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ОСОБЕННОСТИ СОСТОЯНИЯ СИСТЕМЫ КОЛЛАГЕНОЛИЗА И ФАКТОРЫ РИСКА ЕЕ ИЗМЕНЕНИЙ У ПАЦИЕНТОВ ПРИ ДЛИТЕЛЬНОМ ПОСТКОВИДНОМ СИНДРОМЕ

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Features of the State of the Collagenolysis System and risk factors of Its Changes in Patients with Long-Term Post-COVID Syndrome

Резюме

Цель: оценить состояние системы коллагенолиза у пациентов при длительном постковидном синдроме, определить особенности и факторы риска ее изменений. **Материалы и методы исследования.** В исследование было включено 178 пациентов (мужчин — 59, женщин — 119; возраст 57,15±12,4 лет) после перенесенной новой коронавирусной инфекции (НКВИ) 12 и более недель назад. В зависимости от наличия или отсутствия симптомов длительного постковидного синдрома пациенты, перенесшие НКВИ 12 и более недель назад, были разделены на 2 группы: первую группу составили 88 пациентов с симптомами «Long-covid»; вторую группу — 90 обследуемых без каких-либо симптомов «Long-covid». **Результаты.** Средний период после перенесенной НКВИ составил 8,5[3,6;12,4] месяцев. У всех пациентов, перенесших НКВИ, тканевый ингибитор матриксных металлопротеиназ 1 типа (TIMP1) был выше референсного значения (135 пг/мл). У пациентов первой группы TIMP1 был ниже, чем во второй группе: 315,5 [145,0;410,0] пг/мл против 513,5 [415,0; 865,0] пг/мл (p <0,001). Следовательно, при длительном постковидном синдроме развивается коллагенолитический паттерн на фоне увеличения риска формирования фиброза. **Заключение.** У пациентов, перенесших НКВИ, с длительным постковидным синдромом состояние системы коллагенолиза характеризуется развитием коллагенолитического паттерна на фоне преобладающих процессов коллагенообразования в сравнении с бессимптомными пациентами, перенесшими НКВИ 12 недель назад и более, который может быть рассмотрен как патогенетический механизм формирования «Long Covid».

Ключевые слова: *система коллагенолиза, длительный постковидный синдром*

Конфликт интересов

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

Источники финансирования

Авторы заявляют об отсутствии финансирования при проведении исследования

Соответствие принципам этики

До начала исследования было получено одобрение Этического комитета на его проведение, а также на формы информированного согласия больного, которые до включения в исследование были подписаны всеми больными.

Протокол исследования был одобрен локальным этическим комитетом «Пермского краевого клинического госпиталя для ветеранов войн» (№ 137 от 21.04.2020)

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Abstract

Objective: to assess the state of the collagenolysis system in patients with long-term post-COVID syndrome, to determine the features and risk factors of its changes. **Materials and methods.** The study included 178 patients who had had a new coronavirus infection (NCVI) 12 weeks or more ago, depending on the presence or absence of symptoms of long-term post-COVID syndrome, patients who had NCVI 12 or more weeks ago were divided into 2 groups: the first group consisted of 88 patients with Long-covid symptoms; the second group consisted of 90 subjects without any symptoms of Long-covid. **Results.** The median period after NCVI was 8.5 [3.6; 12.4] months. In all patients who underwent NCVI, tissue inhibitor of matrix metalloproteinase type 1 (TIMP1) was higher than the reference value (135 pg/ml). In the patients of the first group, TIMP1 was lower than in the second group: 315.5 [145.0; 620.0] pg/mL vs. 513.5 [220.0; 865.0] pg/mL ($p<0.001$). Therefore, in long-term post-COVID syndrome, a collagenolytic pattern develops against the background of an increased risk of fibrosis. **Conclusion.** In patients who have undergone NCVI with a long-term post-COVID syndrome, the state of the collagenolysis system is characterized by the development of a collagenolytic pattern against the background of the prevailing processes of collagen formation in comparison with asymptomatic patients who had NCVI 12 weeks ago or more, which can be considered as a pathogenetic mechanism for the formation of "Long Covid".

Key words: *collagenolysis system, long-term post-COVID syndrome*

Conflict of interests

The authors declare no conflict of interests

Sources of funding

The authors declare no funding for this study

Conformity with the principles of ethics

The study protocol was approved by the local ethical committee of the «Perm Krai Clinical Hospital for War Veterans» (№ 137 dated 21.04.2020)

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TIMP1 — tissue inhibitor of matrix metalloprotease, type 1, ILAV — indexed left atrium volume, LVMMI — left ventricle myocardium mass index, LVMM — left ventricle myocardium mass, NCVI — novel coronavirus infection, MMP — matrix metalloproteinase, PCR test — polymerase chain reaction, GFR — glomerular filtration rate, CKD — chronic kidney disease, LV EF — left ventricle ejection fraction, LV — left ventricle, echoCG — echocardiography, AUC — Area Under Curve, ROC — Receiver Operating Characteristic, cSPAP — calculated value of systolic pulmonary arterial pressure, WC — waist circumference, OR — odds ratio, RR — relative risk, PWVcf — carotid-femoral pulse wave velocity, CAVI — cardio-ankle vascular index, TNF-α — tumour necrosis factor alpha

Introduction

The long-lasting post-COVID syndrome is a real challenge to the healthcare system [1]. According to various estimates, two (2) to 86% of patients suffer from the long-lasting post-COVID syndrome [2, 3]. There are more and more evidences of new risk factors and pathogenic mechanisms of the so-called long COVID.

