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ORIGINAL RESEARCH

*CYP2C19*17* May Increase the Risk of Death Among Patients with an Acute Coronary Syndrome and Non-Valvular Atrial Fibrillation Who Receive Clopidogrel and Rivaroxaban

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Introduction: The aim of this study is to assess the influence of gene *CYP2C19, CYP3A4, CYP3A5* and *ABCB1* polymorphisms on clopidogrel antiplatelet activity, rivaroxaban concentration equilibrium, and clinical outcomes among patients with acute coronary syndrome and non-valvular atrial fibrillation.

Methods: In the multicenter prospective registry study of the efficacy and safety of a combined antithrombotic therapy 103 patients with non-valvular atrial fibrillation both undergoing or not a percutaneous coronary intervention were enrolled. The trial assessed the primary outcomes (major bleeding, in-hospital death, cardiovascular death, stroke\transient ischaemic attack, death/renal insufficiency) and secondary outcomes (platelet reactivity units (PRU), rivaroxaban concentration).

Results: For none of the clinical outcomes when combined with other covariates, the carriership of polymorphisms *CYP3A5*3 rs776746, CYP2C19*2 rs4244285;*17 rs12248560, ABCB1 3435 C>T, ABCB1 rs4148738* was significant. None of the markers under study (*CYP3A5*3 rs776746, CYP2C19*2 rs4244285, *17 rs12248560, ABCB1 3435 C>T, ABCB1 rs4148738*) has proven to affect rivaroxaban equilibrium concentration in blood plasma among patients with atrial fibrillation and acute coronary syndrome.

Conclusion: In situations of double or triple antithrombotic rivaroxaban and clopidogrel therapy among patients with atrial fibrillation and acute coronary syndrome, the genetic factors associated with bleeding complications risk (*CYP2C19*17*) may prove to be clinically relevant.

Keywords: rivaroxaban, clopidogrel, polymorphism, atrial fibrillation, acute coronary syndrome

Plain Language Summary

- A multicenter prospective registry trial was performed to study the efficacy and safety of a combined antithrombotic therapy: 103 patients with non-valvular atrial fibrillation some undergoing a percutaneous coronary intervention were included.
- The trial assessed the primary outcomes (major bleeding, in-hospital death, cardiovascular death, stroke\transient ischaemic attack, death/renal insufficiency) and secondary outcomes (platelet reactivity units, rivaroxaban concentration).
- In situations of double or triple antithrombotic rivaroxaban and clopidogrel therapy among patients with AF and ACS, the genetic factors associated with bleeding complications risk (*CYP2C19*17*) may prove to be clinically relevant.

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Introduction

All the patients with an acute coronary syndrome regardless of the treatment strategy (with or without a percutaneous coronary intervention) were assigned to a double antiplatelet therapy, which includes aspirin and a P2Y12-inhibitor, in order to prevent thrombotic complications: cardiovascular death, myocardial infarction, stent thrombosis.[1] Nowadays, 3 P2Y12-inhibitors are available per os: clopidogrel, ticagrelor, prasugrel. In some trials the variability in response to clopidogrel was observed, which is connected with numerous factors, CYP2C19-dependent active metabolite production among them.[2,3] Though the presence of low function alleles *CYP2C19*2* (*rs4244285, c.681G>A*) and *CYP2C19*3* (*rs4986893, c.636G>A*) leads to an insufficient platelet activity inhibition and significantly increases the risk of thrombotic complications compared to non-carriers.[2,4] *CYP2C19*17* carriership is connected to an increased risk of bleeding complications on clopidogrel.[3] The number of patients who are resistant to clopidogrel may be up to 35% of the population, which depends on the method of testing and the borders of high residual platelet reactivity.[5] A genotype-based antiplatelet therapy, as observed in a number of observational and randomized trials, is capable of overcoming high residual platelet reactivity[6–9] and may lead towards a decrease in the number of adverse cardiovascular events.[8–16] These results are proven by the results of a meta-analysis of 9000 patients on clopidogrel: the carriership of low function allelic variants increases the risk of major adverse cardiovascular events (MACE) 1.5-fold and the risk of stent thrombosis 2.8-fold.[17] The negative results of later meta-analyses can be explained by the heterogeneity of the population and inclusion in the meta-analyses of patients with a stable coronary disease.[18] Meanwhile, several large randomized clinical trials are in progress (Popular genetics (NCT01761786) – 2700 patients; TAILOR-PCI (NCT01742117) – 5270 patients) and the clinical application of genotype-based antiplatelet therapy remains controversial. Other P2Y12–inhibitors – ticagrelor and prasugrel are not susceptible to the influence of allelic variants of the CYP2C19 gene carriership[19,20] and have appeared to be more potent in terms of decrease in thrombotic complications in comparison with clopidogrel among patients with acute coronary syndrome undergoing percutaneous coronary intervention.[21,22] Nevertheless, the problem of the variability of the response to clopidogrel is still relevant as the therapy with ticagrelor and prasugrel is connected to the higher number of bleeding complications; it is more expensive, which leads to lower compliance among patients and, finally, is not the treatment of choice when used as part of a combined antithrombotic treatment.[23,24]

