23–1.74), $p = 0.376$
INR >3.3 in the first 5 days, No. (%)	3 (7.3%)	2 (4.3%)	5 (5.7%)	1.78 (0.28–11.19), $p = 0.541$
INR >4 during completed follow-up, No. (%)	4 (9.8%)	10 (21.3%)	14 (15.9%)	0.40 (0.12–1.39), $p = 0.149$
INR >5 during completed follow-up, No. (%)	2 (4.9%)	5 (10.6%)	7 (8.0%)	0.43 (0.08–2.35), $p = 0.331$

**Fig. 4** Median INR in the first 10 days of treatment in the loading dose (LDG) and maintenance dose (MDG) groups

6.6% ($\pm 0.2\%$) in the MDG, $p = 0.664$. There was no statistically significant difference between the study groups in terms of thromboembolic or bleeding complications. Further analyses were not done due to the low incidence of both bleeding and thromboembolic complications in the study.

We observed four cases of thromboembolic events. A male aged 78 years in the MDG suffered from recurrent ischaemic stroke (multiple acute infarctions on CT scan) on day 7 of warfarin treatment (INR 3.82) and died 4 days later due to multiple organ failure. Two females, 76 and 60 years, both in the MDG, experienced recurrent ischaemic stroke (TIA in both cases) in the second and third month of warfarin treatment, respectively. A 66-year-old male in the LDG suffered from a non-fatal myocardial infarction 3 months after warfarin treatment initiation.

There were two cases of clinically relevant bleeding complications. Neither were classified as major, and both were classified as clinically relevant non-major bleed according to the ISTH. Both appeared in the loading dose group with an INR below the therapeutic range in the time of bleeding. However, both patients were treated with concomitant bridging low-molecular heparin. A 64-year-old male in the LDG with warfarin treatment initiated 3 days

Table 4 Univariate Cox analysis results for the first INR in range in the first 10 days

Variable	Statistical significance, p	HR	CI 95% lower	CI 95% upper
Age as continuous variable	0.072	0.99	0.97	1.00
Age >80 years	0.028	0.51	0.28	0.93
Sex male	0.526	1.15	0.74	1.79
Height	0.429	1.01	0.99	1.03
Weight	0.606	1.00	0.99	1.02
Loading dose	0.060	1.49	0.82	2.72
Total count of variant alleles	0.674	0.95	0.73	1.22
Dose adjusted	0.520	1.16	0.74	1.80
Hypertension	0.301	0.78	0.48	1.26
Type 2 diabetes mellitus	0.620	1.14	0.69	1.89
Hyperlipidaemia	0.539	0.87	0.56	1.35
Peptic ulcer disease	0.570	1.34	0.49	3.68
Hepatic disease	0.904	0.95	0.41	2.18
Renal insufficiency	0.501	1.29	0.62	2.67
Ischaemic heart disease	0.555	0.87	0.55	1.36
Smoking	0.357	1.29	0.75	2.20
Statin	0.171	0.71	0.43	1.16
Proton pump inhibitors	0.736	1.09	0.66	1.81
Selective serotonin reuptake inhibitors	0.371	0.75	0.40	1.41
Diuretics	0.248	0.74	0.45	1.23
Oral antidiabetic drugs	0.777	1.09	0.60	1.97
Antiplatelet drugs (acetylsalicylic acid)	0.663	0.85	0.41	1.77
Amiodarone	0.808	0.90	0.39	2.08

after cardiac pacemaker implantation had to discontinue treatment after 11 days due to a pocket haematoma (INR 1.5, anti-Xa assay 0.66 IU/mL). Another male, 64 years in the LDG, suffered from a significant blood loss due to upper gastrointestinal bleeding on day 24 (INR 1.24, anti-Xa assay 0.35 IU/mL).

Discussion

To the best of our knowledge, this is the first study comparing a pharmacogenetically individualised loading dose (double the estimated maintenance dose) to the pharmacogenetically predicted maintenance dose solely in this highly vulnerable segment of the population, i.e., stroke survivors. We focused mainly on the very first 10 days of treatment and the efficacy of achieving therapeutic anticoagulation. It is critical to avoid overcoagulation because ischaemic stroke patients have an increased risk of bleeding complications. On the other hand, the pressure to minimize costs leads providers to minimize hospitalization time by achieving therapeutic anticoagulation more quickly.

Previous studies showed that a higher initiation dose gets the patient to the therapeutic range more quickly. However, this implies an increased risk of overcoagulation. When a 10 mg loading dose was administered on the first 2 days of treatment, a considerable number of patients had INR above 3 on day four. This was 36% in the study by Harrison et al. [3], 24% in Crowther et al. [4], and 6% in Fennerty et al. [6]. Our trial used a pharmacogenetic algorithm and only 1 patient (1.1%) had an INR above the therapeutic range on day four. The effect of pharmacogenetically estimated loading dose in our study seems to get patients to therapeutic range more quickly and safely in the first 10 days than the standard 5 mg daily. In the Kovacs study, only 46% of patients were in range by day 5 compared to 61% of patients in our loading dose group [5].

Our results are quite comparable to another study applying pharmacogenetic algorithm. Gong et al. [18] reported that 60.5% patients reached therapeutic range by day six. In our study 68.3% patients had effective treatment by day six. We found no significant difference between the LDG and MDG concerning the 30-day TTR—the mean TTR was 71.5% and 70.0%, respectively. Our results are consistent with high 30-day (or 4 weeks) TTRs in recent studies evaluating pharmacologically guided dosing: 70.2 and 67.5% for PG algorithms in the CoumaGen-II study [19], 54.6% in the genotype-guided group in 4 weeks of treatment in the EU-PACT study [22] and 68.1% in the WRAPID (Warfarin Regimen using A Pharmacogenetics-guided Initiation Dosing) protocol by Gong et al. [18].

Most importantly, only 2 patients encountered bleeding complications over the 90-day follow-up. Even though both cases occurred in the loading dose group, we did not observe an increased risk of overcoagulation because in both cases the INR was below the therapeutic range. There was no difference in the frequency of thromboembolic complications. While more frequent in the MDG these were not statistically significant and mostly appeared after the first 30 days of treatment. The only patient with thromboembolism in the first 10 days of treatment suffered a

recurrent stroke when he was effectively anticoagulated (INR 3.82).

A major limitation of our study is its monocentric design and using a non-stratified simple randomization procedure. The source of potential bias could also be the relatively high percentage of patients discontinuing treatment or with incomplete follow-up. However, we believe that coverage of patient data in the first 10 days of treatment makes our results valid because the primary outcome of our study was TTR in this critical time point. We consider the higher frequency of smokers in the LDG coincidental and with no major influence on the results. Our study was carried out on a completely Caucasian population, and we cannot generalize it to other ethnic groups.

Based on our dataset, we conclude that use of a loading dose guided by pharmacogenetics (double the estimated maintenance dose) after recent cardioembolic stroke improved the efficacy of warfarin initiation compared to the pharmacogenetically predicted maintenance dose without increasing the risk of adverse events. Our presumption that with an adjusted dose-prediction algorithm a loading dose equal double the estimated dose could be safely administered needs to be validated by further studies. Although newer anticoagulant agents (xabans, gatrans, etc.) may overcome warfarin in secondary prevention of cardioembolic stroke due to the non-valvular atrial fibrillation, applying these agents for other indications (mechanical valves, cerebral sinus thrombosis, etc.) is problematic due to the lack of evidence-based data from clinical trials.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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