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Association of human leukocyte antigen class II alleles with epithelial cell apoptosis and extracellular matrix production in acute COVID-19

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Abstract

Introduction: *Pathogenic mechanisms and long-term consequences of COVID-19 require attention in studies on SARS-CoV-2. The association of the severity of COVID-19 with genetic factors, such as human leukocyte antigen (HLA) genes, remains underexplored. Our study assessed the relationships between HLA class II alleles and COVID-19 severity and blood-based indicators of systemic inflammation and organ damage, serum markers of epithelial cell apoptosis such as caspase-cleaved CK18 fragment M30 (CK18-M30) and the extracellular matrix product hyaluronic acid (HA).*

Material and methods: *The study included 101 hospitalized COVID-19 patients (mean age 60 ±14 years). Clinical tests were performed at admission to the hospital. The levels of CK18-M30 and HA were detected in serum by enzyme-linked immunosorbent assay (ELISA). HLA typing was performed in HLA-DRB1, -DQA1, and -DQB1 loci by the polymerase chain reaction with low-resolution sequence-specific primers.*

Results: *Sixty-one patients had a non-severe and 40 had a severe or critical disease course (following the WHO definition). The severity was associated with older age, male gender, higher HA, CK18-M30, and some indicators of inflammation. Despite the lack of direct association between HLA alleles and the severity of COVID-19, the presence of HLA-DRB1*04 and 12 alleles in the genotype was associated with lowered or elevated HA, respectively. The HLA-DQB1*03:01 allele was associated with lowered CK18-M30, aspartate aminotransferase, and ferritin. In addition, HLA-DQB1*06:01 was associated with elevated alanine aminotransferase.*

Conclusions: *Associations of HLA class II alleles with markers of epithelial cell apoptosis and extracellular matrix production indirectly support the influence of HLA genes on acute COVID-19 severity.*

Key words: *liver, hyaluronic acid, cytokeratin 18, HLA class II, COVID-19, apoptosis.*

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Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has a varied acute clinical course with possible development of acute respiratory distress syndrome and multiple organ dysfunction and mortality risk [1, 2]. Some people have long-term health effects that last for weeks or even months after recovery from an acute illness [3, 4] or can develop autoimmune processes after the resolution of infection [5]. Therefore, establishing pathogenic mechanisms and long-term consequences of COVID-19 requires attention in studies on SARS-CoV-2.

Age, male gender, and comorbidities are among the main factors related to the outcome of COVID-19 [1, 2]. The outcome can be primarily age-dependent in patients with a similar initial viral load due to the impaired adaptive immune response to SARS-CoV-2 and possibly facilitating and amplifying hyperinflammation in older people [6]. Additionally, the severity of SARS-CoV-2 infection can be related to genetic factors such as human leukocyte anti-

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gen (HLA) genes [7-10]. HLA genes regulate the immune response by encoding HLA or major histocompatibility complex (MHC) I and II molecules, which bind antigen peptides and present them to T lymphocytes. Therefore, differences in peptide-binding affinities between HLA class I and class II proteins and SARS-CoV-2 peptides [11] may affect the disease course [12].

Since the beginning of the pandemic, the role of HLA genes in COVID-19 severity has been intensively discussed [7-10, 13, 14]. These studies produced inconsistent results due to different sample sizes, genetic variation between populations, and different indicators of COVID-19 severity. For example, a potentially protective effect on COVID-19 severity may be associated with *HLA-DRB1*04:01* [7] and *HLA-DRB1*01:01* and *03:01* [8], but disease severity may be related to *HLA-DRB1*09:01* [10]. Other studies [9, 13, 14] revealed no significant associations between HLA alleles and COVID-19 severity. From a theoretical perspective [15], the infected individuals enter the severe stage with an intense inflammatory response and increased production and deposition of hyaluronic acid (HA) in the lungs when their general health status and HLA haplotype do not eliminate the virus in the early stage. Simultaneously, there is a lack of empirical assessment of the relationship between HLA alleles, inflammation, and the level of serum HA. This study aimed to investigate the relationships between HLA class II alleles and COVID-19 severity and blood-based indicators of systemic inflammation and organ damage.

