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К ВОПРОСУ О РОЛИ ТУЧНЫХ КЛЕТОК И ИХ ПРОТЕАЗ В ТЯЖЕЛОМ ТЕЧЕНИИ НОВОЙ КОРОНАВИРУСНОЙ ИНФЕКЦИИ COVID-19

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On the Role of Mast Cells and Their Proteases in the Severe COVID-19

Резюме

В период пандемии новой коронавирусной инфекции COVID-19 в процессе исследования патогенеза и поиска методов лечения возник вопрос о роли тучных клеток и их протеаз в течении данного заболевания. **Цель** данной работы — определение значения тучных клеток и их протеаз (химазы и триптазы) в патогенезе COVID-19 тяжелого течения. **Материалы и методы.** В исследование включены 55 пациентов: 29 мужчин (52,7 %) и 26 женщин (47,3 %) в возрасте 67 [62;71] лет с установленным диагнозом новой коронавирусной инфекции COVID-19 тяжелого течения с летальным исходом. Проводился анализ микропрепаратов аутопсийного материала легких пациентов с COVID-19 с определением представительства тучных клеток и анализом протеазного профиля и дегрануляционной активности. Проведен корреляционный анализ между показателями тучных клеток и клинико-лабораторными данными пациентов. **Результаты.** Обнаружено увеличение количества тучных клеток и их дегрануляционной активности у пациентов с хронической сердечной недостаточностью, ожирением, хронической болезнью почек, ишемической болезнью сердца и острым нарушением мозгового кровообращения. Отмечено истощение процессов дегрануляции триптаза-позитивных тучных клеток по мере увеличения продолжительности заболевания: содержание одиночных триптаза-позитивных тучных клеток (в %) отрицательно коррелирует с продолжительностью заболевания и госпитализации (p=0,015, r=-0,327 и p=0,006, r=-0,368, соответственно), содержание фрагментов триптаза-позитивных тучных клеток (в %) положительно коррелирует с продолжительностью госпитализации (p=0,007, r=0,357). Установлены положительные взаимосвязи уровней свободного билирубина и аланинаминотрансферазы с содержанием одиночных

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триптаза-позитивных тучных клеток (на мм²) (r=0,340, p<0,05 и r=0,307, p<0,05, соответственно), а также одиночных дегранулирующих триптаза-позитивных тучных клеток (на мм²) (r=0,369, p<0,05 и r=0,363, p<0,01, соответственно), а уровня связанного билирубина с содержанием одиночных триптаза-позитивных тучных клеток (%) (r=0,415, p<0,05). Уровень кальция сыворотки крови коррелирует с абсолютным общим содержанием одиночных триптаза-позитивных тучных клеток (p=0,013, r=0,457), а также — дегранулирующих (p=0,017, r=0,441). Также обнаружена отрицательная корреляция уровня калия с относительным содержанием одиночных триптаза-позитивных тучных клеток без признаков дегрануляции (p=0,014, r=-0,352). Обнаружены положительные связи уровня общего билирубина на момент поступления и в динамике с содержанием одиночных дегранулирующих химаза-позитивных тучных клеток (на мм²) (p=0,043, r=0,277 и p=0,027, r=0,317, соответственно). Показатели мочевины при поступлении положительно коррелируют с абсолютным общим содержанием одиночных химаза-позитивных тучных клеток (p=0,045, r=0,277), а также отдельно с признаками дегрануляции (p=0,04, r=0,283). Содержание натрия в крови коррелирует с общим содержанием совместно прилежащих химаза-позитивных тучных клеток (p <0,05, r=0,388), а также с содержанием совместно прилежащих химаза-позитивных тучных клеток с признаками дегрануляции (p <0,05, r=0,388). **Заключение.** Отмечаются значимые взаимосвязи между показателями тучных клеток и продолжительностями заболевания и госпитализации, наличием сопутствующих заболеваний, уровнями свободного и связанного билирубина, АЛТ, мочевины, общего белка, натрия, калия, кальция крови. Обнаружено увеличение количества тучных клеток и их дегрануляционной активности у пациентов с коморбидностью: хронической сердечной недостаточностью, ожирением, хронической болезнью почек, ишемической болезнью сердца и перенесенным в прошлом острым нарушением мозгового кровообращения. Выявлено истощение процессов дегрануляции триптаза-позитивных тучных клеток по мере увеличения продолжительности заболевания. Наблюдается участие химазы и триптазы тучных клеток в развитии поражения печени и почек у пациентов с COVID-19, что подтверждает их значение в тяжелом течении заболевания и в перспективе может рассматриваться для разработки патогенетической терапии.

