DOI: https://doi.org/10.17816/RCF635362

Specifics of drug-drug interactions between rivaroxaban and a P-glycoprotein inhibitor depending on the *CYP3A4/A5* gene polymorphism in patients aged 80 years and older with non-valvular atrial fibrillation

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ABSTRACT

BACKGROUND: An increased risk of bleeding during rivaroxaban administration is associated with polymorphism of genes involved in its biotransformation, as well as with the use of drugs inhibiting shared metabolic pathways. However, the data are inconsistent.

AIM: To study specifics of drug-drug interactions between rivaroxaban and a P-glycoprotein inhibitor (using verapamil as an example) depending on the polymorphism of the *CYP3A4* (*rs35599367*) and *CYP3A5* (*rs776746*) genes in patients aged 80 years and older with non-valvular atrial fibrillation.

MATERIALS AND METHODS: A total of 128 patients (median age 87.5 years [83; 90], 75% women) were examined. All patients underwent genotyping for the studied gene variants, determination of the minimum steady-state concentration of rivaroxaban ($C_{min,\ ss}$), standardization of the minimum steady-state concentration of rivaroxaban per daily dose ($C_{min,\ ss}/D$), determination of prothrombin time in plasma, and analysis of medical documentation for the occurrence of clinically significant minor bleeding.

RESULTS: Compared to patients receiving rivaroxaban without calcium channel blockers, co-administration of rivaroxaban and verapamil in carriers of the CC variant of the *CYP3A4* gene resulted in higher values of $C_{min,\ ss}$ (73.8 [49; 113.5] vs. 40.5 [25.6; 73.3] ng/mL), $C_{min,\ ss}/D$ (2.5 [1.7; 4.0] vs. 4.7 [2.9; 7.7] ng/mL/mg), prothrombin time (14.8 [13.3; 17.3] vs. 14.0 [12.6; 14.5] s) and clinically significant minor bleeding [10/30 (33.3%) vs. 6/45 (13.3%) cases], $p < 0.05$. In carriers of the GG variant of the *CYP3A5* gene, the same regimen resulted in higher values of $C_{min,\ ss}$ (74.7 [50.6; 108.8] vs. 40.2 [25.7; 72.3] ng/mL), $C_{min,\ ss}/D$ (4.6 [3.0; 7.3] vs. 2.5 [1.7; 4.0] ng/mL × mg), prothrombin time (14.6 [12.8; 15.2] vs. 14.0 [12.6; 14.5] s) and clinically significant minor bleeding [10/27 (37%) vs. 5/40 (12.5%) cases], $p < 0.05$. Furthermore, in carriers of the GA+AA variant of the *CYP3A5* gene, this regimen resulted in higher values of $C_{min,\ ss}$ (88.1 [5.5; 88.1] vs. 52.8 [25.0; 77.2] ng/mL), $C_{min,\ ss}/D$ (5.7 [0.4; 5.7] vs. 3.5 [1.7; 5.2] ng/mL × mg), $p < 0.05$. The combined use of rivaroxaban with verapamil in carriers of the CT variant of the *CYP3A4* gene was not observed in our sample.

CONCLUSIONS: Carriers of the homozygous wild-type *CYP3A4/A5* genotype showed high pharmacokinetic variability to the administration of verapamil (a strong P-glycoprotein inhibitor and a moderate CYP3A4 inhibitor).

Keywords: drug-drug interactions; rivaroxaban; P-glycoprotein inhibitor; CYP3A4/5; pharmacogenetics; elderly patient; bleeding.

To cite this article

Cherniaeva MS, Konstantinova IA, Baranovskaya EK, Golovina OV, Sinitsyna II, Bochkov PO, Abdullaev ShP, Denisenko NP, Sozaeva ZhA, Mirzaev KB, Lomakin NV, Sychev DA. Specifics of drug-drug interactions between rivaroxaban and a P-glycoprotein inhibitor depending on the *CYP3A4/A5* gene polymorphism in patients aged 80 years and older with non-valvular atrial fibrillation. *Reviews on Clinical Pharmacology and Drug Therapy.* 2024;22(4):425–438. DOI: https://doi.org/10.17816/RCF635362

Received: 23.08.2024 **Accepted:** 12.11.2024 **Published:** 30.12.2024

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УДК 615.036
DOI: https://doi.org/10.17816/RCF635362

Особенности межлекарственного взаимодействия ривароксабана и ингибитора Р-гликопротеина в зависимости от полиморфизма генов *CYP3A4/A5* у пациентов 80 лет и старше с неклапанной фибрилляцией предсердий

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АННОТАЦИЯ

Актуальность. Повышение риска кровотечений на фоне приема ривароксабана связывают с полиморфизмом генов, участвующих в его биотрансформации, а также с применением лекарственных средств, которые ингибируют совместные пути метаболизма. Однако данные противоречивы.

Цель — изучить особенности межлекарственного взаимодействия ривароксабана и ингибитора Р-гликопротеина (на примере верапамила) в зависимости от полиморфизма генов *CYP3A4* (rs35599367) и *CYP3A5* (rs776746) у пациентов 80 лет и старше с неклапанной фибрилляцией предсердий.

Материалы и методы. Обследованы 128 пациентов (медиана возраста 87,5 лет [83; 90], 75 % женщин). Всем пациентам проведены генотипирование по исследуемым вариантам генов, определение минимальной равновесной концентрации ривароксабана ($C_{min,\ ss}$), стандартизация минимальной равновесной концентрации ривароксабана на суточную дозу ($C_{min,\ ss}/D$), определение протромбинового времени в плазме и анализ медицинской документации на наличие клинически значимых небольших кровотечений.

Результаты. Совместное применение ривароксабана с верапамилом в сравнении с пациентами на фоне приема ривароксабана без блокаторов кальциевых каналов у носителей CC гена *CYP3A4* приводило к более высоким значениям $C_{min,\ ss}$ (73,8 [49; 113,5] vs 40,5 [25,6; 73,3] нг/мл), $C_{min,\ ss}/D$ (2,5 [1,7; 4,0] vs 4,7 [2,9; 7,7] нг/мл/мг), протромбинового времени (14,8 [13,3; 17,3] vs 14,0 [12,6; 14,5] с) и клинически значимых небольших кровотечений [10/30 (33,3 %) vs 6/45 (13,3 %) случаев], $p < 0,05$. У носителей GG гена *CYP3A5* приводило к более высоким значениям $C_{min,\ ss}$ (74,7 [50,6; 108,8] vs 40,2 [25,7; 72,3] нг/мл), $C_{min,\ ss}/D$ (4,6 [3,0; 7,3] vs 2,5 [1,7; 4,0] нг/мл × мг), протромбинового времени (14,6 [12,8; 15,2] vs 14,0 [12,6; 14,5] с) и клинически значимых небольших кровотечений [10/27 (37 %) vs 5/40 (12,5 %) случаев], $p < 0,05$. И у носителей GA+AA гена *CYP3A5* приводило к более высоким значениям $C_{min,\ ss}$ (88,1 [5,5; 88,1] vs 52,8 [25,0; 77,2] нг/мл), $C_{min,\ ss}/D$ (5,7 [0,4; 5,7] vs 3,5 [1,7; 5,2] нг/мл × мг), $p < 0,05$. У носителей CT гена *CYP3A4* совместное применение ривароксабана с верапамилом в нашей выборке не встречалось.