To analyze the association of HLA class II alleles with systemic inflammation and organ damage, we selected indicators reflecting the pathogenetic mechanisms of COVID-19. They were lymphocyte count, C-reactive protein (CRP), interleukin 6 (IL-6), ferritin, D-dimer, and serum enzymes, such as lactate dehydrogenase (LDH), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), as widely used non-specific early predictors of a poor outcome [16-19]. Recent studies [20, 21] have shown that serum markers of epithelial cell apoptosis, such as caspase-cleaved cytokeratin 18 fragment M30 (CK18-M30) [22, 23], and serum markers of increased extracellular matrix production, such as HA [15], were previously observed in a higher concentration in severe than mild COVID-19 cases [20, 21]. Increased CK18-M30 in acute COVID-19 can be explained by the direct damage of epithelial cells expressing angiotensin-converting enzyme 2 (ACE2) receptors in the lungs, kidneys, liver, and other organs by the virus [24] and inducing infected cell apoptosis [25, 26]. Intensive cytokine production and inflammation during acute COVID-19 facilitate the production of HA in tissues that can lead to acute respiratory distress syndrome [15, 27]. Increased HA can also be detected in the blood, and HA association with acute COVID-19 severity was observed in the context of developing fibrotic processes in the lungs [21] and liver [28]. Therefore, using HA and CK-18 as indicators of COVID-19 severity can extend our understanding of the pathogenetic mechanism of SARS-CoV-2. Considering previous findings [20, 21, 28], we can hypothesize an intercorrelation between CK18-M30 and HA. Additionally, based on previously described links between COVID-19 severity and serum levels of CK18-M30 and HA [20, 21] and the association between COVID-19 severity and HLA profile [7, 8, 10], we can hypothesize associations between HLA class alleles and indicators of COVID-19 severity, including epithelial cell apoptosis and extracellular matrix production.

Material and methods

Study design and participants

The one-center cross-sectional study was conducted from September to December 2020. Permissions of the Central Medical Ethics Committee, Riga, Latvia (protocol No. 01-29.1/2429) and the Ethics Committee of Riga Stradiņš University, Latvia (protocol No. 6-1/07/14) were obtained for the genetic analysis. All patients were hospitalized at Riga East Clinical University Hospital (Latvia) and signed an agreement for participation in the study.

The study group included 101 patients with confirmed SARS-CoV-2 infection by positive real-time reverse-transcription polymerase chain reaction in the nasopharyngeal swab. Chest X-rays and blood gas analysis at admission were performed for all patients. Chest computed tomography was performed for some patients according to clinical indications. Based on clinical and radiological data and the minimal level of blood oxygen saturation (minimal SpO_2) during hospitalization in medical records, all cases were retrospectively classified as non-severe (cases without or with evidence of pneumonia and $SpO_2 \geq 90\%$ on room air), severe (with pneumonia and $SpO_2 < 90\%$ on room air), or critical (with additional evidence of respiratory failure, multiple organ dysfunction, septic shock) according to the World Health Organization COVID-19 severity definition [29]. For statistical analysis, patients with severe and critical diseases were combined into one group.

For all patients, venous blood samples were obtained on an empty stomach within 24 hours after admission to the hospital. Venous blood samples were centrifuged at 4000 × g for 10 min, and serum was stored at –80°C until additional analysis. For genetic analysis, 5 ml of venous blood with EDTA was used and stored at –80°C.

Routine clinical tests

Routine clinical tests included leukocyte, lymphocyte, and platelet counts and levels of CRP, IL-6, ferritin, ALT, AST, LDH, γ-glutamyl transferase (GGT), D-dimer, and creatinine. We also analyzed data on the minimal SpO_2 during hospitalization.

