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Cell-free nucleic acids as a non-invasive biomarker for predicting COVID-19 disease severity and outcome: a retrospective cohort study

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Abstract

Background: The COVID-19 pandemic highlighted the need for novel biomarkers to identify patients at risk of developing severe disease. Circulating cell-free nucleic acids (cfNAs) are released from injured host cells or can be derived from pathogens. As cfNA is suggested to be a useful biomarker in oncologic, autoimmune, and other diseases, the aim of our study was to investigate the role of serum cfNA in predicting the course and outcome of COVID-19 disease.

Methods: We conducted a retrospective cohort study at Vilnius University Hospital Santaros Klinikos, utilizing serum samples collected upon admission

and accompanying health information obtained from the Vilnius Santaros Klinikos Biobank. A total of 108 adult COVID-19 patients hospitalized between November 24, 2020, and November 10, 2021, and 24 healthy controls were enrolled. cfDNA concentration was measured using capillary electrophoresis. cfRNA was detected using quantitative real-time PCR.

Results: cfDNA concentration was higher in COVID-19 patients compared with controls (4.28 vs. 0.51 ng/µL, $p<0.001$) and increased with disease severity: from 1.06 ng/µL in mild to 2.65 ng/µL in severe, and 6.68 ng/µL in critical disease. cfDNA levels were higher in intensive care unit (ICU) patients (6.68 vs. 2.30 ng/µL, $p<0.001$), patients requiring advanced respiratory support (ARS) (7.11 vs. 2.74 ng/µL, $p<0.001$), and non-survivors (16.68 vs. 3.44 ng/µL, $p<0.001$). cfDNA showed high predictive values for ICU admission (AUC 0.79), ARS (AUC 0.77), and lethal outcome (AUC 0.81), outperforming other routine biomarkers. In logistic regression analysis, cfDNA remained an independent predictor for ICU admission (OR 1.21, 95%CI 1.08–1.36), ARS requirement (OR 1.15, 95%CI 1.03–1.28), and in-hospital mortality (OR 1.08, 95%CI 1.02–1.14), while serum SARS-CoV-2 RNAemia remained an independent predictor of ARS requirement (OR 4.18, 95%CI 1.15–15.20).

Conclusions: Higher serum cfDNA concentrations were independently associated with greater COVID-19 disease severity, increased likelihood of requiring intensive care, ARS, and in-hospital mortality. cfDNA demonstrated superior prognostic performance, surpassing established biomarkers. Serum SARS-CoV-2 RNAemia was associated with the need for ARS. This indicates that cfNAs may serve as clinically valuable biomarkers for early risk stratification and outcome prediction in COVID-19 patients. Further validation in independent cohorts is required to confirm generalizability.

Keywords: serum cell-free NA, serum cell-free DNA, serum cell-free RNA, cfDNA, cfRNA, cfNA, SARS-CoV-2 RNAemia, COVID-19, COVID-19 severity predictor, COVID-19 biomarker.

Background

The coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in more than 7 million deaths worldwide as of January 26, 2026, since its emergence in 2019 [1]. Despite advances in the understanding of COVID-19 pathophysiology, predicting disease progression and clinical outcomes remains challenging due to marked heterogeneity in clinical presentation, ranging from asymptomatic infection to severe pneumonia, acute respiratory distress syndrome, multiple organ dysfunction syndrome, or death [2]. Early and accurate risk stratification of hospitalized patients is therefore essential to guide clinical decision-making and optimize the use of healthcare resources. A wide range of prognostic markers has been investigated, including demographic characteristics, comorbidities, and routinely available laboratory parameters such as C-reactive protein (CRP), ferritin, lactate dehydrogenase (LDH), D-dimers [3–5], creatinine, urea [6,7], and troponin I [8–10]. While these biomarkers are widely used in everyday clinical practice, they lack specificity for COVID-19 infection, and their optimal threshold values for predicting its course remain insufficiently defined [3–5].

In recent years, increasing attention has been given to novel biomarkers reflecting the underlying pathophysiological mechanisms of COVID-19, including those identified through advanced analytical approaches such as metabolomics, proteomics, and genomics [11–15]. These approaches have

provided insights into molecular alterations associated with disease severity and clinical outcomes. However, their application in routine clinical practice remains limited, underscoring the need for accessible and reliable biomarkers suitable for clinical use.

In this context, circulating cell-free nucleic acids (cfNAs), including cell-free DNA (cfDNA) and cell-free RNA (cfRNA), have emerged as promising minimally invasive biomarkers that reflect both tissue and underlying disease process and are increasingly being widely investigated across various fields of medicine [16–18]. cfDNA and cfRNA refer to extracellular fragments of DNA and RNA, respectively, that can be found in many body fluids during both physiological and pathological processes [19,20]. cfDNA is released from cells into the blood circulation upon their death, either through apoptosis (programmed cell death), necrosis, or NETosis (pathogen-induced cell death, which includes the release of neutrophil extracellular traps (NETs)), or during active secretion processes [20,21]. In the infectious disease scenario, despite the host releasing cfNAs, pathogen-derived cfNAs can also be a source of blood cfNAs [22]. Recent studies suggest that cfNA can be a useful biomarker in the investigation and monitoring of patients with oncologic [23,24] and autoimmune diseases [25,26], as well as after transplantation [27,28] or severe trauma [29]. In the infectious disease context, elevated cfDNA levels are found in sepsis [30,31], and increased concentration of cell-free mitochondrial DNA (cf-mtDNA) is common in people living with HIV (PLWH) [32].

Individual studies suggest that plasma cfDNA concentrations are higher in COVID-19 cases, which may be related to a poor prognosis [33,34]. As data on the importance of cfNAs in predicting COVID-19 disease severity continue to

accumulate, the aim of our study was to investigate the role of serum cfNA in predicting the course and outcome of COVID-19 disease.

Methods

Study design and participants

This cohort study using retrospective samples and accompanying health information was conducted at Vilnius University Hospital Santaros Klinikos. All participants included in this study had signed informed written consent forms for biobanking at Vilnius Santaros Klinikos Biobank. COVID-19 positive patients were hospitalized in Vilnius University Hospital Santaros Klinikos between November 24, 2020, and November 10, 2021. Pseudonymized participants' health information and serum samples taken on admission were obtained from Vilnius Santaros Klinikos Biobank.

Inclusion criteria of the COVID-19 positive group were: patients ≥ 18 years of age; diagnosed with COVID-19 confirmed by SARS-CoV-2 real-time quantitative polymerase chain reaction (RT-qPCR) or by a rapid antigen test; during the COVID-19 disease episode were hospitalized at Vilnius University Hospital Santaros Klinikos; had signed a written, informed consent form for participation in the activity of Vilnius Santaros Klinikos Biobank; and their residual blood samples were stored in the Vilnius Santaros Klinikos Biobank.

Criteria for the inclusion of the control group were: individuals ≥ 18 years of age; had signed a written, informed consent form for participation in the activity of Vilnius Santaros Klinikos Biobank; on the day of the blood collection, the person did not have any respiratory symptoms characteristic of COVID-19 infection; and their blood samples were stored in the Vilnius Santaros Klinikos Biobank.

Exclusion criteria were: patients with oncological or haematological diseases, patients after organ transplantation, PLWH, and vulnerable persons specified in the law on Ethics of Biomedical Research of the Republic of Lithuania.

Study outcomes and groups

In this study, participants were divided into two groups: the COVID-19 positive group and the control group (according to the inclusion criteria detailed in the "Study design and population" section).

The COVID-19 positive patients were divided into subgroups based on study outcomes: COVID-19 disease severity (mild/severe/critical) and COVID-19 disease outcome (survivors/non-survivors). A mild COVID-19 course was described as a COVID-19 course that did not meet the criteria of severe and critical COVID-19; a severe COVID-19 course – a COVID-19 course requiring any oxygen therapy but without the need for treatment in the intensive care unit (ICU); a critical COVID-19 course – a COVID-19 course requiring treatment in the ICU.

During the statistical analysis, the COVID-19 positive patient group was also divided into the following subgroups: mild and severe vs. critical disease course (non-ICU group vs. ICU group), and advanced respiratory support (ARS) group (patients who received non-invasive ventilation (NIV), invasive mechanical ventilation (IMV), or extracorporeal membrane oxygenation (ECMO)) vs. no ARS group.

Blood serum samples preparation

In the Biobank, gel vacutainers containing blood samples were centrifuged for 8 minutes at 3600 g (relative centrifugal force, RCF). Subsequently, 500 µL of serum was aliquoted into separate tubes (one or two aliquots depending on the serum volume), encoded, and stored in a freezer at -80 °C. Haemolysis or

leukocyte lysis was not documented. Samples were thawed only once and used immediately for cfNA extraction.

cfNA extraction from blood serum

cfNA was extracted using QIAamp Circulating Nucleic Acid Kit following the 1 mL blood serum extraction protocol according to the manufacturer's recommendations. The nucleic acids were eluted with 50 µL of the provided elution buffer (Sample to Insight 16 QIAamp ® Circulating Nucleic Acid Handbook 2019). The extracted cfNA samples were coded using letter and number combinations and then stored in a -80 °C freezer for further analysis.

cfDNA capillary electrophoresis

cfDNA samples were analysed by Agilent Technologies high-resolution automated DNA electrophoresis in the Bioanalyzer 2100 system using the Agilent DNA 1000 kit. If the chip could not measure the samples due to small concentrations of nucleic acids, the High Sensitivity DNA kit was used.

Quantitative PCR

To detect cell-free SARS-CoV-2 RNA (cfRNA) in extracted samples, the Quantitative PCR method was used in the QuantStudio Real-Time PCR System. The total volume of each reaction component was calculated according to the TaqPath 1-Step Multiplex Master Mix protocol. A single-reaction mixture consisted of: (i) 6.25 µL 4X TaqPath Master Mix; (ii) 1.25 µL Assay Multiplex; (iii) 12.5 µL nuclease-free water.

