INTERNAL DISEASES

ROLE OF *ITGB3*, *GP1B1*, AND *ITGA2* GENE POLYMORPHISMS IN PLATELET DYSFUNCTION IN PATIENTS WITH COVID-19-ASSOCIATED LUNG DAMAGE

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ABSTRACT

The aim of the work. *To investigate platelet aggregation, polymorphism in the genes that ensure its implementation, and the association between these indicators in patients with COVID-19-associated lung damage, depending on the severity of the clinical course.*

Methodology. *The study involved 75 patients with COVID-19, which, depending on the severity of lung involvement, were divided into two groups: patients with damage of up to 50 % of the lung parenchyma (n = 48) and with damage of more than 50 % (n = 27), respectively. The control group consisted of healthy people (n = 24), comparable in gender and age. In all individuals, the number of platelets, platelet aggregation induced by ADP, collagen and ristomycin were studied; polymorphisms rs6065 in the GP1BA gene, rs1126643 in the ITGA2 gene, and rs5918 in the ITGB3 gene were determined by polymerase chain reaction. Analysis of the data obtained was executed using the IBM SPSS Statistics v. 23 (IMB Corp., USA).*

Results and discussion. *In patients with moderate and severe COVID-19-associated lung damage, platelet aggregation induced by ADP, collagen, and ristomycin accelerated; in severe cases, the number of platelets decreased. The frequency of variants of the rs6065 polymorphism did not change, the frequency of occurrence of the T/C genotype of the rs5918 polymorphism increased; with moderate severity, the frequency of occurrence of the C/T and T/T genotypes of the rs1126643 polymorphism increased; with severe lung damage, the frequency of occurrence of the mutant C/C genotype polymorphism rs5918 increased. In moderate lung damage, the presence of the mutant T/T polymorphism rs1126643 accelerated collagen-induced platelet aggregation; in severe cases, the presence of mutant C/C and heterozygous variant C/T polymorphism rs5918 accelerated ADP-induced platelet aggregation. There was no effect of the rs6065 polymorphism on platelet aggregation. The data obtained indicate the possible role of genetic predisposition in the activation of platelet aggregation in patients with COVID-19-associated lung damage.*

Key words: *COVID-19, platelets, aggregation, polymorphism, GP1BA, ITGA2, ITGB3*

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РОЛЬ ПОЛИМОРФИЗМА ГЕНОВ *ITGB3*, *GP1B1* И *ITGA2* В ПАТОГЕНЕЗЕ ГИПЕРРЕАКТИВНОСТИ ТРОМБОЦИТОВ ПРИ COVID-19-АССОЦИИРОВАННОМ ПОРАЖЕНИИ ЛЁГКИХ СРЕДНЕЙ И ТЯЖЁЛОЙ СТЕПЕНИ ТЯЖЕСТИ

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РЕЗЮМЕ

Цель работы. *Исследовать агрегацию тромбоцитов, полиморфизм в генах, обеспечивающих её реализацию, и ассоциацию между данными показателями у пациентов с COVID-19 при среднем и тяжёлом течении заболевания.*

Методология. *В исследовании принимали участие 75 больных COVID-19, которые в зависимости от объёма поражения лёгочной паренхимы разделены на две группы в зависимости от объёма поражения паренхимы лёгких. Контрольная группа – практически здоровые люди (n = 24). У всех лиц исследованы количество тромбоцитов в крови и агрегация тромбоцитов, индуцированная аденозиндифосфатом (АДФ), коллагеном и ристомицином; методом полимеразной цепной реакции определяли полиморфизмы rs6065 в гене GP1BA, rs1126643 в гене ITGA2, rs5918 в гене ITGB3.Анализ полученных данных проводили с помощью пакета прикладных программ IBM SPSS Statistics v. 23 (IBM Corp., США).*