The main pathophysiological hypotheses to explain persistent post-COVID-19 symptoms are direct viral toxicity, endothelial damage, dysregulated immune response, hyperinflammation, hypercoagulation and poor adaptation of angiotensin converting enzyme [4].

One of the most speculative areas pertaining to the pathological pathways of development of long-lasting post-COVID syndrome, is the state of the collagen disorganisation system and its changes, determined on the basis of the ratio between matrix metalloproteinases (MMPs) and their inhibitors. There are two contrary hypotheses: some researchers present evidences of profibrotic process progression, while others mention collagen disorganisation activation and an increase in MMPs [5, 6].

In this study, we attempted to demonstrate the collagen disorganisation status of post-NCVI patients, who have long-lasting post-COVID syndrome, and asymptomatic patients, using the concentration of an integrated index, i.e., tissue inhibitor of matrix metalloprotease, type 1.

Objective of the study is to evaluate the collagen disorganisation status of patients with post-COVID syndrome; to identify the features and risk factors of its changes.

Materials and Methods

Before commencement, the study was approved by the Ethics Committee, and all informed consent forms were signed by all patients.

An observational, cross-sectional clinical study was conducted for a period from 2021 to 2024 in outpatient settings. Out of 802 patients, who needed medical attention, 178 patients were enrolled in the study (59 males, 119 females; age: 57.15 ± 12.40 years old); they had NCVI at least 12 weeks before enrolment, met the inclusion criteria and did not have any of the non-inclusion criteria.

Inclusion criteria: outpatient patients aged 18+ years old, who had NCVI confirmed with polymerase chain reaction test (PCR test) and smear test for SARS-CoV-2.

Non-inclusion criteria were any acute infections; pneumonia; exacerbation of chronic bronchopulmonary diseases; myocardial infarction or unstable angina, pulmonary embolism, acute decompensated cardiac insufficiency within three months before the enrolment; congenital and acquired cardiac defects; severe renal conditions; chronic kidney disease (CKD), stage 4–5; haematologic and rheumatologic diseases; history of malignancies and active tumours; acute inflammation; cognitive disorders and mental conditions; refusal to sign the informed consent form.

Depending on the presence or absence of the symptoms of long-lasting post-COVID syndrome, patients, who had NCVI at least 12 weeks ago, were divided into two groups: the first group were 88 patients with the long COVID symptoms (30 males, 58 females; age: 56.39 ± 12.8 years old); the second group comprised 90 subjects without any long COVID symptoms (29 males, 61 females; age: 55.69 ± 10.97 years old).

The long-lasting post-COVID syndrome (long COVID) was verified in accordance with the recommendations of the World Health Organisation [7].

In addition to the routine clinical, laboratory and instrumental examinations, all patients underwent iron exchange assessment.

All patients underwent echocardiography (echoCG) using Vivid S5 device (General Electric, USA), and the following parameters were measured: Simpson's left ventricle ejection fraction (LV EF); the ratio of E mean (the highest velocity of early LV filling) to e' mean (early diastolic velocity of fibrous ring) based on tissue Doppler sonography; LV myocardial mass index (LVMMI) for individuals with the normal body weight (LVMM/body surface area) and obese individuals (LVMM/height^{2,7}); indexed left atrium volume (ILAV); calculated value of systolic pulmonary arterial pressure (cSPAP); tricuspid annular plane systolic excursion (TAPSE); right atrium arterial interference (RAAI) as TAPSE to cSPAP ratio.

All patients underwent 3D sphygmoplethysmography using VaSeraVS-1000 device (Fucuda Denshi, Japan), and the following parameters were measured: mean cardio-ankle vascular index (CAVI1); pulse wave velocity (PWV) in the carotid-femoral segment (PWVcf) (right and left); PWV in the shoulder-ankle segment (right and left) (R-PWV and L-PWV); B-PWV in the brachial artery (B-PWV); PWV in the aorta (PWVa); C-PWV in the carotid (C-PWV); augmentation index (R-AI).

In order to evaluate the calculated renal filtration function, blood creatinine and cystatin C levels were measured; glomerular filtration rate (CKD-EPIcre and CKD-EPIcys using an online calculator) and morning urine albumin/protein to creatinine ratio were calculated [8]. Serum cystatin C concentration was determined by ELISA using Expert Plus Microplate reader (Biochrom Ltd., UK) and Vector-Best reagent kit (Novosibirsk, Russia).

The inflammatory process was evaluated by measuring tumour necrosis factor alpha (TNF-α) and interleukin-1β by ELISA using Vector-Best reagent kit (Novosibirsk, Russia) and Lazurite reader (Dynex Technologies Inc., USA).

The collagen disorganisation status was assessed by measuring the concentration of the integrated index, i.e., tissue inhibitor of matrix metalloprotease, type 1 (TIMP1), by ELISA using SEA 552Hu kit from Cloud-Clone Corp. (USA/China) and Stat Fax 2100 photometer (Awareness Technology, USA).

