

The Effect of *ABCB1* and *CES1* Polymorphisms on Plasma Levels of Dabigatran and Risk of Hemorrhagic Complications in Ischemic Stroke Patients

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Background: Dabigatran directly inhibits thrombin and is used in primary and secondary stroke prevention in individuals with nonvalvular atrial fibrillation. The prodrug dabigatran etexilate is absorbed by enteral P-glycoprotein (*ABCB1*) and then activated by hepatic and intestinal carboxylesterases (*CES1*) to produce active metabolites. Variations in dabigatran metabolism because of genetics may affect concentration levels and clinical outcomes.

Study Question: We conducted a study to assess how polymorphisms in the *CES1* (rs2244613) and *ABCB1* (rs4148738) genes affect the through plasma level ($c_{\min}$) of dabigatran and its correlation to clinical outcomes.

Study Design: Retrospective multicentric study of consecutive patients on dabigatran therapy. Examination of *CES1* rs2244613 and *ABCB1* rs4148738 polymorphisms, $c_{\min}$ 12 hours after administration, clinical follow-up (ischemic stroke, major or clinically relevant hemorrhage, myocardial infarction, other thromboembolism, and death).

Measures and Outcomes: A total of 432 patients received treatment for an average of 19.78 months (SD of 20.165). The sex distribution of the patients was 56.5% male, and the average age was 67.56 years (SD of 14.7). The *ABCB1* variant genotype was present in 67.8% of patients, whereas 37.5% carried the *CES1* polymorphism.

Results: Compared with wild-type patients, patients with the *CES1* variant had significantly lower dabigatran plasma levels (with a mean difference of 16.986; 95% confidence interval, 5.794–28.178 ng/mL, $P = 0.003$). We also found a significant risk of major bleeding in patients carrying the *ABCB1* rs4148738 allele (hazard ratio = 1.99, confidence interval 95% 1.10 to 3.59, $P = 0.024$).

Conclusions: The *CES1* variant genotype rs2244613 is closely linked with reduced $c_{\min}$ of dabigatran. Carriers of the *ABCB1* rs4148738 polymorphism exhibit a tendency toward higher plasma levels of dabigatran, which leads to a significantly increased risk of bleeding.

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INTRODUCTION

According to a recent report of the World Health Organization, ischemic stroke ranked as the second leading cause of mortality and long-term disability worldwide.¹ Anticoagulation is recommended for all patients with nonvalvular atrial fibrillation (NVAF), accounting for more than 30% of all ischemic stroke cases.² Anticoagulation is also recommended for patients with cerebral venous thrombosis, those who have undergone bioprosthetic valve replacement, and to prevent embolization from intracardiac thrombi.^{3–5}

Direct oral anticoagulants (DOACs) such as gatrans and xabans are the preferred option for anticoagulation in primary or secondary prevention of ischemic stroke if indicated according to guidelines. Direct thrombin inhibitor dabigatran has demonstrated efficacy in preventing ischemic stroke and systemic embolization in the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) trial compared with warfarin.⁶ Although various studies indicate that high or low plasma levels of DOACs can increase the risk of complications such as bleeding or ischemic events,^{7–10} it is not recommended to measure these plasma levels as a preventive measure. The measurement of dabigatran plasma levels is only necessary and advised in cases of treatment complications, such as bleeding or suspected overdose, or treatment failure before administering intravenous thrombolysis for ischemic stroke, during acute surgery, or in cases of noncompliance suspicion events.⁴

Dabigatran etexilate, a prodrug of the direct thrombin inhibitor dabigatran, is taken orally and absorbed in the intestines by the ATP-binding cassette subfamily B member 1 (ABCB1) protein, which serves as an efflux transporter membrane protein and is encoded by the *ABCB1* gene. Dabigatran etexilate undergoes metabolism through 2 enzymes, CES1 and CES2 isoforms, primarily present in the liver and small intestine. CES2 is predominantly located in the small intestine, whereas CES1 metabolizes dabigatran etexilate in the endoplasmic reticulum of liver cells.^{11,12} This leads to the production of the metabolites BIBR 951 and BIBR 1087, which are then hydrolyzed to form active dabigatran.¹³

Genetic variations in the *ABCB1* and *CES1* genes may affect the metabolism of dabigatran, resulting in varying drug levels among individuals. Such variability can heighten the risk of ischemic events, such as stroke in lower plasma levels; excessively high levels

can lead to bleeding complications. Clinical trials so far have conflicting results concerning levels and clinical outcomes^{12,14–17}; thus, we aimed to evaluate the impact of *ABCB1* and *CES1* gene polymorphisms on drug levels and clinical outcomes, such as stroke recurrence, transient ischemic attack (TIA), and clinically relevant and major bleeding complications.