Результаты и обсуждение. *У больных с COVID-19-ассоциированным поражением лёгких среднего и тяжёлого течения ускоряется агрегация тромбоцитов, индуцированная АДФ, коллагеном, ристомицином; при тяжёлом течении снижается количество тромбоцитов. Не изменяется частота встречаемости вариантов полиморфизма rs6065, повышается частота встречаемости генотипа T/C полиморфизма rs5918; при средней тяжести повышается частота встречаемости генотипов C/T и T/T полиморфизма rs1126643; при тяжёлом поражении лёгких повышается частота встречаемости мутантного генотипа C/C полиморфизма rs5918. При поражении лёгких средней степени тяжести наличие мутантного варианта T/T полиморфизма rs1126643 ускоряет коллаген-индуцированную агрегацию тромбоцитов; при тяжёлой степени тяжести наличие мутантного C/C и гетерозиготного C/T вариантов полиморфизма rs5918 ускоряет АДФ-индуцированную агрегацию тромбоцитов. Не выявлено влияния полиморфизма rs6065 на агрегацию тромбоцитов. Полученные данные указывают на возможную роль генетической предрасположенности в активации агрегации тромбоцитов у больных с COVID-19-ассоциированным поражением лёгких.*

Ключевые слова: *COVID-19, тромбоциты, агрегация, полиморфизм, GP1BA, ITGA2, ITGB3*

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INTRODUCTION

Changes in hemostasis leading to thrombotic complications are common in hospitalized patients with COVID-19 (coronavirus disease 2019). Given that platelets are key participants and regulators of thrombosis and inflammation, they are an important source of mediators in the pathogenesis of COVID-19 [1, 2]. Patients with COVID-19-associated lung damage have an increased risk of thrombotic complications and mortality, also due to hyperreactivity of platelets [3–5]. Platelets are involved in the pathogenesis of COVID-19 in different ways. SARS-CoV-2 (severe acute respiratory syndrome-related coronavirus 2) infects bone marrow megakaryocytes; the presence of virions in peripheral blood platelets has been shown to directly increase their aggregation capacity [6]. It has been established that markers of platelet activity (platelet size and maturity) are significantly associated with both disease severity and mortality, even taking into account the presence of comorbidities, medications, and other laboratory parameters, including biomarkers of inflammation and thrombosis (e.g., D-dimer). Platelets in patients with COVID-19 were studied and showed activation of metabolic processes, including oxidative phosphorylation and glycolysis, which increases their aggregation [7]. Multiple platelet activation pathways initiate and maintain thrombus formation. Platelets extracted from patients with COVID-19 show a greater degree of aggregation with various agonists (adenosine triphosphate, adrenaline, collagen, and ristomycin), which may be due to genetic factors [8–10]. A limited number of studies have examined the impact of individual prothrombotic risk factors, both genetic and acquired, on the severity of COVID-19.

Genetic factors include the 4G/5G polymorphism of the plasminogen activator inhibitor-I (PAI-I) gene: it was found to enhance thrombosis-mediated osteonecrosis after COVID-19 infection. A strong correlation between the presence of the C677T polymorphism of the methylenetetrahydrofolate reductase (*MTHFR*) gene and the severity of COVID-19 course has been described [11]. However, the complexity of the clinical course and major complications in patients with severe COVID-19 suggests that a number of other genetic risk factors may be involved in the pathogenesis of COVID-19. This is the reason for the relevance of studies of protein genes involved in hemostasis and cardiovascular complications in patients with COVID-19. These genes include *GP1BA* (glycoprotein Ib-alpha), *ITGB3* (integrin beta 3), and *ITGA2* (integrin alpha 2), which mediate the triggering of various platelet activation mechanisms. The connection of the named genes with increased platelet activation by aggregation inducers in COVID-19 patients has not been systematically studied. The high rate of thrombotic complications in severe forms of COVID-19-associated lung damage may be related to platelet hyperreactivity in conditions of genetic predisposition.

THE AIM OF THE STUDY

To analyze platelet aggregation and polymorphisms in genes providing its realization in patients with COVID-19-associated lung damage, depending on the severity of clinical course.

