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Abnormal proteolytic activity profile in plasma of blood donors according to anti-SARS-CoV-2 IgG titer

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Abstract

This work aims to study whether there is a relationship between titer values of anti-SARS-CoV-2 IgG and changes in proteolytic processes. To confirm this hypothesis, we analyzed the content and activity of Matrix Metalloproteinases (MMPs) as well as the concentration and composition of circulating peptide pools in the plasma of blood donors divided into groups on the basis of anti-SARS-CoV-2 IgG titers. The results of gelatin zymography showed the presence of active MMP-2 in donors' plasma who recovered from COVID-19. In contrast, collagenases and their complexes were detected in the plasma of donors with no anti-SARS-CoV-2 IgG, while their activity was undetectable in some groups of COVID-19 convalescent individuals. The content of MMPs also differed among the donors with different titers of anti-SARS-CoV-2 IgG. Plasma peptide content was identical among the donors' groups, but there were more peptide fractions in plasma peptide pools of COVID-19 convalescent individuals; furthermore, they all were characterized by the presence of peptides with molecular weights less than 920 Da and greater than 1530 Da. We hypothesized a link between proteolytic alterations and peptide fraction composition. Our data need further validation to confirm the relationship between the titer values of anti-SARS-CoV-2 IgG and the severity of the proteolytic imbalance.

Introduction

Matrix Metalloproteinases (MMPs) are a large family of zinc-dependent endopeptidases that play a crucial role in the degradation of Extracellular Matrix (ECM) components.[1] Recent findings indicate that MMPs are involved in different physiological processes, including embryogenesis, morphogenesis, cell migration, and proliferation.[1,2] Additionally, these molecules are involved in pathophysiological processes such as wound healing, inflammation, and cancer.[2,3] Growing evidence implies that, under physiological conditions, there are low levels of most MMPs in the bloodstream, while during pathological reactions, dramatic changes in both the concentration and activity of circulating MMPs are observed.[4,5]

The role of MMPs in infection-associated pathologies has also been extensively studied.[6,7] Available data suggest that MMPs are involved in the inflammatory response not only by regulating cytokine signaling but also by contributing to disruption of the tissue-blood vessel barrier and migration of infectious agents and immune cells during infectious diseases.[6,7]

Recent studies have focused on the relationship between tissue proteolytic imbalance and the pathogenesis of coronavirus disease (COVID-19), caused by the SARS-CoV-2 virus. Recent clinical data have revealed that MMPs may induce an inflammatory cascade, generate tissue damage, and aggravate the complications of COVID-19.[8] It has been shown that MMP levels were significantly elevated in the blood circulation of COVID-19 patients, compared to healthy subjects and gradually increased with the disease stage.[8,9] Furthermore, we hypothesized that activation of tissue MMPs may cause the accumulation of degradation products, which can influence cell behavior by transducing cell signals from the extracellular environment and trigger specific disease-related processes leading to the worsening of COVID-19 symptoms.[10,11] Unfortunately, there is limited data on the role of MMPs in the development of delayed complications in the post-acute period of SARS-CoV-2 infection.[8,12]

In this study, we focused on the characteristics of MMPs in the blood plasma of individuals who recovered from COVID-19. We investigated the proteolytic parameters depending on the current titer of anti-SARS-CoV-2 IgG in plasma of blood donors and tried to predict the influence of antibodies on the development of proteolytic imbalance. We also investigated the quantitative and qualitative compositions of the blood plasma peptide fraction.

Materials and Methods

Participants and study design

The study comprised a group of 180 blood donors, of which 160 were COVID-19 convalescent patients (the average age was 37.8 ± 11.5 years and 54% were men) who were positive in the past 3-6-month period and 20 did not suffer from COVID-19 previously (the average age was 35.3 ± 9.4 years and 50% were men). COVID-19 cases were laboratory-confirmed by real-time reverse transcriptase-polymerase chain reaction of the respiratory specimen (nasopharyngeal and oropharyngeal swabs) in certified labs. At the time of blood sampling, all participants did not have any symptoms of infectious diseases and were SARS-CoV-2 negative by nasopharyngeal swab. Donor plasma samples were collected at "BIOPHARMA-PLASMA" (Kyiv, Ukraine) and analyzed using the Anti-SARS-CoV-2 Antibody IgG Titer Serologic Assay kit (AMSBIO, USA, Cat. No AMS.TAS-K002) for the quantitative detection of antibodies to the SARS-CoV-2 spike protein receptor binding domain. We identified 9 groups based on anti-SARS-CoV-2 IgG level: group 1 had no anti-SARS-CoV-2 IgG (control group, n = 20); IgG titer values of other groups were: group 2 10 ± 3 ng/mL (n=20), group 3: 55 ± 5 ng/mL (n=20), group 4: 65 ± 5 ng/mL (n=20), group 5: 75

± 5 ng/mL (n=20), group 6: 85 ± 5 ng/mL (n=20), group 7: 95 ± 5 ng/mL (n=20), group 8: 125 ± 5 ng/mL (n=20) and group 9: 175 ± 5 ng/mL (n=20).

