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Posted Date: 25 April 2025

doi: 10.20944/preprints202504.2160.v1

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 doi:10.20944/preprints202504.2160.v1

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Antiviral Intervention of COVID-19: Linkage of Disease Severity with Genetic Markers FGB (rs1800790), NOS3 (rs2070744) and TMPRSS2 (rs12329760)

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Abstract: The purpose of this study was to investigate polymorphic variants of the genes FGB (rs1800790), NOS3 (rs2070744) and TMPRSS2 (rs12329760) in patients with coronavirus infection and to determine their role in the development of clinical forms of COVID-19 on the background of antiviral therapy. Real-time polymerase chain reaction (RT-PCR) was used to genotype the polymorphism of the selected genes. GS-5734 (Remdesivir) was prescribed as the basic antiviral drug. Binary logistic regression confirmed a low probability of developing COVID-19 in carriers of the mutational A-allele of the FGB gene. The highest probability of developing moderate and severe clinical forms of COVID-19 among residents of Central Ukraine was found in carriers of the G-allele (especially the GG genotype) of the FGB gene (rs1800790) and the T-allele of the TMPRSS2 gene (rs12329760). The administration of the antiviral drug GS-5734 (Remdesivir) and anti-inflammatory therapy reduces the blood level of TMPRSS2 in moderate and IL-6 in severe COVID-19. The proposed treatment does not significantly affect the concentration of endothelin-1, but a decrease in procalcitonin associated with additional antibacterial use was observed, especially in severe COVID-19.

Keywords: COVID-19; antiviral treatmen; genes; polymorphism; FGB (rs1800790); NOS3 (rs2070744); TMPRSS2 (rs12329760)

1. Introduction

Recent studies of coronavirus-associated phenotypes (SARS, MERS, and COVID-19) have shown that susceptibility to coronavirus infection and its severity can also be influenced by the characteristics of the host genome [1,2].

The first genome-wide association study (GWAS) of COVID-19 (Severe Covid-19 GWAS Group Study) identified two loci associated with the severity of the disease in Italians and Spaniards: the

 doi:10.20944/preprints202504.2160.v1

3p21.31 locus, which contains several immune genes, and the ABO locus, which determines the ABO blood group [3,4]. In this regard, the COVID-19 Host Genetics Initiative (HGI) was established to unite global efforts to clarify the role of host genetic factors in susceptibility to SARS-CoV-2 virus and the severity of the pandemic [5]. However, COVID-19 genetic studies reported are mainly based on European populations. It is therefore unknown whether these findings can be applied to other populations.

At this stage of the study of infection factors for coronavirus infection, it has been established that the FV Leiden polymorphism (rs6025) promotes COVID-19 infection, while the ACE2 rs41303171 polymorphism plays a protective role, and the MTHFR gene SNP (rs1801131) correlates with a milder course of COVID-19. In addition, in patients with severe COVID-19, the inflammatory marker NLR, circulating hemostatic proteins vWF and fibrinogen are associated with death [6,7].

Previous studies have found that mRNA levels for regulators of the kappa-kinin pathway (C1-inhibitor), coagulation (thrombomodulin, endothelial protein C receptor), and fibrinolytic pathways (urokinase and urokinase receptor) were significantly reduced in patients with COVID-19. While the transcripts for several coagulation proteins were increased, the protein encoding tissue factor and coagulation-initiating factor, whose expression is often upregulated in inflammatory diseases, did not increase in bronchoalveolar lavage in patients with COVID-19. These data indicate that increased coagulation and decreased fibrinolysis are the causes of coagulopathy in the lungs in COVID-19 [8,9].

In this regard, it became necessary to investigate polymorphic variants of the genes fibrinogen beta (FGB, rs1800790), endothelial nitric oxide synthase (NOS3, rs2070744) and transmembrane serine protease 2 (TMPRSS2, rs12329760) in the context of the severity and clinical and laboratory features of COVID- in order to identify high-risk groups with a more severe course of the disease and a greater susceptibility to COVID-19, as well as to assess the impact of antiviral therapy.

