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Level of cell-free DNA in plasma as an early marker of hospital course of covid-19 in patients with type 2 diabetes and obesity

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The progression of COVID-19 varies significantly among individuals; the severity and mortality rate are correlated with obesity, pre-existing type 2 diabetes and hypertension. However, not all patients from high-risk groups are characterized by severe passing of disease. Finding precise and rapid prognostic indicators is one of the main aims of our study. The study used plasma samples from 103 patients and volunteers. A retrospective analysis was performed on 93 COVID-19 patients classified by the severity of the disease as mild (n = 14), moderate (n = 42), and severe (n = 37). The distribution into groups was conducted after the completion of treatment in patients. The severity of COVID-19 was retrospectively assessed at the time of hospital discharge. Upon admission, all patients presented with similar symptoms of the disease. Glucocorticosteroids were not administered during the pre-hospital treatment phase. Demographic data and parametric indicators were registered. Clinical analysis and quantification of extracellular DNA (cfDNA) were performed and the levels of NETosis, the concentration of human myeloperoxidase (MPO) and human neutrophil elastase in blood plasma were measured. Statistical analyses were provided in the R environment (www.R-project.org, V.4.0). Our data illustrates that the severity of coronavirus disease among subjects largely correlates with the initial cell-free DNA in plasma and NETs formation activity. The plasma cfDNA levels in the early period of the disease were statistically significantly different in the subsequent course of the disease in patients with diabetes. The cfDNA value of 4297 ng/ml and higher corresponded to a more severe passing of disease in patients with COVID-19 from a high-risk group with 82% sensitivity and 72% specificity (AUC0.856, 95% CI 0.778-0.935, P < 0.001). The level of elastase in the blood plasma of patients with severe COVID-19 shows a statistically significant difference from the level of mild and moderate patients (P < 0.001), as well as compared with the level of elastase in healthy donors. In addition, the data demonstrate statistically significant differences in MPO levels between all groups of patients with COVID-19. We found an association between circulating NET formation markers at the disease's initial stage and clinical outcome. This demonstrates the potential importance of assessing plasma levels of cfDNA for clinical decision-making in the early stages of the disease.

Key words: cfDNA; neutrophils; COVID-19; NETosis; diabetes; predictors.

INTRODUCTION

The progression of COVID-19 varies significantly among individuals, and it is well established that the severity and mortality rate is correlated with age, pre-existing type 2 diabetes, obesity, and hypertension. However, not all patients from high-risk groups are characterized by severe passing of the disease and the majority recover without oxygen therapy and intensive care. Finding precise and rapid prognostic indicators is one of the main aims of COVID-19 management. Well-known criteria of disease severity are red blood cell distribution width, low albumin levels, high lymphocyte number, and high levels of D-dimer, ferritin, procalcitonin, IL-6, C-reactive protein, lactic acid, LDH, the number of polymorphonuclear leukocytes, plasma extracellular DNA (cfDNA) and other neutrophil extracellular traps (NETs) indicators [1-4].

Neutrophils release networks formed from their DNA in response to many different stimuli (microorganisms and their products and chemokines). The traps created by decondensed chromatin contain enzymes such as elastase, proteinase 3, myeloperoxidase, cathepsin G, defensins, and other substances with cytotoxic effects. Extracellular trap formation, distribution of the substances mentioned before, and further interaction with their targets, including the formed elements of blood, can lead to thrombosis [5] and tissue damage. Some studies have shown that NETs are formed under the influence of viruses [6-7].

Activation of neutrophil granulocytes with excessive NETs formation is considered a critical pathogenetic mechanism of different pathological processes. It has been described in patients with cardiovascular pathologies, diabetes mellitus, or obesity [9-11]. These processes also play a significant role in the pathogenesis of respiratory diseases. The Songa study showed that neutrophils form NETs in acute lung injury [12], and the number of NETs is increased in the alveolar spaces of patients with ventilator-associated pneumonia [13-14].

In 2020, there was evidence that NETs play an essential role in the pathogenesis of coronavirus disease [18]. Neutrophil granulocyte activation with NETs formation is one of the fundamental pathogenetic mechanisms of ARDS (acute respiratory distress syndrome) [15-16], which is the leading cause of death from COVID-19. It was also shown that the number of NETs in bronchopulmonary lavage correlated with the severity of the pathological process in patients with ARDS [16]. In addition to direct damaging effects, NETs lead to the polarization of alveolar macrophages and the formation of the M1 phenotype with proinflammatory activity. When triggered by platelets, NETosis can become dysregulated and cause NET-mediated tissue damage, hypercoagulability, and thrombosis [17].