Determination of MMP activity in blood plasma

Zymography was performed according to the method described by Ostapchenko et al [13]. The separating gel (12%) was polymerized in the presence of gelatin or collagen at a rate of 1 mg/mL. Equal volumes of plasma samples were applied onto the gel. After electrophoretic separation, the gels were washed in 2.5% Triton X-100 for an hour to remove residual sodium dodecyl sulfate. Then, to remove Triton X-100, the gels were washed with distilled water and incubated in 0.05 M Tris-HCl buffer, pH 7.4, containing 0.13 M NaCl (pH 7.4) for 12 hours. Fixation and staining of the gels were performed according to a standard protocol for SDS-PAGE. The digested bands were visualized as the non-stained regions on the zymograms. The electropherograms were analyzed using the TotalLab v. 2.04 program.

Determination of MMP and TIMP-1 content

Levels of gelatinases (MMP-2, -9), collagenases (MMP-1, -8) and stromelysins (MMP-3, -10), and TIMP-1 were determined using an Enzyme-Linked Immunosorbent Assay (ELISA).[14] ELISA plates were coated with a sample of donor plasma diluted 1:100 with 0.05 M Tris-HCl buffer (pH 7.4) and incubated at 37°C for 1 hour. The plates were washed with 0.05 M Tris-HCl buffer (pH 7.4) containing 0.05% Tween-20, then coated with 3% nonfat dry milk to block any free binding sites and incubated overnight. Then, the plates were washed again, coated with monoclonal antibodies against the targeted antigens (Santa Cruz Biotechnology, Inc., Dallas, USA) and incubated at 37°C for 1 hour. The microplates were then washed and incubated with the corresponding secondary antibodies (Sigma-Aldrich, St Louis, USA), which were conjugated to horseradish peroxidase at 37°C for 1 hour. The reaction was visualized using horseradish peroxidase substrate (*o*-phenylenediamine and hydrogen peroxide). The reaction was stopped using 2.5 M H_2SO_4. The absorbance was determined at 492 nm using a microplate spectrophotometer (BioTek, Vermont, USA).

Determination of peptide content

The peptide pool was obtained from plasma, according to the method described previously.[15] The whole procedure was performed on ice, with 15-minute breaks between each stage. Cold 1.2 M $HClO_4$ was added to the plasms (1:1, v/v). After centrifugation at 10,000 g for 20 min at +4 °C, the resultant supernatant was neutralized with 5 N KOH to pH 7.0 and centrifuged again. The remaining protein in the resultant supernatant was precipitated with 4 volumes of cold 96% ethanol. After centrifugation, the supernatant was gathered, and its optical density was measured at 210 nm

using the spectrophotometer (Smart SpecTMPlus, BioRad, Hercules, USA). The peptide concentration was calculated using a calibration curve prepared with CBZ-glycyl-glycine dipeptide (0.26 kDa) as a standard.

Size-exclusion chromatography analysis

Peptide fraction separation and mass determination were performed using a Sephadex G15 column (Bio-Rad, Hercules, USA) by means of a low-pressure chromatography system (BioRad, Hercules, USA). Column pre-equilibration was achieved with 0.05 M Tris-HCl (pH 7.4) containing 0.13 M NaCl. Samples were loaded at a flow rate of 30 mL/hour. The mass of peptides was determined by a calibration curve calculated using a standard mixture containing lysozyme (14.3 kDa), insulin (5.7 kDa), and vitamin B12 (1.35 kDa).

Statistical analysis

Statistical analysis was performed using the STATISTICA Package, version 12.0 (StatSoft, Inc., Tulsa, USA). For the studied variables, we reported medians with Interquartile Ranges (IQRs). The hypothesis of a normal distribution was checked using the Shapiro-Wilk test. All donor groups showed a non-normal distribution. Therefore, a Kruskal-Wallis test followed by Dunn's test was performed to determine whether or not there was a statistically significant difference between the medians of different variables. In the current study, we compared the data of the case groups (groups 2–9 of convalescent COVID-19 donors) to the values of the control group. We used group 1, the group of blood donors who did not suffer from COVID-19 previously, as the control group. A $p < 0.05$ was considered statistically significant.

