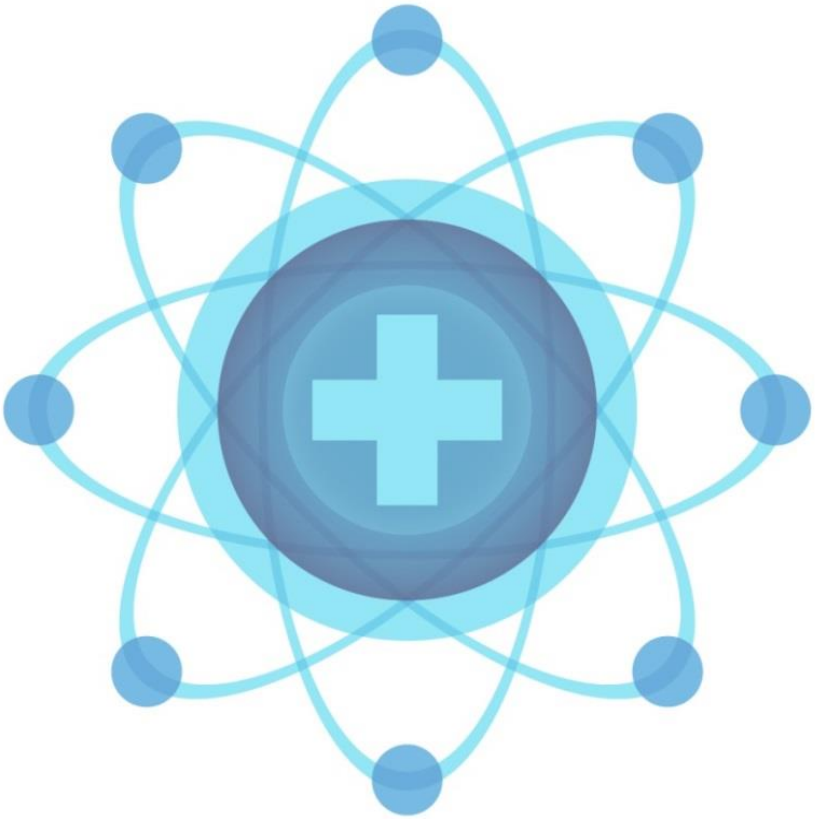

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THE SIGNIFICANCE OF FOLATE CYCLE GENE POLYMORPHISM IN THE DEVELOPMENT OF OSTEONECOSIS OF THE FEMORAL HEAD ASSOCIATED WITH COVID-19

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Abstract. It is known that the frequency of aseptic necrosis of the femoral head increased significantly during the COVID-19 pandemic. The use of corticosteroid drugs in the acute phase of COVID-19, chronic hypercoagulopathy caused by COVID-19, lifestyle (pernicious habits) of patients, genetic predisposition are of great importance in the development of the disease. It is known that microvascular circulatory disorders are considered an important pathogenetic factor in the development of aseptic necrosis. Therefore, thrombophilia genes are designed to study polymorphisms in patients with aseptic necrosis, such as the gene MTHFR A1298C (rs1801131), C677T (rs1801133), the gene MTR A2756G (rs1805087) and the gene MTRR A66G (rs1801394), in the Uzbek population. These polymorphisms were studied to determine the significance of minor alleles in the development of COVID-19-related disease. The selection of people prone to the development of this disease was carried out, and the combined influence of additional exogenous factors, such as negative habits, was studied. By selecting individuals who are carriers of the minor allele, special preventive and therapeutic measures can be carried out in order to reduce the frequency of aseptic necrosis associated with COVID-19, as well as a possible reduction in the incidence rate.

Keywords: Aseptic necrosis of the femoral head, gene polymorphism, MTHFR, MTR, MTRR, hyperhomocysteinemia.

Entry. Osteonecrosis of the femoral head, also known as avascular necrosis, is a pathological condition that leads to the death of osteocytes, increased demineralization and decreased resorption of spongy bone tissue, as well as changes in the trabecular architecture of the femoral head due to insufficient vascularization of the subchondral bone [1, 2]. The main cause of avascular necrosis of the femoral head is death bone cells due to circulatory disorders. Violation of microcirculation can occur by the following mechanisms:

1. Mechanical damage to blood vessels.
2. Obstruction of normal blood flow in the veins due to intravascular factors.
3. Obstruction of normal blood flow in the vessels due to extravascular factors [3, 4].

The pathophysiology of avascular necrosis of the femoral head is that it is a typical example of cell death due to a violation of normal blood flow [5]. Early pathological signs include necrosis of hematopoietic cells and adipocytes, as well as interstitial bone marrow damage and edema. Bone necrosis can develop due to a violation of arterial blood flow, blockage of venous blood flow, obstruction of intracapillary lumen or compression of capillaries in the bone marrow cavity.

