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https://orcid.org/0000-0002-4220-6053

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Ieva Kubiliūtė

Research on Predictors of Disease Severity and Clinical Outcomes in Hospitalised COVID-19 Patients

DOCTORAL DISSERTATION

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The dissertation was prepared between 2021 and 2025 at Vilnius University Faculty of Medicine, Institute of Clinical Medicine, Clinic of Infectious Diseases and Dermatovenerology.

Academic Supervisor
Prof. Dr. Ligita Jančorienė (Vilnius University, Medicine and Health Sciences, Medicine – M 001).

Academic Consultant
Dr. Jurgita Urbonienė (Vilnius University Hospital Santaros Klinikos, Medicine and Health Sciences, Medicine – M 001).

This doctoral dissertation will be defended in a public meeting of the Dissertation Defence Panel:

Chairperson – Prof. Dr. Vytautas Kasiulevičius (Vilnius University, Medicine and Health Sciences, Medicine – M 001).

Members:
Assoc. Prof. Dr. Agnė Kirkliauskienė (Vilnius University, Medicine and Health Sciences, Medicine – M 001);
Prof. Dr. Violeta Kvedarienė (Vilnius University, Medicine and Health Sciences, Medicine – M 001);
Assoc. Prof. Dr. Ieva Tolmane (University of Latvia, Medicine and Health Sciences, Medicine – M 001);
Assoc. Prof. Dr. Danguolė Važnaisienė (Lithuanian University of Health Sciences, Medicine and Health Sciences, Medicine – M 001).

The dissertation shall be defended at a public meeting of the Dissertation Defence Panel at 13:00 on 28 August 2026 in the Large Auditorium of the Faculty of Medicine, Vilnius University.
Address: Čiurlionio 21, Room 203, Vilnius, Lithuania.
Tel. +370 5 239 879; e-mail: mf@mf.vu.lt

https://doi.org/10.15388/vu.thesis.969
https://orcid.org/0000-0002-4220-6053

VILNIAUS UNIVERSITETAS

Ieva Kubiliūtė

Hospitalizuotų pacientų sunkios COVID-19 ligos ir jos baigčių prognostinių veiksnių tyrimas

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Mokslinė vadovė – prof. dr. Ligita Jančorienė (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina – M 001).

Mokslinė konsultantė – dr. Jurgita Urbonienė (Vilniaus universiteto ligoninė Santaros klinikos, medicinos ir sveikatos mokslai, medicina – M 001).

Gynimo taryba:
pirmininkas – prof. dr. Vytautas Kasiulevičius (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina – M 001).

Nariai:
doc. dr. Agnė Kirkliauskienė (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina – M 001);
prof. dr. Violeta Kvedarienė (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina – M 001);
doc. dr. Ieva Tolmane (Latvijos universitetas, medicinos ir sveikatos mokslai, medicina – M 001);
doc. dr. Danguolė Važnaisienė (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001).

Disertacija ginama viešame Gynimo tarybos posėdyje 2026 m. rugpjūčio 28 d. 13 val. Vilniaus universiteto Medicinos fakulteto Didžiojoje auditorijoje.
Adresas: M. K. Čiurlionio g. 21, Vilnius.
Tel. +370 5 239 879; el. paštas: mf@mf.vu.lt

LIST OF ABBREVIATIONS

ACE2	–	Angiotensin-converting enzyme 2
ALP	–	Alkaline phosphatase
ALT	–	Alanine aminotransferase
ARDS	–	Acute respiratory distress syndrome
ARS	–	Advanced respiratory support
AST	–	Aspartate aminotransferase
BMI	–	Body mass index
cfDNA	–	Cell-free DNA
cf-mtDNA	–	Cell-free mitochondrial DNA
cfNA	–	Cell-free nucleic acid
cfRNA	–	Cell-free RNA
CI	–	Confidence interval
COPD	–	Chronic obstructive pulmonary disease
COVID-19	–	Coronavirus disease 2019
CRP	–	C-reactive protein
CT	–	Computed tomography
DNA	–	Deoxyribonucleic acid
DTT	–	Dithiothreitol
DVT	–	Deep vein thrombosis
ECMO	–	Extracorporeal membrane oxygenation
eGFR	–	Estimated glomerular filtration rate
E protein	–	Envelope protein
GGT	–	Gamma-glutamyl transferase
HbA1c	–	glycated haemoglobin
HDU	–	High Dependency Unit
HIV	–	Human immunodeficiency virus
HR	–	Hazard ratio
ICD-10-AM	–	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision, Australian Modification
ICTV	–	International Committee on Taxonomy of Viruses

ICU	–	Intensive care unit
IL-6	–	Interleukin 6
IL-8	–	Interleukin 8
IMV	–	Invasive mechanical ventilation
INR	–	International normalised ratio
IQR	–	Interquartile range
LDH	–	Lactate dehydrogenase
M protein	–	Membrane protein
MCP-1	–	Monocyte chemoattractant protein-1
N protein	–	Nucleocapsid protein
NETs	–	Neutrophil extracellular traps
NIV	–	Non-invasive ventilation
NLR	–	Neutrophil-to-lymphocyte ratio
NT-proBNP	–	N-terminal pro B-type natriuretic peptide
OR	–	Odds ratio
PLWH	–	People living with HIV
PT	–	Prothrombin time
qPCR	–	Quantitative polymerase chain reaction
RNA	–	Ribonucleic acid
ROC	–	Receiver operating characteristic
RT-PCR	–	Reverse transcription polymerase chain reaction
SARS-CoV-2	–	Severe acute respiratory syndrome coronavirus 2
SpO_2	–	Peripheral oxygen saturation
S protein	–	Spike protein
TMPRSS2	–	Transmembrane serine protease 2
TNF-α	–	Tumour necrosis factor-alpha
VOC	–	variant of concern
WHO	–	World Health Organisation

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1. INTRODUCTION AND LITERATURE REVIEW

1.1. COVID-19 Disease: Background and its Burden

In December 2019, a cluster of cases of atypical pneumonia of an unknown aetiology was first identified in Wuhan City, People's Republic of China [1]. The causative agent was subsequently identified as a novel betacoronavirus and officially designated Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses (ICTV), while the associated disease was named Coronavirus Disease 19 (COVID-19) [2,3]. Due to its high transmissibility and rapid global spread, the outbreak was declared a pandemic by the World Health Organisation (WHO) on 11 March 2020 [4]. Since then, COVID-19 has posed an unprecedented challenge to global health systems. As of June 30, 2026, more than 779 million confirmed cases and over 7 million deaths have been reported worldwide [5], thus underscoring the substantial and ongoing burden of the disease. In Lithuania, the first case of SARS-CoV-2 infection was officially reported on 28 February 2020, and the first case of COVID-19 infection in Vilnius Region was reported on 13 March 2020 [6]. Overall, more than 1.4 million confirmed cases and more than 9,900 deaths have been reported until 30 June 2026, [5] in Lithuania.

This emphasises the burden of COVID-19, especially at the beginning of the pandemic. During the first pandemic year, up to 44% of hospitalised patients were admitted to the intensive care unit, and at least one third of them died [7–10]. In-hospital mortality rates varied considerably across countries and over time, reflecting differences in patient characteristics, testing strategies, admission criteria, and the healthcare system capacity [11,12]. Reported in-hospital mortality ranged from approximately 5% in some European and United States cohorts to over 25% in others [11,13–16], with notable fluctuations between pandemic waves and populations [17–19]. COVID-19 mortality rates declined substantially over time, with a marked reduction observed during the Omicron-dominant period compared with earlier waves such as Delta [20,21], including the risk of hospital attendance and admission [22,23]. However, despite the lower case fatality rates, the markedly higher number of cases during the Omicron wave resulted in an overall increase in absolute deaths, particularly among older individuals [21]. These findings highlight the persistent burden of COVID-19 on healthcare systems and ongoing healthcare resource utilisation despite apparent attenuation in the disease severity, decreased virulence of the SARS-CoV-2 virus, widespread vaccination, and the development of antiviral treatments [21].

1.2. Aetiology and Pathogenesis of COVID-19

SARS-CoV-2 is an enveloped, positive-sense single-stranded RNA virus that encodes four major structural proteins: nucleocapsid (N), membrane (M), envelope (E), and spike (S) proteins [24,25]. The E protein contributes to infection-associated immunopathology, the M protein is essential for maintaining the viral structure and facilitating the viral assembly and release, while the N protein participates in viral genome replication [26]. The spike protein consists of two functional subunits, S1 and S2, which are essential for viral entry into host cells. The S1 subunit mediates binding to the angiotensin-converting enzyme 2 (ACE2) receptor, while the S2 subunit is activated by transmembrane serine protease 2 (TMPRSS2), enabling fusion of the viral and cellular membranes [24,25]. Elevated expression of ACE2 receptors facilitates more efficient viral entry and enhances replication rates [26]. As ACE2 and TMPRSS2 are co-expressed in most epithelial cells of the human respiratory tract, the virus primarily targets tissues of both the upper and lower airways [27,28].

SARS-CoV-2 infection activates both innate and adaptive immune responses, with airway epithelial cells playing a central role in initiating and amplifying inflammation through interactions with immune cells [29]. Viral infection triggers the release of proinflammatory cytokines and chemokines (e.g., tumour necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), interleukin-8 (IL-8), monocyte chemoattractant protein-1 (MCP-1)), which contribute to immune cell recruitment and, in severe cases, lead to dysregulated inflammatory responses and a cytokine storm [30–32]. Emerging evidence further highlights that the complexity and imbalance of host immune responses are key determinants of the disease severity and progression [32]. This exaggerated immune activation may lead to tissue damage and disease progression, highlighting the critical role of host-virus interactions in determining COVID-19 severity and clinical outcomes [29,31]. This excessive and dysregulated inflammation leads to multiorgan involvement and can affect the heart, liver, kidneys, and the central nervous system, potentially resulting in sepsis, shock, or multiple organ failure [26,33].

1.3. Variants of COVID-19

Similarly to other single-stranded RNA viruses characterised by high mutation rates [34] driven by error-prone replication mechanisms [35], SARS-CoV-2 has demonstrated a remarkable capacity for genetic evolution, driven

by the accumulation of mutations, particularly in the spike (S) protein, a key determinant of viral infectivity and immune recognition [36–38].

During the initial phase of the COVID-19 pandemic, SARS-CoV-2 demonstrated a relatively low genetic variability, with limited observable evolution [37]. However, approximately eight months after its emergence, SARS-CoV-2 underwent a notable evolutionary shift with the appearance of the first divergent lineages. The initial variants of concern (VOCs) – Alpha, Beta, and Gamma – emerged independently and were characterised by accelerated evolutionary rates and the accumulation of numerous non-synonymous mutations. This tendency became more pronounced with the emergence of Omicron, another VOC, which harboured a markedly higher number of mutations in the spike (S) protein [37,39].

The emergence of Omicron in late November 2021 signalled a new phase of the pandemic, characterised by successive sub-lineages rather than highly divergent variants [40]. Notably, Omicron demonstrates the highest genetic diversity among VOCs and continues to diversify into multiple sub-lineages [41] with shared spike (S) protein mutations [42]. These mutations have been associated with an enhanced transmissibility, altered pathogenicity, and an increased potential for immune evasion, particularly in the context of prior infection or vaccination [43].

Despite the ongoing viral evolution resulting in the continuous emergence of new variants with distinct biological and clinical properties [43], their direct impact on individual disease severity remains complex, thus underscoring the continued importance of host-related factors and clinically applicable predictors in determining the disease outcomes.

1.4. Clinical Heterogeneity of COVID-19

SARS-CoV-2 is mainly transmitted between people through respiratory droplets and aerosols [44,45]. Clinical manifestations and outcomes of COVID-19 vary widely from asymptomatic to severe disease with acute respiratory distress syndrome (ARDS), multiple organ failure, and even death [27,44]. Patients with mild or moderate COVID-19 most commonly experience fever, cough, fatigue [46] and myalgia [47]. Among hospitalised patients, fever, cough, dyspnoea, fatigue, confusion, diarrhoea, and vomiting are the most frequent symptoms [48], thereby emphasising that patients with COVID-19 experience not only respiratory system but also other systems (gastrointestinal, neurological) and systemic symptoms. Moreover, although anosmia and ageusia are not the most prevalent symptoms, they are highly specific to COVID-19 [49].

Pulmonary involvement in COVID-19 most commonly presents with fever, dry cough, and dyspnoea [50]. While the majority of patients exhibit mild symptoms, a considerable proportion progress to more severe disease, including viral pneumonia and ARDS [51,52]. Studies indicate that lung injury is predominantly characterised by diffuse alveolar damage, thus supporting the central role of ARDS in COVID-19-related mortality [53–55].

The massive pro-inflammatory response in COVID-19 is associated with significant vascular injury, including endothelial damage, disruption of endothelial cell integrity, and widespread thrombosis with microangiopathy, which may ultimately contribute to multiorgan failure [56,57]. Endothelial dysfunction observed across multiple vascular systems – including lungs, kidneys, heart, liver, and the gastrointestinal tract – can promote thromboinflammation by increasing thrombin production, impairing fibrinolysis, and activating complement pathways, leading to microvascular dysfunction and microthrombi formation [58–60]. These processes contribute to damage in multiple organ systems, including not only ARDS, but also acute kidney injury, cardiovascular complications such as myocardial infarction, myocarditis, heart failure, cardiogenic shock, liver dysfunction, and other forms of organ dysfunction leading to multiple organ dysfunction syndrome [61–64].

1.5. Predictors of COVID-19 Disease Course: Demographic, Clinical, and Laboratory Determinants

Despite significant advances in understanding the pathophysiology of COVID-19, predicting disease progression and clinical outcomes remains challenging due to the marked heterogeneity in clinical presentation. Early and accurate risk stratification of hospitalised patients is therefore essential to guide clinical decision-making and optimise the allocation of healthcare resources. A wide range of prognostic markers has been investigated, including demographic characteristics, comorbidities, and routinely available laboratory parameters. Established risk factors for severe COVID-19 and adverse clinical outcomes – such as Intensive Care Unit (ICU) admission, development of ARDS or other complications, and in-hospital mortality – include the male sex [65], an older age [52,66,67], a lower socioeconomic status, ethnicity, the country of birth [66], as well as chronic conditions, including obesity, arterial hypertension, diabetes mellitus, and other comorbidities [52,67–69]. Comorbidities may contribute to disease severity through multiple mechanisms, including dysregulation of the immune response and substantially increased ACE2 expression, which accelerates the

pathogenic virus binding to its target cells. This may enhance the inflammatory response, ultimately leading to a cytokine storm and a more severe disease course [67,70–73].

In addition to demographic factors and comorbidities, a wide range of routinely available laboratory parameters have been identified as potential predictors of disease severity and adverse outcomes in COVID-19. Higher lactate dehydrogenase (LDH) levels have been consistently associated with an increased risk of developing severe disease [74], higher mortality, prolonged need for invasive mechanical ventilation [75], and development of ARDS [76]. Similarly, higher levels of inflammatory markers, including C-reactive protein (CRP), IL-6 [77,78], ferritin [79,80], and neutrophil count [80,81], as well as decreased lymphocyte counts [81,82], have been observed in patients with more severe and fatal COVID-19, reflecting an enhanced inflammatory response.

Furthermore, elevated concentrations of aspartate aminotransferase (AST) [75], D-dimers [75,77–81], and troponin I (TnI) [75,81,83,84] have been associated with an increased disease severity and mortality in COVID-19, reflecting underlying tissue injury and activation of intravascular coagulation pathways. Markers of renal function, including urea [85] and creatinine [75,85], have also been reported to be elevated in more severe cases and in patients with poor and lethal outcomes. Although these biomarkers are routinely used in clinical practice, they lack specificity for COVID-19 infection, and their optimal threshold values for predicting its course, progression, and outcomes remain insufficiently defined [79,81,86].

1.6. Emerging Biomarkers

For predicting the COVID-19 course, 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 [87–91]. These approaches have provided insights into molecular alterations associated with the disease severity and clinical outcomes. However, despite their considerable promise, the omics and multi-omics approaches remain challenging to implement in routine clinical practice due to data complexity, limited standardisation, and the need for specialised expertise, thus underscoring the ongoing need for accessible, clinically applicable biomarkers [92–94].

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 [95–97]. 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 [98,99]. 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 [99,100]. In the infectious disease scenario, despite the host releasing cfNAs, pathogen-derived cfNAs can also be a source of blood cfNAs [101]. Recent studies suggest that cfNA can be a useful biomarker in the investigation and monitoring of patients with oncologic [102,103] and autoimmune diseases [104,105], as well as after transplantation [106,107] or severe trauma [108]. In the infectious disease context, elevated cfDNA levels are found in sepsis [109,110], and an increased concentration of cell-free mitochondrial DNA (cf-mtDNA) is common in people living with HIV (PLWH) [111]. Individual studies suggest that plasma cfDNA concentrations are higher in COVID-19 cases, which may be related to a poor prognosis [112,113]. Moreover, cfDNA has emerged as a clinically validated, minimally invasive biomarker that is readily applicable in routine clinical settings, providing real-time, reproducible insights into disease dynamics [99,114–116].

1.7. Rationale of the Study

At the onset of the COVID-19 pandemic in 2020, healthcare systems were rapidly overwhelmed, and clinical decision-making was hindered by a scarce and evolving evidence base, particularly regarding reliable predictors of the disease severity and mortality. In response, multiple demographic, clinical, and laboratory predictors were identified, and numerous predictive models were developed by using both traditional statistical methods and advanced machine learning approaches [52,65–69,74–78,117,118]. However, no consensus had been established regarding a unified set of predictors. Furthermore, the clinical utility of many proposed biomarkers varied according to their availability, the timing of assessment, and threshold values used in clinical decision-making [119,120]. Therefore, there was a need to identify clinically relevant predictors readily available at hospital admission so that to enable timely risk stratification and guide the treatment and patient management strategies.

Importantly, the performance and applicability of prognostic models were strongly influenced by population-specific and healthcare-related factors, including the genetic background, demographic characteristics, healthcare infrastructure, clinical practice patterns, and vaccination strategies [121]. Predictive models developed in Western Europe, Asia, or North America might not have been directly applicable to the Baltic region. To date, data from Eastern and Central Europe remain limited, despite the unique challenges faced by healthcare systems in these regions during the pandemic [122,123]. Therefore, generating region-specific evidence was essential for identifying clinically relevant predictors of disease severity and outcomes.

Although the global burden of severe COVID-19 has declined over time, largely due to increasing population immunity and the emergence of less virulent viral variants, substantial variability in disease severity persists. A comprehensive understanding of factors associated with severe disease and mortality remains essential not only for optimising the management of current SARS-CoV-2 infection but also for improving preparedness for future outbreaks of respiratory pathogens. In addition, the identification of accessible and clinically applicable novel biomarkers with an improved predictive performance remains important for timely risk stratification and supporting routine clinical decision-making.

The novelty of this study lies in its multidimensional approach towards characterising hospitalised COVID-19 patients and identifying the predictors of disease severity and adverse outcomes. In addition to analysing the established demographic, clinical, laboratory, and radiological risk factors, this study investigates the prognostic value of non-inflammatory biomarkers, namely, TnI and hyperglycaemia, which may provide additional insights into the systemic effects of COVID-19. The assessment of serum cfNAs as potential biomarkers for predicting the disease severity and lethal outcomes represents an innovative aspect of the study.

2. STUDY AIM AND OBJECTIVES

2.1. Aim of the Study

This study aims to assess demographic, clinical, and laboratory factors predicting severe COVID-19 disease and lethal outcomes in hospitalised COVID-19 patients.

2.2. Objectives of the Study

1. To describe demographic, clinical, laboratory, and radiological characteristics of hospitalised COVID-19 patients.
2. To identify predictors associated with severe COVID-19 disease in hospitalised patients.
3. To identify predictors associated with in-hospital mortality in COVID-19 patients.
4. To evaluate non-inflammatory biomarkers – troponin I and hyperglycaemia – as potential predictors of in-hospital mortality in COVID-19 patients.
5. To assess serum cell-free nucleic acids as potential biomarkers for predicting COVID-19 disease severity and the lethal outcome.

3. METHODS

3.1. Study Design, Population, and Inclusion Criteria

This study was composed of three parts:

(1) A retrospective cohort study of a depersonalised database of COVID-19 positive adults hospitalised in Vilnius University Hospital Santaros Klinikos between 1 March 2020 and 30 May 2021, serving to identify risk factors for in-hospital mortality due to COVID-19. A total of 2,794 patients were included.

In the evaluation of the predictive value of TnI, only individuals who had undergone TnI testing upon admission were included in the analysis. Patients with end-stage chronic kidney disease, defined by an estimated glomerular filtration rate (eGFR) < 15 mL/min/1.73 m^2, and those who developed myocardial infarction were excluded.

In the assessment of hyperglycaemia as a possible predictor of in-hospital mortality in COVID-19 patients, only individuals with blood glucose measured at admission were included. Patients who had pre-existing diabetes mellitus or met the criteria for newly diagnosed diabetes during their hospitalisation were excluded.

(2) An ambispective observational cohort study of COVID-19 positive adults hospitalised in the Infectious Diseases Department of Vilnius University Hospital Santaros Klinikos to describe demographic, clinical, laboratory, and radiological characteristics of hospitalised COVID-19 patients and identify risk factors for severe COVID-19 disease in hospitalised patients. This part included retrospectively collected data (13 March – 30 June 2020) and prospectively collected data (1 July 2020 – 31 December 2021). Exclusion criteria were hospitalisation ≤ 24 hours, refusal to participate, or inability to provide informed consent. In total, 495 patients were included, of whom 258 (52.1%) were enrolled retrospectively and 237 (47.9%) prospectively.

(3) A retrospective cohort study using stored serum samples along with accompanying health information of patients hospitalised in Vilnius University Hospital Santaros Klinikos between 24 November 2020 and 10 November 2021 intended to evaluate serum cfNAs as possible prognostic biomarkers for COVID-19 disease severity and the lethal outcome. Exclusion criteria included oncological or haematological diseases, prior organ transplantation, HIV infection, and vulnerable individuals as defined by the Lithuanian biomedical research ethics law. A total of 132 individuals were included: 108 patients with COVID-19 and 24 controls.

Eligible participants across all study parts were adults (≥18 years) with confirmed COVID-19 disease requiring hospitalisation. COVID-19 disease was confirmed by a positive SARS-CoV-2 reverse transcriptase polymerase chain reaction (RT-PCR) or a rapid antigen test using a nasopharyngeal sample. Rapid antigen testing was performed in symptomatic patients within 5 days of the symptom onset.

3.2. Data Collection

In a retrospective cohort study (Part 1), depersonalised patient data were retrieved from the electronic medical records of Vilnius University Hospital Santaros Klinikos and provided by the Informatics and development centre of Vilnius University Hospital Santaros Klinikos in accordance with hospital-approved procedures. The retrieved data included: demographic characteristics (age, sex), comorbidities coded according to the International Statistical Classification of Diseases and Related Health Problems, Tenth Revision, Australian Modification (ICD-10-AM) in medical records – arterial hypertension (I10; I11.0; I11.9), diabetes mellitus without complications (E10.9, E11.9), diabetes mellitus with complications (E10, E11 except for E10.9, E11.9), obesity (E66, E66.0, E66.2, E66.8, E66.9), chronic kidney disease (N18.1, N18.2, N18.3, N18.4, N18.5, N18.9), heart failure (I50.0, I50.1, I50.9), HIV infection (B23.8, B20), viral hepatitis C (B18.2), viral hepatitis B (B18.1), chronic obstructive pulmonary disease (COPD) (J44.0, J44.1, J44.8, J44.9), previous stroke (I69), previous myocardial infarction (I25.2), coronary artery disease (I20.0, I20.2, I20.8, I20.9), organ transplantation (Z94.0, Z94.1, Z94.4), complications during COVID-19 hospitalisation (sepsis (A02.1, A22.7, A26.7, A32.7, A40, A41, A42.7), acute kidney injury (N17), acute myocardial infarction (I21), acute pulmonary embolism (I26), acute stroke (I61, I63)), utilization of antibiotics, systemic steroids, antivirals, the need for invasive mechanical ventilation (IMV), in-hospital mortality and length of hospitalisation [124–126].

In the ambispective observational cohort study (Part 2), data were collected from the electronic medical records of Vilnius University Hospital Santaros Klinikos, whereas, for the prospective component, the data were supplemented by conducting direct patient interviews. Both sources provided information on demographic characteristics, underlying medical conditions, and other clinical data. Demographic variables included the age (in years) and sex (male/female). Comorbidities covered anaemia or other haematological diseases, asthma, COPD, other pulmonary diseases, arterial hypertension, other cardiovascular diseases (including coronary artery disease, heart failure,

atrial fibrillation, and prior myocardial infarction), non-haematological malignancy, dementia, diabetes mellitus, immunodeficiency, chronic liver disease (including cirrhosis), chronic kidney disease, rheumatological disease, neuromuscular disease, prior stroke, tuberculosis, and obesity. Additional variables included the smoking status (never/former/current), the body mass index (BMI, kg/m²), the pregnancy status, and the COVID-19 vaccination status (vaccinated/unvaccinated). The 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. Clinical presentation at admission was recorded as present or absent for symptoms such as a sudden onset of symptoms, subfebrile and febrile fever, chills, tachypnoea, malaise, headache, dizziness, confusion, myalgia, sore throat, coryza, cough, dyspnoea, chest pain, palpitations, general deterioration, nausea, vomiting, diarrhoea, abdominal pain, ageusia, anosmia, conjunctivitis, rash, and other dermatological manifestations. Objective findings included the body temperature (°C), respiratory rate (breaths/min), and peripheral oxygen saturation (SpO_2, %). Radiological data consisted of chest X-ray and Computed Tomography (CT) findings. Information on in-hospital management included the need for respiratory support: conventional oxygen therapy (nasal cannula or face mask), high-flow oxygen therapy, non-invasive ventilation (NIV), IMV, and extracorporeal membrane oxygenation (ECMO). In-hospital complications included pneumonia, ARDS, bronchiolitis, secondary bacterial infections (including culture-confirmed pneumonia), other secondary bacterial infections (confirmed by positive culture), sepsis, acute kidney injury, heart failure, multiple organ dysfunction, critical illness myopathy, pulmonary embolism, deep vein thrombosis (DVT), stroke, dermatological complications, and other unspecified complications. Information about the transmission to the ICU or High Dependency Unit (HDU) (yes/no), the length of stay in these units, overall length of hospitalisation (in days), and the hospital discharge status (discharged/transferred, died) was also obtained [127].

In the retrospective cohort study using stored serum samples (Part 3), 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 the age in years and sex (male/female), comorbidities (arterial hypertension, coronary artery disease, congestive heart failure, atrial fibrillation, previous myocardial infarction, COPD, obesity, diabetes mellitus, chronic kidney disease, and sequelae of prior stroke), weight and height (in kilograms and meters, respectively), and the BMI value was subsequently calculated.

Moreover, the data on complications developed during the hospitalisation were obtained; it included pneumonia, acute respiratory failure, pulmonary embolism, DVT, stroke, acute kidney injury, and sepsis. The data on the need for any oxygen therapy, including NIV, IMV, and ECMO, were also extracted. The length of stay (in days) and the result of hospitalisation (survivors/non-survivors) were also collected. For the control group, we collected data on sex and age that were available in the Biobank dataset [128].

For all three study parts, data on the laboratory test results obtained on the day of hospitalisation were collected. These included the complete blood count (white blood cell count, neutrophils, lymphocytes, haemoglobin, platelets), glucose, creatinine, eGFR, urea, sodium, potassium, alanine aminotransferase (ALT), AST, gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), LDH, lactate, CRP, procalcitonin, ferritin, IL-6, D-dimer, fibrinogen, TnI, N-terminal pro B-type natriuretic peptide (NT-proBNP), total protein, prothrombin time (PT), and international normalised ratio (INR).

3.3. Laboratory Procedures for Cell-Free Nucleic Acids Extraction and Analysis

Cell-free nucleic acid extraction and analysis for cfDNA and cell-free SARS-CoV-2 RNA (cfRNA) were performed for Part 3 of the study [128]. These analytes were measured by analysing blood serum samples collected on the day of hospitalisation.

Blood serum samples preparation: in the Biobank, gel vacutainers containing blood samples were centrifuged for 8 minutes at 3600 g (relative centrifugal force). Subsequently, 500 µL of serum was aliquoted into separate tubes (one or two aliquots, depending on the serum volume), encoded, and stored at -80 °C. Haemolysis or leukocyte lysis was not documented. The samples were thawed only once and used immediately for cfNA extraction.

cfNA extraction from blood serum: cfNA was extracted by 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 by using letter and number combinations and stored at -80 °C 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 low nucleic acid concentrations, the High Sensitivity DNA kit was used.

Quantitative PCR: in order to detect cfRNA in the extracted samples, the Quantitative PCR method was used on 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 was used. Total reaction volume thus was 25 µL. The first stage of the quantitative PCR method involved incubating uracil-N-glycosylase for one cycle, 2 minutes, at a temperature of 25 °C. The second reverse transcription consisted of 1 cycle, 10 minutes, 53 °C; the third polymerase activation consisted of 1 cycle, 2 minutes, 95 °C; the fourth amplification lasted for 40 cycles, 3 seconds at 95 °C and 30 seconds at 60 °C. The results were analysed by 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 the C24 SARS-CoV-2 genome was used; for the negative control, 4 µL of H_2O was used. 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.

3.4. Analysed Groups and Outcomes

Severe COVID-19 was defined as a clinical course requiring any form of oxygen therapy during hospitalisation. Critical COVID-19 was defined as a disease course requiring ICU admission. Non-severe (mild) disease was defined as not requiring oxygen therapy.

For analysis, the patients were grouped as non-ICU versus ICU, and as requiring advanced respiratory support (ARS), defined as NIV, IMV, or ECMO, versus no ARS.

The outcome for identifying risk factors for in-hospital mortality due to COVID-19 was in-hospital mortality: patients who died during hospitalisation were referred to the lethal outcome group, and those who were discharged or transferred were referred to the non-lethal group. In the cfNA analysis, the COVID-19 positive group was also compared with the control group.

For troponin I analysis, patients were stratified by admission troponin I levels into three groups: <19 ng/L, 19–100 ng/L, and >100 ng/L.

For hyperglycaemia analysis, the patients without diabetes were categorised by admission glucose levels into four groups: hypoglycaemia (<4.0 mmol/L), normoglycaemia (≥4.0 to <6.1 mmol/L), mild hyperglycaemia (≥6.1 to <7.8 mmol/L), and intermittent hyperglycaemia (≥7.8 to <11.1 mmol/L).

3.5. Statistical Analysis

Descriptive statistics were used to characterise the baseline demographic, clinical, laboratory, radiological characteristics, complications, and the outcome data. Continuous variables were presented as medians with interquartile ranges (IQR), while categorical and binary variables were presented as absolute numbers and percentages.

Normality was assessed by using the Shapiro–Wilk test; as most variables were non-normally distributed, non-parametric tests were applied. Log transformation was used to reduce the skewness of cfDNA values.

Mann-Whitney U test or the Kruskal–Wallis test, followed by Dunn's *post-hoc* pairwise comparisons with Bonferroni correction wherever appropriate, were used to compare continuous variables, and χ^2 test, or Fisher's exact test, was used to compare categorical and binary variables. Associations between cfDNA and laboratory parameters were assessed by using Spearman's rank correlation.

The predictive performance of laboratory parameters and cfDNA was assessed by using receiver operating characteristic (ROC) curve analysis, including variables that differed significantly between the outcome categories.

Optimal cut-off values, along with their corresponding sensitivity and specificity, were obtained by calculating the Youden index (sensitivity + specificity –1).

To identify the predictors of severe COVID-19, ICU admission, need for IMV, ARS, and in-hospital mortality, univariable and multivariable binary logistic regression models were constructed. Univariable models included demographic and clinical characteristics, as well as laboratory values (entered either as continuous variables or dichotomised according to established cut-off values). Variables that were statistically significant in univariable analyses were reviewed by clinical experts for inclusion in the multivariable model. Odds ratios (ORs) with 95% confidence intervals (CIs) were reported. Multivariable logistic regression models were fitted by using either the enter method or stepwise backward conditional selection, as appropriate. Model performance was assessed by using Cox & Snell and Nagelkerke R^2, whereas calibration was implemented with the Hosmer – Lemeshow test, and classification was accomplished by using a 0.50 probability threshold. Internal validation of the regression model was performed by using bootstrap resampling (1000 samples) to assess the stability of the identified predictors.

Survival analysis was performed by using Cox proportional hazards models to assess the effects of glucose and TnI levels on 30-day in-hospital mortality, while adjusting for age, sex, relevant comorbidities, and treatment. Survival curves were plotted to illustrate the outcomes stratified by glucose and troponin I levels.

Due to the retrospective design and routine clinical testing, some data on comorbidities and laboratory parameters were unavailable for all patients. Missing data were handled by using a complete-case approach, with cases that contained missing values being excluded from the respective analyses. ROC and multivariable regression analyses were restricted to laboratory variables with <20% missing data.

A p-value of <0.05 was considered statistically significant. All analyses were performed by using *IBM SPSS Statistics*, version 30.0 (*IBM Corp*., USA) and *Microsoft Excel*.

3.6. Ethical Aspects

All three study parts were conducted in accordance with the Declaration of Helsinki.

In Part 1 of the study – a retrospective cohort study of a depersonalised database of COVID-19 positive adults hospitalised at Vilnius University Hospital Santaros Klinikos – only depersonalised and pseudonymised data

were analysed. Therefore, no bioethics approval was required, and the need for participant consent was waived. Approval for publication of the study results was granted by Vilnius University Hospital Santaros Klinikos (2022-12-13; No. SR-7021).

For Part 2 of the study – an ambispective observational cohort study of COVID-19 positive adults hospitalised in Vilnius University Hospital Santaros Klinikos – the approval from the Lithuanian Bioethics Committee was obtained on July 3, 2020 (approval number L-20-3/1, with subsequent amendments approved on July 25, 2022, January 25, 2023, December 13, 2023, and December 20, 2024). The requirement for written informed consent was waived for participants enrolled retrospectively (until July 2020), whereas all participants recruited prospectively provided written informed consent.

Part 3 of the study – a retrospective cohort study using stored serum samples and accompanying health information – was conducted under the approval granted for the study "*Study on Predicting the Course of COVID-19 Disease Based on Blood Markers*" by Vilnius Regional Biomedical Research Ethics Committee on June 6, 2023 (approval number 2023/6-1521-982; whereas, further amendments were approved on November 21, 2024, and March 7, 2025). All participants had previously provided written informed consent for biobanking at Vilnius Santaros Klinikos Biobank; therefore, the requirement for additional study-specific written informed consent was waived.

3.7. Funding

The ambispective observational cohort study of COVID-19 positive adults hospitalised at Vilnius University Hospital Santaros Klinikos was funded by the *Horizon 2020* program of the European Commission, project *I-MOVE-COVID-19*, grant agreement number 101003673.

The study "*Study on Predicting the Course of COVID-19 Disease Based on Blood Markers*" was funded by Vilnius University Hospital Santaros Klinikos. This research received the contribution from the *EuCARE Project* funded by the European Union's Horizon Europe Research and Innovation Program under Grant Agreement No. 101046016.

4. RESULTS

4.1. Demographic, Clinical, Laboratory, and Radiological Characteristics of Hospitalised COVID-19 Patients

Demographic, clinical, laboratory, and radiological data are presented from an ambispective cohort study with 495 participants [127]. Out of the 495 hospitalised patients, 52.9% were male. The median age was 55 (IQR 43–65) years, and 44.6% of the sample was composed of individuals aged 50–69 years. More than half of the patients (61.2%) had at least one comorbidity. The most frequent underlying conditions were arterial hypertension (51.5%), other cardiovascular diseases (20.8%), and diabetes mellitus (11.1%). Among patients with available BMI data, obesity was identified in 50.5% of the cases (Table 1). Initial laboratory test results performed upon admission are summarised in Table 1.

Table 1. Demographic characteristics, prevalence of comorbidities and initial laboratory parameters of hospitalised COVID-19 patients

Characteristic	N	n (%) / Median (IQR)
Age in years, median (IQR)	495	55 (43–65)
Age groups, years		
18–29	39	39 (7.9)
30–39	56	56 (11.3)
40–49	99	99 (20.0)
50–59	116	116 (23.4)
60–69	105	105 (21.2)
70–79	45	45 (9.1)
≥ 80	35	35 (7.1)
Male sex	495	262 (52.9)
Obesity	271	137 (50.5)
Comorbidities	495	303 (61.2)
Anaemia or any other haematological disease	495	40 (8.1)
Asthma	495	17 (3.4)
COPD	495	14 (2.8)
Other pulmonary diseases, not asthma or COPD	495	11 (2.2)
Non-haematological oncological disease	495	28 (5.7)
Arterial hypertension	495	255 (51.5)
Other cardiovascular disease (not arterial hypertension)	495	103 (20.8)
Dementia	495	13 (2.6)
Diabetes mellitus	495	55 (11.1)

Characteristic	N	n (%) / Median (IQR)
Immunodeficiency	495	15 (3.0)
Chronic liver disease, including cirrhosis	495	18 (3.6)
Neuromuscular disease	495	12 (2.4)
Chronic kidney disease	495	32 (6.5)
Rheumatological disease	495	17 (3.4)
Prior stroke	495	18 (3.6)
Tuberculosis	495	3 (0.6)
Length of hospitalization in days, median (IQR)	495	10 (7–14)
Laboratory parameters		
WBC, $x10^9$/L	481	5.6 (4.3–7.1)
Neutrophils, $x10^9$/L	481	3.8 (2.7–5.2)
Lymphocytes, $x10^9$/L	481	1.2 (0.8–1.6)
Platelets, $x10^9$/L	481	187 (149–236)
ALP, U/L	392	64.0 (52.0–80.3)
ALT, U/L	443	30.0 (18.0–48.0)
AST, U/L	430	33.0 (24.0–50.0)
Ferritin, µg/L	398	358.7 (168.6–775.5)
IL-6, ng/L	401	25.0 (11.0–50.6)
LDH, U/L	407	269.0 (208.0–348.6)
D-dimer, µg/L	395	375.0 (215.0–640.0)
Fibrinogen, g/L	360	5.1 (4.2–6.1)
CRP, mg/L	479	29.9 (6.8–84.1)
Lactate, mmol/L	367	1.3 (1.0–1.8)
Troponin I, ng/L	387	6.6 (3.0–13.0)
NT-proBNP, ng/L	339	147.2 (71.6–386.0)
GGT, U/L	391	34.0 (18.0–76.0)
PT, %	387	90.0 (78.0–104.0)
INR	393	1.04 (0.98–1.11)
Creatinine, µmol/L	467	77.6 (64.0–95.0)
Urea, mmol/L	376	4.7 (3.6–6.4)

Note. Abbreviations in use: ALP – alkaline phosphatase; ALT – alanine aminotransferase; AST – aspartate aminotransferase; COPD – chronic obstructive pulmonary disease; CRP – C-reactive protein; GGT – gamma-glutamyl transferase; IL-6 – interleukin 6; INR – international normalised ratio; IQR – interquartile range; LDH – lactate dehydrogenase; NT-proBNP – N-terminal pro-B-type natriuretic peptide; PT – prothrombin time; WBC – white blood cell count.

Adapted from: Kubiliute, I. et al. *Predicting severe COVID-19 disease in adults: A single-centre cohort study during the first three pandemic waves in 2020–2021 in Vilnius, Lithuania*. PLoS ONE. 2026;21(5):e0350112 [127].

The clinical symptoms reported by patients at hospital admission are detailed in Table 2. Most patients (81.5%) experienced the sudden onset of the disease. The most frequently reported symptoms were malaise (77.1%), cough (69.7%), subfebrile fever (65.9%), and shortness of breath (40.5%).

Table 2. Clinical symptoms at hospital admission in COVID-19 patients (patient-reported)

Symptoms	N	n (%)
Sudden onset of symptoms	482	393 (81.5)
Subfebrile fever	495	326 (65.9)
Febrile fever	495	179 (36.2)
Chills	490	143 (29.2)
Tachypnoea	495	91 (18.4)
Malaise	488	376 (77.1)
Headache	488	152 (31.2)
Dizziness	488	85 (17.4)
Confusion	491	24 (4.9)
Myalgia	488	142 (29.1)
Sore throat	488	101 (20.7)
Coryza	488	73 (15.0)
Cough	489	341 (69.7)
Shortness of breath	489	198 (40.5)
Chest pain	488	114 (23.4)
Palpitations	488	41 (8.4)
General deterioration	492	151 (30.7)
Nausea	488	54 (11.1)
Vomiting	495	12 (2.4)
Diarrhoea	491	62 (12.6)
Abdominal pain	488	28 (5.7)
Ageusia	487	75 (15.4)
Anosmia	487	109 (22.4)
Conjunctivitis	495	11 (2.2)
Rash/other dermatological manifestation	495	4 (0.8)

Adapted from: Kubiliute, I. et al. *Predicting severe COVID-19 disease in adults: A single-centre cohort study during the first three pandemic waves in 2020–2021 in Vilnius, Lithuania*. PLoS ONE. 2026;21(5):e0350112 [127].

Radiological imaging findings, characteristics of oxygen therapy, the need for treatment in ICU and HDU, and documented complications are summarised in Table 3. The majority of hospitalised patients underwent radiological evaluation. Pneumonia was diagnosed in 82.1% of the patients,

with bilateral involvement in 84.8% of them versus unilateral involvement in 15.2%. Among those patients who underwent chest X-ray, pulmonary infiltrates were identified in 75.0%, and infiltration opacity of more than 50% of the lung fields was observed in 18.4% of the cases. Whereas, stasis was reported in 12.3% of the cases, and pleural effusion in 6.6%. Chest CT was performed in 25.5% of the patients. Among these, ground-glass opacities were detected in 65.1%, peripheral opacities in 51.6%, and infiltration in 49.2% of the patients.

Table 3. Radiological investigation findings, oxygen therapy, and complications of COVID-19 patients

Characteristic	n (%)
Chest X-ray	473 (95.6)
Infiltration	354 (75.0)
Pleural effusion	31 (6.6)
Infiltration opacity > 50%	45 (18.4)
Stasis	58 (12.3)
Chest computed tomography	126 (25.5)
Peripheral opacity	65 (51.6)
Ground-glass opacity	82 (65.1)
Consolidation	31 (24.6)
Infiltration	62 (49.2)
Pleural effusion	15 (11.9)
Pneumonia	395 (82.1)
Unilateral	60 (15.2)
Bilateral	335 (84.8)
Oxygen therapy	291 (58.8)
Nasal cannula	87 (17.6)
Face mask	218 (44.0)
High-flow therapy	29 (5.9)
Non-invasive ventilation	5 (1.0)
Mechanical ventilation	14 (2.8)
ECMO	3 (0.6)
Complications	
Any complication	114 (25.1)
Acute respiratory distress syndrome	46 (9.3)
Bronchiolitis	1 (0.2)
Secondary bacterial pneumonia (confirmed by positive bronchoalveolar lavage culture)	10 (2.0)
Other secondary bacterial infection (confirmed by positive culture)	60 (12.1)
Sepsis	18 (3.6)

Characteristic	n (%)
Acute renal failure	18 (3.6)
Heart failure	5 (1.0)
Multiple organ dysfunction	14 (2.8)
Dermatological complications	3 (0.6)
Critical illness myopathy	1 (0.2)
Pulmonary embolism	8 (1.6)
Other complication	27 (5.5)
Transferred to ICU or HDU	54 (10.9)
Transferred to ICU	49 (9.9)
Transferred to HDU	11 (2.2)

Note. Abbreviations in use: ECMO – extracorporeal membrane oxygenation; HDU – High Dependency Unit; ICU – Intensive Care Unit.
Adapted from: Kubiliute, I. et al. *Predicting severe COVID-19 disease in adults: A single-centre cohort study during the first three pandemic waves in 2020–2021 in Vilnius, Lithuania*. PLoS ONE. 2026;21(5):e0350112 [127].

The most frequently administered oxygen therapy modalities among patients with severe COVID-19 were a face mask (74.9%) and a nasal cannula (29.9%). High-flow oxygen therapy was utilised for 10.0% of severe patients. Fewer patients required NIV (1.7%) or IMV (4.8%). ECMO was used in 1.0% of the patients with severe disease.

Overall, 114 patients (25.1%) developed at least one complication during hospitalisation. The most frequent complications were ARDS (9.3%) and other secondary bacterial infections (not pneumonia) (12.1%). Sepsis and acute renal failure were each observed in 3.6% of the patients, while multiple organ dysfunction occurred in 2.8% of the cases. Less common complications included pulmonary embolism (1.6%), heart failure (1.0%), dermatological complications (0.6%), bronchiolitis (0.2%), and critical illness myopathy (0.2%). Secondary bacterial pneumonia confirmed by bronchoalveolar lavage was identified in 2.0% of cases.

In total, 54 patients (10.9%) required transfer to a higher level of care, including 49 (9.9%) to the ICU and 11 (2.2%) to HDU. The median length of hospitalisation was 10 days (IQR 7–14).