According to the current guidelines and expert opinions, patients with an acute coronary syndrome (ACS) with atrial fibrillation (AF) need to be treated with combined antithrombotic therapy: antiplatelet therapy plus oral anticoagulants.[24] Although the prescription of anticoagulant therapy is regulated by the guidelines for treatment of AF aimed to prevent cardioembolic complications, a combined antithrombotic therapy is connected to an increase in the bleeding events risk.[24–27] Nevertheless, the results of previous randomized clinical trials PIONEER AF-PCI, RE-DUAL PCI[28,29] which included patients with ACS and AF undergoing PCI and meta-analyses[30,31] showed the superiority of double antithrombotic treatment (oral anticoagulant+ P2Y12 inhibitor) over triple antithrombotic therapy in terms of decrease in the number of bleeding complications without significant differences in the effectiveness. Apart from AF treatment, prescription of oral anticoagulants may be pathophysiologically justified in ACS treatment. Adhesion and activation of platelets after erosion and rupture of the atherosclerosis plaque plays a key role in the initialization and development of atherothrombosis.[32] Activation of coagulation usually contributes less to the development of acute arterial thrombosis in comparison to antiplatelet action. Rivaroxaban, a direct inhibitor of Xa-factor, does not directly affect the antiplatelet aggregation, caused by collagen, ADP, thromboxane A2 and thrombin.[33,34] At the same time, it was shown that rivaroxaban, which is capable of total inhibition of thrombin formation, may suppress antiplatelet aggregation inhibiting thrombin generation, through the related tissue factor.[35] The main part of thrombin is generated after the primary clot formation, which explains that thrombin plays an important role in clot stabilization rather than its initialization at an early stage of atherothrombosis.[36] In previous studies, increased procoagulant activity at an acute stage of ACS was revealed with a significant increase of thrombin formation, which can remain for several months after the event increasing the risk of thrombotic complications.[37–40] The analysis of randomized clinical trials of the third phase and postmarket studies have shown that high concentrations of DOACs in the blood plasma correlate with the increased rate of bleeding events.[41,42] Similar studies have shown the connection between low plasma concentrations of DOACs measured in the first month of therapy and

thromboembolic events.[43,44] In addition, the data from the centers have shown that a 15-fold variation of plasma concentration of rivaroxaban can be observed with an average level of 40 ng/mL. And almost 40% of the patients were beyond the therapeutic range.[45] In the development of such a variability of pharmacokinetic parameters pharmacogenetics can play a significant role. Rivaroxaban is a substrate of transmembrane transporter P-glycoprotein and is metabolized by P450 enzymes (CYP3A and CYP2J2).[46] Consequently, the carriership of gene polymorphisms which encode the P-glycoprotein formation (*ABCB1)* and isoenzyme CYP3A4 and CYP3A5 may exert influence on pharmacokinetic, pharmacodynamic parameters and clinical outcomes on rivaroxaban therapy.[47]

That is why the search for safety and effectiveness biomarkers of a combined antithrombotic therapy is of particular interest, and the aim of this study is to assess the influence of gene *CYP2C19, CYP3A4, CYP3A5* and *ABCB1* polymorphisms on primary and secondary clinical outcomes among patients with ACS and non-valvular AF who received clopidogrel and rivaroxaban.