MATERIAL AND METHODS

Study settings and design

We conducted a retrospective observational multicentric study enrolling patients treated with dabigatran etexilate between 2019 and 2022. Study subjects were recruited from consecutive consenting patients referred from Comprehensive stroke centers (Department of Neurology in University Hospital Motol and Military University Hospital, Prague, Czech Republic) for pharmacogenetic testing to the molecular genetics laboratory (Hospital Na Homolce, Prague, Czech Republic). This study was approved by the Ethics Committee of the University Hospital Motol and 2nd Faculty of Medicine, Charles University in Prague (EK-1263.1.25/19). All patients signed informed consent.

Study population

To be eligible for inclusion, patients needed to have been diagnosed with ischemic stroke verified on magnetic resonance of brain (acute lesion with restriction of diffusion), and the patient was started on dabigatran in the secondary prevention of stroke. Dabigatran was initiated after the ischemic stroke according to the “Diener rule” depending on the severity of the stroke.¹⁸ Patients were older than 18 years, and other criteria strictly followed therapeutic indications according to European Medicines Agency.⁴ All patients received treatment in adherence to the national guidelines.¹⁹ If through dabigatran levels were detected to be above or below the therapeutic range (50–200 ng/mL), the patient’s safety was ensured by modifying the therapy. This may include reducing the dosage or changing to a different DOAC.^{4,20–22}

Detailed clinical characteristics of the patients were collected, including arterial hypertension, diabetes mellitus, dyslipidemia, NVAF before the stroke, ischemic heart failure, myocardial infarction, peripheral arterial disease, and coadministration of dabigatran inhibitors or inductors, as well as coadministration of antiplatelet

medication (Table 1). Renal function was evaluated using the Cockcroft–Gault equation to determine creatinine clearance. Mortality, thromboembolic complications, such as stroke, TIA, myocardial infarction, or deep vein thrombosis, as well as bleeding events, were documented during dabigatran treatment. The clinically relevant bleeding events were defined as signs of hemorrhage that require medical intervention by a health care professional, leading to hospitalization, increased level of care, or prompting a face-to-face evaluation. These criteria, together with the International Society on Thrombosis and Haemostasis definition of major bleeding, are

recommended in atrial fibrillation and nonsurgical venous thromboembolism studies.²³

The primary outcome was the occurrence of hemorrhage, including intracranial. The secondary outcome was any ischemic event, defined as a composite of ischemic stroke/TIA, systemic embolism, myocardial infarction, deep vein thrombosis, and pulmonary embolism.

Selection of SNPs and genotyping, dabigatran plasma concentration analysis

The single nucleotide polymorphisms (SNPs) were selected based on studies that indicate their potential

Table 1. Demographics and clinical characteristics.

Parameter	Total cohort (n = 432)	Without major bleeding (n = 407)	Patients with major bleeding (n = 25)	Statistical significance— major bleeding versus without major bleeding, <i>P</i> —Fisher, chi, <i>t</i> test
Male, n (%)	244 (56.5%)	233 (57.2%)	11 (44.0%)	0.195
Age (years), (SD), range	67.56 (14.7), 18–96	66.9 (14.8)	78.1 (8.3)	<0.001*
Follow-up (months), (SD)	19.78 (20.165)	19.88 (19.99)	18.08 (23.27)	0.708
Death from any cause, n (%)	52 (12.0)	45 (11.1)	7 (28.0)	0.021*
Reduced dose of dabigatran (110 mg twice a day)*, n (%)	141 (32.6)	131 (32.2)	10 (40)	0.419
Weight (kg), (SD)	83.48 (16.79)	83.73 (16.8)	79.48 (15.2)	0.187
Creatinine clearance (mL/s), (SD)	1.66 (0.63)	1.69 (0.63)	1.26 (0.47)	<0.001*
History of hypertension, n (%)	328 (75.9)	308 (75.7)	20 (80)	0.624
History of diabetes mellitus, n (%)	98 (22.7)	87 (21.4)	10 (40)	0.030*
History of dyslipidemia, n (%)	211 (48.8)	196 (48.2)	15 (60)	0.250
History of afib before stroke, n (%)	159 (36.8)	148 (36.4)	11 (44)	0.442
History of ischemic heart failure, n (%)	38 (8.8)	35 (8.6)	3 (12)	0.560
History of myocardial infarction, n (%)	96 (22.2)	89 (21.9)	7 (28)	0.474
History of peripheral arterial disease, n (%)	29 (6.7)	24 (5.6)	5 (20)	0.006*
Coadministration inhibitors, n (%)	14 (3.2)	13 (5.9)	1 (4)	0.572
Coadministration of aspirin, n (%)	2 (0.5)	1 (0.24)	1 (4)	0.079
Coadministration of clopidogrel, n (%)	18 (4.2)	16 (3.9)	2 (8)	0.374
Trough concentration (ng/ mL) (SD)	91.84 (62.25)	89.59 (60.10)	128.4 (83.655)	0.031*