Выводы. Носители гомозиготного дикого генотипа *CYP3A4/A5* показали высокую фармакокинетическую вариабельность к приему верапамила (сильный ингибитор Р-гликопротеина и умеренный ингибитор CYP3A4).

Ключевые слова: межлекарственное взаимодействие; ривароксабан; ингибитор Р-гликопротеина; CYP3A4/5; фармакогенетика; пожилой пациент; кровотечения.

Как цитировать

Черняева М.С., Константинова И.А., Барановская Е.К., Головина О.В., Синицина И.И., Бочков П.О., Абдуллаев Ш.П., Денисенко Н.П., Созаева Ж.А., Мирзаев К.Б., Ломакин Н.В., Сычев Д.А. Особенности межлекарственного взаимодействия ривароксабана и ингибитора Р-гликопротеина в зависимости от полиморфизма генов *CYP3A4/A5* у пациентов 80 лет и старше с неклапанной фибрилляцией предсердий // Обзоры по клинической фармакологии и лекарственной терапии. 2024. Т. 22. № 4. С. 425–438. DOI: https://doi.org/10.17816/RCF635362

Рукопись получена: 23.08.2024 **Рукопись одобрена:** 12.11.2024 **Опубликована:** 30.12.2024

ЭКО•ВЕКТОР

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BACKGROUND

Over the last 50 years, there has been an upward trend in the number of older adults due to improvements in healthcare and quality of life. Experts predict that by 2050, the number of people over 80 years will increase fourfold. The number of diseases, including atrial fibrillation (AF), increases with age. The prevalence of AF in the general population is 1%–2%, and its incidence increases with age from <0.5% at age 40–50 years to 5%–15% at an age 80 years [1]. Pharmacotherapy of elderly patients has some peculiarities, including changes in pharmacokinetics due to deterioration of liver and kidney function, sarcopenia, impaired water balance and other complications, as well as changes in pharmacodynamics, such as impaired receptor density and sensitivity, post-receptor impairments and impaired regulation of homeostasis mechanisms. It is worth considering that elderly people are often polymorbid, which increases the risk of polypharmacy. Polypharmacy leads to drug-drug interactions and an increased risk of adverse drug reactions (ADRs) [2]. To prevent thromboembolic events, patients with AF are prescribed direct oral anticoagulants, one of which is rivaroxaban. Rivaroxaban is a direct-acting anticoagulant that inhibits factor Xa, resulting in disruption of the coagulation cascade and thrombosis. It has a dose-dependent effect on prothrombin time (PT), which is well-correlated with plasma concentration of the drug [3, 4]. Moreover, 2/3 of rivaroxaban dose are excreted from the body by the kidneys, and the other 1/3 is excreted by the liver with the participation of cytochromes CYP3A4, CYP2J2. In the study by Zhao et al. [5] on the effect of cytochromes P450 on rivaroxaban metabolism *in vitro* the authors distributed CYP isoforms by rivaroxaban hydroxylation activity as follows: CYP2J2>3A4>2D6>4F3>1A1>3A5>3A7>2A6>2E1>2C9>2C19. However, *in vivo* studies show conflicting results regarding the contribution of these gene polymorphisms to rivaroxaban metabolism [6–12].

Rivaroxaban is a substrate for the transporter proteins BCRP (breast cancer resistance protein) and P-glycoprotein (P-gp). A recent meta-analysis by Mardi et al. [13] showed that mutations in the *ABCB1* gene (rs1045642), encoding P-gp, are a potential factor that increases serum concentration of rivaroxaban and can be considered as a marker of rivaroxaban metabolism. Co-administration of the P-gp substrate with its inhibitor shows different degree of bleeding risk. Thus, a meta-analysis by Li et al. [14] included 4,417,195 cases, revealing 11,967 bleeding events associated with P-gp inhibitors and a higher risk of bleeding when combining rivaroxaban with dronedarone [1] and diltiazem.

In addition, an increased risk of bleeding is noted with concomitant use of P-gp and CYP3A4 inhibitors. In a retrospective study by Grymonprez et al. [15] among 193,072 patients with AF taking oral anticoagulants, 23.9% (46,194) of patients co-administered P-gp/CYP3A4 inhibitors. In the analysis, an increased risk of major bleeding was observed with rivaroxaban co-administered with amiodarone [relative risk (RR) 1.27, 95% confidence interval (CI) 1.21–1.34], diltiazem (RR1.28, 95% CI 1.13–1.46), verapamil (RR1.36, 95% CI 1.03–1.80), ticagrelor (RR1.50, 95% CI 1.20–1.87), and clarithromycin (RR1.55, 95% CI 1.14–2.11). In a meta-analysis of 11 studies including 37,973 patients by Yang et al. [16], dabigatran, apixaban and edoxaban [2] were associated with a significantly lower risk of major bleeding compared to rivaroxaban when co-administered with P-pg/CYP3A4 inhibitors.

Since the data regarding the contribution of genetic factors related to the transport and metabolism of rivaroxaban and the influence of P-gp inhibitors on its pharmacokinetic profile are scarce and contradictory, in continuation of our previous study [17], we analyzed the peculiarities of drug-drug interaction between rivaroxaban and P-gp inhibitors depending on the polymorphism of *CYP3A4* and *CYP3A5* genes.

The study aimed to investigate the characteristics of drug-drug interaction between rivaroxaban and P-gp inhibitors (using verapamil as an example) depending on polymorphism of *CYP3A4* (rs35599367) and *CYP3A5* (rs776746) genes in patients with non-valvular atrial fibrillation aged 80 years and older.