One of the consequences (markers) of NETosis activation is an increase in cell-free DNA (cfDNA) levels in blood plasma and bronchopulmonary lavage. It was shown that the level of free DNA conjugated to myeloperoxidase (MPO-DNA) and citrullinated histone H3 (Cit-H3) - the primary highly specific markers of NETs – is increased in the plasma of patients with COVID-19 [19]. Reham Hammad retrospectively showed that cfDNA levels correlate with the severity of COVID-19 [20]. In another work, it was shown a positive correlation between the level of NETosis and the amount of cfDNA with other markers of severity of COVID-19, while the level of citrullinated histone H3 did not increase in patients who were on mechanical ventilation [21].

The main goal of our work was to determine the level of cfDNA significance in the plasma of high-risk patients during the pre-hospital (or early hospital) period as a possible predictor and the prognostic marker of the severity of COVID-19. For this purpose, we conducted research by determining the level of cfDNA in plasma exactly in aged patients with type 2 diabetes and obesity.

METHODS

Study of the clinical characteristics of patients. The study used plasma samples from 93 patients (mean age, 61 ± 15.39 years) overweight (BMI >30), received antidiabetic drugs, and hospitalized in Kyiv City Clinical Hospital No 4, infectious disease department. All hospitalized patients were confirmed to have COVID-19 in the laboratory using real-time PCR. The control group included 10 almost healthy volunteers (mean age, 59 ± 12.3 years) with BMI <25 without any diabetes signs.

Data included patients' demographics, comorbidities, inpatient medications, laboratory studies, treatment, and outcomes.

A retrospective analysis was performed on 93 COVID-19 patients. They were subsequently divided into three groups according to the severity of the disease and classified as mild (n = 14), moderate (n = 42), and severe (n = 37) accord-

ing to the Guidelines released by the National Health Commission of Ukraine. The severity of COVID-19 was retrospectively assessed at the time of hospital discharge. Upon admission, all patients presented with similar symptoms of the disease. Glucocorticosteroids were not administered during the pre-hospital treatment phase.

Clinically, the severity of the disease is defined due to the level of saturation and duration of oxygen dependence according to the WHO classification. Mild disease was considered to be a disease without signs of viral pneumonia or hypoxia, moderate disease was with clinical signs of pneumonia (fever, cough, dyspnoea, fast breathing) but no signs of severe pneumonia, including $SpO_2 \geq 90\%$ on room air. Severe disease was considered to be a disease with clinical symptoms of pneumonia (fever, cough, dyspnoea, fast breathing) plus one of the following: respiratory rate >30 breaths/min; severe respiratory distress; or SpO_2 <90% on room air [22].

Institutional Review Boards approval. All participants provided written informed consent. The study was reviewed and approved by appropriate national competent authorities: the Research Ethics Committee (No. 6/20 dated 11.11.2020.) and the Hospital Ethics Committee (No. 104/1-284 dated 13/05/2021) used to obtain patient and healthy donor samples.

Blood collection. Blood samples were collected from patients immediately after hospitalization for 6-9 days from the onset of the disease (using blood sampling system S-Monovette Sarstedt, stabilizer anticoagulant EDTA). After collection, 2 ml of venous blood was used to obtain blood plasma by centrifugation for 10 min at 400g. Another 1 ml of venous blood was used to isolate neutrophil granulocytes. The following clinical parameters were also determined: glucose level, hemoglobin, hematocrit, erythrocytes, leukocytes, platelets, segmented neutrophils, band neutrophils, lymphocytes, monocytes, eosinophils, alanine transaminase, aspartate transaminase, total protein, creatinine, urea, fibrinogen, D-dimer, C-reactive protein.