2. Materials and Methods

2.1. Sample Collection

The cohort study involved 197 patients with COVID-19 who were hospitalized at the Uman Central City Hospital, Infectious Diseases Department during 2021-2023. Diagnosis, laboratory examination and treatment were carried out in accordance with the current Protocol "Provision of Medical Care for the Treatment of Coronavirus Disease (COVID-19)" (Order of the Ministry of Health of Ukraine of 17. 05.2023 No. 913) [10], the Standards of Medical Care "Coronavirus Disease (COVID-19)" (Order of the Ministry of Health of Ukraine No. 2122 of 17.09.2020) [11], as well as WHO, CDC and global standards for the diagnosis, treatment and prevention of COVID-19 [12]. All patients were hospitalized in the infectious diseases department with covid-associated community-acquired pneumonia of moderate (21.97%) and severe (78.03%) severity.

Patients were divided by the severity of COVID-19 into moderate (n=55) and severe (n=142) (Table 1).

Table 1. Clinical and demographic characteristics of patients with COVID-19, taking into account the severity of the course.

Individual factors	Middle course n=55	Severe course n=142	χ^2	p
Age, years (M±m)	63,97±10,58	68,78±11,09	-	0,140
Women, n=100 (%)	21 (38,18)	79 (55,63)	4,83	0,028
Men, n=97 (%)	34 (61,82)	63 (44,37)		
Vaccinated, n=75	19 (34,55)	56 (39,44)	0,4	0,527
Unvaccinated, n=122	36 (65,45)	86 (60,56)		
Non-invasive oxygen therapy, n=172	30 (54,56)	142 (100,0)	73,93	<0,001
No oxygen therapy, n=25	25 (45,45)	0		
SBP, mm/Hg	148,47±3,70	142,78±3,66	-	0,077
DBP, mm/Hg	88,69±3,19	87,92±3,17	-	0,184
BMI, kg/m2	30,23±1,15	29,09±0,88	-	0,211
SpO 2, %	0,90±0,04	0,81±0,05	-	0,025
T2DM, n=52	13 (23,64)	39 (27,46)	0,3	0,584

 doi:10.20944/preprints202504.2160.v1

Smoking, n=50	25 (45,45)	25 (17,60)	16,23	<0,001

Notes. T2DM - type 2 diabetes mellitus; SBP, DBP - systolic, diastolic blood pressure; BMI - body mass index.

Patients in both groups did not differ in age, as well as in SBP, DBP, and BMI (slightly worse indicators were observed in moderate severity). The sex distribution was parity: men/women (n=97/n=100), but the relative frequency of women dominated in severe COVID-19 and men in moderate COVID-19 by 17.45% (χ2=4.83; p=0.028). The majority (87.31% of patients) received non-invasive oxygen therapy as a basic treatment: 100% (n=142) of patients with severe COVID-19 and almost 55% (n=30) of patients with moderate severity of the disease (χ2=73.93; p<0.001). SpO2 in the group of patients with severe disease was lower than in those with moderate disease - by 10.0% (p=0.025). The absolute number of smokers was equally divided between the groups (n=25 in each), but the relative number prevailed in moderate than in severe disease - by 27.85% (χ2=16.23; p<0.001). The number of unvaccinated persons was almost twice as high as the number of vaccinated persons in each group, without a significant difference between them (χ2=0.4; p=0.527).

GS-5734 (Remdesivir), recommended by the FDA for the treatment of COVID-19 (2020) and approved by the WHO, was prescribed as the basic antiviral drug [13], according to the treatment regimen described in the drug's instructions. Remdesivir was administered within the first 5 days of the onset of symptoms, but if the patient was hospitalized later, at any time if clinically indicated, as allowed by the clinical protocol. On the first day, a loading dose of 200 mg once daily (IV over 30-120 minutes), and from the second day, a maintenance dose of 100 mg once daily (IV over 30-120 minutes). The treatment duration was 5 days. Careful monitoring of remdesivir toxicity was performed: Before starting therapy and daily during the use of remdesivir in adult patients, the estimated glomerular filtration rate (eGFR) was determined. Remdesivir was not used in patients with eGFR < 30 ml/min/1.73 m2. Such patients were not included in the study. Prior to treatment, the functional state of the liver was analyzed and monitored throughout the treatment period (if the increase in blood alanine aminotransferase (ALT) was more than 5 times, the drug was discontinued).

Blood samples were taken before treatment (on the first day of hospital admission) and before discharge from the hospital (on the 12-15th day of hospitalization).