Ключевые слова: *тучные клетки, COVID-19, новая коронавирусная инфекция, химаза, триптаза*

Конфликт интересов

Авторы заявляют, что данная работа, её тема, предмет и содержание не затрагивают конкурирующих интересов

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Abstract

During the pandemic of the new coronavirus infection COVID-19 the question about the importance of mast cells and their proteases arose. **The aim** of this study is to determine the role of mast cells and their proteases chymase and tryptase in the pathogenesis of severe COVID-19. **Materials and methods.** The study included 55 patients: 29 male (52,7 %) and 26 female (47,3 %) aged 67 [62;71] years with severe COVID-19 and fatal outcome. An analysis of postmortem lung biopsies of patients with COVID-19 was carried out, determining the representation of mast cells, protease profile and degranulation activity. A correlation analysis was carried out between mast cell and clinical and laboratory parameters of patients. **Results.** Increased number of mast cells and their degranulation activity were found in patients with chronic heart failure, obesity, chronic kidney disease, coronary heart disease and acute cerebrovascular accident. Degranulation of tryptase-positive mast cells are depleted as the duration of the disease increases: the content of single tryptase-positive mast cells (%) negatively correlates with the duration of the disease and hospitalization (p=0,015, r=-0,327 and p=0,006, r= -0,368, respectively), the content of tryptase-positive mast cells fragments (%)correlates with the duration of hospitalization (p=0,007, r=0,357). Correlations were established between the levels of non-conjugated bilirubin and alanine aminotransferase with the content of single tryptase-positive mast cells (per mm2) (r=0,340, p <0,05 and r=0,307, p <0,05, respectively), as well as single degranulated tryptase-positive mast cells (per mm2) (r=0,369, p <0,05 and r=0,363, p <0,01, respectively), and the level of conjugated bilirubin with the content of single tryptase-positive mast cells (%) (r= 0,415, p <0,05). The blood calcium level correlates with the absolute total content of single tryptase-positive mast cells (p=0,013, r=0,457), as well as degranulated (p=0,017, r=0,441). A negative correlation was also found between potassium level and the relative content of single non-degranulated tryptase-positive mast cells (p=0,014, r=-0,352). Correlations were found between the level of total bilirubin at the time of admission and over time with the content of single degranulated chymase-positive mast cells (per mm²) (p=0,043, r=0,277 and p=0,027, r=0,317, respectively). Urea level upon admission positively correlates with the absolute total content of single chymase-positive mast cells (p=0,045, r=0,277), as well as degranulated (p=0,04, r=0,283). The potassium level in the blood correlates with the total content of co-adjacent chymase-positive mast cells (p <0,05, r=0,388), as well as content of co-adjacent degranulated chymase-positive mast cells (p <0,05, r=0,388). **Conclusion.** Significant correlations were noted between mast cells parameters and duration of the disease and hospitalization, the presence of comorbidities, unconjugated and conjugated bilirubin, ALT, urea, total protein, sodium, potassium and calcium blood levels. An increase in the number of mast cells and their degranulation activity has been found in patients with comorbidities: chronic heart failure, obesity, chronic kidney disease, ischemic heart disease and previous stroke. The revealed depletion of degranulation processes of tryptase-positive mast cells as the duration of the disease increases indicates their role in lung damage. We noted participation of mast cells and their proteases chymase and tryptase in the development of liver and kidney damage in patients with COVID-19, which confirms their importance in the severe course of the disease and may be considered in the future for the development of pathogenetic therapy.