4.2. Predictors of Severe COVID-19 Disease in Hospitalised Patients

A total of 291 of 495 patients (58.8%) developed severe COVID-19 [127]. Compared with non-severe cases, these patients were more often male (57.4% vs. 46.6%, $p = 0.018$) and approximately 10 years older. Comorbidities were more prevalent among patients with severe disease (68.4% vs. 51.0%, p

< 0.001). In particular, COPD (4.5% vs. 0.5%, p = 0.009), arterial hypertension (59.1% vs. 40.7%, $p < 0.001$), and other cardiovascular diseases (24.7% vs. 15.2%, p = 0.010) were significantly more common in severe cases.

Patients with severe COVID-19 were significantly more likely to present with febrile fever (45.7% vs. 22.6%, $p < 0.001$), cough (74.4% vs. 63.0%, p = 0.007), shortness of breath (56.1% vs. 18.0%, $p < 0.001$), tachypnoea (28.9% vs. 3.4%, $p < 0.001$), chest pain (28.5% vs. 16.0%, p = 0.001) and malaise (87.5% vs. 62.0%, $p < 0.001$) on admission. Other symptoms that were also more common in severe cases included chills (35.3% vs. 20.4%, $p < 0.001$), dizziness (24.3% vs. 7.5%, $p < 0.001$), confusion (6.6% vs. 2.5%, p = 0.036), general deterioration (39.5% vs. 18.2%, $p < 0.001$), and diarrhoea (16.2% vs. 7.5%, p = 0.004).

On admission, inflammatory markers were substantially higher in those patients who developed severe COVID-19 compared to non-severe cases: CRP (56.0 vs. 6.2 mg/L, $p < 0.001$), IL-6 (33.4 vs. 9.9 ng/L, $p < 0.001$), ferritin (480.0 vs. 215.0 µg/L, $p < 0.001$) and fibrinogen (5.4 vs. 4.4 g/L, $p < 0.001$). Differences in haematological parameters included a lower lymphocyte count (1.1 vs. 1.4 $\times 10^9$/L, $p < 0.001$) and a higher neutrophil count (4.1 vs 3.3 $\times 10^9$/L, $p < 0.001$) in the severe group. In addition, several other laboratory analytes were significantly higher among the severe cases, including LDH (303.0 vs. 206.4 U/L, $p < 0.001$), AST (38.0 vs. 24.0 U/L, $p < 0.001$), ALT (34.0 vs. 24.0 U/L, $p < 0.001$), lactate (1.4 vs. 1.1 mmol/L, $p < 0.001$), D-dimer (425.0 vs. 265.0 µg/L, $p < 0.001$), troponin I (8.0 vs. 3.8 ng/L, p < 0.001), NT-proBNP (170.3 vs. 98.0 ng/L, $p < 0.001$), urea (5.0 vs. 4.3 mmol/L, p <0.001), and creatinine (80.0 vs. 74.4 µmol/L, p = 0.007).

In order to identify factors associated with severe COVID-19, logistic regression analysis was performed. In univariable analysis, multiple demographic, clinical, and laboratory variables were significantly associated with severe disease, including an older age, the male sex, obesity, arterial hypertension, other cardiovascular disease, COPD, subfebrile and febrile fever, chills, tachypnoea, malaise, dizziness, confusion, cough, dyspnoea, chest pain, general deterioration, diarrhoea, lymphocyte and neutrophil counts, ALT, AST, ferritin, IL–6, LDH, D-dimer, fibrinogen, CRP, lactate, creatinine, and urea.

Multivariable logistic regression identified six independent predictors of severe COVID-19, five of which remained significant after internal validation (Figure 1). An older age was independently associated with a higher likelihood of severe disease (OR 1.04 per year, 95% CI 1.00–1.08, p = 0.040), whereas obesity increased the odds 3.55 times compared with non-obese

individuals (OR 3.55, 95% CI 1.35–9.30, p = 0.010). Among symptoms presenting at admission, febrile fever was associated with severe COVID-19 (OR 3.41, 95% CI 1.09–10.73, p = 0.036). Lymphopenia on admission also emerged as a significant predictor (OR 3.70, 95% CI 1.37–9.99, p = 0.010). Furthermore, higher LDH and CRP concentrations were independently associated with COVID-19 severity (LDH: OR 1.008, 95% CI 1.00–1.01, p = 0.022; CRP: OR 1.021, 95% CI 1.01–1.04, p = 0.002), with each 10 U/L increase in LDH and each 10 mg/L increase in CRP corresponding to an 8% and 21% increase in odds of severe COVID-19, respectively. Bootstrap-based internal validation confirmed the robustness of age, obesity, lymphopenia, LDH, and CRP as independent predictors of severe COVID-19 (Figure 1).

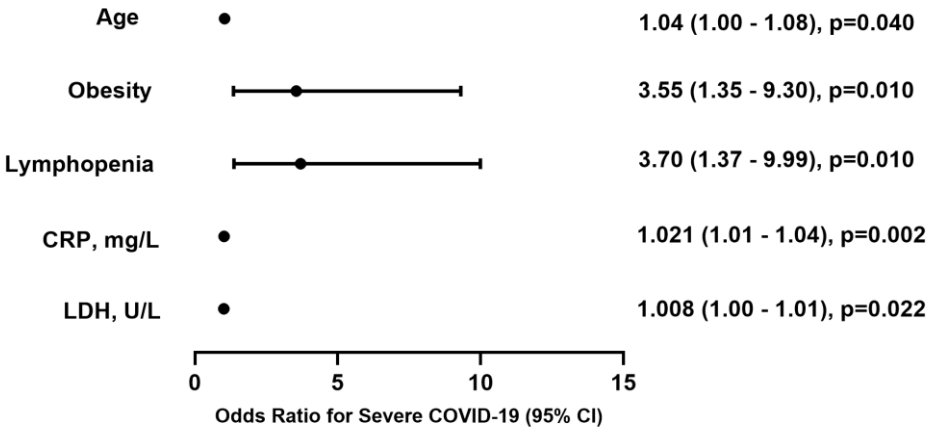

Figure 1. Significant predictors of severe COVID-19 disease course, a multivariable logistic regression model after bootstrap-based internal validation

Note. Abbreviations in use: CI – confidence interval; CRP – C-reactive protein; LDH – lactate dehydrogenase.
Reprinted from: Kubiliute, I. et al. *Predicting severe COVID-19 disease in adults: A single-centre cohort study during the first three pandemic waves in 2020–2021 in Vilnius, Lithuania*. PLoS ONE. 2026;21(5):e0350112 [127].

4.3. Predictors of In-Hospital Mortality in COVID-19 Patients

The overall in-hospital mortality rate among patients with COVID-19 was 12.7%, and it demonstrated an age-related increase, rising from 1.6% among patients younger than 40 years to 29.7% among those aged ≥ 70 years. Patients deceased during hospitalization were significantly older (72.5 vs. 58

years, $p < 0.001$), and had a higher prevalence of comorbidities such as arterial hypertension (54.8% vs 34.2%, $p < 0.001$), coronary artery disease (9.0% vs. 3.0%, $p < 0.001$), congestive heart failure (28.9% vs. 4.8%, $p < 0.001$), diabetes mellitus (23.9% vs. 12.2%, $p < 0.001$), obesity (11.5% vs. 3.6%, $p < 0.001$), COPD (4.8% vs. 1.1%, $p < 0.001$), chronic kidney disease (17.4% vs. 6.3%, $p < 0.001$), and prior stroke (5.6% vs. 0.8%, $p < 0.001$) compared with patients in the non-lethal group (Table 4) [124].

At hospital admission, non-survivors exhibited significantly higher levels of WBC, neutrophil count, neutrophil-to-lymphocyte ratio (NLR), glucose, creatinine, urea, potassium, AST-to-ALT ratio, LDH, CRP, ferritin, IL-6, D-dimer, and TnI than those who recovered. In contrast, haemoglobin concentration, lymphocyte count, platelets, and sodium concentration were significantly lower in non-survivors (Table 4).

Table 4. Demographic, clinical, and therapeutic characteristics of hospitalised COVID-19 patients by lethal outcome

Characteristic	Non-lethal outcome (N=2438), n (%)	Lethal outcome (N=356), n (%)	*p*-value
Age, years, median (IQR)	58 (47–67)	72.5 (62–81)	<0.001
Male	1324 (54.3)	196 (55.1)	0.791
Comorbidities			
Any underlying condition	1045 (42.9)	278 (78.1)	<0.001
Arterial hypertension	835 (34.2)	195 (54.8)	<0.001
Coronary artery disease	73 (3.0)	32 (9.0)	<0.001
Congestive heart failure	117 (4.8)	103 (28.9)	<0.001
Diabetes mellitus	297 (12.2)	85 (23.9)	<0.001
Obesity	88 (3.6)	41 (11.5)	<0.001
COPD	27 (1.1)	17 (4.8)	<0.001
Chronic kidney disease	153 (6.3)	62 (17.4)	<0.001
Previous stroke	19 (0.8)	20 (5.6)	<0.001
Laboratory parameters			
Haemoglobin, g/L	138 (125–149)	127 (111–145)	<0.001
WBC, $x10^9$/L	6.43 (4.83–8.77)	8.56 (5.67–12.00)	<0.001
Neutrophils, $x10^9$/L	4.67 (3.24–6.80)	6.96 (4.38–10.28)	<0.001
Lymphocytes, $x10^9$/L	1.04 (0.75–1.48)	0.72 (0.50–1.10)	<0.001
NLR	4.40 (2.69–7.28)	8.85 (4.61–17.70)	<0.001
Platelets, $x10^9$/L	200.5 (157.0–260.0)	192.0 (143.0–255.8)	0.010
Glucose, mmol/L	6.11 (5.45–7.11)	7.16 (6.03–9.39)	<0.001
Creatinine, µmol/L	80.00 (65.24–97.66)	110.00 (78.00–170.29)	<0.001

Characteristic	Non-lethal outcome (N=2438), n (%)	Lethal outcome (N=356), n (%)	*p*-value
Urea, mmol/L	5.38 (3.99–7.66)	11.67 (7.10–20.68)	<0.001
Sodium, mmol/L	140.00 (137.00–143.00)	138.00 (134.95–142.00)	<0.001
Potassium, mmol/L	4.2 (3.9–4.5)	4.3 (3.9–4.8)	0.003
ALT, U/L	31.20 (19.85–51.96)	31.00 (17.00–55.00)	0.669
AST, U/L	35.00 (25.00–53.00)	51.81 (31.09–78.75)	<0.001
AST to ALT ratio	1.13 (0.85–1.57)	1.69 (1.19–2.49)	<0.001
LDH, U/L	294.00 (231.00–392.00)	417.00 (298.00–626.00)	<0.001
CRP, mg/L	53.30 (17.50–111.80)	118.40 (58.53–183.43)	<0.001
Ferritin, µg/L	447.51 (224.00–954.00)	717.00 (366.60–1687.24)	<0.001
IL-6, ng/L	27.00 (13.50–52.25)	55.15 (26.18–122.50)	<0.001
D-dimer, µg/L	480.00 (290.00–888.75)	1080.00 (560.00–2158.75)	<0.001
Fibrinogen, g/L	5.39 (4.51–6.46)	5.68 (4.12–6.93)	0.400
Troponin I, ng/L	9.00 (5.00–20.65)	41.00 (15.75–173.50)	<0.001
Length of hospitalization, days, median (IQR)	11 (7–16)	12 (5.25–19)	0.360

Note. Abbreviations in use: ALT – alanine aminotransferase; AST – aspartate aminotransferase; COPD – chronic obstructive pulmonary disease; CRP – C-reactive protein; IL-6 – interleukin 6; IQR – interquartile range; LDH – lactate dehydrogenase; NLR – neutrophil-to-lymphocyte ratio; WBC – white blood cell count.

Adapted from: Kubiliute, I. et al. *Clinical characteristics and predictors for in-hospital mortality in adult COVID-19 patients: A retrospective single center cohort study in Vilnius, Lithuania*. PLoS ONE. 2023;18(8):e0290656 [124].

Univariable analysis identified age, several comorbid conditions (arterial hypertension, coronary artery disease, congestive heart failure, diabetes mellitus, obesity, COPD, chronic kidney disease, and previous stroke), and multiple laboratory analytes as factors associated with in-hospital mortality in COVID-19 patients.

Multivariable analysis confirmed that independent predictors for in-hospital mortality of COVID-19 patients were age (OR 1.04, 95% CI 1.02–1.06), congestive heart failure (OR 3.06, 95% CI 1.96–4.77), obesity (OR 3.90, 95% CI 2.12–7.16), COPD (OR 2.92, 95% CI 1.12–7.60), previous stroke (OR 5.80, 95% CI 2.07–16.21) and the following laboratory parameters: urea > 7.01 mmol/l (OR 2.32, 95% CI 1.47–3.67), AST to ALT ratio > 1.49 (OR 1.54, 95% CI 1.08–2.21), LDH > 452.5 U/l (OR 2.60, 95% CI 1.74–3.88), CRP > 92.68 mg/l (OR 1.58, 95% CI 1.06–2.35), IL-6 > 69.55 ng/l (OR 1.62, 95% CI 1.10–2.40), and TnI > 18.95 ng/l (OR 2.04, 95% CI 1.38–3.02) (Figure 2).

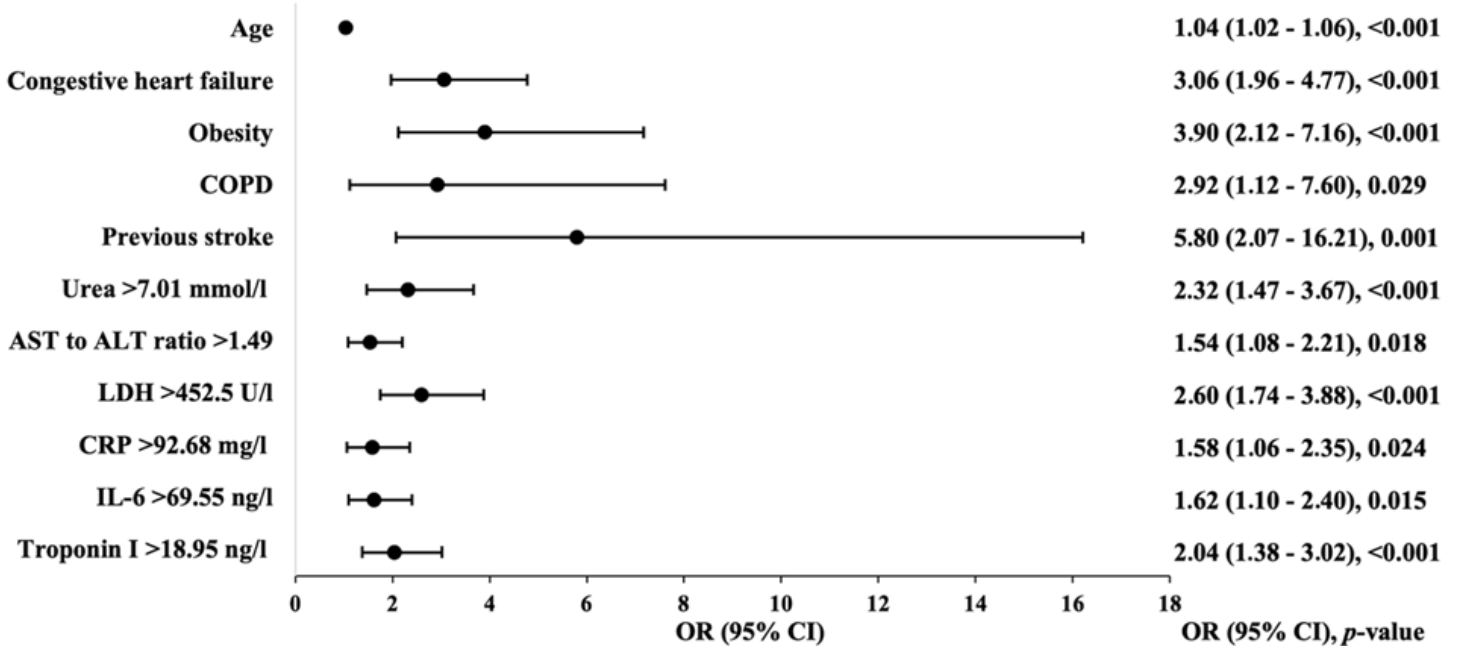

Figure 2. Significant predictors associated with in-hospital mortality in patients with COVID-19, a multivariable logistic regression model

Note. Abbreviations in use: ALT – alanine aminotransferase; AST – aspartate aminotransferase; CI – confidence interval; COPD – chronic obstructive pulmonary disease; CRP – C-reactive protein; IL-6 – interleukin 6; LDH – lactate dehydrogenase; OR – odds ratio.
Reprinted from: Kubiliute, I. et al. *Clinical characteristics and predictors for in-hospital mortality in adult COVID-19 patients: A retrospective single center cohort study in Vilnius, Lithuania*. PLoS ONE. 2023;18(8):e0290656 [124].

4.4. Non-Inflammatory Biomarkers – Troponin I and Hyperglycaemia – Evaluation as Potential Predictors of In-Hospital Mortality in COVID-19 Patients

We analysed non-inflammatory biomarkers – troponin I and hyperglycaemia – as potential predictors of in-hospital mortality in patients with COVID-19. Among 2,019 patients included in the analysis, 603 patients (29.9%) had acute myocardial injury, defined by elevated TnI levels. Cox regression analysis showed that patients with admission TnI levels of 19–100 ng/L had a 2.58-fold higher risk of 30-day in-hospital mortality (hazard ratio (HR) 2.58; 95% CI 1.83–3.62; $p < 0.001$), while those with levels >100 ng/L had a 2.97-fold higher risk (HR 2.97; 95% CI 2.01–4.39; $p < 0.001$), compared with patients with normal TnI levels (Figure 3) [126].

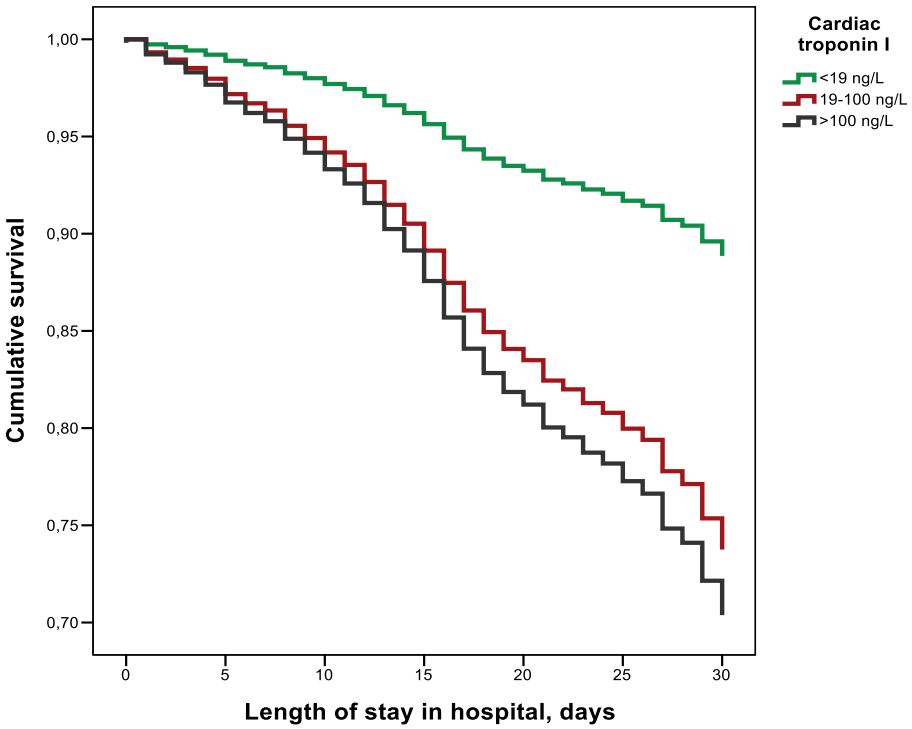

Figure 3. Cumulative survival of hospitalised COVID-19 patients stratified by cardiac troponin I concentration on admission

Adapted from: Kubiliute, I. et al. *Elevated Cardiac Troponin I as a Mortality Predictor in Hospitalised COVID-19 Patients*. Medicina. 2024;60:842 [126].

Out of a total of 1945 patients without diabetes, 1078 (55.4%) had a normal glucose level, 651 (33.5%) had mild hyperglycaemia, and 196 (10.1%) of patients had intermittent hyperglycaemia at admission. Additionally, 20 (1.0%) of the patients without diabetes had hypoglycaemia upon admission. The highest in-hospital mortality rate was noted in patients with intermittent hyperglycaemia at admission, reaching 22.4% compared to those with mild hyperglycaemia (9.4%) and normoglycaemia (5.3%). Cox regression analysis demonstrated that patients with mild hyperglycaemia on admission had a 1.62-fold higher hazard of 30-day in-hospital mortality (HR 1.62; 95% CI 1.10–2.39; $p = 0.015$), while patients with intermittent hyperglycaemia had a 3.04-fold higher hazard (HR 3.04; 95% CI 2.01–4.60; $p < 0.001$) compared with patients with normoglycaemia (Figure 4) [125].

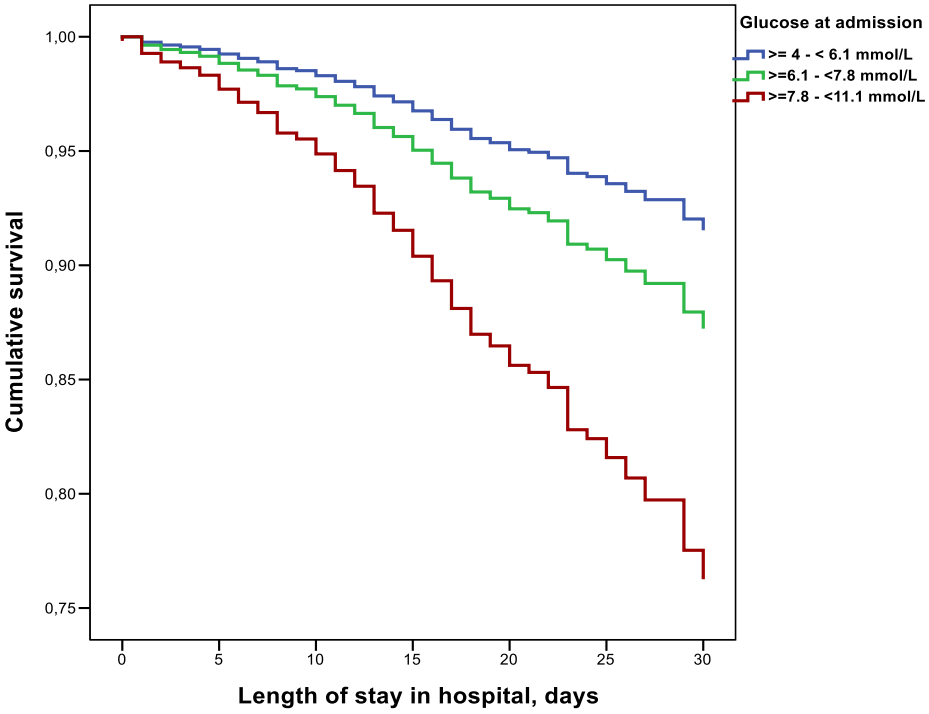

Figure 4. Cumulative survival of hospitalised patients without diabetes stratified by glycaemia levels at admission

Adapted from: Zabuliene, L. et al. *Hyperglycaemia and Its Prognostic Value in Patients with COVID-19 Admitted to the Hospital in Lithuania*. Biomedicines. 2024;12(1):55 [125].

4.5. Cell-Free Nucleic Acids as Potential Biomarkers Predicting COVID-19 Disease Severity and Lethal Outcome

To evaluate serum cfNAs' (cfDNA and SARS-CoV-2 RNA) value in predicting COVID-19 disease severity and the lethal outcome, overall, 132 individuals were enrolled, comprising 108 patients with confirmed COVID-19 and 24 controls [128]. The control group consisted of healthy males with a median age of 30.5 years (IQR 23.25–38.75).

The median cfDNA concentration among COVID-19 patients was 4.28 ng/µL (IQR 1.62–8.78). A total of 108 COVID-19 positive patients were stratified into three groups according to their COVID-19 severity: 12 with mild, 45 with severe, and 51 with critical disease. A progressive increase in cfDNA levels was observed with an increasing disease severity, rising from 1.06 ng/µL in mild cases to 2.65 ng/µL in severe cases, and 6.68 ng/µL in the critical group ($p < 0.001$ compared severe and critical groups to the mild disease group) (Figure 5A).

In the control group, the median cfDNA concentration was 0.51 ng/µL (IQR 0.27–0.75), which was significantly lower than in COVID-19 patients (0.51 vs. 4.28 ng/µL, $p < 0.001$) (Figure 5B), including those with severe (0.51 vs. 2.65 ng/µL, $p < 0.001$) and critical disease (0.51 vs. 6.68 ng/µL, $p < 0.001$) (Figure 5A). As the control group was younger than the COVID-19 cohort, an age-adjusted analysis was performed, thus confirming that cfDNA concentrations remained significantly higher in COVID-19 patients compared with the controls ($p = 0.018$).

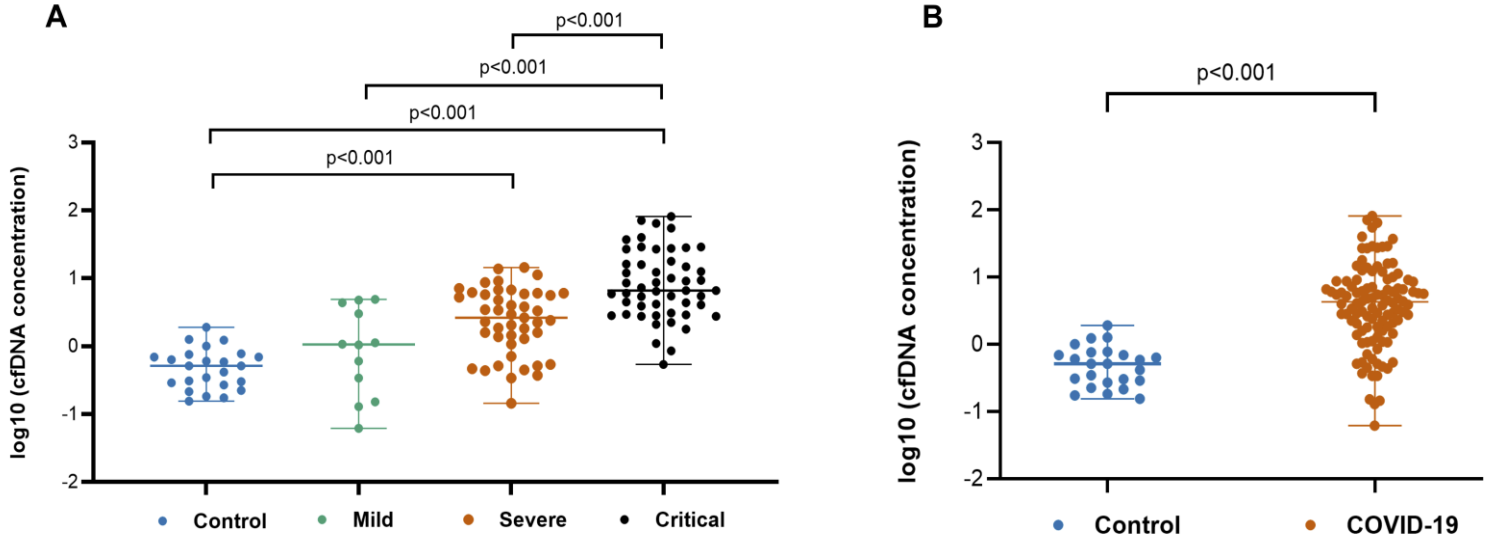

Figure 5. Comparison of cfDNA concentrations among healthy controls and COVID-19 patients by disease severity (A) and healthy controls and COVID-19 patients (B)

Note. cfDNA concentrations are shown on a log10-transformed scale.

Reprinted from: Kubiliute, I. et al. *Cell-free nucleic acids as a non-invasive biomarker for predicting COVID-19 disease severity and outcome: a retrospective cohort study*. BMC Infectious Diseases. 2026;26:1010 [128].

A total of 51 patients developed critical COVID-19 requiring ICU admission. cfDNA concentrations were significantly higher in the ICU group, with median levels of 6.68 ng/µL compared with 2.30 ng/µL in non-ICU patients ($p < 0.001$) (Figure 6).

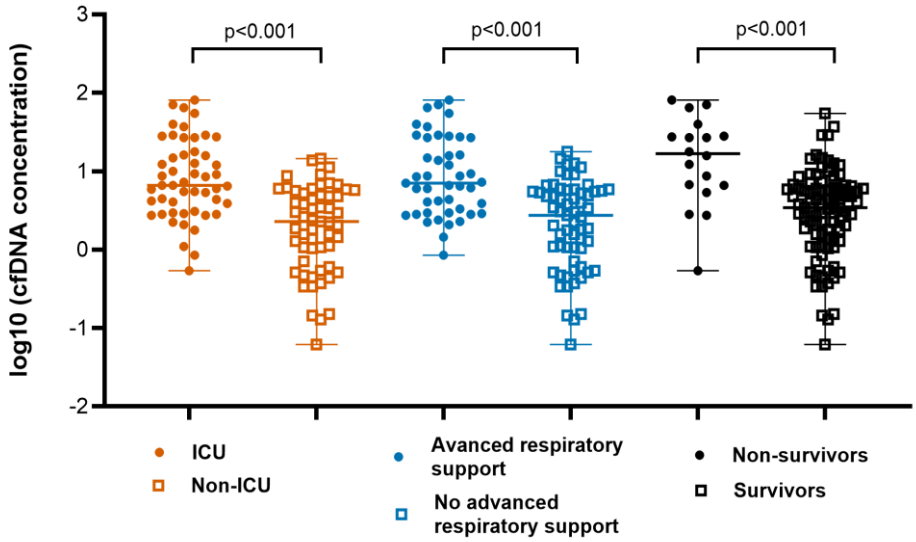

Figure 6. cfDNA concentrations in relation to ICU treatment, the need for advanced respiratory support, and survival in hospitalised COVID-19 patients

Note. cfDNA concentrations are shown on a log10-transformed scale.
Reprinted from: Kubiliute, I. et al. *Cell-free nucleic acids as a non-invasive biomarker for predicting COVID-19 disease severity and outcome: a retrospective cohort study*. BMC Infectious Diseases. 2026;26:1010 [128].

Overall, 45 patients required ARS: NIV, IMV, or ECMO. Admission cfDNA levels were markedly higher in patients requiring ARS compared with those who did not require it (7.11 vs. 2.74 ng/µL, $p < 0.001$) (Figure 6).

Among 108 patients, 18 (16.7%) died during hospitalisation. Non-survivors exhibited substantially higher cfDNA concentrations than survivors (16.68 vs. 3.44 ng/µL, $p < 0.001$) (Figure 6).

SARS-CoV-2 was detected by the quantitative polymerase chain reaction (qPCR) in the serum of 31 (28.7%) COVID-19 patients, while all control samples were negative. The presence of SARS-CoV-2 RNA in serum was more common in the patients with more severe disease. SARS-CoV-2 RNA positivity in serum was significantly higher among ICU patients compared with non-ICU patients (43.1% vs. 15.8%, $p = 0.002$) and among

those requiring ARS compared with those who did not require it (46.7% vs. 15.9%, $p < 0.001$). A higher proportion of non-survivors had SARS-CoV-2 RNA detected in serum than the survivors (50.0% vs. 24.4%, $p = 0.029$). Furthermore, 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], $p < 0.001$). 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 hospitalisation.

ROC analysis demonstrated that cfDNA was the strongest predictor among all evaluated laboratory parameters for critical COVID-19 requiring ICU admission, the need for ARS, and in-hospital mortality. For predicting critical COVID-19 requiring ICU treatment, cfDNA showed an AUC of 0.79 (95% CI 0.71–0.87, $p < 0.001$), with an optimal cfDNA threshold of 2.69 ng/μL, thus outperforming other laboratory parameters, including AST, LDH, ferritin, and urea, which had AUC values ranging from 0.62 to 0.64. Similarly, cfDNA exhibited superior predictive performance for the need for ARS, with an AUC of 0.77 (95% CI 0.68–0.87, $p < 0.001$) and an optimal cutoff of 5.93 ng/μL, thus exceeding the performance of other biomarkers, including neutrophil count, AST, ALT, and LDH (AUC range 0.62–0.69). cfDNA also demonstrated the highest 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 threshold of 6.47 ng/μL, surpassing other laboratory parameters, such as WBC, neutrophil count, potassium, urea, and NT-proBNP, which showed AUC values between 0.65 and 0.68.

To determine independent predictors of critical COVID-19, the need for ARS, and in-hospital mortality, multivariable logistic regression models were constructed, incorporating age, sex, obesity, presence of any other comorbidity, other laboratory variables significant in univariable regression analysis, SARS-CoV-2 RNA positivity, and cfDNA as covariates. In these models, cfDNA remained an independent predictor of critical COVID-19 requiring ICU treatment, the need for ARS, and in-hospital mortality after adjustment for the age, sex, and SARS-CoV-2 RNA positivity (Table 5). 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 likelihood 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, $p = 0.009$).

Table 5. Prognostic significance of cfDNA and clinical covariates for predicting critical illness, advanced respiratory support, and mortality in hospitalised COVID-19 patients

Predictor	Odds ratio (95% CI)	*p*-value
Critical COVID-19		
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

Note. Abbreviations in use: cfDNA – cell-free DNA; CI – confidence interval; ICU – Intensive Care Unit; LDH – lactate dehydrogenase; NT-proBNP – N-terminal pro B-type natriuretic peptide; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2. Reprinted from: Kubiliute, I. et al. *Cell-free nucleic acids as a non-invasive biomarker for predicting COVID-19 disease severity and outcome: a retrospective cohort study*. BMC Infectious Diseases. 2026;26:1010 [128].

Serum SARS-CoV-2 RNA positivity was independently associated only with the requirement for ARS, increasing the odds by 4.18 (95% CI 1.15–15.20) times, while no significant associations were observed with a critical COVID-19 or in-hospital mortality (Table 5).

5. DISCUSSION

In this doctoral research, clinical characteristics and prognostic factors associated with severe COVID-19 and adverse outcomes among hospitalised patients were comprehensively evaluated. Older age, obesity, and markers of systemic inflammation and organ dysfunction, such as elevated CRP and LDH, were identified as consistent predictors of both disease severity and in-hospital mortality. These findings reinforce the central role of host-related factors and inflammatory burden in determining the disease progression. In addition, selected non-inflammatory (troponin I, hyperglycaemia) and emerging biomarkers demonstrated an independent prognostic value for in-hospital mortality, further supporting a multifactorial model of COVID-19 outcomes. Notably, circulating cfDNA demonstrated strong predictive performance for critical illness, the need for ARS, and mortality, highlighting its potential as a clinically relevant biomarker.

In this study, the demographic and clinical profile of hospitalised patients with COVID-19 was characterised by a predominance of individuals aged 50–69 years, with a slightly higher proportion of men than women, and a substantial burden of comorbidities, particularly arterial hypertension, other cardiovascular diseases, and diabetes mellitus. Similar demographic patterns have been consistently reported in large international cohorts, where an advanced age and comorbidity burden are key determinants of hospitalisation and adverse outcomes [11,118]. The prevalence of underlying conditions increased markedly with age, underscoring the heightened vulnerability of elderly patients also noted in other studies [118]. The clinical presentation was consistent with an acute viral respiratory infection, with most patients experiencing sudden symptom onset and commonly reporting malaise, fever, cough, and dyspnoea, which is in line with previous reports [11,46,48,118,129]. Radiological findings demonstrated extensive pulmonary involvement, as the majority of patients developed pneumonia, most frequently bilateral pneumonia, with characteristic features such as ground-glass and peripheral opacities, which are well-established hallmarks of COVID-19-related lung injury [130–132]. A considerable proportion of patients required oxygen therapy, while only a minority progressed to advanced respiratory support, thus reflecting the heterogeneity of disease severity even among hospitalised individuals. Nevertheless, complications were frequent, affecting approximately one-quarter of the patients, with ARDS and secondary infections being the most prevalent, which is consistent with prior studies highlighting the systemic and multi-organ nature and

damage of severe COVID-19 [58,133]. A subset of patients (approximately one-tenth) required escalation to intensive or high-dependency care, thus illustrating the significant strain placed on healthcare systems during the pandemic. Overall, in-hospital mortality was 12.7%, with a pronounced age-dependent gradient. Given that in-hospital mortality rates for COVID-19 vary substantially across countries and healthcare settings, reaching up to 30% in some reports [11,13–16,134], the results observed in our cohort are consistent with the overall trends reported in the literature and do not deviate from the established epidemiological patterns.

Severe COVID-19, defined by the need for oxygen therapy, was independently predicted by older age, obesity, lymphopenia, and elevated CRP and LDH concentrations in our study, in line with previous findings [74–77,82,135–143]. The association of advanced age with severe disease likely reflects immunosenescence and chronic low-grade inflammation [135–138], whereas obesity may contribute through impaired respiratory mechanics, metabolic dysregulation, and a pro-inflammatory state [139]. Although the male sex, COPD, arterial hypertension, and cardiovascular disease have been associated with poor COVID-19 outcomes in other studies [72,140–144], these factors were more frequent among severe cases in our research but did not remain independent predictors after adjustment for confounding variables. Moreover, routinely available laboratory biomarkers provided substantial prognostic value. Elevated CRP and LDH levels, along with lymphopenia, were independently associated with severe disease, reflecting the central roles of systemic inflammation and immune dysregulation in COVID-19 identified in other studies [74–77]. Notably, lymphopenia increased the odds of severe disease more than threefold, which is consistent with previous meta-analyses linking reduced lymphocyte counts with severe COVID-19 disease, ICU admission, ARDS, and adverse clinical outcomes [82,145,146].

Clinical symptoms at admission demonstrated limited prognostic utility. While systemic and lower respiratory tract manifestations were more common in severe disease, febrile fever was the only symptom initially associated with severe COVID-19 in multivariable analysis, and this association did not persist after internal validation. These findings support previous evidence indicating that symptoms alone are insufficient to reliably predict the disease progression [147–152]. Although dyspnoea has been consistently associated with severe COVID-19 outcomes in previous studies [147–152], it remains uncertain whether shortness of breath represents an independent prognostic factor or primarily reflects established respiratory compromise and ongoing clinical deterioration.

In-hospital mortality among hospitalised COVID-19 patients was associated with multiple clinical characteristics and routinely available laboratory parameters. Consistent with previous studies [15,118,153–156], an advanced age emerged as an independent risk factor, which was associated with a 4% increase in the odds ratio per year, thus likely reflecting the combined effects of the comorbidity burden and immunosenescence in the older population [67,73,135]. Comorbid conditions were also associated with an increased in-hospital mortality risk. Congestive heart failure was identified to increase the odds of in-hospital mortality by 3.06 times, obesity by 3.90 times, COPD by 2.93 times, and a prior stroke by 5.80 times, thus supporting earlier findings that underlying diseases substantially contribute to adverse outcomes [118,157–159]. In addition, patients with fatal outcomes exhibited pronounced systemic inflammation, organ dysfunction, and metabolic disturbances, as reflected by elevated levels of urea, AST-to-ALT ratio, LDH, CRP, IL-6, and TnI on admission, which remained independent predictors in multivariable analysis, which is in line with previous studies [74,78,80,83–85,160–165]. We identified that urea concentration > 7.01 mmol/L increased the odds of in-hospital mortality by 2.32 times, LDH > 452.5 U/L by 2.6 times, TnI > 18.95 ng/L by 2.04 times, CRP > 92.68 mg/L by 58%, IL-6 > 69.55 ng/L by 62%, and AST to ALT ratio >1.49 by 54%. These findings highlight the clinical utility of combining the patient characteristics and history with routinely available laboratory parameters on admission for an early risk stratification.

In our cohort, myocardial injury was a frequent finding and a strong independent predictor of adverse outcomes. Increasing TnI levels were associated with a markedly higher risk of 30-day in-hospital mortality, consistent with previous studies demonstrating the prognostic value of troponin elevation in COVID-19 [166–168]. We identified that patients presenting with TnI levels of 19–100 ng/L on admission had a 2.58-fold increased risk of 30-day in-hospital mortality, while those with levels exceeding 100 ng/L had a 2.97-fold higher risk, compared with patients with normal TnI concentrations. These findings likely reflect the combined effects of direct viral injury and systemic inflammation, as myocardial injury was also associated with elevated inflammatory and prothrombotic markers, which is in line with the already existing evidence [168–171]. Overall, our results highlight the clinical importance of myocardial injury and TnI as an accessible and robust marker for early risk stratification of in-hospital mortality in hospitalised COVID-19 patients.

Moreover, we evaluated hyperglycaemia, another non-inflammatory marker, as a predictor of 30-day in-hospital mortality in COVID-19 patients

without pre-existing diabetes. Admission hyperglycaemia was associated with an increase in 30-day in-hospital mortality risk, with HRs of 1.62 for mild and 3.04 for intermittent hyperglycaemia compared with normoglycaemia. These findings are consistent with previous studies demonstrating an independent association between hyperglycaemia and mortality in COVID-19 patients [172–177]. This relationship is likely multifactorial, reflecting infection-induced metabolic dysregulation, systemic inflammation, immune dysfunction, and vascular injury. Infection disrupts glucose homeostasis by increasing hepatic production and reducing peripheral uptake, mediated by neurohumoral activation, counterregulatory hormones, and proinflammatory pathways [178–181]. In turn, hyperglycaemia exacerbates inflammation [182], impairs immune responses [183], and promotes endothelial dysfunction and thrombosis [184], while frequently coexisting with an older age and comorbidities that further increase the mortality risk [11,182,185,186].

In our research, higher serum cfDNA concentrations were observed in COVID-19 patients compared with healthy controls and increased progressively with the disease severity, being higher in critically ill patients, those requiring ARS, and non-survivors, which is consistent with previous studies evaluating plasma cfDNA concentrations [112,113,187–190]. This likely reflects enhanced cell death and NETosis, which have been implicated in COVID-19 pathophysiology and are more pronounced in severe disease [188,191–193]. Moreover, cfDNA concentrations have been shown to be significantly higher in COVID-19 patients compared with individuals infected with other respiratory viruses, such as influenza and respiratory syncytial virus [187], thus suggesting a potentially greater contribution of NETosis and other cell death mechanisms to COVID-19 pathophysiology than in other viral respiratory infections. Notably, cfDNA demonstrated the strongest prognostic performance among evaluated laboratory markers and remained an independent predictor of critical illness requiring ICU treatment (AUC 0.79, OR 1.21), need for ARS (AUC 0.77, OR 1.15), and in-hospital mortality (AUC 0.81, OR 1.08), which is in line with other studies [112,113,187,194]. These findings support its potential role as an early and clinically applicable biomarker reflecting both tissue injury and a dysregulated host response. Despite the consistency of our findings with previous studies, this field remains limited by a lack of standardised methodologies for cfDNA measurement, data reporting [99,187,188] and reference interval determination [195], which complicates direct comparison across studies. In contrast, although SARS-CoV-2 RNAemia was more frequently detected in patients with severe disease, it showed a limited independent prognostic value, remaining significantly associated only with the need for ARS, increasing its

odds by 4.18 times. Similar associations between RNAemia and disease severity have been reported previously [196–200], although its role as an independent predictor remains inconsistent across studies. In addition, long-range SARS-CoV-2 PCR was performed on serum samples; however, only one patient tested positive. For this reason, further analysis of the association between long-range PCR positivity and disease severity was not feasible. Nevertheless, these findings may suggest that the presence of the entire SARS-CoV-2 genome in serum is uncommon, supporting evidence that infectious virus is rarely detected in the bloodstream [201]. Taken together, these findings highlight cfDNA as a particularly promising and clinically relevant biomarker for early risk stratification in COVID-19, whereas the prognostic utility of SARS-CoV-2 RNAemia appears more limited.

6. STRENGTHS AND LIMITATIONS

6.1. Strengths of the Doctoral Work

This study represents the first comprehensive analysis of demographic, clinical, laboratory, and radiological characteristics and their association with COVID-19 outcomes in the Lithuanian population, thereby contributing valuable data from an underrepresented region and enhancing the global understanding of population-specific disease patterns. In addition to routinely used laboratory parameters, this study also evaluated novel biomarkers – cfNAs – and identified them as promising predictors of disease severity and mortality, while highlighting their potential for future integration into routine clinical practice.

A broad spectrum of clinical and laboratory analytes was evaluated, enabling a comprehensive characterisation of COVID-19 disease in the studied population. Although the single-centre design imposes certain limitations, it ensured the use of standardised protocols, thereby reducing interinstitutional variability and enhancing the internal consistency of the findings.

To evaluate predictors of in-hospital mortality, a large cohort was analysed, thereby enhancing the reliability and robustness of the findings. The ambispective study design was chosen for assessing predictors of the COVID-19 disease severity to enable more detailed data collection, including clinical symptoms not captured in depersonalised datasets, thereby improving the overall accuracy and depth of the analysis.

The study assessed novel biomarkers, including both host- and pathogen-derived cfNAs, namely, cfDNA and SARS-CoV-2 RNA. In addition to serum qPCR testing, long-range SARS-CoV-2 PCR was also performed with the objective to evaluate viremia and its potential role in COVID-19. Moreover, serum cfDNA was analysed, for which, studies are scarce, suggesting that serum cfDNA results can be applied similarly to plasma cfDNA analysis. To minimise confounding bias, patients with conditions known to influence cfDNA levels, including oncological or haematological diseases, organ transplantation and PLWH, were not included in this study, thereby ensuring a more accurate assessment of cfDNA in the context of COVID-19.