The study was conducted in accordance with the moral and ethical standards of bioethics in accordance with the ICH/GCP, the Helsinki Declaration of Human Rights (1964), the Council of Europe Convention on Human Rights and Biomedicine (1997), and the current legislation of Ukraine. The study protocol was approved by the Bioethics Committee of Bukovinian State Medical University (Protocol No. 7 of April 2025). All subjects signed a written informed consent to participate in the study.

2.2. Laboratory and Clinical Data

A comprehensive laboratory examination was performed, including the determination of oxygen saturation (SpO 2, %), transmembrane serine protease 2 (TMPRSS2), endothelin-1 (ET-1), interleukin-6 (IL-6), procalcitonin (PCT).

2.3. Identifying Genetic Polymorphisms

For the isolation of genomic DNA, peripheral blood leukocytes were used using a commercial kit (Thermo Scientific™ GeneJET™ Whole Blood Genomic DNA Purification Mini Kit). 200 μL of whole blood from each participant was digested with proteinase K followed by the addition of lysis buffer. The next steps included washing and elution of the purified DNA.

Real-time polymerase chain reaction (RT-PCR) was used to genotype the polymorphism of the FGB (rs1800790), NOS3 (rs2070744), and TMPRSS2 (rs12329760) genes. For this purpose, the CFX96™ real-time PCR detection system (Bio-Rad Laboratories, Inc., USA) was used. Specific TaqMan™ kits were used for each target SNP. Genotyping was performed using TaqMan® probes and TaqMan® Genotyping Master Mix (4371355) in combination with the CFX96™ Real-Time PCR Detection System. The PCR protocol was strictly followed the manufacturer's instructions (Applied Biosystems, USA). The TaqMan® Genotyping Master Mix includes AmpliTaq Gold® DNA polymerase, dNTPs, ROX™ reference dye, and optimized reaction buffers. For the identification of gene alleles, TaqMan® probes were used, which are allele-specific oligonucleotides with reporter dyes (VIC® for allele 1 and 6-FAM™ for allele 2) attached to the 5' end and a non-fluorescent quencher (NFQ) at the 3' end. Genomic DNA (10 μL) was amplified in a reaction mixture containing primers, probes, master mix,

 doi:10.20944/preprints202504.2160.v1

and target DNA. For genotyping, allele discrimination based on relative fluorescence units (RFUs) was used using CFX-Manager™ software. PCR cycle conditions were as follows: Initial denaturation: 95 °C for 10 minutes; amplification cycles (49 cycles); denaturation: 95 °C for 15 seconds; annealing: 60 °C for 1:10 minutes; final melting curve analysis: Increase in temperature to 95 °C. Genotype determination was based on melting curve analysis using CFX96™ Real-Time PCR Basic software (Bio-Rad Laboratories, Inc., USA).

2.4. Statistical Analysis

Statistically, the results were processed in accordance with modern requirements, using the Statistica 13.0 program (StatSoft Inc, USA, license number JPZ804I382130ARCN10-J). The reliability of data for independent samples with an array distribution close to normal was calculated by the Student's t-test, and in case of uneven distribution - by the Wilcoxon-Mann-Whitney U test. Differences were considered significant at $p<0.05$.

3. Results

The distribution of alleles and genotypes of the FGB (rs1800790), NOS3 (rs2070744), and TMPRSS2 (rs12329760) genes in patients with COVID-19 is shown in Table 2.

Table 2. Distribution of alleles and genotypes of FGB (rs1800790), NOS3 (rs2070744) and TMPRSS2 (rs12329760) genes in patients with COVID-19.