Key words: *mast cells, COVID-19, new coronavirus infection, chymase, tryptase*

Conflict of interests

The authors declare no conflict of interests

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ALT — alanine aminotransferase, AST — aspartate aminotransferase, NCVI — novel coronavirus infection, AKI — acute kidney injury, MC — mast cells, TNF-α — tumour necrosis factor alpha, CKD — chronic kidney disease, CHF — chronic heart failure, COVID-19 — COronaVIrus Disease 2019.

Introduction

Late in 2019, an outbreak of the novel coronavirus infection (NCVI) COVID-19 (COronaVIrus Disease 2019) was recorded in the People's Republic of China, which spread and gave rise to a pandemic. The search for relevant methods for diagnosis, progression forecasting and therapy for COVID-19 led to numerous cytology and histology examinations both of intravital and autopsy materials of patients. Mast cells (MC) are of interest for researchers due to their versatile participation in pathogenesis of NCVI [1,2].

MCs are myeloid immune cells, which regulate the function of other immune cells, attract them to an area of inflammation, secreting chemokines, and synthesising numerous cytokines and proteases. MCs participate in development of allergic reactions, infections and inflammations, pathogenesis of atherosclerosis and myocardial infarction, bronchial asthma and chronic obstructive pulmonary disease, obesity and gastrointestinal disturbances, a lot of malignancies, etc. [3,4]. Activated MCs express over 1,000 mediators, including heparin, histamine, serotonin, chondroitin sulfate A and C, proteases (chymase, tryptase and carboxypeptidase A3), interleukin-6, interleukin-1-β, interleukin-31, interleukin-33, tumour necrosis factor alpha (TNF-α), prostaglandins D2 and E2, leukotrienes B4 and C4, etc., a number of which are associated with inflammation and cytokine storm observed in COVID-19 [1].

The main symptom of COVID-19 is known to be pulmonary involvement, represented by acute respiratory distress syndrome (ARDS) [5]. According to various sources, acute hepatic damage is observed in 10–65 % cases and is caused both by direct cytopathic action of the virus on hepatic cells and cytokine storm-mediated damage [6]. Besides, there is evidence of kidney involvement in 25–50 % of COVID-19 cases; 15 % are associated with acute kidney injury (AKI), with disputable information on the pathogenic role of mast cells and their proteases [7,8].

The purpose of this paper is to identify the role of mast cells and their proteases — chymase and tryptase — in the pathogenesis of severe COVID-19 by assessing the degranulatory activity of MCs in pulmonary autopsy materials of patients, depending on clinical and laboratory characteristics of patients.

Materials and Methods

The study included 55 patients: 29 men (52.7 %) and 26 women (47.3 %) aged 67 [62;71] years old with confirmed severe COVID-19, community-acquired bilateral multisegmental pneumonia, acute respiratory distress syndrome, who were treated in COVID-19 units at the Budgetary Healthcare Institution of the Voronezh Region Voronezh City Clinical Emergency Care Hospital No. 1 and Budgetary Healthcare Institution of the Voronezh Region Voronezh Regional Clinical Hospital No. 1 over the period from September 2021 to June 2022, but died.

Disease duration was 15 [12; 22.5] days; hospitalisation lasted for 9 [5; 14.5] days. The patients had a history of concomitant diseases presented in Table 1. We performed a biochemistry assay of blood taken upon admission and just before death (indirect and direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, creatinine, urea, total protein, blood electrolytes (K, Na, Ca)).