Results

We identified the potential proteolytic activity and content of various MMPs, including gelatinases (MMP-2, -9), collagenases (MMP-1, -8), and stromelysins (MMP-3, -10), in donors' blood plasma (Table 1). The analysis of donors' blood plasma by zymography method demonstrated the presence of light zones of proteolytic activity of enzymes with molecular weights of 72 kDa and 92 kDa, which, according to the literature,[16] may correspond to MMP-2 (72 kDa) and MMP-9 (92 kDa). The comparative analysis of proteolytic activity using gelatin as a substrate helped determine differences between donor groups. Plasma samples of donors with no anti-SARS-CoV-2 IgG had MMP-9 activity but did not show proteolytic activity in the region of MMP-2. Generally, MMP-9 activity was shown in each donor group with titers of anti-SARS-CoV-2 IgG greater than 10 ng/mL. We detected MMP-2 activity only in two donor groups, namely groups 4 and 5, with titers of anti-SARS-CoV-2 IgG of 65 ± 5 mg/mL and 75 ± 5 mg/mL, respectively. In addition, four donor groups with titers of anti-SARS-CoV-2 IgG of 10 ± 3, 55 ± 5, 65 ± 5, and 75 ± 5 mg/mL had proteolytic

activity in zones that corresponded to enzymes (or their complexes) with molecular weights above 100 kDa. Interestingly, there were no light bands of proteolytic activity above 100 kDa in the plasma of donors with no detected anti-SARS-CoV-2 IgG. The described results are represented in Table 1.

Figure 1 shows the data of the immunoassay performed for quantitative analysis of MMP-2 and MMP-9 in donors' blood plasma. As can be seen from the results, there were statistically significant differences in MMP-2 levels in plasma samples of donors with no anti-SARS-CoV-2 IgG (group 1) and the other groups (Figure 1A). Plasma MMP-2 levels were significantly higher in donors of group 5 than in controls (group 1), while the same parameter was significantly decreased in groups 6 and 8, compared to group 1. The other study groups showed a slight increase in plasma MMP-2 content compared to donors with no detected anti-SARS-CoV-2 IgG (group 1).

The results of plasma MMP-9 measurements are represented in Figure 1B. A significant difference was observed for group 2 with a titer of anti-SARS-CoV-2 IgG of 10 ± 3 ng/mL, compared to donors with no anti-SARS-CoV-2 IgG (group 1).

Collagen-substrate zymography was used for the detection of enzymes with collagenase activity (MMP-1, -8, and -13) in biological samples. The obtained zymogram data illustrated the presence of light zones in two regions, between 40 and 55 kDa and between 90 and 100 kDa, in plasma samples of donor groups (Table 2). In particular, active bands ranging from 40 to 55 kDa were detected in samples of donors from groups 1, 4, 6, and 7, which, according to the literature,[17] may correspond to MMP-1 (proMMP-1 has ~49 kDa; active MMP-1 has 49-51 kDa), MMP-8 (proMMP-8 has ~57 kDa) and MMP-13 (proMMP-13 has ~59 kDa; active MMP-13 has 46 kDa). In addition, in all studied groups, except in group 5, we observed enzyme activity zones in a region between 90 and 100 kDa (Table 2), which can be explained by the presence of enzymes in the form of complexes with their inhibitors.

The levels of MMP-1 and -8 have been investigated in the plasma samples of donors' groups by means of the ELISA technique (Figure 2). A remarkable increase in MMP-1 content was observed in plasma samples of three donor groups with titers of anti-SARS-CoV-2 IgG of 65 ± 5, 75 ± 5, and 123 ± 5 mg/mL, compared to donors with no anti-SARS-CoV-2 IgG. The other groups, except group 7, showed a small but statistically significant difference compared to the control group (Figure 4A). The analysis of MMP-8 plasma levels between donor groups detected statistically significant changes between donor group with no anti-SARS-CoV-2 IgG (group 1) and groups with antibody titers of 10 ± 3; 55 ± 5, 65 ± 5, and 75 ± 5 ng/mL (Figure 2B). In particular, the MMP-8 content was remarkably increased in groups 3, 4, and 5, while in group 2 this parameter was decreased when compared to the control.