6.2. Limitations of the Doctoral Work

This doctoral work consisted of three complementary parts, which, together, enabled the analysis of a larger and more diverse patient population, particularly by utilising a depersonalised database. However, this multi-part research model also led to some heterogeneity, which limited direct comparability across the individual study components. Furthermore, variations in the level of detail of the available data – ranging from comprehensive clinical information in the ambispective cohort study to more limited datasets in retrospective analyses – may have influenced the consistency of the study findings.

Moreover, this study was conducted at a single tertiary care centre – the largest hospital in the capital of Lithuania – which may introduce selection bias and limit the generalisability of the findings to other healthcare settings. Since this is a tertiary referral centre with specific admission criteria, it is likely that a higher proportion of patients with more severe disease was included, particularly during the peak pandemic periods. Furthermore, institutional differences in patient demographics, clinical practices, and treatment protocols may not fully reflect differences in the broader population. Therefore, external validation in independent, more diverse cohorts is warranted to confirm the reproducibility and broader applicability of these results. In addition, the results of the study on cfNAs remain limited as a relatively small sample size was involved in this part. Thereby, larger multicentre studies are needed to better define their prognostic role in both COVID-19 and future infectious disease cases.

As the study components evaluating predictors of a severe disease course and in-hospital mortality included adult COVID-19 patients with a broad range of underlying comorbidities, certain laboratory parameters (e.g., urea, troponin I, AST, ALT, LDH, etc.) may have been influenced not only by SARS-CoV-2 infection but also by the underlying conditions. Consequently, the identified predictors likely reflect both COVID-19-related pathophysiological processes and the patients' overall clinical status. While the identified predictors remain valuable indicators of disease outcomes, their interpretation should consider the potential impact of pre-existing comorbidities, particularly in a hospitalised population.

A fully or partially retrospective study design led to incomplete data capture, resulting in substantial proportions of missing values for several laboratory variables, as investigations at admission were performed according to clinical indications rather than based on a standardised research protocol. Consequently, patients with more severe disease were more likely to undergo

more extensive laboratory evaluation, subsequently introducing potential selection bias. This resulted in smaller sample sizes for certain analyses, and some variables that were significant in univariable analyses could not be included in multivariable models, thus potentially limiting the robustness and completeness of the findings.

Furthermore, the assessment of in-hospital mortality risk factors was based on strictly pseudonymised and depersonalised electronic medical records, relying on coded clinical data. As a result, some patient information may have been incomplete, inaccurately recorded, or not captured within the extracted dataset. Additionally, variables such as substance use, BMI, vaccination status, and symptoms could not be included in this analysis. In the analysis of hyperglycaemia, the lack of data on the patients' fasting status at admission and glycated haemoglobin (HbA1c) levels limited the ability to accurately distinguish stress-induced hyperglycaemia from pre-existing hyperglycaemia, which may have led to classification bias. Furthermore, the lack of information on pre-admission glucocorticoids and other medication use may have confounded the observed associations, as these factors could have influenced blood glucose levels and clinical outcomes. However, since glucose levels were assessed on the day of hospital admission, glucocorticoids administered during hospitalisation did not influence these measurements or results.

Upon assessing the value of cfNAs in predicting the COVID-19 disease course, the control group differed from the COVID-19 cohort in several aspects that may have influenced cfDNA comparisons. The controls were younger, exclusively male, and lacked available comorbidity data, thus potentially introducing age-, sex-, and health status-related confounding, despite age-adjusted analyses that were performed. The relatively small size of the control group further limits the robustness of comparisons. Preanalytical factors (e.g., sample handling and processing) may have affected the absolute cfDNA levels; however, standardised procedures across all samples likely minimised this bias. These limitations should be considered when interpreting cfDNA differences and highlight the need for external validation and the establishment of context-specific reference ranges. Moreover, a larger sample size could help more accurately assess SARS-CoV-2 viremia.

The study spanned several pandemic waves (predominantly Alpha, Beta, and Delta variants, inferred from their temporal prevalence in Lithuania), during which, clinical management strategies and hospitalisation criteria evolved. In the later phases of the pandemic, hospitalisation was increasingly reserved for patients with more severe disease, potentially influencing the distribution of clinical characteristics and outcomes over time.

This shift may have limited comparability between milder and severe disease presentations, as the number of mild cases was smaller and most likely occurred during the earlier period of the pandemic. Furthermore, since SARS-CoV-2 sequencing was not performed, it was not possible to conduct outcome analyses specific to particular variants. Additionally, treatment regimens, as they changed over time, were not included in the final analysis. The treatment administered could have potentially influenced the disease outcomes, and thus could have been a source of residual confounding. However, it should be noted that the study was conducted at a single centre, where uniform treatment protocols were applied according to the pandemic stage.

7. CONCLUSIONS

1. Among hospitalised adults with COVID-19, individuals aged 50–69 years constituted the largest proportion of the study population. More than half of the patients had at least one pre-existing comorbidity. The clinical presentation was consistent with an acute viral respiratory infection, most commonly manifesting as malaise, fever, cough, and dyspnoea. Radiological evaluation demonstrated a high prevalence of pneumonia, predominantly bilateral pneumonia. Laboratory findings reflected key pathophysiological mechanisms of COVID-19, including systemic inflammation, immune dysregulation, and coagulopathy, thus highlighting the multisystem nature of the disease. More than half of the patients required oxygen therapy, whereas advanced respiratory support was necessary in only a minority of the cases, thus indicating substantial heterogeneity in the disease severity. Complications were observed in approximately one-quarter of the patients.
2. An older age, obesity, lymphopenia, and higher CRP and LDH levels on admission were identified as independent predictors for a severe COVID-19 disease course, defined by the requirement for any form of oxygen therapy.
3. The overall in-hospital mortality was 12.7%. In-hospital mortality increased with an advancing age, reaching 29.7% among patients aged 70 years and older. Independent predictors of in-hospital mortality among COVID-19 patients were identified to be age, congestive heart failure, obesity, COPD, prior stroke, as well as elevated admission levels of urea, LDH, CRP, IL-6, troponin I, and an increased ALT-to-AST ratio.
4. Assessment of non-inflammatory biomarkers demonstrated that elevated troponin I levels and admission hyperglycaemia were independently associated with an increased risk of in-hospital mortality, thus highlighting their added value in risk stratification of patients with COVID-19 beyond inflammatory markers.
5. Higher circulating cfDNA levels in serum were independently associated with more severe COVID-19, a higher probability of intensive care admission, advanced respiratory support, and increased in-hospital mortality. In contrast, SARS-CoV-2 RNAemia was significantly associated only with the need for advanced respiratory support. Notably, cfDNA exhibited superior prognostic accuracy compared with conventional biomarkers. Collectively, these findings

support the clinical relevance of circulating cell-free nucleic acids, particularly cfDNA, as robust and potentially implementable biomarkers for early risk stratification and outcome prediction in hospitalised COVID-19 patients.

8. RECOMMENDATIONS

1. In the context of emergency department triage and hospital admission decisions, the patient age, comorbidity burden (particularly the presence of obesity, congestive heart failure, COPD, and prior stroke), and routinely available laboratory parameters, including lymphopenia, an elevated neutrophil-to-lymphocyte ratio, hyperglycaemia, and elevated CRP, LDH, urea, and the AST-to-ALT ratio, should be systematically incorporated into clinical assessment and interpreted in an integrated manner. In the hospital setting, IL-6 assessment may provide an additional prognostic value for risk stratification. These factors provide clinically relevant information for the early identification of patients at an increased risk of severe disease and may support timely clinical decision-making along with the appropriate allocation of healthcare resources.
2. Given that myocardial injury, defined by elevated troponin I levels, was observed in approximately 30% of hospitalised patients with COVID-19 in our study, routine assessment of troponin I at hospital admission should be considered, particularly in patients with underlying cardiovascular disease. Patients presenting with elevated troponin I levels should be considered at an increased risk of adverse outcomes and may benefit from closer clinical monitoring and earlier escalation of care due to their substantially higher risk of requiring invasive mechanical ventilation and in-hospital mortality.
3. Although further validation studies are warranted, circulating serum cfDNA represents a promising biomarker with superior prognostic performance compared to routine laboratory analytes. Its integration into routine clinical practice could enhance early risk stratification of COVID-19 patients and improve the identification of individuals who are at risk for severe disease progression. Additionally, further research is recommended to evaluate the applicability of cfDNA as a prognostic tool in other infectious diseases, thus potentially extending its utility beyond COVID-19.

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10. SUMMARY IN LITHUANIAN (SANTRAUKA LIETUVIŲ KALBA)

ĮVADAS IR LITERATŪROS APŽVALGA

COVID-19 liga: epidemiologinis kontekstas

2019 m. gruodžio mėn. Uhane, Kinijoje, kilo nežinomos etiologijos pneumonijos protrūkis. Kaip jos sukėlėjas vėliau identifikuotas naujas betakoronavirusas – sunkaus ūminio respiracinio sindromo koronavirusas 2 (SARS-CoV-2), sukeliantis koronaviruso infekciją 2019 (COVID-19) [1–3]. Dėl spartaus viruso plitimo 2020 m. kovo 11 d. Pasaulio sveikatos organizacija (PSO) paskelbė COVID-19 pandemiją [4]. Nuo to laiko COVID-19 tapo vienu didžiausių iššūkių viso pasaulio sveikatos priežiūros sistemoms. 2026 m. birželio 30 d. duomenimis, pasaulyje registruota daugiau kaip 779 mln. COVID-19 atvejų ir daugiau kaip 7 mln. mirčių [5]. Lietuvoje pirmasis SARS-CoV-2 infekcijos atvejis nustatytas 2020 m. vasario 28 d. [6], o iki 2026 m. birželio 30 d. šalyje registruota daugiau kaip 1,4 mln. atvejų ir daugiau kaip 9 900 mirčių nuo COVID-19 [5].

Pandemijos pradžioje COVID-19 našta buvo didžiausia – pirmaisiais pandemijos metais iki 44 proc. hospitalizuotų pacientų buvo gydomi intensyviosios terapijos skyriuose (ITS), o daugiau kaip trečdalis jų mirė [7–10]. Hospitalizuotų pacientų mirštamumas skyrėsi tarp šalių ir skirtingais pandemijos bangų laikotarpiais – svyravo nuo 5 proc. kai kuriose Europos ir Jungtinių Amerikos Valstijų iki daugiau kaip 25 proc. kitų regionų kohortose [11, 13–19]. Vėlesniais pandemijos etapais, ypač Omikron atmainos dominavimo laikotarpiu, kreipimosi į ligonines dažnis, hospitalizacijos mastas bei mirštamumas sumažėjo [20–23]. Tačiau, nepaisant mažesnio mirštamumo, dėl didesnio susirgimų skaičiaus Omikron bangos metu absoliutus mirčių nuo COVID-19 skaičius padidėjo, ypač tarp vyresnių asmenų [21]. Šie duomenys pabrėžia, kad COVID-19 našta sveikatos priežiūros sistemoms ir nuolatinis sveikatos priežiūros išteklių naudojimas išlieka, nepaisant sumažėjusio SARS-CoV-2 virulentiškumo, vakcinacijos ir antivirusinio gydymo pažangos [21].

COVID-19 ligos etiologija ir patogenezė

SARS-CoV-2 yra apvalkalą turintis viengrandės teigiamos krypties RNR virusas, koduojantis keturis pagrindinius struktūrinius baltymus: nukleokapsidės (N), matrikso (M), apvalkalo (E) ir paviršiaus glikoproteino (smaigo) (S) baltymus [24, 25]. Smaigo baltymas, sudarytas iš S1 ir S2

subvienetų, kurie yra būtini virusui patekti į šeimininko ląstelę: S1 subvienetas jungiasi prie angiotenziną konvertuojančio fermento 2 (AKF2) receptoriaus, o S2 yra aktyvinamas transmembraninės serino proteazės 2 (TMPRSS2), leidžiančios įvykti viruso ir ląstelės membranų susiliejimui [24, 25]. Kadangi daugelis žmogaus kvėpavimo takų epitelio ląstelių ekspresuoja AKF2 ir TMPRSS2, SARS-CoV-2 pirmiausia pažeidžia viršutinius ir apatinius kvėpavimo takus [27, 28].

SARS-CoV-2 infekcija aktyvina tiek įgimtą, tiek įgytą imuninį atsaką [29], sukelia prouždegiminių citokinų ir chemokinų, tokių kaip navikų nekrozės faktoriaus α (TNF α), interleukino 6 (IL-6), interleukino 8 (IL-8) ir monocitus pritraukiančio baltymo-1 (MCP-1), išsiskyrimą. Sunkiais ligos atvejais išsivysto neadekvatus uždegiminis atsakas ir citokinų audra, sukeliantys audinių pažeidimą ir ligos progresavimą [30–32]. Nauji mokslo įrodymai dar labiau pabrėžia, kad šeimininko imuninių reakcijų kompleksiškumas ir jų disbalansas yra pagrindiniai COVID-19 ligos sunkumo ir progresavimo veiksniai [32]. Pernelyg intensyvus sisteminis uždegimas gali sukelti įvairių organų, įskaitant širdį, kepenis, inkstus ir centrinę nervų sistemą, pažeidimą, o sunkiausiais atvejais – sepsį, šoką ar dauginį organų nepakankamumą [26, 33].

SARS-CoV-2 variantai

SARS-CoV-2, kaip ir kiti viengrandės RNR virusai, pasižymi genetiniu kintamumu [34]. Šį procesą daugiausia lemia mutacijų kaupimasis, ypač smaigo (S) baltymo gene, kuris yra svarbus viruso užkrečiamumui ir imuniniam atpažinimui [36–38].

Pandemijos pradžioje SARS-CoV-2 pasižymėjo santykinai nedideliu genetiniu kintamumu [37], tačiau maždaug po aštuonių mėnesių atsirado pirmieji susirūpinimą keliantys variantai – Alfa, Beta ir Gama, pasižymėję spartesniais evoliuciniais pokyčiais bei gausiu mutacijų kaupimu [37, 39]. Vėliau išplitęs Omikron variantas, kuriam būdingas ypač didelis mutacijų skaičius bei didžiausia genetinė įvairovė tarp susirūpinimą keliančių SARS-CoV-2 variantų, žymėjo naują pandemijos etapą [40, 41], susijusį su didesniu Omikron subvariantų užkrečiamumu, pakitusiu patogeniškumu ir gebėjimu išvengti imuninio atsako, ypač po buvusios infekcijos ar vakcinacijos [43].

Nepaisant nuolatinės SARS-CoV-2 evoliucijos ir naujų variantų, pasižyminčių skirtingomis biologinėmis ir klinikinėmis savybėmis [43], atsiradimo, jų poveikis ligos eigai sudėtingas ir dar nevisiškai išsiaiškintas, o tai parodo su šeimininku susijusių veiksnių ir klinikinėje praktikoje taikomų rodiklių svarbą ligos eigai ir baigčiai prognozuoti.

Klinikinis COVID-19 ligos heterogeniškumas

SARS-CoV-2 daugiausia plinta oro lašeliniu būdu ir per aerozolius [44, 45]. COVID-19 klinikinė eiga yra labai heterogeniška – nuo besimptomės infekcijos iki sunkios ligos, galinčios komplikuotis ūminiu respiracinio distreso sindromu (ŪRDS), dauginiu organų nepakankamumu ir mirtimi [27, 44]. Lengva ar vidutinio sunkumo COVID-19 liga dažniausiai pasireiškia karščiavimu, kosuliu, nuovargiu [46] ir raumenų skausmu [47], o hospitalizuoti pacientai dažnai skundžiasi dusuliu, viduriavimu, vėmimu, produktyviu kosuliu, jiems gali pasireikšti sąmonės sutrikimai [48]. Anosmija ir ageuzija, nors nėra dažniausiai pasireiškiantys simptomai, laikomi gana specifiniais COVID-19 ligos požymiais [49].

Plaučių pažeidimas yra viena dažniausių COVID-19 ligos klinikinių išraiškų ir paprastai pasireiškia karščiavimu, sausu kosuliu bei dusuliu [50]. Nors daugumai pacientų simptomai yra lengvi, daliai pacientų liga progresuoja į virusinę pneumoniją ir ŪRDS, kuris laikomas viena pagrindinių su COVID-19 liga susijusio mirštamumo priežasčių [51–55].

COVID-19 ligos metu išsivystantis intensyvus uždegiminis atsakas sukelia endotelio pažeidimą, trombozę ir mikrovaskulinius sutrikimus, prisidedančius prie dauginio organų pažeidimo [56–60]. Šie procesai gali lemti ne tik ŪRDS, bet ir ūminį inkstų pažeidimą, širdies ir kraujagyslių sistemos komplikacijas, kepenų funkcijos sutrikimą ir kitų organų disfunkciją [61–64].

COVID-19 ligos eigos prognostiniai veiksniai: demografiniai, klinikiniai ir laboratoriniai rodikliai

Dėl didelio COVID-19 ligos klinikinės eigos heterogeniškumo ankstyvas pacientų rizikos stratifikavimas yra svarbus priimant klinikinius sprendimus ir siekiant optimizuoti sveikatos priežiūros išteklių naudojimą. Iki šiol pasaulyje plačiai tirti įvairūs prognostiniai žymenys, įskaitant demografinius, klinikinius veiksnius, rutininėje klinikinėje praktikoje atliekamus laboratorinius tyrimus. Sunkios COVID-19 ligos formos, ŪRDS išsivystymo, gydymo ITS ir mirštamumo rizika siejamos su vyriškąja lytimi [65], vyresniu amžiumi [52, 66, 67], žemesne socialine ir ekonomine padėtimi, etnine kilme, gimimo šalimi [66] bei gretutinėmis ligomis, tokiomis kaip nutukimas, pirminė arterinė hipertenzija (PAH), cukrinis diabetas (CD) ir kitomis [52, 67–69]. Gretutinės ligos gali lemti sunkesnę COVID-19 ligos eigą dėl įvairių patofiziologinių mechanizmų, įskaitant imuninio atsako disbalansą, padidėjusią AKF2 receptorių ekspresiją, pagreitinančią viruso patekimą į ląsteles ir skatinančią uždegiminį atsaką [67, 70–73].

Įvairūs įprastai klinikinėje praktikoje atliekami laboratoriniai tyrimai taip pat pripažinti galimais COVID-19 ligos sunkumo ir nepalankių baigčių prognostiniais rodikliais. Didesnė laktatdehidrogenazės (LDH) [74–76], C reaktyviojo baltymo (CRB), IL-6 [77, 78], feritino [79, 80] koncentracija, padidėjęs neutrofilų [80, 81] ir sumažėjęs limfocitų skaičius [81, 82], atspindintys stipresnį uždegiminį atsaką, dažniau nustatyti pacientams, sergantiems sunkesne COVID-19 ligos forma. Didesnė D-dimerų [75, 77–81], troponino I [75, 81, 83, 84] bei asparagininės aminotransferazės (AST) [75] koncentracija, atspindinti audinių pažeidimą bei koaguliacijos aktyvaciją, yra susijusi su sunkesne ligos eiga bei didesniu mirštamumu. Taip pat nustatyta, kad sunkesniais ligos atvejais dažniau padidėja šlapalo [85] ir kreatinino [75, 85] koncentracija, rodanti inkstų funkcijos sutrikimą. Nors šie laboratoriniai rodikliai plačiai taikomi klinikinėje praktikoje, jų specifiškumas COVID-19 infekcijai yra ribotas, o prognostinės ribinės vertės vis dar nėra galutinai apibrėžtos [79, 81, 86].

Nauji biologiniai žymenys

Siekiant prognozuoti COVID-19 ligos eigą, vis daugiau dėmesio skiriama naujiems biožymenims. Metabolomikos, proteomikos ir genomikos tyrimai suteikė svarbių įžvalgų apie molekulinius pokyčius, susijusius su COVID-19 ligos sunkumu ir klinikinėmis baigtimis [87–91]. Vis dėlto, nepaisant didelio potencialo, multiomikos metodų taikymą rutininėje klinikinėje praktikoje riboja duomenų sudėtingumas, standartizacijos stoka ir specializuotų žinių poreikis [92–94], todėl tebėra aktualu nustatyti prieinamus ir klinikinėje praktikoje pritaikomus biožymenis.

Dėl šios priežasties laisvai cirkuliuojančios nukleorūgštys (lcNR), įskaitant laisvai cirkuliuojančią DNR (lcDNR) ir RNR (lcRNR), išskiriamos kaip perspektyvūs minimaliai invaziniai biožymenys, atspindintys tiek audinių pažeidimą, tiek ligos patofiziologinius procesus [95–97]. lcDNR ir lcRNR – tai atitinkamai DNR ir RNR ekstraląsteliniai fragmentai, kurių gali būti randama daugelyje kūno skysčių tiek fiziologinių, tiek patologinių procesų metu [98, 99]. lcDNR į kraujotaką išsiskiria ir ląstelių žūties, vykstant apoptozei, nekrozei, netozei, ir aktyvios sekrecijos metu [98–100]. Infekcinių ligų atveju kraujyje gali būti aptinkama ne tik šeimininko, bet ir patogeno nukleorūgščių [101].

Naujausi tyrimai rodo, kad lcNR gali būti naudingas biožymuo tiriant ir stebint pacientus, sergančius onkologinėmis [102, 103], autoimuninėmis ligomis [104, 105], po transplantacijų [106, 107] ar sunkios traumos [108]. Didesnė lcDNR koncentracija nustatoma sergant sepsiu [109, 110], ŽIV

infekcija [111]. Pavienių tyrimų rezultatai rodo, kad COVID-19 pacientams būdinga didesnė lcDNR koncentracija, o tai gali būti susiję su blogesne ligos prognoze [112, 113]. Be to, dėl minimalaus invazyvumo, gero atkuriamumo bei lengvo pritaikomumo rutininėje klinikinėje praktikoje lcDNR laikomas perspektyviu klinikiniu biožymeniu [99, 114–116].

Mokslinio darbo aktualumas ir naujumas

COVID-19 pandemijos pradžioje sveikatos priežiūros sistemos susidūrė su didžiuliu pacientų srautu ir poreikiu laiku identifikuoti didesnės rizikos pacientus, įvertinti galimą ligos sunkumą bei užtikrinti tinkamos apimties medicininę pagalbą ir racionalų sveikatos priežiūros išteklių paskirstymą. Klinikinių sprendimų priėmimą apsunkino ribotos žinios apie patikimus ligos sunkumą ir mirštamumą prognozuojančius veiksnius. Nors buvo identifikuota daug demografinių, klinikinių ir laboratorinių rodiklių bei sukurta įvairių prognostinių modelių, taikant tiek tradicinius statistinius metodus, tiek pažangius mašininio mokymosi algoritmus [52, 65–69, 74–78, 117, 118], iki šiol nėra nustatyto bendro ir plačiai pritaikomo prognostinių veiksnių rinkinio. Be to, daugelio siūlomų biožymenų klinikinė vertė priklauso nuo jų prieinamumo, atlikimo laiko ir taikomų ribinių verčių [119, 120]. Todėl iki šiol išlieka poreikis nustatyti lengvai prieinamus ir kliniškai reikšmingus veiksnius, tinkamus ankstyvai COVID-19 rizikos stratifikacijai priimant sprendimus dėl pacientų hospitalizavimo.

Prognozinių modelių tikslumui ir pritaikomumui didelę įtaką turi populiacijos genetiniai, demografiniai ir sveikatos priežiūros sistemų ypatumai [121], todėl Vakarų Europoje, Azijoje ar Šiaurės Amerikoje įdiegti modeliai ne visada gali būti tiesiogiai pritaikomi Baltijos regionui. Iki šiol duomenų iš Vidurio ir Rytų Europos yra nedaug [122, 123], todėl regioninių tyrimų atlikimas yra svarbus siekiant nustatyti kliniškai reikšmingus COVID-19 ligos eigos prediktorius Lietuvos populiacijoje.

Šio darbo naujumą sudaro kompleksiškas hospitalizuotų COVID-19 pacientų charakteristikų ir ligos baigčių vertinimas. Be įprastinių demografinių, klinikinių, laboratorinių ir radiologinių rodiklių analizės, tyrime vertinta neuždegiminių biožymenų – troponino I ir hiperglikemijos – prognostinė reikšmė, taip pat lcNR potencialas prognozuojant sunkią COVID-19 ligos eigą ir mirštamumą. Be to, šiame tyrime analizuojami išsamūs vienos didžiausių Lietuvos gydymo įstaigų duomenys, suteikiantys vertingų įžvalgų apie hospitalizuotų COVID-19 pacientų charakteristikas ir ligos baigtis Lietuvos asmens sveikatos priežiūros sistemos kontekste.

MOKSLINIO DARBO TIKSLAS IR UŽDAVINIAI

Mokslinio darbo tikslas

Šio tyrimo tikslas – nustatyti ir įvertinti hospitalizuotų COVID-19 pacientų demografinius, klinikinius bei laboratorinius veiksnius, prognozuojančius sunkią COVID-19 ligos formą ir letalią baigtį.

Mokslinio darbo uždaviniai

1. Apibūdinti hospitalizuotų COVID-19 liga sergančių pacientų demografinius, klinikinius, laboratorinius ir radiologinius ypatumus.
2. Nustatyti veiksnius, prognozuojančius sunkios COVID-19 ligos išsivystymą hospitalizuotiems pacientams.
3. Nustatyti veiksnius, susijusius su hospitalizuotų COVID-19 pacientų mirštamumu.
4. Įvertinti neuždegiminius kraujo rodiklius – troponiną I ir hiperglikemiją – kaip letalią COVID-19 ligos baigtį prognozuojančius žymenis hospitalizuotiems pacientams.
5. Įvertinti kraujo serumo laisvai cirkuliuojančias nukleorūgštis kaip COVID-19 ligos sunkumą ir letalią baigtį prognozuojančius žymenis.

TYRIMO METODAI

Tyrimo dizainas ir tiriamųjų populiacija

Disertacinį darbą sudarė trys dalys:

1. Retrospektyvusis kohortinis tyrimas, kuriame analizuoti Vilniaus universiteto ligoninės Santaros klinikose (VULSK) nuo 2020 m. kovo 1 d. iki 2021 m. gegužės 30 d. hospitalizuotų COVID-19 liga sergančių suaugusiųjų nuasmeninti duomenys siekiant nustatyti veiksnius, susijusius su COVID-19 pacientų mirštamumu, ir įvertinti neuždegiminius žymenis – troponiną I ir hiperglikemiją – kaip galimus letalios baigties prediktorius. Į tyrimą iš viso įtraukti 2794 pacientai. Į troponino I analizę nebuvo įtraukti pacientai, kuriems hospitalizavimo metu nebuvo atliktas troponino I tyrimas, sergantys 5 stadijos lėtine inkstų liga (LIL), ir tie, kuriems hospitalizacijos metu pasireiškė miokardo infarktas. Hiperglikemijos analizėje vertinti pacientai, kurie nesirgo CD ir kuriems hospitalizavimo metu buvo atliktas glikemijos tyrimas.

2. Ambispektyvusis kohortinis tyrimas, atliktas VULSK Infekcinių ligų skyriuje, skirtas įvertinti hospitalizuotų COVID-19 pacientų demografinius, klinikinius, laboratorinius ir radiologinius požymius bei nustatyti veiksnius, susijusius su sunkios COVID-19 ligos formos išsivystymu. Retrospektyvieji duomenys rinkti nuo 2020 m. kovo 13 d. iki birželio 30 d., perspektyvieji – nuo 2020 m. liepos 1 d. iki 2021 m. gruodžio 31 d. Iš viso įtraukti 495 pacientai, iš kurių 258 (52,1 proc.) įtraukti retrospektyviai ir 237 (47,9 proc.) – perspektyviai.
3. Retrospektyvusis kohortinis tyrimas, kuriame naudoti Santaros klinikų Biobanke sukaupti kraujo serumo mėginiai ir Biobanko veikloje sutikusių dalyvauti COVID-19 pacientų, hospitalizuotų VULSK nuo 2020 m. lapkričio 24 d. iki 2021 m. lapkričio 10 d., bei kontrolinės grupės asmenų klinikiniai duomenys siekiant nustatyti lcNR vertę COVID-19 ligos sunkumui ir letaliai baigčiai prognozuoti. Neįtraukti pacientai, sirgę onkologinėmis ar hematologinėmis ligomis, infekuoti ŽIV, po organų transplantacijos bei pažeidžiami asmenys. Iš viso įtraukti 132 asmenys: 108 COVID-19 pacientai ir 24 kontrolinės grupės tiriamieji.

Į visas disertacinio darbo tyrimų dalis buvo įtraukti ≥ 18 metų pacientai, hospitalizuoti dėl polimerazės grandininės reakcijos (PGR) ar greitojo antigeno testu patvirtintos COVID-19 ligos.

Duomenų rinkimas

Retrospektyviajame kohortiniame tyrime (1 dalis) [124–126] nuasmeninti pacientų duomenys buvo gauti iš VULSK elektroninių medicininių įrašų vadovaujantis ligoninės patvirtintomis procedūromis. Rinkti pacientų demografiniai duomenys, informacija apie gretutines ligas, hospitalizacijos metu išsivysčiusias komplikacijas, taikytą gydymą antibiotikais, sisteminiais gliukokortikoidais, antivirusiniais vaistais, dirbtinės plaučių ventiliacijos (DPV) taikymą, hospitalizacijos trukmę ir hospitalizacijos baigtį (letali / neletali). Analizuotos gretutinės ligos: PAH, CD, nutukimas, LIL, širdies nepakankamumas, lėtinės plaučių ligos, širdies ir kraujagyslių ligos, virusiniai hepatitai, ŽIV infekcija bei organų transplantacija.

Ambispektyviajame kohortiniame tyrime (2 dalis) [127] duomenys buvo renkami iš elektroninių medicininių įrašų, o perspektyvinėje dalyje papildomai atliktos ir pacientų apklausos. Vertintos tiriamųjų demografinės charakteristikos, gretutinės ligos, rūkymas, kūno masės indeksas (KMI), vakcinacija nuo COVID-19 ir klinikiniai simptomai hospitalizavimo metu.

Taip pat rinkti objektyvūs klinikiniai rodikliai, radiologinių tyrimų rezultatai, duomenys apie kvėpavimo palaikymo priemonių (deguonies terapija, neinvazinė plaučių ventiliacija (NIV), DPV, ekstrakorporinė membraninė oksigenacija (EKMO)) taikymą, hospitalizacijos metu išsivysčiusias komplikacijas bei hospitalizacijos trukmę.

Retrospektyviajame tyrime, kuriame naudoti Vilniaus Santaros klinikų Biobanko sukaupti serumo mėginiai (3 dalis) [128], naudoti iš elektroninės VULSK sistemos surinkti ir iš Vilniaus Santaros klinikų Biobanko gauti klinikiniai duomenys. Surinkta informacija apie demografinius rodiklius, gretutines ligas, KMI, hospitalizacijos metu išsivysčiusias komplikacijas, kvėpavimo palaikymo priemonių (deguonies terapija, NIV, DPV, EKMO) taikymą, hospitalizacijos trukmę ir jos baigtį. Kontrolinės grupės tiriamųjų surinkti amžiaus ir lyties duomenys, kurie buvo prieinami Biobanke.

Visose trijose dalyse buvo rinkti hospitalizavimo metu atliktų laboratorinių tyrimų rezultatai: bendrojo kraujo tyrimo rodikliai, gliukozės, kreatinino, glomerulų filtracijos greičio, šlapalo, natrio, kalio, alanininės aminotransferazės (ALT), AST, šarminės fosfatazės (ŠF), LDH, laktato, CRB, prokalcitonino, feritino, IL-6, D-dimerų, fibrinogeno, troponino I, N-terminalinio pro-B natriuretinio peptido (NT-proBNP), bendro baltymo, protrombino laiko (PT) ir tarptautinio normalizuoto santykio (INR) duomenys.

Laisvai cirkuliuojančių nukleorūgščių laboratoriniai išskyrimo ir analizės metodai

Laisvai cirkuliuojančių nukleorūgščių – lcDNR ir SARS-CoV-2 RNR – išskyrimas ir analizė buvo atlikti trečiojoje disertacinio darbo dalyje [128]. Šios analitės vertintos tiriant hospitalizavimo metu surinktus kraujo serumo mėginius.

Kraujo serumo mėginių paruošimas. Biobanke kraujo serumo mėginiai buvo centrifuguojami, išpilstomi po 500 µl į atskirus mėgintuvėlius, užkoduojami ir laikomi –80 °C temperatūroje šaldiklyje. Mėginiai buvo atšildyti vieną kartą ir naudojami lcNR gryninimui.

lcNR gryninimas iš kraujo serumo. lcNR iš kraujo serumo buvo išskiriamos naudojant „QIAamp Circulating Nucleic Acid Kit“ laikantis gamintojo nurodymų. Išgryninti lcNR mėginiai buvo užkoduoti raidžių ir skaitmenų kombinacija ir užšaldyti –80 °C šaldiklyje tolesniems tyrimams.

lcDNR kapiliarinė elektroforezė. lcDNR koncentracija buvo analizuojama kapiliarinės elektroforezės metodu naudojant „Agilent Technologies“ didelės skiriamosios gebos automatizuotą DNR elektroforezės

sistemą „Bioanalyzer 2100“ su „Agilent DNR 1000“ rinkiniu. Tais atvejais, kai dėl mažos nukleorūgščių koncentracijos mėginių nebuvo galima išmatuoti, naudotas „Agilent High Sensitivity DNR“ rinkinys.

Kiekybinis SARS-CoV-2 RNR nustatymas. SARS-CoV-2 lcRNR nustatyta kiekybiniu PGR metodu („QuantStudio Real-Time PCR“ sistema), naudojant standartizuotą amplifikacijos protokolą. Gauti rezultatai analizuoti naudojant „Thermo Fisher Scientific QuantStudio“ rinkinį.

Ilgų sekų PGR. SARS-CoV-2 RNR vientisumui išgrynintuose mėginiuose įvertinti taikytas ilgų sekų PGR metodas „QuantStudio Real-Time PCR“ sistemoje. Po atvirkštinės transkripcijos reakcijos mėginiai analizuoti kiekybine PGR, taikant standartizuotą amplifikacijos protokolą.

Tiriamųjų grupavimas ir vertintos baigtys

Sunki COVID-19 ligos forma apibrėžta kaip COVID-19 liga, kai paciento gydymui buvo taikyta deguonies terapija. Kritiškai sunki COVID-19 ligos forma apibrėžta kaip COVID-19 ligos forma, kurios metu pacientui buvo reikalingas gydymas ITS. Lengva ligos eiga apibrėžta kaip COVID-19 liga, kurios gydymui nebuvo skirta deguonies terapija.

Statistinėje analizėje tiriamieji taip pat buvo suskirstyti į gydytų ir negydytų ITS grupes, į pacientų, kuriems taikytos ir netaikytos pažangios kvėpavimo palaikymo priemonės (PKPP), t. y. NIV, DPV ar EKMO, grupes. Vertinant veiksnius, susijusius su COVID-19 pacientų mirštamumu, pagrindinė baigtis buvo mirtis hospitalizacijos metu. lcNR analizėje COVID-19 pacientų grupė buvo lyginama su kontroline grupe.

Troponino I analizėje tiriamieji buvo suskirstyti į tris grupes pagal troponino I koncentraciją hospitalizavimo metu: < 19 ng/l, 19–100 ng/l ir > 100 ng/l.

Hiperglikemijos analizėje tiriamieji pagal gliukozės koncentraciją hospitalizavimo metu suskirstyti į keturias grupes: hipoglikemijos (< 4,0 mmol/l), normoglikemijos (≥ 4,0 ir < 6,1 mmol/l), lengvos hiperglikemijos (≥ 6,1 ir < 7,8 mmol/l) ir ženklios hiperglikemijos (≥ 7,8 ir < 11,1 mmol/l).

Etiniai aspektai

Visos trys disertacinio darbo dalys buvo atliktos laikantis Helsinkio deklaracijos principų.

Pirmojoje dalyje – retrospektyviajame kohortiniame tyrime – buvo analizuojami tik nuasmeninti ir pseudonimizuoti duomenys, gauti iš VULSK Informatikos ir plėtros centro pagal ligoninės patvirtintas procedūras. Kadangi

buvo naudojami tik nuasmeninti duomenys, bioetikos leidimas šiai tyrimo daliai nebuvo reikalingas. Leidimas publikuoti tyrimo rezultatus išduotas Vilniaus universiteto ligoninės Santaros klinikų (2022-12-13, Nr. SR-7021).

Antrajai daliai – ambispektyviajam kohortiniam tyrimui – 2020 m. liepos 3 d. gautas Lietuvos bioetikos komiteto leidimas (Nr. L-20-3/1). Retrospektyviai įtrauktiems pacientams informuoto asmens sutikimo formos (IASF) reikalavimas nebuvo taikytas, o visi perspektyviai įtraukti tiriamieji (nuo 2020 m. liepos mėn.) pasirašė IASF.

Trečiajai daliai – retrospektyviajam kohortiniam tyrimui, naudojant Biobanko sukauptus kraujo serumo ėminius ir klinikinius duomenis, – 2023 m. birželio 6 d. buvo gautas Vilniaus regioninio biomedicininių tyrimų etikos komiteto leidimas (Nr. 2023/6-1521-982). Visi tiriamieji buvo pasirašę dalyvavimo Vilniaus Santaros klinikų Biobanko veikloje sutikimus, todėl nuo papildomo tyrimui skirto IASF pasirašymo buvo atleisti.

Statistinė analizė

Duomenims apibūdinti taikyta aprašomoji statistika. Aprašant duomenis pateikta kiekybinių kintamųjų mediana ir tarpkvartilinis plotis (TKP), kokybinių – absoliutus ir santykinis dažniai (proc.). Daugumos kintamųjų pasiskirstymas neatitiko normaliojo skirstinio, buvo taikyti neparametriniai statistinės analizės metodai. Siekiant sumažinti lcDNR pasiskirstymo asimetriją buvo taikyta logaritminė reikšmių transformacija. Kiekybinių kintamųjų palyginimui buvo naudojamas Mano ir Vitnio (*Mann–Whitney U*) arba Kruskalo ir Voliso (*Kruskal–Wallis*) testas, taikyti aposterioriniai poriniai palyginimai su Bonferonio (Bonferroni) p korekcija. Kategorinių kintamųjų palyginimui buvo naudotas χ^2 testas arba Fišerio (*Fisher*) tikslusis testas. Ryšiams tarp lcDNR ir laboratorinių rodiklių vertinti taikytas Spirmeno (*Spearman*) koreliacijos metodas.

Laboratorinių rodiklių ir lcDNR prognostinio tikslumo vertinimui sudarytos ROC (*Receiver operator characteristic*) kreivės, įtraukiant kintamuosius, kurie statistiškai reikšmingai skyrėsi tarp lyginamų baigčių grupių. Optimalios ribinės vertės, jų jautrumas ir specifiškumas apskaičiuoti taikant Judeno (*Youden*) indeksą.

Siekiant nustatyti sunkios COVID-19 ligos, gydymo ITS, PKPP taikymo poreikio ir stacionarinio mirštamumo prognostinius veiksnius, buvo sudaryti vienaveiksnės ir daugiaveiksnės logistinės regresijos modeliai. Demografiniai, klinikiniai bei laboratoriniai rodikliai buvo įtraukti į vienaveiksnės regresijos modelius. Kintamieji, kurie buvo statistiškai reikšmingi vienaveiksnėje analizėje, buvo peržiūrėti, įvertinti ir įtraukti į

daugiaveiksnės regresijos modelį. Rezultatai pateikti kaip šansų santykiai (ŠS) su 95 proc. pasikliautinaisiais intervalais (PI). Siekiant įvertinti troponino I ir gliukozės koncentracijų ryšį su 30 dienų išgyvenamumu naudota Kokso (*Cox*) proporcingų rizikų regresijos analizė, koreguojant pagal amžių, lytį, reikšmingas gretutines ligas ir gydymą.

Į analizes buvo įtraukti tik tiriamieji, kurių duomenys buvo išsamūs. Į ROC ir daugiaveiksnes regresines analizes buvo įtraukti tik tie laboratoriniai kintamieji, kurių trūkstamų duomenų dalis buvo mažesnė nei 20 proc.

Rezultatai buvo laikomi statistiškai reikšmingais, kai $p < 0,05$. Statistinė analizė atlikta naudojant „IBM SPSS Statistics 30.0“ versiją (IBM Corp., JAV) ir „Microsoft Excel“.

Finansavimas

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Tyrimas „COVID-19 ligos eigos prognozavimo pagal kraujo žymenis tyrimas“ buvo finansuojamas Vilniaus universiteto ligoninės Santaros klinikų lėšomis. Tyrimas taip pat gavo finansinę paramą iš Europos Sąjungos mokslinių tyrimų ir inovacijų programos „Horizon Europe“ projekto „EuCARE“ (dotacijos sutartis Nr. 101046016).

REZULTATAI

Hospitalizuotų COVID-19 pacientų demografinės, klinikinės, laboratorinės ir radiologinės charakteristikos

Demografiniai, klinikiniai, laboratoriniai ir radiologiniai hospitalizuotų COVID-19 pacientų duomenys pateikiami vadovaujantis ambispektyviuoju kohortiniu tyrimu, kuriame dalyvavo 495 pacientai [127]. Iš 495 hospitalizuotų pacientų 52,9 proc. buvo vyrai. Amžiaus mediana – 55 (TKP 43–65) metai. Beveik du trečdaliai pacientų (61,2 proc.) sirgo bent viena gretutine liga, dažniausiai PAH (51,5 proc.), kitomis širdies ir kraujagyslių ligomis (20,8 proc.) ir CD (11,1 proc.) (1 lentelė). Hospitalizavimo metu atliktų laboratorinių tyrimų rezultatai pateikiami 1 lentelėje.

1 lentelė. Hospitalizuotų COVID-19 liga sergančių pacientų demografinės charakteristikos, gretutinių ligų paplitimas ir laboratoriniai rodikliai hospitalizavimo metu

Kintamasis	N	n (proc.) / mediana (TKP)
Amžius, metais (TKP)	495	55 (43–65)
Amžiaus grupės, metais		
18–29	39	39 (7,9)
30–39	56	56 (11,3)
40–49	99	99 (20,0)
50–59	116	116 (23,4)
60–69	105	105 (21,2)
70–79	45	45 (9,1)
≥ 80	35	35 (7,1)
Vyras	495	262 (52,9)
Nutukimas	271	137 (50,5)
Gretutinės ligos	495	303 (61,2)
Anemija ar kita hematologinė liga	495	40 (8,1)
Bronchų astma	495	17 (3,4)
LOPL	495	14 (2,8)
Kita plaučių liga (ne bronchų astma ar LOPL)	495	11 (2,2)
Nehematologinė onkologinė liga	495	28 (5,7)
PAH	495	255 (51,5)
Kita širdies ir kraujagyslių sistemos liga (ne PAH)	495	103 (20,8)
Demencija	495	13 (2,6)
CD	495	55 (11,1)
Imunodeficitas	495	15 (3,0)
Lėtinė kepenų liga, įskaitant kepenų cirozę	495	18 (3,6)
Nervų ir raumenų liga	495	12 (2,4)
LIL	495	32 (6,5)
Reumatologinė liga	495	17 (3,4)
Buvęs insultas	495	18 (3,6)
Tuberkuliozė	495	3 (0,6)
Hospitalizacijos trukmė dienomis, mediana (TKP)	495	10 (7–14)
Laboratoriniai rodikliai		
Leukocitai, $x10^9$/l	481	5,6 (4,3–7,1)
Neutrofilai, $x10^9$/l	481	3,8 (2,7–5,2)
Limfocitai, $x10^9$/l	481	1,2 (0,8–1,6)
Trombocitai, $x10^9$/l	481	187 (149–236)
Šarminė fosfatazė, U/l	392	64,0 (52,0–80,3)
ALT, U/l	443	30,0 (18,0–48,0)
AST, U/l	430	33,0 (24,0–50,0)

Kintamasis	N	n (proc.) / mediana (TKP)
Feritinas, µg/l	398	358,7 (168,6–775,5)
IL-6, ng/l	401	25,0 (11,0–50,6)
LDH, U/l	407	269,0 (208,0–348,6)
D-dimerai, µg/l	395	375,0 (215,0–640,0)
Fibrinogenas, g/l	360	5,1 (4,2–6,1)
CRB, mg/l	479	29,9 (6,8–84,1)
Laktatas, mmol/l	367	1,3 (1,0–1,8)
Troponinas I, ng/l	387	6,6 (3,0–13,0)
NT-proBNP, ng/l	339	147,2 (71,6–386,0)
GGT, U/l	391	34,0 (18,0–76,0)
Protrombino laikas, proc.	387	90,0 (78,0–104,0)
TNS	393	1,04 (0,98–1,11)
Kreatininas, µmol/l	467	77,6 (64,0–95,0)
Šlapalas, mmol/l	376	4,7 (3,6–6,4)

ALT – alanininė aminotransferazė; AST – asparagininė aminotransferazė; CD – cukrinis diabetas; CRB – C reaktyvusis baltymas; GGT – gama gliutamiltransferazė; IL-6 – interleukinas 6; LDH – laktatdehidrogenazė; LIL – lėtinė inkstų liga; LOPL – lėtinė obstrukcinė plaučių liga; NT-proBNP – N-terminalinis pro-B natriuretinis peptidas; PAH – pirminė arterinė hipertenzija; TKP – tarpkvartilinis plotis; TNS – tarptautinis normalizuotas santykis.