The study did not include patients with chronic pulmonary diseases (bronchial asthma; chronic obstructive pulmonary disease); chronic bronchitis, occupational pulmonary diseases); other pulmonary infections (other than COVID-19) (pneumonia of other origin, TB, etc.); pulmonary embolism; cancer, including oncohaematological conditions; hepatitis; hepatic cirrhosis; chronic heart failure (CHF) of at least stage IIA according to the Vasilenko — Strazhesko classification; venostasis in the lesser circulation; hydrothorax, type 1 and type 2 diabetes mellitus; a history of smoking; chronic kidney disease (CKD) (prior to NCVI), with GFR below 60 mL/min/1.73 m^2; and developed acute kidney injury.

The study was approved by the Ethics Committee at the N. N. Burdenko Voronezh State Medical University of the Ministry of Health of Russia (Minutes No. 8 dated November 17, 2021).

After the patients died, autopsy materials (a representative area of lung parenchyma) were collected within 24 hours in pathoanatomical units of Voronezh City Clinical Emergency Care Hospital No. 1 and Voronezh Regional Clinical Hospital No. 1. Autopsy materials were preserved with 10 % neutral buffered formalin and embedded in paraffin; later, 5 μm sections were prepared for H&E and Giemsa staining, and ultrathin 2 μm sections were prepared for immunohistochemistry assay. Immunohistochemistry staining was performed under a standard protocol to identify MC tryptase and chymase. Proteases were identified using primary murine Anti-Mast Cell Tryptase antibody (clone AA1, #ab2378, diluted to 1 : 4,000) and Anti-Mast Cell Chymase antibody (#ab233103, diluted to 1 : 1,000). Secondary antibodies were goat anti-rabbit antibodies #AS-R1-HRP, which were visualised with ImmPACTTM DAB Peroxidase Substrat Kit (#SK-4105) under the protocol stated in the instructions.

Table 1. *Baseline clinical characteristics of COVID-19 patients in the study*

Clinical characteristic	Number
Duration of disease, days	15 [12; 22,5]
Duration of hospitalization, days	9 [5; 14,5]
Time to hospitalization, days	7 [4,5; 10]
Comorbidities:	
Arterial hypertension, n (%)	45 (82)
Coronary artery disease, n (%)	6 (11)
Acute cerebrovascular accident, acute period, n (%)	6 (11)
Previous acute cerebrovascular accident n (%)	4 (7)
Congestive heart failure, n (%)	15 (27)
Stage I*	6 (11)
Stage IIA*	9 (16)
Obesity, total, n (%)	14 (25)
Class I	11 (20)
Class II	1 (2)
Class III	2 (4)
Chronic tubulointerstitial nephritis, n (%)	8 (15)
Chronic glomerulonephritis, n (%)	3 (5)
Chronic kidney disease, n (%) (C1-C2 stages)	11 (20)
Treatment:	
Anticoagulant therapy, n patients (%)	55 (100 %)
Glucocorticosteroids, n patients (%)	53 (96 %)
Favipiravir, n patients (%)	27 (49 %)
IL-6 Inhibitors, n patients (%)	16 (29 %)
Janus Kinase Inhibitors, n patients (%)	2 (4 %)
Monoclonal antibody against IL-6, n patients (%)	2 (4 %)
Convalescent plasma, n patients (%)	2 (4 %)

Note: * according to the classification of N. D. Strazhesko and V. Kh. Vasilenko

MC activation was assessed based on the quantity of tryptase- and chymase-positive mast cells; their degranulation parameters were assessed as well.

Microsections were analysed at the Scientific Research Institute of Experimental Biology and Medicine of N. N. Burdenko Voronezh State Medical University using ZEISS Axio Imager.A2 microscope; images were processed in ZEN 2.3 (Carl Zeiss, Germany). MCs were counted with ×40 zooming and analysed at least at 50 HPF. An analysis of microsections included total MC count, with a distribution depending on the presence of degranulation, as well as quantification of protease profile (tryptase, chymase) per mm^2 and as % of the total amount of mast cells.