Osteocyte necrosis manifests itself approximately 2-3 hours after anoxia, and histological signs appear approximately 24-72 hours after hypoxia [12, 13]. Osteocyte necrosis begins with pyknosis of cell nuclei, and then empty osteocytic lacunae appear. Reactive hyperemia and capillary revascularization are observed along the periphery of the necrosis zone, which indicates the restoration of blood flow, bone resorption and partial replacement of dead cells with living bone cells. Although the mechanism of development of aseptic necrosis of the femoral head associated with SARS-CoV-2 is complex and has not yet been fully studied, hypercoagulating conditions caused by the SARS-CoV-2 virus and the widespread use of corticosteroids in the acute phase of the disease are the main risk factors [8]. In addition, there is a positive correlation between the development of the disease and the level of alcohol consumption.

The significance of hyperergic inflammation and hypercoagulopathy caused by COVID-19 in the origin and development of ANGBN. Many pro-inflammatory factors - IL-1β, IL-1PA, IL-2, IL-4, IL-6, IL-8, IL-9, IL-13, IL-17, FGFß in the body of patients with COVID-19, G-CSF, GM-CSF, CXCL10, INF-g, IP-10, MIP-1a, MIP-1b, TNFa (tumor necrosis factor), granulocyte colony stimulating factor and a sharp increase in the number of other cytokines and growth factors cause a "cytokine storm" [9, 10, 11]. Avascular necrosis of the femur occurs as a result of impaired blood supply to the femoral head, cell death, and increased systemic inflammation mediated by cytokines, including CXCL10, IL-17, and TNF-alpha, which leads to a decrease in osteoblast proliferation and differentiation [16]. Hypersecretion of proinflammatory cytokines, especially TNF-α, leads to the activation of nuclear factor kappa-B in osteoblasts and osteocytes, which negatively affects bone formation and factors supporting normal osteogenesis, such as fibroblast growth factor-2, TGF-β1 and bone morphogenetic protein-2 [17]. In addition, it inhibits classical WNT signaling, such as TGF-α, and suppresses osteoblast differentiation and, on the other hand, promotes the differentiation of osteoclast precursors into mature osteoclasts [18]. In addition, the SARS-CoV virus can enhance the inflammatory process by activating the activity of NLPR3 inflammasomes. The resulting intense inflammation causes more secondary damage to healthy cells than it helps fight the virus. The inflammasome is a multimolecular complex that activates procaspase-1, turning it into caspase-1. Caspase-1 is pro-IL-1ß and pro-IL-18 cleave cytokines and convert them to the active form. An increase in the amount of IL-1, IL-18 activates macrophages, causes apoptosis of epithelial cells.With hyperactivation of the Inflammasome-NLPR3, structural protein E in the virus (by opening ion channels in the Golgi cell complex and causing ion imbalance) and non-structural proteins Orf8b, Orf3a (respectively, in the cell EPR causes cellular stress, forming insoluble aggregates, plays a role, disrupting the passage of substances from them as a result of oligomerization of lysosomal and endosomal membranes [12, 13]. Modulation of the SARS-CoV-2 immune system through the above mechanisms causes the occurrence of a specific hyperergic inflammatory process. For this reason, impaired normal control of the inflammatory response has a detrimental effect on the normal function and restoration of bones and the ratio of osteoblasts/osteoclasts. In the case of excessive inflammation, the production of reactive oxygen species and the activation of proteases is not complete without damage to normal tissues [14].]. Excessive inflammation also stimulates differentiation and activation of osteoclasts, which leads to osteolysis caused by inflammation [15]. It is believed that due to the hyperproduction of cytokines induced by COVID-19 as a result of a violation of the ratio of procoagulant and anticoagulant factors, the formation of thrombosis and normal blood supply to the femur increases (indirectly) due

to a violation of the endothelial system, thereby causing ischemia and necrosis of the femoral head [19, 20].

The significance of the abuse of corticosteroid drugs in the origin and development of ANGBN disease. Systemic glucocorticoids, such as dexamethasone and methylprednisolone, are used in the treatment of hospitalized patients with severe COVID-19, in particular, to prevent or reduce the "cytokine storm", reduce the need for artificial ventilation and reduce the length of hospital stay and the likelihood of death [25].

Several potential mechanisms have been proposed to explain this relationship, including dose-dependent glucocorticoid degeneration of bone matrix and cartilage, increased apoptosis of stem cells important for femoral regeneration, impaired lipid metabolism, increased development of fat emboli, abnormal coagulation and changes in blood. delivery may come [26]. Meta-analysis showed that in patients receiving high doses of corticosteroids, the risk of developing ANGBI was 10-fold, and the risk of osteonecrosis was 2—fold when the cumulative dose exceeded 10 g [27]. It has also been shown that corticosteroids cause osteoblast death and a decrease in osteoblast proliferation, as well as disruption of regeneration and repair processes in necrosis zones [26].