STATISTICA 10.0 (v.10.0.1011) was used for statistical processing of results. For the quantitative attributes, the arithmetic mean value (M) ± standard deviation (SD) or the median value with the lower and upper quartile (Me [LQ; UQ]) were calculated, depending on their degree of normality of distribution. The type of distribution was analysed using Shapiro-Wilk and Kolmogorov-Smirnov tests. For the qualitative attributes, the absolute frequency and percentage (%) were calculated. For the comparison of data in both groups depending on their distribution, both parametric and non-parametric statistical methods were used: for quantitative attributes — Student t-test or Mann-Whitney test; for qualitative attributes — χ^2 test. The significance level of the zero statistic hypothesis was $p < 0.05$. In order to analyse the relationship between the quantitative attributes outside the normal distribution, Spearman's rank correlation was used; for qualitative

Table 1. *Characteristics of patients included in the study (n=178)*

Indicator		Significance
Sex (total included in the study, n — 178), n (%)	men	56/31,5
	female	122/68,5
Age (total included in the study, n — 178), ages, MS±D	men	55 [45,75;62,25]
	female	60 [48,0;66,75]
Sex (with the symptoms of «Long-covid», n — 88), n (%)	men	30/34,1
	female	58/65,9
Age (with the symptoms of «Long-covid», n — 88), ages, Me[LQ; UQ]	men	57 [46,25;65,5]
	female	60 [47,25;67,75]
Sex (without the symptoms of «Long-covid», n — 90), n (%)	men	26/32,2
	female	64/67,8
Age (without the symptoms of «Long-covid», n — 90), ages, Me[LQ; UQ]	men	54 [45,5;61,0]
	female	59 [48,0;66,0]
Comorbid pathologies:	GD + BA, abs./%	24/13,5
	GD + BA + type 2 DM, abs./%	9/5,1
	GD + BA + COPD, abs./%	8/4,5
Obesity:	I degree, abs./%	54/30,3
	II degree, abs./%	26/14,6
	III degree, abs./%	10/5,6
Smoking:	quantity, abs./%	40/22,5
	seniority, ages, Me[LQ; UQ]	12 [7;22,5]
Have been immunized against COVID-19, abs./%		22/12,4
	Therapies received by patients	
Beta-adrenoblockers, abs./%		61/34,3
Thiazides / thiazide-like/loop diuretics, abs./%		28/15,7
iACE, abs./%		26/14,6
ARBs, abs./%		52/29,2

Note. GD — hypertensive disease, BA — bronchial asthma, type 2 DM — type 2 diabetes mellitus, COPD — chronic obstructive pulmonary disease, iACE — angiotensin-converting enzyme inhibitors, ARBs — angiotensin receptor blockers

attributes, the mutual contingency coefficient developed by A. A. Chuprov was used [9]. The significance level of the zero statistic hypotheses in the evaluation of relationships was $p < 0.05$. In order to select a TIMP-1 value as a parameter representing the collagen disorganisation status, to identify the risk factors of its changes in patients with long post-COVID syndrome, the cutoff point was identified using the ROC curve for all values, with the calculation of the area under curve (AUC) of > 0.5 at $p < 0.05$ and operational characteristics of sensitivity and specificity. To determine odds ratios (OR), relative risk (RR) and 95% CI for OR and RR, 2×2 contingency tables were made; χ^2 value was calculated with determination of the achieved significance level with Yates' correction for continuity.

Results and Discussion

The mean period after the past NCVI was 8.5 [3.6;12.4] months. Evaluation of the integrated index TIMP1 demonstrated compromised collagen disorganisation status in all post-NCVI patients, manifesting mostly as its transformation towards collagen production with respective TIMP1 levels above the reference threshold (135 pg/mL). However, in group 1 patients with long-lasting post-COVID syndrome, TIMP1 exceeded the reference values, still it was lower than in group 2, i.e., asymptomatic patients: 315.5 [145.0;410.0] pg/mL vs. 513.5 [415.0; 865.0] pg/mL ($p < 0.001$). The data show that patients with long-lasting COVID-syndrome develop a collagenolytic pattern caused by an increased risk of fibrosis.

In order to further analyse fibrotic processes in patients with long post-COVID syndrome, a decision was made to find a cut-off point for all TIMP1 values by plotting a ROC curve and to perform classification depending on the presence of long-lasting post-COVID syndrome (Fig. 1).

For TIMP1 values in all post-NCVI patients, the obtained cut-off point was between ≤ 410 pg/mL and > 135 pg/mL, with sensitivity of 78.3% and specificity of 73.7% (AUC=0.786, p=0.001). In group 1, 57 (64.8%) patients had TIPM1 value between ≤ 410 pg/mL and > 135 pg/mL, in group 2 — 18 (20.0%) patients (p < 0.001). Thus, if TIPM1 is between ≤ 410 pg/mL and > 135 pg/mL, OR of long-lasting post-COVID syndrome increases 7.4 times (OR=7.355, 95% CI=3.555;15.382), RR — 3.2 times (RR=3.234, 95% CI=2.093;5.154).

In order to identify the risk factors of collagenolytic pattern development due to prevailing fibrotic processes in patients with long-lasting post-COVID syndrome, group 1 patients were divided into two subgroups: subgroup 1 included 57 patients with 135 < TIMP1 ≤ 410 pg/mL, subgroup 2 comprised 31 patients with TIMP1 > 410 pg/mL.

The clinical and anamnestic characteristic of patient subgroups with long-lasting post-COVID syndrome is presented in Table 2.

The correlation analysis did not reveal any correlation between waist circumference (WC) and body mass index (BMI) with TIMP1 concentrations.

The course of NCVI and long-lasting post-COVID syndrome in subgroups of patients is presented in Table 3.

The correlation analysis demonstrated medium inverse correlation for long COVID in any variants: weakness (r=–0.315, p=0.031), feeling generally unwell (r=–0.315, p=0.022), fatigue, impaired attention concentration and memory (r=–0.315, p=0.045), shortness of breath (r=–0.344, p=0.021), lower quality of life (r=–0.364, p=0.001), with TIMP1 concentrations (r=–0.298, p=0.005); and strong direct correlation between NCVI vaccination and TIMP1 concentration (r=0.538, p=0.038).