MATERIALS AND METHODS

Study design and ethics

This study is a cross-sectional study of data from patients with non-valvular AF aged 80 years and older that were collected from January 2019 to February 2020. The study was approved by the Ethical Committee of the Russian Medical Academy of Continuing Professional Education (Protocol No. 1 dated January 22, 2019) and conducted in accordance with the Declaration of Helsinki and in compliance with the Rules of Good Clinical Practice. Verbal and written informed consent were obtained from all the participants included in the study.

Patients

We examined 128 Caucasian patients with non-valvular AF aged 80 years and older (median age 87.5 years [83–90 years], 75% women), who were treated in a multidisciplinary hospital in Moscow. Patients were included in the study if they met the inclusion criteria.

Inclusion criteria: 1) patients with non-valvular AF of both sexes; 2) age at the time of inclusion in the study: 80 years and older; 3) duration of previous use of rivaroxaban with verapamil, amlodipine or without calcium

[1] The drug product is not approved in the Russian Federation.

[2] Same.

DOI: https://doi.org/10.17816/RCF635362

channel blockers (CCBs): at least 1 year from the moment of inclusion in the study; 4) voluntary informed consent for participation in the study.

The main non-inclusion criteria were: 1) age less than 80 years; 2) concomitant drug therapy with known drug-drug interactions with rivaroxaban (fluconazole, ketoconazole and other azole antifungal drugs; ritonavir and other human immunodeficiency virus protease inhibitors; amiodarone, clarithromycin, erythromycin; platelet aggregation inhibitors (including acetylsalicylic acid); non-steroidal anti-inflammatory drugs; selective serotonin and norepinephrine reuptake inhibitors; rifampicin, phenytoin, carbamazepine, phenobarbital, *Hypericum perforatum* preparations; 3) patient's violation of the examination and treatment plan procedures; 4) refusal to participate in the study.

All patients were taking rivaroxaban (once daily) for ischemic stroke prophylaxis at a dose of 15 mg/day (86.7% of patients) and 20 mg/day (13.3% of patients). Each patient was genotyped according to the studied polymorphisms, and the minimum equilibrium concentration of rivaroxaban ($C_{min, ss}$) was determined. In addition, the minimum equilibrium concentration of rivaroxaban was standardized to the daily drug dose ($C_{min, ss}/D$). All patients underwent coagulation tests with determination of plasma prothrombin time (PT) and analysis of medical records for the presence of adverse drug reactions (ADR) in the form of clinically relevant non-major bleeding (CRNMB). The International Society on Thrombosis and Haemostasis (ISTH) bleeding criteria were used in the study [18].

Genotyping

The material for DNA isolation was venous blood, which was collected in 4 mL Vacuette vacuum tubes with K3 EDTA anticoagulant. Genotyping for rs35599367 of the *CYP3A4* gene and rs776746 of the *CYP3A5* gene was performed using real-time polymerase chain reaction (PCR) using a CFX96 Touch™ Real-Time PCR Detection System DNA amplifier (Bio-Rad Laboratories, Inc., USA) at the Research Institute of Molecular and Personalized Medicine, Russian Medical Academy of Continuing Professional Education, Ministry of Health of Russia.

Determination of plasma rivaroxaban concentration

Venous blood was collected for determination of $C_{min, ss}$ of rivaroxaban on Day 7 of treatment with a fixed dose of anticoagulant (at least after 5 half-lives) immediately before the next dose of the drug. Determination of $C_{min, ss}$ of rivaroxaban in blood was performed by high-performance liquid chromatography with mass spectrometric detection. Samples were analyzed on Agilent 1200 liquid chromatograph (USA; includes four-channel pump, mobile phase degasser, chromatographic column thermostat). Agilent Extend-C18 column (length 100 mm, inner diameter 2.1 mm, grain size 3.5 μm) was used. The separation was performed at a column temperature of 40 °C. The mobile phase: solution "A" (50 mL of 0.1 M ammonium acetate solution and 5 mL of formic acid diluted with deionized water to a total volume of 1 L) and solution "B" (50 mL of 0.1 M ammonium acetate solution and 5 mL of formic acid diluted with acetonitrile to a total volume of 1 L). Chromatographic separation was carried out in isocratic elution mode at the ratio of components "A" and "B" of 70:30. The flow rate of the mobile phase was 0.3 mL/min. The volume of the injected sample was 10 μL. The analysis was carried out within 7 min. Agilent Triple Quad LC/MS6410 mass spectrometer (triple quadrupole type) with electrospray ionization in positive ionization mode was used. Rivaroxaban spectra were recorded in multiple molecular reaction mode. The atomizer gas pressure was 35 psi. The volume rate of drying gas was 11 L/min, and the temperature was 350 °C. The fragmentation voltage value was 135 V and the collision cell voltage was 25 V. Sample preparation was carried out by precipitation of blood plasma proteins. Plasma samples were thawed at room temperature. Then 100 μL of plasma was transferred into plastic Eppendorf tubes; 250 μL of a methanol mixture with 0.1% hydrochloric acid 9:1 was added, mixed on a Vortex shaker, left for 10 min and mixed again. After that, the obtained samples were centrifuged at 10,000 rpm for 10 min. The supernatant was transferred to chromatographic vials and placed on the autosampler of the chromatograph.

Laboratory tests

Venous blood for coagulation tests was collected simultaneously with the collection of blood for determination of $C_{min, ss}$ of rivaroxaban. Coagulation tests were performed using automatic device ACL Elite Pro (Instrumentation Laboratory, USA); complete blood count analysis was performed using hematology analyzer Advia 2120i (Siemens, USA), biochemical blood analysis was performed using integrated analyzer for biochemical, immunochemical and electrolyte analysis Siemens Dimension X and Plus (Siemens, USA), urinalysis was performed using automatic urine analyzer Aution Max AX 4280 (ARKRAY Factory Inc., Russia). All studies were performed in accordance with the manufacturer's instructions.

Statistical analysis

Statistical analysis of the data was performed in the program package Statistics v. 26. The frequency of occurrence of the studied genotypes was checked for compliance with the Hardy–Weinberg principle using the online Hardy–Weinberg equilibrium calculator [19]. The sample was described by calculating the median (*Me*) and interquartile range as 25th and 75th percentiles [Q_1; Q_2] for non-normally distributed parameters, and by

determining the mean value (*M*) with standard deviation (SD) for normally distributed parameters. The normality of the distribution of the obtained parameters was assessed using the Shapiro–Wilk criteria. For non-normally distributed parameters, the nonparametric Mann–Whitney *U*-test was used. Differences were considered significant at $p < 0.05$.

RESULTS

It was impossible to calculate the distribution of *CYP3A4* (rs35599367) gene genotypes for compliance with the Hardy–Weinberg equilibrium due to the absence of carriers of homozygous genotype (TT) of *CYP3A4* (rs35599367) in the studied group of patients. The distribution of genotypes of *CYP3A5* gene (rs776746) did not deviate from the Hardy–Weinberg equilibrium (Table 1).