Adaptuota pagal: Kubiliute I. ir kt. *Predicting severe COVID-19 disease in adults: A single-centre cohort study during the first three pandemic waves in 2020–2021 in Vilnius, Lithuania*. PLoS ONE. 2026; 21(5): e0350112 [127].

Hospitalizavimo metu pacientų nurodyti klinikiniai simptomai pateikiami 2 lentelėje. Daugumai pacientų (81,5 proc.) liga prasidėjo staiga. Dažniausiai nustatyti simptomai buvo bendras silpnumas (77,1 proc.), kosulys (69,7 proc.), subfebrilus karščiavimas (65,9 proc.) ir dusulys (40,5 proc.).

2 lentelė. COVID-19 liga sergančių pacientų klinikiniai simptomai hospitalizavimo metu

Simptomai	N	n (proc.)
Staigus simptomų atsiradimas	482	393 (81,5)
Subfebrilus karščiavimas	495	326 (65,9)
Febrilus karščiavimas	495	179 (36,2)
Šaltkrėtis	490	143 (29,2)
Padažnėjęs kvėpavimas	495	91 (18,4)
Bendras silpnumas	488	376 (77,1)
Galvos skausmas	488	152 (31,2)

Simptomai	N	n (proc.)
Galvos svaigimas	488	85 (17,4)
Sumišimas	491	24 (4,9)
Raumenų skausmas	488	142 (29,1)
Gerklės skausmas	488	101 (20,7)
Sloga	488	73 (15,0)
Kosulys	489	341 (69,7)
Dusulys	489	198 (40,5)
Krūtinės skausmas	488	114 (23,4)
Širdies permušimai	488	41 (8,4)
Bendras savijautos pablogėjimas	492	151 (30,7)
Pykinimas	488	54 (11,1)
Vėmimas	495	12 (2,4)
Viduriavimas	491	62 (12,6)
Pilvo skausmas	488	28 (5,7)
Skonio praradimas	487	75 (15,4)
Uoslės praradimas	487	109 (22,4)
Konjunktyvitas	495	11 (2,2)
Bėrimai ir kiti odos pakitimai	495	4 (0,8)

Adaptuota pagal: Kubiliute I. ir kt. *Predicting severe COVID-19 disease in adults: A single-centre cohort study during the first three pandemic waves in 2020–2021 in Vilnius, Lithuania*. PLoS ONE. 2026; 21(5): e0350112 [127].

Radiologinių tyrimų radiniai, taikyta deguonies terapija, gydymas ITS ir IPS bei hospitalizacijos metu išsivysčiusios komplikacijos pateikiami 3 lentelėje. Pneumonija nustatyta 82,1 proc. pacientų, iš kurių 84,8 proc. atvejų – abipusė, o 15,2 proc. – vienpusė. Krūtinės ląstos rentgenogramose plaučių infiltracija nustatyta 75,0 proc. pacientų, 18,4 proc. pacientų infiltracija apėmė daugiau nei 50 proc. plaučių. Krūtinės ląstos kompiuterinė tomografija atlikta 25,5 proc. pacientų. Dažniausi radiniai buvo matinio stiklo vaizdas (65,1 proc.), periferiniai pritemimai (51,6 proc.) ir infiltracija (49,2 proc.). Dažniausiai taikyti deguonies terapijos būdai pacientams, sirgusiems sunkia COVID-19 ligos forma, buvo deguonies terapija per veido kaukę (74,9 proc.) ir nosines kaniules (29,9 proc.). Didelės tėkmės deguonies terapija taikyta 10,0 proc., o NIV, DPV ir EKMO prireikė atitinkamai 1,7 proc., 4,8 proc. ir 1,0 proc. sunkia COVID-19 ligos forma sirgusių pacientų. Bent viena komplikacija hospitalizacijos metu nustatyta 25,1 proc. pacientų. Dažniausios komplikacijos buvo ŪRDS (9,3 proc.) ir kitos antrinės bakterinės infekcijos (ne bakterinė pneumonija) (12,1 proc.).

Iš viso 10,9 proc. pacientų reikėjo aukštesnio lygio priežiūros: 9,9 proc. buvo gydyti ITS, o 2,2 proc. – IPS.

3 lentelė. COVID-19 liga sergančių pacientų radiologinių tyrimų radiniai, taikyta deguonies terapija ir komplikacijos

Kintamasis	n (proc.)
Krūtinės ląstos rentgenografija	473 (95,6)
Infiltracija	354 (75,0)
Skystis pleuros ertmėje	31 (6,6)
Plaučių infiltracija > 50 proc.	45 (18,4)
Stazė	58 (12,3)
Krūtinės ląstos kompiuterinė tomografija	126 (25,5)
Periferiniai pritemimai	65 (51,6)
Matinio stiklo vaizdas	82 (65,1)
Konsolidacija	31 (24,6)
Infiltracija	62 (49,2)
Skystis pleuros ertmėje	15 (11,9)
Pneumonija	395 (82,1)
Vienpusė	60 (15,2)
Abipusė	335 (84,8)
Deguonies terapija	291 (58,8)
Per nosies kaniules	87 (17,6)
Per veido kaukę	218 (44,0)
Didelės tėkmės deguonies terapija	29 (5,9)
Neinvazinė ventiliacija	5 (1,0)
DPV	14 (2,8)
EKMO	3 (0,6)
Komplikacijos	
Bet kokia komplikacija	114 (25,1)
ŪRDS	46 (9,3)
Bronchiolitas	1 (0,2)
Antrinė bakterinė pneumonija (patvirtinta bronchoalveolinio lavažo pasėliu)	10 (2,0)
Kita antrinė bakterinė infekcija (patvirtinta pasėliu)	60 (12,1)
Sepsis	18 (3,6)
Ūminis inkstų pažeidimas	18 (3,6)
Širdies nepakankamumas	5 (1,0)
Dauginis organų pažeidimas	14 (2,8)
Dermatologinės komplikacijos	3 (0,6)
Kritinių būklių miopatija	1 (0,2)
Plaučių arterijos tromboembolija	8 (1,6)

Kintamasis	n (proc.)
Kitos komplikacijos	27 (5,5)
Perkėlimas į ITS ar IPS	54 (10,9)
Perkėlimas į ITS	49 (9,9)
Perkėlimas į IPS	11 (2,2)

DPV – dirbtinė plaučių ventiliacija; EKMO – ekstrakorporinė membraninė oksigenacija; IPS – intensyviosios pulmonologijos skyrius; ITS – intensyviosios terapijos skyrius; ŪRDS – ūminis respiracinis distreso sindromas.

Adaptuota pagal: Kubiliute I. ir kt. *Predicting severe COVID-19 disease in adults: A single-centre cohort study during the first three pandemic waves in 2020–2021 in Vilnius, Lithuania*. PLoS ONE. 2026; 21(5): e0350112 [127].

Veiksniai, prognozuojantys hospitalizuotų pacientų sunkios COVID-19 ligos išsivystymą

Iš 495 pacientų 291 (58,8 proc.) išsivystė sunki COVID-19 ligos forma [127]. Sunkia ligos forma sirgusieji dažniau buvo vyrai (57,4 proc. *vs* 46,6 proc.; $p = 0,018$), apytiksliai 10 metų vyresni ir sergantieji gretutinėmis ligomis (68,4 proc. *vs* 51,0 proc.; $p < 0,001$). Dažniau sunkia COVID-19 ligos forma sirgo sergantieji lėtine obstrukcine plaučių liga (LOPL) (4,5 proc. *vs* 0,5 proc.; $p = 0,009$), PAH (59,1 proc. *vs* 40,7 proc.; $p < 0,001$) ir kitomis širdies ir kraujagyslių ligomis (24,7 proc. *vs* 15,2 proc.; $p = 0,010$).

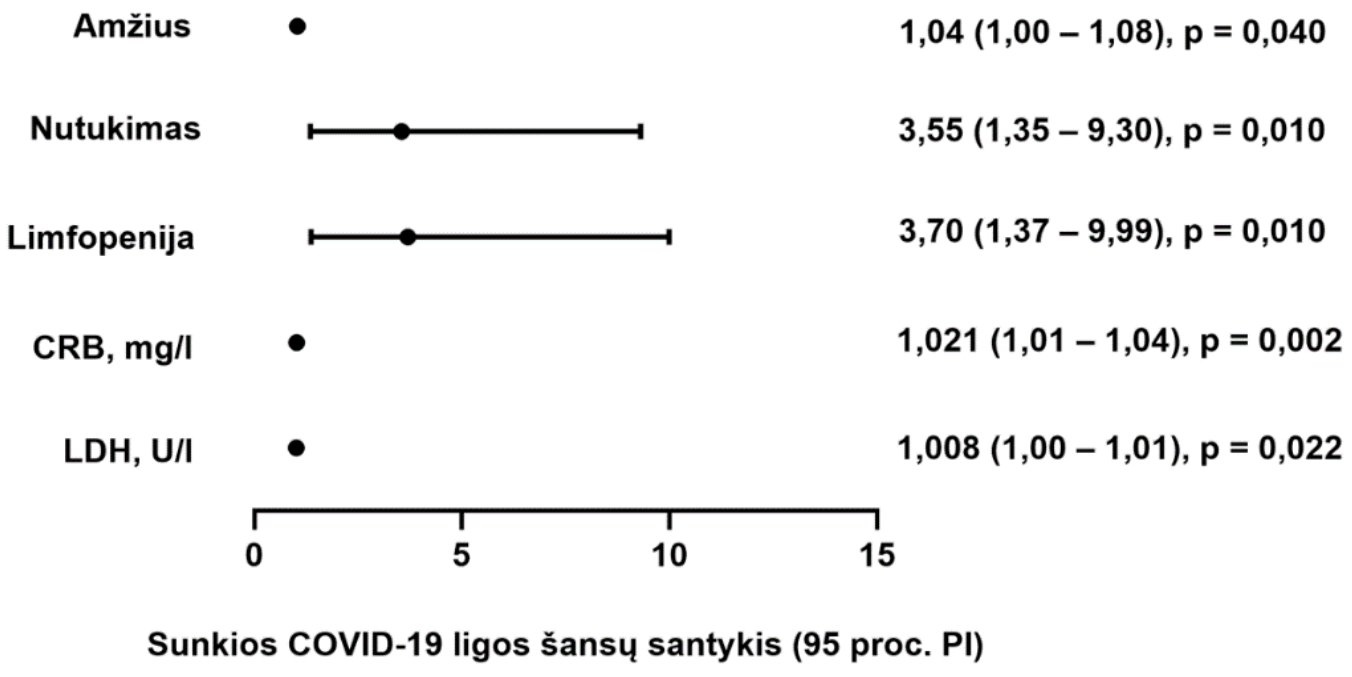
Pacientams, kuriems išsivystė sunki COVID-19 ligos forma, hospitalizavimo metu reikšmingai dažniau pasireiškė febrilus karščiavimas (45,7 proc. *vs* 22,6 proc., $p < 0,001$), kosulys (74,4 proc. *vs* 63,0 proc., $p = 0,007$), dusulys (56,1 proc. *vs* 18,0 proc., $p < 0,001$), padažnėjęs kvėpavimas (28,9 proc. *vs* 3,4 proc., $p < 0,001$), krūtinės skausmas (28,5 proc. *vs* 16,0 proc., $p = 0,001$) ir bendras silpnumas (87,5 proc. *vs* 62,0 proc., $p < 0,001$). Taip pat sunkios ligos grupėje dažniau nustatyti šaltkrėtis (35,3 proc. *vs* 20,4 proc., $p < 0,001$), galvos svaigimas (24,3 proc. *vs* 7,5 proc., $p < 0,001$), sumišimas (6,6 proc. *vs* 2,5 proc., $p = 0,036$), bendros būklės blogėjimas (39,5 proc. *vs* 18,2 proc., $p < 0,001$) ir viduriavimas (16,2 proc. *vs* 7,5 proc., $p = 0,004$).

Pacientų, kuriems COVID-19 ligos eiga buvo sunki, uždegimo rodiklių – CRB (56,0 *vs* 6,2 mg/l, $p < 0,001$), IL-6 (33,4 *vs* 9,9 ng/l, $p < 0,001$), feritino (480,0 *vs* 215,0 µg/l, $p < 0,001$) ir fibrinogeno (5,4 *vs* 4,4 g/l, $p < 0,001$) – koncentracija buvo statistiškai reikšmingai didesnė, palyginti su sirgusiųjų lengva COVID-19 ligos forma. Šių pacientų kraujo tyrimuose nustatytas mažesnis limfocitų (1,1 *vs* 1,4 $\times 10^9$/l, $p < 0,001$) ir didesnis neutrofilų (4,1 *vs* 3,3 $\times 10^9$/l, $p < 0,001$) skaičius. Be to, sunkios ligos atvejais nustatyta didesnė

LDH (303,0 *vs* 206,4 U/l, p < 0,001), AST (38,0 *vs* 24,0 U/l, p < 0,001), ALT (34,0 *vs* 24,0 U/l, p < 0,001), laktato (1,4 *vs* 1,1 mmol/l, p < 0,001), D-dimerų (425,0 *vs* 265,0 µg/l, p < 0,001), troponino I (8,0 *vs* 3,8 ng/l, p < 0,001), NT-proBNP (170,3 *vs* 98,0 ng/l, p < 0,001), šlapalo (5,0 *vs* 4,3 mmol/l, p < 0,001) ir kreatinino (80,0 *vs* 74,4 µmol/l, p = 0,007) koncentracija.

Siekiant nustatyti veiksnius, susijusius su sunkia COVID-19 ligos eiga, atlikta logistinė regresinė analizė. Vienaveiksnėje regresijoje sunki COVID-19 ligos eiga buvo reikšmingai susijusi su vyresniu amžiumi, vyriškąja lytimi, nutukimu, PAH, širdies ir kraujagyslių ligomis, LOPL, įvairiais klinikiniais simptomais (subfebriliu ir febriliu karščiavimu, šaltkrėčiu, padažnėjusiu kvėpavimu, bendru silpnumu, galvos svaigimu, sumišimu, kosuliu, dusuliu, krūtinės skausmu, bendra bloga būkle) ir laboratoriniais rodikliais (ALT, AST, feritino, IL-6, CRB, laktato, kreatinino, šlapalo koncentracijos padidėjimu).

Daugiaveiksnėje binarinėje logistinėje regresijoje nustatyti šeši sunkios COVID-19 ligos prediktoriai, iš kurių penki išliko reikšmingi po vidinės validacijos (1 pav.). Vyresnis amžius buvo susijęs su sunkia ligos forma (ŠS 1,04 kiekvieniems metams; 95 proc. PI 1,00–1,08; p = 0,040), o nutukimas šiuos šansus padidino 3,55 karto (ŠS 3,55; 95 proc. PI 1,35–9,30; p = 0,010). Iš klinikinių simptomų reikšmingas prediktorius sunkiai COVID-19 ligai buvo febrilus karščiavimas (ŠS 3,41; 95 proc. PI 1,09–10,73; p = 0,036). Taip pat su sunkia COVID-19 liga buvo susijusi limfopenija (ŠS 3,70; 95 proc. PI 1,37–9,99; p = 0,010), padidėjusi LDH ir CRB koncentracija. LDH koncentracijos padidėjimas 10 U/l, o CRB koncentracijos padidėjimas 10 mg/l buvo susijęs atitinkamai su 8 proc. ir 21 proc. didesniais sunkios COVID-19 ligos šansais.



1 paveikslas. Reikšmingi sunkios COVID-19 ligos formos prediktoriai: daugiaveiksnės binarinės logistinės regresijos modelis po boostrap vidinės validacijos

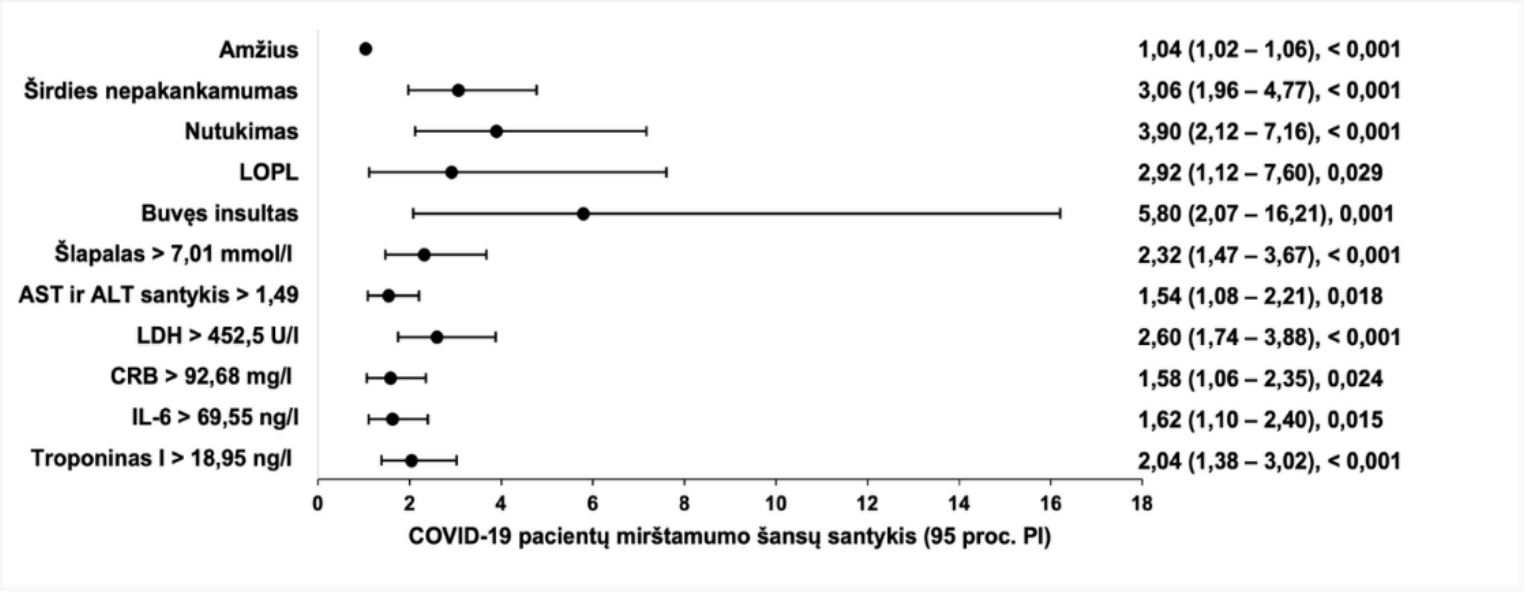
CRB – C reaktyvusis baltymas; LDH – laktatdehidrogenazė; PI – pasikliautinasis intervalas.
Adaptuota pagal: Kubiliute I. ir kt. *Predicting severe COVID-19 disease in adults: A single-centre cohort study during the first three pandemic waves in 2020–2021 in Vilnius, Lithuania*. PLoS ONE. 2026; 21(5): e0350112 [127].

Bendras COVID-19 pacientų mirštamumas stacionare siekė 12,7 proc. ir didėjo su amžiumi – nuo 1,6 proc. tarp jaunesnių nei 40 metų pacientų iki 29,7 proc. tarp ≥ 70 metų pacientų. Hospitalizacijos metu mirę pacientai buvo vyresni nei išgyvenusieji (72,5 *vs* 58 metai; p < 0,001), taip pat jiems dažniau nustatytos gretutinės ligos: PAH (54,8 proc. *vs* 34,2 proc., p < 0.001), koronarinė širdies liga (9,0 proc. *vs* 3,0 proc., p < 0,001), širdies nepakankamumas (28,9 proc. *vs* 4,8 proc., p < 0,001), CD (23,9 proc. *vs* 12,2 proc., p < 0,001), nutukimas (11,5 proc. *vs* 3,6 proc., p < 0,001), LOPL (4,8 proc. *vs* 1,1 proc., p < 0,001), LIL (17,4 proc. *vs* 6,3 proc., p < 0,001) bei buvęs insultas (5,6 proc. *vs* 0,8 proc., p < 0.001) [124].

Neišgyvenusiems pacientams hospitalizavimo metu rasti statistiškai reikšmingai didesnis leukocitų (8,6 *vs* 6,4 ×10⁹/l, p < 0,001), neutrofilų (7,0 *vs* 4,7 ×10⁹/l, p < 0,001) skaičius, neutrofilų ir limfocitų santykis (8,9 *vs* 4,4, p < 0,001), didesnė gliukozės (7,2 *vs* 6,1 mmol/l, p < 0,001), kreatinino (110,0 *vs* 80,0 µmol/L, p < 0,001), šlapalo (11,7 *vs* 5,4, p < 0,001), kalio (4,3 *vs* 4,2 mmol/l, p = 0,003), LDH (417,0 *vs* 294,0 U/l, p < 0,001), CRB (118,4 *vs* 53,5 mg/L, p < 0,001), feritino (717,0 *vs* 447,5 µg/L, p < 0,001), IL-6 (55,2 *vs* 27,0 ng/l, p < 0,001), D-dimerų (1080,0 *vs* 480,0 µg/L, p < 0,001) bei troponino I (41,0 *vs* 9,0 ng/l, p < 0,001) koncentracija, didesnis AST ir ALT santykis (1,7 *vs* 1,1, p < 0,001), tačiau hemoglobino koncentracija (127 *vs* 138 g/l, p < 0,001), limfocitų (0,7 *vs* 1,0 ×10⁹/l, p < 0,001) ir trombocitų (192,0 *vs* 200,5 ×10⁹/l, p = 0,010) skaičius bei natrio koncentracija (138,0 *vs* 140,0 mmol/l, p < 0,001) buvo statistiškai reikšmingai mažesni nei išgyvenusių pacientų.

Vienaveiksnė analizė parodė, kad stacionarinis mirštamumas nuo COVID-19 ligos buvo susijęs su vyresniu amžiumi, gretutinėmis ligomis (PAH, koronarine širdies liga, širdies nepakankamumu, CD, nutukimu, LOPL, LIL ir buvusiu insultu) bei įvairiais laboratoriniais rodikliais.

Atliekant daugiaveiksnę binarinę logistinę regresiją nustatyta, kad nepriklausomi COVID-19 pacientų mirštamumo prediktoriai buvo vyresnis amžius (ŠS 1,04; 95 proc., PI 1,02–1,06), širdies nepakankamumas (ŠS 3,06; 95 proc. PI 1,96–4,77), nutukimas (ŠS 3,90; 95 proc. PI 2,12–7,16), LOPL (ŠS 2,92; 95 proc. PI 1,12–7,60), buvęs insultas (ŠS 5,80; 95 proc. PI 2,07–16,21) ir laboratoriniai rodikliai: šlapalo koncentracija > 7,01 mmol/l (ŠS 2,32; 95 proc. PI 1,47–3,67), AST ir ALT santykis > 1,49 (ŠS 1,54; 95 proc. PI 1,08–2,21), LDH koncentracija > 452,5 U/l (ŠS 2,60; 95 proc. PI 1,74–3,88), CRB koncentracija > 92,68 mg/l (ŠS 1,58; 95 proc. PI 1,06–2,35), IL-6 koncentracija > 69,55 ng/l (ŠS 1,62, 95 proc. PI 1,10–2,40), ir troponino I koncentracija >18,95 ng/l (ŠS 2,04, 95 proc. PI 1,38–3,02) (2 pav.).



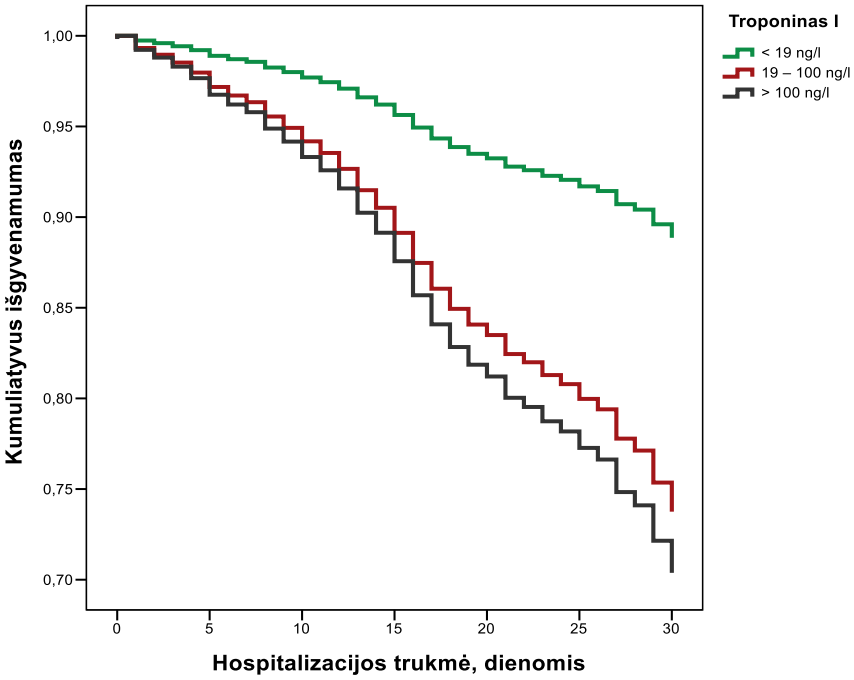
2 paveikslas. Reikšmingi hospitalizuotų COVID-19 pacientų mirštamumo prediktoriai: daugiaveiksnės binarinės logistinės regresijos modelis

ALT – alanininė aminotransferazė; AST – asparagininė aminotransferazė; CRB – C reaktyvusis baltymas; IL-6 – interleukinas 6; LDH – laktatdehidrogenazė; LOPL – lėtinė obstrukcinė plaučių liga; PI – pasikliautinasis intervalas.

Adaptuota pagal: Kubiliute I. ir kt. *Clinical characteristics and predictors for in-hospital mortality in adult COVID-19 patients: A retrospective single center cohort study in Vilnius, Lithuania*. PLoS ONE. 2023; 18(8): e0290656 [124].

Neuždegiminių biožymenų – troponino I ir hiperglikemijos – kaip galimų stacionarinio COVID-19 mirštamumo prognostinių veiksnių vertinimas

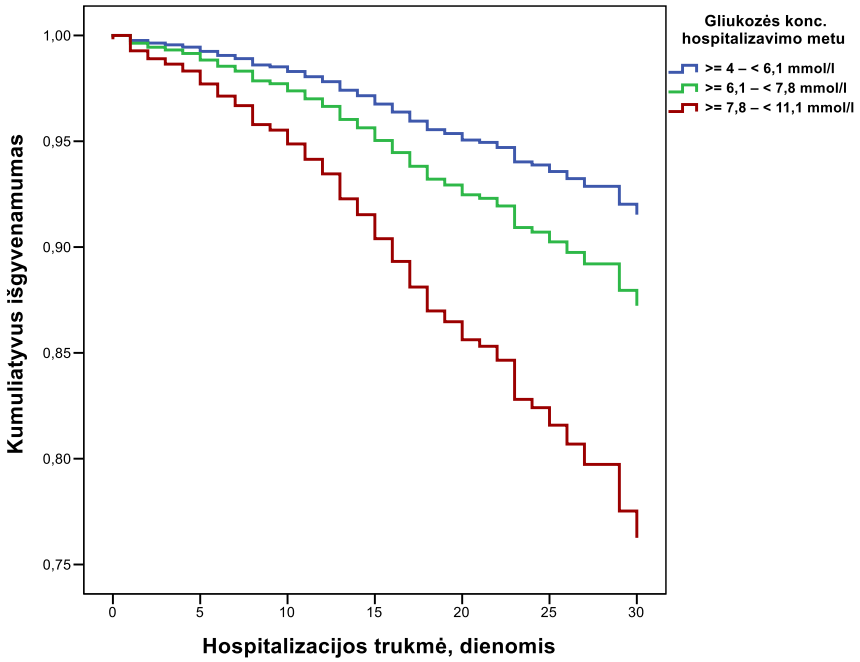
Disertaciniame darbe analizuoti neuždegiminiai rodikliai – troponinas I ir hiperglikemija – kaip potencialūs pacientų, sergančių COVID-19 liga, mirštamumo prediktoriai. Išanalizavus 2019 pacientų duomenis rasta, kad hospitalizavimo metu 603 pacientams (29,9 proc.) nustatyta ūminė miokardo pažaida, kuri pasireiškė padidėjusia troponino I koncentracija. Pacientų, kurių troponino I koncentracija hospitalizavimo metu buvo 19–100 ng/l, 30 dienų rizika mirti hospitalizacijos metu buvo 2,58 karto didesnė (SR 2,58; 95 proc. PI 1,83–3,62; $p < 0,001$), o pacientų, kurių troponino I koncentracija viršijo 100 ng/l – 2,97 karto didesnė (SR 2,97; 95 proc. PI 2,01–4,39; $p < 0,001$), palyginti su pacientais, kurių troponino I koncentracija buvo normali (3 pav.) [126].



3 paveikslas. Hospitalizuotų COVID-19 pacientų Kokso regresijos išgyvenamumo analizė pagal troponino I koncentraciją pirmą hospitalizacijos dieną

Adaptuota pagal: Kubiliute I. ir kt. *Elevated Cardiac Troponin I as a Mortality Predictor in Hospitalised COVID-19 Patients*. Medicina. 2024; 60: 842 [126].

Iš 1945 pacientų, nesirgusių CD, 55,4 proc. hospitalizavimo metu nustatyta normoglikemija, 33,5 proc. – lengva hiperglikemija, o 10,1 proc. – ženkli hiperglikemija. Hipoglikemija nustatyta 20 pacientų (1,0 proc.). Didžiausias, 22,4 proc., mirštamumas hospitalizacijos metu nustatytas pacientų, kuriems buvo pasireiškusi ženkli hiperglikemija. Pacientų, kuriems hospitalizavimo metu buvo lengva hiperglikemija, mirštamumas siekė 9,4 proc., o kuriems normoglikemija – 5,3 proc. Nustatyta, kad lengva hiperglikemija buvo susijusi su 1,62 karto didesne 30 dienų mirštamumo rizika hospitalizacijos metu (SR 1,62; 95 proc. PI 1,10–2,39; p = 0,015), o ženkli hiperglikemija – su 3,04 karto didesne rizika (SR 3,04; 95 proc. PI 2,01–4,60; p < 0,001), palyginti su pacientais, kuriems hospitalizavimo metu rasta normoglikemija (4 pav.) [125].



4 paveikslas. Hospitalizuotų COVID-19 pacientų Kokso regresijos išgyvenamumo analizė pagal gliukozės koncentraciją pirmą hospitalizacijos dieną

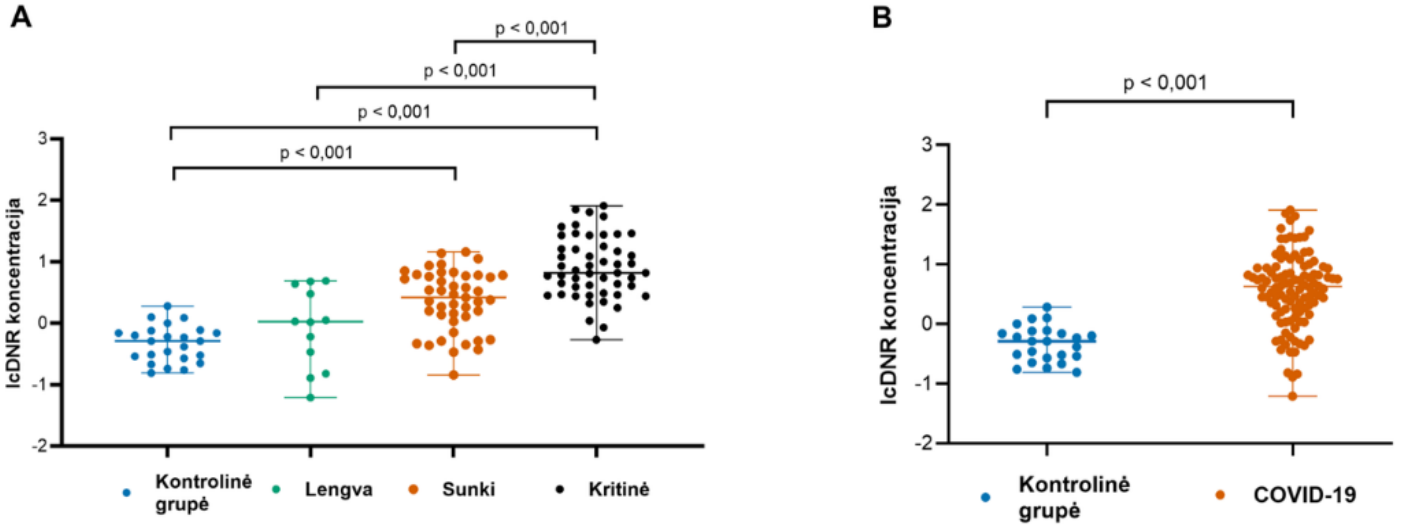
Adaptuota pagal: Zabuliene L. ir kt. *Hyperglycaemia and Its Prognostic Value in Patients with COVID-19 Admitted to the Hospital in Lithuania*. Biomedicines. 2024; 12(1): 55 [125].

Laisvai cirkuliuojančios nukleorūgštys kraujo serume kaip galimi COVID-19 ligos sunkumą ir letalią baigtį prognozuojantys biožymenys

Siekiant įvertinti serumo lcNR (lcDNR ir SARS-CoV-2 RNR) prognostinę reikšmę COVID-19 ligos sunkumui ir mirštamumui, į tyrimą įtraukti 132 asmenys: 108 hospitalizuoti COVID-19 pacientai ir 24 kontrolinės grupės tiriamieji [128]. Kontrolinę grupę sudarė sveiki vyrai, kurių amžiaus mediana buvo 30,5 metų (TKP 23,25–38,75).

COVID-19 pacientų lcDNR koncentracijos mediana buvo 4,28 ng/µl (TKP 1,62–8,78). Pagal ligos sunkumą pacientai suskirstyti į tris grupes: 12 pacientų sirgo lengva, 45 – sunkia, o 51 – kritiškai sunkia COVID-19 liga. Nustatyta, kad lcDNR koncentracija didėjo sunkėjant COVID-19 ligos eigai – nuo 1,06 ng/µl lengvos ligos atvejais iki 2,65 ng/µl sunkios ir 6,68 ng/µl kritiškai sunkios ligos grupėje ($p < 0,001$, lyginant sunkios ir kritiškai sunkios COVID-19 ligos grupes su lengvos ligos grupe) (5 pav., A).

Kontrolinės grupės lcDNR koncentracija buvo reikšmingai mažesnė nei COVID-19 pacientų (0,51 *vs* 4,28 ng/µl; $p < 0,001$) (5 pav., B), taip pat mažesnė nei sunkia (0,51 *vs* 2,65 ng/µl; $p < 0,001$) ir kritiškai sunkia (0,51 *vs* 6,68 ng/µl; $p < 0,001$) COVID-19 ligos forma sirgusių pacientų grupėse (5 pav., A). Kadangi kontrolinės grupės tiriamieji buvo jaunesni nei COVID-19 pacientai, atlikta pagal amžių koreguota analizė, kuri patvirtino, kad lcDNR koncentracija COVID-19 pacientų grupėje išliko reikšmingai didesnė ($p = 0,018$).



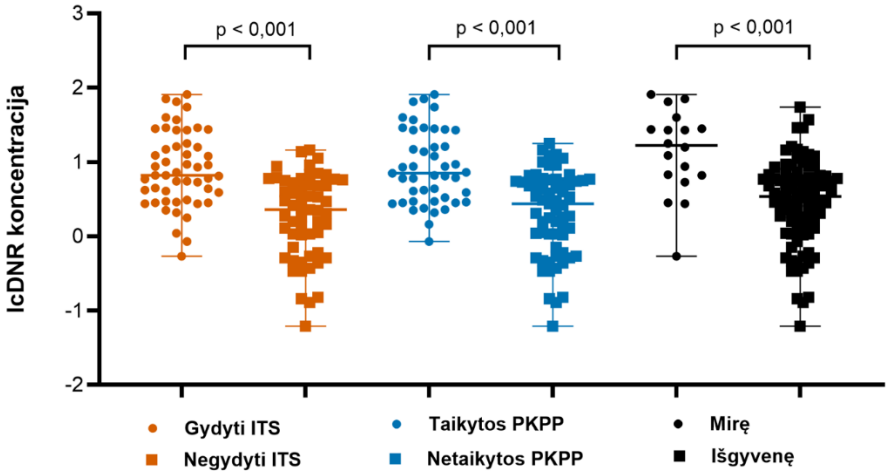
5 paveikslas. Skirtingo sunkumo COVID-19 pacientų ir sveikų kontrolinės grupės tiriamųjų (A) bei COVID-19 pacientų ir kontrolinės grupės tiriamųjų lcDNR koncentracijų palyginimas (B)

lcDNR koncentracijos pateiktos pritaikius logaritminę transformaciją.
Adaptuota pagal: Kubiliute I. ir kt. *Cell-free nucleic acids as a non-invasive biomarker for predicting COVID-19 disease severity and outcome: a retrospective cohort study*. BMC Infectious Diseases. 2026; 26: 1010 [128].

Iš viso 51 pacientui išsivystė kritiškai sunki COVID-19 ligos forma, dėl kurios prireikė gydymo ITS. Šių pacientų lcDNR koncentracija buvo statistiškai reikšmingai didesnė nei negydytų ITS pacientų grupėje (6,68 *vs* 2,30 ng/µl; $p < 0,001$) (6 pav.).

PKPP (NIV, DPV arba EKMO) prireikė iš viso 45 pacientams. Pacientų, kuriems buvo taikyta PKPP, lcDNR koncentracija hospitalizavimo metu buvo reikšmingai didesnė nei pacientų, kuriems PKPP neprireikė (7,11 *vs* 2,74 ng/µl; $p < 0,001$) (6 pav.).

Iš 108 pacientų 18 (16,7 proc.) hospitalizacijos metu mirė. Neišgyvenusių pacientų lcDNR koncentracija buvo reikšmingai didesnė nei išgyvenusių (16,68 *vs* 3,44 ng/µl; $p < 0,001$) (6 pav.).



6 paveikslas. Hospitalizuotų COVID-19 pacientų lcDNR koncentracijų palyginimas pagal gydymo ITS poreikį, PKPP poreikį ir mirštamumą hospitalizacijos metu
lcDNR koncentracijos pateiktos pritaikius logaritminę transformaciją.

Adaptuota pagal: Kubiliute I. et al. *Cell-free nucleic acids as a non-invasive biomarker for predicting COVID-19 disease severity and outcome: a retrospective cohort study*. BMC Infectious Diseases. 2026;26:1010 [128].

SARS-CoV-2 RNR serume kiekybinės PGR metodu nustatyta 31 (28,7 proc.) COVID-19 pacientui, o visų kontrolinės grupės tiriamųjų serumo mėginiai buvo neigiami. SARS-CoV-2 RNR serume dažniau nustatyta sunkesne ligos forma sirgusiems pacientams. SARS-CoV-2 RNR statistiškai reikšmingai dažniau nustatyta ITS gydytiems pacientams, palyginti su negydytais ITS (43,1 proc. *vs* 15,8 proc.; $p = 0,002$), taip pat pacientams,

kuriems taikytos PKPP, palyginti su pacientais, kuriems PKPP neprireikė (46,7 proc. *vs* 15,9 proc.; p < 0,001). Be to, SARS-CoV-2 RNR serume dažniau nustatyta neišgyvenusiems pacientams nei išgyvenusiems (50,0 proc. *vs* 24,4 proc.; p = 0,029). Pacientams, kuriems kraujo serume buvo aptikta SARS-CoV-2 RNR, nustatyta statistiškai reikšmingai didesnė lcDNR koncentracija (6,68 *vs* 3,07 ng/µl; p < 0,001).

ROC analizė parodė, kad lcDNR pasižymėjo didžiausia prognostine verte iš visų tirtų laboratorinių rodiklių numatant kritiškai sunkią COVID-19 ligos formą, PKPP poreikį ir mirštamumą hospitalizacijos metu. Prognozuojant kritiškai sunkią COVID-19 ligą, lcDNR AUC siekė 0,79 (95 proc. PI 0,71–0,87; p < 0,001), o optimali riba buvo 2,69 ng/µl. Prognozuojant PKPP poreikį, lcDNR AUC buvo 0,77 (95 proc. PI 0,68–0,87; p < 0,001), optimali riba – 5,93 ng/µl. Vertinant mirštamumą hospitalizacijos metu, lcDNR prognostinė vertė buvo didžiausia (AUC 0,81; 95 proc. PI 0,69–0,93; p < 0,001), o optimali riba siekė 6,47 ng/µl. Visais atvejais lcDNR prognostinis tikslumas viršijo kitų įprastinių laboratorinių rodiklių rezultatų tikslumą.

Siekiant nustatyti nepriklausomus kritiškai sunkios COVID-19 ligos, PKPP poreikio ir mirštamumo hospitalizacijos metu prognostinius veiksnius, sudaryti daugiaveiksnės binarinės logistinės regresijos modeliai, į kuriuos įtraukti amžius, lytis, nutukimas, gretutinės ligos, reikšmingi laboratoriniai rodikliai, SARS-CoV-2 RNR aptikimas serume ir lcDNR koncentracija. Visuose modeliuose lcDNR buvo nepriklausomas kritiškai sunkios COVID-19 ligos, PKPP ir mirštamumo hospitalizacijos metu predikorius, nepriklausomai nuo amžiaus, lyties ir SARS-CoV-2 RNR nustatymo serume (4 lentelė). Kiekvienas lcDNR koncentracijos padidėjimas 1 ng/µl buvo susijęs su 21 proc. didesniais kritiškai sunkios COVID-19 ligos šansais (ŠS 1,21; 95 proc. PI 1,08–1,36; p = 0,001), 15 proc. didesniais PKPP poreikio šansais (ŠS 1,15; 95 proc. PI 1,03–1,28; p = 0,014) ir 8 proc. didesniais letalios baigties šansais (ŠS 1,08; 95 proc. PI 1,02–1,14; p = 0,009).

4 lentelė. Hospitalizuotų COVID-19 pacientų lcDNR ir klinikinių veiksnių sąsaja prognozuojant kritiškai sunkią COVID-19 ligą, pažangių kvėpavimo palaikymo priemonių poreikį ir mirštamumą hospitalizacijos metu

Prediktorius	**Šansų santykis (95 proc. PI)**	**p reikšmė**
Kritiškai sunki COVID-19 liga		
lcDNR	1,21 (1,08–1,36)	0,001
Amžius	0,98 (0,94–1,01)	0,173

Prediktorius	Šansų santykis (95 proc. PI)	p reikšmė
Lytis	0,66 (0,26–1,67)	0,376
Nutukimas	1,65 (0,44–6,14)	0,455
Bet kokia gretutinė liga	0,82 (0,26–2,55)	0,732
SARS-CoV-2 RNR serume	2,57 (0,86–7,64)	0,090
Pažangių kvėpavimo palaikymo priemonių poreikis		
lcDNR	1,15 (1,03–1,28)	0,014
Amžius	0,98 (0,94–1,03)	0,496
Lytis	0,39 (0,13–1,20)	0,100
Nutukimas	7,08 (1,55–32,25)	0,011
Bet kokia gretutinė liga	1,61 (0,40–6,55)	0,505
SARS-CoV-2 RNR serume	4,18 (1,15–15,20)	0,030
Neutrofilai	1,05 (0,83–1,32)	0,703
LDH	1,001 (0,998–1,004)	0,647
Mirtis hospitalizacijos metu		
lcDNR	1,08 (1,02–1,14)	0,009
Amžius	1,07 (0,98–1,18)	0,136
Lytis	0,37 (0,05–2,57)	0,314
Nutukimas	1,36 (0,08–23,25)	0,830
Bet kokia gretutinė liga	3,24 (0,24–43,24)	0,375
SARS-CoV-2 RNR serume	4,77 (0,78–29,01)	0,090
Neutrofilai	1,12 (0,76–1,64)	0,582
Trombocitai	1,01 (0,998–1,02)	0,097
Šlapalas	0,91 (0,69–1,19)	0,493
D-dimeai	1,00 (1,000–1,001)	0,686
NT-proBNP	1,00 (1,000–1,001)	0,203

ITS – intensyviosios terapijos skyrius; lcDNA – laisvai cirkuliujanti deoksiribonukleorūgštis; LDH – laktatdehidrogenazė; NT-proBNP – N-terminalinis pro-B natriuretinis peptidas; PI – pasikliautinasis intervalas; RNR – ribonukleorūgštis; SARS-CoV-2 – sunkaus ūminio respiracinio sindromo koronavirusas 2.

Adaptuota pagal: Kubiliute I. et al. *Cell-free nucleic acids as a non-invasive biomarker for predicting COVID-19 disease severity and outcome: a retrospective cohort study*. BMC Infectious Diseases. 2026;26:1010 [128].

SARS-CoV-2 RNR aptikimas serume buvo susijęs tik su PKPP poreikiu, padidindamas šansus 4,18 karto (95 proc. PI 1,15–15,20), tačiau reikšmingų sąsajų su kritiškai sunkia COVID-19 ligos forma ar letalia baigtimi nenustatyta (4 lentelė).