The importance of alcohol in the origin and development of aseptic necrosis of the femoral head (ANGB). An inverse positive relationship with excessive alcohol consumption was found in 10-40% of SSN cases [43]. Matsuo and his colleagues found that people who consumed more than 400 ml of alcohol per week had a 9.8-fold higher risk of developing ANGB compared to those who did not drink alcohol, and those who consumed more than 1000 ml of alcohol per week had a 17.9-fold higher risk of developing ANGB [21].

It is believed that alcohol affects changes in lipid metabolism and increased adipogenesis [22]. Increased formation of lipids leads to blockage of blood vessels by fat emboli. In addition, an increased level of lipids in the blood can cause bone marrow coagulation, increased intraosseous pressure and decreased blood flow. Alcohol can also lead to the death of osteocytes [23]. The mechanism of this is that the cortisol level is higher in patients with alcoholic osteonecrosis than in patients with idiopathic osteonecrosis, which indicates the important role of the steroid pathway in alcoholic osteonecrosis [24].

The significance of hyperhomocysteinemia in the origin and development of aseptic necrosis of the femoral head. Another significant factor that increases the risk of hypercoagulopathy and ANGB is hyperhomocysteinemia (HGS), which can develop due to a lack of vitamins and folate cycle cofactors or a deficiency of enzymes involved in the folate cycle (MTHFR, MTR and MTRR). HGS is characterized by a high level of homocysteine in the blood and is associated with an increased risk of cardiovascular diseases, stroke and venous thromboembolism. Several studies have shown a positive association between HHS-related hypercoagulopathy and ANGB. One of the mechanisms through which HGS can contribute to hypercoagulopathy and ANGB is its effect on the coagulation cascade. Homocysteine has been shown to interfere with the normal function of anticoagulant proteins, including protein C and antithrombin III, which leads to an increase in the procoagulant state. In addition, GGS increases the concentration of fibrinogen, which is involved in the formation of blood clots. Homocysteine affects the biology of bones, as it plays an important role in the modulation of osteoblastogenesis, osteoclastogenesis and their functions [28, 29]. Homocysteine is an inflammatory molecule that activates macrophages and acts as a pro-

inflammatory factor. Homocysteine is known to inhibit the activity of NOS, an important enzyme necessary for the functioning of blood vessels, and has a vasodilating effect on blood vessels [30]. Inhibition of NOS leads not only to a decrease in blood flow, but also to an increase in osteoclastogenesis [31]. Homocysteine promotes blood clotting, causing activation of endotheliocytes and imbalance between procoagulant and anticoagulant factors. This can lead to the formation of blood clots and disruption of blood flow in organs and tissues, including the femoral head, which leads to ischemia and necrosis [32]. However, several studies have shown a positive correlation between hyperhomocysteinemia and ANGBN [32, 34].

Material and methods. For clinical studies conducted during 2021-2023, 98 patients with aseptic necrosis of the femoral head after an acute period of COVID-19 disease were taken, who were treated in the department of Adult Orthopedics "Republican Specialized Traumatological and Orthopedic Scientific and Practical Medical Center".

Patients with ANGBC disease were determined by radiography, MRI, densitometry and ultrasound. According to him, 5.1% (n=5) of the total number of patients (n=98) had bilateral ANGBC stage 1-2, 14.3% (n=14) - bilateral ANGBC stage 2-3, 7, In 14% (n= 7) unilateral ANGBC stage 3-4, in 69.4% (n=68) bilateral ANGBC stage 3-4 and 4.1% (n=4) bilateral ANGBC stage 4-5. Considering that negative habits, in particular alcohol abuse and smoking, are recognized as risk factors for the development of aseptic bone necrosis, the main group of patients (98 people) was regrouped, 48 of them chronic smokers of alcohol and tobacco products - the first group and 50 patients who do not drink alcohol and do not smoke - in the second group. 96 conditionally healthy patients with a history of COVID-19, but who did not develop ANGBC, were selected as a control group. Polymorphisms A1298C (rs1801131) and C677T (rs1801133) of the MTHFR gene, polymorphism A2756G (rs1805087) of the MTR gene, polymorphism A66G (rs1801394) of the MTRR gene revealed single-nucleotide polymorphism using polymerase chain reaction in the DT-Lite 48 amplifier using reagents. DNA technology (Russia). In addition, taking into account that the abuse of corticosteroid drugs is an important risk factor for the development of ANGBC associated with COVID-19, the total amount of dexamethasone taken during the acute period of COVID-19 disease in general patients was studied (see Fig. 1).