Then 5 µL of the cfNA sample was pipetted into each well. For the positive control, 5 µL of the C24 SARS-CoV-2 genome was used; for the negative control, 5 µL of H_2O. Total reaction volume 25 µL. The first stage of the quantitative PCR method involved incubating uracil-N-glycosylase (UNG) for one cycle, 2 minutes, at a temperature of 25 °C. The second reverse transcription – 1 cycle,

10 minutes, 53 °C, the third polymerase activation – 1 cycle, 2 minutes, 95 °C, the fourth amplification, which lasted for 40 cycles, 3 seconds at 95 °C and 30 seconds at 60 °C. The results were analysed using the Thermo Fisher Scientific QuantStudio design and analysis tool.

Long-range PCR

To determine the integrity of SARS-CoV-2 RNA in extracted samples, the Long-range PCR method was used on the QuantStudio Real-Time PCR System. The total primer mixture for one sample consisted of: (i) 1.4 µL purified cfDNA sample; (ii) 2 µL 50 µM oligo(dT)20; (iii) 5.4 µL nuclease-free water.

Then 7.4 µl of the prepared primer mix was aliquoted into PCR strip tubes. Afterwards, 4 µL of the cfNA sample was pipetted into each PCR strip tube: for the positive control, 4 µL of C24 SARS-CoV-2 genome was used; for the negative control, 4 µL of H_2O. The prepared PCR tubes were placed in the ProFlex PCR system for 3 minutes at 95 °C and then at 4 °C. In the meantime, the enzyme mixture was prepared, the components of which for one reaction consisted of: (i) 1.4 µL 5X reverse transcriptase buffer; (ii) 1 µL 10mM (each) dNTPs; (ii) 0.25 µL reverse transcriptase; (iii) 2 µL dithiothreitol (DTT); (iv) 1.35 µL nuclease-free water.

After the incubation, PCR strip tubes and enzyme mixes were spun for 10 seconds. Afterwards, 8.6 µL of the prepared enzyme mix was pipetted into each PCR strip tube and mixed by pipetting 3 times. The prepared PCR strip tubes were placed in the ProFlex PCR system for 1 hour at 48 °C.

Following the incubation in the ProFlex PCR system, the reaction mixture was used in quantitative PCR, as described in the ‘Quantitative PCR’ section above. The stages of quantitative PCR were as follows: (i) 1 cycle for 2 minutes at 95 °C; (ii) 40 cycles for 3 seconds at 95 °C, and 30 seconds at 60 °C.

The schematic overview of the study cohort, sample processing, and analytical workflow is shown in Figure 1.

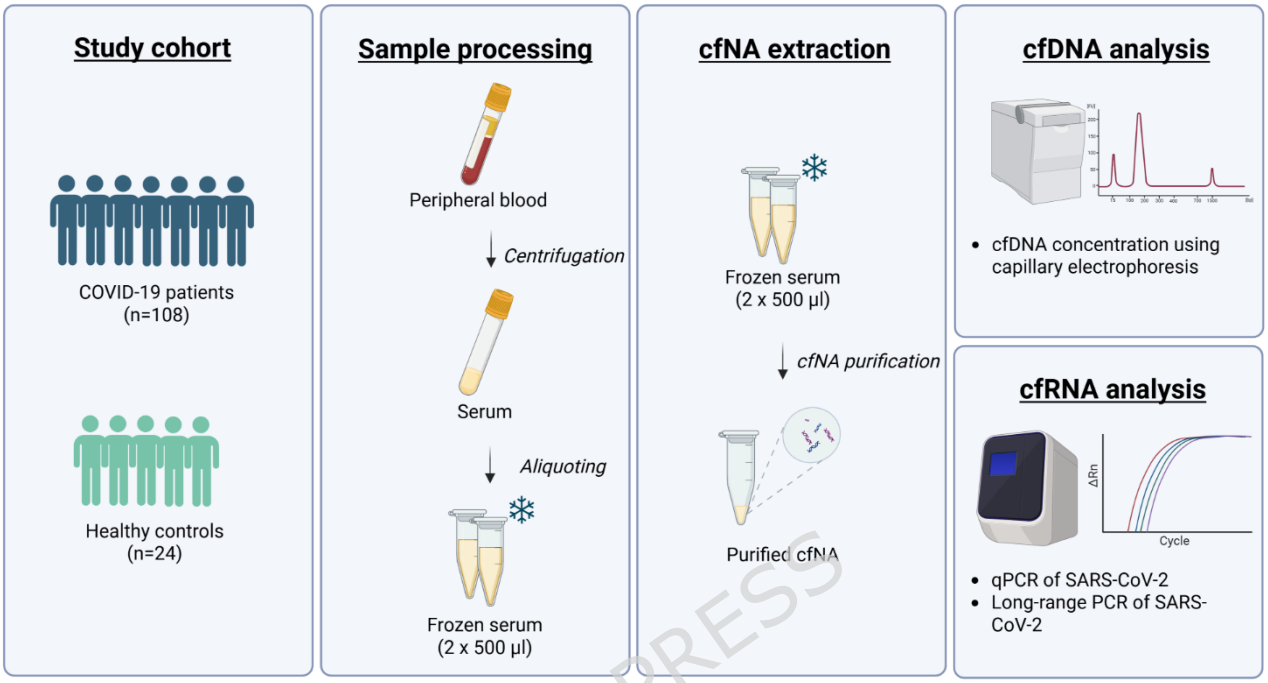

Figure 1. Schematic overview of study cohort, sample processing, and analytical workflow. Created in BioRender. Juozapaite, D. (2026) https://BioRender.com/s0moadk.

Health data collection

Data related to the participants were collected from the Vilnius University Hospital Santaros Klinikos electronic health record system and obtained from the Vilnius Santaros Klinikos Biobank.

The following information was collected for the COVID-19 positive participants: demographic data included age in years and sex (male/female). Comorbidities were recorded as categorical variables and included arterial hypertension, coronary artery disease, congestive heart failure, atrial fibrillation, previous myocardial infarction, chronic obstructive pulmonary disease (COPD), obesity,

diabetes mellitus, chronic kidney disease, and sequelae of prior stroke. Weight and height were also collected (in kilograms and metres, respectively), from which the body mass index (BMI) was calculated. Participants were defined as obese if they had a BMI ≥ 30 kg/m^2 or a diagnosis of obesity was noted in their medical records. Moreover, the data on complications developed during the hospitalization were obtained and included pneumonia, acute respiratory failure, pulmonary embolism, deep vein thrombosis (DVT), stroke, acute kidney injury, and sepsis. We also extracted data on the need for any oxygen therapy, including NIV, IMV, and ECMO. The length of stay (in days) and the result of hospitalization (survivors/non-survivors) data were also collected. Furthermore, the results of the following laboratory tests performed on the first day of hospitalization were extracted from the system: white blood cell count (WBC); neutrophils, lymphocytes, haemoglobin, platelets, sodium, potassium, glucose, creatinine, urea, alanine aminotransferase (ALT), aspartate aminotransferase (AST), CRP, LDH, interleukin-6 (IL-6), ferritin, fibrinogen, D-dimer, troponin I, and N-terminal pro-B-type natriuretic peptide (NT-proBNP).

For the control group, we collected data on sex and age that were available in the Biobank dataset.

Statistical analysis

Descriptive statistics were used to characterize baseline demographic, clinical, laboratory, and outcome data. Continuous variables were presented as medians with interquartile ranges (IQR), and categorical variables were presented as absolute numbers and percentages. The Shapiro–Wilk test was used to assess the normality of continuous variables, and most variables did not meet the assumption of normal distribution.

For continuous variables, group comparisons were performed using the Mann–Whitney U test or the Kruskal–Wallis test, followed by Dunn's post-hoc pairwise comparisons with Bonferroni correction where appropriate. To account for the age difference between the control group and patients with COVID-19, an age-adjusted analysis was performed using a general linear model with log10-transformed cfDNA as the dependent variable, group as the fixed factor, and age as a covariate. Log transformation was applied to reduce the skewness of cfDNA values. Comparisons of categorical variables were made using the χ^2 test or Fisher's exact test. Associations between cfDNA concentrations and laboratory parameters were evaluated using Spearman's rank correlation coefficient.

The predictive performance of cfDNA and other laboratory parameters for critical COVID-19, the need for ARS, and in-hospital mortality was assessed using receiver operating characteristic (ROC) curve analysis. Laboratory parameters that differed significantly between groups were included in the ROC analysis. Optimal cut-off values, along with their corresponding sensitivity and specificity, were determined using the Youden index.

To identify independent predictors of critical COVID-19, the need for ARS, and in-hospital mortality, multivariable binary logistic regression models were constructed, including age, sex, obesity, presence of any comorbidity, SARS-CoV-2 RNA positivity, cfDNA, and laboratory variables showing $p < 0.05$ in univariable regression analysis as covariates. Odds ratios (ORs) with 95% confidence intervals (CIs) were reported. A p-value < 0.05 was considered statistically significant.

Missing data were handled using a complete-case approach. Because the study was retrospective and laboratory tests were performed according to routine

clinical practice, some laboratory parameters were not available for all patients. Cases with missing values were excluded from the respective analyses. ROC and multivariable regression analyses were restricted to variables with less than 20% missing data.

All analyses were performed using IBM SPSS Statistics, version 30.0 (IBM Corp., USA). Figure 1 was created in the BioRender Services (see the legend of Figure 1).

Results

Baseline characteristics

A total of 132 individuals were enrolled in this study: 108 patients with confirmed COVID-19 and 24 controls. The control group consisted of healthy men with a median age of 30.5 years (IQR 23.25–38.75).