DISERTACINIO DARBO STIPRIOSIOS SAVYBĖS IR TRŪKŪMAI

Disertacinio darbo stiprybės

Šis tyrimas yra vienas pirmųjų išsamus demografinių, klinikinių, laboratorinių ir radiologinių rodiklių bei jų sąsajų su COVID-19 ligos baigtimis tyrimas Lietuvoje. Tyrimas papildė ribotus duomenis iš Rytų ir Vidurio Europos regiono bei prisidėjo prie geresnio populiacinių COVID-19 ligos ypatumų supratimo.

Tyrime vertintas platus klinikinių ir laboratorinių rodiklių spektras, leidęs išsamiai apibūdinti COVID-19 ligos eigą tiriamojoje populiacijoje. Nors tyrimo atlikimas viename centre turėjo tam tikrų ribotumų, toks tyrimo dizainas užtikrino vieningų diagnostikos ir gydymo protokolų taikymą, sumažino tarpinstitucinį kintamumą ir padidino rezultatų patikimumą.

Letalios COVID-19 ligos baigties prognostinių veiksnių vertinimas atliktas didelėje pacientų kohortoje, o sunkios COVID-19 formos rizikos veiksnių vertinimui pasirinktas ambispektyvusis tyrimo dizainas leido surinkti detalesnius klinikinius duomenis, įskaitant simptomus, kurie nebuvo prieinami nuasmenintose duomenų bazėse.

Svarbi šio darbo stiprybė – naujų biologinių žymenų tyrimas. Be įprastinių laboratorinių rodiklių, tyrime buvo vertintos tiek šeimininko, tiek patogeno kilmės laisvai cirkuliuojančios nukleorūgštys (lcDNR ir SARS-CoV-2 RNR). Be kiekybinio SARS-CoV-2 PGR tyrimo serume, buvo atliktas ir ilgų sekų SARS-CoV-2 PGR tyrimas, siekiant įvertinti viremiją ir jos reikšmę COVID-19 ligos eigai. Serumo lcNR vertinančių tyrimų iki šiol atlikta nedaug, todėl gauti rezultatai prisideda prie šios srities žinių plėtros ir rodo šių biožymenų potencialą ateityje būti integruotiems į klinikinę praktiką.

Disertacinio darbo trūkumai

Šį disertacinį darbą sudarė trys viena kitą papildančios tyrimo dalys, todėl skirtingi duomenų šaltiniai ir duomenų rinkimo metodai lėmė tyrimo heterogeniškumą ir apribojo tiesioginį atskirų rezultatų palyginamumą.

Tyrimas atliktas viename tretinio lygio sveikatos priežiūros centre, todėl galimas atrankos šališkumas ir ribotas rezultatų pritaikomumas kitose pacientų populiacijose. Kadangi tai buvo referencinis centras, tikėtina, kad šiame centre, ypač pandemijos pikų metu, galėjo būti hospitalizuota santykinai daugiau sunkesnės būklės pacientų, kuriems dažniau buvo atliekami išsamesni laboratoriniai tyrimai, o tai galėjo lemti sisteminę duomenų rinkimo paklaidą. Dėl retrospektyvaus tyrimo dalių dizaino

nepavyko surinkti visų pacientų išsamių duomenų, sumažėjo kai kurių analizių imtys, o tai galėjo apriboti rezultatų patikimumą ir jų interpretavimo išsamumą.

Sunkios COVID-19 ligos eigos ir stacionarinio mirštamumo prognostinių veiksnių analizėje dalis laboratorinių rodiklių galėjo būti susiję ne tik su SARS-CoV-2 infekcija, bet ir su pacientų gretutinėmis ligomis, todėl rezultatų interpretaciją būtina vertinti atsižvelgiant į galimą liekamąjį klaidinančių veiksnių poveikį.

Laisvai cirkuliuojančių nukleorūgščių tyrimo dalį ribojo santykinai nedidelė tiriamųjų ir kontrolinės grupės imtis bei grupių skirtumai pagal amžių, lytį, duomenų apie kontrolinės grupės gretutines ligas Biobanko sistemoje nebuvimas. Be to, absoliučioms lcDNR koncentracijoms įtakos galėjo turėti preanalitiniai veiksniai, nors standartizuotos mėginių apdorojimo procedūros šią įtaką, tikėtina, sumažino. Interpretuojant lcDNR skirtumus būtina atsižvelgti į šiuos ribotumus, o rezultatų patvirtinimui reikalingi platesni tyrimai ir pamatinių verčių nustatymas. Be to, didesnė imtis leistų tiksliau įvertinti SARS-CoV-2 viremiją kaip galimą COVID-19 sunkumą ir baigtis prognozuojantį veiksnį.

Tyrimas apėmė skirtingus COVID-19 pandemijos etapus, kurių metu keitėsi hospitalizavimo kriterijai, gydymo strategijos ir cirkuliuojantys SARS-CoV-2 variantai. SARS-CoV-2 sekoskaita tyrime nebuvo atlikta, todėl nebuvo galimybės analizuoti skirtingų viruso variantų įtaką ligos baigtims. Be to, į galutinę analizę nebuvo įtrauktas pasikeitęs COVID-19 ligos gydymas, kuris galėjo turėti įtakos klinikinėms baigtims. Vis dėlto šis tyrimas atliktas viename centre, kuriame, atsižvelgiant į pandemijos etapą, buvo taikomi standartizuoti gydymo protokolai, todėl tikėtina, kad šio klaidinančio veiksnio poveikis buvo bent iš dalies sumažintas.

IŠVADOS

1. Hospitalizuotų suaugusių COVID-19 pacientų populiacijoje didžiausią dalį sudarė asmenys nuo 50 iki 69 metų. Daugiau nei pusė pacientų sirgo bent viena gretutine liga. COVID-19 ligos klinikiniam pasireiškimui buvo būdingi ūminės virusinės kvėpavimo sistemos infekcijos požymiai – vyraujantys simptomai buvo bendras silpnumas, karščiavimas, kosulys ir dusulys. Radiologiniuose tyrimuose dažniausiai nustatyta abipusė pneumonija. Laboratoriniai tyrimai atspindėjo sisteminį uždegimą, imuninės sistemos disbalansą, audinių pažeidimą bei koagulopatiją, išryškindami daugiasistemį COVID-19 ligos pobūdį. Daugiau nei pusei pacientų prireikė deguonies terapijos,

o pažangios kvėpavimo palaikymo priemonės taikytos tik mažai pacientų daliai, ketvirtadaliui pacientų išsivystė komplikacijos.

2. Vyresnis amžius, nutukimas, limfopenija bei padidėjusi CRB ir LDH koncentracija hospitalizavimo metu buvo nepriklausomi sunkios COVID-19 ligos, apibrėžtos kaip bet kokios deguonies terapijos poreikis, prognostiniai veiksniai.
3. Bendras mirštamumas stacionare siekė 12,7 proc. Mirštamumas didėjo didėjant amžiui ir 70 metų bei vyresnių pacientų grupėje siekė 29,7 proc. Vyresnis amžius, širdies nepakankamumas, nutukimas, LOPL, buvęs insultas, taip pat padidėjusi šlapalo, LDH, CRB, IL-6, troponino I koncentracija bei padidėjęs AST ir ALT santykis buvo susiję su hospitalizuotų COVID-19 liga sergančių pacientų mirštamumu.
4. Neuždegiminių biožymenų vertinimas parodė, kad hospitalizavimo metu padidėjusi troponino I koncentracija ir hiperglikemija buvo susijusios su didesne stacionarinio mirštamumo rizika, ir pabrėžė jų papildomą vertę greta uždegiminių rodiklių prognozuojant COVID-19 ligos baigtis.
5. Didesnė laisvai cirkuliuojančios DNR koncentracija kraujo serume buvo nepriklausomai susijusi su sunkesne COVID-19 liga – didesniu gydymo intensyviosios terapijos skyriuje ir pažangių kvėpavimo palaikymo priemonių poreikiu bei didesniu mirštamumu hospitalizacijos metu. SARS-CoV-2 RNR nustatymas kraujo serume buvo susijęs su pažangių kvėpavimo palaikymo priemonių poreikiu. Laisvai cirkuliuojanti DNR pasižymėjo didesniu prognostiniu tikslumu nei įprastiniai biožymenys. Šie rezultatai parodo laisvai cirkuliuojančių nukleorūgščių, ypač DNR, kaip perspektyvių biožymenų, klinikinę reikšmę ankstyvai hospitalizuotų COVID-19 pacientų rizikos stratifikacijai ir ligos baigčių prognozavimui.

REKOMENDACIJOS

1. Priėmimo-skubiosios pagalbos skyriuose rūšiuojant pacientus ir priimant sprendimus dėl hospitalizavimo tikslinga atsižvelgti į paciento amžių, turimas gretutines ligas (ypač nutukimą, širdies nepakankamumą, LOPL ir buvusį insultą), taip pat įprastinių laboratorinių rodiklių pokyčius, būtent limfopeniją, padidėjusį neutrofilų-limfocitų santykį, hiperglikemiją, padidėjusią CRB, LDH, šlapalo koncentraciją, AST ir ALT santykį. Stacionare gydomiems pacientams papildomą prognostinę vertę gali suteikti IL-6 koncentracijos vertinimas. Šių rodiklių integruotas vertinimas gali

padėti anksti nustatyti pacientus, kuriems yra didesnė sunkios COVID-19 ligos rizika, bei optimizuoti priimamus klinikinius sprendimus ir sveikatos priežiūros išteklių paskirstymą.

2. Kadangi beveik trečdaliui hospitalizuotų COVID-19 pacientų buvo nustatyta miokardo pažaida radus padidėjusią troponino I koncentraciją, rekomenduojama šį rodiklį rutiniškai tirti hospitalizavimo metu, ypač pacientams, sergantiems širdies ir kraujagyslių ligomis. Pacientai, kurių troponino I koncentracija hospitalizavimo metu yra padidėjusi, turėtų būti vertinami kaip turintys didesnę nepalankios COVID-19 ligos eigos, dirbtinės plaučių ventiliacijos ir mirties riziką. Šiems pacientams tikslinga taikyti intensyvesnį stebėjimą ir ankstesnį gydymo eskalavimą.
3. Nors reikalingi patvirtinantys tyrimai, laisvai cirkuliuojanti DNR yra perspektyvus biožymuo, pasižymintis didesniu prognostiniu tikslumu nei įprastiniai šiuo metu naudojami laboratoriniai rodikliai. Ateityje šio biožymens integravimas į klinikinę praktiką galėtų pagerinti ankstyvą COVID-19 pacientų rizikos vertinimą, padėtų anksti nustatyti pacientus, kuriems gresia sunki ligos eiga. Taip pat tikslingi tolesni tyrimai, vertinantys laisvai cirkuliuojančios DNR pritaikomumą kitų infekcinių ligų prognozavimui.

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12. BRIEF CURRICULUM VITAE

Ieva Kubiliūtė graduated from the Faculty of Medicine of Vilnius University in 2019, obtaining the degree of Master of Medicine (*Magna Cum Laude*) and the qualification of Medical Doctor. In 2023, she completed her residency training in Infectious Diseases at Vilnius University and obtained the professional qualification of Infectious Diseases Physician. From 2021 to 2025, she pursued doctoral studies in Medicine at Vilnius University.

Ieva Kubiliūtė combines clinical practice, research, and teaching activities. Since 2023, she has been working as an Infectious Diseases Physician at Vilnius University Hospital Santaros Klinikos. She holds the positions of Junior Researcher and Junior Assistant at Vilnius University and supervises student research projects. In 2025–2026, she served as Head of the Infectious Diseases Group of the Student Scientific Activity Network at the Faculty of Medicine, Vilnius University. From 2023 to 2025, she worked as an Assistant at Riga Stradiņš University. She is a member of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), the European AIDS Clinical Society (EACS), and the Lithuanian Society for Infectious Diseases (*Lietuvos infektologų draugija* – LID).

She continuously develops her professional and scientific competencies through participation in international training courses and scientific conferences. In 2023, she attended the *Eureka International Summer Course on Translational Medicine* at the Utrecht Summer School, the Netherlands, and, in 2024, she participated in the *EACS HIV Summer School* (Research Module), Prague, Czech Republic.

Ieva Kubiliūtė is actively involved in research in the field of infectious diseases. To date, she has been involved in eight biomedical research projects, including one in which she served as the principal investigator. She has presented the results of her research in more than 15 poster presentations at international scientific conferences. Ieva Kubiliūtė is a co-author of 12 scientific publications indexed in the *Clarivate Analytics Web of Science* database, five of which she authored as the first author.

13. LIST OF PUBLICATIONS

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14. LIST OF CONFERENCE PRESENTATIONS

1. Kubiliūtė, Ieva; Kunigonytė, Monika; Lubytė, Karolina; Svetikas, Linas; Rudėnaitė, Akvilė; Zablockienė, Birutė; Gefenaitė, Giedrė; Jančorienė, Ligita. Obesity and overweight associations with COVID-19 severity among adults hospitalized in the university hospital, Lithuania // European journal of case reports in internal medicine: vol. 9, suppl. 1: 20th European Congress of Internal Medicine, 9–11 June in Malaga, Spain: abstract book. Milano : SMC media. ISSN 2284-2594. 2022, vol. 9, no. 1, p. 52. doi: 10.12890/2022_V9Sup1.

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15. APPENDICES

PLOS ONE

RESEARCH ARTICLE

Clinical characteristics and predictors for in-hospital mortality in adult COVID-19 patients: A retrospective single center cohort study in Vilnius, Lithuania

Ieva Kubiliute[1☯]*, Monika Vitkauskaite[2☯], Jurgita Urboniene[3☯], Linas Svetikas[1☯], Birute Zablockiene[1☯], Ligita Jancoriene[1☯]

1 Clinic of Infectious Diseases and Dermatovenerology, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania, **2** Faculty of Medicine, Vilnius University, Vilnius, Lithuania, **3** Center of Infectious Diseases, Vilnius University Hospital Santaros Clinics, Vilnius, Lithuania

☯ These authors contributed equally to this work.
* ieva.kubiliute@santa.lt

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Abstract

Background

The COVID-19 infection had spread worldwide causing many deaths. Mortality rates and patients' characteristics varied within and between countries, making it important to understand the peculiarities of different populations. The aim of this study was to identify the main predictors associated with in-hospital mortality due to COVID-19 in Vilnius, Lithuania.

Materials and methods

This was a retrospective observational cohort study conducted at Vilnius University Hospital Santaros Clinics, Lithuania. The study included SARS-CoV-2 positive patients aged over 18 years and hospitalized between March 2020 and May 2021. Depersonalized data were retrieved from electronic medical records. The predictive values of laboratory parameters were evaluated using ROC analysis. Multivariable binary logistic regression was performed to reveal predictors of in-hospital mortality due to COVID-19.

Results

Among 2794 patients, 54.4% were male, the age median was 59 years (IQR 48–70), 47.4% had at least one comorbidity. The most common comorbidities were arterial hypertension (36.9%) and diabetes mellitus (13.7%). Overall, 12.7% of patients died. Multivariable regression revealed that age (OR 1.04, 95%CI 1.02–1.06), congestive heart failure (OR 3.06, 95% CI 1.96–4.77), obesity (OR 3.90, 95%CI 2.12–7.16), COPD (OR 2.92, 95%CI 1.12–7.60), previous stroke (OR 5.80, 95%CI 2.07–16.21), urea >7.01 mmol/l (OR 2.32, 95%CI 1.47–3.67), AST/ALT >1.49 (OR 1.54, 95%CI 1.08–2.21), LDH >452.5 U/l (OR 2.60, 95%CI 1.74–3.88), CRP >92.68 mg/l (OR 1.58, 95%CI 1.06–2.35), IL-6 >69.55 ng/l (OR 1.62, 95%

CI 1.10–2.40), and troponin I >18.95 ng/l (OR 2.04, 95%CI 1.38–3.02), were associated with increased risk for in-hospital mortality in COVID-19 patients.

Conclusions

Age, congestive heart failure, obesity, COPD, prior stroke, and increased concentration of urea, LDH, CRP, IL-6, troponin I, ALT to AST ratio were identified to be the predictors for in-hospital mortality of COVID-19 patients.

Introduction

In December 2019, the first atypical pneumonia cases of unknown etiology were identified in Wuhan City, China [1, 2]. The virus causing this disease was subsequently confirmed as belonging to the β group of coronaviruses and was described as Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses (ICTV) [3, 4]. Due to its rapid global spread, the World Health Organization (WHO) declared the outbreak of the novel coronavirus a pandemic on 11 March 2020 [5]. Over 169 million cases of COVID-19 infection have been confirmed worldwide by 1 June 2021, including more than 3.53 million deaths [6].

SARS-CoV-2 is mainly transmitted between people through respiratory droplets and aerosols [7]. The virus primarily targets tissues of both the upper and lower airway by utilizing angiotensin-converting enzyme (ACE2) and the transmembrane protease serine 2 (TMPRSS2) which are co-expressed in most epithelial cells of the human respiratory tract. COVID-19 has a broad spectrum of clinical manifestation, ranging from asymptomatic to severe disease with multiple organ failure [7, 8]. Hospitalization rates vary between 4 and 7% in different population settings [7]. Moreover, studies have found that up to 44% of hospitalized patients were admitted to the intense care unit and at least one third of them died between March 2020 and January 2021 [9–12]. Patient characteristics and mortality rates vary substantially within and between countries and over time due to a lack of standardized testing strategies, different hospital admission criteria and unequal capacities of health care systems [13, 14]. The geriatric population, people with major comorbidities, and males were considered to be at higher risk of poor clinical outcome, longer hospital stays and higher mortality rate [15–17]. Patients with hypertension, diabetes mellitus, obesity and chronic kidney disease have been observed at the high risk for mortality in COVID-19 [13, 18]. These comorbidities are not only associated with a downregulation of immune system but also with a substantially increased expression of ACE2. The latter is known to accelerate the binding of the pathogenic virus to their target cells and enhance inflammatory response leading to a cytokine storm [18].

In Lithuania, the first case of SARS-CoV-2 infection was officially reported on 28 February 2020, and the first case of COVID-19 infection in the Vilnius region was reported on 13 March 2020 [19]. A total of 274 783 confirmed cases and nearly 4300 deaths have been reported until 1 June 2021 [20]. It is important to know clinical characteristics and factors associated with poor outcomes of COVID-19 patients in every region and country to create the effective patient monitoring and treatment strategy and provide individually and economically appropriate healthcare. To the best of the author's knowledge, this kind of data of Lithuanian population has not been published yet. Therefore, the aim of our study was to identify the main predictors associated with in-hospital mortality due to COVID-19 infection in Vilnius, Lithuania.

Materials and methods

Study design and setting

This is a retrospective observational cohort study conducted at a tertiary care university hospital Vilnius University Hospital Santaros Clinics, Vilnius, Lithuania. The objectives of our study were: to describe main clinical and initial laboratory characteristics of hospitalized COVID-19 positive patients, to obtain optimal cut-off values and predictive accuracy for in-hospital mortality due to COVID-19 infection of initial laboratory tests performed on admission and to identify the main predictors associated with in-hospital mortality due to COVID-19 infection.

Participants

Inclusion criteria were people older than 18 years who were hospitalized to any COVID-19 unit, including standard care, high dependency, or intensive care unit, in Vilnius University Hospital Santaros Clinics with confirmed COVID-19 infection between March 2020 and May 2021. The infection was confirmed by positive SARS-CoV-2 reverse transcriptase polymerase chain reaction or rapid antigen test in nasopharyngeal sample. The antigen test was used for symptomatic patients within 5 days from the onset of COVID-19 symptoms.

Data collection and variables

Depersonalized data were retrieved from electronic Vilnius University Hospital Santaros Clinics medical records and were provided by Informatics and development center of Vilnius University Hospital Santaros Clinics in accordance with hospital-approved procedures.

Demographic variables included gender and age. Data about comorbidities included: arterial hypertension (coded as I10; I11.0; I11.9 according to the International Statistical Classification of Diseases and Related Health Problems, Tenth Revision, Australian Modification (ICD-10-AM)), diabetes mellitus without complications (E10.9, E11.9), diabetes mellitus with complications (E10, E11 except for E10.9, E11.9), obesity (E66, E66.0, E66.2, E66.8, E66.9), chronic kidney disease (N18.1, N18.2, N18.3, N18.4, N18.5, N18.9), heart failure (I50.0, I50.1, I50.9), HIV infection (B23.8, B20), viral hepatitis C (B18.2), viral hepatitis B (B18.1), chronic obstructive pulmonary disease (COPD) (J44.0, J44.1, J44.8, J44.9), previous stroke (I69), previous myocardial infarction (I25.2), coronary artery disease (I20.0, I20.2, I20.8, I20.9), organ transplantation (Z94.0, Z94.1, Z94.4). If underlying condition was not registered by these ICD-10-AM codes in patient medical records during hospitalization due to COVID-19, the patient was considered as not having that condition.

Information about used medications (antibiotics, systemic steroids, antivirals), required invasive mechanical ventilation, and length of hospitalization was also obtained from depersonalized electronic medical records.

The results of initial laboratory tests that were performed upon admission to hospital, including complete blood count, glucose, creatinine, urea, sodium, potassium, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), C-reactive protein (CRP), ferritin, interleukin 6 (IL-6), D-dimer, fibrinogen, troponin I, were also extracted and evaluated.

The main outcome in this study was in-hospital mortality. Therefore, the patients were distributed into two groups according to their outcome. Patients who died during the hospitalization due to COVID-19 infection were included in the 'Lethal outcome' group. Patients who were discharged or transferred to another hospital or nursing care facility were included into 'Non-lethal outcome' group.

Statistical analysis

Continuous variables are presented as median (interquartile range (IQR)). For categorical variables, absolute and relative frequencies were calculated. Mann-Whitney U test was used to compare continuous variables, and χ^2; test, or Fisher's exact test was used to compare categorical variables. The predictive value of laboratory parameters was evaluated by measuring the area under the receiver operating characteristic (ROC) curve. The optimal cut-off value was obtained by calculating the Youden index (sensitivity + specificity–1). To explore the predictors associated with in-hospital mortality due to COVID-19, univariable and multivariable binary logistic regression models were created. Comorbidities statistically significantly associated with in-hospital mortality in univariable analysis, age, gender, and concentration of laboratory tests analytes with highest established predictive accuracy (as a categorical variable dichotomized according to the cut-off value) were included into multivariable analysis. Odds ratio (OR), 95% confidence interval (CI) were reported for logistic regression. Sensitivity analysis was conducted by calculating E-value for OR and lower limit of CI [21]. p-value <0.05 indicated statistical significance. IBM Statistical Package for the Social Sciences software version 20.0 was used for statistical analysis.

Ethical aspects

The study was conducted in accordance with the Declaration of Helsinki. In this study, only depersonalized and pseudonymized data provided by Informatics and development center of Vilnius University Hospital Santaros Clinics in accordance with hospital-approved procedures were analyzed. For this reason, no bioethics approval is required for this study. The need for participant consent was waived because only depersonalized and strictly pseudonymized data was analyzed. Vilnius University Hospital Santaros Clinics have confirmed that data processing for this biomedical research was carried out and this publication was released under the Law on the Ethics of Biomedical Research of the Republic of Lithuania (2022-12-13; No. SR-7021).

Results

Demographic, clinical, and laboratory characteristics of hospitalized COVID-19 patients

Overall, 2794 hospitalized COVID-19 positive adults were included in this study. The flow chart of patient selection is presented in Fig 1.

Among 2794 hospitalized patients, 54.4% were male. The median age was 59 (IQR 48–70) years, and 49.4% of patients were 60 years old and older (Fig 2).

Almost half of the patients (n = 1323/2794, 47.4%) had at least one underlying medical condition. The most frequent comorbidities were arterial hypertension (n = 1030/2794, 36.9%) and diabetes mellitus (n = 382/2794, 13.7%) (Table 1). The proportion of patients with underlying medical conditions increased with age reaching 75% of patients at age of 80 years and older (Fig 2). Prevalence of arterial hypertension, coronary artery disease, congestive heart failure, COPD, chronic kidney disease, stroke, previous transplant, HIV infection, viral hepatitis B and C did not differ between males and females. Obesity and diabetes mellitus were more prevalent among females than males (obesity 5.5% *vs* 3.9%, p = 0.043 and diabetes mellitus 16.1% *vs* 11.6%, p = 0.001, respectively).

A total of 1931/2794 (69.1%) patients were treated with antibiotics (Table 1). The combinations of amoxicillin/clavulanic acid and piperacillin/tazobactam were the most frequently administered antibiotics given to 81.2% (1568/1931) and 32.5% (627/1931) of patients

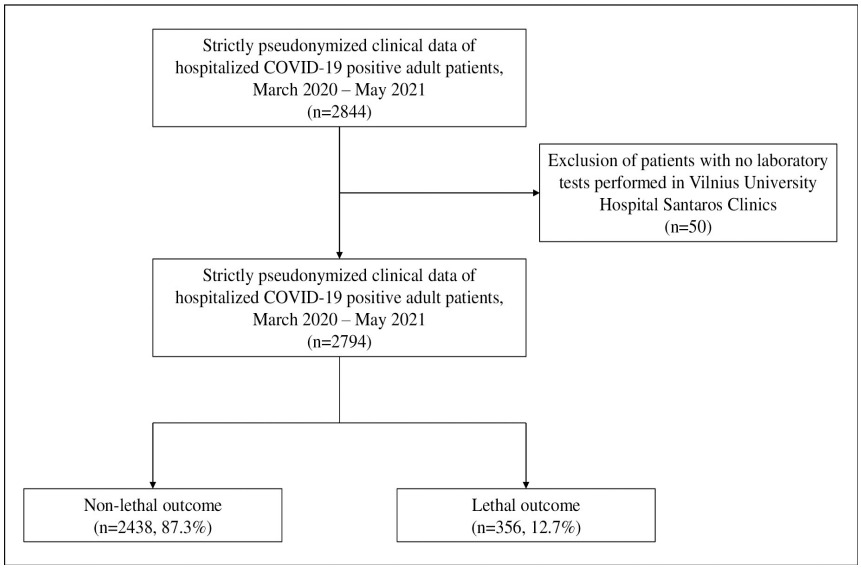

Fig 1. The flow chart of patient selection.

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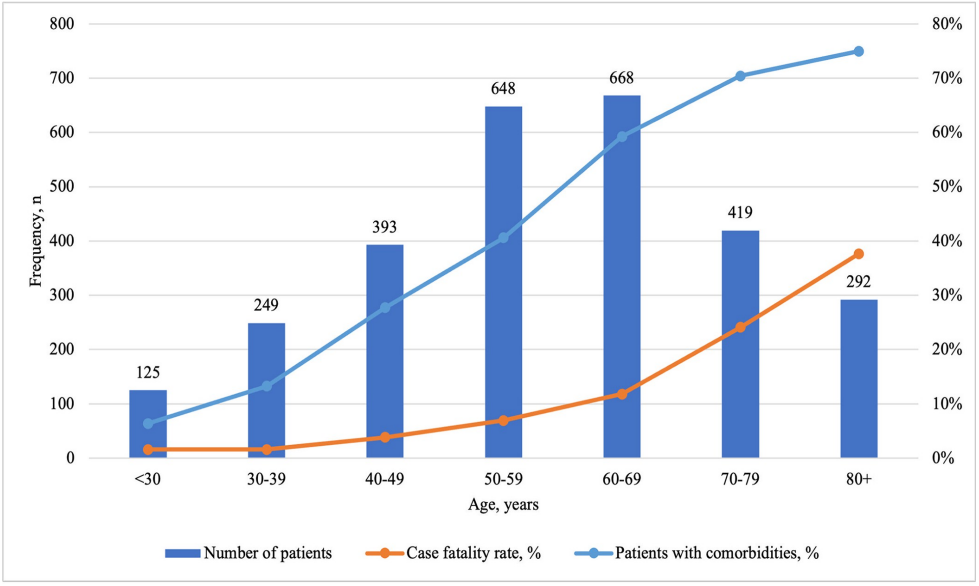

Fig 2. Distribution of patients and comorbidities, and case fatality rate of patients by age.

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Table 1. Demographic, clinical, and therapeutic characteristics of hospitalized COVID-19 patients by outcome.

Characteristic	Total (N = 2794), n (%)	Non-lethal outcome (N = 2438), n (%)	Lethal outcome (N = 356), n (%)	*p*-value
Age in years, median (IQR)	59 (48–70)	58 (47–67)	72.5 (62–81)	<0.001
Male	1520 (54.4)	1324 (54.3)	196 (55.1)	0.791
Female	1274 (45.6)	1114 (45.7)	160 (44.9)	0.791
Comorbidities				
Any underlying condition	1323 (47.4)	1045 (42.9)	278 (78.1)	<0.001
Arterial hypertension	1030 (36.9)	835 (34.2)	195 (54.8)	<0.001
Coronary artery disease	105 (3.8)	73 (3.0)	32 (9.0)	<0.001
Congestive heart failure	220 (7.9)	117 (4.8)	103 (28.9)	<0.001
Diabetes mellitus	382 (13.7)	297 (12.2)	85 (23.9)	<0.001
Diabetes mellitus with no complications	111 (4.0)	105 (4.3)	6 (1.7)	0.018
Diabetes mellitus with complications	272 (9.7)	193 (7.9)	79 (22.2)	<0.001
Obesity	129 (4.6)	88 (3.6)	41 (11.5)	<0.001
COPD	44 (1.6)	27 (1.1)	17 (4.8)	<0.001
Chronic kidney disease	215 (7.7)	153 (6.3)	62 (17.4)	<0.001
Previous stroke	39 (1.4)	19 (0.8)	20 (5.6)	<0.001
Transplant recipient	32 (1.1)	26 (1.1)	6 (1.7)	0.287
HIV infection	4 (0.1)	4 (0.2)	0 (0.0)	1.000
Viral hepatitis B	14 (0.5)	12 (0.5)	2 (0.6)	0.697
Viral hepatitis C	6 (0.2)	6 (0.2)	0 (0.0)	1.000
Complications				
Sepsis	219 (7.8)	67 (2.7)	152 (42.7)	<0.001
Acute kidney injury	296 (10.6)	112 (4.6)	184 (51.7)	<0.001
Acute myocardial infarction	47 (1.7)	40 (1.6)	7 (2.0)	0.655
Acute pulmonary embolism	63 (2.3)	45 (1.8)	18 (5.1)	<0.001
Acute stroke	39 (1.4)	30 (1.2)	9 (2.5)	0.052
Treatment				
Invasive mechanical ventilation	225 (8.1)	56 (2.3)	169 (47.5)	<0.001
Antibiotics	1931 (69.1)	1659 (68.0)	272 (76.4)	0.001
None	863 (30.9)	779 (32.0)	84 (23.6)	<0.001
One	1165 (41.7)	1060 (43.5)	105 (29.5)	
Two	471 (16.9)	400 (16.4)	71 (19.9)	
Three or more	295 (10.6)	199 (8.2)	96 (27.0)	
Antivirals (Remdesivir)	820 (29.3)	752 (30.8)	68 (19.1)	<0.001
Systemic steroids	1679 (60.1)	1452 (59.6)	227 (63.8)	0.130
Length of hospitalization in days, median (IQR)	11 (7–16)	11 (7–16)	12 (5.25–19)	0.360

COPD–chronic obstructive pulmonary disease; HIV–human immunodeficiency virus; IQR–interquartile range.

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receiving antibiotics, respectively. Median duration of treatment with amoxicillin/clavulanic acid was 7 (IQR 4–10) days, and with piperacillin/tazobactam– 8 (IQR 5–11) days. Sixty percent of patients (1679/2794, 60.1%) received systemic steroids, 96.8% (1625/1679) of them received dexamethasone. Median duration of treatment with dexamethasone was 8 (IQR 5–10) days. Almost one-third of patients (820/2794, 29.3%) were treated with remdesivir for 5 (IQR 5–5) days. Remdesivir was administered intravenously as a 200 mg dose on day 1, followed by a 100 mg dose on days 2 through 5. Out of all the patients treated with remdesivir, 78% (640/820) completed full treatment regimen. Among 2794 hospitalized patients, 8.1% (225/2794) required invasive mechanical ventilation. Sepsis was diagnosed in 7.8% (219/2794)

Table 2. Initial laboratory characteristics of hospitalized COVID-19 patients by outcome.

Variable	All patients		Non-lethal outcome		Lethal outcome		*p*-value
	n	Value, median (IQR)	n	Value, median (IQR)	n	Value, median (IQR)	
Hemoglobin, g/L	2794	137 (123–149)	2438	138 (125–149)	356	127 (111–145)	<0.001
WBC, x10^9/L	2794	6.58 (4.89–9.25)	2438	6.43 (4.83–8.77)	356	8.56 (5.67–12.00)	<0.001
Neutrophils, x10^9/L	2794	4.84 (3.33–7.30)	2438	4.67 (3.24–6.80)	356	6.96 (4.38–10.28)	<0.001
Lymphocytes, x10^9/L	2794	1.00 (0.70–1.41)	2438	1.04 (0.75–1.48)	356	0.72 (0.50–1.10)	<0.001
NLR	2791	4.69 (2.80–8.12)	2437	4.40 (2.69–7.28)	354	8.85 (4.61–17.70)	<0.001
Platelets, x10^9/L	2794	199.0 (155.0–259.0)	2438	200.5 (157.0–260.0)	356	192.0 (143.0–255.8)	0.010
Glucose, mmol/L	2589	6.18 (5.50–7.35)	2268	6.11 (5.45–7.11)	321	7.16 (6.03–9.39)	<0.001
Creatinine, μmol/L	2741	81.00 (66.00–104.11)	2386	80.00 (65.24–97.66)	355	110.00 (78.00–170.29)	<0.001
Urea, mmol/L	2509	5.77 (4.14–8.90)	2157	5.38 (3.99–7.66)	352	11.67 (7.10–20.68)	<0.001
Sodium, mmol/L	2743	140.00 (137.00–143.00)	2386	140.00 (137.00–143.00)	357	138.00 (134.95–142.00)	<0.001
Potassium, mmol/L	2743	4.2 (3.9–4.6)	2386	4.2 (3.9–4.5)	357	4.3 (3.9–4.8)	0.003
ALT, U/L	2647	31.04 (19.00–52.00)	2312	31.20 (19.85–51.96)	335	31.00 (17.00–55.00)	0.669
AST, U/L	2629	36.00 (25.21–57.00)	2293	35.00 (25.00–53.00)	336	51.81 (31.09–78.75)	<0.001
AST to ALT ratio	2629	1.18 (0.88–1.68)	2292	1.13 (0.85–1.57)	334	1.69 (1.19–2.49)	<0.001
LDH, U/L	2404	303 (235–411)	2113	294 (231–392)	291	417 (298–626)	<0.001
CRP, mg/L	2775	59.60 (19.90–123.00)	2419	53.30 (17.50–111.80)	356	118.40 (58.53–183.43)	<0.001
Ferritin, μg/L	2351	481.50 (236.26–1019.25)	2143	447.51 (224.00–954.00)	319	717.00 (366.60–1687.24)	<0.001
IL-6, ng/L	2351	29.50 (14.30–57.40)	2041	27.00 (13.50–52.25)	310	55.15 (26.18–122.50)	<0.001
D-dimer, μg/L	2436	522.50 (310.00–1045.00)	2112	480.00 (290.00–888.75)	324	1080.00 (560.00–2158.75)	<0.001
Fibrinogen, g/L	2146	5.42 (4.47–6.53)	1853	5.39 (4.51–6.46)	293	5.68 (4.12–6.93)	0.400
Troponin I, ng/L	2179	10.00 (5.18–27.00)	1897	9.00 (5.00–20.65)	282	41.00 (15.75–173.50)	<0.001

ALT–alanine aminotransferase; AST–aspartate aminotransferase; CRP–C-reactive protein; IL-6 –interleukin 6; IQR–interquartile range; LDH–lactate dehydrogenase; NLR–neutrophil-to-lymphocyte ratio; WBC–white blood cell count.

Reference values: hemoglobin: 128–160 g/L (for males), 117–145 g/L (for females); WBC: 4.0–9.8 x10^9/L; neutrophils: 1.5–6.0 x10^9/L; lymphocytes: 1.0–4.0 x10^9/L; NLR– 1–2 [22]; platelets: 140–450 x10^9/L; glucose: 4.2–6.1 mmol/L; creatinine: 64–104 μmol/L (for males), 49–90 μmol/L (for females); urea: 2.5–7.5 mmol/L; sodium: 134–145 mmol/L; potassium: 3.8–5.3 mmol/L; ALT: ≤40 U/l; AST: ≤40 U/L; AST to ALT ratio: <1; LDH: 125–243 U/L; CRP: <5 mg/L; ferritin: 25–350 μg/L (for men), 13–232 μg/L (for women); IL-6: 0–7 ng/L; D-dimer: <250 μg/L; fibrinogen: 2–4 g/L; troponin I: <19 ng/L.

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of patients, acute kidney injury occurred in 10.6% (296/2794), acute myocardial infarction–in 1.7% (47/2794), acute pulmonary embolism–in 2.3% (63/2794), and stroke affected 1.4% (39/2794) of patients during hospitalization.

The median length of hospitalization was 11 (IQR 7–16) days. Four-fifths of patients (2240/2794, 80.2%) were discharged home, 7.1% (198/2794) of patients were transferred to another hospital or nursing home, 12.7% (356/2794) of patients died. Case fatality rate increased with age from 1.6% in patients under the age of forty to 37.1% in patients at age of eighty years and older (Fig 2).

Initial laboratory results and the comparison of their medians by outcome groups are summarized in Table 2.

Clinical and laboratory characteristics associated with lethal outcome of COVID-19 patients

Compared with patients in non-lethal group, patients with lethal outcome during hospitalization were older (72.5 (62–81) years *vs* 58 (47–67) years, p<0.001), and more often had comorbidities such as arterial hypertension (54.8% *vs* 34.2%, p<0.001), coronary artery disease (9.0% *vs* 3.0%, p<0.001), congestive heart failure (28.9% *vs* 4.8%, p<0.001), diabetes mellitus (23.9%

vs 12.2%, p<0.001), obesity (11.5% *vs* 3.6%, p<0.001), COPD (4.8% *vs* 1.1%, p<0.001), chronic kidney disease (17.4% *vs* 6.3%, p<0.001), and prior stroke (5.6% *vs* 0.8%, p<0.001) (Table 1).

On admission, patients with lethal outcome had higher levels of white blood cell count (WBC), neutrophil count, neutrophil-to-lymphocyte ratio (NLR), glucose, creatinine, urea, potassium, AST to ALT ratio, LDH, CRP, ferritin, IL-6, D-dimer, and troponin I compared to those in non-lethal group. Whereas, haemoglobin concentration, lymphocytes count, platelets, and sodium concentration were significantly lower in non-survivors. Only the medians of ALT and fibrinogen concentrations did not differ between these groups. These differences are detailed and presented in Table 2.

The area under the ROC curve (AUC) were calculated to evaluate predictive accuracy of laboratory tests for lethal outcome. These results are presented in Table 3 and Fig 3. Urea possessed the highest predictive accuracy with AUC 0.80 (95% confidence interval (CI) 0.77–0.82), NLR, creatinine, AST to ALT ratio, LDH, D-dimer, and troponin I demonstrated good predictive accuracy with AUC ≥0.70, CRP, and IL-6 showed fair predictive accuracy with AUC 0.68 (95% CI 0.65–0.71) and 0.69 (95% CI 0.66–0.72), respectively. Optimal cut-off values were calculated and are shown in Table 3.

Predictors for COVID-19 in-hospital mortality

Univariable analysis revealed that age, arterial hypertension, coronary artery disease, congestive heart failure, diabetes mellitus, obesity, COPD, chronic kidney disease, and previous stroke were associated with in-hospital mortality in patients with COVID-19 (Table 4).

Table 3. Predictive accuracy for in-hospital mortality of initial laboratory tests performed upon admission.

Variable	AUC (95% CI)	*p*-value	Cut-off value	Reference value
Hemoglobin, g/L	0.40 (0.36–0.43)	<0.001	172.5	128–160 (for males) 117–145 (for females)
WBC, $x10^9$/L	0.63 (0.60–0.67)	<0.001	8.09	4.0–9.8
Neutrophils, $x10^9$/L	0.66 (0.62–0.69)	<0.001	6.16	1.5–6.0
Lymphocytes, $x10^9$/L	0.34 (0.31–0.37)	<0.001	3.07	1.0–4.0
NLR	0.71 (0.68–0.74)	<0.001	5.71	1–2 [22]
Platelets, $x10^9$/L	0.46 (0.42–0.49)	0.010	332.5	140–450
Glucose, mmol/L	0.66 (0.62–0.69)	<0.001	7.07	4.2–6.1
Creatinine, μmol/L	0.70 (0.66–0.73)	<0.001	98.97	64–104 (for males) 49–90 (for females)
Urea, mmol/L	0.80 (0.77–0.82)	<0.001	7.01	2.5–7.5
Sodium, mmol/L	0.40 (0.36–0.43)	<0.001	150.7	134–145
Potassium, mmol/L	0.55 (0.51–0.58)	0.003	4.68	3.8–5.3
ALT, U/L	0.49 (0.46–0.53)	0.669	102.5	≤40
AST, U/L	0.65 (0.62–0.68)	<0.001	55.92	≤40
AST to ALT ratio	0.70 (0.67–0.73)	<0.001	1.49	<1
LDH, U/L	0.70 (0.67–0.74)	<0.001	452.50	125–243
CRP, mg/L	0.68 (0.65–0.71)	<0.001	92.86	<5
Ferritin, μg/L	0.63 (0.59–0.66)	<0.001	483.34	25–350 (for men) 13–232 (for women)
IL-6, ng/L	0.69 (0.66–0.72)	<0.001	69.55	0–7
D-dimer, μg/L	0.72 (0.69–0.75)	<0.001	687.5	<250
Fibrinogen, g/L	0.52 (0.48–0.56)	0.400	7.51	2–4
Troponin I, ng/L	0.77 (0.74–0.80)	<0.001	18.95	<19

ALT–alanine aminotransferase; AST–aspartate aminotransferase; AUC—area under the ROC curve; CI–confidence interval; CRP–C-reactive protein; IL-6 –interleukin 6; LDH–lactate dehydrogenase; NLR–neutrophil-to-lymphocyte ratio; WBC–white blood cell count.

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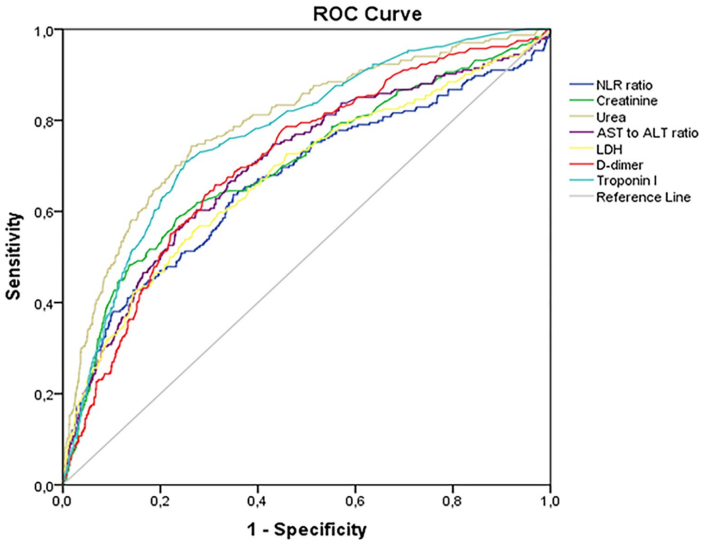

Fig 3. Predictive accuracy for in-hospital mortality of initial laboratory tests performed upon admission, ROC curve. ALT–alanine aminotransferase; AST–aspartate aminotransferase; LDH–lactate dehydrogenase; NLR–neutrophil-to-lymphocyte ratio.

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Multivariable analysis confirmed that age (OR 1.04, 95% CI 1.02–1.06), congestive heart failure (OR 3.06, 95% CI 1.96–4.77), obesity (OR 3.90, 95% CI 2.12–7.16), COPD (OR 2.92, 95% CI 1.12–7.60), previous stroke (OR 5.80, 95% CI 2.07–16.21) and the following laboratory parameters: urea >7.01 mmol/l (OR 2.32, 95% CI 1.47–3.67), AST to ALT ratio >1.49 (OR 1.54, 95% CI 1.08–2.21), LDH >452.5 U/l (OR 2.60, 95% CI 1.74–3.88), CRP > 92.68 mg/l (OR 1.58, 95% CI 1.06–2.35), IL-6 > 69.55 ng/l (OR 1.62, 95% CI 1.10–2.40), and troponin I >18.95 ng/l (OR 2.04, 95% CI 1.38–3.02) are independent predictors for in-hospital mortality of patients with COVID-19 (Table 4, Fig 4). The E-value was calculated and attested the robustness of predictors and in-hospital mortality association regarding bias caused by potential unmeasured confounders (Table 4).

Univariable and multivariable analyses were repeated in subgroups stratified by sex and by age (Fig 5). In multivariable analysis age, obesity, urea >7.01 mmol/L, and LDH >452.5 U/L were associated with higher risk for in-hospital mortality in patients of both genders, while congestive heart failure (OR 4.83, CI 95% 2.68–8.71) and COPD (OR 4.29, CI 95% 1.44–12.78) were identified as predictors only in male COVID-19 patients, and previous stroke (OR 10.46, CI 95% 2.26–48.32), IL-6 > 69.55 ng/L (OR 2.31, CI 95% 1.23–4.30) and troponin I >18.95 ng/L (OR 2.98, CI 95% 1.64–5.42)–only in female patients.