Polymorphic variants of FGB genes		Patients, n=72 (%)	Control, n=48 (%)	χ^2	p
FGB gene (455G>A; rs1800790)					
FGB (*455G>A*), n (%)	*GG*	36 (50,0)	12 (25,0)	7,50	0,006
	AG	28 (38,89)	30 (62,50)	6,43	0,011
	AA	8 (11,11)	6 (12,50)	0,05	0,823
χ^2; p		χ^2=5,84*; p=0,016		-	
FGB (*455G>A*), n (%)	Allele *G*	100 (69,44)	54 (56,25)	4,36	0,037
	Allele *A*	44 (30,56)	42 (43,75)		
NOS3 gene (T-786C; rs2070744)					
NOS3 (*T-786C*), n (%)	*TT*	28 (38,89)	18 (37,50)	0,02	0,887
	TC	30 (41,67)	21 (43,75)	0,05	0,823
	CC	14 (19,44)	9 (18,75)	0,01	0,920
χ^2; p		χ^2=0,05; p=0,823		-	-
NOS3 (*T-786C*), n(%)	Allele *T*	86 (59,72)	57 (59,37)	0	1,0
	Allele *C*	58 (40,28)	39 (40,62)		
TMPRSS2 gene (Val160Met C/T; rs12329760)					
TMPRSS2 (*Val160Met C/T*), n (%)	*CC*	40 (55,56)	18 (37,50)	3,76	0,05
	CT	26 (36,11)	25 (52,08)	3,01	0,061
	TT	6 (8,33)	5 (10,42)	0,04	0,467
χ^2; p		χ^2=2,62; p=0,105		-	-
TMPRSS2 (*Val160Met C/T*), n (%)	Allele *C*	106 (74,03)	61 (63,54)	2,76	0,065
	Allele *T*	38 (25,97)	35 (36,46)		

Notes. * - for df=1 $\chi2$ Yates with continuity correction ($\chi2$ Pearson without continuity correction =7.87; P=0.005); $\chi2$ - Pearson coefficient.

The distribution of FGB gene (rs1800790) genotypes between the groups differed (Pearson $\chi2=7.87$; $p=0.005$, without adjustment for continuity): the relative frequency of the wild-type G-allele and GG genotype significantly prevailed in the patient group, and the A-allele, on the contrary, in the control group by 13.19% ($\chi2=4.36$; $p=0.037$) and 25.0% ($\chi2=7.50$; $p=0.006$), respectively. In addition, the relative frequency of the AG genotype also dominated in the control group by 23.61% ($\chi2=6.43$; $p=0.011$). The G-allele prevailed in both groups over the A-allele, but only in patients with COVID-19 - by 38.88% ($\chi2=43.56$; $p<0.001$).

 doi:10.20944/preprints202504.2160.v1

Among the alleles of the endothelial nitric oxide synthase (NOS3) gene dpSNP: rs2070744 in patients with COVID-19 of the experimental and control groups, the wild T-allele dominated over the C-allele: in patients - by 19.44% ($\chi2$=10.89; p<0.001), in healthy patients - by 18.75% ($\chi2$=6.75; p=0.009). The distribution of genotypes and alleles between the groups did not differ.

The frequency of the C-allele of the TMPRSS2 gene (rs12329760) in the homozygous state was almost higher among patients with COVID-19 than in the control group - by 18.06% ($\chi2$=3.76; p=0.05). In contrast, the frequency of the T-allele (especially the TC genotype) dominated in the control group, but not significantly - by 10.49% ($\chi2$=2.76; p=0.065) and 15.97% ($\chi2$=3.01; p=0.061). In both groups, the wild-type C allele prevailed over the mutational T allele by 48.06% ($\chi2$=64.22; p<0.001) and 27.08% ($\chi2$=14.08; p<0.001), respectively. In general, there were no statistically significant differences in the distribution of genotypes between the groups ($\chi2$=2.62; p=0.105).

The analysis of inheritance patterns of susceptibility/susceptibility to COVID-19, taking into account the 455G>A polymorphism of the FGB gene (dpSNP: rs1800790), is shown in Table 3.

Table 3. Models of inheritance of susceptibility/susceptibility to COVID-19 based on the 455G>A polymorphism of the FGB gene (dpSNP: rs1800790).

Genotypes	Experiment, n=72 (%)	Control, n=48 (%)	OR [95% CI]	p	KA
The codominant model					
GG	36 (50,0)	12 (25,0)	1,00	0,02	17,68
AG	28 (38,89)	30 (62,50)	0,31 [0,13 – 0,70]		
AA	8 (11,11)	6 (12,50)	0,44 [0,13 – 1,59]		
The dominant model					
GG	36 (50,0)	12 (25,0)	1,00	0,01	16,03
AG + AA	36 (50,0)	36 (75,0)	0,33 [0,15 – 0,73]		
Recessive model					
GG + AG	64 (88,89)	42 (87,50)	1,00	0,82	23,70
AA	8 (11,11)	6 (12,50)	0,87 [0,28 – 2,83]		
Super-dominant model, df=2					
GG + AA	44 (61,11)	18 (37,50)	1,00	0,01	17,27
AG	28 (38,89)	30 (62,50)	0,38 [0,18 – 0,80]		
Additive model					
GG	36 (50,0)	12 (25,0)	1,00	0,03	19,14
2AA + AG	44	42	0,54 [0,30 – 0,95]		

Notes. OR - odds ratio; CI - confidence interval; df - degrees of freedom; (in the superdominant model df=2, in other models df=1); KA - Akaike's coefficient.