MATERIALS AND METHODS

The study involved 75 patients with COVID-19 (44 women and 31 men) aged 44 to 75 years, hospitalized in Chelyabinsk Regional Clinical Hospital No. 3, who did not take drugs affecting platelet function before hospitalization and were not related to each other. The control group consisted of 24 clinically healthy volunteers (Group 1), comparable in gender and age to COVID-19 patients and unrelated to each other. Depending on the volume of lung damage, patients with COVID-19 were divided into groups: Group 2 with up to 50 % damage – medium severity (n = 48); Group 3 with more than 50 % damage – severe severity (n = 27), – in accordance with the guidelines of the Ministry of Health of Russia "Prevention, diagnosis and treatment of new coronavirus infection (COVID-19)" [12]. The inclusion criteria for Groups 2 and 3 were the presence of COVID-19 confirmed by detection of SARS-CoV-2 RNA virus on the mucous membranes of the pharynx and nasal cavity using polymerase chain reaction (RealBest SARS-CoV-2 RNA; Vector-Best, Russia). Exclusion criteria were presence of previously detected oncological diseases, chronic diseases of cardiovascular, respiratory, nervous systems and gastrointestinal tract organs; extremely severe course of combined pathology requiring hospitalization of the patient in the intensive care unit; presence of arterial hypertension (stage 2 and higher); body mass index over 30 kg/m^2; anemia. All patients signed an informed consent. The study was approved by the Ethical Committee of the South Ural State Medical University (Protocol No. 4 dated May 25, 2021). In all patients, multispiral computed tomography of the chest (SOMATOM Definition AS 64; Siemens, Germany) revealed bilateral lung damage corresponding to pathognomonic changes in COVID-19: the ground-glass opacity and consolidation type combined with reticular changes.

Blood sampling was performed on day 1 of the patient's admission to the hospital. Standard thromboprophylaxis with unfractionated heparin started after blood sampling for the study. In addition to anticoagulant therapy, patients received standard antiviral therapy and glucocorticosteroids, antibacterial therapy, according to the temporary guidelines of the Ministry of Health of Russia "Prevention, diagnosis and treatment of new coronavirus infection (COVID-19)", revision 15 dated February 22, 2022.

The number of platelets in the blood was counted using Fonio's method. Platelet aggregation was evaluated using a laser platelet aggregation analyzer "ALAT-2" (BIOLA, Russia). Adenosine diphosphate (ADP;

2.5 mmol/ml), collagen (3.3 μg/ml), and ristomycin (7.5 mg/ml) (Technologia Standard, Russia) were used as inducers. The number of aggregates (units) of average size per minute (units/minute) was counted. The average radius of platelets before the onset of aggregation was taken as the unit radius.

Genetic studies were performed using real-time polymerase chain reaction (PCR) (Roche LightCycler 96; Roche Molecular Systems, USA); buccal epithelial scrape was used as the material. We used "SNP-express-cardiogenetics" reagent kits for detection of polymorphisms in genes (Lytech Research and Production Company, Russia). The following polymorphisms were determined: rs6065 (Thr145Met) in the *GP1BA* gene, rs1126643 (Phe253Phe) in the *ITGA2* gene and rs5918 (Leu33Pro) in the *ITGB3* gene. Results were presented as homozygous wild-type variant: C/C for rs6065 and rs1126643 polymorphisms, T/T for rs5918 polymorphism; heterozygous variant: C/T for rs6065 and rs1126643, T/C for rs5918; homozygous mutant variant: T/T for rs6065 and rs1126643, C/C for rs5918.

Statistical processing was performed using IBM SPSS Statistics v. 23 (IBM Corp., USA). Sample characteristics are presented in *Me* (Q_{25}–Q_{75}) format, where *Me* is the median; Q_{25} and Q_{75} are the values of the lower and upper quartiles, respectively. Statistical hypothesis testing in groups was performed using nonparametric Mann – Whitney criteria. Fisher's exact test was used to compare proportions (percentages). Bonferroni correction was introduced in case of multiple comparisons. Differences were considered statistically significant at the level of $p \leq 0.05$. Allele frequencies were estimated using the gene count method, and the χ^2 criterion was used to detect deviations from Hardy – Weinberg equilibrium.

RESULTS

The content of platelets in the blood of patients with moderate lung damage did not change statistically significantly during the study. Group 2 showed statistically significant acceleration of ADP-, collagen-, and ristomycin-induced platelet aggregation by 9 % ($p = 0.038$), 23 % ($p = 0.027$), and 8 % ($p = 0.042$) at the median, respectively, compared to the control group. The number of platelets in blood in patients with severe lung tissue damage was statistically significantly lower by 37 % ($p = 0.002$) at the median compared to the control group. Group 3 showed statistically significant acceleration of ADP-, collagen- and ristomycin-induced platelet aggregation by 21 % ($p = 0.024$), 38 % ($p = 0.003$) and 16 % ($p = 0.019$) respectively compared to the control group. Compared to the group of patients with COVID-19-associated moderate lung damage, the number of platelets in the blood was 30 % lower ($p = 0.007$); ADP-, collagen-, and ristomycin-induced platelet aggregation was accelerated by 16 % ($p = 0.011$), 24 % ($p = 0.004$), and 10 % ($p = 0.009$) at the median, respectively (Fig. 1).