Analysis of the effect of *CYP3A4* gene polymorphism (rs35599367) on pharmacokinetics of rivaroxaban in the study group (in routine clinical practice)

In our study sample there were patients with wild type CC genotype (96.1%) and patients with heterozygous ST genotype (3.9%), and no patients carrying homozygous mutated TT genotype of *CYP3A4* gene (rs35599367). Initially, the patients were comparable by sex, age, comorbidities and number of drugs used. There were no significant differences in $C_{min, ss}$ of rivaroxaban, $C_{min, ss}/D$ of rivaroxaban, PT and the number of ADRs in the form of CRNMB in patients carrying the CC genotype compared to patients carrying the ST genotype ($p > 0.05$; Table 2).

Analysis of the effect of drugs with potential drug-drug interactions on the pharmacokinetics of rivaroxaban in patients with different variants of the *CYP3A4* gene (rs35599367)

As previously stated, there is a risk of potential drug-drug interactions during co-administration of rivaroxaban with a P-gp inhibitor, which may affect the pharmacokinetics of the P-gp substrate and thus have a clinical impact on the patient. Supporting this statement, our previous analysis showed that co-administration of verapamil (a potent P-gp inhibitor and moderate CYP3A4 inhibitor) in combination with rivaroxaban, resulted in a higher $C_{min, ss}$ of rivaroxaban, and consequently, more frequent ADRs in the form of CRNMB [18].

Therefore, we separately analyzed the pharmacokinetic profile of carriers of each of the CC and ST genotypes of the *CYP3A4* gene (rs35599367) depending on concomitant therapy with calcium channel blockers (CCBs), where co-administration of rivaroxaban (P-gp substrate) with amlodipine (dihydropyridine CCB, DCCB;

Table 1. Distribution of rs35599367 (*CYP3A4*) and rs776746 (*CYP3A5*) genotypes
Таблица 1. Распределение генотипов rs35599367 (*CYP3A4*) и rs776746 (*CYP3A5*)

Genotype	Number of patients	Frequency	Hardy–Weinberg equilibrium	
			χ^2	*p*
rs35599367 (*CYP3A4*)				
CC	123	0.961	–*	–*
CT	5	0.039		
rs776746 (*CYP3A5*)				
GG	110	0.859		
AG	16	0.125	2.237	0.134
AA	2	0.016		

*It is not possible to calculate the distribution of *CYP3A4* (rs35599367) gene genotypes for compliance with Hardy–Weinberg equilibrium
*Нет возможности рассчитать распределение генотипов гена *CYP3A4* (rs35599367) на соответствие равновесию Харди–Вайнберга

Table 2. Effects of *CYP3A4* (rs35599367) gene polymorphism on various parameters
Таблица 2. Влияние rs35599367 гена *CYP3A4* на различные параметры

Parameter	All patients ($n = 128$)	Genotype		*p*
		CC ($n = 123$)	CT ($n = 5$)	
$C_{min, ss}$ of rivaroxaban, *Me* [Q_1; Q_2], ng/mL	52.3 [28.5; 81.9]	52.1 [28.9; 86.6]	54.5 [22.9; 65.6]	0.483
$C_{min, ss}/D$ of rivaroxaban, *Me* [Q_1; Q_2], ng/mL × mg	3.4 [1.9; 5.1]	3.4 [1.9; 5.2]	3.6 [1.4; 4.4]	0.539
Prothrombin time, *Me* [Q_1; Q_2], sec	13.9 [12.6; 14.7]	14.0 [12.6; 14.9]	12.8 [11.9; 13.9]	0.157
Mild clinically significant bleeding, abs. (%)	23/128 (18)	22/123 (17.9)	1/5 (20)	0.904

P-gp substrate) may lead to drug-drug interactions due to competition of the substrates for binding sites on cell membranes, and co-administration of rivaroxaban with verapamil (non-DCCB; potent P-gp inhibitor and moderate CYP3A4 inhibitor) may lead to drug-drug interactions due to inhibition of the leading transport and metabolic pathways of rivaroxaban. Patients were initially comparable by sex, age, comorbidities and number of drugs used.

When analyzing the pharmacokinetic profile of carriers of the CC genotype of *CYP3A4* gene (rs35599367), the $C_{min, ss}$ level of rivaroxaban was significantly higher in the group of patients taking rivaroxaban + verapamil compared to the group of patients taking rivaroxaban without CCBs (73.8 [49; 113.5] vs. 40.5 [25.8; 75.4] ng/mL; $p = 0.005$), and compared to the group of patients taking rivaroxaban without CCBs and rivaroxaban + amlodipine (vs. 46.7 [28.3; 78.8] ng/mL, $p = 0.013$). Similar results were obtained when analyzing the $C_{min, ss}/D$ of rivaroxaban: in the group of patients taking rivaroxaban + verapamil, the $C_{min, ss}/D$ level of rivaroxaban was significantly higher compared to the group of patients taking rivaroxaban without CCBs (4.7 [2.9; 7.7] vs. 2.5 [1.7; 4.2] ng/mL × mg, $p = 0.004$) and compared to the group of patients taking rivaroxaban without CCBs and rivaroxaban + amlodipine (vs. 3.0 [1.9; 4.6] ng/mL × mg, $p = 0.010$). The PT level in the group of patients taking rivaroxaban + verapamil was higher than in the group of patients taking rivaroxaban without CCBs (14.8 [13.3; 17.3] vs. 14.0 [12.6; 14.5] s, $p = 0.014$), higher than in the group of patients taking rivaroxaban + amlodipine (13.3 [12.4; 14.6] sec, $p = 0.009$) and higher than in the group of patients taking rivaroxaban without CCBs and rivaroxaban + amlodipine (13.7 [12.6; 14.6] sec, $p = 0.005$). ADRs in the form of CRNMB were more frequent in patients taking rivaroxaban + verapamil than in the group of patients taking rivaroxaban without CCBs (33.3% vs. 13.3%, $p = 0.038$), more frequent than in the group of patients taking rivaroxaban + amlodipine (12.5%, $p = 0.027$) and more frequent than in the group of patients taking rivaroxaban without CCBs and rivaroxaban + amlodipine (12.9%, $p = 0.011$; Table 3).