Table 1. Demographic characteristics, routine clinical tests, hyaluronic acid, and cytokeratin 18 fragment M30 at admission to the hospital in patients with different COVID-19 severity

Parameters	COVID-19 severity				Mann-Whitney *U* test
	Non-severe		Severe and critical		
	n	Median (IQR)	*n*	Median (IQR)	
Age, years	61	61.0 (49.5; 67.0)	40	67.5 (58.0;73.0)	1552.0*
Females (%)	61	62%	40	33%	8.58** [a]
Min SpO_2 (%)	61	95 (92; 96)	39	87 (79; 88)	0.0***
Leucocytes ($\times 10^3$/µl)	61	5.04 (4.09; 7.49)	40	5.77 (4.80; 7.29)	1399.5
Lymphocytes ($\times 10^3$/µl)	59	0.97 (0.70; 1.42)	37	0.92 (0.70; 1.20)	967.0
Platelets ($\times 10^3$/µl)	60	200 (169; 263)	40	183 (134; 230)	941.0
CRP (mg/l)	61	29.6 (12.0; 93.3)	40	61.6 (29.6; 125.3)	1574.5*
IL-6 (pg/ml)	51	11.9 (6.4; 24.8)	30	39.9 (15.4; 50.0)	1163.0***
Ferritin (ng/ml)	60	387.7 (145.5; 788.9)	35	614.0 (277.5; 1219.7)	1259.0
ALT (U/l)	57	25 (17; 41)	39	27 (18; 43)	1160.0
AST (U/l)	46	30 (20; 45)	34	39 (27; 67)	980.5
LDH (U/l)	58	241 (195; 316)	38	383 (266; 445)	1664.5***
GGT (U/l)	44	31 (16; 58)	27	59 (31; 94)	797.5*
Creatinine (µmol/l)	60	72.5 (64.0; 84.3)	39	87.0 (73.0; 108.0)	1636.5**
D-dimer (µg/l)	61	0.48 (0.41; 0.73)	37	0.54 (0.41; 0.73)	1262.0
HA (ng/ml)	61	62.1 (34.9; 93.5)	40	87.8 (46.9; 197.5)	1524.5*
CK18-M30 (U/l)	53	217.0 (143.5; 266.5)	32	246.0 (207.8; 336.3)	1071.0*

*[a] Assessed with chi-square test. IQR – interquartile range, Min SpO_2 – lowest oxygen saturation during hospitalization, CRP – C-reactive protein, IL-6 – interleukin 6, ALT – alanine aminotransferase, AST – aspartate aminotransferase, LDH – lactate dehydrogenase, GGT – γ-glutamyl transferase, HA – hyaluronic acid, CK18-M30 – caspase-cleaved cytokeratin 18, n – number of patients. *p < 0.05, **p < 0.01, ***p < 0.001.*

Hyaluronic acid

Hyaluronic acid was detected by an enzyme-linked immunosorbent assay kit (Hyaluronan Quantikine ELISA Kit, R&D Systems) following the manufacturer's instructions. HA higher than 75 ng/ml was considered elevated [29].

Caspase-cleaved cytokeratin 18 fragment (CK18-M30)

Caspase-cleaved cytokeratin 18 fragment M-30 (CK18-M30) was detected in serum by an enzyme-linked immunosorbent assay kit (M30 Apoptosense ELISA Kit, Peviva) following the manufacturer's instructions. CK18-M30 higher than 200 U/l was considered elevated [22].

HLA typing

DNA extraction was performed using the QIAamp DNA Blood Kit (QIAGEN). DNA quality and quantity were checked using a Qubit fluorometer (Invitrogen). HLA typing was performed in *HLA-DRB1*, *-DQA1*, and *-DQB1* loci by a polymerase chain reaction with sequence-specific primers (SSP-PCR) according to the manufacturer's instructions (DNA-Technology). Amplification was performed using a thermocycler with four channels and 48 wells.