Materials and Methods

Design and Study Population

A multicenter prospective registry trial was performed to study the efficacy and safety of a combined antithrombotic therapy: 103 patients with non-valvular atrial fibrillation some undergoing a percutaneous coronary intervention were consecutively involved.

The study protocol was approved by the Ethics Committee of Russian Medical Academy of Continuous Professional Education, Moscow, Russian Federation (resolution no. KM-634), it was conducted in accordance with the Declaration of Helsinki, and all patients gave written informed consent for participation.

Inclusion criteria: presence of an informed written consent, age above 18 years, ACS event less than 7 days, non-valvular atrial fibrillation, registered, and which needs anticoagulant treatment (≥2 CHA2DS2-VASC score for men and ≥ 3 CHA2DS2-VASC score for women); first registered atrial fibrillation which needs anticoagulant prescription. Exclusion criteria: pregnancy, lactation, active internal bleeding, liver cirrhosis with C Child-Pugh liver insufficiency; chronic renal disease, HIV-infection, alcohol/drug addiction; mitral valve stenosis moderate and severe, mechanical heart valves, severe psychiatric disorders, allergic reactions/drug intolerance. The follow-up period was 12 months with telephone interviews every 3 months. The trial assessed the primary outcomes (major bleeding, in-hospital death, cardiovascular death, stroke \transient ischaemic attack, death/renal insufficiency) and secondary outcomes (platelet reactivity units, rivaroxaban concentration)

Assessment of Platelet Activity

The assessment of residual platelet reactivity was performed utilizing VerifyNow assay («Accumetrics», US). For assessment of platelet activity venous blood was used, drained 7 days after the beginning of clopidogrel therapy in vacuum vials 2 mL with 3.2% sodium citrate. Platelets are activated by ADP and interact with fibrinogen on the microparticles in the solution. The more the aggregation is - the less is the optical density of the solution. The level of aggregation of platelets is measured in P2Y12 Reactivity Units or percentage of inhibition. The study was conducted within 1 hr (it is permissible to conduct the study within 4 hrs) after the whole venous blood sample was drained. The therapeutic range of P2Y12-inhibitors when measured with VerifyNow P2Y12 assay are as follows: PRU>208 – the risk of thrombotic events, PRU<95 – bleeding risk, 95<PRU<208 – adequate response to clopidogrel.

Measurement of Rivaroxaban Plasma Concentration

The blood plasma sampling utilizing VACUETTE® (Greiner Bio-One, Austria) with heparin sodium was performed to assess minimal equilibrium concentration of rivaroxaban in the blood plasma before a regular dose of rivaroxaban on the 5th–6th day of the initiation of the therapy. Rivaroxaban concentration determination was carried out by high-performance liquid chromatography with mass-spectrometry detection (HPLC-MS). Agilent 1200 HPLC – system consists of 4-channel pump, mobile phase degasser and chromatographic columns thermostat. The chromatographic separation was done using Agilent Extend-C18 (2.1*100 mm, 3.5 μm) column at temperature 40°C. The mobile phase: Solution "A" (50 mL 0.1 M solution ammonium acetate and 5 mL formic acid were diluted with unionized water up to 1 liter) and solution "B" (50 mL 0.1 M solution ammonium acetate and 5 mL formic acid were diluted with acetonitrile up to 1 liter). Chromatographic separation was performed in the