The genotype frequency distribution of *ITGA2*, *GP1BA* and *ITGB3* genes conformed to the expected Hardy – Weinberg equilibrium in both the control group ($p = 0.51$, $p = 0.95$ and $p = 0.81$, respectively) and in the group of patients with COVID-19-associated moderate lung damage ($p = 0.50$, $p = 0.87$ and $p = 0.82$, respectively). The combination of two mutations in one individual was detected in 5 (21 %) patients from the control group and 13 (27 %) patients from Group 2. The combination of three mutations was detected in 1 (4.8 %) patient from the control group and in 4 (8.3 %) patients from Group 2. In the group of patients with COVID-19-associated moderate lung damage the C allele of the rs5918 polymorphism in the *ITGB3* gene is statistically significantly more frequent compared to the control group, and, accordingly, the T allele was less frequent ($p = 0.009$). A lower frequency ($p = 0.012$) of the T/T variant of the rs5918 polymorphic locus of the *ITGB3* gene (64.5 % of observations) and a higher frequency ($p = 0.007$) of the T/C variant (29.2 % of observations) were determined in Group 2 compared to the control group. The frequency of the C/C variant did not change statistically significantly. No statistically significant differences with Group 1 were found in Group 2, when analyzing the frequency of alleles and genotypes of the rs6065 polymorphic locus in the *GP1BA* gene, including the C/C mutant variant. When the frequency of the rs1126643 polymorphism in the *ITGA2* gene was analyzed, it was found that the C allele was less frequent ($p = 0.022$), and the T allele was more frequent ($p = 0.014$) than the corresponding alleles in the control group. The C/C variant of this polymorphism was found in 39.6 % of observations, statistically significantly less frequent ($p = 0.002$) than in the control group. C/T and T/T variants were found in 43.8 and 16.6 % of observations, respectively, which was more frequent ($p = 0.031$ and $p = 0.042$, respectively) than in the control group (Table 1).

The genotype frequency distribution of the *ITGA2*, *GP1BA*, and *ITGB3* genes in Group 3 was consistent with the expected Hardy – Weinberg equilibrium ($p = 0.47$, $p = 0.82$, and $p = 0.71$, respectively). The combination of two mutations was found in 6 (22.2 %) patients, three mutations – in 3 (11 %) people. When analyzing the rs5918 polymorphism of the *ITGB3* gene, the C allele was statistically significantly more frequent and, accordingly, the T allele was less frequent both in comparison with the control and in comparison with Group 2 (Table 1). The frequency of T/T variant of the rs5918 polymorphic locus of the *ITGB3* gene in Group 3 was 51.9 %, which was statistically significantly lower than in Groups 1 and 2 ($p = 0.030$ and $p = 0.038$, respectively), while T/C variant was detected in 29.6 % of observations, which was statistically significantly higher than in the control group, but did not differ from the values of Group 2. The frequency of the C/C mutant variant in this group was 18.5 %, which was statistically significantly higher ($p = 0.043$) compared to the control group and Group 2. When analyzing the frequency distribution of alleles and genotypes of the rs6065 polymorphic locus in the *GP1BA* gene in Group 3, no statistically significant differences with the control group