Since stromelysins (MMP-3 and -10) play a crucial role in the regulation of other MMP activity,[18] we investigated their levels in donors' plasma by means of the ELISA technique using the MMP-3/10 antibody (Santa Cruz Biotech, Inc., Dallas, USA). Three of the studied groups, namely groups 3, 4, and 8, showed a significant decrease in plasma MMP-3/10 content, while groups 7 and 9 had elevated plasma levels of MMP-3/10 compared to the control group. Three other groups, which had titers of anti-SARS-CoV-2 IgG of 10 ± 3, 75 ± 5, and 85 ± 5 ng/mL, did not show significant changes in plasma MMP-3/10 content compared to the group with no anti-SARS-CoV-2 IgG (Figure 3).

In addition, we measured the levels of TIMP-1, which is a key inhibitor of MMPs such as MMP-1, -2, -3, -7, -8, -9, -10, -11, -12, -13, and -16.[19] We detected that the levels of TIMP-1 in donor groups with titers of anti-SARS-CoV-2 IgG of 55 ± 5, 65 ± 5, 75 ± 5, 85 ± 5, and 175 ± 5 differ from those of the control group (Figure 4).

Since any changes in proteolytic balance are reflected in the composition of the tissue peptide pool, in the next stage of our study we performed quantitative and qualitative analysis of the circulating peptide fraction in each donor's group. As can be seen from the results (Figure 5A), there were no differences in peptide content in plasma between all donors' groups. On the other hand, we detected the different compositions of the peptide pools in the studied groups (Figure 5B). In particular, all groups with titers of anti-SARS-CoV-2 IgG greater than 10 ± 3 ng/mL had more chromatographic peaks than the group with no anti-SARS-CoV-2 IgG (group 1). Also, we established that all SARS-CoV-2 IgG-positive groups are characterized by the presence of peaks of peptides with molecular weights less than 920 Da and greater than 1530 Da, which were not observed in the control group (Figure 5B).

Discussion

Dysregulation of the proteolytic balance is often associated with the development of many pathological conditions, including cancer, Alzheimer's disease, arthritis, blood clotting disorders, allergies, and viral and bacterial infections.[1-3,6,20] The latest data confirmed the participation of proteolytic enzymes, particularly MMPs, in the pathogenesis of COVID-19.[8,9,21] MMPs seem to be involved in pro- and anti-inflammatory reactions, oxidative stress, and metabolic alterations during SARS-CoV-2 infection.[8] Studies of proteolytic biomarkers have shown a consistent upregulation of MMP-1, -7, -10, and -12 in post-COVID-19 individuals.[9,22] Active MMPs can promote inflammation and the accumulation of protein degradation products, which may be related to the development of post-COVID-19 complications.

The primary task of this study was to determine the content and activity of MMPs in plasma samples of individuals who have been divided into groups based on anti-SARS-CoV-2 IgG titer values. Firstly, we investigated the potential proteolytic activity in plasma samples from donors'

groups. By means of gelatin- and collagen-substrate zymography, the potential activities of MMP-1, -2, -8, -9, and -13 were analyzed. Our results indicated that there were different proteolytic activity profiles in donors with different anti-SARS-CoV-2 IgG titers. We speculate that there may be a relationship between anti-SARS-CoV-2 antibody levels and the severity of proteolytic imbalance. Our hypothesis was confirmed by the difference between levels of various MMPs in the plasma of donor groups. Furthermore, in some groups of COVID-19 convalescent individuals, the proteolytic activity in high-molecular-weight zones was observed. Such results can be explained by the accumulation of MMPs in the blood circulation of donors in different forms, including pro-MMP, complexes of MMP with their inhibitors, and active forms. In addition, the active proteolytic zones in the region higher than 110 kDa may correspond to complexes of MMP with other proteins. Indeed, MMP-9 can bind with microglobin (25 kDa) to form a complex with a molecular weight of 125 kDa, a dimer (215 kDa) or even a multimer (240 kDa).[23]

MMP-3 and -10 belong to the stromelysin family, which regulates gelatinase and collagenase activity. They play a crucial role in activating other MMPs, including pro-MMP-1, -3, -7, -8, -9, and -13.[24] Interestingly, we noted multidirectional changes in plasma MMP-3/10 content in groups of COVID-19 convalescent individuals compared to the control group. However, more research is needed to better understand the full impact of stromelysins on MMP activity and the development of proteolytic imbalance.