Determination of levels of spontaneous and induced NETs. Blood fractionation was performed in a Percoll gradient. Stabilized blood was diluted with 0.9% sodium chloride solution in a ratio of 1:1 and then poured into a preprepared gradient solution of percoll, which consisted of 4 layers with a relative density of 72%, 63%, 54%, and 45%. The concentration of percoll was obtained with a change of 9 parts of percoll and 1 part of 10-fold Hanks' solution (pH 7.0). It is necessary to reach further down to the dilution of 0.9% sodium chloride. The centrifugation of the blood was carried out in two stages:

At 400g for 5 min, for which the first supernatant ball was removed, with an additional amount of 0.9% sodium chloride.

At 800g for 15 min, then blood cells were taken between the density layers: 45 and 54% (monocytes), 54 and 63% (lymphocytes), 63 and 72% (neutrophilic granulocytes).

The neutrophil granulocyte fraction was centrifuged separately at 1200g for 5 min.

The supernatant of the percoll gradient was replaced with culture medium (RPMI-1640 Medium, "Sigma-Aldrich", USA), and the neutrophil precipitate was carefully diluted therein. After washing, the number of cells was counted in Goryaev's chamber. Neutrophil granulocytes were placed in plates, at the bottom of which were prepared slides and RPMI medium (800 µl per well). Cells were placed on round slides (d = 12 mm) at a density of 140000-160000/cm^2 and incubated for three hours at a temperature of 37°C. Isolated neutrophils were divided into control to determine the spontaneous NETosis level (incubation without exposure to additional substances) and induced by incubation (3 h) with the activator of the neutrophil traps formation phorbol-12-myristate-13-acetate (PMA, 20 µmol/l).

Double staining with Hoechst 33342 (1.62 µmol/l) and Propidium Iodide (1 µmol/l) was used to assess the activity of the NETs formation. Neutrophil traps and intact neutrophils were detected and imaged with the fluorescence microscope Nikon Eclipse E200 and camera DS-Fi1.0

Measurement of the concentration of cell-free DNA in blood plasma. The concentration

of cell-free DNA was measured in blood plasma on a Hitachi4000 spectrophotometer using the Quant-iT PicoGreen dsDNA test kit (Invitrogen). Standard λ DNA provided at 100 μg/ml concentration in Quant-iT™ PicoGreen® kits was used to prepare the calibration curve. It was diluted 50 times with a TE buffer to obtain a 2 μg/ml concentration working solution. The DNA concentration was confirmed using an absorption wave of 260 nm (A260) in a cuvette with a path length of 1 cm, A260 0.04 corresponding to 2 μg/ml solution of a double-stranded DNA molecule.

A standard curve was created using five points with 1 ng/ml concentrations to 1 μg/ml, then 1.0 ml of a working aqueous solution of Quant-iT ™ PicoGreen® reagent (after predilution of the effluent 200 times) was added to the cuvette.

Blood plasma was placed into the cuvette of the spectrofluorometer, and 1.0 ml of an aqueous working solution of Quant-iT ™ PicoGreen® reagent was added. The solution was stirred well and incubated for 2–5 min at room temperature in the dark. Fluorescence was measured at the following wavelengths: excitation ~ 480 nm and emission ~ 520 nm. The concentration of free DNA in the samples was recalculated using the constructed calibration curve.

Measurement of the concentration of Human Myeloperoxidase (MPO) in blood plasma. All reagents, working standards, and samples were prepared according to the manufacturer's instructions (Abcam, ab119605). After completing all preparations, 100 μl of prepared standards and diluted samples were added to the appropriate wells. The sealed plate was incubated at 37°C for 90 min. After removing the contents of each well, 100 μl of Biotinylated anti-Human Myeloperoxidase antibody was added to each well, and the plate was sealed and incubated at 37°C for 60 min. After incubation, the plate was washed three times with 300 μl of 0.01 M PBS. Subsequently, 100 μl of Avidin-Biotin-Peroxidase Complex working solution was added to each well, and the plate was incubated at 37°C for 30 min. After that, the plate was washed five times with 0.01 M PBS. Then 90 μl of prepared TMB color-developing agent was added to each well, and the plate was sealed and incubated at 37°C in the dark for 25-30 min. Finally, 100 μl of the prepared TMB Stop Solution was added to each well. O.D. absorbance at 450 nm was read in a microplate reader within 30 min after the addition of the stop solution.