Statistical analysis

The IBM SPSS 22.0 program was used for data analysis. The analysis of the total frequency of alleles and their distribution in groups of patients was allele-based and counted every allele. Therefore, the total number of alleles was 202 for each HLA locus under investigation. We applied Pearson's chi-square (χ^2) test and the Cochran-Mantel-Haenszel test to compare allele distribution in their association with disease severity. Comparisons of clinical markers and correlation analysis were patient-focused, and data on allele-positive or allele-negative patients were analyzed in 101 people. We used the nonparametric Mann-Whitney test and Spearman rank correlation coefficient for this task.

Results

Clinical characteristics of study groups

There were 101 patients aged between 26 and 85 (M = 60 ±14 years) in the study. Comorbidities were

reported in 80% of patients. The most common comorbidities were arterial hypertension (57 patients) and diabetes mellitus (18 patients). The less commonly reported comorbidities were arrhythmias (11 patients), chronic heart failure (10 patients), chronic obstructive pulmonary disease (2 patients), asthma (7 patients), chronic kidney disease (5 patients), adiposity (7 patients), cerebrovascular disease (8 patients), oncological disease (5 patients), and liver diseases (8 patients), including fatty liver disease (5 patients) and chronic viral hepatitis B or C (3 patients).

Patients were admitted to the hospital on day 8 ±4 of illness. Eight patients were hospitalized in the intensive care unit. Remdesivir was used for 18 patients, steroids for 57, vasopressors for three patients, oxygen supplementation without mechanical ventilation for 56 patients and mechanical ventilation for one patient. A retrospective analysis of COVID-19 severity showed that 61 patients had non-severe COVID-19, and 40 had severe and critical disease courses. Table 1 presents the differences between groups.

Patients with severe and critical COVID-19 were older than patients with non-severe disease ($p < 0.05$). Male gender was associated with COVID-19 severity ($p < 0.01$). Patients with severe and critical COVID-19 had a lower level of SpO_2 during the hospitalization period than patients with non-severe COVID-19.

To confirm clinical differences at admission to the hospital among patients with non-severe and severe and critical COVID-19, we analyzed routine clinical tests. The analysis (Table 1) revealed higher levels of the inflammatory markers CRP (61.6 mg/l, IQR [29.6, 125.3] vs. 29.6 mg/l, IQR [12.0, 93.3]), IL-6 (39.9 pg/ml, IQR [15.4, 50.0] vs. 11.9 pg/ml, IQR [6.4, 24.8]), and higher levels of the serum enzymes LDH (383 U/l, IQR [266, 445] vs. 241 U/l, IQR [195, 316]) and GGT (59 U/l, IQR [31, 94] vs. 31 U/l, IQR [16, 58]) in patients with severe and critical COVID-19 than in patients with non-severe COVID-19, respectively. Patients with severe and critical COVID-19 also had a higher level of creatinine (87.0 µmol/l, IQR [73.0, 108.0] vs. 72.5 µmol/l, IQR [64.0, 84.3]).

In addition, we found that patients with severe and critical COVID-19 at admission had a higher level of HA (87.8 ng/ml, IQR [46.9, 197.5] vs. 62.1 ng/ml, IQR [34.9, 93.5]), and CK18-M30 (246.0 U/l, [207.8, 336.3] vs. 217.0 U/l, IQR [143.5, 266.5]). Based on cut-off levels [22, 29], we detected that 47% of all patients had increased HA (≥ 75 ng/ml), and 66% had increased CK18-M30 (≥ 200 U/l).