Baseline characteristics of COVID-19 patients are summarized in Table 1. The median age of the COVID-19 positive group was 59 years (IQR 49–66), and 49.1% were male. The most common comorbidities were arterial hypertension (60.2%), obesity (48.2%), diabetes mellitus (16.7%), and atrial fibrillation (11.1%). Less frequent conditions included congestive heart failure (9.3%), chronic kidney disease (7.4%), and coronary artery disease (2.8%).

Pneumonia was diagnosed in 94.4% of hospitalized patients. NIV was required in 37.0% of COVID-19 cases, 9.2% required IMV, and 5.6% ECMO. The median length of hospital stay was 16 days (IQR, 10–26), and the in-hospital mortality rate was 16.7%. SARS-CoV-2 qPCR in serum was positive in 31 patients (27.7%). The median cfDNA concentration was 4.28 ng/μL (IQR 1.62–8.78). Laboratory parameters of COVID-19 patients obtained on the first day of hospitalization are presented in Table 2.

Table 1. Baseline characteristics, comorbidities, and complications of hospitalized COVID-19 patients.

Characteristic	N	Value
Age in years, median (IQR)	108	59.0 (49.0–66.0)
Male, %	108	53 (49.1%)
BMI, kg/m^2, median (IQR)	72	32.6 (27.8–36.4)
Comorbidities, N (%)		
Arterial hypertension	108	65 (60.2)
Coronary artery disease	108	3 (2.8)
Congestive heart failure	108	10 (9.3)
Atrial fibrillation	108	12 (11.1)
Previous myocardial infarction	108	2 (1.9)
Obesity	108	52 (48.2)
Diabetes mellitus	108	18 (16.7)
Chronic kidney disease	108	8 (7.4)
Sequelae of stroke	108	1 (0.9)
COPD	108	0 (0.0)
Complications of COVID-19, N (%)		
Pneumonia	108	102 (94.4)
Acute respiratory failure	108	54 (50.0)
Pulmonary embolism	108	1 (0.9)
DVT	108	1 (0.9)
Stroke	108	1 (0.9)
Acute kidney injury	108	8 (7.4)

Sepsis	108	11 (10.2)
Respiratory support and hospitalization outcomes		
Non-invasive ventilation, N (%)	108	40 (37.0)
Invasive mechanical ventilation, N (%)	108	10 (9.2)
Extracorporeal membrane oxygenation, N (%)	108	6 (5.6)
Length of stay in hospital, days	108	16.0 (10.0–26.0)
In-hospital mortality, N (%)	108	18 (16.7)

BMI – body mass index; COPD – chronic obstructive pulmonary disease; DVT – deep vein thrombosis; IQR – interquartile range.

Table 2. Laboratory parameters of COVID-19 patients on the day of hospitalization.

Laboratory parameter	N	Value, median (IQR)
WBC, $x10^9$/L	108	5.75 (4.34–7.68)
Neutrophils, $x10^9$/L	108	4.74 (3.37–5.84)
Lymphocytes, $x10^9$/L	108	0.95 (0.59–1.20)
Haemoglobin, g/L	108	141.50 (127.00–151.00)
Platelets, $x10^9$/L	108	190.00 (144.25–254.75)
Sodium, mmol/L	108	139.00 (135.25–141.00)
Potassium, mmol/L	108	4.00 (3.80–4.48)
Glucose, mmol/L	67	8.20 (6.50–9.90)
Creatinine, µmol/L	88	75.00 (61.00–85.75)
Urea, mmol/L	104	5.70 (4.33–8.18)
ALT, U/L	107	35.00 (26.00–53.00)

AST, U/L	107	43.00 (30.00–62.00)
CRP, mg/L	108	95.45 (50.60–144.98)
LDH, U/L	98	389.00 (300.00–501.75)
IL-6, ng/L	89	58.80 (33.85–88.75)
Ferritin, µg/L	101	856.00 (473.50–1,846.50)
Fibrinogen, g/L	74	5.71 (4.87–6.89)
D-dimer, µg/L	102	565.00 (318.75–882.50)
Troponin I, ng/L	95	10.00 (6.00–21.00)
NT-proBNP, ng/L	89	237.00 (124.00–561.00)
SARS-CoV-2 positive by qPCR in serum, N (%)	108	31 (28.7)
cfDNA in serum, ng/µL	108	4.28 (1.62–8.78)

ALT – alanine aminotransferase; AST – aspartate aminotransferase; cfDNA – cell-free DNA; CRP – C-reactive protein; IL-6 – interleukin 6; IQR – interquartile range; LDH – lactate dehydrogenase; NT-proBNP – N-terminal pro-B-type natriuretic peptide; qPCR – quantitative polymerase chain reaction; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2; WBC – white blood cell.

Associations between cell-free DNA levels and COVID-19 disease severity and outcomes

A total of 108 COVID-19 positive patients were divided into three groups according to COVID-19 severity: 12 with mild, 45 with severe, and 51 with critical disease. The groups were comparable in age and sex distribution ($p > 0.05$). The prevalence of obesity increased with disease severity, being 16.7%

in mild, 44.4% in severe, and 58.8% in critical cases (p = 0.009 for mild vs. critical). There were no significant differences between groups in the prevalence of other concomitant diseases (Additional file 1).

cfDNA levels increased markedly with disease severity: from 1.06 ng/µL in mild to 2.65 ng/µL in severe, and 6.68 ng/µL in critical disease groups (p < 0.001 compared severe and critical groups to the mild disease group) (Figure 2A, Additional file 1).

cfDNA was also measured in a control group. Median cfDNA concentration in healthy controls was 0.51 ng/µL (IQR 0.27–0.75), which was significantly lower compared with patients with COVID-19 (0.51 vs. 4.28 ng/µL, p < 0.001), including those with severe (0.51 vs. 2.65 ng/µL, p < 0.001) and critical disease course (0.51 vs. 6.68 ng/µL, p < 0.001) (Figure 2B). Because the control group was younger than the COVID-19 cohort, an age-adjusted analysis was performed. After adjustment for age, cfDNA levels remained significantly higher in COVID-19 patients compared with controls (p = 0.018).

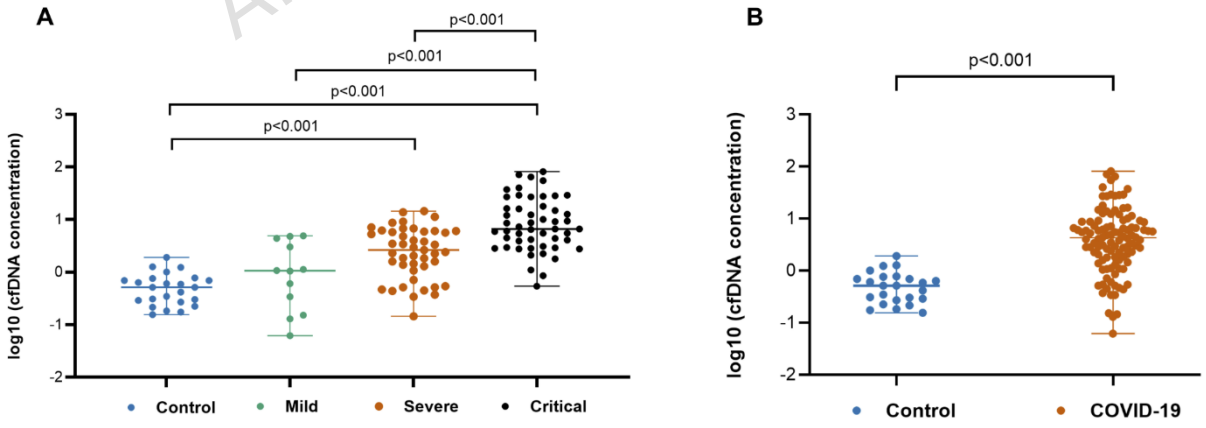

Figure 2. Comparison of cfDNA concentrations among healthy controls and COVID-19 patients by disease severity (A) and healthy controls and COVID-19 patients (B).

cfDNA concentrations are shown on a log10-transformed scale.

A total of 51 patients had critical COVID-19 and required treatment in the ICU. Age and the prevalence of comorbidities did not differ significantly between patients who required and those who did not require ICU care, except for obesity, which was more common among ICU patients (58.8% vs. 38.6%, $p = 0.036$). Patients admitted to the ICU demonstrated higher levels of tissue injury and inflammation biomarkers, including AST (53.0 vs. 41.0 U/L, $p = 0.013$), LDH (415.0 vs. 355.0 U/L, $p = 0.046$), ferritin (1,085.0 vs. 633.0 µg/L, $p = 0.021$), and urea (6.16 vs. 5.21 mmol/L, $p = 0.023$), compared with non-ICU patients. cfDNA levels were markedly elevated in the ICU group, reaching 6.68 ng/µL versus 2.30 ng/µL in non-ICU patients ($p < 0.001$) (Figure 3, Additional file 2).

A total of 45 patients required ARS: NIV, IMV, or ECMO. Age and the prevalence of comorbidities did not differ significantly between groups. Patients who required ARS showed higher neutrophil counts (5.19 vs. 4.50 $\times 10^9$/L, $p = 0.034$), elevated ALT (39.5 vs. 32.0 U/L, $p = 0.022$), AST (54 vs. 40 U/L, $p < 0.001$), and LDH (463.5 vs. 346.5 U/L, $p = 0.008$) on admission day. cfDNA levels on admission were significantly higher in the ARS group compared with those who did not require ARS during hospitalization (7.11 vs. 2.74 ng/µL, $p < 0.001$) (Figure 3, Additional file 3).