Analysis of the pharmacokinetic profile of 5 carriers of heterozygous ST type of *CYP3A4* gene (rs35599367) included 2 patients taking rivaroxaban without CCBs and 3 patients taking rivaroxaban in combination with amlodipine, there were no carriers of ST genotype of *CYP3A4* gene (rs35599367) taking rivaroxaban in combination with verapamil in the sample. The analysis showed that patients taking rivaroxaban in combination with amlodipine had higher values of the studied parameters ($C_{min, ss}$ of rivaroxaban, $C_{min, ss}/D$ of rivaroxaban, PT and ADRs as CRNMB) compared to patients taking rivaroxaban without CCBs, but these values did not reach statistical significance ($p > 0.05$) (Table 3).

Analysis of the effect of *CYP3A5* gene polymorphism (rs776746) on the pharmacokinetics of rivaroxaban in the study group (in routine clinical practice)

In the study sample, wild type GG was found in 85.9% (110/128) of patients, heterozygous type GA in 12.5% (16/128), homozygous mutant type AA in 1.6% (2/128) of patients. For further analysis, we combined heterozygous and homozygous mutant type (GA + AA) carriers into one group of patients 14.1% (18/128). Initially, patients were comparable by sex, age, comorbidities and number of drugs used. No significant differences in $C_{min, ss}$ of rivaroxaban, $C_{min, ss}/D$ of rivaroxaban, PT, and number of ADRs as CRNMB were observed in GG type carriers compared to GA+AA type carriers ($p > 0.05$; Table 4).

Analysis of the effect of drugs with potential drug-drug interactions on pharmacokinetics of rivaroxaban in patients with different variants of *CYP3A5* gene (rs776746)

We separately analyzed the pharmacokinetic profile of carriers of each of the GG and GA + AA genotypes depending on concomitant CCBs therapy, where co-administration of rivaroxaban (P-gp substrate) with amlodipine (dihydropyridine CCB, DCCB; P-gp substrate) may lead to drug-drug interactions due to competition of the substrates for binding sites on cell membranes, and co-administration of rivaroxaban with verapamil (non-DCCB; strong P-gp inhibitor and moderate CYP3A4 inhibitor) may lead to drug-drug interactions due to inhibition of the leading transport and metabolic pathways of rivaroxaban.

When analyzing the pharmacokinetic profile of wild-type (GG) *CYP3A5* gene (rs776746) carriers, the $C_{min, ss}$ levels of rivaroxaban were significantly higher in the group of patients taking rivaroxaban + verapamil compared to the group of patients taking rivaroxaban without CCBs (74.7 [50.6; 108.8] vs. 40.2 [25.7; 72.3] ng/mL; $p = 0.003$) and the group of patients taking rivaroxaban + amlodipine (vs. 49.2 [28.9; 68.8] ng/mL, $p = 0.036$). The level of $C_{min, ss}$ of rivaroxaban was also significantly higher in the group of patients taking rivaroxaban without CCBs and rivaroxaban + amlodipine (vs. 45.6 [28.2; 68.8] ng/mL, $p = 0.005$). Similar results were obtained when analyzing the $C_{min, ss}/D$ of rivaroxaban: in the group of patients taking rivaroxaban + verapamil, the $C_{min, ss}/D$ level of rivaroxaban was significantly higher than in the group of patients taking rivaroxaban without CCBs (4.6 [3.0; 7.3] vs. 2.5 [1.7; 4.0] ng/mL × mg, $p = 0.002$) and the group of patients who took rivaroxaban + amlodipine (3.3 [1.9; 4.6] ng/mL × mg, $p = 0.032$); $C_{min, ss}/D$ of rivaroxaban was significantly higher in the group of patients who took rivaroxaban without CCBs and rivaroxaban + amlodipine (vs. 3.0 [1.9; 3.0] ng/mL × mg, $p = 0.004$). The PT level in the group of patients taking rivaroxaban + verapamil was significantly higher than in the group of patients taking rivaroxaban without CCBs (14.6 [13.4; 17.4] vs. 14.0

Table 3. Effects of a drug with potential drug-drug interactions (depending on the *CYP3A4* (rs35599367) gene polymorphism) on various parameters

Таблица 3. Влияние лекарственного средства, с потенциально возможным межлекарственным взаимодействием в зависимости от варианта гена *CYP3A4* (rs35599367), на различные параметры

Parameter	Rivaroxaban without CCB	Rivaroxaban and amlodipine	Rivaroxaban and verapamil	*p*
CC genotype (*n* = 123) of *CYP3A4* gene (rs35599367)				
Number of patients, abs. (%)	47/128 (36.7)	51/128 (39.8)	30/128 (23.5)	–
$C_{min, ss}$ of rivaroxaban, *Me* [Q_1; Q_2], ng/mL	40.5 [25.6; 73.3]	55.2 [28.9; 91.3]	73.8 [49; 113.5]	$p_1 = 0.120$ $p_2 = 0.004$ $p_3 = 0.079$
$C_{min, ss}/D$ of rivaroxaban, *Me* [Q_1; Q_2], ng/mL × mg	2.5 [1.7; 4.0]	3.3 [1.9; 5.0]	4.7 [2.9; 7.7]	$p_1 = 0.110$ $p_2 = 0.003$ $p_3 = 0.066$
Prothrombin time, *Me* [Q_1; Q_2], sec	14.0 [12.6; 14.5]	13.3 [12.4; 14.6]	14.8 [13.3; 17.3]	$p_1 = 0.791$ $p_2 = 0.014$ $p_3 = 0.009$
Mild clinically significant bleeding, abs. (%)	6/45 (13.3)	6/48 (12.5)	10/30 (33.3)	$p_1 = 0.905$ $p_2 = 0.038$ $p_3 = 0.027$
CT genotype (*n* = 5) of *CYP3A4* gene (rs35599367)				
Number of patients, abs. (%)	2/5 (40)	3/5 (60)	0/5	–
$C_{min, ss}$ of rivaroxaban, *Me* [Q_1; Q_2], ng/mL	39.9 [25.2; 39.9]	61.7 [20.6; 61.7]	–	$p_1 = 0.800$
$C_{min, ss}/D$ of rivaroxaban, *Me* [Q_1; Q_2], ng/mL/mg	2.7 [1.7; 2.7]	4.1 [1.0; 4.1]	–	$p_1 = 0.800$
Prothrombin time, *Me* [Q_1; Q_2], sec	11.9 [11.4; 11.9]	13.4 [12.8; 13.4]	–	$p_1 = 0.200$
Mild clinically significant bleeding, abs. (%)	–	1/3 (33.3)	–	$p_1 = 0.361$

Note. p_1, differences between the group of patients taking rivaroxaban without CCB and the group of patients taking rivaroxaban in combination with amlodipine; p_2, differences between the group of patients taking rivaroxaban without CCB and the group of patients taking rivaroxaban in combination with verapamil; p_3, differences between the group of patients taking rivaroxaban in combination with amlodipine and the group of patients taking rivaroxaban in combination with verapamil. CCB, calcium channel blockers.