The correlation analysis revealed an intercorrelation between HA and CK18-M30 ($r_s = 0.56$, $p < 0.001$). HA correlated with the inflammatory markers IL-6 ($r_s = 0.34$, $p < 0.01$) and ferritin ($r_s = 0.23$, $p < 0.05$), and the markers of tissue damage ALT ($r_s = 0.38$, $p < 0.001$), AST ($r_s = 0.43$, $p < 0.001$), LDH ($r_s = 0.49$, $p < 0.001$), and GGT ($r_s = 0.25$, $p < 0.05$). Similarly, CK18-M30 positively correlated with ferritin ($r_s = 0.39$, $p < 0.001$), ALT ($r_s = 0.45$, $p < 0.001$), AST ($r_s = 0.47$, $p < 0.001$), LDH ($r_s = 0.45$, $p < 0.001$), and GGT ($r_s = 0.331$, $p < 0.05$). Both HA and CK18-M30 negatively correlated with SpO_2 during hospitalization ($r_s = –0.32$, $p < 0.01$ for HA; $r_s = –0.39$, $p < 0.001$ for CK18-M30). HA and CK18-M30 demonstrated no correlation with creatinine or gender, while HA was positively related to patients' age ($r_s = 0.39$, $p < 0.01$).

HLA class II alleles and their associations with severity and clinical markers

Table 2 presents HLA class II allele frequencies in patients with different COVID-19 severity. The allele-based analysis revealed no significant associations between *HLA-DRB1**, *-DQA1**, and *-DQB1** alleles and COVID-19 severity following the WHO definition. The most common *HLA-DRB1** allele – *HLA-DRB1*13* – was similarly distributed between severe and critical and non-severe groups (25.0% vs. 16.4%, respectively). The most common *HLA-DQA1** allele – *HLA-DQA1*05:01* – also demonstrated similar distribution in severe and critical and non-severe groups (31.3% vs. 24.6%, respectively). In the *HLA-DQB1* locus, the most common allele was *HLA-DQB1*03:01*. It also demonstrated no significant differences between severe and critical and non-severe groups (23.8% vs. 24.6%, respectively).

Patient-focused analysis revealed several alleles linked to the serum levels of HA, CK18-M30, ferritin, ALT, and AST. Patients with the presence of the *HLA-DRB1*12* allele in the genotype had a higher level of HA, $U = 62.0$, $p < 0.05$ (320.0 ng/ml, IQR [109.0; 320.0]), than patients without *HLA-DRB1*12* (66.1 ng/ml, IQR [37.6; 113.1]). Patients with the *HLA-DRB1*04* allele had a lower level of HA, $U = 610.5$, $p < 0.05$ (49.8 ng/ml, IQR [29.2; 75.3]), than patients without this allele (81.2 ng/ml, IQR [44.9; 133.6]).

Patients with *HLA-DQB1*03:01* had lower levels of CK18-M30, $U = 647.5$, $p < 0.05$ (207.0 U/l, IQR [127.5; 260.5] vs. 240 U/l, IQR [196; 343.8]), a lower level of ferritin, $U = 765.0$, $p < 0.01$ (322.7 ng/ml, IQR [146.0; 619.4] vs. 675.0 ng/ml, IQR [262.6; 1160.4]), and a lower level of AST, $U = 527.0$, $p < 0.05$ (29 U/l, IQR [21; 41] vs. 41 U/l, IQR [26; 72]), than patients without it. In addition, patients with *HLA-DQB1*06:01* showed a higher level of ALT, $U = 174.5$, $p < 0.05$ (50 U/l, IQR [36; 79]), than patients without this allele in the genotype (25 U/l, IQR [17; 40]).