*Patients not taking 110 mg were treated with dose of 150 mg twice a day.

impact on plasma levels and clinical outcomes. These include *CES1* rs2244613 and *ABCB1* rs4148738.^{12,15,24,25}

Genomic DNA was extracted from 300 mL of whole-blood ethylenediaminetetraacetic acid (EDTA)/K2 samples obtained from patients using the PureGene Blood kit under the manufacturer's instructions. DNA concentrations and purity were measured by DeNovix spectrophotometer using the dsDNA program. The genotyping of polymorphisms *CES1* rs2244613 and *ABCB1* rs4148738 was performed by restriction fragment length polymorphism analysis combined with high resolution melting visualization. The polymerase chain reaction was performed in 15-mL reactions using LightScanner Master Mix under the manufacturer's instructions, with subsequent restriction with BstNI and BsrI restriction endonucleases. The 5'-3' primers for *CES1* rs224613 were F: TTCACCATTGAGGCAGGA and R: AGCAATGCACTGAAATAGATT for *ABCB1* rs4148738 F: GGACTGTTGGATCTACTTTA and R: TTCATTGTAAAGACTAGGGTTG. The reaction temperature for denaturation for *CES1* was 95°C, for annealing 68°C and extension 72°C, each lasting 30 minutes, in total 40 cycles. The same procedure applied to *ABCB1*; only annealing was performed at 62°C.

The trough plasma level (C_{trough}) of dabigatran was detected using the liquid chromatography-tandem mass spectrometry (LC-MS/MS) method precisely 12 hours after the last dose intake. The concentrations of dabigatran in human plasma were measured using high-performance liquid chromatography—tandem mass spectrometry. The method uses protein precipitation as a sample preparation technique, reversed-phase liquid chromatographic separation, and tandem mass spectrometric detection. Briefly, plasma proteins were precipitated directly with the solution of isotopically labeled dabigatran, and after centrifugation, the supernatant was diluted with ammonium acetate solution. The separation was performed on a column packed with C18 stationary phase; the mobile phase consisted of methanol—20-mM ammonium acetate (30:70 vol/vol). Ionization of analytes was performed using electrospray ionization technique with positive polarity, and selected reaction monitoring was used to monitor the transitions m/z 472.2 $\rightarrow$ 289.0 for dabigatran and m/z 475.2 $\rightarrow$ 292.0 for dabigatran-D₃, respectively.

The method was validated according to the internationally accepted guidelines: Intra-assay and inter-assay precision and accuracy were better than 8%, and the linearity range was 18.7–1900 ng/mL. Stability tests indicated no significant concentration changes during sample preparation and storage. Moreover, the laboratory participated in the external quality

assessment of the dabigatran measurement with satisfactory results.

Statistical analysis

Predefined subgroups based on the polymorphisms rs4148738 in gene *ABCB1* and rs2244613 in gene *CES1* were wild-type (WT), heterozygote, homozygote, and variant allele carrier. Using χ^2 tests for categorical variables and Mann–Whitney tests for continuous variables, baseline characteristics were compared between the major bleeding and control groups. Analyzed continuous variables had normal distribution and hence were presented as mean and SD as appropriate. The occurrence of outcome events was reported as incidence rates, events per 100 patient-years. We used the χ^2 test to check whether the allele frequencies of variants were in Hardy–Weinberg equilibrium. Plasmatic dabigatran levels among the respective genotypes were compared using one-way analysis of covariance test with testing for relevant assumptions (homogeneity of variances, distribution). The Bonferroni post hoc test was used for all subgroup pairwise comparisons.