Примечание. p_1 — Различия между группой пациентов, принимающих ривароксабан без БКК и группой пациентов, принимающих ривароксабан в сочетании с амлодипином; p_2 — различия между группой пациентов, принимающих ривароксабан без БКК и группой пациентов, принимающих ривароксабан в сочетании с верапамилом; p_3 — различия между группой пациентов, принимающих ривароксабан в сочетании с амлодипином, и группой пациентов, принимающих ривароксабан в сочетании с верапамилом. БКК — блокаторы кальциевых каналов.

Table 4. Effects of *CYP3A5* (rs776746) gene polymorphism on various parameters

Таблица 4. Влияние генотипов гена *CYP3A5* (rs776746) на различные параметры

Parameter	All patients (*n* = 128)	Genotype		*p*
		GG (*n* = 110)	GA + AA (*n* = 18)	
$C_{min, ss}$ of rivaroxaban, ng/mL, *Me* [Q_1; Q_2]	52.3 [28.5; 81.9]	50.8 [29.2; 80.8]	74.0 [25.7; 106.8]	$p_1 = 0.497$
$C_{min, ss}/D$ of rivaroxaban, ng/mL/mg, *Me* [Q_1; Q_2]	3.4 [1.9; 5.1]	3.8 [2.0; 5.0]	4.4 [1.7; 7.1]	$p_1 = 0.451$
Prothrombin time, sec, *Me* [Q_1; Q_2]	13.9 [12.6; 14.7]	14.9 [12.7; 14.8]	13.2 [12.4; 14.9]	$p_1 = 0.461$
Mild clinically significant bleeding, abs. (%)	23/128 (18)	22/110 (20)	1/18 (5.6)	$p_1 = 0.139$

[12.6; 14.5] sec, $p = 0.025$) and in the group of patients taking rivaroxaban + amlodipine (13.3 [12.4; 14.6] sec, $p = 0.012$). ADRs in the form of CRNMB were more frequent in patients taking rivaroxaban + verapamil than in the group of patients taking rivaroxaban without CCBs (37% vs. 12.5%, $p = 0.018$) and in the group of patients taking rivaroxaban + amlodipine (16.3%, $p = 0.049$; Table 5).

When we analyzed the pharmacokinetic profile of patients carrying mutant types GA + AA of *CYP3A5* gene (rs776746), the levels of $C_{min, ss}$ of rivaroxaban and $C_{min, ss}/D$ of rivaroxaban were significantly higher in patients taking rivaroxaban in combination with verapamil compared to patients taking rivaroxaban without CCBs (88.1 [5.5; 88.1] vs. 52.8 [25.0;77.2] ng/mL, $p = 0.040$, and 5.7 [0.4; 5.7] vs. 3.5 [1.7; 5.2] ng/mL × mg, $p = 0.030$, respectively). No significant differences were found for the other parameters, $p > 0.05$ (Table 5).

DISCUSSION

Our data showed that in patients aged 80 years and older with non-valvular AF there is pharmacokinetic variability of drug-drug interactions of rivaroxaban with P-gp inhibitors depending on the variants of *CYP3A4/A5* genes. Thus, in carriers of the CC genotype of *CYP3A4* gene (rs35599367) co-administration of rivaroxaban with verapamil resulted in higher values of rivaroxaban concentration in serum and ADRs in the form of CRNMB. There were no carriers of the heterozygous ST genotype taking rivaroxaban with verapamil in the sample; however, co-administration of rivaroxaban with amlodipine to the heterozygous ST genotype carriers resulted in higher values of rivaroxaban serum concentration, PT and ADRs in the form of CRNMB, but they were statistically insignificant. In patients carrying the wild-type GG genotype of *CYP3A5* gene (rs776746), co-administration of rivaroxaban with verapamil resulted in higher values of rivaroxaban serum concentrations, PT and ADRs as CRNMB, and in carriers of the GA + AA mutant genotypes co-administration of rivaroxaban with verapamil resulted in higher values of rivaroxaban serum concentration compared to patients taking rivaroxaban without BCC.

Notably, when analyzing the effect of *CYP3A4* and *CYP3A5* gene polymorphism on rivaroxaban pharmacokinetics and the development of ADRs for genotypes that included the mutant allele, there were higher values of rivaroxaban serum concentration and ADRs as CRNMB in the ST type of *CYP3A4* gene and higher values of rivaroxaban serum concentration in the GA + AA type carriers of *CYP3A5* gene. Although these values did not reach statistical significance, this trend cannot be neglected. This may have been due to the small sample of patients in the groups for the studied mutant gene variants (5 vs. 123 for *CYP3A4* and 18 vs. 110 for *CYP3A5*), so larger and more carefully designed controlled studies are needed to obtain statistically significant differences.

Our results on the variability of the pharmacologic response of rivaroxaban when co-administered with a P-gp inhibitor are comparable to the study by Gouin-Thibault et al. [6], where co-administration of rivaroxaban with a P-gp/CYP3A4 inhibitor (clarithromycin) was shown to increase AUC (area under the curve) of rivaroxaban by 94% ($p < 0.0001$) and its maximum concentration ($C_{max, ss}$) by 92% ($p < 0.0001$): geometric mean ratios were 1.94 (95% CI 1.42–2.63) and 1.92 (95% CI 1.60–2.28) for AUC and $C_{max, ss}$, respectively. Whereas the ABCB1 genotype was not a significant determinant of individual variability in rivaroxaban pharmacokinetics.

Similar results were obtained regarding the influence of *CYP3A4* and *CYP3A5* gene variants on the development of ADRs with rivaroxaban administration by Campos-Staffico et al. [7], who conducted a single-center retrospective cohort study with 2,364 Caucasian outpatients with non-valvular AF with rivaroxaban administration (mean age 68.3 ± 13.6 years, 32% women). Although the authors found no statistically significant differences in the risk of bleeding during rivaroxaban treatment between *CYP3A4* (rs35599367) [AA vs. AG vs. GG, relative risk (RR) 0.876 (95% CI 0.691–1.110, $p > 0.05$] and *CYP3A5* [GG vs. GA vs. AA, RR0.960 (95% CI 0.685–1.347), $p > 0.05$] genotypes. In the exploratory analysis, there was a trend for an association between carriers of the G allele of *CYP3A5* gene and a reduced risk of bleeding on oral anticoagulants ($p < 0.05$), which may indirectly confirm the contribution of the mutant A allele of *CYP3A5* gene to the development of bleeding. The authors suggested that to obtain more accurate and meaningful results, future pharmacogenetic studies should be performed in African patients, in whom the A allele is much more common (greater than 70%).