Measurement of the concentration of Human Neutrophil Elastase in blood plasma. To determine the level of Human Neutrophil Elastase, the Direct Detection Sandwich ELISA method was used. First, basic preparations of plasma samples and serial dilution of the standard in reagent diluent were performed. Then, 50 μl of samples were dispensed into the wells, where they were incubated for 2 h at 4°C. After incubation, each well was aspirated and washed with 300 μl of wash buffer. Then 50 μl of Biotinylated Human Elastase Antibody was dispensed into each well, and the plate was incubated for 1 hour at room temperature. After washing, 50 μl of SP Conjugate was added to each well and incubated for 30 min. After washing again with buffer, 50 μl of Chromogen Substrate was added to the appropriate wells. The plate was incubated for 7 min at room temperature or until the optimal blue color density developed. After color development, 50 μl of stop solution was dispensed into each well to stop the enzymatic reaction. As a result, the absorbance of each well was measured using a spectrophotometer/plate reader with the appropriate absorbance setting.

Statistical analysis. Statistical analyses were provided in the R environment (www.R-project.org, V.4.0). Receiver operating characteristic curves (ROC) were calculated by R package "pROC." The area under the ROC curve (AUC) and cut-off values of selected parameters were used to distinguish between mild and severe cases. One-way analysis of variance (ANOVA) was used to compare group means. The equality of variances was analyzed using the Levine test. Tukey HSD was used for multiple

comparisons. When two groups were present, normally distributed data were analyzed by the two-sided t-test, and the Mann–Whitney test analyzed skewed data. The normality of data was tested using Shapiro-Wilk. Pearson's correlation coefficient tested correlations in normally distributed data and Spearman's correlation coefficient in skewed data. The data are presented as Mean ± SEM. SEM is the standard deviation of the mean of random samples drawn from the original population. Statistically, a significant result was considered with $P < 0.05$

RESULTS

Neutrophils isolated from COVID-19 patients produced more NETs in a steady-state (without stimulation) than neutrophils from healthy individuals. In healthy donors, the level of spontaneous NETosis was 1.92%. The level of PMA- stimulated NETosis) was 10.14%. The spontaneous NETosis of patients with COVID-19 in the early period (the first 6-8 days of the disease) was four times higher and reached 7.57%. The induced NETosis increased by 65% and amounted to 16.74% in this group of patients. No statistically significant difference in spontaneous and stimulated NETosis between the groups was observed after dividing patients with COVID-19 into groups according to the severity of the disease (moderate and severe). Representative fluorescence microscopy data in healthy volunteers and patients with COVID-19 are presented in Fig 1.

The level of cfDNA in patients' blood plasma fluctuated substantially from 1650.94 to 30691.73 ng/ml. The cfDNA level was 2489.47 ± 142.62 ng/ml in the group of healthy donors. It was shown that the plasma cfDNA among patients with mild passing was 3054.9 ± 255.94 ng/ml, in the group of patients with moderate passing - 4466.15 ± 286.14 ng/ml, in patients with severe passing 8631.29 ± 983.62 ng/ml. The data presented in this way illustrates a statistically significant difference between the level of free DNA and the severity of COVID-19 (Fig.2).

We used the Direct Detection Sandwich ELISA method to evaluate the level of elastase and MPO in human blood plasma. The level of plasma elastase was 9.68 ± 1.04 ng/ml in the group of healthy donors. It was estimated that the level of plasma elastase among patients with mild passing was 10.59 ± 0.56 ng/ml, in the group of patients with moderate passing was 11.29 ± 0.567 ng/ml, in patients with severe passing was 17.06 ± 1.01 ng/ml. The levels of elastase in the blood plasma of patients with severe COVID-19 show a statistically significant difference from the levels of mild and moderate patients ($P < 0.001$), as well as compared with the levels of elastase in healthy donors (Fig. 3A).

It was shown that the levels of plasma MPO were 4031.85 ± 606.09 ng/ml among patients with mild passing of COVID-19, in the group of patients with moderate passing were 9068.889 ± 699.257 ng/ml in patients with severe passing were 16423.36 ± 1120.57 ng/ml. Thus, the data demonstrate statistically significant differences in MPO levels between all groups of patients with COVID-19 (Fig. 3B).