OR and RR values for long COVID were calculated for the following variants: presence of weakness, feeling generally unwell, fatigue, shortness of breath, lower quality of life with the development of the collagenolytic pattern due to prevailing fibrotic processes. It is reported that OR was 5.3 (OR=5.334, 95% CI=1.726; 17.216, p=0.035), RR — 2.9 (RR=2.901, (95% CI=1.379; 7.168 p=0.015). OR for long COVID in the variant of impaired attention concentration and memory is 6.3 (OR=6.314, 95% CI=1.562; 29.600, p=0.023), RR — 4.2 (RR=4.170, 95% CI=1.378; 16.960 p=0.025).

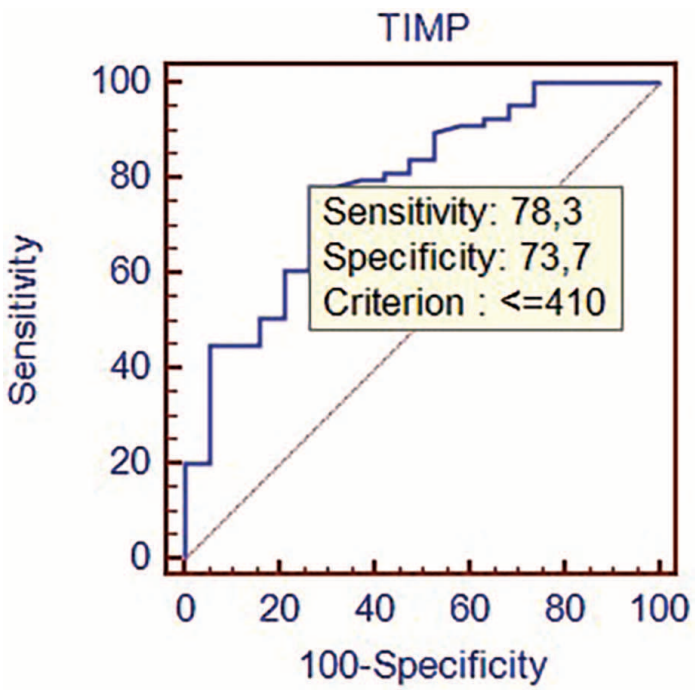

Figure 1. *ROC curve for 135< tissue inhibitor of matrix metalloproteinase type 1(TIMP1) ≤410 pg/mL in new coronavirus infection patients as an integral index reflecting the state of the collagenolysis system*

Table 2. *General clinical characteristics by subgroups of patients with long-term post-COVID syndrome (n=88)*

Indicator	First subgroup (135<TIMP1 ≤ 410 пг/мл, *n* = 57)	Second subgroup (TIMP1> 410 пг/мл, *n* = 31)	*P*
Sex, abs, m/w, abs. /%	17 (29,8)/ 40 (70,2)	13 (41,9)/ 18 (58,1)	0,566/ 0,729
Age, years Me[LQ; UQ]	57,11±12,92	55,10±12,56	0,485
Smoking, abs. /%	9/15,8	8/25,8	0,516
Body circumference, sm Me[LQ; UQ]	88 [70,0;105,0]	67 [67,0;95,0]	0,034
BMI > 30 kg/m^2, абс. /%	6/10,5	11/35,5	0,045
BMI, kg/m^2	27,2±6,10	29,02±5,21	0,123
AH, abs. /%	26/45,6	8/25,8	0,305
Angina pectoris, abs. /%	8/14,0	1/3,2	0,219
ACS history, abs. /%	5/8,8	0/0,0	0,497
PCI/CB history, abs. /%	3/5,3	0/0,0	0,494
Stable CHF, abs. /%	7/12,3	1/3,2	0,307
FP, abs. /%	5/8,8	1/3,2	0,587
VRD, abs. /%	11/19,3	6/19,4	0,997
Stroke/TIA, abs. /%	2/3,5	0/0,0	0,729
type 2 DM, abs. /%	6/10,5	0/0,0	0,184
BA, abs. /%	9/15,8	0/0,0	0,075
COPD, abs. /%	2/3,5	0/0,0	0,776
iACE/ARA/ARNI, abs. /%	22/38,6	12/38,7	0,838
Aldosterone antagonists, abs. /%	3/5,3	0/0,0	0,494
Beta-adrenoblockers, abs. /%	17/29,8	5/16,1	0,388
Thiazides / thiazide-like/loop diuretics, abs. /%	8/14,0	2/6,5	0,540
AC, abs. /%	11/19,3	7/22,6	0,931
Statins, abs. /%	11/19,3	5/16,1	0,938
Antiagregants/anticoagulants, abs. /%	12/21,1	3/9,7	0,291
Sugar-reducing medications, abs. /%	6/10,5	0/0,0	0,154
Inhalation HCs, abs. /%	6/10,5	0/0,0	0,154

Abbreviations: TIMP — Tissue Inhibitor of Matrix Metalloproteinases, BMI — body mass index, AH — arterial hypertension, ACS — acute coronary syndrome, PCI — percutaneous coronary intervention, CB — coronary bypass, CHF — chronic heart failure, FP — atrial fibrillation, VRD — ventricular rhythm disturbances, TIA — transient ischemic attack, DM — diabetes, BA — bronchial asthma, COPD — chronic obstructive pulmonary disease, iACE — angiotensin-converting enzyme inhibitors, ARA — angiotensin receptor antagonists, ARNI — angiotensin II receptor antagonists and neprilysin inhibitors, AC — calcium antagonists, HCs — glucocorticoids