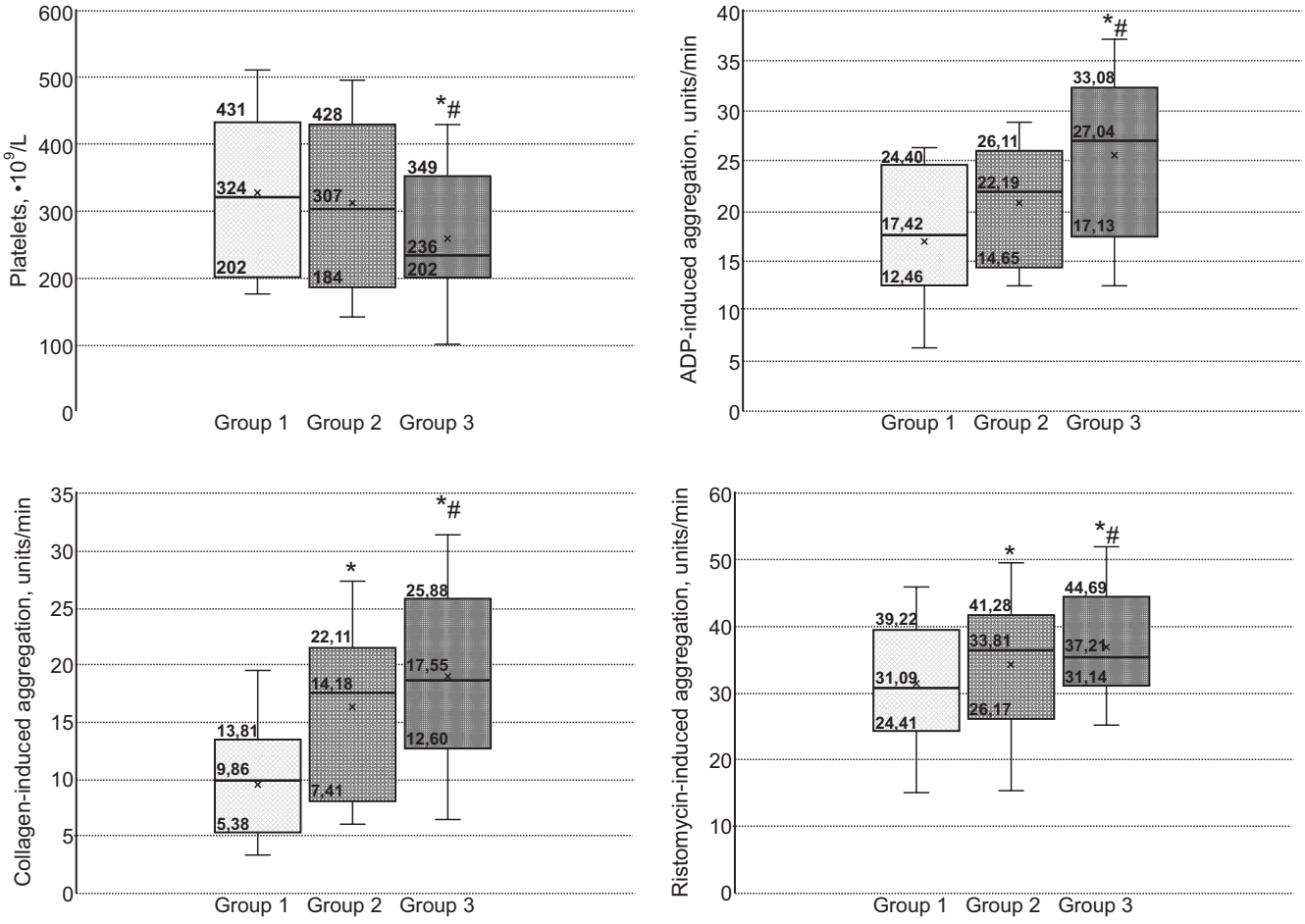

FIG. 1.

*Platelet blood count and their aggregation rate in COVID-19-associated lung damage depending on the severity of the disease. ——— – median; ▭ – 25th–75th percentiles; × – arithmetic mean; * – statistically significant ($p < 0.05$) differences with Group 1; # – statistically significant ($p < 0.05$) differences with Group 2*

and with Group 2 were found. When analyzing the frequency of the rs1126643 polymorphism in the *ITGA2* gene, no differences with the control group were found, but it was found that the C allele was more frequent, and the T allele was less frequent than the corresponding alleles in Group 2. The C/C variant of the rs1126643 polymorphism was found in 59.3 % of cases, which did not differ from the control group but was statistically significantly more frequent than in Group 2 ($p = 0.024$). The C/T variant was found in 29.6 % of observations, which was less frequent ($p = 0.033$) than the same variant in Group 2 and not statistically significantly different from the control group. The T/T variant was found in 11.1 % of cases, which was not statistically significant when compared with Groups 1 and 2.