The associations between variant genotypes and the primary outcome event were evaluated using survival analysis techniques. We used Cox proportional hazards models to compute a hazard ratio (HR) with a 95% confidence interval (CI), both unadjusted and adjusted for the potential confounding covariates. Because of the possibility of numerically unstable estimates and large standard error, we did not include all available covariates in the final Cox proportional hazards model. For potentially clinically meaningful comparisons, age was assessed as a categorical variable with age below and above 75 years as it is the age indicated for a reduced dose of dabigatran. A backward stepwise elimination algorithm with likelihood ratio statistic to minimize the exclusion of predictors involved in suppressor effects was therefore used. Variables with a value of $P \leq 0.1$ on univariate testing were included in the elimination algorithm. In all analyses, the follow-up time was restricted to 97 months. In addition, 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's assumption. All statistical analysis were performed using IBM SPSS Statistics 27.0.0 (IBM) software.

RESULTS

A total of 432 patients after ischemic stroke were enrolled in the study. Of the total, 56.5% were male, and the average age was 67.56 years (SD of 14.7). The

mean duration of dabigatran use, which determined the follow-up time, was 19.78 months (SD of 20.165). The total follow-up time of the cohort was 804 patient-years. All patients received treatment with dabigatran following the guidelines, with 32.6% receiving a reduced dose (110 mg twice a day [BID]). In majority of cases (397 patients, 91.9%), stroke was caused by NVAf and cardioembolization, the rest by dissection of carotid or vertebral arteries (15 patients, 3.5%) and cerebral venous sinus thrombosis (20 patients, 4.6%). The group's mean weight was 83.48 kg (SD of 16.79), and the creatinine clearance was 1.66 mL/s (SD of 0.63). Table 1 describes all other characteristics.

During the follow-up time, the incidence of major bleeding events in patients treated with dabigatran was 3.1 (95% CI, 2.0–4.6) per 100 patient-years. Of 25 recorded clinically relevant bleeding incidents, 12 patients experienced gastrointestinal bleeding, 4 had severe hematuria, 3 had intracranial bleeding (including 1 instance of subarachnoid hemorrhage), 2 had hemoptysis, 3 experienced severe subcutaneous bleeding and suffusions leading to the discontinuation of treatment, and 1 patient had gynecological bleeding. Patients who had clinically significant bleeding were treated with dabigatran for a median duration of 14 months before this end point (SD 19.16, interquartile range [IQR] 6–22 months).

The studied population revealed an occurrence of 4.7 (95% CI, 3.4–6.5) thromboembolic events (including ischemic stroke, TIA, myocardial infarction, or deep vein thrombosis) per 100 patient-years, 38 events in total during follow-up time. Of these patients, 32 had a stroke or TIA, 5 had a myocardial infarction, and only 1 reported having deep vein thrombosis. Ischemic event patients were treated with dabigatran for a median of 16.5 months (SD 24.12, IQR 8–40 months) before reaching the end point.

Mortality was observed in 4.9 (95% CI, 3.5–6.5) per 100 patient-years. Of 39 reported deaths during dabigatran therapy, 22 (56.4%) were attributed to other specific causes, such as sepsis, COVID-19, generalized cancer, and hepatic or renal failure. Nine deaths (23.1%) were classified as undetermined causes. The remaining deaths were caused by heart failure or malignant arrhythmia (3, 7.7%), ischemic stroke (3, 7.7%), or intracranial hemorrhage (2, 5.1%). In the group of patients with bleeding, there were in total 3 deaths (12%)—2 of them caused by intracranial hemorrhage (8%) and 1 by sepsis (with gastrointestinal tract hemorrhage as a complication). These patients experienced hemorrhage after being on dabigatran therapy for 5, 21, and 24 months after minor strokes (The National Institutes of Health Stroke Scale 1, 3, and 5 points).

Among the 407 patients in the group without bleeding, 36 patients died. Of those, 21 died because of another specific cause (5%), and 9 died because of an unknown cause (2.2%). In addition, 3 patients died because of arrhythmia or heart failure, and the same number died because of ischemic stroke (0.7%). Patients without observed hemorrhage were treated with dabigatran for a median of 18.5 months (SD 21.66, IQR 7.5–30.5) before death.

When the plasmatic level of dabigatran was measured, 80% of all patients were kept on the original dose of dabigatran. Only 5% of patients experienced high dabigatran levels and were subsequently prescribed a lower dosage. On the other hand, 3% of patients who were initially taking 110 mg BID and had low levels were prescribed 150 mg BID. The remaining 12% were prescribed alternative medication because of high levels on 110 mg BID, low levels on 150 mg BID, or adverse effects from the treatment. In case of a switching to different medication, patients were excluded from further follow-up.