Eighteen patients (16.7%) died during hospitalization, while 90 (83.3%) survived. Non-survivors were older than survivors (64.0 vs. 57.5 years, $p = 0.028$), and sex distribution did not differ significantly between groups. The prevalence of comorbidities was generally similar between groups, except for congestive heart failure, which was more common among non-survivors (27.8% vs. 5.6%, $p = 0.011$). Severe complications occurred more often in non-survivors, including acute respiratory failure (72.2% vs. 45.6%, $p = 0.039$),

acute kidney injury (27.8% vs. 3.3%, p = 0.003), and sepsis (33.3% vs. 5.6%, p = 0.003). Non-survivors required NIV (72.2% vs. 30.0%, p = 0.001) and IMV (38.9% vs. 6.7%, p = 0.001) significantly more frequently than survivors. Laboratory analyses on admission showed higher total white blood cell (6.99 vs. 5.47 $\times 10^9$/L, p = 0.015) and neutrophil (5.41 vs. 4.52 $\times 10^9$/L, p = 0.018) counts, serum potassium (4.25 vs. 3.97 mmol/L, p = 0.042), worse renal function markers, including higher creatinine (88.3 vs. 71.3 µmol/L, p = 0.037), and urea (7.15 vs. 5.46 mmol/L, p = 0.021), and elevated NT-proBNP levels (532 vs. 224 ng/L, p = 0.025). cfDNA concentrations were markedly higher in non-survivors compared with survivors (16.68 vs. 3.44 ng/µL, p < 0.001) (Figure 3, Additional file 4).

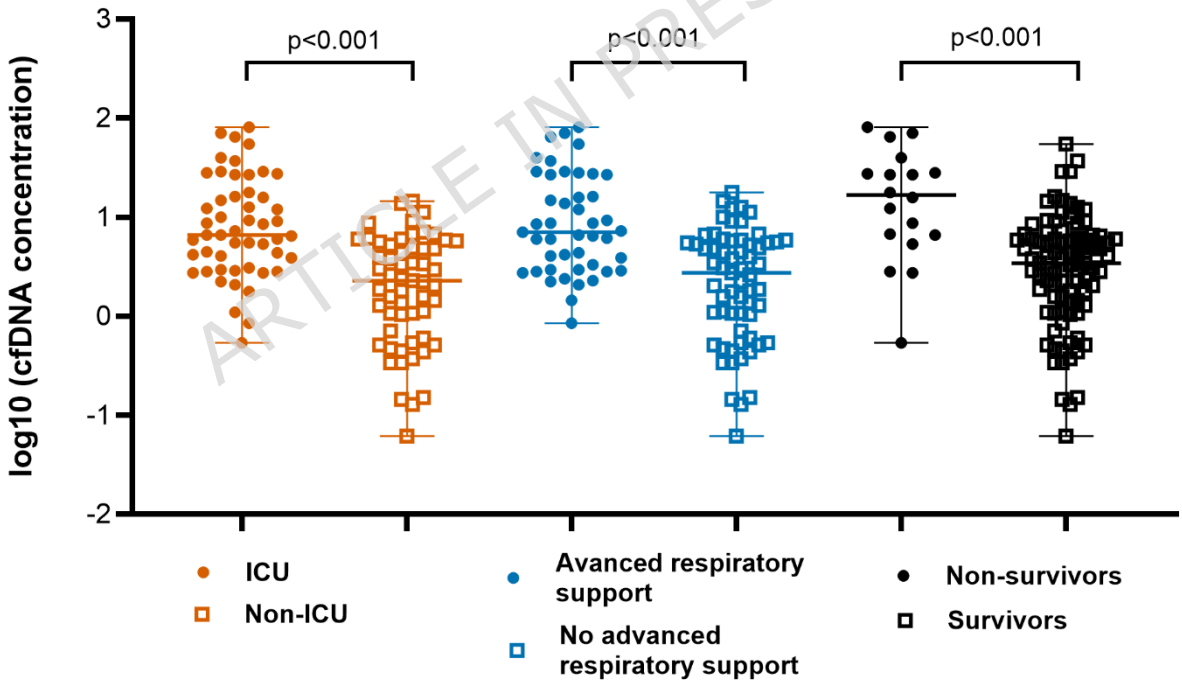

Figure 3. cfDNA concentrations in relation to ICU treatment, the need for advanced respiratory support, and survival in hospitalized COVID-19 patients.

cfDNA concentrations are shown on a log10-transformed scale.

Associations of cfDNA with laboratory parameters and serum SARS-CoV-2 RNA status

In hospitalized patients with COVID-19, cfDNA concentrations showed weak positive correlations with WBC ($\rho = 0.31$, $p = 0.001$), neutrophil count ($\rho = 0.35$, $p < 0.001$), and CRP ($\rho = 0.29$, $p = 0.003$). Weak positive correlations were also observed with tissue injury biomarkers, including ALT ($\rho = 0.25$, $p < 0.010$), AST ($\rho = 0.40$, $p < 0.001$), LDH ($\rho = 0.39$, $p < 0.001$), and NT-proBNP ($\rho = 0.26$, $p = 0.016$) (Additional file 5).

Positive serum SARS-CoV-2 RNA results were more frequent in patients with higher disease severity. The prevalence of SARS-CoV-2 RNA positivity in serum was significantly higher among ICU patients compared with non-ICU patients (43.1% vs. 15.8%, $p = 0.002$) (Additional file 2) and among those requiring ARS compared with those who did not (46.7% vs. 15.9%, $p < 0.001$) (Additional file 3). Non-survivors also showed a higher rate of SARS-CoV-2 RNA positivity compared with survivors (50.0% vs. 24.4%, $p = 0.029$) (Additional file 4). cfDNA concentrations were significantly higher in patients with positive SARS-CoV-2 RNA results compared with those who tested negative (6.68 ng/µL [IQR 3.80 - 15.75] vs. 3.07 ng/µL [IQR 1.08–6.28], $p < 0.001$).

While SARS-CoV-2 RNA was detected by qPCR in the serum of 31 (28.7%) patients, long-range SARS-CoV-2 PCR yielded a positive result in only one patient, who required ICU treatment and experienced a non-fatal outcome of the COVID-19 disease during hospitalization. In the control group, all SARS-CoV-2 qPCRs were negative.

Prognostic performance of cfDNA in predicting disease severity, requirement of advanced respiratory support, and in-hospital mortality in COVID-19 disease

ROC analysis identified cfDNA as the strongest predictor, among all laboratory evaluated parameters, of critical COVID-19 requiring ICU treatment, the need for ARS, and in-hospital mortality (Table 3).

Table 3. Prognostic performance of cfDNA and other laboratory parameters in predicting critical COVID-19 disease requiring ICU admission, ARS, and in-hospital mortality in hospitalized COVID-19 patients.

Predictor	AUC	95% CI	p-value	Optimal threshold	Sensitivity, %	Specificity, %
Critical COVID-19 disease requiring ICU admission						
cfDNA	0.790	0.707–0.873	<0.001	2.69	88.2	54.4
Urea	0.629	0.522–0.736	0.018	7.20	41.2	83.0
AST	0.640	0.534–0.745	0.010	51.00	52.9	75.0
LDH	0.617	0.505–0.729	0.041	462.00	48.9	78.4
Ferritin	0.633	0.525–0.741	0.016	715.50	72.9	54.7
Advanced respiratory support						

cfDNA	0.771	0.684–0.869	<0.001	5.93	62.2	81.0
Neutrophils	0.620	0.512–0.727	0.029	5.35	48.9	74.6
ALT	0.630	0.526–0.735	0.015	27.5	88.6	42.9
AST	0.692	0.592–0.792	<0.001	44	68.9	66.1
LDH	0.660	0.549–0.770	0.005	355.5	76.3	55.0
In-hospital mortality						
cfDNA	0.810	0.690–0.930	<0.001	6.47	77.8	76.7
WBC	0.682	0.548–0.816	0.008	6.04	72.2	64.4
Neutrophils	0.677	0.536–0.819	0.014	4.98	72.2	64.4
Potassium	0.652	0.523–0.781	0.021	3.81	94.4	36.7
Creatinine	0.668	0.497–0.838	0.055	-	-	-
Urea	0.673	0.535–0.812	0.014	6.29	61.1	67.4
NT-proBNP	0.684	0.525–0.843	0.024	446.5	60.0	73.0

Receiver operating characteristic (ROC) curve analysis was performed to assess diagnostic performance. The optimal thresholds were determined based on the Youden Index.

ALT – alanine aminotransferase; AST – aspartate aminotransferase; AUC – area under the ROC curve; cfDNA – cell-free DNA; CI – confidence interval; ICU – intensive care unit; LDH – lactate dehydrogenase; NT-proBNP – N-terminal pro-B-type natriuretic peptide; WBC – white blood cell.

For predicting critical COVID-19 requiring ICU treatment, the AUC was 0.79 (95% CI 0.71–0.87, $p < 0.001$), with an optimal cfDNA threshold of 2.69 ng/µL providing 88.2% sensitivity and 54.4% specificity (Table 3). This performance exceeded that of other laboratory parameters, including AST, LDH, ferritin, and urea, which showed AUC values ranging from 0.62 to 0.64 (Figure 4A, Table 3).