During the analysis of platelet aggregation in Group 2 depending on the rs1126643 polymorphism of the *ITGA2* gene, it was noted that the T/T mutant variant accelerated collagen-induced platelet aggregation by 31 % ($p = 0.011$) and 23 % ($p = 0.019$) compared to the C/C and C/T variants, respectively. Statistical analysis revealed no differences in induced platelet aggregation in this group depending on polymorphisms of *ITGB3* and *ITGA2* genes (Table 2).

As a result of platelet aggregation analysis in Group 3 in the presence of the rs5918 polymorphism of the *ITGB3* gene, it was noticed that a statistically significant acceleration of ADP-induced platelet aggregation by 13 % ($p = 0.022$) was found in the T/C variant compared to the T/T variant. In the C/C variant, ADP-induced platelet aggregation was accelerated by 18 % ($p = 0.017$) compared to the T/T variant and did not change compared to the T/C variant. Statistical analysis revealed no statistically significant connections of other studied genotypes of the *ITGB3* and *ITGA2* genes with platelet aggregation in Group 3. The study showed no effect of the rs6065 polymorphism of the *GP1BA* gene on platelet aggregation ability.

DISCUSSION

Decrease in the number of platelets in patients with COVID-19-associated lung damage is present in 58–

TABLE 1

FREQUENCY OF OCCURRENCE OF GENOTYPES IN THE ANALYSIS OF *ITGB3*, *GP1B1*, AND *ITGA2* GENE POLYMORPHISMS IN COVID-19-ASSOCIATED LUNG DAMAGE, Me (Q25; Q75)

Polymorphisms	Genotypes/alleles		Genotype frequency		
			Group 1 (n = 24)	Group 2 (n = 48)	Group 3 (n = 27)
rs5918 in the *ITGB3* gene	genotypes	*0 (T/T)*	19 (79.0 %)	**31 (64.5 %)***	**14 (51.9 %)*#**
		I (T/C)	4 (16.7 %)	**14 (29.2 %)***	**8 (29.6 %)**
		II (C/C)	1 (4.3 %)	3 (6.3 %)	**5 (18.5 %)*;#**
	alleles	*T*	87.3 %	**79.1 %***	**66.7 %*;#**
		C	12.7 %	**20.9 %***	**33.3 %*;#**
rs6065 in the *GP1BA* gene	genotypes	*0 (C/C)*	20 (83.4 %)	38 (79.1 %)	22 (81.4 %)
		I (C/T)	4 (16.6 %)	9 (18.8 %)	5 (18.6 %)
		II (T/T)	0	1 (2.1 %)	0
	alleles	*C*	91.7 %	88.5 %	90.7 %
		T	8.3 %	11.5 %	9.3 %
rs1126643 in the *ITGA2* gene	genotypes	*0 (C/C)*	16 (66.7 %)	**19 (39.6 %)***	**16 (59.3 %)#**
		I (C/T)	6 (25.0 %)	**21 (43.8 %)***	**8 (29.6 %)#**
		II (T/T)	2 (8.3 %)	**8 (16.6 %)***	3 (11.1 %)
	alleles	*C*	79.2 %	**61.5 %***	**74.1 %#**
		T	20.8 %	**38.5 %***	**25.9 %#**

Note. * – statistically significant ($p < 0.05$) differences with Group 1; # – statistically significant ($p < 0.05$) differences with Group 2; 0 – homozygous wild-type variant; I – heterozygous variant; II – homozygous mutant variant..

TABLE 2

THE RATE OF PLATELET AGGREGATION IN COVID-19-ASSOCIATED LUNG DAMAGE DEPENDING ON POLYMORPHISMS OF *ITGB3*, *GP1B1* AND *ITGA2* GENES, Me (Q25; Q75)