Statistical processing of research results. The $\chi 2$ criterion was used to evaluate genotypes and compare the distribution of genotypes and alleles, taking into account the Hardy-Weinberg equilibrium. If the criterion $\chi 2$ was used to confirm the presence of predisposition to the studied pathology through the association of alleles and genotypes, then the pathogenetic significance of alleles and genotypes in the studied disease was confirmed by the ratio of relative risk (RR) and the ratio of chances (OR) with a 95% confidence interval (95% CI:). The $p < 0.05$ level was considered statistically significant. Statistical data processing was carried out using the Statistics 6.1 program (StatSoft, USA).

Results and discussion. The molecular genetic analysis included 98 cases of aseptic necrosis of the femoral head (ANGBC) associated with COVID-19 as the main group, as well as 96 healthy individuals who underwent COVID-19 but did not have ANGBN, as the control group polymorphisms A1298C (rs1801131) and C677T (rs1801133) of the gene MTHFR, polymorphism A2756G (rs1805087) of the MTR gene, polymorphism A66G (rs1801394) of the MTRR gene revealed the distribution of alleles and genotypes, as shown in Table 1.

Table 1.

Distribution of various gene polymorphisms (SNP) by alleles and genotypes in the main and control groups.

Poly-morphisms	Main group					Control group				
	Alleles		Genotypes			Alleles		Genotypes		
	Wild allele (%)	Minor allele (%)	Homo-zygous (%)	Hetero-zygote (%)	Homo-zygous not wild (%)	Wild allele (%)	Minor allele (%)	Homo-zygous (%)	Hetero-zygote (%)	Homo-zygous not wild (%)
MTHFR rs1801133	69,3	30,7	51,0	38,7	11.2	78,6	21,3	63,5	30,2	6.2
MTHFR rs1801131	68,9	31.1	47,9	41,8	10.2	72,4	27,6	51,0	42,7	6.2
MTR rs1805087	63,8	36,2	44,8	37,7	17,3	72,9	27.1	54,2	37,5	8.3
MTRR rs1801394	63,3	36,7	43,8	38,7	17,3	74,0	26,0	57,3	33,3	9.4

The distribution of the results of polymorphisms determined in the main and control groups by alleles was checked on the basis of the Hardy-Weinberg law in order to verify the adequacy of the distribution of alleles at the population level. And according to the results obtained in the main and control groups, there was no statistically significant deviation from the expected or observed empirical results or theoretical results for all identified polymorphisms ($\chi 2<3.85?P>0.05$) (2- and 3 - see tables). This shows that the results obtained during the study violate the Hardy-Weinberg law.

Table 2.

The results of testing various polymorphisms of genes in the main group according to the Hardy-Weinberg law.7

Poly-morphisms	Main group							
	Observed			Expected			$\chi 2$	p-valu
	Homo-	Hetero-	Homo-	Homo-	Hetero	Homo-		

	zygous wild	zygote	zygous not wild	zygous wild	- zygote	zygous not wild		e
MTHFR rs1801133	0,5	0,39	0,11	0,48	0,42	0,09	0,75	0,37
MTHFR rs1801131	0,48	0,42	0,1	0,47	0,43	0,1	0,06	0,774
MTR rs1805087	0,45	0,38	0,17	0,41	0,46	0,13	3,28	0,071
MTRR rs1801394	0,44	0,39	0,17	0,4	0,46	0,13	2,69	0,096

Hint: df=1

Table 3

The results of testing various polymorphisms of genes in the control group according to the Hardy-Weinberg law.

	Control group							
	Observed			Expected			$\chi 2$	p-value
Polymorphisms	Homo-zygous wild	Hetero-zygote	Homo-zygous not wild	Homo-zygous wild	Hetero - zygote	Homo-zygous not wild		
MTHFR rs1801133	0,64	0,3	0,06	0,62	0,34	0,05	0,97	0,312
MTHFR rs1801131	0,51	0,43	0,06	0,52	0,4	0,08	0,45	0,477
MTR rs1805087	0,54	0,38	0,08	0,53	0,39	0,07	0,25	0,592
MTRR rs1801394	0,57	0,33	0,09	0,55	0,39	0,07	1,74	0,181

Hint: df=1

In the main and control groups, the results of a molecular genetic study were analyzed, and the distribution of minor alleles was checked, thus checking the correlation of each polymorphism with the studied disease. In particular, in the gene MTHFR 1298A>C (rs1801131), according to the results of the polymorphism test, the control group was dominated by a minor allele - C with a low percentage of frequency ($\chi 2<3.85$, $p>0.05$). Accordingly, the homozygous wild–type genotype was a risk factor for the disease (RR=1.6; 95% CI: 0.73-3.63), and the odds ratio (OR=1.7; 95% CI: 0.6–4, 84) was recognized as an inductor. diseases. On the other hand, the homozygous wild-type genotype reduces the risk of developing the disease (R=0.90; 95% CI: 0.54-1.62), has a protective effect on the development of the disease. But these indicators were not statistically significant, since the difference between the main and control groups in the distribution of genotypes was not statistically significant. ($\chi 2<3.85$, $p>0.05$) (See Table 4).