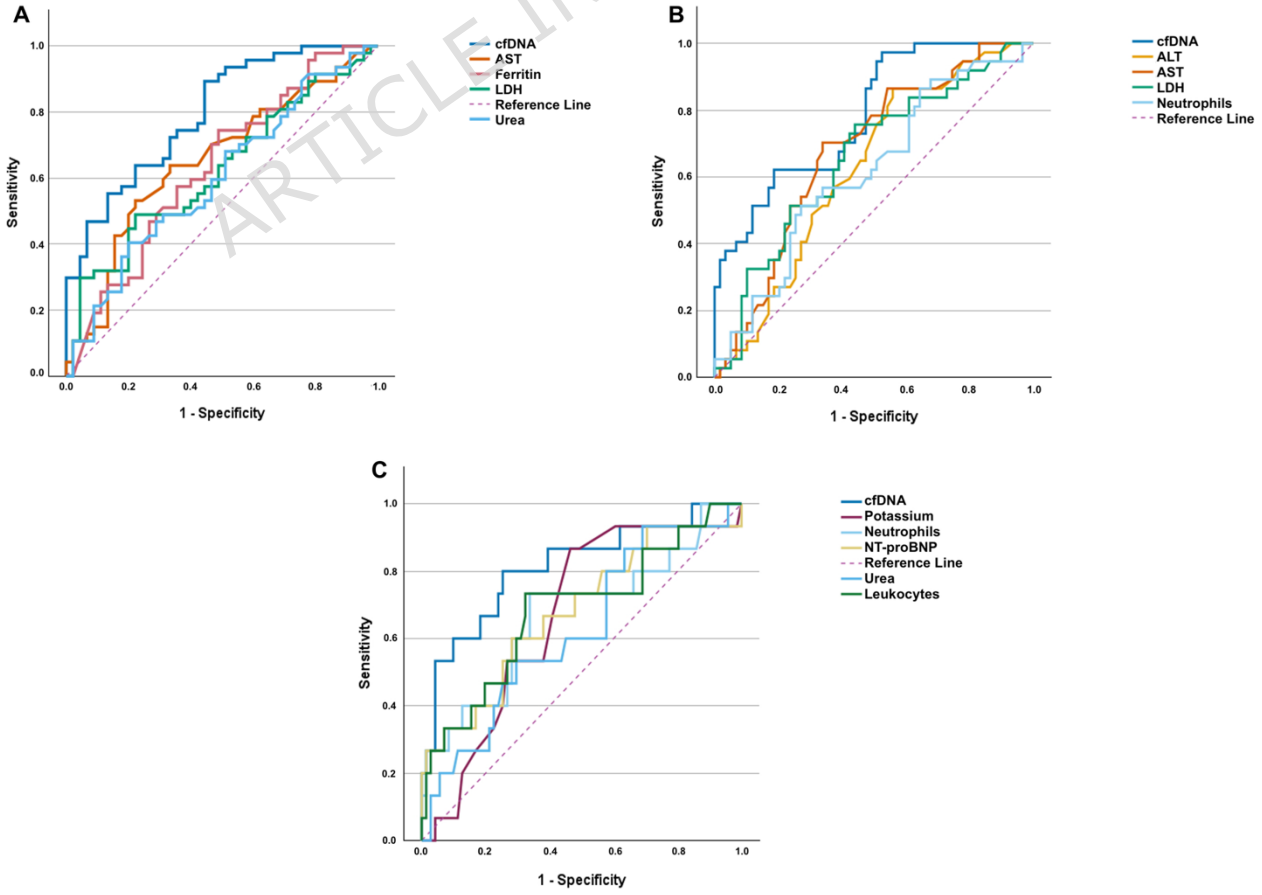

Figure 4. Prognostic performance of cfDNA and other laboratory parameters in predicting critical disease requiring ICU admission (A), the need for ARS (B), and in-hospital mortality (C) in hospitalized COVID-19 patients.

ALT – alanine aminotransferase; AST – aspartate aminotransferase; cfDNA – cell-free DNA; LDH – lactate dehydrogenase; NT-proBNP – N-terminal pro-B-type natriuretic peptide.

The predictive value of cfDNA for the need for ARS was similarly high, with an AUC of 0.77 (95% CI 0.68–0.87, $p < 0.001$), outperforming other biomarkers, such as neutrophil count, AST, ALT, and LDH (AUC range 0.62–0.69). The optimal threshold of 5.93 ng/μL yielded 62.2% sensitivity and 81.0% specificity (Figure 4B, Table 3).

cfDNA also demonstrated the strongest prognostic performance for in-hospital mortality, with an AUC of 0.81 (95% CI 0.69–0.93, $p < 0.001$) and the optimal cfDNA cutoff of 6.47 ng/μL provided 77.8% sensitivity and 76.7% specificity, surpassing other laboratory parameters, including WBC, neutrophil count, potassium, urea, and NT-proBNP, which showed AUC values ranging from 0.65 to 0.68 (Figure 4C, Table 3).

In multivariable logistic regression analysis, cfDNA concentration remained an independent predictor of critical COVID-19 requiring ICU treatment, the need for ARS, and in-hospital mortality after adjustment for age, sex, obesity, presence of any other comorbidity, other laboratory variables significant in univariable regression analysis (Additional file 6), and SARS-CoV-2 RNA positivity (Table 4). Each unit increase in cfDNA was associated with a 21% increase in the odds of critical disease (OR 1.21, 95% CI 1.08–1.36, $p = 0.001$),

a 15% increase in the odds of requiring ARS (OR 1.15, 95% CI 1.03–1.28, p = 0.014), and an 8% increase in the odds of in-hospital mortality (OR 1.08, 95% CI 1.02–1.14, p = 0.009). Serum SARS-CoV-2 RNA positivity remained an independent predictor of the requirement for ARS, increasing the odds by 4.18 times. In contrast, no statistically significant associations were observed with a critical COVID-19 course or in-hospital mortality (Table 4).

Table 4. Prognostic significance of cfDNA and clinical covariates for predicting critical illness, advanced respiratory support, and mortality in hospitalized COVID-19 patients.

Predictor	Odds ratio (95% CI)	p-value
Critical COVID-19 requiring treatment in ICU		
cfDNA	1.21 (1.08–1.36)	0.001
Age	0.98 (0.94–1.01)	0.173
Sex	0.66 (0.26–1.67)	0.376
Obesity	1.65 (0.44–6.14)	0.455
Presence of any other comorbidity	0.82 (0.26–2.55)	0.732
SARS-CoV-2 RNA positivity in serum	2.57 (0.86–7.64)	0.090
Advanced respiratory support		
cfDNA	1.15 (1.03–1.28)	0.014
Age	0.98 (0.94–1.03)	0.496
Sex	0.39 (0.13–1.20)	0.100
Obesity	7.08 (1.55–32.25)	0.011

Presence of any other comorbidity	1.61 (0.40–6.55)	0.505
SARS-CoV-2 RNA positivity in serum	4.18 (1.15–15.20)	0.030
Neutrophils	1.05 (0.83–1.32)	0.703
LDH	1.001 (0.998–1.004)	0.647
In-hospital mortality		
cfDNA	1.08 (1.02–1.14)	0.009
Age	1.07 (0.98–1.18)	0.136
Sex	0.37 (0.05–2.57)	0.314
Obesity	1.36 (0.08–23.25)	0.830
Presence of comorbidities	3.24 (0.24–43.24)	0.375
SARS-CoV-2 RNA positivity in serum	4.77 (0.78–29.01)	0.090
Neutrophils	1.12 (0.76–1.64)	0.582
Platelets	1.01 (0.998–1.02)	0.097
Urea	0.91 (0.69–1.19)	0.493
D-dimer	1.00 (1.000–1.001)	0.686
NT-proBNP	1.00 (1.000–1.001)	0.203

Multivariable logistic regression, adjusted for age, sex, obesity, presence of any other comorbidity, and laboratory variables significant in the univariable regression analysis, was performed.

cfDNA – cell-free DNA; CI – confidence interval; ICU – intensive care unit; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2.

Discussion

In this study, we evaluated serum cfNAs (cfDNA and SARS-CoV-2 RNA) as predictors of COVID-19 disease severity and lethal outcome in a tertiary care hospital in Vilnius, Lithuania. We identified cfDNA as an independent predictor of a critical COVID-19 disease course, requiring treatment in the ICU, the need for ARS (NIV, IVM, or ECMO), and in-hospital mortality. Moreover, cfDNA showed the highest prognostic performance for these outcomes in ROC analysis compared with other laboratory analytes. In contrast, serum SARS-CoV-2 RNA positivity persisted as an independent predictor only of the requirement for ARS. No statistically significant associations were found in predicting the need for ICU treatment or in-hospital mortality. Due to the novelty of this field of investigation, no standardized protocol currently exists for measuring cfDNA concentration, reporting data [20,35,36], or evaluating the reference interval [37], which makes it difficult to compare studies and their results.

Higher serum cfDNA concentrations were observed in the COVID-19 positive population compared with healthy controls in our study. Although plasma (not serum) cfDNA is assessed more frequently in other studies, the same tendency comparing healthy controls and COVID-19 patients was identified by other authors [35,38,39]. Moreover, we found higher cfDNA levels in the critical COVID-19 patient group (ICU patients) compared with the mild and severe COVID-19 groups, defined as those requiring oxygen therapy. Furthermore, we identified that patients who required ARS during hospitalization, upon admission, had higher cfDNA concentrations, as well as non-survivors. These findings are consistent with other recent studies. Hoeter et al., in their pilot study, also identified gradually increasing levels of cfDNA with the progression of COVID-19 disease [38]. Other authors observed higher cfDNA levels in

COVID-19 cases requiring oxygen therapy [34], requiring IMV [34,36], and those with fatal outcomes [33].

We identified weak positive correlations between cfDNA and inflammatory markers, including WBC and neutrophil counts, CRP, and tissue injury biomarkers, represented by ALT, AST, LDH, and NT-proBNP. Although cfDNA levels were significantly elevated in patients with severe disease and those requiring intensive care or ARS, their correlations with conventional biochemical and inflammatory markers were weak. Other studies have shown similar findings – weak to moderate positive correlations between cfDNA and inflammatory and tissue damage markers, including CRP [33,35,38–41], ferritin, neutrophils [33,35,39–41] and the neutrophil-to-lymphocyte ratio [33,40], LDH [33,34,36,41], D-dimers [34–36,38,39], IL-6 [33,41], fibrinogen [41], brain natriuretic peptide (BNP) [34]. These findings suggest that cfDNA reflects a distinct pathophysiological process, representing global cell injury and death (apoptosis, necrosis, NETosis) [20,21], rather than specific inflammatory or organ-specific responses captured by markers such as CRP, LDH, or AST.