isocratic elution mode with the components ratio "A":"B" = 70:30. The velocity of the chromatographic mobile phase stream was 0.3 mL/min. The volume of the sample was 10 µL. The analysis was performed within 7 mins. Agilent Triple Quad LC/MS 6410 mass-spectrometer was used (triple quadrupole type) with electrospray ionization in the positive ionization mode. The detection of rivaroxaban mass-spectra was performed in the multiple reaction monitoring mode. The nebulizer gas pressure was 35 psi. The volume velocity of the dry gas flow was 11 L/min, desolvation temperature was 350°C. The value of fragmentation voltage was 135 V, the collision cell voltage was 25 V. Sample preparation was performed using plasma protein precipitation method. The blood plasma samples thawed at room temperature. Then 100 µL of plasma was placed in Eppendorf vials, 250 µL of methanol was added, as well as 0.1% hydrogen chloride 9:1 mixture, mixed with Vortex, left alone for 10 mins and mixed once again. Then the acquired samples were centrifuged at 9000 RCF for 10 mins. The supernatant was transferred into chromatographic vials and placed on autosampler. The technique was validated in the 5–1000 ng/mL concentration range. Precision and accuracy values within-run and between runs did not exceed 15% CV (lower limit of quantitation values were lower 20% CV). The samples were stable after freeze-thaw cycle and a month of storage.

Genotyping

Venous blood collected into vacuum vials VACUETTE® (Greiner Bio-One, Austria) with ethylene diamine tetra acetate (EDTA) was used for genotyping. Utilizing real-time polymerase chain reaction with commercially available assays («Sintol», Russia; Thermo Fisher Scientific, USA) utilizing Real-Time CFX96 Touch amplifier (Bio-Rad Laboratories, Inc., USA), the carriership of *CYP2C19, CYP3A4, CYP3A5* and *ABCB1* polymorphisms was detected.

Statistics

Data analysis was carried out in the statistical package IBM SPSS Statistics 23.0. All quantitative variables were tested for normal distribution by the Shapiro–Wilk criterion, resulting in abnormal data distribution ($Z<1.0$; $p<0.0001$). For the subsequent analysis of quantitative variables between subgroups, nonparametric criteria (Mann–Whitney, Kruskal-Walles) were applied. Comparison of frequencies of categorical variables was carried out by means of Pearson's Chi-square. In order to establish the predictive role of genetic polymorphisms and clinical parameters for the outcomes, binomial logistic regression was carried out. For the regression analysis, the following variables were selected as the dependent variables: serious bleeding, in-hospital death, death by renal failure, stroke, cardiovascular death. The regression model always included genetic polymorphisms *CYP3A5*3, CYP2C19*2, *17, ABCB1* and DAPT\TATT variables as covariates. Parameters which showed significant differences when comparing subgroups by outcomes were added as covariates in relevant regression model. Covariates were included in the model using the Wald reverse step-by-step selection method. There were no differences from Hardy-Weinberg equilibrium (Table 1).

Results

The baseline characteristics of the population are featured in Table 2. In the studied patient group 54.5% male, mean age 73±9.8. PCI, CABG (Coronary artery bypass graft), Myocardial infarction, Heart Failure and bleeding were observed among 32.0%, 6.8%, 46.6%, 35.9% and 19.4%, respectively. Among the major adverse cardiovascular events during the 12-months observation period major bleeding – 11 (10.6%), cardiovascular death – 4 (3.9%), acute cerebrovascular event – 2 (2.0%). The number of patients with resistance to clopidogrel treatment (PRU >208) and who have the risk of bleeding events (PRU<95): 12 (11.7%) and 25 (24.3%), respectively.

Table 3 features the results of the assessment of the influence of polymorphism carriership *CYP2C19, CYP3A4, CYP3A5* and *ABCB1* among patients with ACS and non-valvular AF on secondary (clopidogrel antiplatelet activity), rivaroxaban equilibrium concentration clinical outcomes. When comparability assessment was performed between the groups of patients, statistically significant differences in terms of factors which could affect the primary and secondary outcomes were observed for *ABCB1 3435 C>T* (T-carriers had higher HAS-BLED, CHA2DS2VASC scores and mean age) and *CYP2C19*2* (*2-carriers had lower Killip scores).