Polymorphisms	Genotypes	Group 2 (n = 48)	Group 3 (n = 27)
	ADP-induced aggregation (units/min)		
rs5918 in the *ITGB3* gene	*0 (T/T)*	19.84 (14.45; 23.32)	20.19 (17.11; 23.17)
	I (T/C)	20.71 (15.81; 25.02)	**23.19 (17.31; 25.72)***
	II (C/C)	21.22 (18.35; 24.11)	**24.74(16.91; 28.12)***
	Ristomycin-induced aggregation (units/min)		
rs6065 in the *GP1BA* gene	*0 (C/C)*	33.72 (26.17; 39.03)	38.58 (31.14; 44.69)
	I (C/T)	34.06 (28.44; 40.28)	36.41 (32.78; 39.90)
	II (T/T)	27.18	–
	Collagen-induced aggregation (units/min)		
rs1126643 in the *ITGA2* gene	*0 (C/C)*	13.17 (7.31; 19.63)	17.17 (12.60; 24.11)
	I (C/T)	14.02 (8.67; 24.15)	18.24 (13.41; 22.58)
	II (T/T)	**17.25 (9.42; 22.14)*;#**	16.22 (13.01; 19.28)

Note. * – statistically significant ($p < 0.05$) differences with homozygous wild-type variant within a group; # – statistically significant ($p < 0.05$) differences with heterozygous variant within a group; 0 – homozygous wild-type variant; I – heterozygous variant; II – homozygous mutant variant.

95 % of cases and can be associated with multiple factors. Thrombocytopenia (low platelet count) in the early stages of COVID-19 is usually due to platelet destruction and increased platelet consumption, but decreased platelet production may be evident in the later stages of the disease [9]. Previously, an association between blood platelet count, severity of disease course, and increased risk of mortality in hospitalized patients with COVID-19 has been demonstrated [13]. In COVID-19, platelet activation is carried out by inflammatory mediators, antigen-antibody complexes, and damaged endothelium. The resulting activated platelets are removed from the bloodstream by mononuclear phagocytes. SARS-CoV-2 is able to inhibit thrombopoiesis through direct interaction with megakaryocytes and also has a direct effect on platelet through binding to ACE2 receptors, which causes escalation of oxidative stress in platelets, their increased consumption, impaired thrombopoiesis in bone marrow, triggering and autoimmune reactions [14]. The products of cells (primarily endotheliocytes) destroyed by SARS-CoV-2 may be another cause of accelerated platelet aggregation, which leads to thrombosis and thrombocytopenia. Individual genotype characteristics, including polymorphisms of the *GP1BA*, *ITGA2*, and *ITGB3* genes, are other factors that have a prominent effect on platelet aggregation activity.

The *GP1BA* gene encodes the α-subunit of glycoprotein Ib involved in the formation of the platelet receptor GpIb/IX/V. The main ligand of the receptor is von Willebrand factor (vWF), which binds platelets to the site of vascular damage [15]. The strength of the binding formed between them depends largely on the configuration of the receptor, vWF structure and blood flow velocity. The rs6065 polymorphism of the *GP1BA* gene is connected with the cytosine(C)-to-thymidine(T) substitution near the initiation of genetic transcription, which results in threonine-to-methionine substitution in the receptor site responsible for binding to vWF. As a result, carriers of the C/C genotype have a higher concentration of platelet membrane glycoprotein Ib than those with other genotype variants. The mutant homozygous T/T form (frequency in the population is about 1.5 %) sharply increases the risks of thrombosis. In case of heterozygous C/T variant, the expression of GpIb/IX/V receptors on platelets is not so strongly expressed, but some studies have revealed an increased risk of thrombosis in carriers of this gene variant [16]. Several studies show that carriers of the C allele have an increased risk of coronary thrombosis, ischemic stroke, and a decreased age of its onset [17].

In the presented study, there are no data indicating an effect of the rs6065 polymorphism of the *GP1BA* gene on the acceleration of ristomycin-induced platelet aggregation, reflecting the interaction of vWF with the GpIb receptor. A possible explanation is the absence of a significant number of carriers of the homozygous mutant variant of the T/T gene in the study population and small sample size. In addition, there is a cooperativity effect in the binding of vWF to platelet GpIb: glycoproteins IX and V, whose structure and function may remain intact, participate in the formation of collagen complex. In addition, the GpIb/IX/V receptor serves more for platelet adhesion. Its role in aggregation is less prominent [18]. Thus, the acceleration of ristomycin-induced platelet aggregation in patients with COVID-19-associated lung damage cannot be explained by the *GP1BA* gene rs6065 polymorphism. It is probably influenced by other genetic and non-genetic factors.