Table 3. *Variants of the course of Covid-19 and long-term post-COVID syndrome by subgroups of patients (n = 88)*

Indicator	First subgroup (135<TIMP1 ≤ 410 пг/мл, *n* = 57)	Second subgroup (TIMP1> 410 пг/мл, *n* = 31)	*P*
Covid-19 without hospitalization, abs. /%	33/57,9	12/38,7	0,422
Covid-19 + pneumonia, abs. /%	32/56,1	5/16,1	0,776
Covid-19 + pneumonia + hospitalization, abs. /%	24/42,1	19/61,3	0,424
Covid-19 + pneumonia+PIT, abs. /%	6/10,5	0/0,0	0,184
Covid-19 + other hospital complications, abs. /%	1/1,7	0/0,0	0,749
Vaccination from Covid-19, abs. /%	**6/10,5**	**19/61,3**	**<0,001**
«Long Covid» (weakness), abs. /%	**32/56,1**	**6/19,4**	**0,047**
«Long Covid» (general malaise), abs. /%	**32/56,1**	**6/19,4**	**0,047**
«Long Covid» (fatigue), abs. /%	**32/56,1**	**6/19,4**	**0,047**
«Long Covid» (impaired concentration and memory), abs. /%	**23/40,4**	**3/9,7**	**0,038**
«Long Covid» (dyspnea), abs. /% abs	**32/56,1**	**6/19,4**	**0,003**
«Long Covid» (reduced quality of life), абс. /%	**32/56,1**	**6/19,4**	**0,047**
Pulmonary fibrosis by CT, % Me[LQ; UQ]	41,0 [10,0;64,0]	19,0 [10,0,0;30,0]	0,076

Abbreviations: TIMP — тканевый ингибитор матриксных металлопротеиназ (Tissue Inhibitor of Matrix Metalloproteinases), Covid-19 — new coronavirus infection, PIT — intensive care room, CT- computed tomography

NCVI vaccination reduces OR of development of the collagenolytic pattern due to prevailing fibrotic processes in post-NCVI patients, thus, of long-lasting post-COVID syndrome, by 92.5% (OR=0.075, 95% CI=0.021; 0.254 p=0.013), RR — by 82.8% (RR=0.172, 95% CI=0.070; 0.391, p=0.005).

An analysis of laboratory parameters demonstrated that subgroup 1 patients have lower platelet concentrations (p=0.040) within the normal range, a higher lymphocyte count (p=0.029) and higher D-dimer levels (p=0.048). There were no statistically significant correlations with TIMP1 levels for all parameters (complete blood count: WBC; ESR; blood biochemistry: CRP, fasting plasma glucose, HbA1c, total cholesterol, LDL, HDL, TG, uric acid, potassium, sodium, urea, NT-proBNP) between subgroups.

In echoCG, statistically significant differences between subgroups were obtained only for TAPSE; however, there was no correlation between this parameter and TIMP1 levels.

The renal filtration function in subgroups was evaluated. Cystatin C levels were significantly higher in subgroup 1 vs. subgroup 2 (0.84 [0.71;0.99] vs. 0.72 [0.58;0.89], p=0.025). There were no differences in eGFR based on CKD-EPI creatinine (group 1 77.0 [66.3;85.9], group 2 85.0 [72.3;92.8], p=0.533), and eGFR based on CKD-EPI (cystatin C) (group 1 71.0[56.5;88.75], group 2 78.0[67.0;88.0], p=0.329).

The correlation analysis made it possible to identify medium direct correlation between TIMP1 levels and cystatin C concentrations in blood (r=0.297, p=0.025). ROC curve plotting for all cystatin C concentrations allowed obtaining a cut-off point of ≥ 0.736 mg/L, which makes it possible to treat this parameter as a risk factor of collagenolytic pattern due to prevailing fibrotic processes in patients with long post-COVID syndrome, with sensitivity of 52.6% and specificity of 71.0% (AUC=0.633, p=0.034) (Fig. 2).

Cystatin C values of ≥0.74 mg/L increase OR of collagenolytic pattern due to prevailing fibrotic processes in patients with long-lasting post-COVID syndrome by 3.2 times (OR=3.245, 95% CI=2.317; 7.343, p=0.019), RR — by 2.0 times (RR=2.045, 95% CI=1.501; 13.412, p=0.03).

An analysis of 3D sphygmoplethysmography results demonstrated higher PWVcf (p=0.044) and CAVI1 index (p=0.037) in subgroup 1 (13.40[10.35;15.7]) vs. subgroup 2 (12.3[10.23;13.7]).

A correlation analysis demonstrated medium reverse correlation between TIMP1 levels and PWVcf (r=–0.355, p=0.027). ROC curve plotting for all PWVcf values allowed obtaining a cut-off point of ≥ 11.6 m/s,