Tissue Inhibitors of Metalloproteinases (TIMPs) play a central regulatory role as inhibitors of MMPs.[25] Since TIMP-1 is a strong inhibitor of almost all MMPs, we decided to study TIMP-1 content in plasma samples of donors. According to the results obtained, TIMP-1 levels increased among donor groups, with titers of anti-SARS-CoV-2 IgG higher than 10 ± 3 ng/mL. Such results may be explained by overexpression of TIMP-1, that may be a part of the pathological process during COVID-19.[26]

It is well known that proteases, including MMPs, are involved in the generation of components of "tissue-specific peptide pools".[27,28] There are several ways in which peptides can be generated. First, cell-specific proteinases can break down proteins inside living cells, whereupon peptides release into extracellular medium.[29] Second, tissue-specific proteinases such as MMPs can degrade the proteins in the extracellular matrix.[30] In both of these cases, proteolytic enzymes catalyze the cleavage of cell- or tissue-specific proteins, which can be either proteins that have already accomplished their function or proteins that were modified under pathophysiological reactions. Our findings showed that the peptide pool's quality composition changed in individuals who recovered from COVID-19. We assumed that peptides that were not present in the plasma of control individuals could show up for two reasons: either proteolytic processes accelerated, or the processes linked to cell disruption got more intense. Given the changes in the content and activity of MMPs in plasma samples of donors who recovered from COVID-19, it is possible that many of the peptides

produced under the pathological condition were synthesized as byproducts of protein catabolism. However, some of the formed peptides could be produced by the direct translation of small mRNA sequences.[31] Peptides may have a wide biological impact by influencing cell signaling both inside and outside of cells. These atypical peptides can also be produced expressly for a certain function and act either as triggers of pathological processes or modulators that may help preserve homeostasis.

We hypothesized a correlation between the degree of proteolytic imbalance and anti-SARS-CoV-2 antibody. An increasing number of studies demonstrate the accumulation of both anti-SARS-CoV-2 IgG and autoantibodies in the post-COVID-19 period due to the destabilization of the immune system caused by the strong immune response against the infection agent.[32,33] Previous studies report on the appearance of autoantibodies against MMPs in various diseases, including systemic sclerosis, cancer, and arthritis.[34-37] Thus, depending on the behavior of antibodies in the bloodstream, destabilization of proteolytic processes may obviously occur in the post-COVID-19 period. We think all detected complications may have the same origin from the influence of antibody subpopulations. We cannot directly confirm the relationship between the titer values of anti-SARS-CoV-2 IgG and the severity of the proteolytic imbalance. We believe that further experiments using purified IgG fractions obtained from donors recovered from COVID-19 will help us to better understand the mechanisms related to the control of proteolysis and how MMPs affect pathogenesis, contributing to disease onset and progression.

The findings of the current research would be beneficial to complete with an analysis of correlation relationships between the levels of anti-SARS-CoV-2 antibodies and the severity of plasma proteolytic imbalance. To draw a final conclusion on the association between SARS-CoV-2 infection and either proteolytic abnormalities or changes in peptidome composition, more patients should be recruited for the study. Larger sample sizes allow us to control the risk of reporting false findings, providing greater statistical power and producing more precise estimates.

Conclusions

In this study, we estimated the proteolytic process in the blood plasma of donor groups depending on the titers of anti-SARS-CoV-2 antibodies. Obtained findings revealed differences in activity and plasma levels of MMPs as well as qualitative changes in peptide pools' composition in donor groups. The coronavirus seems to trigger the alterations in plasma proteolysis, and the severity of proteolytic imbalance may be associated with levels of anti-SARS-CoV-2 IgG titers. Furthermore, some previous studies showed the effector functions of IgG on particular pathophysiological processes, allowing us to put forward a hypothesis about the direct involvement of antibodies in the disease progression and development of new complications. Since we only carried out a preliminary

study on this issue, we currently cannot provide an explanation regarding the mechanism by which IgG subpopulations affect proteolysis. We believe that further experiments using purified IgG fractions from donors who recovered from coronavirus disease will help to advance our knowledge on the role of antibodies in post-COVID-19 syndrome. Thus, the isolation and purification of the IgG fraction from donors' plasma are prospective stages for future research regarding the investigation of the effects of antibodies by means of *in vitro* model systems.

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Table 1. Proteolytic activity profiles in donors' blood plasma mapped by zymography using gelatin as a substrate.