Multivariable analysis of subgroups by age revealed that congestive heart failure, obesity, urea >7.01 mmol/L, and troponin I >18.95 ng/L were associated with in-hospital mortality of COVID-19 patients in both groups–patients aged 60 years or younger and patients over 60 years old. Coronary artery disease (OR 15.98, 95% CI 1.56–163.93), IL-6 > 69.55 ng/L (OR 4.81, 95% CI 2.06–11.27) were identified as predictors for in-hospital mortality in COVID-19 patients aged 60 years and below, while age (OR 1.05, 95% CI 1.03–1.08), COPD (OR 2.69, 95% CI 1.02–7.11), previous stroke (OR 5.59, 95% CI 2.03–15.35), AST to ALT ratio >1.49

Table 4. Predictors associated with in-hospital mortality of hospitalized patients with COVID-19.

	Univariable regression		Multivariable regression		
Characteristic	**OR (95% CI)**	***p*-value**	**OR (95% CI)**	***p*-value**	**E-value for OR; for CI lower limit**
Age in years	1.07 (1.06–1.08)	<0.001	1.04 (1.02–1.06)	<0.001	1.24; 1.16
Male	1.03 (0.82–1.29)	0.791	0.84 (0.58–1.20)	0.329	-
Comorbidities					
Arterial hypertension	2.33 (1.86–2.91)	<0.001	0.79 (0.55–1.15)	0.224	-
Coronary artery disease	3.20 (2.08–4.93)	<0.001	1.19 (0.62–2.26)	0.601	-
Congestive heart failure	8.08 (6.01–10.85)	<0.001	3.06 (1.96–4.77)	<0.001	5.57; 3.33
Diabetes mellitus	2.26 (1.72–2.97)	<0.001	0.92 (0.60–1.43)	0.717	-
Obesity	3.48 (2.36–5.13)	<0.001	3.90 (2.12–7.16)	<0.001	7.26; 3.66
COPD	4.48 (2.42–8.30)	<0.001	2.92 (1.12–7.60)	0.029	5.29; 1.49
Chronic kidney disease	3.15 (2.29–4.33)	<0.001	1.00 (0.59–1.68)	0.997	-
Previous stroke	7.58 (4.00–14.35)	<0.001	5.80 (2.07–16.21)	<0.001	11.08; 3.56
Transplant recipient	1.59 (0.65–3.89)	0.309	-	-	-
Viral hepatitis B	1.15 (0.26–5.18)	0.851	-	-	-
Laboratory analytes					
NLR > 5.71	4.10 (3.22–5.22)	<0.001	1.09 (0.75–1.60)	0.647	-
Creatinine > 98.97, μmol/L	4.58 (3.64–5.78)	<0.001	1.23 (0.79–1.90)	0.361	-
Urea > 7.01, mmol/L	7.72 (5.94–10.03)	<0.001	2.32 (1.47–3.67)	<0.001	4.07; 2.30
AST to ALT ratio > 1.49	3.92 (3.09–4.97)	<0.001	1.54 (1.08–2.21)	0.018	2.54; 1.37
LDH > 452.5, U/L	4.82 (3.72–6.25)	<0.001	2.60 (1.74–3.88)	<0.001	4.64; 2.87
CRP > 92.68, mg/L	3.60 (2.86–4.53)	<0.001	1.58 (1.06–2.35)	0.024	2.54; 1.31
IL-6 > 69.55, ng/L	4.42 (3.43–5.70)	<0.001	1.62 (1.10–2.40)	0.015	2.62; 1.43
D-dimer > 687.5, μg/L	4.67 (3.62–6.02)	<0.001	1.14 (0.78–1.66)	0.492	-
Troponin I > 18.95, ng/L	7.11 (5.38–9.41)	<0.001	2.04 (1.38–3.02)	<0.001	3.5; 2.1

ALT–alanine aminotransferase; AST–aspartate aminotransferase; CI–confidence interval; COPD–chronic obstructive pulmonary disease; CRP–C-reactive protein; IL-6 –interleukin 6; LDH–lactate dehydrogenase; NLR–neutrophil-to-lymphocyte ratio; OR–odds ratio.

Reference values: NLR– 1–2 [22]; creatinine: 64–104 μmol/L (for males), 49–90 μmol/L (for females); urea: 2.5–7.5 mmol/L; AST to ALT ratio: <1; LDH: 125–243 U/L; CRP: <5 mg/L; IL-6: 0–7 ng/L; D-dimer: <250 μg/L; troponin I: <19 ng/L.

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(OR 1.73, 95% CI 1.15–2.58), LDH >452.5 U/L (OR 2.81, 95% CI 1.75–4.49), and CRP > 92.68 mg/L (OR 1.70, 95% CI 1.08–2.70) were also associated with in-hospital mortality in patients older than 60 years old.

Discussion

In this retrospective study, we report the patient characteristics and predictors associated with in-hospital mortality among hospitalized COVID-19 patients in one of the main tertiary care hospitals in the country. This is one of the few studies on this topic conducted in Eastern Europe and particularly in the Baltic region. This study contributes to the knowledge of the natural course of SARS-CoV-2 infection and how gender, age, underlying conditions, and laboratory characteristics are associated with the outcomes of the disease. We identified age, congestive heart failure, obesity, COPD, prior stroke, and increased concentration of urea (> 7.01 mmol/L), LDH (> 452.5 U/L), CRP (> 92.68 mg/L), IL-6 (> 69.55, ng/L), troponin I (> 18.95 ng/L), ALT to AST ratio (> 1.49) to be predictors for in-hospital mortality of COVID-19 patients. These predictors slightly differed between patient groups by sex and age.

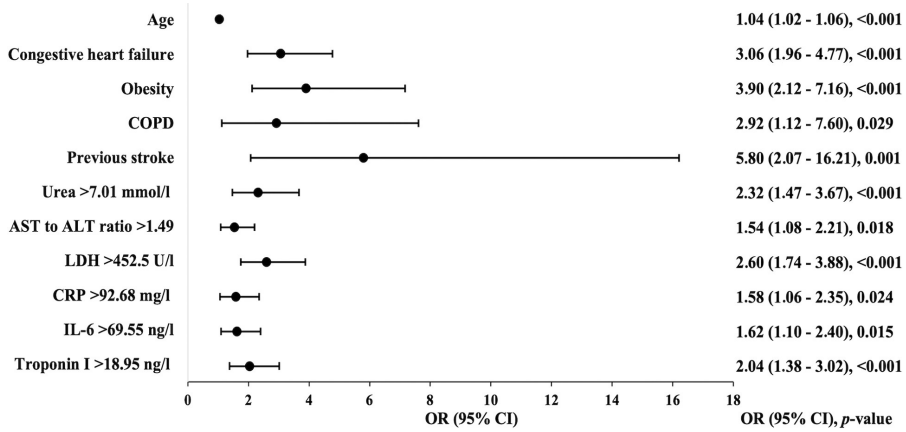

Fig 4. Significant predictors associated with in-hospital mortality in patients with COVID-19, multivariable logistic regression model. ALT–alanine aminotransferase; AST–aspartate aminotransferase; CI–confidence interval; COPD–chronic obstructive pulmonary disease; CRP–C-reactive protein; IL-6 –interleukin 6; LDH–lactate dehydrogenase; OR–odds ratio.

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We found the in-hospital mortality to be 12.7%, which was close to Fakih et al. research (13%) conducted in the US [23] and lower than published by Gujski et al. in Poland (18.4%) [24]. Other studies done in the Netherlands, United Kingdom, Germany reported in-hospital mortality to be 24.6%, 26%, 17.9%, respectively [13, 25, 26]. It also should be noted that in-hospital mortality differed during separate COVID-19 waves and in different populations [27–29].

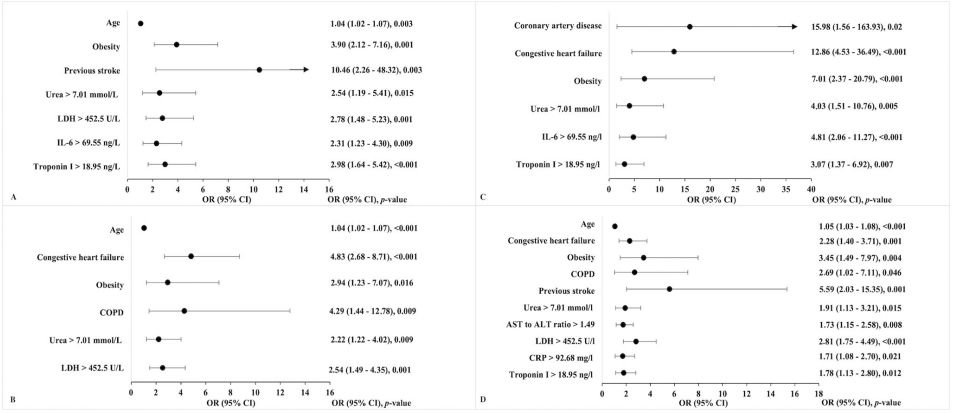

Fig 5. Significant predictors associated with in-hospital mortality in patients with COVID-19, stratified by age and sex, multivariable logistic regression models. (A) Predictors associated with in-hospital mortality in female COVID-19 patients. (B) Predictors associated with in-hospital mortality in male COVID-19 patients. (C) Predictors associated with in-hospital mortality in COVID-19 patients aged 60 years and younger. (D) Predictors associated with in-hospital mortality in COVID-19 patients aged over 60 years. ALT–alanine aminotransferase; AST–aspartate aminotransferase; CI–confidence interval; COPD–chronic obstructive pulmonary disease; CRP–C-reactive protein; IL-6 –interleukin 6; LDH–lactate dehydrogenase; OR–odds ratio.

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Gray et al. revealed that in-hospital mortality rates were lower in the second wave compared to the first for all socioeconomic and demographic groups [27]. In addition, in-hospital mortality was 15.1%, 12.6%, 12.8% during the first, the second and the third waves of COVID-19 in Spain, and 9.5%, 10.2%, 5.4% in Switzerland, respectively [28, 29]. On the contrary, Gujski et al. noted a higher fatality rate during the second COVID-19 wave in Poland (8.3% *vs* 21%) [24], which could be linked to an increased number of hospitalized patients during the second wave. Unfortunately, we could not distinguish the separate COVID-19 waves to compare the mortality rates and patient characteristics, since we did not identify different SARS-CoV-2 strains and we can only hypothesize that our study period included B.1.1.280, B.1.177.60, alpha (B.1.1.7), and B.1.620 variants.

Lower mortality could be a possible result of introduction of interventions such as remdesivir, dexamethasone, high-use of thromboprophylaxis, as well as ventilation management, and the start of COVID-19 vaccination program [30, 31]. Despite declining mortality rates, advanced age, male sex, and pre-existing comorbidity remained a key mortality risk factors among COVID-19 patients [27–29].

Consistent with other studies [25, 32–35], we found that advanced age is an independent predictor for in-hospital mortality associated with 4% increase in odds ratio per each year. This tendency could be explained by the fact that older people have more comorbidities which themselves are risk factors for in-hospital mortality of COVID-19 patients [18, 36]. This also could be due to immunosenescence, characterized by impaired age-dependent defects in T-cell and B-cell function, which weaken immune responses to most viruses, including SARS-CoV-2 [37]. Although, numerous studies have reported that males are at higher risk of severe COVID-19 infection, as well as of death, especially over 65 years [33, 38, 39], we found that 55.1% of fatal cases were male but males did not appear to have a significantly higher in-hospital mortality risk.

Docherty et al. established that up to 77.5% of hospitalized COVID-19 patients had at least one comorbidity [13]. Additionally, Thakur et al. disclosed that arterial hypertension, obesity, and diabetes mellitus were the most prevalent comorbidities [40], accounting for up to 51.3%, 35.0%, and 49.8% of cases, respectively [23, 41]. In our study, 47.4% of patients had at least one underlying condition with the most prevalent being arterial hypertension (36.9%) and diabetes mellitus a second (13.7%). Different from other researches [23, 32, 42], the prevalence of obesity in our study was considerably lower (4.6%). This difference could be related to the fact that we analyzed depersonalized database and obesity might be not encoded in patients' medical records during this hospitalization. Patients with comorbidities are not only more vulnerable to COVID-19 infection, but underlying conditions act as triggers for increased risk of fatality [18, 36]. We observed that comorbidity was significantly associated with fatal outcome. Moreover, multivariable analysis identified congestive heart failure, obesity, COPD, and previous stroke as predictors for in-hospital mortality. While analysing the results stratified by sex, we discovered that cardiovascular and chronic lung diseases were associated with higher in-hospital mortality in men. These results are in contrast to the findings of Fernández-Martínez et al. [43] and could be linked to gender-related lifestyle differences.

We found that the presence of congestive heart failure 3.06 times increased the risk of in-hospital mortality in general population and in both groups stratified by age. Increased mortality in congestive heart failure patients is associated with dysregulation of intracellular calcium handling system, therefore COVID-19 infection causes hypoxia induced excessive intracellular calcium that culminate in apoptosis of cardiac myocyte [44]. A global meta-analysis by Popkin et al. demonstrated that obesity increased COVID-19 mortality by 1.48 times [45], whereas we found that in-hospital mortality was 3.90-fold higher in obese patients. Obesity also remained significant in subgroup analysis by sex and by age. Not only does obesity lead to an increased

expression of inflammatory molecules, but also reduces thoracic wall compliance and functional residual capacity, promoting the development of acute respiratory distress syndrome related lung damage in patients with COVID-19 [46–48]. Our study showed that COPD increased in-hospital mortality in COVID-19 patients by 2.92-fold and in male patients population–by 4.29-fold. Pranata et al. also found that COVID-19 patients with COPD had a 4.36 times higher rate of death [49]. This is due to an increased expression of ACE2 in COPD epithelial cells, reduced antiviral responses (especially interferons), and the potential for secondary bacterial infection [50, 51]. We also found that previous stroke increased in-hospital mortality by 5.80 times, however the results must be taken with caution given the wide confidence interval. The relationship between death and previous stroke might be due to lasting disabilities in this group [52]. Tehrani et al. suggested that patients with a history of stroke carry a predisposition that, under the impact of COVID-19 induced coagulopathy, may lead to vascular events causing fatal outcome [52].

In this study, there were significant differences in the initial laboratory parameters between patients with lethal and non-lethal outcomes, indicating higher levels of inflammation, cell-turnover and metabolic dysregulation in the first group [53]. Many studies have investigated the role of various biomarkers in determining prognosis for patients with COVID-19 and evidenced that routine laboratory parameters have important clinical application value in predicting the course of COVID-19 [54–56]. Since the beginning of the pandemic, CPR, ferritin, LDH and D-dimer have been widely used for risk stratification, however there is marked diversity in cut-off values [39, 57, 58].

Our study results demonstrated that NLR, urea, creatinine, AST to ALT ratio, LDH, D-dimer, CRP, IL-6 and troponin I demonstrated good predictive accuracy for in-hospital mortality. Multivariable analysis confirmed elevated urea, AST to ALT ratio, LDH, CRP, IL-6, and troponin I together with age and presence of comorbidities described above to be predictors for in-hospital mortality.

The results of this study indicated that increased concentration of urea (above 7.01 mmol/L) was significantly associated with in-hospital mortality increasing these odds 2.32 times and it also remained a significant predictor in further subgroup analysis by sex and age. A study conducted in China on 12,413 patients had evidenced association of elevated blood urea nitrogen (BUN) and elevated serum creatinine with all-cause in-hospital mortality risk, respectively BUN adjusted hazard ratio (aHR) was 6.27, and serum creatinine aHR was 2.65 [59]. Moreover, Liu YM et al. reported elevated BUN level to show a more significant association with adverse outcomes than serum creatine suggesting that the elevation in BUN level not only indicates a kidney dysfunction, but it can also reflect inflammatory status, catabolism, and renal hypoperfusion from hypovolemia, sepsis, or reduced cardiac output while serum creatinine mainly represents a status of kidney injury and metabolic disturbance [59].

Furthermore, our research demonstrated that AST to ALT ratio has better predictive accuracy for in-hospital mortality than AST or ALT separately, and patients with AST to ALT ratio >1.49 had 54% higher odds for lethal outcome. Consistent results were reported in other studies [60–62].

This study revealed elevated LDH, CRP and IL-6 to be also associated with an increased risk of in-hospital mortality. Patients with LDH >452.5 U/L on admission had 2.6 times higher odds for in-hospital mortality. In a pooled analysis of nine published studies conducted by Henry et al. which included 1532 patients with COVID-19, elevated LDH levels were associated with 6-fold increased odds of developing severe disease and 16-fold increased odds of mortality that is much higher compared to our results [63]. Our findings indicated that CRP >92.68 mg/L and IL-6 >69.55 ng/L were the optimal cut-off values discriminating patients with lethal and non-lethal outcome. Both CRP and IL-6 remained predictors for in-hospital

mortality in multivariable analysis that increased this risk for 58% and 62%, respectively. In multiple studies exploring routine laboratory parameters in COVID-19 patients, increased CRP levels and IL-6 were also reported in severe patients when compared to non-severe patients [64–67].

Moreover, we identified that patients with troponin I concentration above 18.95 ng/L had 2.04 times higher risk for in-hospital lethal outcome. Other studies also found out increased level of troponin to be associated with this outcome [68, 69]. The metanalysis of 12,262 COVID-19 patients indicated that increased troponin concentration was detected in 31% of patients and they had 4.75 times higher odds for mortality [70].

The combination of patient medical history and routine laboratory tests are simple, and cost-effective indicators that can be used to identify COVID-19 patients at a greater risk of death. The presence of these predictors might assist in choosing of medical care and treatment for COVID-19 patients individually.

Bias/limitations

Data for this study was retrieved from strictly pseudonymized and depersonalized electronic medical records based on encoded medical information. For this reason, there is a probability that some information about patients could be not mentioned or missed in their medical records or were encoded in any other code that was not extracted from electronic database. Furthermore, we could not extract the data about addictions, vaccination status, COVID-19 pandemic wave etc. and include these variables into analysis. It indicates the need of further prospective research of COVID-19 positive patients' cohorts to get more accurate and extensive results.

As in this study we included all hospitalized COVID-19 positive adults despite their comorbidities, some laboratory test results (e.g., urea, troponin I, AST to ALT ratio) could be increased not only because of the COVID-19 infection itself, but also due to comorbidities. Therefore, the identified predictors of in-hospital mortality could be determined not only by factors caused by COVID-19 disease, but the consequences of the general condition. In the context of COVID-19 disease, laboratory markers (e.g., urea, creatinine, AST, ALT, CRB, LDH, troponin I) serve as indicators of disease severity and prognosis, while it should be recognized that these markers can be influenced by other underlying conditions, especially in hospitalized patients with COVID-19.

Conclusions

The predictors for in-hospital mortality of COVID-19 patients were identified to be age, congestive heart failure, obesity, COPD, prior stroke, and increased concentration of urea, LDH, CRP, IL-6, troponin I, ALT to AST ratio. This study evidenced that the history of comorbidities and routine laboratory tests can be beneficial identifying COVID-19 patients at increased risk of death and choosing the best medical care strategy.

Supporting information

S1 Data.
(XLS)

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Author Contributions

Conceptualization: Ieva Kubiliute, Jurgita Urboniene, Ligita Jancoriene.

Data curation: Jurgita Urboniene.

Formal analysis: Ieva Kubiliute, Jurgita Urboniene.

Investigation: Ieva Kubiliute, Jurgita Urboniene.

Methodology: Ieva Kubiliute, Jurgita Urboniene.

Supervision: Ligita Jancoriene.

Visualization: Ieva Kubiliute, Jurgita Urboniene.

Writing – original draft: Ieva Kubiliute, Monika Vitkauskaite, Jurgita Urboniene.

Writing – review & editing: Jurgita Urboniene, Linas Svetikas, Birute Zablockiene, Ligita Jancoriene.

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Hyperglycaemia and Its Prognostic Value in Patients with COVID-19 Admitted to the Hospital in Lithuania

Lina Zabuliene [1], Ieva Kubiliute [2], Mykolas Urbonas [3], Ligita Jancoriene [2], Jurgita Urboniene [4] and Ioannis Ilias [5,*]

[1] Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, 03101 Vilnius, Lithuania; lina.zabuliene@mf.vu.lt
[2] Clinic of Infectious Diseases and Dermatovenerology, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, 08661 Vilnius, Lithuania; ieva.kubiliute@santa.lt (I.K.); ligita.jancoriene@mf.vu.lt (L.J.)
[3] Faculty of Medicine, Vilnius University, 03101 Vilnius, Lithuania
[4] Center of Infectious Diseases, Vilnius University Hospital Santaros Klinikos, 08661 Vilnius, Lithuania; jurgita.urboniene@santa.lt
[5] Department of Endocrinology, Diabetes and Metabolism, Elena Venizelou Hospital, 11521 Athens, Greece
* Correspondence: iiliasmd@yahoo.com

Abstract: Background and objectives: Increased blood glucose levels atadmission are frequently observed in COVID-19 patients, even in those without pre-existing diabetes. Hyperglycaemia is associated with an increased incidence of severe COVID-19 infection. The aim of this study was to evaluate the association between hyperglycaemia at admission with the need for invasive mechanical ventilation (IMV) and in-hospital mortality in patients without diabetes who were hospitalized for COVID-19 infection. Materials and methods: This retrospective observational study was conducted at Vilnius University Hospital Santaros Clinics, Lithuania with adult patients who tested positive for severe acute respiratory syndrome coronavirus 2 SARS-CoV-2 and were hospitalized between March 2020 and May 2021. Depersonalized data were retrieved from electronic medical records. Based on blood glucose levels on the day of admission, patients without diabetes were divided into 4 groups: patients with hypoglycaemia (blood glucose below 4.0 mmol/L), patients with normoglycaemia (blood glucose between ≥4.0 mmol/L and <6.1 mmol/L), patients with mild hyperglycaemia (blood glucose between ≥6.1 mmol/L and <7.8 mmol/L), and patients with intermittent hyperglycaemia (blood glucose levels ≥7.8 mmol/L and <11.1 mmol/L). A multivariable binary logistic regression model was created to determine the association between hyperglycaemia and the need for IMV. Survival analysis was performed to assess the effect of hyperglycaemia on outcome within 30 days of hospitalization. Results: Among 1945 patients without diabetes at admission, 1078 (55.4%) had normal glucose levels, 651 (33.5%) had mild hyperglycaemia, 196 (10.1%) had intermittent hyperglycaemia, and 20 (1.0%) had hypoglycaemia. The oddsratio (OR) for IMV in patients with intermittent hyperglycaemia was 4.82 (95% CI 2.70–8.61, $p < 0.001$), and the OR was 2.00 (95% CI 1.21–3.31, $p = 0.007$) in those with mild hyperglycaemia compared to patients presenting normal glucose levels. The hazardratio (HR) for 30-day in-hospital mortality in patients with mild hyperglycaemia was 1.62 (95% CI 1.10–2.39, $p = 0.015$), while the HR was 3.04 (95% CI 2.01–4.60, $p < 0.001$) in patients with intermittent hyperglycaemia compared to those with normoglycaemia at admission. Conclusions: In COVID-19 patients without pre-existing diabetes, the presence of hyperglycaemia at admission is indicative of COVID-19-induced alterations in glucose metabolism and stress hyperglycaemia. Hyperglycaemia at admission in COVID-19 patients without diabetes is associated with an increased risk of invasive mechanical ventilation and in-hospital mortality. This finding highlights the importance for clinicians to carefully consider and select optimal support and treatment strategies for these patients. Further studies on the long-term consequences of hyperglycaemia in this specific population are warranted.

Keywords: coronavirus; SARS-CoV-2; COVID-19; glucose on admission; hyperglycaemia; in-hospital mortality

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1. Introduction

Since the beginning of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, diabetes mellitus has emerged as one of the most common comorbidities predisposing to coronavirus disease 2019 (COVID-19) and an important risk factor for hospitalization and mortality [1–3]. People with diabetes face a compromised immune system, making them more susceptible to severe outcomes of COVID-19. Diabetes is known for causing a pro-inflammatory syndrome, so patients with diabetes are more vulnerable to increased production of cytokines, which consequently lead to the deterioration of COVID-19 due to the development of acute respiratory distress syndrome and shock. Moreover, the hyperactivation of the coagulation cascade in COVID-19, particularly in the presence of pre-existing pro-thrombotic hypercoagulability associated with diabetes, intensifies the risk of severe thromboembolic complications [3]. Increased glucose levels at admission are frequently observed in COVID-19 patients, even in those without a history of diabetes.

Stress hyperglycaemia is a common condition in patients with any acute illness when the interaction of various cytokines and stress hormones leads to a state of insulin resistance and increased glucose production [4]. Inflammatory cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumour necrosis factor-α (TNF-α), suppress insulin release and induce insulin resistance, and elevated levels of IL-6 promote hyperglycaemia by releasing glucose from hepatic glycogen reserves. Cortisol, catecholamines, glucagon, and growth hormone decrease insulin release by amplifying the activity of pancreatic alpha cells. Catecholamines further limit insulin binding, and, together with growth hormone, prevent insulin activation by suppressing tyrosine kinase activity. Moreover, catecholamines and glucocorticoids restrict glucose uptake in peripheral skeletal muscles through modulation of the glucose transporter type 4 [4].

Recent studies reported that stress hyperglycaemia increases the risk of mortality in critically ill patients [5] and patients with myocardial infarction [6] and stroke [7]. A single-centre study involving 1000 patients admitted to the intensive care unit found that in patients without diabetes, the risk of death increased by 20% for each 1 mmol/l increase in acute glycaemia [5]. Another study with 660 patients experiencing ST elevation myocardial infarction and treated with primary percutaneous coronary intervention showed significantly higher rates of mortality, cardiogenic shock, contrast-induced nephropathy, and the no-reflow phenomenon in the stress hyperglycaemia patient group [6]. A large study involving 8622 patients revealed that stress hyperglycaemia independently predicted severe neurological deficit within 1 year in patients with acute ischemic stroke, regardless of diabetes status. This association with mortality at 1 year is more pronounced in people without diagnosed or underlying diabetes [7]. In New Zealand, a study involving 739,152 ICU patients without a pre-existing diabetes diagnosis demonstrated a clear dose-response relationship between hyperglycaemia and hospital mortality, as well as an extended duration of hospital stay [8].

The appearance of hyperglycaemia in COVID-19 patients without diabetes likely indicates increased systemic stress as well. Furthermore, recent evidence from experimental studies suggests that SARS-CoV-2 infects human pancreatic β-cells and leads to morphological, transcriptional, and functional changes [9].

Hyperglycaemia is associated with a higher incidence of a severe course of COVID-19 infection, including the need for oxygen therapy [10,11], invasive mechanical ventilation (IMV) [10,11], and admission to the intensive care unit (ICU) [10–12] and has been found to be a predictor of in-hospital mortality in COVID-19 patients [12–15].

While extensive research has been conducted on the impact of hyperglycaemia in patients with diabetes and COVID-19, there remains a noticeable gap in our understanding of hyperglycaemia's role in individuals without pre-existing diabetes. The aim of this study was to evaluate the association between hyperglycaemia at admission with the need for IMV and in-hospital mortality in patients without diabetes who were hospitalized for

COVID-19 infection. By addressing this research gap, our study aims to contribute valuable insights that can inform clinical strategies and interventions tailored to patients without pre-existing diabetes, ultimately enhancing our ability to optimize care and improve outcomes in this specific population.

2. Materials and Methods

A single centre, retrospective observational cohort study was conducted at Vilnius University Hospital Santaros Klinikos (VUH SK), Vilnius, Lithuania [16].

2.1. Participants

Adult patients (18 years of age and older) who were hospitalized at VUH SK between March 2020 and May 2021 for confirmed COVID-19 infection and treated in the standard care unit, high dependency unit, or intensive care unit (ICU) were included in the study. The infection was verified based on a positive SARS-CoV-2 reverse transcriptase polymerase chain reaction result or a rapid antigen test conducted on a nasopharyngeal sample for symptomatic patients within 5 days from the onset of COVID-19 symptoms [16]. A total of 2561 hospitalized patients with COVID-19, who had their blood glucose tested during their hospital stay, were included in the analysis. Out of the 2561 patients hospitalized with COVID-19, 2054 had no pre-existing diabetes or a blood glucose concentration meeting the criteria for newly diagnosed diabetes during the hospital stay (Figure 1).

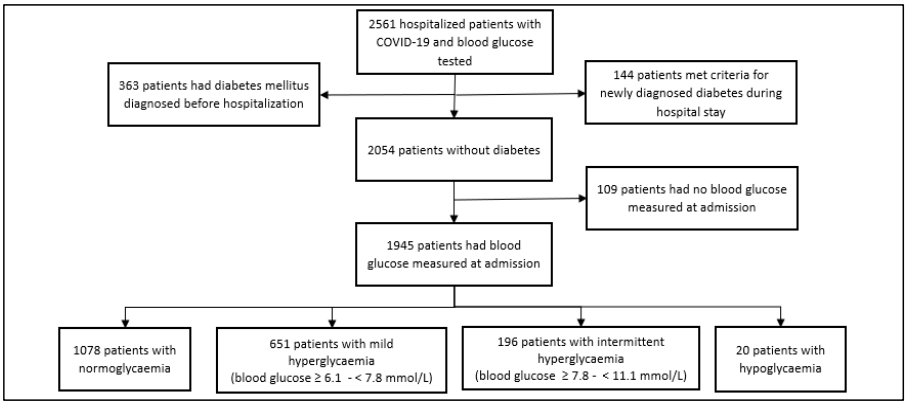

Figure 1. Distribution of patients hospitalized with COVID-19 infection.

2.2. Data Collection and Variables

Depersonalized data were retrieved from the electronic medical records (EMR) of VUH SK and provided by the informatics and development centre in accordance with hospital-approved procedures [16].

Demographic variables included gender and age. Data on comorbidities included arterial hypertension (AH), coronary artery disease (CAD), congestive heart failure (CHF), diabetes mellitus, obesity, chronic obstructive pulmonary disease (COPD), chronic kidney disease (CKD), and previous stroke. Comorbidity data were extracted using the relevant codes from the International Statistical Classification of Diseases and Related Health Problems, Tenth Revision, Australian Modification (ICD-10-AM). AH was defined by the presence of comorbidities with ICD-10-AM codes I10, I11.0, and I11.9; CAD by the presence of comorbidities with ICD-10-AM codes I20.0, I20.2, I20.8, and I20.9; CHF by the presence of comorbidities with ICD-10-AM codes I50.0, I50.1, and I50.9; diabetes by the presence of comorbidities with ICD-10-AM codes E10 and E11; obesity by the presence of comorbidities with ICD-10-AM codes E66, E66.0, E66.2, E66.8, and E66.9; COPD by the

presence of comorbidities with ICD-10-AM codes J44.0, J44.1, J44.8, and J44.9; CKD by the presence of comorbidities with ICD-10-AM codes N18.1, N18.2, N18.3, N18.4, N18.5, and N18.9; and previous stroke by the presence of comorbidities with ICD-10-AM code I69.If the condition was not documented in the patient's EMR during hospitalization for COVID-19 infection, the patient was classified as not having that condition. Information regarding the utilization of antibiotics, systemic steroids, antivirals, IMV, length of hospital stay, and in-hospital mortality data was also extracted from depersonalized EMR.

Data from initial laboratory tests, including complete blood count, creatinine, urea, sodium, estimated glomerular filtration rate (eGFR), potassium, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), C-reactive protein (CRP), ferritin, interleukin 6 (IL-6), D-dimer, and troponin I, were also retrieved and evaluated.The neutrophil-to-lymphocyte ratio (NLR) and AST to ALT ratio was calculated.

Data from blood glucose measurements conducted during hospitalization were obtained. Patients who had a blood glucose level of 11.1 mmol/L or higher during hospitalization were classified as having newly diagnosed diabetes mellitus. Based on the blood glucose level on the day of admission (if multiple blood glucose tests were performed within the first 24 h after admission, the mean glucose value was considered), patients without diabetes were divided into the following 4 groups: patients with hypoglycaemia were defined as those with blood glucose levels at admission below 4.0 mmol/L, patients with normoglycaemia had blood glucose levels at admission between $\geq$4.0 mmol/L and <6.1 mmol/L, patients with mild hyperglycaemia had blood glucose levels at admission was between $\geq$6.1 mmol/L and <7.8 mmol/L, and patients with intermittent hyperglycaemia had blood glucose levels atadmission between $\geq$7.8 mmol/L and <11.1 mmol/L.

Data from 2561 COVID-19 patients were analysed.

2.3. Main Outcome

The main outcome of the study was to evaluate the relationship between glycemia on admission in patients without diabetes and the severity of COVID-19 outcomes, including the need for mechanical ventilation and 30-day in-hospital mortality.

2.4. Statistical Analysis

Continuous and categorical variables were presented as medians (interquartile range [IQR]) and numbers (percentages, %), respectively. The Mann–Whitney U test was used to compare continuous variables, and the χ^2 test was used to compare categorical variables. A multivariable binary logistic regression model was created to determine association of glucose levels and the need for IMV. The model included the need for IMV as the dependent variable, and age, gender, comorbidities which proportions statistically significantly differed between groups as predictors. We conducted survival analysis to assess the effects of glucose level on outcome within 30 days of hospitalization. We created a Cox proportional hazards regression model with in-hospital mortality as the dependent variable and age, gender, comorbidities with proportions that statistically significantly differed between groups, systemic steroids use, and treatment with remdesivir as predictors and plotted curves for survival stratified by glucose level. A two-sided *p*-value less than 0.05 was considered statistically significant. Analysis was performed using IBM Statistical Package for the Social Sciences software version 20.0 (IBM SPSS Statistics for Windows, Version 20.0, 2018, Armonk, NY, USA: IBM Corp.), and Microsoft Excel (Microsoft Corporation, 2018, Microsoft Excel, Available at: https://office.microsoft.com/excel, accessed on 18 December 2023) was used to produce figures.

3. Results

Among 2561 hospitalized adults, 55.1% were men. The median age was 60 years (IQR 49–70). Patients' demographic, clinical characteristics, and initial laboratory parameters are presented in Table 1.

Table 1. Demographic, clinical, and initial laboratory characteristics of patients hospitalized with COVID-19 infection.

Demographic and Clinical Characteristic (2561 Patients)	N (%)	Laboratory Characteristics	N	Median (IQR)
Age, years, median (IQR)	60 (49–70)	Haemoglobin, g/L	2561	138 (124–149)
Male	1412 (55.1)	WBC, $\times 10^9$/L	2561	6.48 (4.84–9.03)
Female	1149 (44.9)	Neutrophils, $\times 10^9$/L	2561	4.76 (3.30–7.13)
Any concommitant condition	1252 (48.9)	Lymphocytes, $\times 10^9$/L	2561	1 (0.70–1.40)
Arterial hypertension	983 (38.4)	NLR	2558	4.70 (2.86–8.05)
Coronary artery disease	90 (3.5)	Platelets, $\times 10^9$/L	2561	198 (153–258)
Congestive heart failure	198 (7.7)	Glucose, mmol/L	2561	6.12 (5.43–7.28)
Diabetes mellitus	363 (14.2)	Creatinine, µmol/L	2557	82 (67–105)
Obesity	123 (4.8)	Urea, mmol/L	2371	5.73 (4.12–8.74)
COPD	42 (1.6)	eGFR, mL/min/1.73 m^2	2513	83 (58.95–96)
Chronic kidney disease	205 (8.0)	Sodium, mmol/L	2551	140 (137–143)
Previous stroke	32 (1.2)	Potassium, mmol/L	2551	4.20 (3.90–4.60)
Invasive mechanical ventilation	192 (7.5)	ALT, U/L	2500	31.42 (19.65–52)
Antibiotics use	1863 (72.7)	AST, U/L	2485	36 (26–57)
Antivirals (remdesivir)	808 (31.6)	AST to ALT ratio	2483	1.17 (0.87–1.67)
Systemic steroids	1630 (63.6)	LDH, U/L	2302	303 (236–408.04)
In-hospital mortality	313 (12.2)	CRP, mg/L	2558	62.15 (22.78–124.88)
Length of hospital stay, days, median (IQR)	11 (7–16)	Ferritin, µg/L	2367	479.85 (236–1009.44)
		IL-6, ng/L	2254	29.60 (14.40–57.30)
		D-dimer, µg/L	2344	510 (305–985)
		Troponin I, ng/L	2122	10 (5–26)

ALT—alanine aminotransferase; AST—aspartate aminotransferase; COPD—chronic obstructive pulmonary disease, CRP—C-reactive protein; eGFR—estimated glomerular filtration rate; IL-6—interleukin 6; IQR—interquartile range; LDH—lactate dehydrogenase; N—number; NLR—neutrophil-to-lymphocyte ratio; WBC—white blood cell count.

Reference values: Haemoglobin: 128–160 g/L (for males), 117–145 g/L (for females); WBC: 4.0–9.8 $\times$ 10^9/L; neutrophils: 1.5–6.0 $\times$ 10^9/L; lymphocytes: 1.0–4.0 $\times$ 10^9/L; NLR—1–2; platelets: 140–450 $\times$ 10^9/L; glucose: 4.2–6.1 mmol/L; creatinine: 64–104 µmol/L (for males), 49–90 µmol/L (for females); urea: 2.5–7.5 mmol/L; sodium: 134–145 mmol/L; potassium: 3.8–5.3 mmol/L; ALT: $\leq$40 U/L; AST: $\leq$40 U/L; AST to ALT ratio: <1; LDH: 125–243 U/L; CRP: <5 mg/L; ferritin: 25–350 µg/L (for men), 13–232 µg/L (for women); IL-6: 0–7 ng/L; D-dimer: <250 µg/L; and troponin I: <19 ng/L.

Out of a total of 2561 patients, 363 (14.2%) had a pre-existing diagnosis of diabetes before hospitalization, and 144 (5.6%) met the diagnostic criteria for newly diagnosed diabetes during their hospital stay.

Glucose levels at admission were measured for 1945 patients without diabetes. Out of a total of 1945 patients without diabetes, 1078 (55.4%) had a normal glucose level at admission, 651 (33.5%) patients had mild hyperglycaemia at admission, and 196 (10.1%) patients had intermittent hyperglycaemia at admission. Additionally, 20 (1.0%) patients without diabetes had hypoglycaemia upon admission (Figure 1).

Patients with mild hyperglycaemia and intermittent hyperglycaemia were older compared to patients with normoglycaemia (Table 2).

Table 2. Demographic and clinical characteristics of COVID-19 patients without diabetes based on glycaemia levels on admission.

Demographic and Clinical Characteristic	Normoglycaemia N = 1078	Mild Hyperglycaemia, N = 651	Intermittent Hyperglycaemia, N = 196	*p*-Value [1]	*p*-Value [2]	*p*-Value [3]
Age in years, median (IQR)	55 (43–66)	60 (50–68)	67 (59–76)	<0.001	<0.001	<0.001
Male	604 (56.0%)	379 (58.2%)	108 (55.1%)	0.373	0.810	0.439
Female	474 (44.0%)	272 (41.8%)	88 (44.9%)	0.373	0.810	0.439
Any underlying condition	373 (34.6%)	307 (47.2%)	119 (60.7%)	<0.001	<0.001	0.001
Arterial hypertension	299 (27.7%)	246 (37.8%)	96 (49.0%)	<0.001	<0.001	0.005
Coronary artery disease	23 (2.1%)	19 (2.9%)	10 (5.1%)	0.304	0.016	0.141
Congestive heart failure	48 (4.5%)	38 (5.8%)	21 (10.7%)	0.200	<0.001	0.019
Obesity	24 (2.2%)	22 (3.4%)	12 (6.1%)	0.149	0.002	0.086
COPD	12 (1.1%)	10 (1.5%)	2 (1.0%)	0.447	1.000	0.743
Chronic kidney disease	58 (5.4%)	30 (4.6%)	18 (9.2%)	0.479	0.039	0.015
Previous stroke	8 (0.7%)	5 (0.8%)	5 (2.6%)	1.000	0.037	0.057
Invasive mechanical ventilation	29 (2.7%)	38 (5.8%)	29 (14.8%)	0.001	<0.001	<0.001
Antibiotics	763 (70.8%)	486 (74.7%)	141 (71.9%)	0.081	0.742	0.447
Antivirals (remdesivir)	326 (30.2%)	242 (37.2%)	64 (32.7%)	0.003	0.500	0.248
Systemic steroids	634 (58.8%)	465 (71.4%)	134 (68.4%)	<0.001	0.012	0.409
In-hospital mortality	57 (5.3%)	61 (9.4%)	44 (22.4%)	0.001	<0.001	<0.001
Length of hospital stay, days, median (IQR)	10 (7–14)	11 (8–16)	11.50 (7–16)	0.001	0.059	0.996

1—*p* value comparing patients with normoglycaemia vs. patients with mild hyperglycaemia. 2—*p* value comparing patients with normoglycaemia vs. patients with intermittent hyperglycaemia. 3—*p* value comparing patients with mild hyperglycaemia vs. patients with intermittent hyperglycaemia.

The prevalence of AH, CAD, CHF, obesity, CKD, and previous stroke was higher in patients with intermittent hyperglycaemia compared to patients with normoglycaemia. The prevalence of AH, CHF, and CKD was higher in patients with intermittent hyperglycaemia compared to patients with mild hyperglycaemia.

The higher proportion of patients with intermittent hyperglycaemia required IMV compared to patients with mild hyperglycaemia (14.8% vs. 5.8%, $p < 0.001$) and normoglycaemia (14.8% vs. 2.7%, $p < 0.001$).

A total of 1233 (64.1%) patients without diabetes received systemic steroids, with 1201 of them treated with dexamethasone for a course lasting 9 (IQR 6–10) days. A higher proportion of patients with mild hyperglycaemia (74.4% vs. 58.8%, $p < 0.001$) and intermittent hyperglycaemia (68.4% vs. 58.8%, $p = 0.012$) received systemic steroids compared to patients with normal glucose levels at admission.

A total of 632 (32.8%) patients without diabetes received remdesivir for a course lasting 5 (IQR 5–5) days. A higher proportion of patients with mild hyperglycaemia (37.2% vs. 30.2%, $p = 0.003$) received systemic steroids compared to patients with normal glucose levels at admission. However, there was no statistically significant difference between the mild and intermittent hyperglycaemia groups regarding the percentage of patients treated with remdesivir.

The highest in-hospital mortality rate was noted in patients with intermittent hyperglycaemia at admission reaching 22.4% compared to those with mild hyperglycaemia (9.4%) and normoglycaemia (5.3%) (Table 2).

Patients with intermittent hyperglycaemia on admission had higher levels of white blood cell (WBC) counts, neutrophil counts, NLR, platelet counts, creatinine, urea, ALT, AST, LDH, CRP, ferritin, IL-6, D-dimer, and troponin I compared to patients with normoglycaemia, whereas lymphocyte count, eGFR, potassium, and sodium concentration were significantly lower (Table 3).

Table 3. Initial laboratory characteristics of COVID-19 patients without diabetes based on glycaemia levels upon admission.

Laboratory Characteristics	Normoglycaemia		Mild Hyperglycaemia		Intermittent Hyperglycaemia		*p*-Value [1]	*p*-Value [2]	*p*-Value [3]
	n	Value, Median (IQR)	n	Value, Median (IQR)	n	Value, Median (IQR)			
Haemoglobin, g/L	1078	139 (125–150)	651	140 (127–151)	196	141 (124–151)	0.120	0.693	0.621
WBC, $\times 10^9$/L	1078	5.87 (4.43–7.62)	651	6.54 (5.05–8.91)	196	8.22 (6.07–11.68)	<0.001	<0.001	<0.001
Neutrophils, $\times 10^9$/L	1078	4.10 (2.90–5.80)	651	5 (3.60–7.16)	196	6.60 (4.50–9.90)	<0.001	<0.001	<0.001
Lymphocytes, $\times 10^9$/L	1078	1.07 (0.80–1.46)	651	0.93 (0.66–1.26)	196	0.90 (0.60–1.33)	<0.001	<0.001	0.732
NLR	1076	3.73 (2.45–5.91)	650	5.20 (3.30–8.46)	196	7.47 (4.50–12.54)	<0.001	<0.001	<0.001
Platelets, $\times 10^9$/L	1078	194 (151–254)	651	195 (153–253)	196	213.50 (168.25–277.75)	0.832	0.001	0.003
Glucose at admission, mmol/L	1078	5.46 (5.09–5.77)	651	6.60 (6.33–7.06)	196	8.70 (8.16–9.57)	<0.001	<0.001	<0.001
Creatinine, µmol/L	1075	78.2 (65–96)	651	81.01 (68–98.93)	196	87 (70.25–130.93)	0.015	<0.001	0.001
Urea, mmol/L	970	4.98 (3.71–6.68)	608	5.60 (4.10–7.82)	182	7.13 (4.88–14.15)	<0.001	<0.001	<0.001
eGFR	1052	88.95 (70–99.50)	638	83.60 (63.28–95.45)	194	68.05 (41–88.15)	<0.001	<0.001	<0.001
Sodium, mmol/L	1073	141 (138–143)	650	140 (137–143)	196	139 (136–142)	0.007	<0.001	0.012
Potassium, mmol/L	1073	4.20 (3.90–4.53)	650	4.10 (3.80–4.44)	196	4.10 (3.77–4.50)	<0.001	0.013	0.882
ALT, U/L	1059	30.11 (19–49)	633	35 (22–55.66)	190	38.50 (21–57)	<0.001	0.002	0.780
AST, U/L	1050	33.12 (24–50)	629	39 (29–60.15)	188	41 (28–76.75)	<0.001	<0.001	0.531
AST to ALT ratio	1050	1.13 (0.86–1.58)	628	1.13 (0.88–1.58)	188	1.25 (0.85–1.73)	0.806	0.123	0.178
LDH, U/L	993	282 (220–359)	592	318 (263–420.81)	165	362 (253.50–534)	<0.001	<0.001	0.024
CRP, mg/L	1077	48.65 (17.1–94.9)	650	68.21 (31.34–129.73)	196	100.05 (42.53–181.85)	<0.001	<0.001	0.001
Ferritin, µg/L	1007	409.33 (204.63–802.70)	612	568.50 (296.25–1223.25)	170	659.98 (294.24–1402.31)	<0.001	<0.001	0.288
IL-6, ng/L	958	26.15 (13.57–48.5)	581	32.40 (15.80–61.24)	161	36.90 (14.5–77.1)	<0.001	0.003	0.454
D-dimer, µg/L	990	420 (260– 726.25)	614	522.50 (320–926.25)	179	780 (365–1745)	<0.001	<0.001	<0.001
Troponin I, ng/L	899	7.97 (4–15)	561	10 (6–22)	161	20.20 (8.25–116)	<0.001	<0.001	<0.001

ALT—alanine aminotransferase; AST—aspartate aminotransferase; CRP—C-reactive protein; IL-6—interleukin 6; IQR—interquartile range; LDH—lactate dehydrogenase; NLR—neutrophil-to-lymphocyte ratio; WBC—white blood cell count. 1—*p* value comparing patients with normoglycaemia vs. patients with mild hyperglycaemia. 2—*p* value comparing patients with normoglycaemia vs. patients with intermittent hyperglycaemia. 3—*p* value comparing patients with mild hyperglycaemia vs. patients with intermittent hyperglycaemia.