A correlation analysis was performed between the parameters of free extracellular DNA in the blood plasma of patients and the level of NETosis. The relationship between the level of spontaneous NETosis and the amount of extracellular free DNA is shown in Fig. 4A. It shows the medium strength of correlation significance ($r = 0.4239$, $P < 0.05$). The graph of the correlation between the level of induced NETosis and the concentration of extracellular free DNA is presented in Fig. 4B, which demonstrates the correlation of medium strength ($r = 0.5319$, $P < 0.05$).

Binary logistic regression was used to determine the most critical predictors associated with the risk of severe disease. The basic model, without including any predictors, allowed 57% classification ability. After all clinical factors were added, a classical model with 85

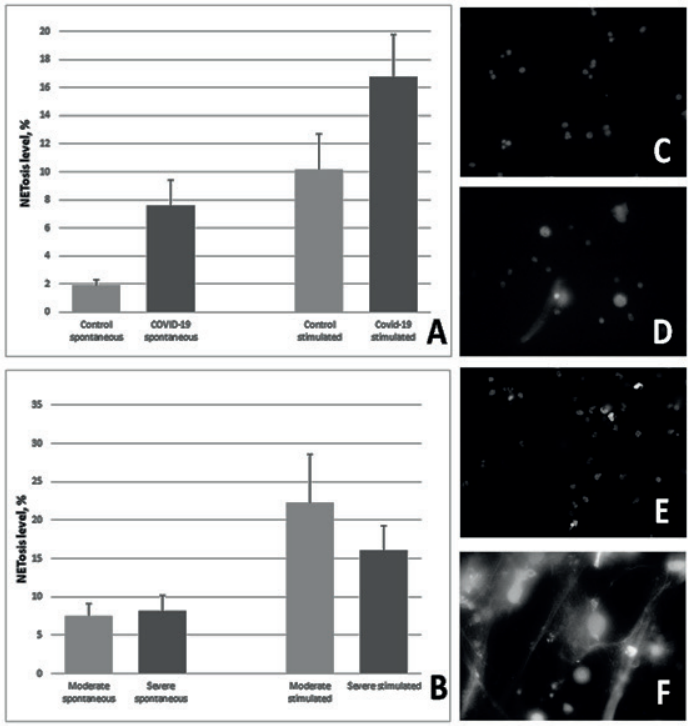

Fig. 1. Spontaneous and induced NETosis of isolated neutrophils. A) NETs formation in the blood of the control group and patients with COVID-19 at an early stage of the disease. The activity of NETs formation in COVID-19 patients was significantly higher at a steady-state and upon stimulation with PMA (20nM). B) NETosis level in patients with different disease severity presented after dividing COVID-19 patients into two groups. No statistically significant difference between the groups was observed in spontaneous and stimulated NETosis. Error bars represent the standard error of the mean. Data were presented as Mean ± SEM. C, D, E, F - Fluorescence microscopy of isolated neutrophils stained with Hoechst 33342 (blue) and Propidium Iodide (red): C) the control patient's neutrophils in a steady state; D) neutrophils of control donors upon stimulation with PMA; E) the COVID-19 patient's neutrophils in a steady-state; F) COVID-19 patient's neutrophils upon stimulation with PMA. *Significant difference from mild severity of the disease ($P < 0.05$). **Significant difference from mild severity of the disease ($P < 0.01$)

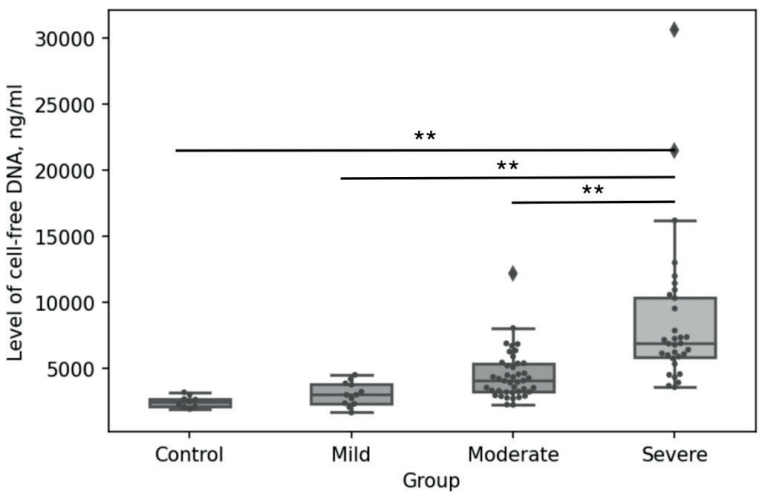

Fig. 2. Plasma from healthy donors and COVID-19 patients was assessed for cell-free DNA. Levels of cell-free DNA in different severities of the disease. **Significant difference from mild severity of the disease ($P < 0.001$)