Group No	**Titer of anti-SARS-CoV-2 IgG, ng/mL**	**Molecular weight of active enzymes**		
		72 kDa	**92 kDa**	**110-120 kDa**
1	0	-	+	-
2	10 ± 3	-	+	+
3	55 ± 5	-	+	+
4	65 ± 5	+	+	+
5	75 ± 5	+	+	+
6	85 ± 5	-	+	-

7	95 ± 5	-	+	-
8	125 ± 5	-	+	-
9	175 ± 5	-	+	-

Table 2. Proteolytic activity profiles in donors' blood plasma mapped by zymography using collagen as a substrate.

Group No	**Titer of anti-SARS-CoV-2 IgG, ng/mL**	**Molecular weight of active enzymes**	
		40-55 kDa	**90-100 kDa**
1	0	+	+
2	10 ± 3	+	+
3	55 ± 5	-	+
4	65 ± 5	-	+
5	75 ± 5	-	-
6	85 ± 5	+	+
7	95 ± 5	+	+
8	125 ± 5	-	+
9	175 ± 5	-	+

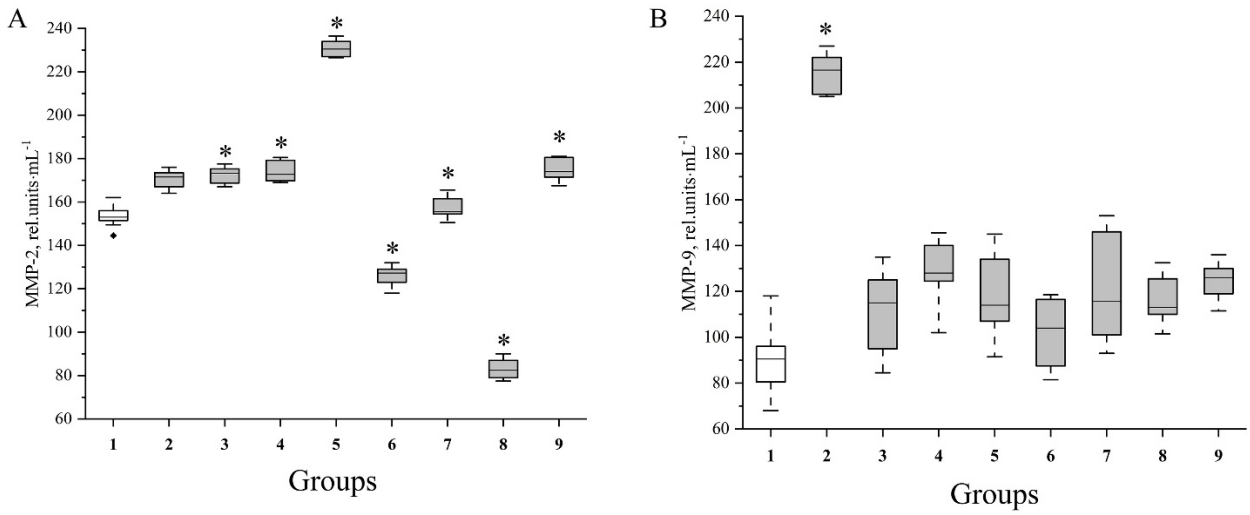

Figure 1. Levels of MMP-2 (A) and MMP-9 (B) in donor groups with different titers of anti-SARS-CoV-2 IgG (ng/mL). **1** – had no anti-SARS-CoV-2 IgG (control group); **2** – 10 ± 3; **3** – 55 ± 5; **4** – 65 ± 5; **5** – 75 ± 5; **6** – 85 ± 5; **7** – 95 ± 5; **8** – 125 ± 5; **9** – 175 ± 5 ng/mL. Data analysis is represented in the form of pairwise comparisons of the case group values (groups of convalescent COVID-19 donors, groups 2-9) to the values of the control group (group 1, individuals who did not

suffer from COVID-19 previously). * $p < 0.05$, significantly different from group 1, group of donors with no anti-SARS-CoV-2 IgG.