Table 4.

In the main and control groups, the MTHFR gene in polymorphism A1298C is the spread and pathogenetic significance of polymorphism.

Alleles and genotypes	Number of alleles and genotypes				χ2	p	RR	95% CI	OR	95% CI
	Main group		Control group							
	n	%	n	%						
A	135	68,9	139	72,4	0,6	p = 0,50	1,0	0,63 - 1,43	0,8	0,54 - 1,31
C	61	31.1	53	27,6	0,6	p = 0,50	1.1	0,67 - 1,65	1,2	0,77 - 1,84
A/A	47	48,0	49	51,0	0,2	p = 0,70	0,9	0,54 - 1,62	0,9	0,5 - 1,55
A/C	41	41,8	41	42,7	0,0	p = 0,95	1,0	0,56 - 1,7	1,0	0,55 - 1,71
C/C	10	10.2	6	6.3	1,0	p = 0,40	1,6	0,73 - 3,63	1,7	0,6 - 4,84

Alleles of polymorphism A1298C of the MTHFR gene and the distribution of genotypes were studied in the main groups, the results of patients with and without negative habits were compared with the results of the control group. According to his data, the distribution of alleles and genotypes in both groups of patients did not differ statistically significantly compared to the control group ($\chi2<3.85$, $p>0.05$) (see Table. 5 and 6). This indicates that there is no connection between the polymorphism A>C of the MTHFR 1298 gene and the SSBON disease associated with COVID-19 in our study.

Table 5.

Patients with negative habits in the main group and in the control group of the MTHFR gene in polymorphism A1298C distribution and pathogenetic significance of polymorphism.

Alleles and genotypes	Number of alleles and genotypes				χ2	p	RR	95% CI	OR	95% CI
	Drinker and smoker		Control group							
	n	%	n	%						
A	66	68,8	139	72,4	0,4	p = 0,60	0,9	0,48 - 1,88	0,8	0,49 - 1,43
C	30	31,3	53	27,6	0,4	p = 0,60	1.1	0,73 - 1,52	1,2	0,7 - 2,04
A/A	23	47,9	49	51,0	0,1	p = 0,80	0,9	0,38 - 2,32	0,9	0,44 - 1,77
A/C	20	41,7	41	42,7	0,0	p = 0,95	1,0	0,39 - 2,45	1,0	0,47 - 1,93
C/C	5	10.4	6	6.3	0,8	p = 0,40	1,7	0,43 - 6,47	1,7	0,51 - 5,96

Table 6.

Patients without bad habits in the main group and in the control group of the MTHFR gene in polymorphism and A1298C spread and pathogenetic significance of polymorphism

Alleles and genotypes	Number of alleles and genotypes				χ2	p	RR	95% CI	OR	95% CI
	Non-drinker and non-smoker		Control group							
	n	%	n	%						
A	69	69,0	139	72,4	0,4	p = 0,60	1,0	0,49 - 1,85	0,8	0,5 - 1,44
C	31	31,0	53	27,6	0,4	p = 0,60	1,0	0,72 - 1,52	1,2	0,69 - 2
A/A	24	48,0	49	51,0	0,1	p = 0,80	0,9	0,39 - 2,27	0,9	0,45 - 1,75
A/C	21	42,0	41	42,7	0,0	p = 0,95	1,0	0,4 - 2,4	1,0	0,49 - 1,94
C/C	5	10,0	6	6.3	0,7	p = 0,50	1,6	0,41 - 6,18	1,7	0,49 - 5,7

Similarly, when analyzing the results of polymorphism C677T (rs1801133) of the MTHFR gene, the minor allele – T was higher in the main group, and the wild allele - C was higher in the control group by allelic parameters, and this difference was statistically significant ($\chi2=4.3$, $p=0.05$). In addition, the wild-type allele or normal allele had a protective effect, reducing the risk of developing ANGBC associated with COVID-19 with a 40% chance ratio (95% CI: 0.39–0.97), while the minor or mutant allele increased the risk by 60% (95% CI: 0.39-0.97 and DCI: 1.03–2.57) had an inducing value ($p = 0.05$). According to the analysis of the results of different genotypes, although wild homozygous genotype - C/C has a protective value in the pathogenesis of the disease (OR=0.6; 95%CI: 0.32 - 1.02), heterozygous genotype C/T and homozygous non-wild genotype - T/T increased the risk of developing the disease (OR=1.5; 95%CI: 0.81-2.65 and OR=1.9; 95% CI: 0.68-5.28) according to the extimolar ratio factor, however, the statistical significance of these indicators was not confirmed ($\chi2<3.85$, $p>0.05$). (See Table 7).