Discussion

We identified associations between HLA class II alleles and serum levels of HA and CK18-M30, which, in turn, were associated with COVID-19 severity in our groups and some previous studies [20, 21, 28]. The levels of HA and CK18-M30 intercorrelated positively, and each of them negatively correlated with the minimum SpO_2 level during hospitalization. These correlations indicate increased ex-

Table 2. Allele frequencies in HLA-DRB1, -DQA1, and -DQB1 loci in patients with different COVID-19 severity

Loci	Allele	COVID-19 severity				Total (2*n* = 202)		Non-severe vs. severe and critical
		Non-severe (2*n* = 122)		Severe and critical (2*n* = 80)				
		n	%	*n*	%	*n*	%	*p*-value
HLA-DRB1	01	14	11.5	7	8.8	21	10.4	0.645
	03	11	9.0	6	7.5	17	8.4	0.690
	04	19	15.6	5	6.3	24	11.9	0.108
	07	12	9.8	9	11.3	21	10.4	0.732
	08	3	2.5	0	0	3	1.5	0.079
	09	0	0	0	0	0	0	NA
	10	0	0	0	0	0	0	NA
	11	15	12.3	13	16.3	28	13.9	0.385
	12	2	1.6	3	3.8	5	2.5	0.346
	13	20	16.4	20	25.0	40	19.8	0.224
	14	5	4.1	6	7.5	11	5.4	0.289
	15	20	16.4	9	11.3	29	14.4	0.690
	16	2	1.6	2	2.5	4	2.0	0.647
HLA-DQA1	01:01	14	11.5	11	13.8	25	12.4	0.682
	01:02	23	18.9	13	16.3	36	17.8	0.593
	01:03	21	17.2	15	18.8	36	17.8	0.752
	02:01	15	12.3	11	13.8	26	12.9	0.648
	03:01	10	8.2	2	2.5	12	5.9	0.456
	04:01	9	7.4	2	2.5	11	5.4	0.384
	05:01	30	24.6	25	31.3	55	27.2	0.193
	06:01	0	0	1	1.3	1	0.5	0.396
HLA-DQB1	02:01-02	24	19.8	18	22.5	42	20.8	0.278
	03:01	30	24.6	19	23.8	49	24.3	0.999
	03:02	10	8.2	4	5.0	14	6.9	0.363
	03:03	1	0.8	0	0	1	0.5	0.999
	03:04	0	0	1	1.3	1	0.5	0.396
	03:05	0	0	0	0	0	0	NA
	04:01-02	5	4.1	1	1.3	6	3.0	0.398
	05:01	17	13.9	10	12.5	27	13.4	0.673
	05:02-04	5	4.1	7	8.8	12	5.9	0.390
	06:01	7	5.7	1	1.3	8	4.0	0.142
	06:02-08	23	18.9	19	23.8	42	20.8	0.618

n – number of patients, NA – not applicable

tracellular matrix production and epithelial cell apoptosis under hypoxia in acute COVID-19 [20, 21, 28]. Moreover, extracellular matrix production and epithelial cell apoptosis occur synchronously. However, we did not find a direct association between HLA and the severity of COVID-19, which was considered severe or critical if the minimal level of SpO_2 during the hospitalization was lower than 90%. In our sample, the presence of the *HLA-DRB1*04* allele in the genotype was associated with a lower serum HA, the presence of *HLA-DRB1*12* with a higher HA, and *HLA-DQB1*03:01* with a lower serum CK18-M30. It indicated possible modulation of extracellular matrix production and epithelial cell apoptosis (as factors associated with COVID-19 severity) during COVID-19 by HLA alleles,

which aligns with our hypothesis. This finding also concurs with a theoretical perspective on associations of HA production with inflammation and HLA [15].

Additionally, we found associations of two HLA class II alleles with liver enzymes and ferritin as an acute phase protein produced by hepatocytes, reflecting inflammation and severity in COVID-19 [16, 17, 19]. There were two additional associations of the *HLA-DQB1*03:01* allele with lower levels of AST and ferritin. The observed association enhances the potentially protective effect of this allele on hepatocellular damage. In contrast, an association of the *HLA-DQB1*06:01* allele with a higher level of ALT may point to the potentially hepatotoxic effect. Even though the SARS-CoV-2 virus predominantly causes lung damage [31], hepatocytes also express ACE-2 receptors and can be infected by the virus or damaged during a pro-inflammatory reaction by cytokines [24]. Therefore, the intercorrelation between HA and CK18-M30 and their correlations with ALT and GGT, as more specific liver enzymes, point to liver involvement in increased production of HA and epithelial cell apoptosis, complementing the involvement of lungs in these processes [20, 21].