However, as already mentioned, data on the effect of gene polymorphisms on rivaroxaban pharmacokinetics are conflicting. For example, in the previous study, we have already analyzed the effect of gene polymorphism of *CYP3A4* (rs35599367), *CYP3A5* (rs776746) and *ABCB1* (rs1045642 and rs4148738) on rivaroxaban pharmacokinetics and prothrombin time dynamics in patients after endoprosthesis of large joints of the lower extremities (hip or knee) taking rivaroxaban at a dose of 10 mg per day (78 patients, mean age 59 ± 11 years) [8]. As a result of the study, patients with mutant genotypes of the studied SNP did not show the expected increase in the peak equilibrium concentration of rivaroxaban. The results of comparison of $C_{max, ss}$ of rivaroxaban between wild-type and mutant genotypes were not statistically significant. There were no statistically significant differences in the $C_{max, ss}$ of rivaroxaban between the wild-type haplotypes [*ABCB1* rs1045642 (CC) — *CYP3A4* rs35599367 (CC) and *ABCB1* rs4148738 (CC) — *CYP3A4* rs35599367 (CC)] and mutant

Table 5. Effects of drugs with potential drug-drug interactions (depending on the *CYP3A5* (rs776746) gene polymorphism) on various parameters

Таблица 5. Влияние лекарственных средств с потенциально возможным межлекарственным взаимодействием в зависимости от вариантов гена *CYP3A5* (rs776746)

Parameter	Rivaroxaban without CCB	Rivaroxaban and amlodipine	Rivaroxaban and verapamil	*p*
GG genotype (*n* = 110) of *CYP3A5* gene (rs776746)				
Number of patients, abs. (%)	40/110 (36.4)	43/110 (39.1)	27/110 (24.5)	–
$C_{min,\ ss}$ of rivaroxaban, ng/mL, *Me* [Q_1; Q_2]	40.2 [25.7; 72.3]	49.2 [28.9; 68.8]	74.7 [50.6; 108.8]	$p_1 = 0.210$ $p_2 = 0.003$ $p_3 = 0.036$
$C_{min,\ ss}/D$ of rivaroxaban, ng/mL × mg, *Me* [Q_1; Q_2]	2.5 [1.7; 4.0]	3.3 [1.9; 4.6]	4.6 [3.0; 7.3]	$p_1 = 0.176$ $p_2 = 0.002$ $p_3 = 0.032$
Prothrombin time, sec, *Me* [Q_1; Q_2]	14.0 [12.6; 14.5]	13.3 [12.4;14.6]	14.6 [12.8; 15.2]	$p_1 = 0.841$ $p_2 = 0.025$ $p_3 = 0.012$
Mild clinically significant bleeding, abs. (%)	5/40 (12.5)	7/43 (16.3)	10/27 (37)	$p_1 = 0.625$ $p_2 = 0.018$ $p_3 = 0.049$
GA + AA genotype (*n* = 18) of *CYP3A5* gene (rs776746)				
Number of patients, abs. (%)	7/18 (39)	8/18 (44)	3/18 (17)	–
$C_{min,\ ss}$ of rivaroxaban, ng/mL, *Me* [Q_1; Q_2]	52.8 [25.0; 77.2]	72.8 [38.2; 98.9]	88.1 [5.5; 88.1]	$p_1 = 0.281$ $p_2 = 0.04$ $p_3 = 0.79$
$C_{min,\ ss}/D$ of rivaroxaban, ng/mL × mg, *Me* [Q_1; Q_2]	3.5 [1.7; 5.2]	4.9 [2.3; 6.3]	5.7 [0.4; 5.7]	$p_1 = 0.336$ $p_2 = 0.03$ $p_3 = 0.066$
Prothrombin time, sec, *Me* [Q_1; Q_2]	13.2 [12.4; 14.4]	13.3 [12.2; 15.7]	16.0 [12.2; 16.0]	$p_1 = 0.536$ $p_2 = 0.517$ $p_3 = 0.376$
Mild clinically significant bleeding, abs. (%)	1/7 (14.3)	–	–	$p_1 = 0.268$ $p_2 = 0.490$

Note: p_1, differences between the group of patients taking rivaroxaban without CCB and the group of patients taking rivaroxaban in combination with amlodipine; p_2, differences between the group of patients taking rivaroxaban without CCB and the group of patients taking rivaroxaban in combination with verapamil; p_3, differences between the group of patients taking rivaroxaban in combination with amlodipine and the group of patients taking rivaroxaban in combination with verapamil. CCB, calcium channel blockers.

Примечание: p_1 — Различия между группой пациентов, принимающих ривароксабан без БКК и группой пациентов, принимающих ривароксабан в сочетании с амлодипином; p_2 — различия между группой пациентов, принимающих ривароксабан без БКК, и группой пациентов, принимающих ривароксабан в сочетании с верапамилом; p_3 — различия между группой пациентов, принимающих ривароксабан в сочетании с амлодипином, и группой пациентов, принимающих ривароксабан в сочетании с верапамилом. БКК — блокаторы кальциевых каналов.

haplotypes [*ABCB1* rs1045642 (CT) — *ABCB1* rs1045642 (TT) — *CYP3A4* rs35599367 (CT) and *ABCB1* rs4148738 (CT) — *ABCB1* rs4148738 (TT) — *CYP3A4* rs35599367 (CT)]. However, a statistically significant mean correlation was found between prothrombin time and $C_{max,\ ss}$ of rivaroxaban ($r = 0.421$; $r_2 = 0.178$; $p < 0.001$).

In another study, where we analyzed the data of 86 patients with AF taking rivaroxaban (mean age 67.24 ± 1.01, 51% women), we also found no statistically significant differences in $C_{min,\ ss}$ of rivaroxaban between the CC and ST genotypes of *CYP3A4* gene (rs35599367) (57.6 vs. 96.35 ng/mL, $p = 0.108$) carriers, in this sample only one patient had the TT genotype. Also, there were no statistically significant differences with respect to $C_{min,\ ss}$ of rivaroxaban between the GG and AG genotypes of *CYP3A5* gene (rs776746) carries (58.6 vs. 117.35 ng/L, $p = 0.272$), only one patient had the AA genotype [9].