The probability of three models in the susceptibility to the development of COVID-19, taking into account the 455G>A polymorphism of the FGB gene, was determined: codominant (p=0.02), dominant (p=0.01), superdominant (p=0.01) and additive (p=0.03), of which the dominant model with the lowest Akaike coefficient (CA=16.03) is the most effective. According to these models, the lowest development of pathology is expected in carriers of the mutational A-allele of the FGB gene (OR=0.31-0.54; 95% CI: 0.13-0.95; p≤0.03-0.01).

The association of the T-786C polymorphism of the NOS3 gene (dpSNP: rs2070744) with the onset of COVID-19 was analyzed using binary logistic regression (Table 4).

Table 4. Models of inheritance of susceptibility/susceptibility to COVID-19 based on the T-786C polymorphism of the NOS3 gene (dpSNP: rs2070744).

Genotypes	Experiment, n=72 (%)	Control, n=48 (%)	OR [95% CI]	p	KA
The codominant model					
TT	28 (38,89)	18 (37,50)	1,00	0,97	18,17
TC	30 (41,67)	21 (43,75)	0,92 [0,40 – 2,07]		
CC	14 (19,44)	9 (18,75)	1,0 [0,36 – 2,85]		
The dominant model					
TT	28 (38,89)	18 (37,50)	1,00	0,88	16,19

 doi:10.20944/preprints202504.2160.v1

TC + CC	44 (61,11)	30 (62,50)	0,94 [0,44– 2,0]		
			Recessive model		
TT + TC	58 (80,56)	39 (81,25)	1,00	0,92	16,21
CC	14 (19,44)	9 (18,75)	1,05 [0,45 – 2,73]		
			Super-dominant model , df=2		
TT + CC	42 (58,33)	27 (56,25)	1,00	0,82	16,17
TC	30 (41,67)	21 (43,75)	0,92 [0,44– 1,93]		
			Additive model		
TT	28 (38,89)	18 (37,50)	1,00	0,96	16,22
2*CC + TC*	58	39	0,99 [0,60 – 1,93]		

Notes. OR - odds ratio; CI - confidence interval; df - degrees of freedom; (in the superdominant model df=2, in other models df=1); KA - Akaike's coefficient.

No model was statistically significant for the allelic state of the NOS3 gene rs2070744. The lowest error of out-of-sample prediction of the disease in the population (Akaike's coefficient) is inherent in the superdominant and subdominant models (CA=16.17 and 16.19; p>0.05).

The analysis of inheritance models of susceptibility to COVID-19, taking into account the Val160Met C/T polymorphism of the TMPRSS2 gene (rs12329760), is shown in Table 5.

Table 5. Models of inheritance of susceptibility/susceptibility to COVID-19 taking into account Val160Met C/T polymorphism of the TMPRSS2 gene (rs12329760).

Genotypes	**Experiment, n=72 (%)**	**Control, n=48 (%)**	**OR [95% CI]**	**p**	**KA**
			The codominant model, df=1		
CC	40 (55,56)	18 (37,50)	1,00	0,15	17,65
CT	26 (36,11)	25 (52,08)	2,14 [0,98 – 4,73]		
TT	6 (8,33)	5 (10,42)	1,85 [0,48 – 6,95]		
			The dominant model, df=1		
CC	40 (55,56)	18 (37,50)	1,00	0,049	15,69
CT+ TT	32 (44,44)	30 (62,50)	2,08 [1,0 – 4,45]		
			Recessive model, df=1		
CC + CT	66 (91,67)	43 (89,58)	1,00	0,70	19,33
TT	6 (8,33)	5 (10,42)	1,28 [0,35 – 4,50]		
			Super-dominant model, df=2		
CC + TT	46 (63,89)	23 (47,92)	1,00	0,08	16,48
CT	26 (36,11)	25 (52,08)	1,92 [0,92– 4,08]		
			Additive model, df=1		
CC	40 (55,56)	18 (37,50)	1,00	0,10	16,72
2*TT + CT*	38 (52,78)	35 (72,92)	1,61 [0,92 – 2,88]		

Notes. OR - odds ratio; CI - confidence interval; df - degrees of freedom; KA - Akaike's coefficient.