Results were statistically processed using Jamovi, version 1.6.23 (Australia). Normality of data distribution was assessed using normalised coefficients of excess and asymmetry, as well as the Shapiro — Wilk test. For correlation analysis, Spearman's rank correlation was used. Correlation relationships were significant at $p < 0.05$. The bonding force at $r = 0.01–0.29$ was weak, at $r = 0.3–0.69$ — moderate, and at $r = 0.7–1.0$ — strong.

Results

We analysed the relationships between MC parameters, medical history, clinical and laboratory results.

MC and concomitant diseases.

The relationships found for MC and concomitant diseased are presented in Tables 2 and 3.

Disease duration and hospitalisation duration.

A relative number of individual tryptase-positive mast cells negatively correlates with disease duration and hospitalisation duration ($p = 0.015$, $r = -0.327$ and $p = 0.006$, $r = -0.368$, respectively). A relative level of tryptase-positive mast cells positively correlates with hospitalisation duration ($p = 0.007$, $r = 0.357$). A relative number of adjacent tryptase-positive mast cells without signs of degranulation positively correlates with disease duration and hospitalisation duration ($p = 0.02$, $r = 0.312$ and $p = 0.016$, $r = 0.324$, respectively). There were no statistically significant relations between parameters of chymase-positive mast cells and duration of disease and hospitalisation.

MC and blood biochemistry parameters.

Statistically significant relations were found between parameters of tryptase-positive mast cells and some blood biochemistry parameters (see Tables 4 and 5). No statistically significant relations were found between MC parameters and AST. An increase in ALT in the

Table 2. *Results of correlation analysis of the presence of concomitant diseases and tryptase-positive mast cells in lung autopsy material per mm²*

Indicators	Single tryptase+MCs			Co-adjacent tryptase + MCs			Tryptase + MCs fragments	Total Tryptase + MCs
	Without degranulation	With degranulation	Total	Without degranulation	With degranulation	Total		
AH	-0,0650	0,1637	0,1068	-0,0603	-0,0269	-0,0479	-0,0325	0,0937
IHD	-0,0899	0,0283	-0,0106	-0,1775	-0,0984	-0,1595	-0,2594	-0,0390
Stroke Acute period	0,1172	0,0616	0,0925	0,0796	-0,0486	0,0173	0,0804	0,0932
Previous stroke (outside the acute period)	-0,0404	0,0221	0,0028	-0,0402	0,0672	0,0130	-0,0817	-0,0024
CHF (I, IIA)**	0,1968	**0,3355***	**0,3406***	-0,0775	0,0233	-0,0346	0,0412	**0,3180***
Obesity	**0,3674***	**0,2867***	**0,3646***	0,1595	-0,0265	0,0754	0,2507	**0,3627***
CKD (C2 and C2)	0,1489	**0,4524***	**0,4163***	-0,0115	0,1636	0,0840	0,2020	**0,4077***

Note: The table shows the Spearman correlation coefficient.
*p <0,05; ** according to the classification of N. D. Strazhesko and V. Kh. Vasilenko
Legends: MCs — mast cells, AH — arterial hypertension, IHD — ischemic heart disease, CHF — chronic heart failure, CKD — chronic kidney disease

Table 3. *Results of correlation analysis of the presence of concomitant diseases and indicators of chymase-positive MCs in lung autopsy material per mm²*

Indicators	Single chymase+ MCs			Co-adjacent chymase + MCs			Chymase + MCs fragments	Total chymase + MCs
	Without degranulation	With degranulation	Total	Without degranulation	With degranulation	Total		
AH	-0,0540	0,0082	-0,0072	-0,0073	0,0641	0,0641	0,0641	-0,0030
IHD	0,1567	**0,5009***	**0,5001***	0,0513	-0,0381	-0,0381	-0,0381	**0,4983***
Stroke Acute period	0,0925	0,0063	0,0311	0,0365	-0,0476	-0,0476	-0,0476	0,0280
Previous stroke (outside the acute period)	0,0321	0,1304	0,1279	-0,1405	**0,4859***	**0,4859***	-0,0381	0,1428
CHF (I, IIA)**	0,2168	**0,3946***	**0,4195***	0,0231	-0,0784	-0,0784	-0,0784	**0,4149***
Obesity	0,1973	0,1031	0,1481	0,0678	-0,0678	-0,0678	0,1284	0,1503
CKD (C2 and C2)	-0,0480	0,0091	-0,0047	0,0634	-0,0301	-0,0301	-0,0301	-0,0067