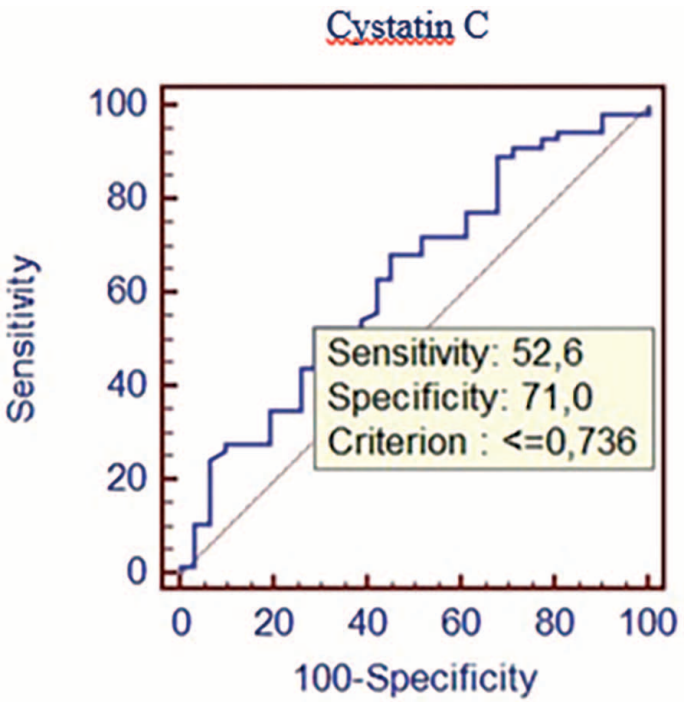

Figure 2. *ROC curve for cystatin C>0.736 mg/l in patients with long-term post-COVID syndrome as a risk factor of the development of a collagenolytic pattern against the background of predominant fibrosis processes*

which makes it possible to treat this parameter as a risk factor of collagenolytic pattern due to prevailing fibrotic processes in patients with long post-COVID syndrome, with sensitivity of 46.9% and specificity of 85.2% (AUC=0.640, p=0.027) (Fig. 3).

Also, there is medium reverse correlation between CAVI1 and TIMP1 levels (r=–0.360, p=0.03). ROC curve plotting for all CAVI1 values allowed obtaining a cut-off point of ≥ 7.1, which makes it possible to treat this parameter as a risk factor of collagenolytic pattern due to prevailing fibrotic processes in patients with long post-COVID syndrome, with sensitivity of 34.7% and specificity of 92.6% (AUC=0.667, p=0.009) (Fig. 3).

PWVcf value of ≥ 11.6 m/s increases OR of collagenolytic pattern due to prevailing fibrotic processes in patients with long-lasting post-COVID syndrome by 3.5 times (OR=3.543, 95% CI=2.589; 11.203, p=0.04), RR — by 2.4 times (RR=2.411, 95% CI=1.827; 9.673, p=0.001). CAVI value of ≥7.1 increases OR of collagenolytic pattern due to prevailing fibrotic processes in patients with long-lasting post-COVID syndrome by 3.0 times (OR=3.002, 95% CI=2.267; 8.406, p=0.02), RR — by 2.1 times (RR=2.146, 95% CI=1.622; 10.157, p=0.04).

Ferrokinetics, apoptosis and inflammation biomarkers were analysed in patients with long-lasting post-COVID syndrome (see Table 4).

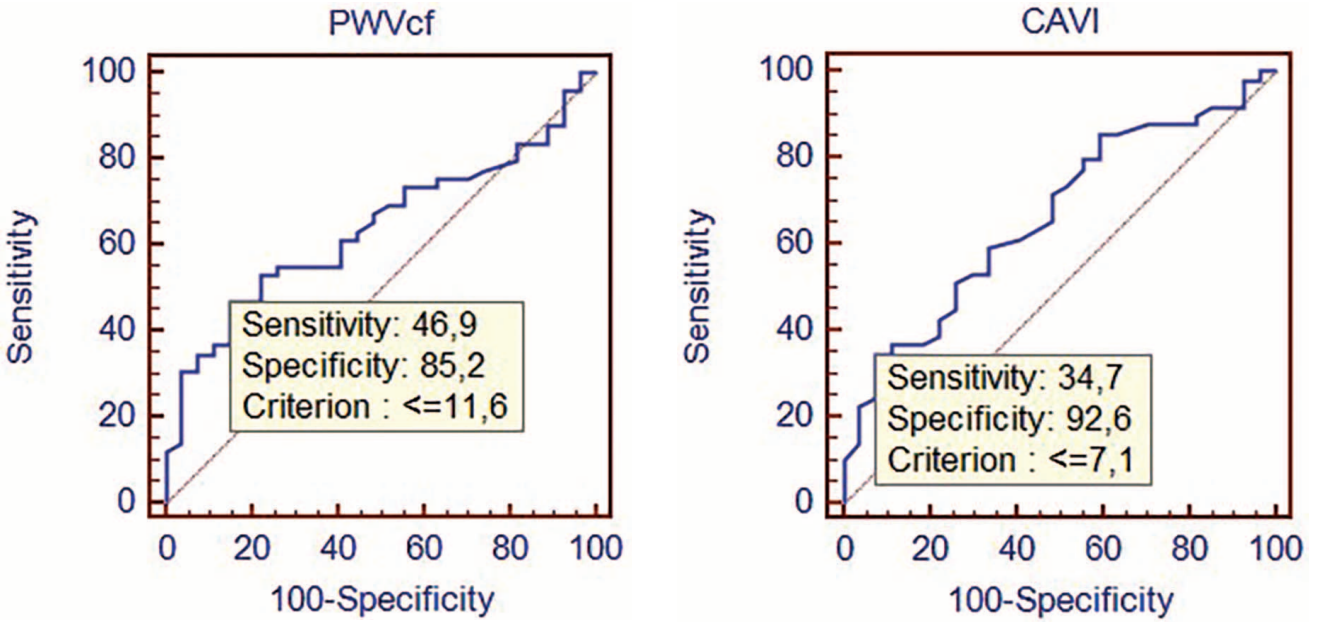

Figure 3. *ROC curves for pulse wave velosity (PWVcf) ≥11.6 m/s and cardio-ankle vascular index (CAVI1) ≥7.1 in patients with long-term post-COVID syndrome as risk factors of the development of a collagenolytic pattern against the background of predominant fibrosis processes*