All enrolled patients were tested for *CES1* rs2244613 and *ABCB1* rs4148738 polymorphisms. A total of 270 patients carried the *CES1* rs2244613 TT genotype, which is considered the WT. In addition, there were 138 individuals with the GT genotype and 24 homozygous carriers of the GG genotype. The *ABCB1* rs4148738 SNP was observed in 139 patients with a WT TT genotype, 217 patients with a CT genotype, and 76 patients with a homozygous CC genotype.^{26,27} The representation of all variants (WT/heterozygote/homozygote) for both genes and dabigatran plasma levels is shown in Table 2. All patients provided blood samples for the trough plasma levels. Using one-way analysis of covariance, we obtained an adjusted plasmatic level of dabigatran for creatinine clearance as a covariate.

After adjustment for creatinine clearance, there was a statistically significant difference in through plasmatic level of dabigatran among the *CES1* genotypes, $F(2, 428) = 4.447$, $P = 0.012$, partial $\eta^2 = 0.020$. Post hoc analysis was performed with a Bonferroni adjustment. Adjusted plasmatic levels were statistically significantly greater in the WT versus the heterozygotes [mean difference of 17.233 (95% CI, 2.804 to 31.663) ng/mL, $P = 0.013$] and nonsignificantly higher than in homozygotes for rs2244613 [mean difference of 15.561 (95% CI, -13.921 to 45.043) ng/mL, $P = 0.616$]. When we considered all *CES1* variant carriers together compared with the WT patients, the plasmatic levels were significantly lower (mean difference of 16.986; 95% CI, 5.794–28.178 ng/mL, $P = 0.003$) (see also Table 2).

The adjusted through plasmatic level of dabigatran among the *ABCB1* genotypes was also significantly

Table 2. Adjusted and unadjusted through plasmatic level of dabigatran for respective genotypes.

Genotype	Patients (percentage of total cohort)	Unadjusted mean through plasmatic level (ng/mL), SD	Adjusted mean through plasmatic level (ng/mL), SD
<i>CES1</i> rs2244613			
WT	270 (62.5%)	98.03 (66.74)	98.21 (3.49)
Hetero	138 (31.9%)	79.75 (49.18)	80.97 (4.89)
Homo	24 (5.6%)	91.62 (69.31)	82.64 (11.76)
Variant allele carriers	162 (37.5%)	81.51 (52.56)	81.22 (4.50)
<i>ABCB1</i> rs4148738			
WT	139 (32.2%)	88.71 (58.38)	88.220 (4.86)
Hetero	217 (50.2%)	87.14 (59.63)	87.714 (3.89)
Homo	76 (17.6%)	110.96 (72.81)	110.212 (6.58)
Variant allele carriers	293 (67.8%)	93.32 (64.25)	93.55 (3.38)

Adjusted for creatinine clearance as covariate using one-way analysis of covariance.

different, $F(2, 428) = 4.742$, $P = 0.009$, partial $\eta^2 = 0.022$. Post hoc analysis Bonferroni adjustment showed statistically significantly greater plasmatic levels of dabigatran in homozygotes for rs4148738 versus the WT (mean difference of 21.992; 95% CI, 2.337–41.647 ng/mL, $P = 0.022$) and heterozygotes (mean difference of 22.498; 95% CI, 4.131–40.865 ng/mL, $P = 0.010$). Variant allele carriers grouped together did not have significantly higher dabigatran plasmatic levels compared with noncarriers (mean difference of 5.335; 95% CI, –17.043 to 6.373 ng/mL, $P = 0.371$) (see also Table 2).

The primary unadjusted Cox proportional hazards model for the risk of major bleeding was significant for higher age (HR = 1.09, 95% CI, 1.04–1.14, $P < 0.001$), chronic renal insufficiency (HR = 3.46, 95% CI, 1.03–11.68, $P = 0.045$), and history of peripheral artery disease (HR = 3.67, 95% CI, 1.36–9.91, $P = 0.010$). Other traditional risk factors for bleeding, including hypertension, history of previous ischemic stroke, chronic heart failure, or gastroduodenal ulcer disease, were not statistically significant (see in more detail Table 3). We observed a significant risk of clinically relevant bleeding for the carriers of the *ABCB1* rs4148738 allele (HR = 1.99, 95% CI, 1.10–3.59, $P = 0.024$). When we analyzed the data according to the allele count, this result was significant for homozygotes and nonsignificant for heterozygotes. We did not see any significant risk in our cohort for carriers of *CES1* rs2244613 variant allele (see in more detail Table 4). In the final adjusted model were included the following covariates: genotype of *ABCB1* rs4148738 allele, age above 75 years, and history of peripheral artery disease. The adjusted HR of major bleeding for carriers of the