Note: The table shows the Spearman correlation coefficient.
*p <0,05; ** according to the classification of N. D. Strazhesko and V. Kh. Vasilenko
Legends: MCs — mast cells, AH — arterial hypertension, IHD — ischemic heart disease, CHF — chronic heart failure, CKD — chronic kidney disease

study group did not exceed x1.5 ULN and that of AST — x2 ULN.

Positive relations were found between individual degranulatory chymase-positive mast cells per mm² and the level of total blood bilirubin upon admission and, over time, in the last intravital sample ($p = 0.043$, $r = 0.277$ and $p = 0.027$, $r = 0.317$, respectively).

Urea levels upon admission positively correlate with the absolute total number of individual chymase-positive mast cells ($p = 0.045$, $r = 0.277$), and with signs of degranulation ($p = 0.04$, $r = 0.283$).

For total bilirubin, indirect bilirubin (before death), direct bilirubin, ALT (before death), creatinine, glucose (before death), no statistically significant correlations were found with parameters of tryptase-positive mast cells per mm².

For total bilirubin, direct bilirubin (before death), ALT, creatinine (before death), glucose, no statistically significant correlations were found with parameters of tryptase-positive mast cells (in %).

There are positive correlations between total blood protein levels and MC parameters. The total blood protein levels positively correlate with the absolute total count of tryptase-positive mast cells ($p = 0.01$, $r = 0.353$), individual tryptase-positive mast cells ($p = 0.013$, $r = 0.340$) and individual degranulatory tryptase-positive mast cells ($p = 0.004$, $r = 0.349$).

Blood sodium levels positively correlate with total adjacent chymase-positive mast cells ($p < 0.05$, $r = 0.388$) and the number of adjacent chymase-positive mast cells with signs of degranulation ($p < 0.05$, $r = 0.388$).

Blood calcium levels positively correlate with the absolute count of individual tryptase-positive mast cells with signs of degranulation ($p = 0.017$, $r = 0.441$) and the absolute count of individual tryptase-positive mast cells ($p = 0.013$, $r = 0.457$). Blood potassium levels negatively correlate with the relative number of individual tryptase-positive mast cells without signs of degranulation ($p = 0.014$, $r = -0.352$).

The variety of relations justifies a multifactor analysis. Later in the study, a multiple regression equation will be generated with due account of the most significant parameters, and the resulting model will be presented.

Table 4. Results of correlation analysis of biochemical blood tests and tryptase-positive MCs (per mm2) in lung autopsy material

Indicators	Single tryptase + MCs without degranulation, mm2	Single tryptase + MCs with degranulation, mm2	Single tryptase+ MCs Total, mm2	Co-adjacent tryptase + MCs without degranulation, mm2	Co-adjacent tryptase + MCs with degranulation, mm2	Co-adjacent tryptase + MCs total, mm2	Tryptase + MCs fragments, mm2	Total amount of tryptase + MCs, mm2
Unconjugated bilirubin bilirubin, μmol/L, No. 1	0,219	**0,369***	**0,340***	-0,233	-0,268	-0,296	-0,161	0,299
Alanine aminotransferase, Ed/l, No1	0,103	**0,363****	**0,307***	-0,007	0,070	0,032	-0,042	**0,284***
Urea, mmol/l, No. 1	0,020	**0,336***	0,255	-0,052	**0,290***	0,121	0,090	0,252
Urea, mmol/l, No. 2	0,129	**0,414****	**0,359***	0,067	0,033	0,057	0,014	**0,334***
Glucose, mmol/l, No. 1	0,084	0,056	0,074	**0,288***	0,235	**0,292***	0,165	0,102

Note: The table shows the Spearman correlation coefficient.
No. 1 — blood test taken upon admission No. 2 — patients' last blood test

Table 5. Results of correlation analysis of biochemical blood tests and tryptase-positive MCs (%) in lung autopsy material.