Table 7.

In the main and control groups of the MTHFR b polymorphism C677T gene, the spread and pathogenetic significance of polymorphism.

Alleles and genotypes	Number of alleles and genotypes				χ2	p	RR	95% CI	OR	95% CI
	Main group		Control group							
	n	%	n	%						
C	136	69,4	151	78,6	4.3	p = 0,05	0,9	0,59 - 1,31	0,6	0,39 - 0,97
T	60	30,6	41	21,4	4.3	p = 0,05	1.1	0,68 - 1,89	1,6	1,03 - 2,57
C/C	49	50,0	61	63,5	3,6	p = 0,10	0,8	0,46 - 1,35	0,6	0,32 - 1,02
C/T	38	38,8	29	30,2	1,6	p = 0,30	1,3	0,74 - 2,22	1,5	0,81 - 2,65
T/T	11	11.2	6	6.3	1,5	p = 0,30	1,8	0,85 - 3,79	1,9	0,68 - 5,28

Polymorphism 677 C>T of the MTHFR gene distribution of alleles and genotypes in patients of the main group when rearranged according to their negative habits, compared with the control group, the minor allele significantly increased the risk of developing the disease in patients with negative

habits (OR=1.8; 95%CI: 1.02-3.03, p=0.05), and wild the allele showed a statistically significant protective (OR=0.6; 95%CI: 0.33-0.98, p=0.05) effect on the development of the disease. On the other hand, when comparing the results of genotypes, no statistically significant relationship was found between the results of patients with negative habits and the control group ($\chi2<3.85$, $p>0.05$) (Table 8). Similarly, in patients with ANGBC disease associated with COVID-19, without negative habits when comparing the results of alleles and There was no statistically significant correlation between the disease and the C677T polymorphism of the MTHFR gene ($\chi2<3.85$, $p>0.05$) with the control group (9- see tables). Thus, the correlation between the minor allele of polymorphism C>T 677 of the MTHFR – T gene and ANGBC disease associated with COVID-19 was statistically significant only in the group of patients with negative habits ($\chi2<4.1$, $p=0.05$). This showed that the minor allele of polymorphism C>T of the MTHFR 677 gene does not independently, but synthropically to the harmful influence of bad habits statistically significantly increases the risk of developing ANGBC associated with COVID-19.

Table 8.

Patients with negative habits in the main group and in the control group of the MTHFR gene in polymorphism C677T spread and pathogenetic significance of polymorphism.

Alleles and genotypes	Number of alleles and genotypes				χ2	p	RR	95% CI	OR	95% CI
	Drinker and smoker		Control group							
	n	%	n	%						
C	65	67,7	151	78,6	4.1	p = 0,05	0,9	0,45 - 1,66	0,6	0,33 - 0,98
T	31	32,3	41	21,4	4.1	p = 0,05	1,2	0,76 - 1,78	1,8	1,02 - 3,03
C/C	23	47,9	61	63,5	3.2	p = 0,10	0,8	0,31 - 1,85	0,5	0,26 - 1,06
C/T	19	39,6	29	30,2	1,3	p = 0,30	1,3	0,53 - 3,25	1,5	0,74 - 3,12
T/T	6	12,5	6	6.3	1,6	p = 0,30	2.0	0,6 - 6,72	2.1	0,67 - 6,89

Table 9.

Patients without bad habits in the main group and in the control group, the MTHFR gene in the C677T polymorphism, the spread and pathogenetic significance of the polymorphism.

Alleles and genotypes	Number of alleles and genotypes				χ2	p	RR	95% CI	OR	95% CI
	Drinker and smoker		Control group							
	n	%	n	%						
C	71	71,0	151	78,6	2.1	p = 0,20	0,9	0,47 - 1,75	0,7	0,38 - 1,15
T	29	29,0	41	21,4	2.1	p = 0,20	1.1	0,72 - 1,69	1,5	0,87 - 2,61
C/C	26	52,0	61	63,5	1,8	p = 0,20	0,8	0,34 - 1,96	0,6	0,31 - 1,24
C/T	19	38,0	29	30,2	0,9	p = 0,40	1,3	0,52 - 3,07	1,4	0,69 - 2,9
T/T	5	10,0	6	6.3	0,7	p = 0,50	1,6	0,41 - 6,18	1,7	0,49 - 5,7