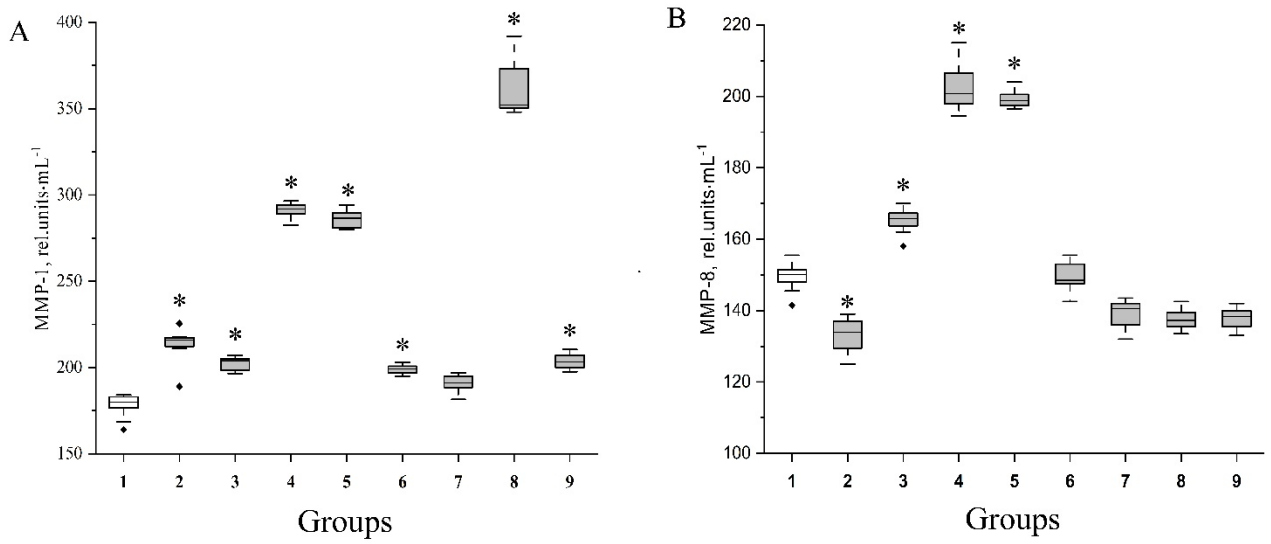

Figure 2. Levels of MMP-1 (A) and MMP-8 (B) in donor groups with different titers of anti-SARS-CoV-2 IgG (ng/mL). **1** – had no anti-SARS-CoV-2 IgG (control group); **2** – 10 ± 3; **3** – 55 ± 5; **4** – 65 ± 5; **5** – 75 ± 5; **6** – 85 ± 5; **7** – 95 ± 5; **8** – 125 ± 5; **9** – 175 ± 5 ng/mL. Data analysis is represented in the form of pairwise comparisons of the case group values (groups of convalescent COVID-19 donors, groups 2-9) to the values of the control group (group 1, individuals who did not suffer from COVID-19 previously). * $p < 0.05$, significantly different from group 1, group of donors with no anti-SARS-CoV-2 IgG.

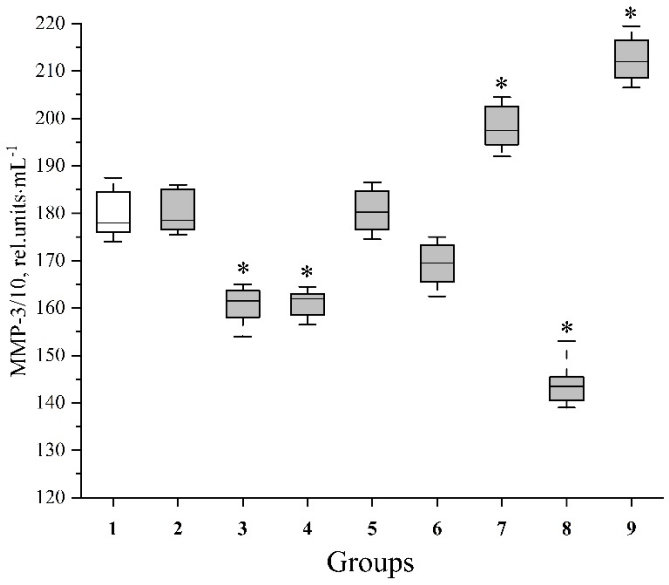

Figure 3. Levels of stromelysins in donor groups with different titers of anti-SARS-CoV-2 IgG (ng/mL). **1** – had no anti-SARS-CoV-2 IgG (control group); **2** – 10 ± 3; **3** – 55 ± 5; **4** – 65 ± 5; **5** – 75 ± 5; **6** – 85 ± 5; **7** – 95 ± 5; **8** – 125 ± 5; **9** – 175 ± 5 ng/mL. Data analysis is represented in the form of pairwise comparisons of the case group values (groups of convalescent COVID-19 donors, groups 2-9) to the values of the control group (group 1, individuals who did not suffer from COVID-19 previously). * $p < 0.05$, significantly different from group 1, group of donors with no anti-SARS-CoV-2 IgG.