Indicators	Single tryptase + MCs without degranulation, %	Single tryptase + MCs with degranulation, %	Single tryptase + MCs total, %	Co-adjacent tryptase + MCs without degranulation, %	Co-adjacent tryptase + MCs with degranulation, %	Co-adjacent tryptase+ MCs total, %	Tryptase + MCs fragments,%
Unconjugated bilirubin, μmol/L, No. 1	-0,055	0,242	**0,381***	-0,209	-0,195	-0,265	**-0,387***
Unconjugated bilirubin, μmol/L, No. 2	-0,014	0,082	0,178	0,019	0,156	0,105	**-0,379***
Conjugated bilirubin, μmol/L, No. 2	0,027	0,136	**0,415***	-0,119	-0,060	-0,131	**-0,382***
Creatinine, μmol/L, No1	**-0,293***	0,189	-0,208	-0,017	**0,306***	0,170	0,193
Urea, mmol/l, No. 1	**-0,317***	0,268	-0,068	-0,093	0,233	0,076	0,043

Note: The table shows the Spearman correlation coefficient. No. 1 — blood test taken upon admission No. 2 — patients' last blood test

Discussion

We found an increase in the number of MCs and their degranulatory activity in patients with CHF, obesity, CKD, IHD and a history of an acute cerebrovascular event. The affinity of the identified positive correlations of adjacent chymase-positive mast cells, as well as individual chymase-positive mast cells with signs of degranulation, with IHD in patients, as well as the number of adjacent chymase-positive mast cells as a whole and with signs of degranulation and a history of ACE can be a result of participation of this protease in development of atherosclerosis. Mast cells are known to take part in metabolism of low-density lipoprotein (LDLP) by stimulating their phagocytosis by macrophages. Activated MCs can metabolise high-density lipoprotein (HDLP) by inducing degradation of HDLP apolipoproteins. When MCs destroy HDLP in vessel intima, macrophages are unable to escape from cholesterol. Thus, MCs can participate in formation of atherosclerosis plaques in vessels [3]. Also, mast cells take part in inflammation processes via cytokines and chemokines; they induce vessel wall infiltration by T cells and macrophages, stimulate smooth muscle cell migration from the middle layer to intima via growth factor synthesis, and platelet growth factor facilitates microthrombosis [3]. Numerous authors emphasise the role of mast cells in the development of IHD, including myocardial infarction [9]. Taking into account that IHD is one of the major causes of CHF, the mentioned mechanisms can explain correlations between MCs and CHF.

Mast cells stimulate expression of inflammatory cytokines by Th1 cells, which activate adipocytes and produce proteases for stimulation of angiogenesis and adipogenesis in fat tissue [5]. During an experiment, scientists found out an increased number of MCs (degranulated MCs prevailed) in thymus gland tissue of obese rats; they explained this fact with an increased proinflammatory activity in obesity and assumed that excessive fat consumption with food causes a specific adaptive reaction of the body, increased activity of phospholipase to boost lipid degradation, in particular cell membrane phospholipids, thus enhancing degranulation process [10].

Taking into account the resulting correlations between MC parameters and duration of disease and

hospitalisation in this paper, it is safe to assume that degranulation of tryptase-positive mast cells weakens with disease duration, given reduction in the total number of individual tryptase-positive mast cells with longer duration of disease and hospitalisation, an increase in MCs without signs of degranulation (probably due to reduction in percentage of degranulatory MCs) and fragments of tryptase-positive mast cells.