NETosis, as a pathophysiology process and effective defence mechanism, as well as an important source of cfDNA, has been described in various bacterial, viral, fungal, and parasitic infectious diseases [42], including influenza, dengue fever, malaria, and bacterial sepsis [43]. Current studies have revealed that NETosis and NETs formation are common processes in COVID-19 infection, especially in its severe cases [44–46]. Some authors have even characterized COVID-19 disease, particularly in hospitalized patients, as a pro-NETotic state [36]. This may be a reason why higher concentrations of cfDNA are found in patients with severe and critical COVID-19. Moreover, other authors have

reported that cfDNA levels are significantly higher in COVID-19 patients than in those infected with other respiratory viruses, including influenza and respiratory syncytial virus (RSV) [35]. These findings suggest that COVID-19 may be associated with more pronounced NETosis and/or other cell death pathways compared with other viral respiratory infections.

Furthermore, in our study, cfDNA was identified as the strongest predictor, among all laboratory evaluated parameters on admission, of critical COVID-19 requiring ICU treatment (AUC 0.79), the need for ARS (AUC 0.77), and in-hospital mortality (AUC 0.81). These findings are consistent with the results of other studies, which likewise reported that cfDNA showed high prognostic performance in predicting the severe disease course [33,40], the need for oxygen therapy or IMV [34], and fatal outcome [33,35]. It is known that NETs drive excessive inflammation and intravascular thrombosis, contributing to tissue and organ damage in severe COVID-19 cases [47,48]. In addition, cfDNA itself functions as a damage-associated molecular pattern (DAMP) that activates an inflammatory response, providing an additional mechanism contributing to tissue damage [49]. As NETosis and cfDNA could be triggers of further inflammation and tissue injury in the course of COVID-19 disease, it may be the reason why, on admission day, this parameter had the highest predictive accuracy among all other laboratory analytes, including inflammatory and tissue damage markers whose concentrations may increase later in the disease course.

Finally, cfDNA, measured on hospitalization day, remained an independent predictor of a critical COVID-19 course requiring treatment in the ICU, the need for ARS, and a fatal outcome of the disease, one-unit elevation in cfDNA increasing these odds by 21%, 15%, and 8%, respectively. A similar role of

cfDNA as an independent risk factor was observed in other studies predicting the need for oxygen therapy or IMV [34] and death [35]. In other research, mitochondrial cfDNA has also been identified as an independent risk factor for ICU admission, IMV, vasopressor use, renal replacement therapy, and deceased outcome [50]. Henry et al. observed that serum cfDNA remained an independent predictor for acute kidney injury in COVID-19 patients [41]. This emphasizes the potential utility of this analyte as an early biomarker for identifying patients at higher risk of developing severe COVID-19. It also highlights its value as a triage tool in the emergency department, particularly when healthcare systems are overwhelmed, as occurred during the early stages of the COVID-19 pandemic.

In addition to analysing the role of cfDNA in predicting severe cases and worse outcomes of COVID-19, we also evaluated serum SARS-CoV-2 RNA as a potential predictor of disease severity in COVID-19. Although viraemia or RNAemia is not a common finding in other respiratory viruses, such as influenza [51], SARS-CoV-2 RNA detected by qPCR was positive in nearly one-third of hospitalized COVID-19 patients. We found that the prevalence of SARS-CoV-2 RNA positivity in serum was significantly higher in ICU patients, those who required ARS, and those with fatal outcomes. Moreover, cfDNA levels were higher in patients with positive SARS-CoV-2 RNA detected by qPCR. Serum SARS-CoV-2 RNA positivity was identified as an independent risk factor only for the requirement for ARS, with an odds ratio of 4.18. Whereas no significant associations were found with a critical course of COVID-19 (treatment in the ICU) or in-hospital mortality. SARS-CoV-2 RNAemia has been reported to be associated with a more severe clinical course of COVID-19, including increased risk of ICU treatment and clinical deterioration [52–55]. Another study, which

evaluated SARS-CoV-2 RNAemia on admission, identified that it was more common in ICU patients and in patients with lethal outcomes. However, as an independent risk factor, it remained significant only for predicting lethal outcome [56]. We also conducted a long-range SARS-CoV-2 PCR assay on serum samples, but only a single patient tested positive. This patient required ICU care but ultimately experienced a non-fatal outcome during hospitalization for COVID-19. Because only a small number of serum samples yielded positive long-range SARS-CoV-2 PCR results, we were unable to perform a more detailed analysis evaluating the association between long-range SARS-CoV-2 PCR positivity and COVID-19 severity. On the other hand, it may indicate that the whole SARS-CoV-2 genome in serum is not a common finding in COVID-19 patients, as the infectious virus is not detected in the blood [57].

Strengths of our study include evaluating cfDNA and SARS-CoV-2 RNA as potential predictors of COVID-19 disease severity and adverse outcomes, thereby assessing both host- and pathogen-derived cfNAs as candidate biomarkers. Moreover, we conducted not only qPCR but also long-range SARS-CoV-2 PCR, aiming to evaluate viremia. We analysed serum cfDNA, for which there is limited data, as most studies have been conducted on plasma cfDNA. However, the results obtained suggest that serum cfDNA results can be applied in the same or at least a similar manner as plasma. In our study, we did not include patients with oncological or haematological diseases, as well as those after organ transplantation and PLWH, conditions known to be associated with elevated levels of cfDNA, to minimize confounding bias.

Our study has several limitations: a larger sample size could help assess viremia in blood more accurately. Due to the small size of the mild COVID-19 patient group, we were unable to evaluate cfNAs' associations with the need for oxygen

therapy. The limited representation of mild cases may also affect comparisons across disease severity groups. This likely reflects the study setting, as the research was conducted at a university referral hospital, where patients with more severe disease are more commonly admitted. The control group was younger than the COVID-19 cohort, which may influence circulating cfDNA levels, as age-related biological variation could contribute to differences between groups. Although we performed an age-adjusted analysis to address this potential confounding factor, residual confounding cannot be excluded. In addition, information on comorbidities was not available for the control group, preventing adjustment for comorbidity burden, which may also affect circulating cfDNA concentrations. Furthermore, the control group included only male participants, whereas the COVID-19 group comprised both sexes, potentially introducing sex-related bias in the comparison of cfDNA levels. The control group was also relatively small, which may limit the precision of comparisons. Healthy controls were included primarily to provide a reference for baseline cfDNA levels in individuals without acute infection; therefore, comparisons between controls and patients should be interpreted with caution. A comparison of cfNAs measurements in plasma versus serum of the same population would have been informative; however, this analysis was not feasible because plasma samples were not available in the Biobank. The total concentration of cfNA in our samples may be elevated due to preanalytical factors, such as processing delays, transport conditions, and haemolysis [58]. However, these factors were likely mitigated in this study as all samples, including controls, were processed under identical laboratory conditions. Nevertheless, laboratories seeking to adopt this methodology should perform independent validation to establish site-specific reference ranges. Moreover,

we did not evaluate the profile of cfDNA that could have provided additional data on COVID-19 pathogenesis. Our study was conducted in a single centre, which may limit the generalizability of the findings. Therefore, validation in independent cohorts from other institutions is warranted to confirm the reproducibility and broader applicability of the results. Because most cfNAs studies have been conducted with relatively small sample sizes, larger cohort studies are needed to clarify the potential role of cfNAs in predicting severe cases, not only in COVID-19 but also in other infectious diseases, which may be useful in dealing with future pandemics.

Overall, the findings of this study demonstrated that serum cfNAs (cfDNA, and SARS-CoV-2 RNA) could be potential biomarkers in predicting more severe COVID-19 disease courses and adverse outcomes. These biomarkers, as non-invasive clinical tools, could be particularly useful early in the disease course, especially during pandemics or other circumstances in which healthcare systems are overwhelmed, and additional triage criteria are needed to guide hospitalization decisions and optimize individualized patient care.

Conclusion

cfDNA appears to reflect a distinct pathophysiological process characterized by extensive cellular injury and death. Elevated serum cfDNA concentrations were independently associated with greater COVID-19 disease severity, an increased likelihood of requiring intensive care and advanced respiratory support, and in-hospital mortality. In contrast, the presence of SARS-CoV-2 RNAemia in serum was associated with the need for advanced respiratory support. cfDNA demonstrated superior prognostic performance, surpassing established biomarkers such as LDH and ferritin. These findings indicate that cfNAs,

especially cfDNA, may serve as clinically valuable biomarkers for early risk stratification and outcome prediction in patients hospitalized with COVID-19. Further validation in independent, multicentre cohorts is warranted to confirm the generalizability of these findings.

Abbreviations

ALT: alanine aminotransferase

ARS: advanced respiratory support

AST: aspartate aminotransferase

AUC: area under the ROC curve

BMI: body mass index

cf-DNA: cell-free deoxyribonucleic acid

cf-DNA: deoxyribonucleic acid

cf-mtDNA: cell-free mitochondrial deoxyribonucleic acid

cfNA: cell-free nucleic acid

cfRNA: cell-free ribonucleic acid

CI: confidence interval

COPD: chronic obstructive pulmonary disease

COVID-19: coronavirus disease 19

CRP: C-reactive protein

DAMP: damage-associated molecular pattern

dNTP: deoxynucleoside triphosphate

DTT: dithiothreitol

DVT: deep vein thrombosis

ECMO: extracorporeal membrane oxygenation

HIV: human immunodeficiency virus

ICU: intensive care unit

IL-6: interleukin-6

IMV: invasive mechanical ventilation

IQR: interquartile range

LDH: lactate dehydrogenase

NETs: neutrophil extracellular traps

NIV: non-invasive ventilation

NT-proBNP: N-terminal pro-B-type natriuretic peptide

OR – odds ratio

PCR: polymerase chain reaction

PLWH: people living with HIV

qPCR: quantitative polymerase chain reaction

RCF: relative centrifugal force

RNA: ribonucleic acid

ROC: receiver operating characteristic

RSV: respiratory syncytial virus

RT-qPCR: real-time polymerase chain reaction

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

UNG: uracil-N-glycosilase

WBC: white blood cell count

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. This study was approved on June 6, 2023, by the Vilnius Regional Biomedical Research Ethics Committee (approval number 2023/6-1521-982, with

amendments approved on November 21, 2024, and March 7, 2025), which waived the requirement for obtaining the study-specific written informed consent form. All participants had signed the informed, written consent form for biobanking in Vilnius Santaros Klinikos Biobank.