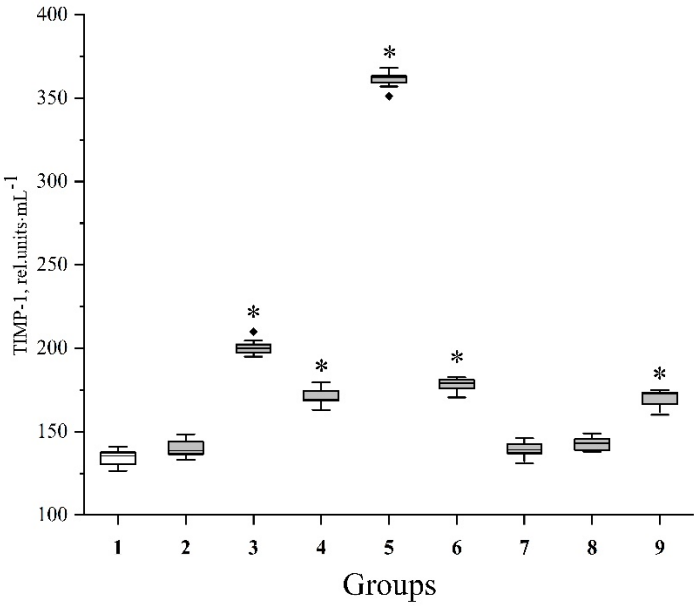

Figure 4. Levels of TIMP-1 in donor groups with different titers of anti-SARS-CoV-2 IgG (ng/mL). **1** – had no anti-SARS-CoV-2 IgG (control group); **2** – 10 ± 3; **3** – 55 ± 5; **4** – 65 ± 5; **5** –

75 ± 5; **6** – 85 ± 5; **7** – 95 ± 5; **8** – 125 ± 5; **9** – 175 ± 5 ng/mL. Data analysis is represented in the form of pairwise comparisons of the case group values (groups of convalescent COVID-19 donors, groups 2-9) to the values of the control group (group 1, individuals who did not suffer from COVID-19 previously). * $p < 0.05$, significantly different from group 1, group of donors with no anti-SARS-CoV-2 IgG.

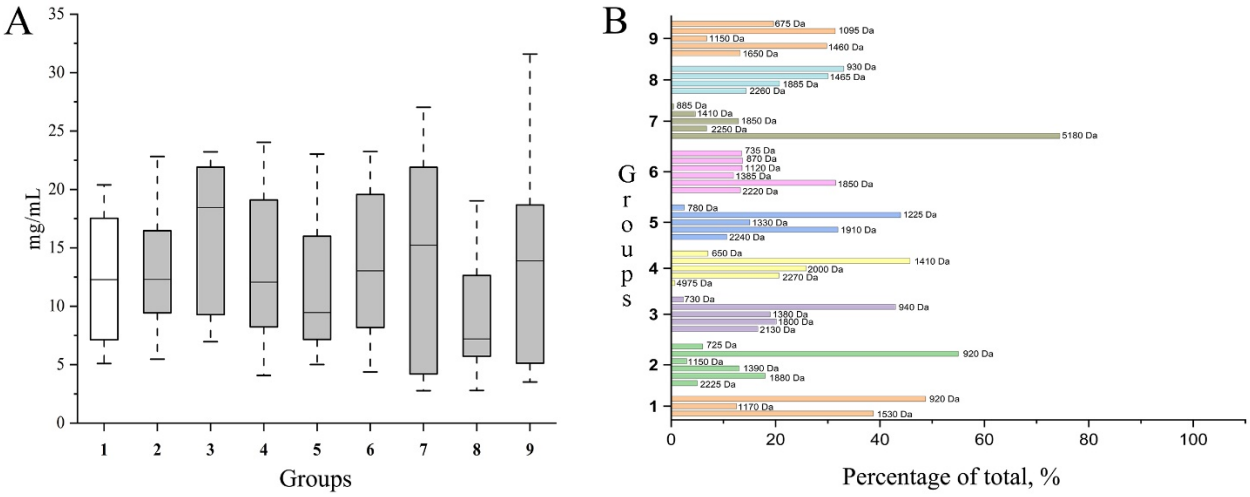

Figure 5. Peptide concentration (A) and peptide pool composition (B) in donor groups with different titers of anti-SARS-CoV-2 IgG (ng/mL). **1** – had no anti-SARS-CoV-2 IgG (control group); **2** – 10 ± 3; **3** – 55 ± 5; **4** – 65 ± 5; **5** – 75 ± 5; **6** – 85 ± 5; **7** – 95 ± 5; **8** – 125 ± 5; **9** – 175 ± 5 ng/mL.