We found positive correlations between indirect bilirubin and ALT levels and the number of tryptase-positive mast cells and their degranulatory activity. Besides, the relative number of individual tryptase-positive mast cells positively correlates with direct bilirubin levels. The absolute count of individual chymase-positive mast cells also changes with changes in total bilirubin levels. In their early hamster experiments with the use of chymase inhibitor, Masubuchi S. et al. (2013) observed reduction in sinusoidal obstruction syndrome, manifesting, among other things, as an increased total bilirubin and ALT levels [11].

The presence of type 2 angiotensine-converting enzyme (ACE2) receptors is known to mediate direct hepatic damage in COVID-19 [6]. Extrapulmonary SARS-CoV-2 was found in liver, it being associated with higher ACE2 expression mostly in cholangiocytes as compared to hepatic cells. We did not assess correlations between MC parameters and levels of gamma glutamyltransferase and alkaline phosphatase; therefore, it is not possible to assess the intensity of cholestasis syndrome. There was no disturbed albumin synthetic ability of the liver observed. Some medicines used in NCVI can be toxic for the liver. Some authors suggest that the main mechanism of liver involvement is systematic release of cytokines, which is proven by the relationship between liver involvement and hypolymphemia and CRP levels. Patients with significantly higher ALT levels often have high CRP, D dimer, ferritin, and IL-6 values [12]. Since we have identified the relationship between MC parameters in lungs and impaired liver function, then most likely this is about the mechanism, which is mediated by systemic impact of proinflammatory cytokines.

We have found positive correlations between total count of tryptase-positive mast cells, as well as individual tryptase-positive mast cells with signs of degranulation, and CKD. There is a unidirectional relationship between creatinine and urea levels and degranulatory activity of chymase-positive mast cells. There is some evidence that kidney disorders are caused by immune disorders, which activate cytokine storm reactions [7]. We have observed significantly higher levels of interleukins 6 and 8, and TNF-α, which are synthesised also by MCs, in patients with CKD vs. controls [7]. Interleukin 8 is known to mediate kidney injury by increasing glomerule permeability and causing proteinuria. Interleukin 6 stimulates mesangial cell proliferation and facilitates glomerulopathy progression. Besides, TNF-α can contribute to glomerule damage [13].

Kidney injury in COVID-19 is caused by a number of mechanisms: epithelial infection of renal tubules and podocytes via ATE2 receptors, kidney injury by proinflammatory cytokines in cytokine storm, artificial lung ventilation (ALV), ischemia due to SARS-Cov-2-induced septic shock, hypoperfusion and high angiotensin II levels, microthrombosis and some other mechanisms [14].

The results of this study regarding chymase-positive MC activity contradict a study by Madjene L.C. et al. (2020), which reports a potent antiinflammatory function of murine MC protease 4 (which is functionally similar to human MC hymase) in ischemic kidney injury, which is the main cause of AKI [8].

This study shows that the blood calcium level positively correlates with the count of tryptase-positive mast cells and their degranulatory activity. These results correlate with available data on the role of calcium as MC degranulation activator, which has been proven experimentally [15]. Established correlations between blood sodium levels and degranulatory activity and intercellular connectivity of chymase-positive mast cells, as well as negative correlations between potassium levels and the relative count of individual tryptase-positive mast cells without signs of degranulation, are still hard-to-explain and require further studies.

Conclusion

We have observed significant relationships between mast cell parameters, clinical-and-laboratory results and medical history of patients: duration of disease and hospitalisation, concomitant diseases, as well as some blood biochemistry parameters (indirect and direct bilirubin, ALT, urea, total protein, sodium, potassium, calcium). We have recorded an increase in the number of MCs and their degranulatory activity in patients with CHF, obesity, CKD, IHD and a history of ACE, which can be an evidence of a higher risk of cytokine storm in patients with the mentioned concomitant diseases.

Established weakening of tryptase-positive mast cell degranulation processes along with an increase in disease duration evidences their significance in pulmonary tissue damage. Besides, mast cells, namely their proteases chymase and tryptase, contribute to the development of liver and kidney injury in patients with COVID-19, thus proving their role in severe COVID-19.

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