Table 4. *Ferrokinetics and biomarker indicators for groups of patients with long-term post-COVID syndrome (n=88)*

Indicator Me[LQ; UQ]	First subgroup (135<TIMP1 ≤ 410 пг/мл, *n* = 57)	Second subgroup (TIMP1> 410 пг/мл, *n* = 31)	*P*
Serum iron, μmol/L	19,0[13,8;20,1]	19,0[13,42;24,47]	0,884
OJSS, μmol/L	**58,0[47,47;71,97]**	**52,0[44,72;58,57]**	**0,033**
Ferritin, ng/mL	**416,0[66,0;675,0]**	**240,0[75,0;347,0]**	**0,005**
CNTJ, %	**30,8[19,2;56,1]**	**36,8[21,4;59,6]**	**<0,001**
Transferrin, mg/mL	3,04[2,06;4,26]	3,08[1,99;3,30]	0,726
Alpha-FNO, pg/mL	**4,9 [0,00;6,35]**	**0,16 [0,00;1,00]**	**0,002**
Interleukin-6, **pg/mL**	2,04[1,18;3,83]	2,00[0,76;4,06]	0,826

Abbreviations: TIMP1 — Tissue Inhibitor of Matrix Metalloproteinases 1, OJSS — total iron-binding capacity of serum, CNTJ — iron saturation factor of transferrin, Alpha-FNO — tumor necrosis factor alpha

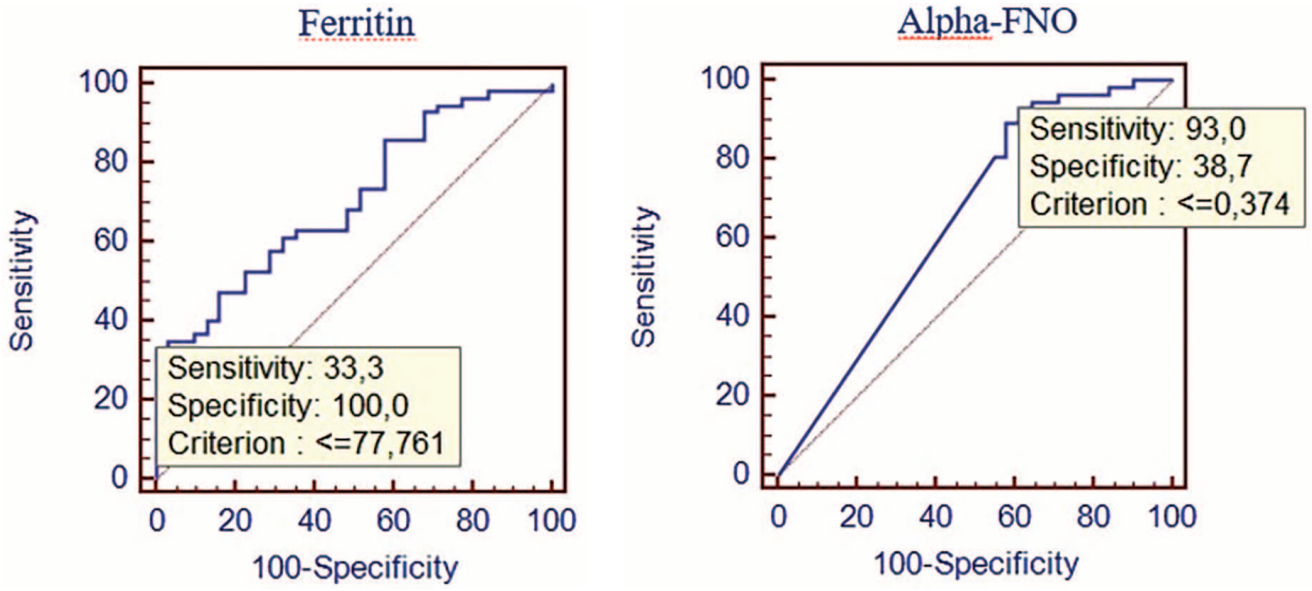

Figure 4. *ROC curves for ferritin >77.76 ng/ml and tumor necrosis factor alpha (alpha -FNO) >0.374 pg/ml in patients with prolonged post-COVID syndrome as risk factors of collagenolytic pattern development against the background of predominant fibrosis processes*

A correlation analysis demonstrated medium reverse correlation between TIMP1 level and ferritin concentration (r=–0.280, p=0.01). ROC curve plotting for all ferritin values allowed obtaining a cut-off point of > 77.76 ng/mL, which makes it possible to treat this parameter as a risk factor of collagenolytic pattern due to prevailing fibrotic processes in patients with long post-COVID syndrome, with sensitivity of 33.3% and specificity of 99.0% (AUC=0.707, p=0.002) (Fig. 4).

Also, there is medium reverse correlation between TNF-α and TIMP1 levels (r=–0.325, p=0.008). ROC curve plotting for all TNF-α values allowed obtaining a cut-off point of ≥ 0.374 ng/mL, which makes it possible to treat this parameter as a risk factor of collagenolytic pattern due to prevailing fibrotic processes in patients with long post-COVID syndrome, with sensitivity of 93.0% and specificity of 38.7% (AUC=0.652, p=0.005) (Fig. 4).