Among the allelic variant carriers *CYP2C19*17* (*CYP2C19*17 CT + CYP2C19*17 TT*) patients had a twice more often increased risk of bleeding: low platelet reactivity (PRU<95) was observed among 17.2% of carriers and 33.3% among non-carriers (OR 0.42 95% CI 0.17–1.05, p = 0.059). In addition, this allelic variant was significantly associated with the risk of in-hospital death (0% vs 6.7%; OR 0.42 95% CI 0.33–0.53 p = 0.046) and cardiovascular death (0% vs 8.9%; OR 0.41

Table 1 Baseline Demographics and Clinical Characteristics (n=103)

Characteristics	Value
Age, years mean ± SD	73±9.8
Men, n (%)	56 (54.5%)
Obesity	51 (49.5)
Smoking	9 (8.7%)
CRUSADE Score	11.4±5.1
Killip Score	1.43±0.74
CHA2DS2VASC Score	5.1±1.5
HAS-BLED Score	2.4±0.9
GFR	53.8±17.7
Previous PCI	33 (32.0%)
Previous CABG	7 (6.8%)
Previous Myocardial Infarction	48 (46.6%)
Heart Failure	37 (35.9%)
Any Bleeding Events	20 (19.4%)
PCI:	76 (73.8%)
1. Angioplasty	3 (3.9%)
2. Drug-Eluting Stent (DES)	41 (53.9%)
3. Bare-Metal Stent (BMS)	32 (42.1%)
AF:	103 (100%)
1. First Detected	13 (12.6%)
2. Paroxysmal	53 (51.5%)
3. Permanent	37 (35.9%)
MI:	103 (100%)
1. STEMI	28 (27.2%)
2. NSTEMI	52 (50.5%)
3. Unstable angina	23 (22.3%)
Major Bleeding	11 (10.6%)
Cardiovascular Death	4 (3.9%)
Cerebrovascular Event	2 (2.0%)
PRU:	
1. PRU >208	12 (11.7%)
2. PRU <95	25 (24.3%)
Combined antithrombotic therapy	
1. Triple	76 (73.7%)
2. Double	27 (26.3%)

Table 2 Hardy-Weinberg Equilibrium Test

Genetic Polymorphism	Genotype	n	%	Chi-Square
*CYP3A4*22 C>T intron 6 rs35599367*	CC CT	102 1	96.2 3.8	0
*CYP3A5*3 rs776746*	GG AG	84 19	81.6 18.4	1.06
ABCB1 rs4148738	CC CT TT	19 46 38	18.4 44.7 36.9	0.58
ABCB1 rs1045642	CC CT TT	30 45 28	29.1 43.7 27.2	1.63
*CYP2C19*2 rs4244285*	AA GA GG	2 29 72	1.9 28.2 69.9	0.22
*CYP2C19*17 rs12248560*	CC CT TT	58 40 5	56.3 38.8 4.9	0.33

95% CI 0.33–0.52 p = 0.021). The *CYP2C19*17* polymorphism carriers had a tendency towards an increase in the frequency of strokes/transient ischaemic attacks (0.0% vs 4.8% OR 0.41 95% CI 0.32–0.52 p = 0.093). Patients with T allelic variant polymorphism *ABCB1 rs4148738* and G allelic variant polymorphism *CYP2C19 * 2* had a tendency towards death by renal insufficiency (15.8% vs 35.7% OR 2.96 95% CI 0.80–11.0, p = 0.093 and 37.5% vs 19.4% OR 0.40 95% CI 0.15–1.10, p = 0.07, respectively). *CYP3A5*3 AG* patients had a tendency towards the increase in the number of major bleeding with double/triple antithrombotic therapy: 22.2% vs 8.5% OR 0.33 95% CI 0.08–1.27, p = 0.093.

The mean platelet reactivity unit values of patients with T allelic variant polymorphism *ABCB1 rs4148738* were significantly higher compared to non-carriers: 113.3 ± 57.1 vs 141.0 ± 62.3 (p = 0.053). Carriers of the allelic variant *CYP2C19 * 17 rs12248560* had higher platelet inhibition values (37.8% ± 28.9 compared to 25.0% ± 25.9; p = 0.013) and, therefore, lower PRU values (121.4 ± 63.5 vs 147.1 ± 59.0; p = 0.044) compared to non-carriers. *CYP3A4*22 rs35599367* polymorphism had only one heterozygous variant, so, statistic analysis was not performed.