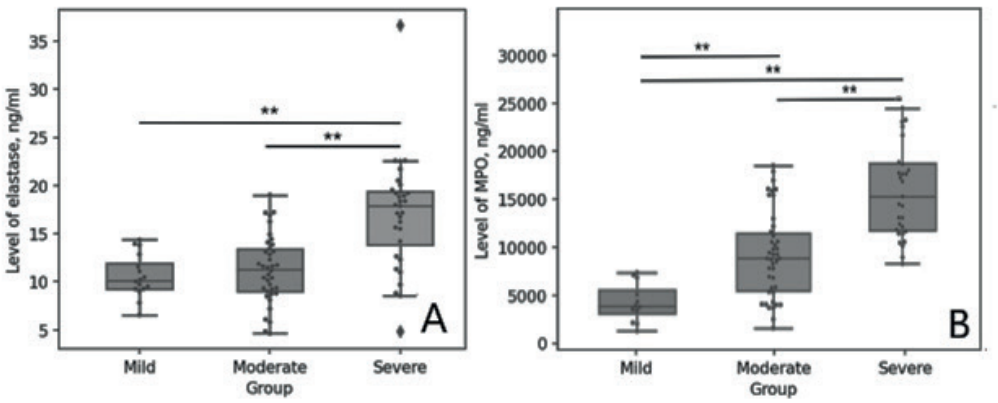

Fig. 3. Plasma from healthy donors and COVID-19 patients was assessed for levels of MPO and elastase. **Significant difference from mild severity of the disease (P < 0.001)

% predictive potential was constructed, which included the following factors: 1) levels of free-cell DNA, ng/ml; 2) glucose; 3) band neutrophils. Thus, after the most significant factors were selected, a model was constructed that has an 85% prediction potential.

To assess the specificity and sensitivity of such a marker as the levels of extracellular free DNA, which can be used to predict the severity of COVID-19 in a high-risk group (patients with obesity, diabetes, hypertension), a ROC Curve was built (Fig. 5). The area under the curve was 85%, making this model reliable. We calculated the cut-off value to determine the value of the marker, which would distinguish and predict the severity of the disease. The value is 4297 ng/ml with a sensitivity of 82% and specificity of 72% (AUC0.856, 95% CI 0.778-0.935, P < 0.001).

DISCUSSION

The primary purpose of this study was to find an answer to the question: "Why do not all high-risk patients have difficulty overcoming SARS-CoV-2?" Patients from the general cohort with elevated plasma NET markers were at higher risk of clinical instability, prolonged length of hospital stay, and 30-day all-cause mortality [14].

Our data illustrates that the severity of coronavirus disease among subjects largely correlates with the initial level of free extracellular DNA. Since plasma collection was performed before the deterioration of patients, but the level of cfDNA in plasma was statistically significantly diverse in different patients at the early period of the disease, this indicator can be used as a prognostic marker in the risk group. It was

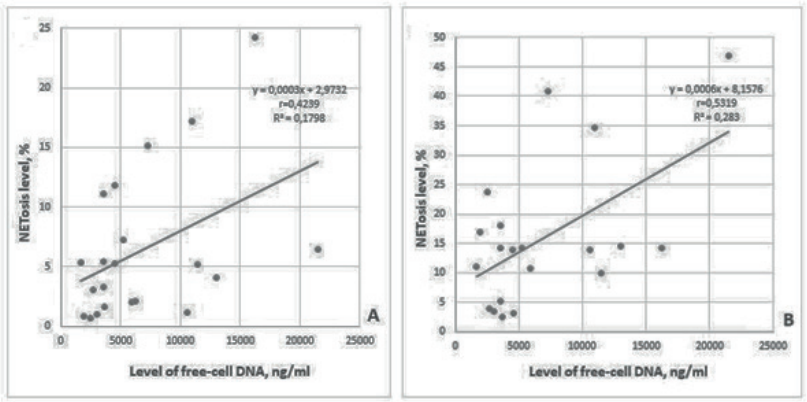

Fig. 4. Dependence between the level of spontaneous (A) and stimulated (B) NETosis and the level of extracellular DNA