Consent for publication

Not applicable.

Data availability

The data supporting the findings of this study are available within the additional files (see Additional file 7).

Competing interests

The authors declare no competing interests.

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Authors’ contributions

IK, DJ, and LJ developed the research question and the study protocol. DT and DJ conducted the laboratory analyses. JU analysed and interpreted the data. IK, JU, DJ, and FM contributed to the final interpretation of the data and the writing of the manuscript. All authors contributed to the editing of the manuscript. All authors read and approved the final manuscript.

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Clinical trial number

Not applicable.

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Study cohort

COVID-19 patients
(n=108)

Healthy controls
(n=24)

Sample processing

Peripheral blood

Centrifugation

Serum

Aliquoting

Frozen serum
(2 x 500 µl)

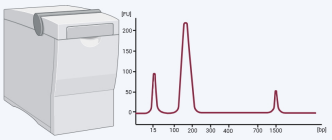
cfNA extraction

Frozen serum
(2 x 500 µl)

cfNA purification

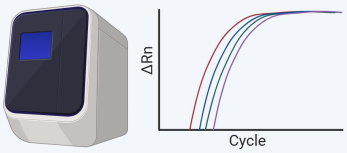
Purified cfNA

cfDNA analysis

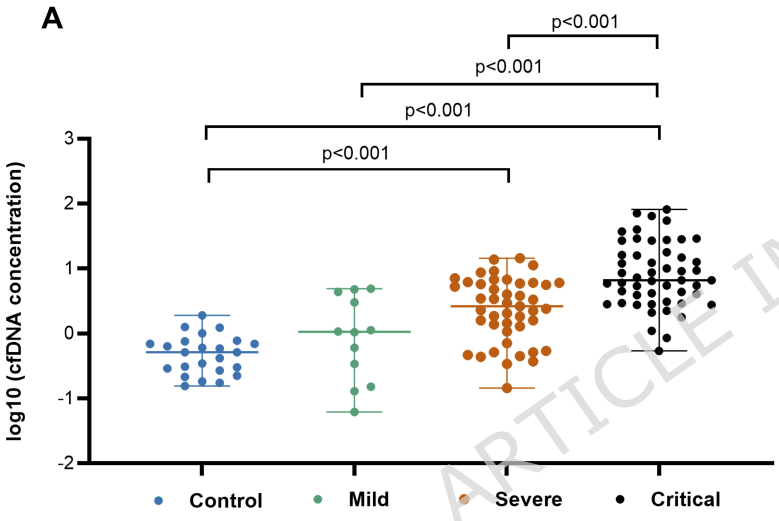


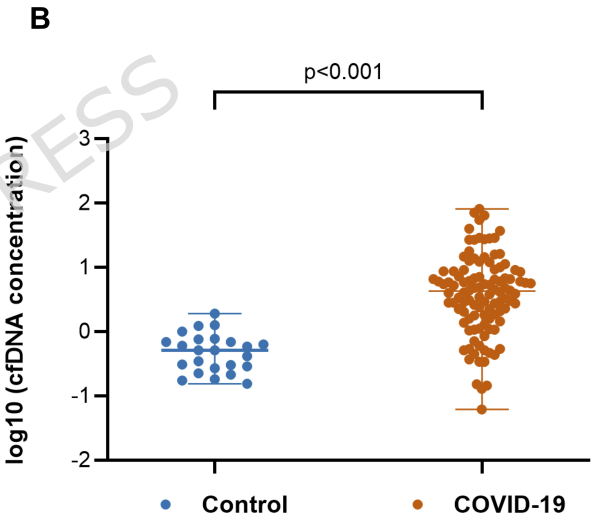
- cfDNA concentration using capillary electrophoresis