Reference values: haemoglobin: 128–160 g/L (for males), 117–145 g/L (for females); WBC: 4.0–9.8 $\times$ 10^9/L; neutrophils: 1.5–6.0 $\times$ 10^9/L; lymphocytes: 1.0–4.0 $\times$ 10^9/L; NLR—1–2; platelets: 140–450 $\times$ 10^9/L; glucose: 4.2–6.1 mmol/L; creatinine: 64–104 µmol/L (for males), 49–90 µmol/L (for females); urea: 2.5–7.5 mmol/L; sodium: 134–145 mmol/L; potassium: 3.8–5.3 mmol/L; ALT: ≤40 U/L; AST: ≤40 U/L; AST to ALT ratio: <1; LDH: 125–243 ULl; CRP: <5 mg/L; ferritin: 25–350 µg/L (for men), 13–232 µg/L (for women); IL-6: 0–7 ng/L; D-dimer: <250 µg/L; and troponin I: <19 ng/L.

Patients with mild hyperglycaemia had higher levels of WBCs, neutrophil counts, NLR, creatinine, urea, ALT, AST, LDH, CRP, ferritin, IL-6, D-dimer, and troponin I compared to patients with normoglycaemia, whereas lymphocyte counts and eGFR, potassium, and sodium concentrations were significantly lower (Table 3).

Among patients with intermittent hyperglycaemia on admission, the odds ratio (OR) for IMV was 4.82 (95% CI 2.70–8.61, $p < 0.001$), while the OR was 2.00 (95% CI 1.21–3.31, $p = 0.007$) in those with mild hyperglycaemia compared to patients presenting normal glucose levels at admission. Other risk factors associated with the need for IMV were male gender, obesity, and CHF (Figure 2).

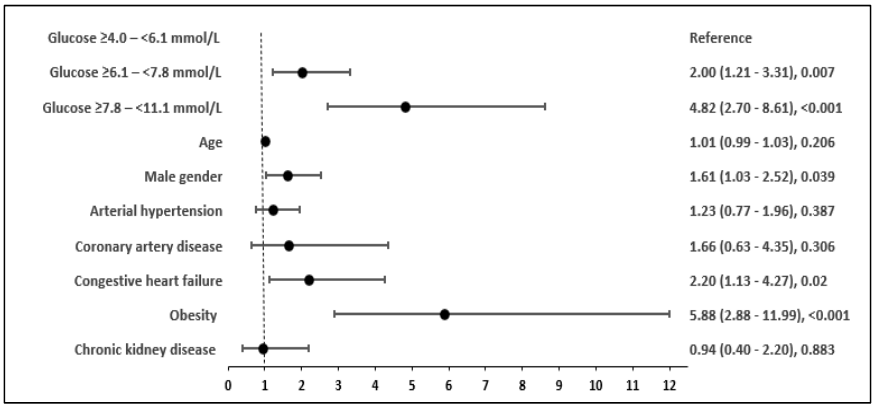

Figure 2. Risk factors and odds ratios (OR) associated with the need for invasive mechanical ventilation in COVID-19 patients without diabetes.

Cox regression analysis revealed that the hazard ratio (HR) for 30-day in-hospital mortality in patients with mild hyperglycaemia on admission was 1.62 (95% CI 1.10–2.39, $p = 0.015$), whilethe HR was 3.04 (95% CI 2.01–4.60, $p < 0.001$) in patients with intermittent hyperglycaemia compared to those with normoglycaemia (Figure 3).

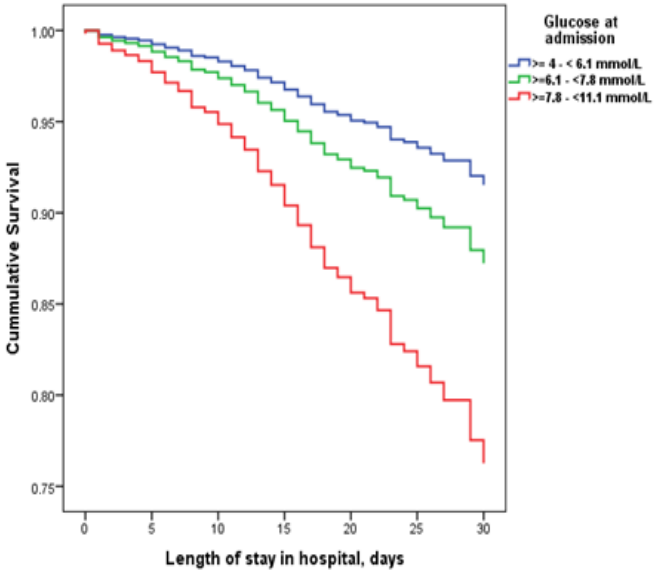

Figure 3. Survival of hospitalized patients without diabetes stratified by glycaemia levels atadmission.

Other risk factors associated with increased HR for 30 days in-hospital mortality were age, obesity, CHF, and previous stroke. Treatment with Remdesivir was associated with reduced HR (0.56 (95% CI 0.37–0.85, $p = 0.006$) for 30-day in-hospital mortality) (Table 4).

Table 4. Hazard ratios for 30-day in-hospital mortality.

Characteristic	In-Hospital Mortality	
	HR (95% CI)	*p*
Normoglycaemia	Reference	
Mild hyperglycaemia	1.62 (1.10–2.39)	0.015
Intermittent hyperglycaemia	3.04 (2.01–4.60)	<0.001
Age in years	1.06 (1.05–1.08)	<0.001
Male gender	1.07 (0.77–1.49)	0.689
Hypertension	0.94 (0.67–1.33)	0.729
Coronary artery disease	1.23 (0.69–2.18)	0.487
Congestive heart failure	1.97 (1.33–2.93)	0.001
Obesity	2.94 (1.56–5.57)	0.001
Previous stroke	3.86 (1.95–7.65)	<0.001
Chronic kidney disease	0.61 (0.36–1.04)	0.069
Antivirals (Remdesivir)	0.56 (0.37–0.85)	0.006
Systemic steroids	1.36 (0.94–1.99)	0.108

4. Discussion

Infection induces significant alterations in glucose metabolism. During infection, there is an upsurge in glucose production attributed to hepatic gluconeogenesis and glycogenolysis accompanied by reduced peripheral glucose uptake due to decreased blood flow in muscles (reversible insulin resistance) and increased anaerobic glycolysis due to hypoxia, resulting in elevated blood glucose levels, regardless of pre-existing diabetes status. Such a response is believed to be influenced by a combination of neurohumoral changes, activation of counterregulatory hormones (glucagon, epinephrine, cortisol, and growth hormone), excessive release of proinflammatory cytokines (IL-6, IL-1β, and tumour necrosis factor (TNF)-α), reactive oxygen species production, impaired immune cell function, and the release of lipid mediators [17–20].

Various viral infections, including cytomegalovirus, enteroviruses, Epstein-Barr virus, hepatitis B and C virus, human immunodeficiency virus, and influenza, use distinct mechanisms to promote hyperglycaemia and can directly affect glucose metabolism and insulin signaling pathways, leading to dysregulation of glucose homeostasis [21]. Recent findings have shed light on the interplay among viral factors, immune response, inflammation, pancreatic function, and the potential role of insulin resistance in driving the occurrence of hyperglycaemia during COVID-19 infection [21–25].

Our study demonstrated that 43.6% of patients without pre-existing diabetes hospitalized for COVID-19 infection had increased blood glucose levels atadmission. Data on the prevalence of hyperglycaemia in COVID-19 patients without diabetes varies and depends on the definition of hyperglycaemia and the population studied. Our study data are comparable to data from Montefusco et al., who reported that 46% of COVID-19 patients had hyperglycaemia, defined as blood glucose levels between 5.6 mmol/L and 11.1 mmol/L or two blood glucose measurements of >5.6 mmol/Land < 7.0 mmol/L [26]. Mamtani et al. reported that 20.6% of COVID-19 patients without pre-existing diabetes had blood glucose levels at admission ≥ 7.78 mmol/L [8]. Zhang et al. found that 20% of COVID-19 patients were in a state of impaired fasting glucose (defined as fasting blood glucose between ≥5.6 mmol/L and <7.0 mmol/L) [15].

The prognostic importance of hyperglycaemia on the outcomes of COVID-19 infection has been emphasized in multiple studies. Hyperglycaemia atadmission in patients without diabetes is associated with a higher incidence of a severe course of COVID-19 infection, need for IMV, admission to the ICU [10–12,24–30], and higher mortality [13,14,27,29,31,32].

Our study showed that a higher proportion of patients with intermittent hyperglycaemia at admission (14.8%) required IMV compared to patients with mild hyperglycaemia (5.8%) and patients with normoglycaemia (2.7%). The risk of IMV in patients with intermittent hyperglycaemia was 4.82-fold higher than in those with normoglycaemia. Iacobellis et al. noted that average blood glucose level at admission was the strongest independent predictor of bilateral pulmonary opacities suggestive of acute respiratory distress in the radiographic imaging of COVID-19 patients [27]. Fadine et al. reported that for every 2 mmol/L increase in glucose level at admission, the probability of severe COVID-19 increased by approximately15% independently from any other clinical-biochemical variables [28]. We determined that the HR for 30-day in-hospital mortality in patients with mild hyperglycaemia was 1.62, while the HR was 3.04in patients with intermittent hyperglycaemia compared to those with normoglycaemia. A meta-analysis including sixteen observational studies with 6386 COVID-19 patients demonstrated that the group of patients with hyperglycaemia at admission had an increased risk of mortality compared to the control group with euglycaemia (OR = 3.45, 95% CI 2.26–5.26) [30]. Morse et al. retrospectively showed that among patients without a pre-existing diabetes diagnosis, any hyperglycaemic value was associated with a substantial increase in the odds of mortality (OR = 3.07, 95% CI 2.79–3.37) compared to patients without hyperglycaemia [33].

The association between hyperglycaemia and increased mortality in COVID-19 patients is likely multifactorial, involving inflammation, immune dysfunction, vascular damage, and underlying comorbidities. Hyperglycaemia is known to contribute to a state of systemic inflammation, which can worsen the severity of infection and outcome in COVID-19 patients [34]. High blood glucose levels have been shown to impair the function of various immune cells, such as T cells and macrophages, thereby compromising the immune response against the virus [35]. Hyperglycaemia can contribute to endothelial dysfunction and arteriopathy, leading to complications such as thrombosis and impaired oxygen delivery, which can further exacerbate the severity of COVID-19 and increase the mortality risk [36]. Furthermore, individuals with newly developed hyperglycaemia during COVID-19 infection may be older and have underlying comorbidities, which can increase vulnerability and in-hospital mortality [34,37–39]. In our study, patients with mild or intermittent hyperglycaemia were older and had more comorbid conditions compared to patients with normoglycaemia. Furthermore, we observed elevated troponin I levels at admission in patients with intermittent hyperglycaemia.

The cytokine storm is a characteristic feature of COVID-19 infection and is strongly associated with disease severity and mortality [40]. The laboratory features of cytokine storms include haematological anomalies, such as leucocytosis or leukopenia, thrombocytopenia, and disseminated intravascular coagulation; high fibrinogen levels; elevated IL-6; and general markers of end-organ dysfunction [41]. We observed a significantly lower lymphocyte count, higher NLR, and higher IL-6 and CRP levels in patients with mild and intermittent hyperglycaemia, presuming that the inflammatory response is more pronounced in patients with increased glucose levels at admission. Our results are comparable to the findings of Coppelli et al., as we also observed a higher neutrophil count, lower lymphocyte count, and higher CRP in patients without known diabetes with hyperglycaemia (glucose level $\geq$ 7.78 mmol/L) at admission compared to patients with normoglycaemia [29].

Beyond the immediate impact on glucose metabolism, investigating the long-term consequences of COVID-19 infection and potential implications for metabolic health emerges as a crucial direction of exploration. Montefusco et al. highlighted the profound impact of COVID-19 on disrupting insulin signalling and beta cell function. They reported significant alterations in hormone profiles, both at basal levels and after stimulation testing, demonstrating elevated insulin, proinsulin, and C-peptide levels in patients who had recovered from COVID-19 compared to healthy controls [26]. In a recent meta-analysis that scrutinized the occurrence of new-onset diabetes and hyperglycaemia after COVID-19 infection in patients without pre-existing diabetes, the proportions of patients with new-onset diabetes was 3% and new-onset hyperglycaemia was 30%. Additionally, the meta-analysis

found that new-onset diabetes and hyperglycaemia were 1.75 times higher in COVID-19 patients compared to non-COVID-19 patients [42]. Recommendations have been made for COVID-19 hyperglycaemic patients, emphasizing the need for follow-up at the first month and at intervals of 3–6 months during the first year post-discharge to discern whether hyperglycaemia is permanent or transient [43].

This study has several limitations. Comorbidities were identified based on ICD-10-AM coding, which may have resulted in some incomplete attributions. Additionally, information about fasting status on admission and glycated haemoglobin (HbA1c) was not available for all patients, potentially biasing the detection of pre-existing hyperglycaemia. Furthermore, the absence of data on body mass index limited our ability to accurately determine obesity in patients. Lastly, we were unable to control for the pre-admission use of glucocorticoids and other medications, which could have influenced our findings. An additional challenge relates to missing information on the duration of COVID-19 symptoms before hospital admission. While our research was conducted at the largest hospital in the capital of Lithuania, limiting the study to a single centre introduces the potential for selection bias and reduces the generalizability of our findings. The healthcare practices, patient demographics, and treatment protocols at this specific centre may not fully represent the broader population. An additional limitation of our retrospective study is the lack of information on the long-term consequences of hyperglycaemia in COVID-19 patients. As a retrospective analysis primarily focused on immediate outcomes during hospitalization, we did not have the opportunity to explore the extended effects of hyperglycaemia beyond the acute phase of the disease. Nonetheless, our study has several strengths, including the comprehensive clinical characterization of all patients who were hospitalized for COVID-19 infection over a period of more than one year in the largest university hospital in the country, as well as the evaluation of multiple laboratory parameters.

5. Conclusions

In COVID-19 patients without pre-existing diabetes, the presence of hyperglycaemia at admission is indicative of COVID-19-induced alterations in glucose metabolism and stress hyperglycaemia. Hyperglycaemia at admission in COVID-19 patients without diabetes is associated with an increased risk of invasive mechanical ventilation and in-hospital mortality. This finding highlights the importance for clinicians to carefully consider and select optimal support and treatment strategies for these patients. Further studies on the long-term consequences of hyperglycaemia in this specific population are warranted.

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Elevated Cardiac Troponin I as a Mortality Predictor in Hospitalised COVID-19 Patients

Ieva Kubiliute [1,*], Jurgita Urboniene [2], Fausta Majauskaite [1], Edgar Bobkov [3], Linas Svetikas [1] and Ligita Jancoriene [1]

[1] Clinic of Infectious Diseases and Dermatovenerology, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, 03101 Vilnius, Lithuania; fausta.majauskaite@santa.lt (F.M.); linas.svetikas@santa.lt (L.S.); ligita.jancoriene@santa.lt (L.J.)
[2] Centre of Infectious Diseases, Vilnius University Hospital Santaros Klinikos, 08661 Vilnius, Lithuania; jurgita.urboniene@santa.lt
[3] Faculty of Medicine, Vilnius University, 03101 Vilnius, Lithuania; edgar.bobkov@santa.lt
* Correspondence: ieva.kubiliute@santa.lt

Abstract: *Background and Objectives:* SARS-CoV-2 affects multiple organ systems, including the cardiovascular system, leading to immediate and long-term cardiovascular complications. Acute myocardial injury is one of the earliest and most common cardiac issues in the acute phase of COVID-19. This study aimed to evaluate the prognostic value of cardiac troponin I (cTnI) levels in predicting in-hospital mortality among hospitalised COVID-19 patients. *Materials and Methods:* A retrospective observational cohort study included 2019 adult patients hospitalised with a confirmed COVID-19 infection stratified by cTnI levels on admission into three groups: <19 ng/L (1416 patients), 19–100 ng/L (431 patients), and >100 ng/L (172 patients). Myocardial injury was defined as blood serum cTnI levels increased above the 99th percentile upper reference limit. Depersonalised datasets were extracted from digital health records. Statistical analysis included multivariable binary logistic and Cox proportional hazards regressions. *Results:* Overall, 29.87% of patients experienced acute myocardial injury, which development was associated with age, male sex, chronic heart failure, arterial hypertension, obesity, and chronic kidney disease. Among patients with cTnI levels of 19–100 ng/L, the odds ratio for requiring invasive mechanical ventilation was 3.18 (95% CI 2.11–4.79) and, for those with cTnI > 100 ng/L, 5.38 (95% CI 3.26–8.88). The hazard ratio for in-hospital mortality for patients with cTnI levels of 19–100 ng/L was 2.58 (95% CI 1.83–3.62) and, for those with cTnI > 100 ng/L, 2.97 (95% CI 2.01–4.39) compared to patients with normal cTnI levels. *Conclusions:* Increased cardiac troponin I, indicating myocardial injury, on admission is associated with a more adverse clinical disease course, including a higher likelihood of requiring invasive mechanical ventilation and increased risk of in-hospital mortality. This indicates cardiac troponin I to be a beneficial biomarker for clinicians trying to identify high-risk COVID-19 patients, choosing the optimal monitoring and treatment strategy for these patients.

Keywords: COVID-19; in-hospital mortality; cardiac troponin I; SARS-CoV-2

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1. Introduction

The COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has emerged as a major global public health crisis. It appears that SARS-CoV-2 affects multiple organ systems, including the cardiovascular system, leading to both immediate and long-term cardiovascular complications. During the acute phase of infection, SARS-CoV-2 can cause direct cardiac injury by interacting with the angiotensin-converting enzyme 2 (ACE-2) receptor on various cardiac cells, such as cardiomyocytes, pericytes, macrophages, fibroblasts, and endothelial cells [1,2]. Autopsy studies of COVID-19 patients have confirmed the presence of the SARS-CoV-2 genome in cardiac tissues [3,4]. Furthermore, the immune response to SARS-CoV-2, resulting in a cytokine storm, may

also cause direct cardiac damage. Indirect pathways of cardiac injury may involve myocardial ischemia, which could be precipitated by conditions like tachycardia, shock, severe respiratory distress (resembling type 2 myocardial infarction), or acute atherothrombotic incidents (resembling type 1 myocardial infarction). Clinical manifestations of cardiac injury include myocarditis, pericarditis, arrhythmia, myocardial infarction, heart failure, right ventricular dysfunction, and Takotsubo cardiomyopathy [5–8]. Acute myocardial injury, characterised by elevated serum cardiac troponin without the typical signs of acute ischemia on electrocardiograms or echocardiograms, is identified as one of the earliest and most common cardiac issues in the acute phase of COVID-19 [5].

This study aimed to evaluate the prognostic value of cardiac troponin I (cTnI) levels in predicting in-hospital mortality among hospitalised COVID-19 patients.

2. Materials and Methods

A retrospective observational cohort study was conducted at Vilnius University Hospital Santaros Klinikos in Lithuania from March 2020 to May 2021 [9].

The study included adult patients, 18 years and older, who were admitted to the hospital with confirmed COVID-19 infection and treated in various units, including standard care, high dependency, or intensive care units. The confirmation of the infection was based on a positive result on either a SARS-CoV-2 reverse transcriptase polymerase chain reaction or a rapid SARS-CoV-2 antigen test conducted on a nasopharyngeal sample. For symptomatic patients, the rapid antigen test was utilised within the first five days of the onset of COVID-19 symptoms. Initially, 2179 patients, who underwent cTnI testing upon admission, were considered for the study. Exclusion criteria included individuals with end-stage chronic kidney disease, defined by an estimated glomerular filtration rate (eGFR) less than 15 mL/min/1.73 m^2, and those who developed myocardial infarction subsequent to admission. After applying these exclusions, the study population was finalised at 2019 patients. These individuals were stratified by cTnI levels on admission into three groups: 1416 patients exhibited cTnI levels below 19 ng/L, 431 patients had cTnI levels within the 19–100 ng/L range, and 172 patients presented with cTnI levels above 100 ng/L (Figure 1). Myocardial injury was defined as blood serum cTnI levels increased above the 99th percentile upper reference limit [10]. The concentration of cTnI was measured using the Snibe MAGLUMI 2000 Chemoluminescence Immunoassay System, Shenzhen New Industries Biomedical Engineering Co., Ltd., Shenzhen, China.

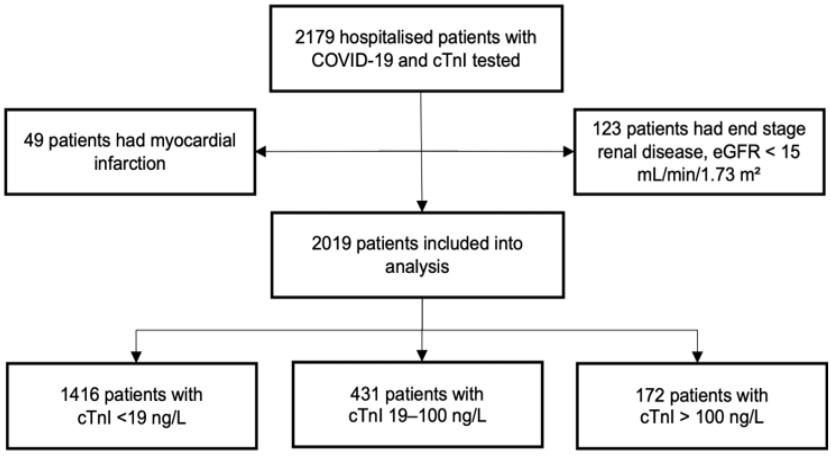

Figure 1. Distribution of patients hospitalised with COVID-19 infection.

Depersonalised datasets were extracted from digital health records, facilitated by the Centre for Informatics and Development, in compliance with the procedures authorised by Vilnius University Hospital Santaros Klinikos. The demographic parameters encompassed sex and age. Comorbid conditions included arterial hypertension (AH), coronary artery disease (CAD), congestive heart failure (CHF), atrial fibrillation (AF), diabetes mellitus, obesity, chronic obstructive pulmonary disease (COPD), chronic kidney disease (CKD), and a history of cerebrovascular accidents. These conditions were identified within the electronic medical records using the corresponding International Classification of Diseases, Tenth Revision (ICD-10) codes. If a medical condition was not documented in the electronic medical records related to the hospitalisation due to COVID-19, the individual was classified as not having that specific comorbidity.

Initial laboratory test results, including a complete blood count, creatinine, urea, eGFR, sodium, potassium, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), C-reactive protein (CRP), ferritin, interleukin 6 (IL-6), D-dimer, and cTnI levels, were extracted from electronic medical records and analysed. Additionally, information regarding the duration of hospitalisation and patient outcomes was gathered from depersonalised medical records.

Statistical analysis was performed using IBM SPSS version 20.0. Continuous and categorical variables were presented as the median (interquartile range (IQR)) and numbers (percentages), respectively. Mann–Whitney *U* test was used to compare continuous variables, and χ^2 test or Fisher's exact test was used to compare categorical variables. Multivariable binary logistic regression was used to determine the association of myocardial injury development with age, sex, and comorbid conditions. Another multivariable binary logistic regression model was created to determine the association of cTnI levels with the need for invasive mechanical ventilation (IMV). The model included the need for IMV as the dependent variable and age; sex; and comorbid conditions (AH, CAD, CHF, AF, diabetes, obesity, COPD, CKD, and previous stroke) as predictors. We conducted a survival analysis to assess the effects of cTnI levels on outcomes within 30 days of hospitalisation. Patients were considered to be right-censored if they were discharged from the hospital alive or remained in the hospital. Cox proportional hazards regression models were created with in-hospital mortality as the dependent variable and age; comorbid conditions (AH, CAD, CHF, AF, diabetes, obesity, COPD, CKD, and previous stroke); treatment with remdesivir; systemic steroids; and antibiotics as predictors. Survival curves were plotted to illustrate the survival rates stratified by the cTnI groups. A two-sided p-value <0.05 was considered significant.

3. Results

Among 2019 adults, 56% were men. The median age was 60 years (IQR 50–69). Table 1 presents the demographic details, clinical features, and initial laboratory findings of the patients included in the analysis.

Table 1. Demographic, clinical, and initial laboratory characteristics of hospitalised COVID-19 patients.

Demographic and Clinical Characteristic (N = 2019)	N (%)	Laboratory Characteristics	N	Median (IQR)
Age, years, median (IQR)	60 (50–69)	Haemoglobin, g/L	2019	140.0 (127.0–150.0)
Male	1130 (55.97)	WBC, $\times 10^9$/L	2019	6.34 (4.76–8.61)
Female	889 (44.03)	Neutrophils, $\times 10^9$/L	2019	4.66 (3.28–6.69)
Any comorbid condition	1000 (49.53)	Lymphocytes, $\times 10^9$/L	2019	1.0 (0.71–1.40)
Arterial hypertension	802 (39.72)	Platelets, $\times 10^9$/L	2019	195.0 (153.0–253.0)
Coronary artery disease	79 (3.91)	cTnI	2019	9.70 (5.0–23.0)

Table 1. *Cont.*

Demographic and Clinical Characteristic (N = 2019)	N (%)	Laboratory Characteristics	N	Median (IQR)
Congestive heart failure	160 (7.92)	Glucose, mmol/L	1984	6.22 (5.54–7.30)
Atrial fibrillation	203 (10.05)	Creatinine, µmol/L	2019	81.0 (67.0–100.0)
Diabetes mellitus	277 (13.72)	eGFR, mL/min/1.73 m^2	2019	84.0 (64.0–96.0)
Obesity	106 (5.25)	Urea, mmol/L	1883	5.50 (4.07–7.91)
COPD	34 (1.68)	Sodium, mmol/L	2019	140.0 (137.0–143.0)
Chronic kidney disease	109 (5.40)	Potassium, mmol/L	2019	4.20 (3.87–4.50)
Previous stroke	23 (1.14)	ALT, U/L	1993	33.0 (21.0–53.0)
Invasive mechanical ventilation	170 (8.42)	AST, U/L	1986	37.0 (26.98–57.0)
Treatment with antibiotics	1514 (74.99)	LDH, U/L	1910	308.0 (243.0–410.0)
Treatment with antivirals (Remdesivir)	711 (35.22)	CRP, mg/L	2018	63.25 (25.45–125.53)
Treatment with systemic steroids	1371 (67.90)	Ferritin, µg/L	1939	471.0 (235.00–1 002.0)
In-hospital mortality	247 (12.23)	IL-6, ng/L	1892	30.40 (15.01–57.30)
Length of hospital stay, days, median (IQR)	11 (8–16)	D-dimer, µg/L	1972	490.0 (295.0–910.0)

ALT: alanine aminotransferase; AST: aspartate aminotransferase; COPD: chronic obstructive pulmonary disease; CRP: C-reactive protein; cTnI: cardiac troponin I; eGFR: estimated glomerular filtration rate; IL-6: interleukin 6; IQR: interquartile range; LDH: lactate dehydrogenase; N: number; WBC: white blood cell count. Reference values: haemoglobin: 128–160 g/L (for males), 117–145 g/L (for females); WBC: 4.0–9.8 $\times 10^9$/L; neutrophils: 1.5–6.0 $\times 10^9$/L; lymphocytes: 1.0–4.0 $\times 10^9$/L; NLR: 1–2; platelets: 140–450 $\times 10^9$/L; glucose: 4.2–6.1 mmol/L; creatinine: 64–104 µmol/L (for males), 49–90 µmol/L (for females); urea: 2.5–7.5 mmol/L; sodium: 134–145 mmol/L; potassium: 3.8–5.3 mmol/L; ALT: ≤40 U/L; AST: ≤40 U/L; LDH: 125–243 U/L; CRP: <5 mg/L; ferritin: 25–350 µg/L (for men), 13–232 µg/L (for women); IL-6: 0–7 ng/L; D-dimer: <250 µg/L.

Out of 2019 patients, 603 (29.87%) experienced acute myocardial injury, characterised by elevated levels of cTnI. Multivariable regression analysis revealed that age, male sex, the presence of CHF, AF, obesity, and CKD were associated with the development of acute myocardial injury (Figure 2).

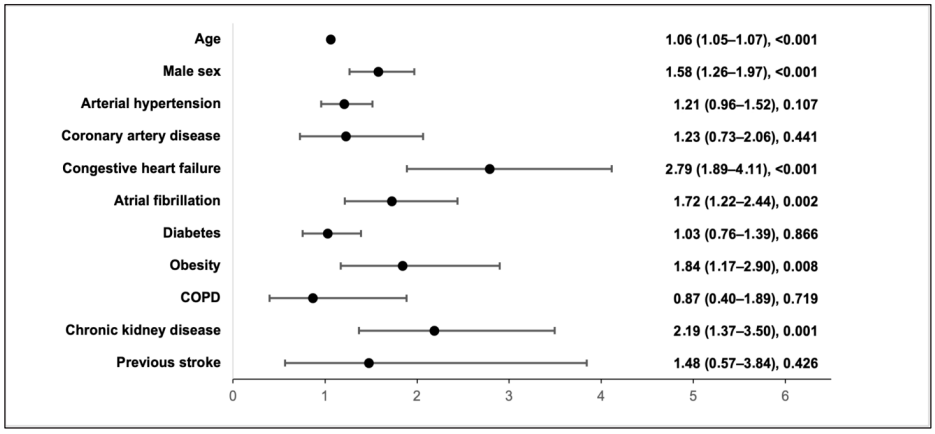

Figure 2. Risk factors and odds ratio (OR) associated with acute myocardial injury in COVID-19 patients.

Out of the 603 patients with elevated cTnI levels, 431 (71.48%) had cTnI levels between 19 and 100 ng/L, and 172 (28.52%) had cTnI levels above 100 ng/L. Patients with elevated cTnI levels were, on average, older than those presenting with normal cTnI levels upon admission. The prevalence of any comorbid condition, including AH, CAD, CHF, AF, diabetes, COPD, CKD, and previous stroke, was higher among patients with cTnI levels of 19–100 ng/L and in those with cTnI levels above 100 ng/L compared to patients with normal cTnI levels. The prevalence of CAD and AF was greater in patients with cTnI levels of 19–100 ng/L than in those with cTnI levels above 100 ng/L (Table 2).

Table 2. Baseline characteristics of hospitalised patients stratified by cardiac troponin I concentration.

Characteristic	cTnI < 19 ng/L (N = 1416), *n* (%)	19 ≤ cTnI ≤ 100 ng/L (N = 431), *n* (%)	cTnI > 100 ng/L (N = 172), *n* (%)	*p*-Value [1]	*p*-Value [2]	*p*-Value [3]
Age in years, median (IQR)	57 (47–65)	67 (58–77)	74 (62–81)	<0.001	<0.001	<0.001
Male	771 (54.45)	253 (58.70)	106 (61.63)	0.120	0.074	0.508
Any underlying condition	584 (41.24)	287 (66.59)	129 (75.00)	<0.001	<0.001	0.044
Hypertension	481 (33.97)	220 (51.04)	101 (58.72)	<0.001	<0.001	0.088
Coronary artery disease	33 (2.33)	27 (6.26)	19 (11.05)	<0.001	<0.001	0.046
Congestive heart failure	50 (3.53)	72 (16.71)	38 (22.09)	<0.001	<0.001	0.122
Atrial fibrillation	74 (5.23)	81 (18.79)	48 (27.91)	<0.001	<0.001	0.014
Diabetes	160 (11.30)	82 (19.03)	35 (20.35)	<0.001	0.001	0.711
Obesity	63 (4.45)	29 (6.73)	14 (8.14)	0.057	0.033	0.543
COPD	17 (1.20)	11 (2.55)	6 (3.49)	0.044	0.031	0.587
Chronic kidney disease	37 (2.61)	46 (10.67)	26 (15.12)	<0.001	<0.001	0.129
Previous stroke	7 (0.49)	10 (2.32)	6 (3.49)	0.002	0.001	0.410
Complications						
Pulmonary embolism	20 (1.41)	17 (3.94)	16 (9.30)	0.001	<0.001	0.009
Stroke	12 (0.85)	15 (3.48)	3 (1.74)	<0.001	0.216	0.258
Remdesivir	513 (36.23)	162 (37.59)	36 (20.93)	0.608	<0.001	<0.001
Systemic steroids	961 (67.87)	315 (73.09)	95 (55.23)	0.040	0.001	<0.001
Antibiotics use	1066 (75.28)	336 (77.96)	112 (65.12)	0.255	0.004	0.001
Invasive ventilation	61 (4.31)	66 (15.31)	43 (25.00)	<0.001	<0.001	0.005
Length of hospital stay, days	11 (7–15)	12 (8–19)	13 (8–21)	<0.001	0.001	0.579
In-hospital mortality	76 (5.37)	106 (24.59)	65 (37.79)	<0.001	<0.001	0.001

(1) *p*-value comparing patients with cTnI < 19 ng/L vs. patients with 19 ≤ cTnI ≤ 100 ng/L. (2) *p*-value comparing patients with cTnI < 19 ng/L vs. patients with cTnI > 100 ng/L. (3) *p*-value comparing patients with 19 ≤ cTnI ≤ 100 ng/L vs. patients with cTnI > 100 ng/L. cTnI: cardiac troponin I; IQR: interquartile range; N or n: number.

Patients with cTnI levels of 19–100 ng/L exhibited statistically significantly higher levels of WBC, neutrophil count, glucose, creatinine, urea, AST, LDH, CRP, ferritin, IL-6, and D-dimer compared to patients with normal cTnI levels. Conversely, their lymphocyte count, eGFR, and sodium concentration were significantly lower (Table 3).

Patients with cTnI levels above 100 ng/L showed higher levels of WBC, neutrophil count, glucose, creatinine, urea, AST, LDH, and D-dimer compared to both patients with normal cTnI levels and those with cTnI levels of 19–100 ng/L. CRP, ferritin, and IL-6 concentrations were higher in patients with cTnI levels above 100 ng/L than in those with normal cTnI levels; however, there was no statistically significant difference in CRP, ferritin, and IL-6 concentrations between patients with cTnI levels above 100 ng/L and those with levels of 19–100 ng/L. Lymphocyte count, eGFR, and sodium concentration

were significantly lower in patients with cTnI levels above 100 ng/L compared to those with normal cTnI levels (Table 3).

Table 3. Laboratory tests of hospitalised COVID-19 patients stratified by cardiac troponin I concentration.

Variable	cTnI < 19 ng/L (N = 1416), Median (IQR)	19 ≤ cTnI ≤ 100 ng/L (N = 431), Median (IQR)	cTnI > 100 ng/L (N = 172), Median (IQR)	*p*-Value [1]	*p*-Value [2]	*p*-Value [3]
Haemoglobin, g/L	142.00 (130.00–151.00)	135.00 (121.00–147.00)	133.00 (116.00–145.75)	<0.001	<0.001	0.164
WBC, $\times 10^9$/L	6.15 (4.65–8.06)	6.72 (5.02–9.41)	8.83 (6.08–12.38)	<0.001	<0.001	<0.001
Neutrophils, $\times 10^9$/L	4.42 (3.14–6.15)	4.95 (3.50–7.53)	7.10 (4.38–10.23)	<0.001	<0.001	<0.001
Lymphocytes, $\times 10^9$/L	1.04 (0.77–1.40)	0.90 (0.60–1.39)	0.80 (0.53–1.30)	<0.001	<0.001	0.071
Platelets, $\times 10^9$/L	195.00 (154.00–252.00)	192.00 (151.00–245.00)	207.00 (150.25–261.25)	0.202	0.495	0.195
Glucose, mmol/L	6.07 (5.46–6.92)	6.67 (5.82–8.23)	7.64 (6.02–9.76)	<0.001	<0.001	0.003
Creatinine, µmol/L	78.00 (65.00–91.00)	92.00 (72.00–125.00)	103.50 (77.00–148.75)	<0.001	<0.001	0.018
eGFR, mL/min/1.73 m^2	88.00 (73.08–98.30)	68.40 (43.60–89.00)	57.20 (34.00–82.00)	<0.001	<0.001	0.002
Urea, mmol/L	4.91 (3.74–6.48)	7.37 (5.24–11.14)	10.17 (6.68–16.25)	<0.001	<0.001	<0.001
Sodium, mmol/L	140.75 (137.88–143.00)	140.00 (136.10–143.00)	139.00 (135.00–142.00)	0.001	0.002	0.386
Potassium, mmol/:	4.15 (3.90–4.50)	4.20 (3.80–4.60)	4.20 (3.85–4.60)	0.468	0.075	0.314
ALT, U/L	33.00 (21.00–53.00)	31.00 (19.35–50.00)	32.50 (19.00–59.75)	0.066	0.676	0.552
AST, U/L	35.00 (25.72–53.00)	40.00 (29.08–61.07)	50.00 (31.00–78.75)	<0.001	<0.001	0.004
LDH, U/L	295.00 (236.00–388.00)	335.00 (264.00–487.00)	385.50 (280.75–564.75)	<0.001	<0.001	0.031
CRP, mg/L	59.00 (24.43–108.90)	85.60 (32.60–157.60)	92.08 (19.88–146.30)	<0.001	0.003	0.486
Ferritin, µg/L	441.00 (225.50–952.55)	543.00 (252.50–1115.50)	543.29 (239.70–1286.73)	0.011	0.046	0.739
IL-6, ng/L	27.70 (13.90–51.05)	42.50 (19.85–71.05)	34.15 (16.85–87.73)	<0.001	0.001	0.739
D-dimer, µg/L	430.00 (270.00–720.00)	677.50 (410.00–1330.00)	1 255.00 (587.50–2360.00)	<0.001	<0.001	<0.001

ALT: alanine aminotransferase; AST: aspartate aminotransferase; CRP: C-reactive protein; cTnI: cardiac troponin I; eGFR: estimated glomerular filtration rate; IL-6: interleukin 6; IQR: interquartile range; LDH: lactate dehydrogenase; N: number; WBC: white blood cell count. (1) *p*-value comparing cTnI < 19 ng/L vs. 19 ≤ cTnI ≤ 100 ng/L. (2) *p*-value comparing cTnI < 19 ng/L vs. cTnI > 100 ng/L. (3) *p*-value comparing 19 ≤ cTnI ≤ 100 ng/L vs. cTnI > 100 ng/L. Reference values: haemoglobin: 128–160 g/L (for males), 117–145 g/L (for females); WBC: 4.0–9.8 $\times 10^9$/L; neutrophils: 1.5–6.0 $\times 10^9$/L; lymphocytes: 1.0–4.0 $\times 10^9$/L; platelets: 140–450 $\times 10^9$/L; glucose: 4.2–6.1 mmol/L; creatinine: 64–104 µmol/L (for males), 49–90 µmol/L (for females); eGFR: >90 mL/min/1.73 m^2; urea: 2.5–7.5 mmol/L; sodium: 134–145 mmol/L; potassium: 3.8–5.3 mmol/L; ALT: ≤40 U/L; AST: ≤40 U/L; LDH: 125–243 U/L; CRP: <5 mg/L; ferritin: 25–350 µg/L (for men), 13–232 µg/L (for women); IL-6: 0–7 ng/L; D-dimer: <250 µg/L.

Remdesivir was administered to 711 (35.22%) patients upon admission or the following day for a standard 5-day course (IQR 5 days). Patients with normal cTnI levels and those with cTnI levels of 19–100 ng/L were more likely to receive remdesivir than those with cTnI levels above 100 ng/L at 36.23% and 37.59%, respectively, compared to 20.93% ($p < 0.001$ for both comparisons).

Systemic steroids were administered to 1371 (67.90%) patients, with the majority (97.67%) being treated with dexamethasone for a median duration of 9 days (IQR 6–10 days).

Fewer patients with cTnI levels greater than 100 ng/L received steroids compared to those with cTnI levels of 19–100 ng/L (55.23% vs. 73.09%, $p < 0.001$) and those with normal cTnI levels (55.23% vs. 67.87%, $p = 0.001$).

A total of 1514 (74.99%) patients received antibiotics. The most commonly administered antibiotics were amoxicillin and clavulanic acid, which were used in 1269 of the 1514 cases (83.82%). Other antibiotics were prescribed less frequently—piperacillin and tazobactam—in 454 cases (29.99%) and azithromycin in 120 cases (7.93%). Antibiotic use was less frequent in patients with cTnI levels greater than 100 ng/L compared to those with cTnI levels of 19–100 ng/L (65.12% vs. 77.96%, $p = 0.001$) and also compared to patients with normal cTnI levels (65.12% vs. 77.96%, $p = 0.004$).

The need for IMV was highest in patients with cTnI levels above 100 ng/L at 25% compared to 15.31% in those with cTnI levels between 19 and 100 ng/L, and 4.31% in patients with normal cTnI levels (Table 2). Among patients with cTnI 19–100 ng/L, the odds ratio (OR) for IMV was 3.18 (95% CI 2.11–4.79, $p < 0.001$); in those with cTnI levels above 100 ng/L, the OR was 5.38 (95% CI 3.26–8.88, $p < 0.001$) compared to patients presenting normal cTnI levels on admission. Other risk factors for IMV were male sex (OR 1.49, 95% CI 1.04–2.12, $p = 0.029$) and CHF (OR 2.85, 95% CI 1.82–4.45, $p < 0.001$).

The in-hospital mortality rate was highest among patients with cTnI levels above 100 ng/L at 37.79% compared to 24.59% in those with cTnI levels of 19–100 ng/L and 5.37% in patients with normal cTnI levels.

Cox regression analysis revealed that the hazard ratio (HR) for 30-day in-hospital mortality for patients with cTnI levels of 19–100 ng/L upon admission was 2.58 (95% CI 1.83–3.62, $p < 0.001$), and for those with cTnI levels above 100 ng/L, the HR was 2.97 (95% CI 2.01–4.39, $p < 0.001$) compared to those with normal cTnI levels (Figure 3).

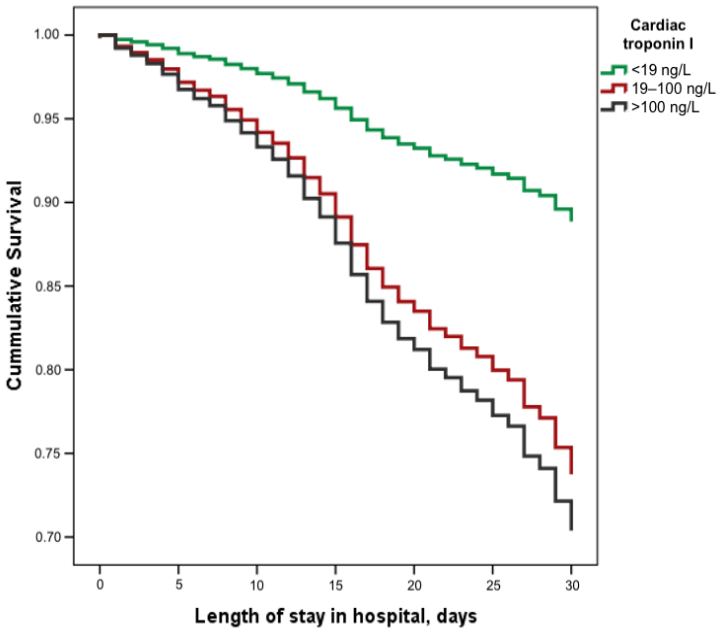

Figure 3. Survival of hospitalised COVID-19 patients stratified by cardiac troponin I concentration.