Ferritin value of ≥ 77.76 ng/mL increases OR of collagenolytic pattern due to prevailing fibrotic processes in patients with long-lasting post-COVID syndrome by 1.9 times (OR=1.904, 95% CI=1.438; 8.659, p=0.015), RR — by 1.5 times (RR=1.504, 95% CI=1.256; 7.238, p=0.023). TNF-α value of > 0.374 pg/mL increases OR of collagenolytic pattern due to prevailing fibrotic processes in patients with long-lasting post-COVID syndrome by 3.5 times (OR=3.518, 95% CI=1.922; 5.361, p=0.038), RR — by 2.3 times (RR=2.344, 95% CI=1.482; 11.006, p=0.02).

Our data on the development of the collagenolytic pattern due to prevailing fibrotic processes in patients with long-lasting post-COVID syndrome correlate with results of a study conducted by Zingaropoli M.A., et al. (2023), reporting that, three months after discharge, patients with COVID19-pneumonia experience reduction in plasma TIMP-1 levels ($p < 0.0001$), while plasma MMP-9 concentration rises (p=0.0088) [6]. Moreover, the authors found positive correlation between plasma TIMP-1 levels and chest CT results (ρ=0.2302, p=0.0160), emphasising its potential use as a fibrotic load biomarker. The authors conclude that increased MMP-9 levels and decreased plasma TIMP-1 concentration show ongoing inflammation and fibrosis three months after NCVI.

An evidence of ongoing inflammation in this study in patients with long COVID and specific collagen disorganisation status was determination of TNF-α values. After reporting long COVID in 67.8% of 333 patients eight months after NCVI, Schultheiß C. et al. (2022) identified a number of inflammatory markers and concluded that long COVID is associated not with autoantibodies, but with increased plasma IL-1β, IL-6 and TNF-α values [10]. The authors assume induction by COVID-19 of these cytokines in pro-inflammatory macrophages of lungs, which creates self-sustained reverse correlation.

Another study reported data, which do not correlate with our results on the presence of a collagenolytic pattern due to prevailing fibrotic processes in patients with long-lasting post-COVID syndrome [11]. Significantly lower MMP-10 and TIMP-1 values were found in both groups of patients with knee osteoarthritis nine months after COVID-19 vs. healthy subjects. It is assumed that reduced, and not increased MMP values are associated with pathogenetic immunosuppressive therapy of arthritis.

In our study, cystatin C is a risk factor of collagen disorganisation system transformation, which demonstrates persistent inflammation in long-lasting post-COVID syndrome. These data correlate with results of the study by Medori M.C. et al. (2023), where patients with long COVID had their serum profiled using mass spectrometry [12]. A multivatiate ROC analysis identified a biomarker panel of 11 proteins, including cystatin C. The authors conclude that the identified biomarkers are associated with inflammatory processes, confirming literature evidences that patients with long COVID develop an inflammation, which damages many tissues, including glomerules, on subclinical level. It is advisable to make clarifications, given that the use of cystatin C as a biomarker to calculate GFR is limited by chronic inflammation (according to Russian guidelines and KDIGO 2024 recommendations).

In this study, risk factors of collagenolytic pattern development due to prevailing fibrotic processes, associated with ongoing inflammatory processes, in patients with long-lasting post-COVID syndrome were arterial stiffness parameters: PVWcf and CAVI1. It is a well known fact that vascular physiology remains impaired for at least 12 months after onset of SARS-CoV-2, even in healthy adults [13, 14]. One recent observational study confirmed evidences of positive linear correlation between C-reactive protein levels and increased arterial stiffness [15].

The results show that long COVID-19 is associated with a number of persistent haematological changes, including altered RBC, anaemia, lymphopenia and increased levels of inflammation markers, such as ferritin, D-dimer and IL-6 [16, 17]. These changes can help understand the pathophysiology of long COVID-19 and associated symptoms better [18]. In this study, increased ferritin levels recorded in patients with long COVID-19 are associated with reduced TIMP1 within higher concentration range, representing more severe inflammation processes, and can have a role in persistence and progression of long COVID-19.

Thus, it has been established that risk factors of collagenolytic pattern development due to prevailing fibrotic processes in patients with long-lasting post-COVID syndrome were higher concentrations of cystatin C, PWVcf, CAVI, ferritin and TNF-α.

Conclusions

In post-NCVI patients with long-lasting post-COVID syndrome, collagen disorganisation status is characterised by the development of collagenolytic pattern due to prevailing fibrotic processes vs. asymptomatic patients, who had NCVI at least 12 weeks ago, which can be seen as a pathogenetic mechanism of long COVID development. In the presence of such a transformation in the collagen disorganisation system in post-NCVI patients, OR of long-lasting post-COVID syndrome increases 7.4-fold, RR — 3.2-fold in any variants of the course of the disease. Risk factors of the development of collagenolytic pattern due to prevailing fibrotic processes in patients with long-lasting post-COVID syndrome were an increase on cyststin C levels to > 0.736 mg/L, PWVcf to $\geq$11.6 m/sc, CAVI to $\geq$7.1, ferritin to > 77.76 ng/mL and TNF-α to > 0.374.

Вклад авторов:

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Полянская Е.А.: проверка общей воспроизводимости результатов критический обзор и редактирование.

Козиолова Н.А.: концептуализация, анализ, редактирование рукописи, общее руководство;

Чернявина А.И.: методология, валидация, исследование, создание черновика;

Author Contribution:

All the authors contributed significantly to the study and the article, read and approved the final version of the article before publication

Masalkina O.V.: Conducting research, preparing, creating and presenting the manuscript, particularly writing the initial draft.

Polyanskaya E.A.: checking the overall reproducibility of the results critical review and editing.

Koziolova N.A.: conceptualization, formal Analysis, writing—review and editing, supervision;

Chernyavina A.I.: methodology, validation, investigation, writing—original draft

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