The *ITGB3* gene regulates the synthesis of the membrane protein integrin β-3, which is involved in interplatelet interactions. Integrin β-3 is a component of glycoprotein IIb/IIIa and recognizes the specific amino acid sequence – glycine-proline-arginine – in a wide range of ligands including prothrombin, fibrinogen, plasminogen, and vWF. Integrin β-3 is a heterodimer composed of noncovalently associated α- and β-subunits. These subunits have a large extracellular part, a transmembrane part and a short cytoplasmic part [19]. The rs5918 polymorphism of the *ITGB3* gene is caused by the thymine (T) to cytosine (C) nucleotide substitution in a certain DNA region, which results in the leucine amino acid-to-proline substitution in position 33 of the protein chain and disruption of the three-dimensional structure of the receptor. Disruption of the receptor structure leads to increased platelet reactivity and contributes to their thrombogenicity [20, 21]. Individuals with the C/C variant of this polymorphism have an increased propensity for platelet aggregation and, consequently, the risk of thrombosis [22]. It is assumed that the effect of the rs5918 polymorphism on platelet characteristics is found only for homozygotes for the C allele [23].

The association of the rs5918 polymorphism of the *ITGB3* gene with the occurrence of thrombotic events has been previously recognized [22–24]. Based on the presence of these adverse events in patients with COVID-19 and the frequency of T/C and C/C variants of the rs5918 polymorphism in these patients, it can be assumed that this genetic defect is of significance in the pathogenesis of COVID-19, especially in disorders of the hemostasis system. We found that the frequency of heterozygotes and mutant homozygotes of the *ITGB3* gene was higher in patients with accelerated ADP-induced aggregation as well as severe COVID-19. Increased mean platelet volume and increased concentration of glycoprotein IIb/IIIa on the platelet membrane are characteristic of mutant homozygote carriers and, to a lesser extent, heterozygotes of the rs5918 polymorphism. As a consequence of such changes, platelet reactivity is increased [25]. However, the exact mechanisms that cause platelet aggregation in the presence of the C/C variant of the rs5918 polymorphism are not thoroughly understood and require further study.

The *ITGA2* gene encodes the protein integrin alpha-2, a membrane glycoprotein GPIa found on the membranes of various cells, including platelets. On the platelet membrane, GPIa forms a complex with GPIIa, which is one of the collagen receptors. The rs1126643 polymorphism of the *ITGA2* gene is caused by the cytosine(C)-to-thymidine(T) substitution. This mutation alters the amino acid sequence, which leads to a correlation between this polymorphism and the level of GPIa expression

on the platelet membrane [26]. In the case of the T/T variant of the rs1126643 polymorphism, platelets bind to collagen more rapidly. Heterozygous individuals with the C/T variant show an intermediate level of receptor expression [27]. The data obtained in this study (acceleration of collagen-induced platelet aggregation) are consistent with the existing data on the function of integrin alpha-2 and may suggest a role for the rs1126643 polymorphism in the pathogenesis of COVID-19, although its mechanism remains unclear.

The obtained data suggest a possible role of genetic predisposition, in particular polymorphisms (rs6065 of *GP1BA* gene, rs1126643 of *ITGA2* gene and rs5918 of *ITGB3* gene), in platelet hyperactivation in patients with COVID-19-associated lung damage. These findings can be used to plan larger studies in order to assess the potential risk of thrombotic complications, to determine further treatment tactics and the choice of anticoagulant and disaggregant therapy. Hemostasis links are controlled by different genes, and the influence of their polymorphisms on the pathogenesis of COVID-19 has not been sufficiently investigated. The association of genetic factors with possible complications and severity of the disease course requires further research.

CONCLUSIONS

Induced platelet aggregation is accelerated in patients with COVID-19-associated lung damage of moderate and severe course. In severe course, the number of platelets in the blood decreases. The frequency of heterozygous C/T and homozygous mutant T/T genotypes of the rs1126643 polymorphism of the *ITGA2* gene is increased in moderate severity of lung damage. The presence of T/T genotype is associated with collagen-induced platelet aggregation. In severe lung damage the frequency of homozygous mutant C/C genotype of the rs5918 polymorphism of the *ITGB3* gene is increased. The presence of C/C and C/T genotypes of the rs5918 polymorphism is associated with ADP-induced platelet aggregation.

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Conflict of interest

The authors of this article declare no conflicts of interest.

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