Additional analysis also revealed that higher platelet inhibition level is connected to higher in-hospital death (63.3% vs 29.6%; p = 0.058), and higher rivaroxaban concentration is associated with higher HAS-BLED scores: 92.3 ± 64.9 if HAS-BLED 3–9 vs 67.2 ± 47.4 if HAS-BLED <3 (p = 0.03). Besides, the correlation analysis revealed a weak negative correlation between haemoglobin levels and rivaroxaban plasma concentration (r=0.3; p=0.002) and platelet activity (0.2; p=0.02).

Linkage Disequilibrium Analysis and Haplotype Analysis

We used "SNPStats' for the analysis of linkage disequilibrium and haplotype analysis. The polymorphisms are considered to be in 'strong LD' if the one-sided upper

Table 3 Association of Gene Polymorphisms *CYP2C19, CYP3A4, CYP3A5* and *ABCB1* with Secondary Outcomes

ABCB1 3435 C>T	**CC**			**CT+TT**			**p-value (Mann–Whitney Test)**
	N	**M**	**SD**	**N**	**M**	**SD**	
Platelet inhibition rate (%)	30	29.8	28.2	73	30.9	27.9	0.839
Platelet reactivity units (PRU)	30	136.8	61.3	73	135.5	32.8	0.910
Rivaroxaban concentration	30	74.7	54.0	73	80.6	59.0	0.596
ABCB1 rs4148738	**CC**			**CT+TT**			**p**
	N	**M**	**SD**	**N**	**M**	**SD**	
Platelet inhibition rate (%)	19	34.5	28.5	84	29.7	27.8	0.416
Platelet reactivity units (PRU)	19	113.3	57.1	84	141.0	62.3	0.053
Rivaroxaban concentration	19	78.8	59.2	84	78.9	57.2	0.865
CYP2C19*2	**GG**			**GA+AA**			**p**
	N	**M**	**SD**	**N**	**M**	**SD**	
Platelet inhibition rate (%)	72	31.4	26.3	31	28.6	31.6	0.275
Platelet reactivity units (PRU)	72	131.9	57.8	31	145.2	71.0	0.276
Rivaroxaban concentration	72	75.7	57.7	31	86.2	56.9	0.219
CYP2C19*17	**CC**			**CT+TT**			**p**
	N	**M**	**SD**	**N**	**M**	**SD**	
Platelet inhibition rate (%)	58	25.0	25.9	45	37.8	28.9	0.013
Platelet reactivity units (PRU)	58	147.1	59.0	45	121.4	63.5	0.044
Rivaroxaban concentration	58	83.5	66.7	45	72.9	42.4	0.821
CYP3A5*3	**GG**			**AG+AA**			**p**
	N	**M**	**SD**	**N**	**M**	**SD**	
Platelet inhibition rate (%)	84	30.6	28.0	19	30.4	28.2	0.993
Platelet reactivity units (PRU)	84	135.0	62.9	19	139.8	60.0	0.75
Rivaroxaban concentration	84	81.6	58.9	19	69.7	55.9	0.430

95% confidence bound on D" is ≥0.98 and the lower bound is ≥0.70.

The polymorphisms in the *CYP2C19* gene (**2 (rs4244285)* and **17 (rs12248560))* were in high linkage disequilibrium (D' = 0.99). There was no AA genotype among the patients who had the bleeding. Major bleeding showed a significant association ($p < 0.05$) with a protective effect of *CYP2C19*2 rs4244285* in the presence of *CYP2C19*17 rs12248560* against major bleeding (Table 4)

Discussion

Although the optimal values of therapeutic range of rivaroxaban concentration in blood plasma have not been