rs4148738 variant allele was 3.88 (95% CI, 1.09–13.77, $P = 0.036$). Kaplan–Meier curve was constructed (Figure 1), and a log-rank test was run to determine whether there were differences in bleeding distribution for the carriers of *ABCB1* rs4148738 polymorphism compared with noncarriers. The distributions of bleeding complications for the 2 groups were statistically different, $\chi^2 = 4.533$, $P = 0.033$.

In the unadjusted Cox proportional hazards model for the risk of all thromboembolic events (recurrent ischemic stroke, myocardial infarction, and pulmonary embolism/deep vein thrombosis), only 2 factors were significant in our cohort—history of peripheral artery disease (HR = 2.49, 95% CI, 1.03–6.04, $P = 0.044$), and concomitant use of antiplatelet medication (HR = 1.64, 95% CI, 1.01–2.64, $P = 0.044$). All other factors did not reach statistical significance, including the variant alleles in genes *CES1* and *ABCB1* (see in more detail in Tables 3 and 4).

DISCUSSION

Our study focused on the correlation between *CES1* rs2244613 and *ABCB1* rs4148738 and the plasma level of dabigatran. In 2013, Paré et al in the pharmacogenetic substudy of the RE-LY trial investigated the inter-individual variability of dabigatran in 1490 European patients with genomewide analysis, followed by the analysis of *CES1* and *ABCB1* gene polymorphisms. The most influential SNP detected was rs2244613 in the intron region of the *CES1* gene (G>T transversion), with heterozygotes for the variant having 15% and homozygotes 28% lower minimum plasma levels

Table 3. Univariate cox regression analysis for major bleeding and ischemic events.

Variable	Major bleeding			Ischemic events (recurrent ischemic stroke, myocardial infarction, and pulmonary embolism/deep vein thrombosis)		
	Hazard ratio, HR	95% CI	Statistical significance, <i>P</i>	Hazard ratio, HR	95% CI	Statistical significance, <i>P</i>
Age >75 yrs	2.867	1.265–6.500	0.012*	1.272	0.665–2.432	0.466
Sex, female	1.715	0.778–3.781	0.181	1.226	0.633–2.373	0.546
Hypertension	0.946	0.351–2.550	0.912	1.932	0.679–5.499	0.217
Diabetes mellitus	1.891	0.835–4.281	0.127	1.301	0.663–2.553	0.444
Previous stroke—before the index stroke	1.005	0.417–2.424	0.990	1.693	0.878–3.265	0.116
Ischemic heart disease	1.220	0.506–2.945	0.658	1.394	0.700–2.779	0.345
Ejection fraction of left ventricle <35%	1.379	0.409–4.645	0.604	1.123	0.397–3.173	0.827
Chronic renal insufficiency	3.462	1.026–11.679	0.045*	2.508	0.764–8.230	0.130
Weight, kg	0.982	0.956–1.008	0.169	1.008	0.989–1.027	0.401
Peripheral artery disease	3.667	1.358–9.905	0.014*	2.489	1.026–6.036	0.044*
Gastroduodenal ulcer disease	2.365	0.533–10.113	0.246	1.876	0.442–7.966	0.394
Concomitant antiplatelet medication	1.571	0.810–3.048	0.181	1.636	1.013–2.641	0.044*
Dabigatran through plasmatic level, ng/mL	1.007	1.002–1.012	0.009*	1.001	0.996–1.006	0.681

*Statistically significant findings.

compared with WT patients, which is consistent with our results.¹² However, subsequent studies have had inconsistent results showing trends without statistical significance, potentially because of a lower number of participants. Our study confirmed that the presence of the *CES1* rs2244613 polymorphism reduces the trough dabigatran levels. We have shown significantly higher plasmatic levels in WT patients when compared with heterozygotes or carriers of *CES1* rs2244613 polymorphism. The trend was also seen in comparison