The analysis of genetic models of inheritance showed a tendency to the Covid-19 in the dominant model, where the presence of a T-allele increases the likelihood of illness by more than 2 times (OR = 2.08; OR95%CI: 1.0-4,45; p = 0.049). This model was statistically significant with the most effective with the lowest coefficient of Akayke (Ka = 15.69). A similar tendency is confirmed in the superimalnt model where the CT-genotype availability increases the risk of Covid-19 in the surveyed population is almost double, but is poor (OR = 1.92; or95%CI: 0.92-4.08; p = 0.08).

The relative frequency of SNP genes FGB (RS1800790), eNOS (RS2070744), TMPRSS2 (RS12329760) is not dependent on the severity of the CVID-19 clinical course and does not affect its risk (Table 6).

Table 6. Distribution of genotypes of polymorphism of FGB genes (455g> a; RS1800790), ENOS (786t> C; RS2070744), TMPRSS2 (Val160met C/T; RS12329760).

Genes	**Genotypes**	**Moderate course, n=36 (%)**	**Severe course, n=36 (%)**	χ^2	**p**
In general, n=197 (%)		55 (27,92)	142 (72,08)	78,64	<0,001

 doi:10.20944/preprints202504.2160.v1

FGB (rs1800790) gene						
FGB (455G>A), n=72 (%)	*GG*	18 (50,0)	18 (50,0)	0	———	1,0
	GA+AA	18 (50,0)	18 (50,0)			
eNOS (rs2070744) gene						
eNOS (786T>C), n=72 (%)	*TT*	16 (44,44)	12 (33,33)	1,1	———	0,294
	CT	13 (36,11)	17 (47,22)			
	CC	7 (19,44)	7 (19,44)			
TMPRSS2 (rs12329760) gene						
TMPRSS2 (C/T), n=72 (%)	*CC*	20 (55,56)	20 (55,56)	0	———	1,0
	CT + TT	16 (44,44)	16 (44,44)			

Epidemiologic analysis confirmed that the risk of severe COVID-19 doubles in women (OR: 2.03; OR 95%CI: 1.07-3.84; p=0.021) and is associated with oxygen therapy (OR: 22.83; OR 95%CI: 8.08-64.49; p<0.001). At the same time, smokers and men were found to have a significantly lower rate of severe COVID-19 (OR=0.26; OR 95%CI: 0.13-0.51; p<0.001 and OR=0.49; OR 95%CI: 0.26-0.93; p=0.038).

Instead, the risk of moderate COVID-19 severity doubles in men (OR: 2.02; OR 95%CI: 1.06-3.80; p=0.041), and almost 4 times in smokers (OR=3.90; OR 95%CI: 1.97-7.73; p<0.001).

To evaluate the effect of treatment, we analyzed the inflammatory response, SpO 2, and blood transmembrane serine protease 2 (Table 7). Under the influence of complex antiviral treatment, a significant decrease in TMPRSS2 and IL-6 was found: in patients with moderate COVID-19 by 16.38% (p=0.014) and 40.62% (p<0.001), in patients with severe COVID-19 - by 11.30% (p=0.049) and 48.24% (p<0.001), respectively. There were no significant changes in the concentration of endothelin-1 in the blood, which did not depend on the severity of the disease, but reached several times higher than the control group - 2.51-3.32 times (p<0.001), which may have negative consequences in terms of the appearance of long-standing covid in the future from the cardiovascular system, or any organ and/or system where the vascular endothelium suffers most (formation of endothelial dysfunction). PCT, as a marker of bacterial burden, mainly decreased under the influence of treatment, as all patients with moderate and severe conditions received additional antibacterial therapy according to the indications, according to the Treatment Protocol: in moderate severity - by 1.93 times (p<0.001), in severe COVID-19 - by 2.33 times (p<0.001), respectively. SpO 2 level significantly improved in all observation groups (by 6.66%; p<0.05 and 14.81%; p<0.001), which made it possible to discharge patients in a compensated state to home for outpatient rehabilitation.

Table 7. Indicators of inflammatory activity and transmembrane serine protease 2 content in patients with COVID-19, taking into account the severity of clinical course and gender.