The MTHFR gene is located on the short arm of chromosome 1 (1q36.3) and consists of 11 exons. The length of the coding gene is about 1980 pairs of nucleotides. As a result of the rs1801133 (C677T) polymorphism of the MTHFR gene, cytosine nucleotide 677 is replaced by thymine, alanine by valine in

the protein-enzyme catalytic domain (p.Ala222Val). As a result, the activity of the enzyme decreases by 70% in the mutant homozygous variant and by 35% in the heterozygous genotype. As a result of a sharp decrease in enzyme activity in the homozygous genotype according to the C677T allele, the concentration of homocysteine in the blood is sharply higher than normal, and in the heterozygous genotype it is much higher than normal. This condition is especially pronounced when the amount of folic acid in the blood is low [35]. Therefore, some studies have shown a positive association between ANGBC and the rs1801133 polymorphism of the MTHFR gene [41, 42]. On the other hand, in the MTR 2756 A>G gene (rs1805087), the type of polymorphism, although the minor allele-G was detected to a greater extent in the main group compared to the control group, its association with ANGBC disease associated with COVID-19 was not statistically significant ($\chi2<3.85$, $P>0.05$). This indicates that there is no association between the development of COVID-19-associated ANGBC and the A>G polymorphism of the MTR 2756 gene ($\chi2<3.85$, $p>0.05$) (see Table 10).

Table 10.

In the main and control group in the MTR gene in the A2756G polymorphism, the distribution and pathogenetic significance of the polymorphism.

Alleles and genotypes	Number of alleles and genotypes				$\chi2$	p	RR	95% CI	OR	95% CI
	Main group		Control group							
	n	%	n	%						
A	125	63,8	140	72,9	3,7	p = 0,10	0,9	0,59 - 1,29	0,7	0,43 - 1,01
G	71	36,2	52	27.1	3,7	p = 0,10	1.1	0,72 - 1,81	1,5	0,99 - 2,35
A/A	44	44,9	52	54,2	1,7	p = 0,20	0,8	0,48 - 1,44	0,7	0,39 - 1,21
A/G	37	37,8	36	37,5	0,0	p = 0,98	1,0	0,57 - 1,77	1,0	0,57 - 1,81
G/G	17	17,3	8	8.3	3,5	p = 0,10	2.1	1,13 - 3,83	2.3	0,96 - 5,54

Similar to the results in the general group, in the group with negative habits, the minor allele and the wild-type homozygous genotype increased the likelihood of developing the disease (respectively OH=1.5; 95%CI: 0.92-2.6, OH=2.2; 95%CI: 0.79-6.16), while the wild allele and wild homozygous genotypes reduce the likelihood of developing the disease (respectively, the wild allele – A: OR=0.6; 95%CI: 0.38-1.09, non-wild homozygous genotype – A/A OR=0.7; 95%CI: 0.33-1.32), the determined parameters were not statistically significant ($\chi2<3.85$, $p>0.05$) (Table 11).

Table 11.

Patients with negative habits in the main group and in the control group in the MTR gene in the A2756G polymorphism: the spread and pathogenetic significance of the polymorphism.

Alleles and genotypes	Number of alleles and genotypes		$\chi2$	p	RR	95% CI	OR	95% CI
	Drinker and smoker	Control group						

	n	%	n	%						
A	61	63,5	140	72,9	2,7	p = 0,20	0,9	0,46 - 1,67	0,6	0,38 - 1,09
G	35	36,5	52	27.1	2,7	p = 0,20	1.1	0,78 - 1,68	1,5	0,92 - 2,6
A/A	21	43,8	52	54,2	1,4	p = 0,30	0,8	0,32 - 2,02	0,7	0,33 - 1,32
A/G	19	39,6	36	37,5	0,1	p = 0,90	1.1	0,42 - 2,66	1.1	0,54 - 2,22
G/G	8	16,7	8	8.3	2.3	p = 0,20	2.0	0,68 - 5,91	2.2	0,79 - 6,16

Also, in groups without negative habits, the minor allele and the wild-type homozygous genotype increased the likelihood of developing the disease (respectively, OR = 1.5; 95% CI: 0.9-2.54, OR = 2.4; 95% CI: 0.89-6.56)., wild allele and wild homozygous genotypes reduce the likelihood of developing the disease (respectively RR = 0.7; 95% CI: 0.39-1.11, OR = 0.7; 95% CI: 0.36-1.43), these indicators were not statistically significant. ($\chi 2<3,85,>0,05$) (Table 12). This suggests that there is no association between the A2756G polymorphism of the MTR gene and COVID-19-related ANGBC disease when comparing the results of the control group and the general group, the group with negative habits and the group without negative habits in our study.

Table 12.

Patients without bad habits in the main group and in the control group in the MTR gene in the A2756G polymorphism: the spread and pathogenetic significance of the polymorphism.