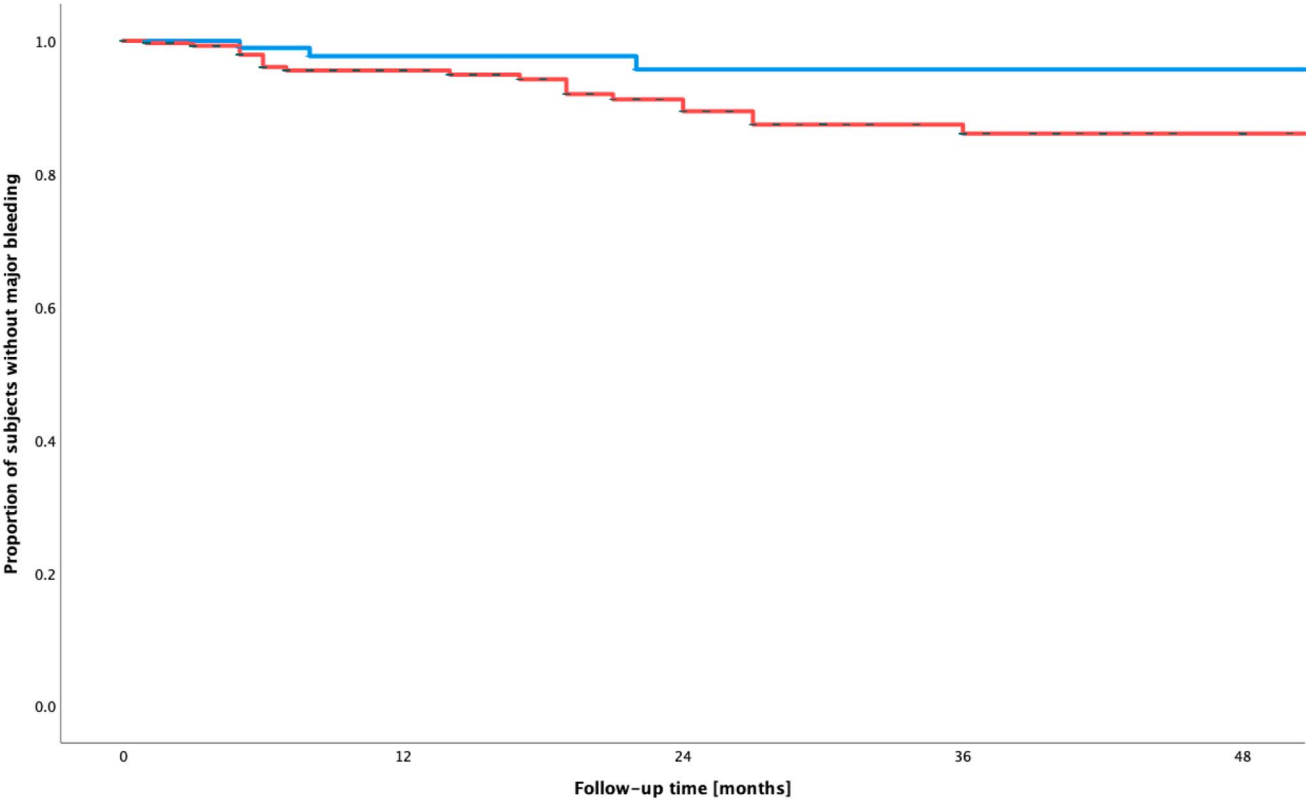
between WT and homozygotes, but it did not reach significance because of the lower number of homozygotes.

The *ABCB1* polymorphisms were studied as well, revealing even more diverse outcomes in individual studies. The individuals with the SNP *ABCB1* rs4148738 variant were found to have either higher peak or trough plasma levels or no statistically significant effect at all.^{14–16,28–30} That is also consistent with our results, showing slightly higher levels of

Table 4. Univariate Cox regression analysis for major bleeding and ischemic events (recurrent ischemic stroke, myocardial infarction, and pulmonary embolism/deep vein thrombosis) according to the genotypes of *CES1* and *ABCB1*.

Genotype	Major bleeding			Ischemic events (recurrent ischemic stroke, myocardial infarction, and pulmonary embolism/deep vein thrombosis)		
	Hazard ratio, HR	95% CI	<i>P</i>	Hazard ratio, HR	95% CI	<i>P</i>
<i>ABCB1</i> rs4148738 carrier	1.985	1.096–3.593	0.024*	1.297	0.628–2.677	0.482
<i>ABCB1</i> rs4148738 heterozygote	2.935	0.848–10.158	0.089	1.205	0.563–2.578	0.631
<i>ABCB1</i> rs4148738 homozygote	4.534	1.160–17.725	0.030*	1.614	0.632–4.121	0.317
<i>CES1</i> rs2244613 carrier	0.828	0.406–1.685	0.602	0.902	0.461–1.765	0.763
<i>CES1</i> rs2244613 heterozygote	0.631	0.333–1.947	0.631	0.894	0.439–1.819	0.757
<i>CES1</i> rs2244613 homozygote	0.739	0.098–5.566	0.769	0.951	0.224–4.040	0.945

Carriers of variant alleles are compared with noncarriers (wild-type) patients.