Indicators		**Control**	**Moderate course**	**Severe course**
TMPRSS2, ng/ml	Before treatment	1,81±0,12	2,87±0,18 p<0,001	2,30±0,19 p=0,003; p_1<0,001
	After treatment		2,40±0,11 p=0,003; p_A=0,014	2,04±0,06 p=0,043; p_1=0,002; p_A=0,049
ET-1, pg\ml	Before treatment	4,03±0,55	13,37±2,97 p<0,001	10,81±3,53 p=0,047
	After treatment		11,56±1,62 p<0,001	10,11±0,95 p=0,002
IL-6, pg\ml	Before treatment	7,79±1,26	42,86±7,48 p<0,001	100,79±4,96 p,p_1<0,001
	After treatment		25,45±3,26 p,p_A<0,001	52,17±2,85 p,p_1,p_A<0,001
PCT, ng/ml	Before treatment	0,1±0,0001	0,29±0,06 p<0,001	0,28±0,06 p<0,001
	After treatment		0,15±0,02 p,p_A<0,001	0,12±0,02 p,p_A<0,001
SpO 2, %	Before treatment	0,98±0,01	0,90±0,04 p<0,001	0,81±0,05 p<0,001; p_1=0,025

 doi:10.20944/preprints202504.2160.v1

	After treatment	0,96±0,01 p_A=0,048	0,93±0,02 p_A<0,001 p=0,013; p_1=0,051

Notes. TMPRSS2: transmembrane serine protease 2; ET-1: endothelin-1; IL-6: interleukin-6; PCT: procalcitonin; p: significance of differences with the control group; p1: significance of differences with moderate severity of COVID-19; pl: significance of differences with the pretreatment state in each group separately.

4. Discussion

Previous studies have found significant differences in allele frequencies in the ACE2 and TMPRSS2 genes (receptor and coreceptor genes for SARS-CoV-2, respectively) between patients with COVID-19 and the general population [14,15]. However, these studies focused only on the regions of these genes. Regulatory regions have not been properly studied. Polymorphic gene variants in regulatory regions, especially enhancers, can disrupt regulatory function, affect the expression of other genes, and thus determine susceptibility to viral infection and the severity of the infectious disease [16,17].

In our study, we found that the distribution of FGB (rs1800790) gene genotypes between groups of patients with coronavirus infection and healthy individuals differed, with the relative frequency of the wild-type G-allele and GG genotype significantly higher in the patient group and the A-allele, on the contrary, in the control group, the G-allele prevailing in both groups over the A-allele. It was determined that the mutation of the NOS3 gene (T-786C, rs2070744) in the homozygous state occurs in almost every 5th subject with the dominance of the wild-type T allele over the mutant allele in both groups, In turn, the mutational T-allele of the TMPRSS2 gene (rs12329760) had no significant differences in frequency between patients with COVID-19 and healthy controls, with a higher frequency of the CC genotype among patients than in the control group.

Given the wide variability in individual response to SARS-CoV2 infection, it is important to understand whether genetic and biological predictors can predispose to infection or determine its severity, including the development of systemic coagulopathy and thrombosis. Cases have been described when, on the contrary, SARS-CoV2 can become a suppressor of anticoagulant or fibrinolytic gene expression [18]. Separate studies in Bergamo (Italy) have investigated the involvement of some gene SNPs in this condition (FII rs1799963, FV rs6025, FV rs118203907, FXIIIA1 rs5985, FGB rs1800790, MTHFR rs1801131, MTHFR rs1801133 [19].

Using binary logistic regression, we confirmed a low probability of developing COVID-19 in carriers of the mutational A-allele of the FGB gene within the codominant, dominant, superdominant, and additive models of pathology inheritance, and also recorded an increased risk of COVID-19 in carriers of the G-allele (especially the GG genotype) of the FGB gene (rs1800790) with a protective role of the A-allele and AG genotype, respectively. In turn, the alleles and genotypes of the NOS3 gene (T-786C, rs2070744) are not predictors of COVID-19 in the study population. Whereas the presence of a mutational T-allele of the TMPRSS2 gene (rs12329760) in the genotype increases the likelihood of the disease by more than 2 times, which confirms the role of the TMPRSS2 gene in increasing the likelihood of clinical development of COVID-19.