The absence of a direct association between HLA and COVID-19 severity is in accordance with some previous studies [9, 13, 14], but not that of Langton *et al*. [7]. However, these studies applied different criteria for defining COVID-19 severity. For example, one study [13] defined a severe disease course as hospitalization (vs. non-hospitalization) without data on SpO_2 monitoring during it. Another study [7] described a severe case as hospitalization with oxygen supplementation. One more study [14] defined severe cases as mechanical ventilation cases, presenting a narrower view than in our study. On the other hand, the definition of severe cases as blood oxygen saturation ≤ 93% [9] was broader than in our study. Despite this, our study revealed similar associations between HLA alleles and indicators of COVID-19 severity. For example, *HLA-DRB1*04* and *HLA-DQB1*06:01* were previously described in patients with COVID-19 [7, 30]. *HLA-DRB1*04* was associated with a non-severe disease course [7], and *HLA-DQB1*06* was described with a higher susceptibility to SARS-CoV-2 infection [30]. Based on the observed relationships, our results indirectly supported the protective effect of *HLA-DRB1*04* and the risk effect of *HLA-DQB1*06:01* in COVID-19. It suggests that the discrepancies in previous observations [7-10, 13, 14] can be explained by the association of HLA class II alleles with common mechanisms of disease rather than different classifications of COVID-19 severity.

These changes during the acute COVID-19 phase can affect tissue remodulation after COVID-19 and lead to lung [21] and liver [24, 33] fibrosis. Based on the revealed association of HLA class II alleles with extracellular matrix production and hepatocellular damage, we can hypothesize an effect of HLA alleles on the development of liver fibrosis after COVID-19 in a long-term period. In further studies, the revealed associations should be tested in a large group to detect genetic predisposition to these complications. It will help identify and stratify individuals with a higher risk of severe COVID-19 and COVID-19-related fibrosis and apply treatment inhibiting the production of HA.

SARS-CoV-2 is an autoimmune virus that induces the production of autoantibodies against different cells, including liver cells [34], which can lead to autoimmune hepatitis. The previously revealed role of particular HLA class II alleles (e.g., *HLA-DRB1*04* and *12*) in the pathogenesis of autoimmune liver diseases and hepatocellular carcinoma [35-37] and associations of *HLA-DRB1*12* with higher HA and *HLA-DQB1*06:01* with a higher level of ALT observed in our study allow us to hypothesize an adverse effect of SARS-CoV-2 infection on possible liver-related autoimmune processes, which should be followed for a longer time.

The main limitation of our study was the relatively small sample size because of a limited number of SARS-CoV-2 infected patients during the project. Additionally, we applied patient-focused analysis for clinical markers because of the small number of homozygotic patients. Therefore, studies with a larger group of COVID-19 patients than in the present study are needed to explain the exact mechanisms underlying the effects of HLA alleles on epithelial tissue damage, fibrosis, disease course, and consequences of COVID-19.

In conclusion, our study revealed associations between HLA class II alleles and levels of epithelial cell apoptosis and extracellular matrix production, identified as indicators of COVID-19 severity. Moreover, HLA alleles may modulate inflammation and hepatocellular damage. We can consider an indirect contribution of HLA genes to acute COVID-19 severity and COVID-19-related consequences such as liver fibrosis that should be tested in the future. In the long-term perspective, patients with the *HLA-DRB1*12* and *HLA-DQB1*06:01* alleles in the genotype may be at risk for liver-related consequences of COVID-19.

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Availability of data and materials

The datasets generated and analyzed during the current study are available in the DataVerse repository, https://doi.org/10.48510/FK2/ATVBQE.

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