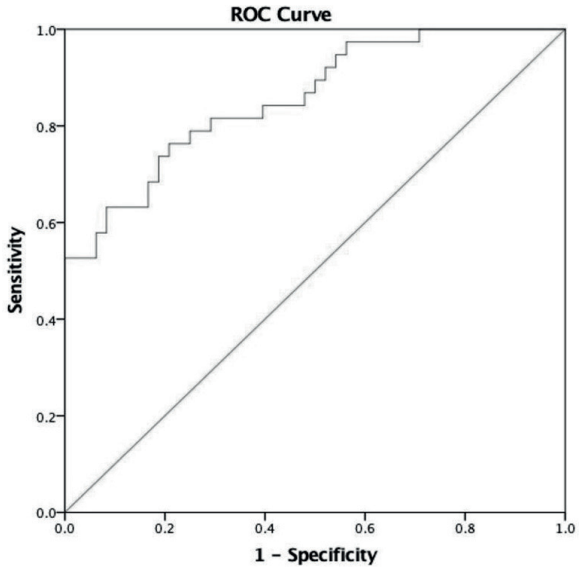

Fig. 5. ROC-curve for determination of specificity and sensitivity of such markers as the concentration of cfDNA in the patient's blood plasma. The cut-off is 4297 ng/ml with a sensitivity of 82% and specificity of 72% (AUC0.856, 95% CI 0.778-0.935, P < 0.001)

confirmed by the created model (Fig. 5). In the study group, at the beginning of the disease, patients with subsequent mild passing had, on average, significantly lower levels of cfDNA in plasma than in the group of patients with severe passing. Since one of the essential factors in the stratification of patients is the saturation of blood with oxygen, it can be argued that the level of cfDNA is associated with the level of saturation. Thus, the level of extracellular free DNA is a vital laboratory indicator evaluation, which allows early screening of patients with SARS-CoV-2 for predicting the future passing of disease severity with oxygen deficiency and early initiation of appropriate therapy.

Interestingly, in seriously ill patients, there is a tendency to decrease the level of stimulated NETosis (Fig. 1B). The severe course of SARS-Cov2 is characterized by the active formation of NETosis in patients' blood. This is evidenced by a significant increase in the level of free DNA in the plasma of this group of patients (Fig. 2); it can be assumed that a relatively large part of neutrophils formed NETs in vivo. Also, the severe course of COVID-19 is accompanied by an acute inflammatory process in the lungs. This suggests a massive migration of activated neutrophils to the areas of inflammation, on the one hand, and active granulopoiesis, on the other hand. In the blood of such patients, we recorded an increase in stab neutrophils by 3-4 times. Accordingly, stab neutrophils cannot form NETosis. Thus, the above resulted in a decrease in the number of neutrophils capable of responding to in vitro stimulation in patients with COVID-19 already at an early stage of the disease.

However, it is essential to consider what happens to the extracellular free DNA. Three options are most likely: thrombus formation, autoimmune damage, and activation of membrane receptors of immunocompetent cells.

The mechanisms by which NETs are formed were presented, and the physiological and pathophysiological consequences of NETs formation were discussed in Sørensen's and Borregaard's review [23]. They concluded that NETs might be more critical in autoimmunity and thrombosis than innate immune defense. Thus, NETs triggering immunothrombosis may explain the prothrombotic clinical presentation in COVID-19, and NETs may represent targets for therapeutic intervention. The thrombosis patients also had higher levels of D-dimer, C-reactive protein, ferritin, and platelets, but not troponin or neutrophils [5]. Finally, there were strong associations between markers of hyperactive neutrophils (calprotectin and cell-free DNA) and D-dimer. Elevated levels of neutrophil activation and NET formation in a group of patients hospitalized with COVID-19 are associated with a higher risk of morbid thrombotic complications [24].

Thus, Kostyuk showed that cfDNA, enriched on GC islands, can cause short-term oxidative stress, increased expression of some proapoptotic genes [25], activation of Toll like receptor 9 and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathways [26]. In addition, there is a hypothesis that cfDNA leads to the development of a cytokine

storm, damage to endothelial cells and various organs, and causes ARDS [27] and thrombosis [17].