The development of antiviral drugs against SASR-CoV-2 is focused on preventing hospitalization, intubation, or death in patients with COVID-19 at high risk of disease progression [20]. One study found that in severe or critical COVID-19, only remdesivir showed significant benefit in clinical improvement and viral suppression without reducing mortality among unvaccinated patients during the initial wave of the SARS-CoV-2 COVID-19 generic strain [21]. Currently, Remdesivir, the only FDA-approved drug for the treatment of patients with COVID-19, is a prodrug of phosphoramidate that is metabolized in cells to form the active NTP analog, which we call remdesivir triphosphate (RTP). Biochemical studies have shown that the RNA-dependent RNA polymerase (RdRp) can utilize RTP as a substrate, leading to the incorporation of remdesivir monophosphate (RMP) into the growing RNA product. Once RMP is incorporated, RdRp elongates the RNA for an additional three nucleotides before it stops. This stalling mechanism is specific to coronaviruses, as Ebola virus RdRp can add five nucleotides of RNA after incorporating RMP before stalling [22]. Thus, these studies explained how RMP is incorporated into RNA instead of AMP. However, they do not explain how remdesivir inhibits RdRp, since RdRp stops only after adding three additional nucleotides to the RNA.

According to the results of the study, we found that the administration of the antiviral drug GS-5734 (Remdesivir) and anti-inflammatory therapy reduces the level of TMPRSS2 in the blood, with

 doi:10.20944/preprints202504.2160.v1

the most significant changes in patients with moderate severity of COVID-19. This proves the direct influence of SARS-Cov-2 viral load on TMPRSS2 synthesis. According to one of our hypotheses, this can be explained by the fact that SARS-Cov-2 is incorporated into the system of GS-5734 (Remdesivir), reducing the stimulation of adhesion protein on the surface of airway epithelial cells, which reduces the need for its production and, by feedback, reduces the expression of the corresponding gene, epigenome activity and proteome. According to another hypothesis, which we believe is less likely to have an impact, general anti-inflammatory pathogenetic therapy (some patients received methylprednisolone, dexamethasone, antiviral, detoxification, membrane stabilizing therapy according to the protocol) reduces the stress of the monocyte-macrophage immune response and the oxidative stress redox system.

Regarding other laboratory results, we found that en-dotlin-1 did not change under the influence of the proposed treatment. Accordingly, it requires further research, as some patients had concomitant cardiovascular disease. High levels of ET-1 may be associated with its high expression in the blood and vascular endothelium and affect the long-term effects of longitudinal vascular injury. It was also found that the level of procalcitonin decreased significantly because all patients received antibacterial therapy in parallel, which is associated with the results of previous studies [23]. It is important to note that IL-6, a proinflammatory marker, in turn, responded best to treatment, but remained elevated several times compared to controls. Therefore, nonspecific anti-inflammatory therapy at the outpatient stage should be continued to avoid the consequences of long-standing COVID.

5. Conclusions

The highest probability of developing moderate and severe clinical forms of COVID-19 among residents of Central Ukraine was found in carriers of the G-allele (especially the GG genotype) of the FGB gene (rs1800790) and the T-allele of the TMPRSS2 gene (rs12329760).

The administration of the antiviral drug GS-5734 (Remdesivir) and anti-inflammatory therapy reduces the blood level of TMPRSS2 in moderate course and IL-6 in severe course of COVID-19. The proposed treatment does not significantly affect the concentration of endothelin-1, but a decrease in procalcitonin associated with additional antibacterial drugs was observed, especially in severe COVID-19.

Author Contributions: Conceptualization and writing—original draft preparation, M.S. and L.S.; writing—review and editing, V.O., R.S., L.S. and I.K.; supervision, O.K., and V.O.; project administration, V.O. and O.K.; visualization, An.S, Al.S, O.S.; funding acquisition, M.S. and L.S. All authors have read and agreed to the published version of the manuscript.

Funding: RECOOP Grant #36—CSMC Senior Scientists (RCSS) "Comprehensive Analysis of Genetic Predictors for MAFLD Development in Patients with COVID-19".

Institutional Review Board Statement: The study protocol met the requirements for biomedical research and was approved by the Local Ethics Committee of the Bukovinian State Medical University as protocol N 7, dated 17 April 2025.

Informed Consent Statement: All patients signed an informed consent for the study.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

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