Alleles and genotypes	Number of alleles and genotypes				$\chi 2$	p	RR	95% CI	OR	95% CI
	Non-drinker and non-smoker		Control group							
	n	%	n	%						
A	64	64,0	140	72,9	2,5	p = 0,20	0,9	0,47 - 1,65	0,7	0,39 - 1,11
G	36	36,0	52	27.1	2,5	p = 0,20	1.1	0,77 - 1,68	1,5	0,9 - 2,54
A/A	23	46,0	52	54,2	0,9	p = 0,40	0,8	0,35 - 2,06	0,7	0,36 - 1,43
A/G	18	36,0	36	37,5	0,0	p = 0,90	1,0	0,38 - 2,41	0,9	0,46 - 1,91
G/G	9	18,0	8	8.3	3.0	p = 0,10	2.2	0,79 - 5,92	2,4	0,89 - 6,56

MTR - methionine synthase, as mentioned above, plays an important role in the folate cycle, methionine synthesis and adequate maintenance of plasma homocysteine levels. The MTR gene is located at the 1q43 locus, and as a result of the rs1805087 polymorphism, the nucleotide from A to G at the 2756 locus replaces aspartate with glycine in the enzyme sequence, thereby changing the protein structure and causing a decrease in enzyme activity [36, 37]. As a result, the function of methionine synthesis is reduced due to the transfer of the methyl group from 5-methyltetrahydrofolate to homocysteine. This can lead to disruption of the folate cycle and hyperhomocysteinemia and hypercoagulopathy and thus to ANGBC.

It was found that the predominance of minor alleles G statistically significantly ($\chi 2 = 4$, $p = 0.05$) in patients with negative habits compared with patients without negative habits increases the likelihood of developing the disease (OR = 1.8, 95% CI 1.01-3.26). On the other hand, the positive allele - A was statistically more common in patients who did not have bad habits ($\chi 2 = 4.0$, $p = 0.05$) and it was found that the wild allele has a protective effect on the pathogenesis of the disease (OR = 0.6, 95% CI 0.31–0.99). On the other hand, the presence of a statistically significant association was not found in the study of differences in the distribution of genotypes in the two

groups (Table 16). Based on the results obtained, it can be concluded that the correlation between A66G polymorphisms of the MTRR gene and minor alleles – G and ANGBC disease associated with COVID-19 is statistically significant only in the group of patients with negative habits ($\chi2<4.1$, $p=0.05$). Also, in patients with a homozygous genotype - G / G and negative habits, the probability of developing ANGBC associated with COVID-19 was statistically significantly high (in terms of odds ratio by 2.9 times) ($\chi2 = 4.9$, $p = 0.05$). This means that the C>T 677 polymorphism of the MTHFR gene is not an independent minor allele that may increase the likelihood of the disease (a statistically significant relationship between the tested polymorphism and the disease is not identified in patients without bad habits), and as a result, the syntropic effect of alcohol consumption and smoking, the development of ANGBC associated with COVID-19 (a statistically significant increase in risk was established).

Similarly, expression of the MT RR gene is important for the storage of active cobolamine cholate, which is a methionine synthase reductase enzyme, a cofactor of the methionine synthase (MTR) enzyme [38, 39]. The rs1801394 polymorphism is located in the 5-untranslated part (5-UTR) of the MT RR gene, and as a result of the rs1801394 polymorphism in the 66 gene sequence Some studies have shown that this SNP causes a decrease in the expression of the MTRR gene in patients with the G allele and a decrease in the concentration of homocysteine methionine in the blood and it has been proven that it causes an increase [38, 39]. The results of our study showed that the probability of developing the disease was statistically significantly increased in patients with negative habits ($p<0.05$), on the other hand, it was statistically insignificant in patients without negative habits ($p>0.05$).

Findings. Among the tested folate cycle gene polymorphisms, a statistically significant positive correlation was found for the COVID-19-related ANGBC disease and the minor C677T polymorphism of the MTHFR gene – the T allele, as well as the minor polymorphism of the A66G polymorphism of the MTRR gene – the G allele ($\chi2>3.85$, $p<0.05$). According to him, the minor allele of the MTHFR C677T gene polymorphism only in the presence of the syntropic effect of negative habits statistically significantly increased the likelihood of developing ANGBC associated with COVID-19 by 80% (OR = 1.8; 95% CI: 1.02 - 3.03, $\chi2 = 4.1$, $p = 0.05$), a similar polymorphism of the MTRR A66G gene minor - the G allele and the homozygous wild genotype - G / G in the group of patients with negative habits, the probability of developing the disease is statistically significant, 2.2 times, respectively (O R = 2.2; 95% CI: 1.32–3.68, $\chi2 = 9.2$, $p = 0.01$) and 2.9 times (O R = 2.9; 95% CI: 1.13–7, 31, $\chi2 = 4.9$, $p = 0.05$) an increase was found. No statistically significant positive association was found between other types of polymorphisms (A2756G polymorphism of the MTR gene, A1298C polymorphism of the MTHFR gene) and the development of COVID-19-related ANGBC ($\chi2<3.85$, $p>0.05$).

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