Similar results were obtained in our study, where we analyzed patients with non-valvular AF and concomitant stage III and IV CKD taking rivaroxaban (*Me* age 82 [74; 86] years, 71.3% women, duration of follow-up 16 weeks) [10]. We divided 122 patients into 3 groups: slow metabolizers [CYP3A5*3 (GG) + CYP3A4*22 (CT)]; intermediate metabolizers [CYP3A5*3 (GG) + CYP3A4*22 (CC) and CYP3A5*3 (GA) + CYP3A4*22 (CT)]; and normal metabolizers [CYP3A5*3 (GA) + CYP3A4*22 (CC)]. The results showed no statistically significant differences in $C_{min,\ ss}$ (62.8 [17.6; 176.2] vs. 51.7 [21.05; 91.75] vs. 46.1 [32.7; 132.7] ng/mL, $p > 0.05$), $C_{min,\ ss}/D$ of rivaroxaban (4.2 [1.08; 8.80] vs. 2.8 [1.29; 5.27] vs. 3.0 [1.67; 8.45] ng/mL × mg, $p > 0.05$), and bleeding episode rates 55 (57.1%) vs. 37 (38.1%) vs. 9 (52.9%) events ($p > 0.05$) between the groups.

No association between the genotypes of *CYP3A4/A5* genes and pharmacokinetics of rivaroxaban, as well as the development of bleeding was found in studies by other authors. Thus, Nakagawa et al. [11] studied the effect of SNP CYP3A5*3 on $C_{min,\ ss}/D$ of rivaroxaban in plasma of 86 Japanese patients, mean age 62.4 ± 10.6 (31 to 82 years) with non-valvular AF who underwent catheter ablation. The results showed that the median (quartile range) $C_{min,\ ss}/D$ of rivaroxaban was 3.39 (2.08–5.21) ng/mL × mg (coefficient of variation: 80.5%), which was not significantly different between CYP3A5*3 genotypes (AA 4.77 [3.83; 7.36] vs. AG 3.58 [2.21; 5.13] vs. GG 2.99 [1.94; 5.34] ng/mL × mg, $p > 0.05$). In the study by Wu et al. [12] they analyzed 95 patients with non-valvular AF taking rivaroxaban (mean age 65.8 ± 12.5 years, 41% women). The results revealed no statistically significant differences in $C_{min,\ ss}/D$ of rivaroxaban between *CYP3A4* (rs2242480) genotype variants (CC0.96 [0.42; 1.98] vs. CT 0.71 [0.29; 1.60] vs. TT 1.10 [0.63; 2.19] ng/mL × mg, $p > 0.05$), *CYP3A4* (rs4646437) (GG 0.81 [0.40; 1.55] vs. GA 0.81 [0.26; 1.71] vs. AA 1.10 [1.03; 1.25] ng/mL × mg, $p > 0.05$) and *CYP3A5* (rs776746) genotypes (TT 1.10 [0.63; 2.19] vs. TC1.01 [0.50; 1.73] vs. CC0.66 [0.36; 1.51] ng/mL × mg, $p > 0.05$). Also, there were no significant differences in the incidence of bleeding events between *CYP3A4* (rs2242480) (6 vs. 9 vs. 1 event, respectively, $p > 0.05$), *CYP3A4* (rs4646437) (11 vs. 4 vs. 1 event, respectively, $p > 0.05$), and *CYP3A5* (rs776746) genotypes (1 vs. 8 vs. 6 events, respectively, $p > 0.05$).

Since all the mentioned studies had different design, indications for rivaroxaban use, age and nationality composition of the sample, and different endpoints, the relationship between genetic variants and pharmacokinetic parameters and the development of bleeding should be further investigated and confirmed in a larger sample size.

CONCLUSION

Our data showed that in routine clinical practice in patients with non-valvular AF aged 80 years and older, carriers of the wild homozygous CC genotype of *CYP3A4* gene (rs35599367) and GG genotype of *CYP3A5* gene (rs776746) have high pharmacokinetic sensitivity to verapamil administration (potent P-gp inhibitor and moderate CYP3A4 inhibitor). Carriers of the ST mutant genotype of *CYP3A4* gene (rs35599367) were showed to have high pharmacokinetic sensitivity to amlodipine administration (possible drug-drug interaction due to substrate competition for binding sites on cell membranes). This knowledge may be useful for the development of a personalized approach to the management of elderly patients, in whom polymorbidity carries a high risk of polypharmacy, which can lead to drug-drug interactions and severe ADRs.

ADDITIONAL INFO

Authors' contribution. All authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study. Personal contribution of each author: M.S. Cherniaeva, I.A. Konstantinova — review of publications on the topic, processing of literary data, creation of a draft; M.S. Cherniaeva, E.K. Baranovskaya, O.V. Golovina, I.I. Sinitsyna, P.O. Bochkov, Sh.P. Abdullaev, N.P. Denisenko, Zh.A. Sozaeva — research, analysis, resources; M.S. Cherniaeva, K.B. Mirzaev, N.V. Lomakin, D.A. Sychev — conceptualization, methodology, resources, editing of the manuscript, general supervision.

Funding source. The study was supported by the Russian Science Foundation (grant No. 22-15-00251 "Personalized use of direct oral anticoagulants based on a pharmacogenomic approach").

Competing interests. The authors declare that they have no competing interests.

Ethics approval. The protocol of the study was approved by the local Ethics Committee of the Russian Medical Academy of Continuing Professional Education (No. 1 dated 2019 Jan 22).

Consent for publication. Written consent was obtained from the patients for publication of relevant medical information within the manuscript.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией. Личный вклад каждого автора: М.С. Черняева, И.А. Константинова — обзор публикаций по теме, обработка литературных данных, создание черновика; М.С. Черняева, Е.К. Барановская, О.В. Головина, И.И. Синицина, П.О. Бочков, Ш.П. Абдуллаев, Н.П. Денисенко, Ж.А. Созаева — исследование, анализ, ресурсы; М.С. Черняева, К.Б. Мирзаев, Н.В. Ломакин, Д.А. Сычев — концептуализация, методология, ресурсы, редактирование рукописи, общее руководство.

Источник финансирования. Работа выполнена при поддержке Российского научного фонда (грант № 22-15-00251 «Персонализированное применение прямых пероральных антикоагулянтов на основе фармакогеномного подхода»).

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Этический комитет. Протокол исследования был одобрен локальным этическим комитетом ФГБОУ ДПО «Российская медицинская академия непрерывного профессионального образования» Министерства здравоохранения Российской Федерации (№ 1 от 22.01.2019).

Информированное согласие на публикацию. Авторы получили письменное согласие пациентов на публикацию медицинских данных.

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