No. at risk	0 months	12 months	24 months	36 months	48 months
ABCB1 rs4148738 carrier	292	156	101	63	32
ABCB1 rs4148738 noncarrier	138	63	44	24	12

FIGURE 1. Kaplan–Meier risk of clinically relevant bleeding during follow-up time.

trough plasma concentration in *ABCB1* rs4148738 polymorphism carriers, but without statistical significance.

Dabigatran, being an anticoagulant, does carry the risk of developing bleeding as a potential adverse effect. Only a few studies examined correlations between patients’ clinical outcomes, drug levels, and individual polymorphisms. In the RE-LY genetic sub-study, only a nonsignificant trend toward a decrease in ischemic stroke and systemic embolization was observed in rs8192935 in the *CES1* gene. New research from 2022 on the *ABCB1* rs4148738 polymorphism has confirmed that carriers are at significant risk of bleeding.¹⁷ We confirmed in our population of stroke

patients the higher risk of hemorrhage for patients with *ABCB1* rs4148738 polymorphism. Based on our findings, no significant correlation was observed between the recurrence of ischemic events and SNPs in the patients we examined.

Numerous studies have been conducted to measure the plasma levels of DOACs (dabigatran, apixaban, and rivaroxaban). These studies have found that patients with high concentrations of DOACs are more likely to experience bleeding complications during DOAC treatment.^{8,9} Conversely, patients with low levels are at an increased risk of thrombotic complications.^{7,8,10} The concentration of dabigatran can vary depending on multiple factors (mainly renal function,

age, weight, coadministration of P-glycoprotein inhibitors/inductors, and others). Since these factors fluctuate over time, it would be necessary to check the c_{min} repeatedly. Genetic testing can reveal a patient's likelihood of having abnormal levels of dabigatran in their plasma. Knowing that these abnormal levels have been linked to complications, having knowledge of the patient's genetic profile can aid in assessing their risk of developing complications. The use of genetic testing in clinical practice has already been established, especially in the case of warfarin and clopidogrel. This has led to numerous advantages, such as increased safety and more precise dosage recommendations. The results of genetic analysis could be used in the process of selecting between different DOACs because analogous research is performed on other drugs within this group (apixaban, rivaroxaban, etc). The implementation of this approach has the potential to significantly improve patient outcomes by enabling informed and personalized drug selection, as well as a more customized dosing methodology that could result in greater flexibility in dabigatran administration.

Our research has limitations concerning the relatively low number of clinically relevant outcomes gathered, particularly in relation to ischemia or bleeding. This issue arose possibly because of the treating physician's decision to react to abnormally elevated levels of dabigatran actively, modifying the dosage or changing to a different anticoagulation medication because of ethical concerns. On the other hand, our patient cohort is genetically representative because it was selected as all consecutive patients with ischemic stroke. It is important to note that the study's retrospective design may serve as a limitation.

Our other limitation is including only dabigatran trough concentrations and not the peak concentrations. Because of the variability in absorption of dabigatran and for the association of lower trough plasma levels with stroke and a correlation between increased dabigatran trough concentrations and bleeding risk, it is recommended to measure the trough concentration rather than the peak concentration.^{13,20} In addition, previous research showed no effect of the *CES1* rs8192935 SNP on peak plasma levels.^{15,30}

The strength of our study lies in being the largest cohort of stroke patients who were tested for dabigatran plasma levels, pharmacogenetics, and clinical outcomes. However, the study was limited to patients with ischemic stroke in a single country, so the results need validation in other cohorts before genetic testing can be implemented into clinical routine.

CONCLUSIONS

The presence of the *CES1* rs8192935 polymorphism is directly linked to decreased trough plasma levels of dabigatran. Individuals with the *ABCB1* rs4148738 polymorphism have shown a tendency toward higher plasma levels, and the statistical significance has been established with respect to an increased risk of clinically relevant bleeding.

It is important to further investigate the correlation between genetic predisposition and anticoagulant therapy to enhance patient safety. Therefore, incorporating genetic testing into clinical practice, similar to what is currently performed with warfarin and clopidogrel, could potentially provide significant benefits and improve the safety of treating patients after a stroke.

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