Apel et al. found that macrophages phagocytosed NETs, components of which translocated from phagolysosomes to the cytosol. In the cytosol, the DNA backbone of the NETs stimulated the innate immune sensor cyclic guanosine monophosphate–adenosine monophosphate, leading to type I interferon secretion both in vitro and in vivo. NETs stimulated IFN production in a cGAS-dependent manner, suggesting that cGAS acts as a sensor of NETs, enabling immune responses during infection [28]. Altogether, it can be argued that the levels of free-cell DNA in the blood plasma are not only a marker of the severity of the course of the disease of COVID-19 but also an essential pathogenetic factor.

CONCLUSIONS

Our data illustrates that the severity of coronavirus disease among patients largely correlates with the initial cell-free DNA amount in plasma, the number of NETs and band neutrophils, and glucose levels. The cfDNA levels in the early period of the disease were statistically significantly different in the subsequent course of the disease in patients with diabetes. It was estimated that the cfDNA cut-off value (4297 ng/ml) showed severe passing of disease in patients with COVID-19 from the high-risk group to have 82% sensitivity and 72% specificity (AUC0.856, 95% CI 0.778-0.935). We discovered an association between circulating markers of NET formation and clinical outcome, demonstrating a potential role of cfDNA levels in clinical decision-making and a likely necessity to consider NETs or cfDNA reception as targets for novel therapeutic interventions in COVID-19.

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The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

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ВМІСТ ПОЗАКЛІТИННОЇ ДНК У ПЛАЗМІ ЯК РАННІЙ МАРКЕР ТЯЖКОСТІ ГОСПІТАЛЬНОГО ПЕРЕБІГУ COVID-19 У ПАЦІЄНТІВ ІЗ ДІАБЕТОМ 2-ГО ТИПУ ТА ОЖИРІННЯМ

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Прогресування COVID-19 значно відрізняється у різних людей; ступінь тяжкості та смертності корелюють з наявним діабетом 2-го типу, ожирінням та гіпертонією. Однак не для всіх пацієнтів із груп ризику характерний важкий перебіг хвороби. метою нашої роботи було знаходження точних і швидких прогностичних показників. У дослідженні використовувалися зразки плазми 103 пацієнтів і добровольців. Проведено ретроспективний аналіз 93 пацієнтів з COVID-19, класифікованих за ступенем тяжкості хвороби на легку (n = 14), середню (n = 42) і важку (n = 37). Розподіл по групах був проведений після закінчення лікування хворих. Важкість перебігу COVID-19 оцінювали ретроспективно на момент виписки з лікарні. На догоспітальному етапі лікування глюкокортикостероїдами не призначалося. Проведено клінічний аналіз та кількісне визначення позаклітинної ДНК (пкДНК), позаклітинних пасток нейтрофілів, концентрацію людської мієлопероксидази (МПО) та нейтрофільної еластази людини в плазмі крові. Наші результати показують, що тяжкість коронавірусної хвороби серед суб'єктів значною мірою корелює з початковим вмістом пкДНК у плазмі та активністю утворення позаклітинних пасток нейтрофілів. Вміст пкДНК у плазмі крові в ранній період захворювання статистично вірогідно відрізнявся від подальшого перебігу захворювання у хворих на ЦД. Значення пкДНК 4297 нг/мл і вище відповідало більш тяжкому перебігу захворювання у пацієнтів із COVID-19 із групи високого ризику з 82%-ю чутливістю та 72%-ю специфічністю (AUC0,856, 95% ДІ 0,778-0,935). Вміст еластази в плазмі крові пацієнтів з тяжким перебігом COVID-19 демонструє статистично значущу різницю з рівнем пацієнтів з легким та середнім

ступенем тяжкості, а також порівняно зі значеннями у здорових донорів. Крім того, спостерігалися вірогідні відмінності вмісту МПО між усіма групами пацієнтів з COVID-19. Це свідчить про потенційну важливість оцінки вмісту пкДНК у плазмі крові для прийняття клінічних рішень на ранніх стадіях захворювання.

Ключові слова: позаклітинна ДНК; нейтрофіли; COVID 19; позаклітинні пастки нейтрофілів; діабет; пре диктори.

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