Table 4 Haplotype Analysis of the *CYP2C19* Polymorphisms for Major Bleeding

Haplotype Association with Response (n=103, Crude Analysis)					
	CYP2C19*2	***CYP2C19*17***	**Freq**	**OR (95% CI)**	**P-value**
1	G	C	0.6101	1.00	–
2	G	T	0.2297	0.13 (0.03–0.60)	0.01
3	A	C	0.1472	0.00 (-Inf - Inf)	1
Global haplotype association p-value: 0.0046					

determined, in previous studies significant associations between equilibrium rivaroxaban concentration and incidence of bleeding among AF patients were revealed (48±30 vs 34 ± 26; p = 0.02).[48] In addition, apart from acknowledged factors (age, renal insufficiency[49,50]), genetic factors such as gene polymorphisms *ABCB1, CYP3A4* may exert influence on rivaroxaban pharmacokinetics and, therefore, on the risk of bleeding complications. This is extremely important in case of antithrombotic therapy among patients with AF and ACS, even more so in light of the correlation of the aforementioned genetic markers with pharmacologic response to clopidogrel,[2] while rivaroxaban indirectly affects platelet aggregation through thrombin generation inhibition.[35] Previous studies assessed primarily non-genetic risk factors of poor outcomes among patients with AF and ACS. Less emphasis was put on the role of genetic markers. Zang L. et al associated major bleeding with rivaroxaban concentration (2.5 mg), sex and prior myocardial revascularization.[51] Several studies assessed the role of genetic factors among patients with AF without ACS. Anne-Laure Sennesael et al report that three patients out of four having major bleeding and blood rivaroxaban concentration above expected (more than 136 nm/mL) were heterozygous *ABCB1 1236C > T, 2677G > T, 3435 C > T* and *rs4148738*.[20] Likewise, *ABCB1 2677G > T* and *3435 C > T* carriership were associated with higher blood plasma rivaroxaban concentration and bleeding complications.[52] Apart from that, there are studies that report no link between *1236C > T, 2677G > T, 3435 C > T* and rivaroxaban pharmacokinetics.[53]

The present study has proven that gene polymorphisms, usually associated with clopidogrel-related bleeding, play an important role in combined antithrombotic therapy: *CYP2C19*17* carriers twice as often had low residual platelet reactivity (PRU less than 95) 17.2% vs 33.3% (p = 0.059).

*CYP2C19*17* carriers turned out to be prone to increased platelet inhibition (37.8% ± 28.9 compared to 25.0% ± 25.9; p = 0.013) and, therefore, lower PRU values (121.4 ± 63.5 vs 147.1 ± 59.0; p = 0.044) compared with non-carriers.

Apart from that, this allelic variant turned out to be the only genetic marker to be significantly associated with in-hospital (0% vs 6.7%; p = 0.046) and cardiovascular death (0% vs 8.9%; p = 0.021).

No genetic marker was connected to clopidogrel resistance (PRU more than 208). The only allelic variant which led towards higher mean PRU values turned out to be *ABCB1 rs4148738*: 113.3 ± 57.1 vs 141.0 ± 62.3 (p = 0.053).

The results can be explained by the fact that in situations of indirect influence of rivaroxaban on platelet reactivity, it is safe to put more emphasis on the risk factors of bleeding complications rather than on well described in literature genetic risk factors for thrombotic complications on clopidogrel therapy (low function alleles *CYP2C19*2 rs4244285, CYP2C19*3 rs4986893*), as these bleeding risk factors do not demand a switch of drug according to the present CPIC guidelines and to a lesser degree disrupt clopidogrel response.[2]

Conclusion

CYP2C19*17 *rs12248560* is significantly associated with in-hospital death of patients with an acute coronary syndrome and non-valvular atrial fibrillation who receive clopidogrel and rivaroxaban.

None of the markers under study (*CYP3A5*3 rs776746, CYP2C19*2 rs4244285, *17 rs12248560, ABCB1 3435 C>T, ABCB1 rs4148738*) has proven to affect rivaroxaban equilibrium concentration in blood plasma among patients with atrial fibrillation and acute coronary syndrome.

In situations of double or triple antithrombotic rivaroxaban and clopidogrel therapy among patients with AF and ACS, the genetic factors associated with bleeding complications risk (*CYP2C19*17 rs12248560*) may prove to be clinically relevant.

Additional results regarding a connection between rivaroxaban concentration and high HAS-BLED scores demonstrate the feasibility of using this marker for personalization of rivaroxaban therapy.

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Disclosure

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