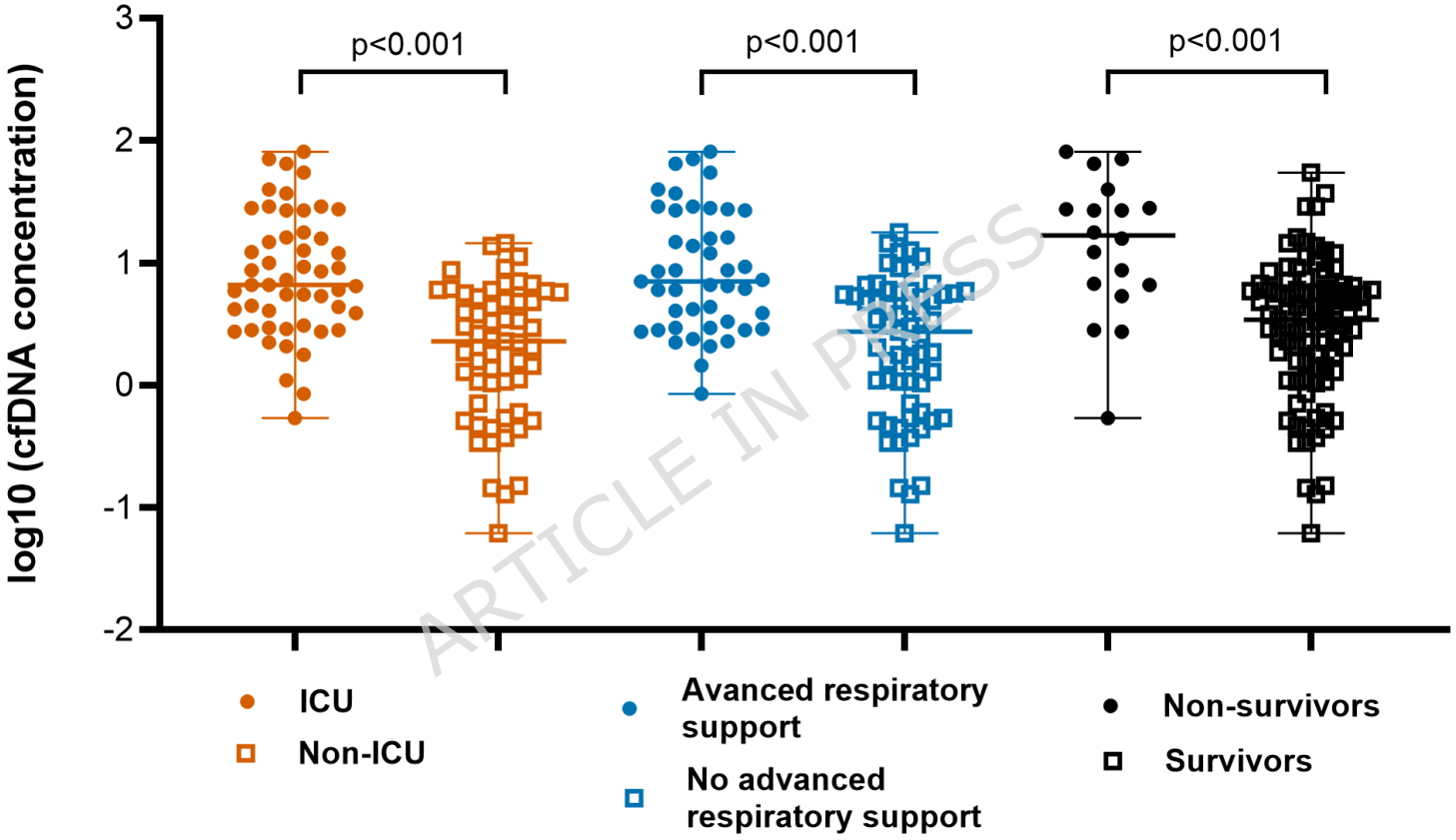
cfRNA analysis



- qPCR of SARS-CoV-2
- Long-range PCR of SARS-CoV-2







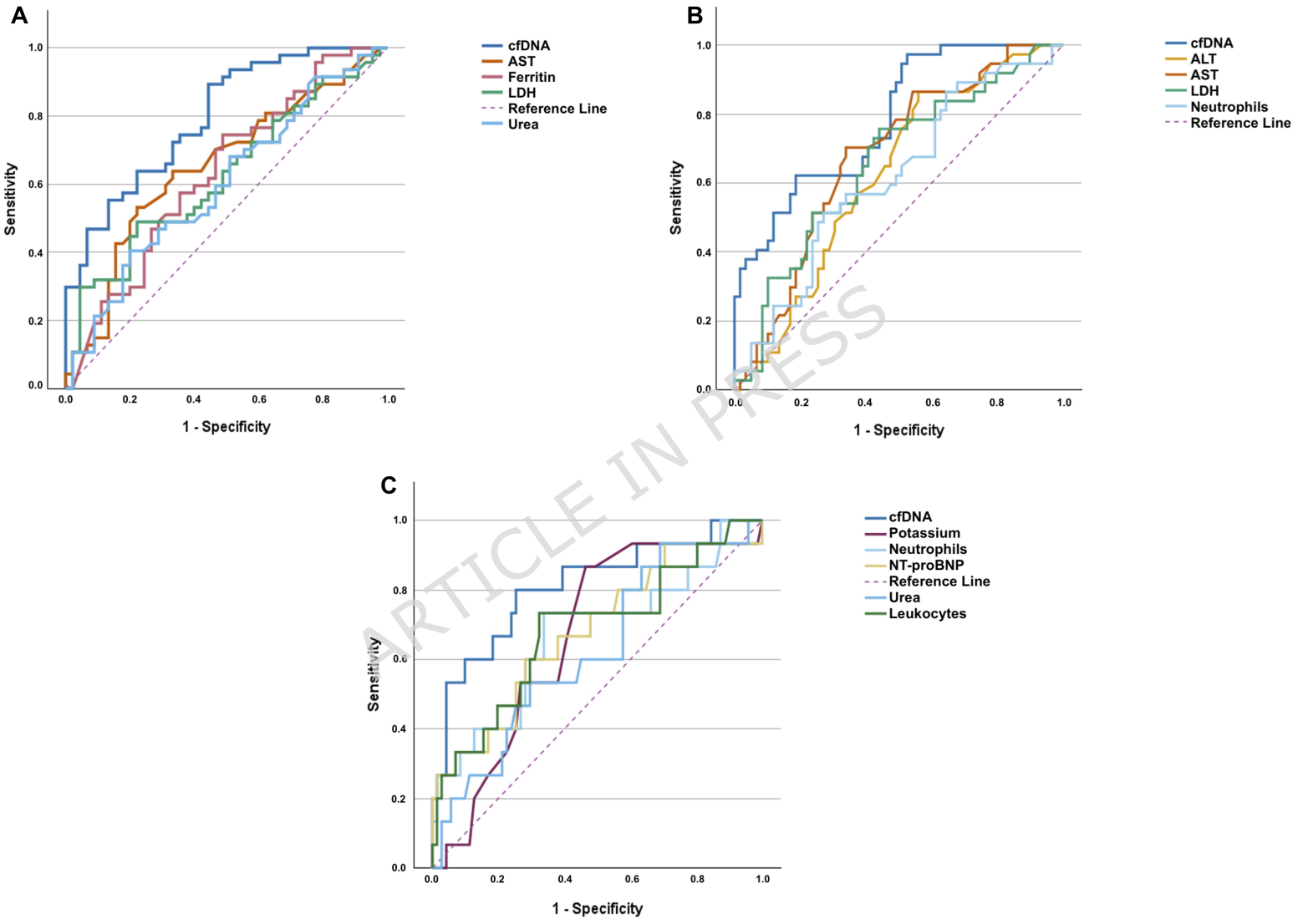


Table 1. Baseline characteristics, comorbidities, and complications of hospitalized COVID-19 patients.

Characteristic	N	Value
Age in years, median (IQR)	108	59.0 (49.0–66.0)
Male, %	108	53 (49.1%)
BMI, kg/m^2, median (IQR)	72	32.6 (27.8–36.4)
Comorbidities, N (%)		
Arterial hypertension	108	65 (60.2)
Coronary artery disease	108	3 (2.8)
Congestive heart failure	108	10 (9.3)
Atrial fibrillation	108	12 (11.1)
Previous myocardial infarction	108	2 (1.9)
Obesity	108	52 (48.2)
Diabetes mellitus	108	18 (16.7)
Chronic kidney disease	108	8 (7.4)
Sequelae of stroke	108	1 (0.9)
COPD	108	0 (0.0)
Complications of COVID-19, N (%)		
Pneumonia	108	102 (94.4)
Acute respiratory failure	108	54 (50.0)
Pulmonary embolism	108	1 (0.9)
DVT	108	1 (0.9)
Stroke	108	1 (0.9)
Acute kidney injury	108	8 (7.4)
Sepsis	108	11 (10.2)
Respiratory support and hospitalization outcomes		
Non-invasive ventilation, N (%)	108	40 (37.0)
Invasive mechanical ventilation, N (%)	108	10 (9.2)
Extracorporeal membrane oxygenation, N (%)	108	6 (5.6)
Length of stay in hospital, days	108	16.0 (10.0–26.0)
In-hospital mortality, N (%)	108	18 (16.7)

BMI – body mass index; COPD – chronic obstructive pulmonary disease; DVT – deep vein thrombosis; IQR – interquartile range.

Table 2. Laboratory parameters of COVID-19 patients on the day of hospitalization.

Laboratory parameter	N	Value, median (IQR)
WBC, x10^9/L	108	5.75 (4.34–7.68)
Neutrophils, x10^9/L	108	4.74 (3.37–5.84)
Lymphocytes, x10^9/L	108	0.95 (0.59–1.20)
Haemoglobin, g/L	108	141.50 (127.00–151.00)
Platelets, x10^9/L	108	190.00 (144.25–254.75)
Sodium, mmol/L	108	139.00 (135.25–141.00)
Potassium, mmol/L	108	4.00 (3.80–4.48)
Glucose, mmol/L	67	8.20 (6.50–9.90)
Creatinine, µmol/L	88	75.00 (61.00–85.75)
Urea, mmol/L	104	5.70 (4.33–8.18)
ALT, U/L	107	35.00 (26.00–53.00)
AST, U/L	107	43.00 (30.00–62.00)
CRP, mg/L	108	95.45 (50.60–144.98)
LDH, U/L	98	389.00 (300.00–501.75)
IL-6, ng/L	89	58.80 (33.85–88.75)
Ferritin, µg/L	101	856.00 (473.50–1,846.50)
Fibrinogen, g/L	74	5.71 (4.87–6.89)
D-dimer, µg/L	102	565.00 (318.75–882.50)
Troponin I, ng/L	95	10.00 (6.00–21.00)
NT-proBNP, ng/L	89	237.00 (124.00–561.00)
SARS-CoV-2 positive by qPCR in serum, N (%)	108	31 (28.7)
cfDNA in serum, ng/µL	108	4.28 (1.62–8.78)

ALT – alanine aminotransferase; AST – aspartate aminotransferase; cfDNA – cell-free DNA; CRP – C-reactive protein; IL-6 – interleukin 6; IQR – interquartile range; LDH – lactate dehydrogenase; NT-proBNP – N-terminal pro-B-type natriuretic peptide; qPCR – quantitative polymerase chain reaction; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2; WBC – white blood cell.

Table 3. Prognostic performance of cfDNA and other laboratory parameters in predicting critical COVID-19 disease requiring ICU admission, ARS, and in-hospital mortality in hospitalized COVID-19 patients.

Predictor	AUC	95% CI	p-value	Optimal threshold	Sensitivity, %	Specificity, %
Critical COVID-19 disease requiring ICU admission						
cfDNA	0.790	0.707–0.873	<0.001	2.69	88.2	54.4
Urea	0.629	0.522–0.736	0.018	7.20	41.2	83.0
AST	0.640	0.534–0.745	0.010	51.00	52.9	75.0
LDH	0.617	0.505–0.729	0.041	462.00	48.9	78.4
Ferritin	0.633	0.525–0.741	0.016	715.50	72.9	54.7
Advanced respiratory support						
cfDNA	0.771	0.684–0.869	<0.001	5.93	62.2	81.0
Neutrophils	0.620	0.512–0.727	0.029	5.35	48.9	74.6
ALT	0.630	0.526–0.735	0.015	27.5	88.6	42.9
AST	0.692	0.592–0.792	<0.001	44	68.9	66.1
LDH	0.660	0.549–0.770	0.005	355.5	76.3	55.0
In-hospital mortality						
cfDNA	0.810	0.690–0.930	<0.001	6.47	77.8	76.7
WBC	0.682	0.548–0.816	0.008	6.04	72.2	64.4
Neutrophils	0.677	0.536–0.819	0.014	4.98	72.2	64.4

Potassium	0.652	0.523 – 0.781	0.021	3.81	94.4	36.7
Creatinine	0.668	0.497 – 0.838	0.055	-	-	-
Urea	0.673	0.535 – 0.812	0.014	6.29	61.1	67.4
NT-proBNP	0.684	0.525 – 0.843	0.024	446.5	60.0	73.0

Receiver operating characteristic (ROC) curve analysis was performed to assess diagnostic performance. The optimal thresholds were determined based on the Youden Index.

ALT – alanine aminotransferase; AST – aspartate aminotransferase; AUC – area under the ROC curve; cfDNA – cell-free DNA; CI – confidence interval; ICU – intensive care unit; LDH – lactate dehydrogenase; NT-proBNP – N-terminal pro-B-type natriuretic peptide; WBC – white blood cell.

Table 4. Prognostic significance of cfDNA and clinical covariates for predicting critical illness, advanced respiratory support, and mortality in hospitalized COVID-19 patients.

Predictor	Odds ratio (95% CI)	p-value
Critical COVID-19 requiring treatment in ICU		
cfDNA	1.21 (1.08–1.36)	0.001
Age	0.98 (0.94–1.01)	0.173
Sex	0.66 (0.26–1.67)	0.376
Obesity	1.65 (0.44–6.14)	0.455
Presence of any other comorbidity	0.82 (0.26–2.55)	0.732
SARS-CoV-2 RNA positivity in serum	2.57 (0.86–7.64)	0.090
Advanced respiratory support		
cfDNA	1.15 (1.03–1.28)	0.014
Age	0.98 (0.94–1.03)	0.496
Sex	0.39 (0.13–1.20)	0.100
Obesity	7.08 (1.55–32.25)	0.011
Presence of any other comorbidity	1.61 (0.40–6.55)	0.505
SARS-CoV-2 RNA positivity in serum	4.18 (1.15–15.20)	0.030
Neutrophils	1.05 (0.83–1.32)	0.703
LDH	1.001 (0.998–1.004)	0.647
In-hospital mortality		
cfDNA	1.08 (1.02–1.14)	0.009
Age	1.07 (0.98–1.18)	0.136
Sex	0.37 (0.05–2.57)	0.314
Obesity	1.36 (0.08–23.25)	0.830
Presence of comorbidities	3.24 (0.24–43.24)	0.375
SARS-CoV-2 RNA positivity in serum	4.77 (0.78–29.01)	0.090
Neutrophils	1.12 (0.76–1.64)	0.582
Platelets	1.01 (0.998–1.02)	0.097
Urea	0.91 (0.69–1.19)	0.493
D-dimer	1.00 (1.000–1.001)	0.686
NT-proBNP	1.00 (1.000–1.001)	0.203

Multivariable logistic regression, adjusted for age, sex, obesity, presence of any other comorbidity, and laboratory variables significant in the univariable regression analysis, was performed.
cfDNA – cell-free DNA; CI – confidence interval; ICU – intensive care unit; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2.