Other risk factors associated with an increased HR for 30-day in-hospital mortality included age, CHF, obesity, and previous stroke. Treatment with remdesivir was associated

with a reduced HR for 30-day in-hospital mortality: HR 0.61 (95% CI 0.43–0.86, $p = 0.005$) (Table 4).

Table 4. Hazards ratio for 30 days in-hospital mortality in hospitalised COVID-19 patients.

Characteristic	In-Hospital Mortality	
	HR (95% CI)	***p*-Value**
TnI < 19 ng/L	Reference	
19 ≤ TnI ≤ 100 ng/L	2.58 (1.83–3.62)	<0.001
TnI > 100 ng/L	2.97 (2.01–4.39)	<0.001
Age in years	1.05 (1.03–1.06)	<0.001
Arterial hypertension	0.71 (0.53–0.95)	0.019
Coronary artery disease	1.03 (0.63–1.67)	0.906
Congestive heart failure	1.81 (1.33–2.46)	<0.001
Atrial fibrillation	1.45 (1.06–1.98)	0.021
Diabetes	0.92 (0.66–1.29)	0.636
Obesity	2.40 (1.50–3.84)	<0.001
COPD	1.75 (0.96–3.18)	0.068
Chronic kidney disease	0.70 (0.46–1.05)	0.083
Previous stroke	1.67 (0.88–3.16)	0.114
Treatment with remdesivir	0.61 (0.43–0.86)	0.005
Treatment with systemic steroids	1.40 (1.00–1.96)	0.051
Antibiotics	0.85 (0.59–1.24)	0.413

COPD: chronic obstructive pulmonary disease; cTnI: cardiac troponin I; HR: hazard ratio.

4. Discussion

Several viral infections are identified as potential causes of heart damage. These include enteroviruses (Coxsackie B viruses, echoviruses, and poliovirus); herpesviruses (human herpes virus 6, Epstein–Barr virus, cytomegalovirus, and varicella zoster virus); parvoviruses (such as parvovirus B19); adenoviruses; influenza viruses; retroviruses (human immunodeficiency virus (HIV)); and coronaviruses [11,12]. In addition, accumulating data suggest that hepatitis C virus may also contribute to heart diseases [13]. Viruses can damage the heart through various mechanisms, including direct virus-mediated damage to cardiomyocytes (Coxsackievirus B, Epstein–Barr virus, cytomegalovirus, and HIV); endothelial cells (human herpes virus 6, parvovirus B19, and cytomegalovirus); and fibroblasts (cytomegalovirus). During the acute phase of infection, SARS-CoV-2 can cause direct cardiac injury by interacting with the ACE-2 receptor on cardiomyocytes, pericytes, macrophages, fibroblasts, and endothelial cells [1,2]. Moreover, viruses can cause heart damage through an immune response, which may result from the development of autoimmune myocarditis or excessive cytokine production [11]. In our research, we focused on myocardial injury caused by SARS-CoV-2 and analysed the data of one of the largest hospitals in Lithuania from March 2020, when the first COVID-19 patient was admitted to the hospital, until May 2021. In the initial stages of the pandemic, until the autumn of 2020, no single SARS-CoV-2 lineage clearly dominated in Lithuania. By the end of 2020, the B.1.177.60 lineage began to spread. Subsequently, this lineage was overtaken by the B.1.1.7 (alpha variant) and its localised version Q.1., which dominated until June 2021 [14].

In our single-centre retrospective cohort study of 2019 hospitalised COVID-19 patients, several key observations were made: (1) myocardial injury was quite common among patients hospitalised with COVID-19, affecting nearly 30% of patients, but in the majority of cases, it was associated with low-level elevations in cTnI concentrations; (2) age, male sex, the presence of CHF, AF, obesity, and CKD were associated with the development of

acute myocardial injury; (3) patients with acute myocardial injury exhibited higher levels of inflammatory biomarkers; and (4) more significant myocardial injury was associated with a five-fold higher risk of requiring invasive mechanical ventilation and nearly a three-fold higher risk of in-hospital mortality.

The detection of elevated myocardial injury markers, particularly cTnI, has garnered considerable scientific interest due to its widespread measurement in various healthcare settings and its clinical significance [15–18]. Numerous studies have reported myocardial injury, defining it as troponin elevation above the 99th percentile of the upper reference limit, with varying prevalence rates based on the population studied. For instance, Lala et al., in a study involving 2736 hospitalised COVID-19 patients, found a 36% prevalence of myocardial injury [16]. De Marzo et al. reported a myocardial injury prevalence of 25.7% among individuals exceeding 60 years of age [17], while Shi et al. documented myocardial injury in 19.7% of their study cohort [18]. In alignment with these findings, our research demonstrated that 29.87% of patients admitted to hospital for COVID-19 infection presented with increased cTnI levels. Despite varying prevalence rates of myocardial injury reported across studies, there is a consensus that myocardial injury occurs more frequently in severe or critically ill COVID-19 patients and non-survivors [19–21].

Furthermore, myocardial injury has been found to occur more frequently among older patients with a greater number of comorbidities, particularly chronic cardiovascular conditions [16,18,22,23]. Lala et al. highlighted that patients with more severe myocardial injury had a higher prevalence of cardiovascular diseases, such as coronary artery disease, atrial fibrillation, and heart failure, compared to those with mildly elevated or normal troponin levels [16]. Our research further quantified this risk, showing that age increased the odds ratio for myocardial injury by 1.06 times, male sex by 1.58 times, the presence of CHF by 2.79 times, AF by 1.72 times, obesity by 1.84 times, and CKD by 2.19 times. Similarly, Papageorgiou et al. identified that age (OR 1.68), hypertension (OR 1.81), and moderate CKD (OR 9.12) were independent predictors of myocardial injury [23]. Additionally, Lombardi and colleagues found significant associations between myocardial injury with heart failure (OR 2.01) and a history of coronary artery disease (OR 2.04) [22]. The existence of numerous comorbidities can exacerbate the severity of COVID-19, resulting in more severe myocardial injury.

Our study revealed that patients with myocardial injury presented with elevated levels of WBC, neutrophil count, glucose, creatinine, urea, AST, LDH, CRP, ferritin, IL-6, and D-dimer in comparison to those with normal cTnI levels. This correlation between myocardial injury and an increase in inflammatory and prothrombotic markers is consistent with findings from other researchers. Specifically, myocardial injury patients demonstrated significant rises in inflammatory biomarkers such as CRP [18,22,24] and thrombotic indicators like D-dimer [22–24]. Moreover, Li et al. identified correlations between high sensitivity cTnI levels and IL-6 ($r = 0.59$ to 0.66, $p < 0.001$), CRP ($r = 0.54$ to 0.62, $p < 0.001$), and D-dimer ($r = 0.54$ to 0.65, $p < 0.001$) at various stages: upon admission, within the first week, and after the first week of hospitalisation, further underscoring the link between cardiac injury and systemic inflammation and thrombosis in this patient population [20].

In our study, 35% of patients received treatment with remdesivir, and this treatment was associated with a 1.64-fold reduction in HR for 30-day in-hospital mortality. These findings are consistent with prior research, indicating that remdesivir administration is linked to improved time to recovery and potentially reduced mortality rates [25]. However, this finding should be approached with caution, as remdesivir was more frequently administered to patients with less severe COVID-19 and less severe acute myocardial injury, taking into consideration the reported potential cardiac effects associated with its use, such as hypotension, bradycardia, and prolonged QTc interval, that are more frequently observed in patients with COVID-19 and concomitant cardiovascular diseases [7,26,27]. Randomised controlled trials have provided strong evidence supporting the beneficial therapeutic effect of corticosteroids in COVID-19 patients requiring oxygen therapy, with no impact on cardiovascular events [28,29]. Our study demonstrated that patients with

more severe acute myocardial injury were less frequently administered systemic steroids and that treatment with systemic steroids had no effect on the HR for 30-day mortality.

Our study revealed a 3.18-fold increase in the odds ratio for IMV and a 2.58-fold increase in the hazard ratio for 30-day in-hospital mortality among patients with cTnI levels between 19 and 100 ng/L. Higher cTnI levels correlated with even greater increases, up to 5.38 times for the OR for IMV and up to 2.97 times for the HR for in-hospital mortality. These findings are in close agreement with those of Lala et al., who observed a 3.03-fold increase in the HR for mortality (95% CI 2.42–3.80) in patients with troponin levels more than three times the upper reference limit, although in an older study population with higher incidences of coronary artery disease (CAD) and heart failure at 16.6% and 10.1%, respectively [16]. Similarly, Shi et al. noted a higher mortality risk in patients with myocardial injury, with a HR of 3.41 [18]. Papageorgiou et al. identified that patients with elevated troponin levels were significantly more likely to require non-invasive and IMV (with ORs of 2.40 and 6.81, respectively) to develop acute kidney injury (with 6.76-times higher odds), to necessitate urgent renal replacement therapy (with 4.14-times higher odds), to experience thromboembolic events (with 11.99-times higher odds), and to die (with 2.40-times higher odds) compared to patients with normal troponin levels [23].

The role of elevated cTnI levels as a critical prognostic marker for severe outcomes in COVID-19, including the necessity for IMV and higher mortality rates, has sparked discussions on its potential integration into routine diagnostics and its implications for tailoring treatment approaches for patients with myocardial injury [30,31]. Sandoval et al. proposed a structured method for leveraging cTnI in risk stratification. An initial cTnI measurement could be instrumental in the early classification of COVID-19 patients, aiding in their preliminary assessment. Patients deemed clinically stable or marginally stable yet presenting with significant laboratory anomalies such as elevated cTnI levels are categorised at an intermediate risk level. Conversely, patients who are clinically unstable with notable laboratory abnormalities fall into the high-risk category and necessitate hospital admission. For hospitalised patients, repeated cTnI monitoring is recommended. Individuals whose cTnI levels remain stable and do not exhibit significant increases might not require further interventions. On the other hand, patients exhibiting escalating cTnI levels should be subjected to more in-depth evaluations and potentially more intensive therapeutic interventions [19].

We acknowledge that our study has several limitations. Firstly, the identification of comorbidities relied on ICD-10-AM codes encoded in medical records, which might not have captured all relevant conditions. Another issue was the absence of data regarding the duration of COVID-19 symptoms prior to hospitalisation, which prevented the specification of the time of myocardial injury and the more precise evaluation of the potential mechanism of the myocardial injury in regard to its relation to virus-mediated or immune-mediated damage and evolution over time. The dataset did not include the dates of hospitalisation, which did not allow for the analysis of acute myocardial damage across various COVID-19 pandemic waves. Conducted in the largest hospital of the capital of Lithuania, the single-centre nature of this study may lead to selection bias and limit the applicability of the results to wider populations. The specific healthcare practices, patient demographics, and treatment approaches of this hospital may not accurately reflect those of the broader population. Furthermore, our retrospective study's design did not allow for the assessment of the long-term effects of acute myocardial injury in COVID-19 patients, as it focused on short-term outcomes during the hospital stay and not addressing the potential prolonged impact of acute myocardial injury beyond the acute phase of the infection.

5. Conclusions

Cardiac troponin I serves as an effective biomarker for risk stratification in COVID-19 patients upon admission. Increased levels of cardiac troponin I, indicating myocardial injury, on admission are associated with a more adverse clinical disease course, including a higher likelihood of the need for invasive mechanical ventilation and increased risk of

in-hospital mortality. This indicates cardiac troponin I to be a beneficial biomarker for clinicians trying to identify high-risk COVID-19 patients, choosing the optimal monitoring and treatment strategy for these patients.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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BMC Infectious Diseases

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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

Ieva Kubiliute[1*], Jurgita Urboniene[2], Fausta Majauskaite[1], Danielius Tamasauskas[3,4], Dovile Juozapaite[3] and Ligita Jancoriene[1]

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

*Correspondence:
Ieva Kubiliute
ieva.kubiliute@santa.lt

Full list of author information is available at the end of the article

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.

Clinical trial number Not applicable.

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 min 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 min, at a temperature of 25 °C. The second reverse transcription – 1 cycle, 10 min, 53 °C, the third polymerase activation – 1 cycle, 2 min, 95 °C, the fourth amplification, which lasted for 40 cycles, 3 s at 95 °C and 30 s 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 min 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 s. 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 h 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 min at 95 °C; (ii) 40 cycles for 3 s at 95 °C, and 30 s at 60 °C.

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

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.

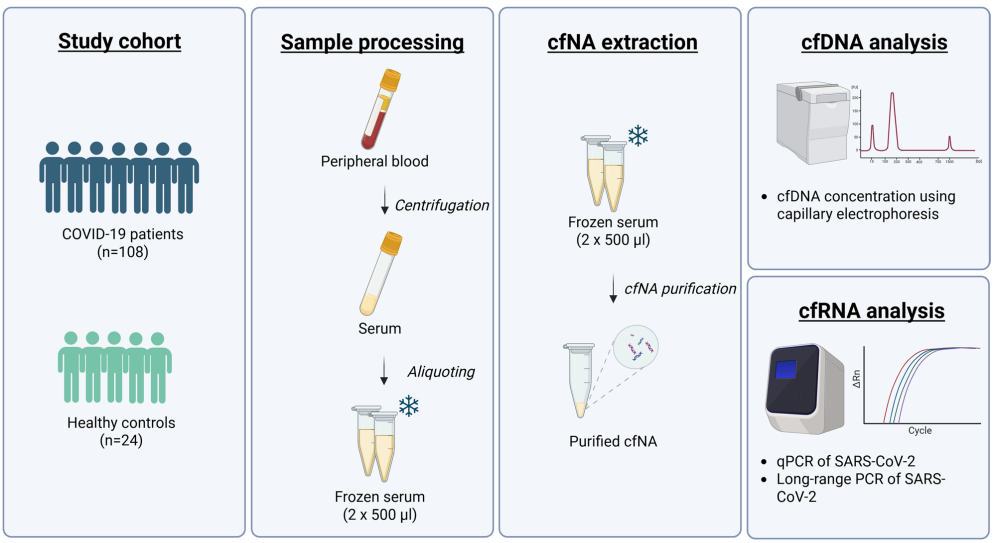

Fig. 1 Schematic overview of study cohort, sample processing, and analytical workflow. Created in BioRender. Juozapaite, D. (2026) https://BioRender.com/s0moadk

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 Fig. 1).

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

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.

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) (Fig. 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, $p < 0.001$) (Fig. 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 ($p = 0.018$).

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

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

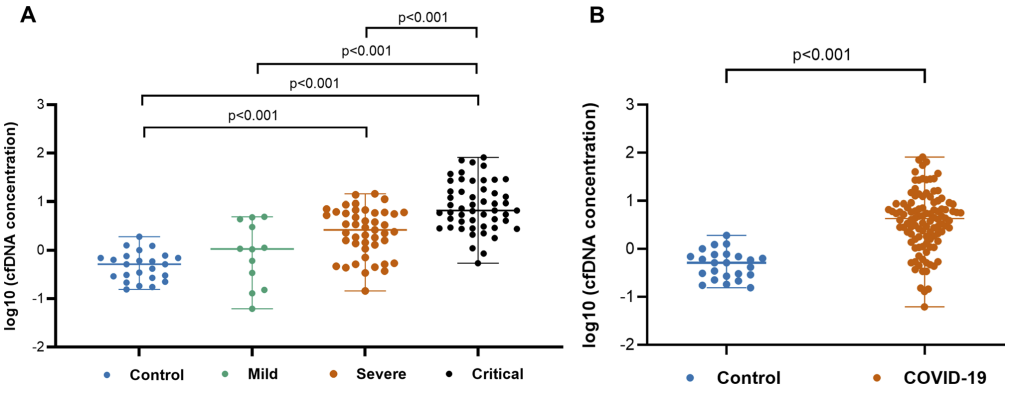

Fig. 2 Comparison of cfDNA concentrations among healthy controls and COVID-19 patients by disease severity (**A**) and healthy controls and COVID-19 patients (**B**). cfDNA concentrations are shown on a log10-transformed scale

(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 ($p < 0.001$) (Fig. 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. 4.50×10^9/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, $p < 0.001$) (Fig. 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. 5.47×10^9/L, $p = 0.015$) and neutrophil (5.41 vs. 4.52×10^9/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, $p < 0.001$) (Fig. 3, Additional file 4).

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], $p < 0.001$).

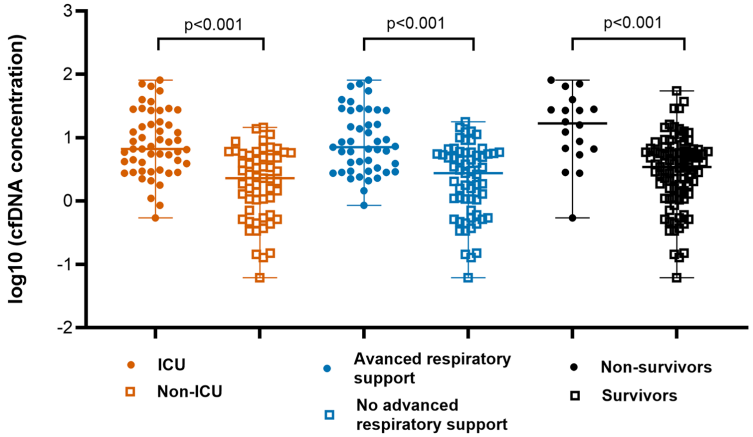

Fig. 3 cfDNA concentrations in relation to ICU treatment, the need for advanced respiratory support, and survival in hospitalized COVID-19 patients. cfDNA concentrations are shown on a log10-transformed scale

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).

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 (Fig. 4A; Table 3).

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 (Fig. 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 (Fig. 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

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

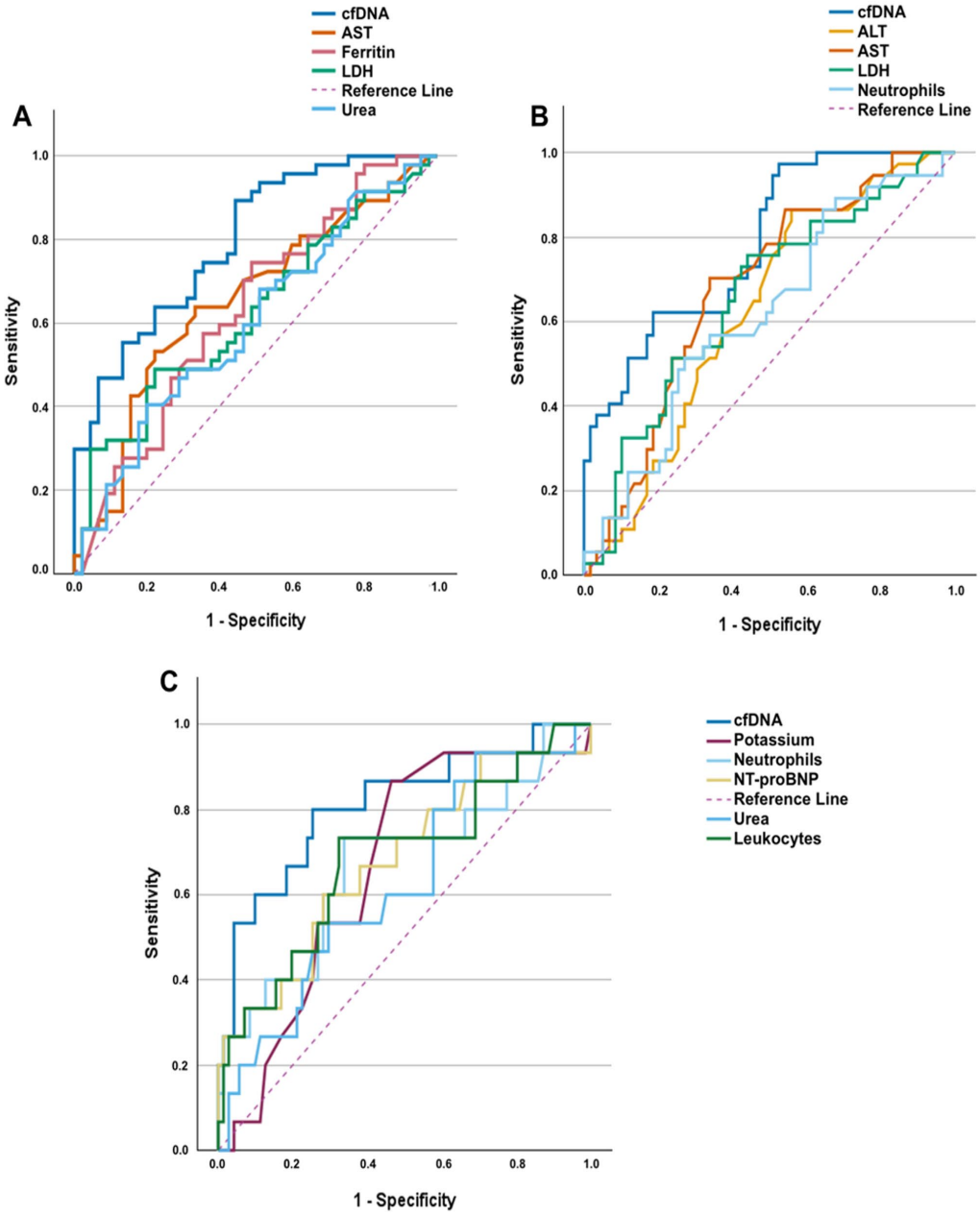

Fig. 4 Prognostic performance of cfDNA and other laboratory parameters in predicting critical disease requiring ICU admission (**A**), the need for ARS (**B**), and in-hospital mortality (**C**) in hospitalized COVID-19 patients. ALT – alanine aminotransferase; AST – aspartate aminotransferase; cfDNA – cell-free DNA; LDH – lactate dehydrogenase; NT-proBNP – N-terminal pro-B-type natriuretic peptide

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

associations were observed with a critical COVID-19 course or in-hospital mortality (Table 4).

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

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-026-13305-7.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

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

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.

Competing interests
The authors declare no competing interests.

Author details
[1]Clinic of Infectious Diseases and Dermatovenerology, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania
[2]Centre of Infectious Diseases, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania
[3]Vilnius Santaros Klinikos Biobank, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania
[4]Life Sciences Center, Vilnius University, Vilnius, Lithuania

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Publisher's note

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RESEARCH ARTICLE

Predicting severe COVID-19 disease in adults: A single-centre cohort study during the first three pandemic waves in 2020–2021 in Vilnius, Lithuania

Ieva Kubiliute[1☯*], Edgaras Zaboras[2☯], Fausta Majauskaite[1☯], Jurgita Urboniene[3☯], Birute Zablockiene[1☯], Giedre Gefenaite[4☯], Aukse Mickiene[5☯], Ligita Jancoriene[1☯]

1 Clinic of Infectious Diseases and Dermatovenerology, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania, **2** Faculty of Medicine, Vilnius University, Vilnius, Lithuania, **3** Centre of Infectious Diseases, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania, **4** Department of Health Sciences, Faculty of Medicine, Lund University, Lund, Sweden, **5** Department of Infectious Diseases, Lithuanian University of Health Sciences, Kaunas, Lithuania

☯ These authors contributed equally to this work.
* ieva.kubiliute@mf.vu.lt

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Abstract

Background

Since its emergence, the COVID-19 infection has led to significant morbidity and mortality worldwide. Early identification of patients at risk for severe disease is essential for more effective triage, timely therapeutic intervention, and optimal resource allocation. Differences in population characteristics may contribute to variability in disease outcomes, which emphasizes the need for regional-level data, especially from underrepresented regions. The main aim of this study was to identify the demographic, clinical, and laboratory predictors of severe COVID-19, defined as the need for oxygen therapy, in Lithuania.

Materials and methods

We conducted an ambispective observational cohort study at Vilnius University Hospital Santaros Klinikos in Vilnius, Lithuania, from March 2020 to December 2021. Adult patients with a confirmed diagnosis of COVID-19 and hospitalized longer than 24 hours were included in this study. Data were collected from the electronic medical records and patient interviews. To identify predictors of severe COVID-19 course, a multivariable binary logistic regression model was performed.

Results

Among 495 patients, 52.9% were male, the median age was 55 years, and 61.2% had at least one underlying condition. The most common symptoms on admission were malaise (77.1%), subfebrile fever (65.9%), and cough (69.7%). CRP

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demonstrated the highest predictive value for severe COVID-19 (AUC = 0.84), followed by LDH (AUC = 0.80). Older age (OR 1.04 per year, 95% CI 1.00–1.08), obesity (OR 3.55, 95% CI 1.35–9.30), lymphopenia (OR 3.70, 95% CI 1.37–9.99), higher LDH (OR 1.008, 95% CI 1.00–1.01) and CRP (OR 1.021, 95% CI 1.01–1.04) levels were identified as the strongest predictors for severe COVID-19 disease course.

Conclusion

Older age, obesity, lymphopenia, and higher CRP and LDH were associated with developing severe COVID-19 disease, indicating that combining patient history and laboratory parameters can provide a practical risk stratification approach to help clinicians identify high-risk patients early upon hospitalisation.

Introduction

Since its emergence in late 2019, the coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to significant morbidity and mortality worldwide. COVID-19 has a wide range of clinical manifestations. While most infected individuals are asymptomatic or experience mild to moderate symptoms, a substantial proportion develop severe or critical illness that is characterized by respiratory failure, multisystem involvement, and deadly complications [1,2]. Early identification of patients at risk for severe disease is essential for appropriate triage, timely initiation of therapy, and optimal resource allocation.

Numerous predictive models have been developed to identify patients at risk of severe disease since the onset of the COVID-19 pandemic. These efforts highlight various demographic, clinical, and laboratory factors like older age, male sex, comorbidities (e.g., cardiovascular disease, diabetes), and specific biomarkers as being associated with worse outcomes [3–6]. However, despite the widespread use of both traditional statistical approaches and advanced machine learning techniques, there is still no clear consensus on a unified set of predictors. Many existing models are limited by limited external validation, heterogeneity in study populations, and methodological constraints, which raises concerns about their generalizability and real-world clinical utility [7–9].

Although the global incidence of severe COVID-19 cases has declined over recent years, especially with the emergence of less virulent variants and increasing immunity through vaccination or prior infection, it remains crucial to understand which clinical factors contributed to the broad spectrum of disease severity. Identifying the predictors of severe COVID-19 can not only inform the management of future SARS-CoV-2 outbreaks but also serve as a reference point for future pandemics caused by novel respiratory pathogens. Early recognition of patients at risk for deterioration enables more effective triage, timely therapeutic intervention, and potentially improved clinical outcomes. For instance, some studies have demonstrated that early warning scores, such as the National Early Warning Score (NEWS), can predict adverse outcomes in COVID-19 patients, facilitating prompt clinical decisions [10,11]. Additionally, research indicates that early identification of high-risk patients allows

targeted therapies to be implemented, which may reduce mortality and the need for intensive care [12]. Moreover, this approach could help reduce the overall burden on healthcare systems by enabling resource prioritization, minimizing preventable complications, and supporting tailored treatment strategies.

Despite the proliferation of risk models for predicting COVID-19 severity, many lack external validation and are heavily influenced by local population characteristics, including genetic background and ethnicity, healthcare infrastructure, diagnostic criteria, and other often unassessed variables [7]. The potential variability of these factors highlights the importance of generating country-specific or at least region-specific data. Differences in population characteristics may contribute to variability in disease outcomes across settings, so this underscores the need for regional-level evidence across different countries [13,14]. Regional data from Eastern and Central Europe remain underrepresented in the global literature, even though healthcare systems in these areas faced unique challenges during the pandemic [15,16]. Thus, region or country-specific studies are crucial in identifying the specific predictive variables in this often-understudied European region. Additionally, the clinical utility of many proposed markers varies depending on the timing of presentation, restricted availability of advanced laboratory marker tests outside major tertiary care centres, and thresholds used for clinical decisions [17,18]. In Lithuania, like in many other countries, the early pandemic period was marked by diagnostic uncertainty and evolving treatment protocols [19]. We therefore designed a study to:

1. Describe the baseline demographic, clinical, laboratory, and radiological characteristics, complications, and hospitalization outcomes of adults hospitalized with COVID-19 in Vilnius, Lithuania, during 2020–2021.
2. Evaluate the predictive accuracy of laboratory parameters for a severe COVID-19 disease course.
3. Identify the strongest demographic, clinical, and laboratory risk factors of severe COVID-19, defined as the need for oxygen therapy.
4. Compare clinical, laboratory, and radiological characteristics, complications, and hospitalization outcomes across different COVID-19 waves.

Methods

Study design and setting

We conducted an ambispective observational cohort study at Vilnius University Hospital Santaros Klinikos, a tertiary care university hospital in Vilnius, Lithuania, combining retrospectively (13 March – 30 June 2020) and prospectively collected data (1 July 2020–31 December 2021).

Participants and recruitment

Individuals aged 18 years and older with a confirmed diagnosis of COVID-19 infection who were hospitalized for more than 24 hours in the COVID-19 department of Vilnius University Hospital Santaros Klinikos were included in this study. COVID-19 diagnosis was confirmed by a positive SARS-CoV-2 reverse transcriptase polymerase chain reaction test or a rapid antigen test on a nasopharyngeal swab sample. The antigen test was administered to symptomatic patients within 5 days of the onset of COVID-19 symptoms. Exclusion criteria were hospitalization for ≤ 24 hours, as well as refusal to participate, or inability to communicate and provide informed consent for participants enrolled prospectively. In total, 495 eligible patients were included in the study, of which 258 (52.1%) were included retrospectively and 237 (47.9%) prospectively.

Data collection and variables

Data were collected from the electronic medical records of Vilnius University Hospital Santaros Klinikos and through direct patient interviews. Both sources provided information about demographic factors, underlying medical conditions, and other

clinical data. Information on objective findings, laboratory results on admission, radiological findings, details of in-hospital complications, intensive care unit (ICU) or high dependency unit (HDU) admissions, length of hospitalization, and hospital discharge status (discharged, transferred, or died) were extracted from the medical records.

Demographic variables were age (in years) and sex (male/female). Comorbidities, recorded as binary variables (yes/no), comprised anaemia or other haematological disease, asthma, chronic obstructive pulmonary disease (COPD), other pulmonary disease (excluding asthma and COPD), non-haematological malignancy, arterial hypertension, other cardiovascular disease (excluding arterial hypertension), dementia, diabetes mellitus, immunodeficiency, chronic liver disease (including cirrhosis), neuromuscular disease, chronic kidney disease, rheumatological disease, prior stroke, tuberculosis, obesity, and malnutrition. Additional medical information consisted of smoking status (never smoked/ former smoker/ current smoker), body mass index (BMI; numeric, kg/m²), pregnancy status (yes/no), and history of COVID-19 vaccination (vaccinated/unvaccinated).

The presence of COVID-19 symptoms at admission was recorded as present or absent for each of the following: sudden onset of symptoms, subfebrile fever, febrile fever, chills, tachypnoea, malaise, headache, dizziness, confusion, myalgia, sore throat, coryza, cough, shortness of breath, chest pain, palpitations, general deterioration, nausea, vomiting, diarrhoea, abdominal pain, ageusia, anosmia, conjunctivitis, rash, or other dermatological manifestation.

Collected objective findings on admission were body temperature (°C), respiratory rate (breaths per minute), and oxygen saturation (SpO_2; %). The risk of patients' clinical deterioration was classified using the National Early Warning Score (NEWS) as low risk (0–4), medium risk (5–6), or high risk (≥ 7).

Initial laboratory test results performed at the time of hospitalization, all treated as numeric variables, and included complete blood count, C-reactive protein (CRP), ferritin, interleukin-6 (IL-6), lactate dehydrogenase (LDH), D-dimer, fibrinogen, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), lactate, troponin I, N-terminal pro-B-type natriuretic peptide (NT-proBNP), creatinine, urea, prothrombin time (PT), and international normalized ratio (INR).

From the medical records, we also obtained information on chest X-ray and computed tomography (CT) findings, development of pneumonia, the required oxygen therapy (through nasal cannula and face mask), high-flow therapy, non-invasive ventilation, invasive mechanical ventilation, and extracorporeal membrane oxygenation (ECMO), all recorded as binary variables.

Furthermore, we collected the data on complications developed during the hospitalization, that included acute respiratory distress syndrome (ARDS), bronchiolitis, secondary bacterial pneumonia (confirmed by positive bronchoalveolar lavage culture), other secondary bacterial infection (confirmed by positive culture), sepsis, acute renal failure, heart failure, multiple organ dysfunction, critical illness myopathy, pulmonary embolism, dermatological complications and any other complication not mentioned above. All complications were recorded as binary variables.

Information about the transmission to the ICU or HDU (yes/no), length of stay in these units, overall length of hospitalization (in days), and hospital discharge status (discharged/transferred, died) was also obtained.

The COVID-19 pandemic in Lithuania in 2020–2021 was marked by distinct periods of SARS-CoV-2 variant dominance: the Alpha variant until September 30, 2020, the Beta variant from October 1, 2020, to July 1, 2021; the Delta variant until December 31, 2021.

The outcome of this study was severe COVID-19 disease, defined as a clinical course requiring any form of oxygen therapy during hospitalization, while non-severe disease was defined as a clinical course in which oxygen therapy was not needed.

Statistical analysis

Descriptive statistics were used to summarize baseline demographic, clinical, laboratory, and radiological characteristics, complications, and hospitalization outcomes. Numeric variables were presented as medians with interquartile ranges (IQR), while categorical variables were summarized using absolute and relative frequencies.

Group comparisons were conducted to analyse differences between severe and non-severe COVID-19 cases and across COVID-19 waves. The Shapiro–Wilk test was used to assess the normality of continuous variables, and none met the assumption of normal distribution. Therefore, group comparisons for numeric variables were performed using the Mann–Whitney U test or the Kruskal–Wallis test with Bonferroni correction for multiple comparisons. Comparisons for categorical and binary variables were made using the χ^2 test or Fisher's exact test, as appropriate. A cumulative distribution curve was used to illustrate the overall distribution of oxygen saturation values.

To address the second study objective, the predictive performance of laboratory parameters was assessed by calculating the area under the receiver operating characteristic (ROC) curve.

To address the third objective – identifying risk factors associated with the development of severe COVID-19 – univariable and multivariable binary logistic regression models were constructed. The univariable models included demographic, clinical characteristics, and laboratory values measured at admission. Variables that were statistically significant in the univariable analysis were reviewed by clinical experts for inclusion in the multivariable regression model. Highly subjective clinical symptoms (e.g., malaise, dizziness, general deterioration, confusion), highly correlated variables (ALT, which was strongly correlated with AST; $r = 0.803$) were excluded from the multivariable model. Tachypnoea, SpO_2, and NEWS were also not included in the model as they directly reflect the need for oxygen therapy. The final multivariable logistic regression model included age, male sex, subfebrile fever, febrile fever, chills, cough, shortness of breath, chest pain, diarrhoea, obesity, COPD, arterial hypertension, other cardiovascular disease (excluding arterial hypertension), lymphocyte count (dichotomized at 1.0×10^9/L), and the following laboratory analytes: neutrophil count, AST, ferritin, IL-6, LDH, D-dimer, and CRP. Odds ratios (OR) with 95% confidence intervals (CI) were reported. Regression model performance was evaluated by assessing explanatory power using Cox & Snell and Nagelkerke R^2 statistics. Model calibration was examined using the Hosmer-Lemeshow goodness-of-fit test. The classification performance of the model was evaluated using a probability cut-off value of 0.50. Internal validation of the regression model was performed using bootstrap resampling (1000 samples) to assess the stability of the identified predictors.

Missing data were evaluated by examining the proportion of missing values for each variable. Cases with missing data were excluded from group comparisons. Laboratory variables with a high proportion of missing values (> 20%) were excluded from ROC and multivariable regression analyses. For the remaining variables, analyses were performed using available cases.

A p-value < 0.05 was considered statistically significant. All analyses were performed using IBM SPSS Statistics, version 30.0.

Ethical aspects

This study was approved on July 3, 2020, by the Lithuanian Bioethics Committee (approval number L-20–3/1), with amendments approved on July 25, 2022, January 25, 2023, December 13, 2023, and December 20, 2024. Signing informed consent was waived for retrospectively enrolled participants (from March 13, 2020, to June 30, 2020), whereas from July 1, 2020, to December 31, 2021, only participants who signed the written informed consent were admitted to the current study. During this study period (from July 3, 2020, to December 31, 2021), after obtaining approval from the Lithuanian Bioethics Committee, only the researchers had access to information that could identify individual participants included in the study (retrospectively and prospectively), allowing them to collect the required data in accordance with the study protocol. After this period, once the necessary data had been collected, only depersonalized data was used for further analysis.

Results

Baseline characteristics

A total of 495 adult patients hospitalized with laboratory-confirmed COVID-19 were included in the study. Of these, 52.9% were male. The median age was 55 years, and 37.4% of patients were aged 60 years or older. Almost two-thirds of the

patients (61.2%) had at least one underlying medical condition. The most frequent comorbidities were arterial hypertension (51.5%), other cardiovascular diseases (20.8%), and diabetes mellitus (11.1%). The median BMI was 29.7 kg/m^2. Half of the participants were obese (n = 137/271, 50.6%). While information on smoking was missing for 257 participants, among those assessed, 29.4% were identified as former smokers, and 7.1% as current smokers. Almost 6% of patients were vaccinated against COVID-19 (Table 1).

Two hundred ninety-one patients (58.8%) developed severe COVID-19 disease. Severe cases were more often male, about 10 years older, and had statistically significantly higher BMI compared with non-severe cases (Table 1).

Comorbidities were significantly more common among those with severe disease (68.4% vs. 51.0%). COPD (4.5% vs. 0.5%), arterial hypertension (59.1% vs. 40.7%), and other cardiovascular diseases (24.7% vs. 15.2%) were more prevalent among severe cases compared to those with non-severe COVID-19. Diabetes mellitus was also more frequent in the severe COVID-19 disease course group (13.4% vs. 7.8%), though this did not reach statistical significance (Table 1).

Clinical symptoms

The clinical symptoms reported by patients at the time of hospital admission are compiled in Table 2. Throughout the entire sample, the most frequently reported symptoms were malaise (77.0%), subfebrile fever (65.9%), cough (69.7%), and shortness of breath (40.5%). Most patients (81.5%) experienced the sudden onset of the disease.

Patients who developed severe COVID-19 disease were significantly more likely to present with general malaise, cough, fever, shortness of breath, and tachypnoea. Other symptoms that were also more common in severe cases included chills, chest pain, dizziness, confusion, general deterioration, and diarrhoea. Symptoms such as headache, myalgia, sore throat, coryza, palpitations, nausea, vomiting, ageusia, and anosmia did not differ significantly between these two groups (Table 2).

Regarding timing, patients with severe COVID-19 were admitted to the hospital later after the onset of symptoms compared to the non-severe patient group. The median time from symptom onset to hospitalization was 7 days (IQR: 4–9) in the severe group and 4 days (IQR: 2–8) in the non-severe group ($p < 0.001$).

Objective clinical findings and laboratory parameters on admission

Patients with severe disease had slightly higher body temperature (36.9°C vs. 36.8°C, $p = 0.025$) compared with patients in the non-severe group. The median respiratory rate was similar between groups (both 16 breaths/min), but the distribution was significantly broader in the severe group ($p < 0.001$). Additionally, the NEWS, a composite indicator of clinical deterioration, was notably higher in the severe COVID-19 group (median 3 vs. 1, $p < 0.001$).

The median oxygen saturation on admission was significantly lower in the severe COVID-19 group (95%, IQR: 92–96) compared to the non-severe group (97%, IQR: 96–98; $p < 0.001$), and the leftward shift of the cumulative distribution curve in the severe COVID-19 group demonstrated consistently lower oxygen saturation values across the entire distribution (Fig 1).

At the time of hospitalization, among patients who later developed severe COVID-19, 84.2% were classified as low risk based on a NEWS score, 11.7% as medium risk, and 4.1% as high risk. All patients with non-severe COVID-19 on admission were classified as low risk.

On admission, inflammatory markers were substantially higher in patients who developed a severe COVID-19 disease course. Median CRP levels were over nine times higher in the severe group (56.0 vs. 6.2 mg/L), and IL-6 levels were more than three times higher (33.4 vs. 9.9 ng/L). The concentrations of other inflammatory markers, such as ferritin (480.0 vs. 215.0 µg/L), fibrinogen (5.4 vs. 4.4 g/L), were also significantly higher in the severe COVID-19 course group.

Differences in haematological parameters at the time of hospitalization included a lower lymphocyte count (1.05 vs. 1.40×10^9/L) and higher neutrophil count (4.14 vs. 3.30×10^9/L) in the severe group. Total leukocyte and platelet count did not differ significantly between the groups.

Table 1. Baseline characteristics of hospitalized COVID-19 patients for the total sample and by disease severity.

Characteristic	N	Total (N = 495), n (%)	Non-severe COVID-19 (N = 204), n (%)	Severe COVID-19 (N = 291), n (%)	p-value
Age in years, median (IQR)	495	55 (43–65)	48 (35–61.75)	58 (48–68)	<0.001
Age groups, years					
18–29	39	39 (7.9)	33 (16.2)	6 (2.1)	<0.001
30–39	56	56 (11.3)	34 (16.7)	22 (7.6)	
40–49	99	99 (20.0)	47 (23.0)	52 (17.9)	
50–59	116	116 (23.4)	34 (16.7)	82 (28.2)	
60–69	105	105 (21.2)	32 (15.7)	73 (25.1)	
70–79	45	45 (9.1)	13 (6.4)	32 (11.0)	
≥ 80	35	35 (7.1)	11 (5.4)	24 (8.3)	
Male sex	495	262 (52.9)	95 (46.6)	167 (57.4)	0.018
Smoking					
Never smoked	238	151 (63.5)	39 (66.1)	112 (62.6)	0.006
Former smoker		70 (29.4)	11 (18.6)	59 (33.0)	
Current smoker		17 (7.1)	9 (15.3)	8 (4.5)	
Obesity	271	137 (50.5)	24 (29.6)	113 (59.5)	<0.001
Any comorbidities	495	303 (61.2)	104 (51.0)	199 (68.4)	<0.001
Comorbidities					
None	495	192 (38.8)	100 (49.0)	92 (31.6)	<0.001
One	495	137 (27.7)	51 (25.0)	86 (29.6)	
Two or more	495	166 (33.5)	53 (26.0)	113 (38.8)	
Pregnancy (only female)	233	5 (0.02)	0 (0.0)	5 (0.02)	–
Anaemia or any other haematological disease	495	40 (8.1)	13 (6.4)	27 (9.3)	0.243
Asthma	495	17 (3.4)	6 (2.9)	11 (3.8)	0.614
COPD	495	14 (2.8)	1 (0.5)	13 (4.5)	0.009
Other pulmonary diseases, not asthma or COPD	495	11 (2.2)	3 (1.5)	8 (2.8)	0.266
Non-haematological oncological disease	495	28 (5.7)	8 (3.9)	20 (6.9)	0.159
Arterial hypertension	495	255 (51.5)	83 (40.7)	172 (59.1)	<0.001
Other cardiovascular disease (not arterial hypertension)	495	103 (20.8)	31 (15.2)	72 (24.7)	0.010
Dementia	495	13 (2.6)	5 (2.5)	8 (2.8)	0.838
Diabetes mellitus	495	55 (11.1)	16 (7.8)	39 (13.4)	0.053
Immunodeficiency	495	15 (3.0)	3 (1.5)	12 (4.1)	0.090
Chronic liver disease, including cirrhosis	495	18 (3.6)	9 (4.4)	9 (3.1)	0.440
Neuromuscular disease	495	12 (2.4)	4 (2.0)	8 (2.8)	0.403
Chronic kidney disease	495	32 (6.5)	10 (4.9)	22 (7.6)	0.236
Rheumatological disease	495	17 (3.4)	5 (2.5)	12 (4.1)	0.314
Prior stroke	495	18 (3.6)	9 (4.4)	9 (3.1)	0.440
Tuberculosis	495	3 (0.6)	3 (1.5)	0 (0.0)	0.069
Vaccinated against COVID-19	495	29 (5.9)	5 (2.5)	24 (8.3)	0.007

Mann-Whitney U test; χ^2 test or Fisher's exact test, as appropriate, were used for calculations.

IQR – interquartile range; COPD – chronic obstructive pulmonary disease.

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There were also other laboratory analytes that concentrations were statistically significantly higher among severe cases: LDH (303.0 vs. 206.0 U/L), AST (38.0 vs. 24.0 U/L), and ALT (34.0 vs. 24.0 U/L), lactate (1.4 vs. 1.1 mmol/L), D-dimer (425.0 vs. 265.0 μg/L), fibrinogen (5.4 vs. 4.4 g/L,), troponin I (8.0 vs. 3.8 ng/L), NT-proBNP (170.3 vs. 98.0 ng/L),

Table 2. Clinical symptoms of COVID-19 patients at the time of hospitalization by disease severity (symptoms were reported by patients).

Symptoms	N	Total (N = 495), n (%)	Non-severe COVID-19 (N = 204), n (%)	Severe COVID-19 (N = 291), n (%)	p-value
Sudden onset of symptoms	482	393 (81.5)	168 (84.4)	225 (79.5)	0.171
Subfebrile fever	495	326 (65.9)	122 (59.8)	204 (70.1)	0.017
Febrile fever	495	179 (36.2)	46 (22.6)	133 (45.7)	<0.001
Chills	490	143 (29.2)	41 (20.4)	102 (35.3)	<0.001
Tachypnoea	495	91 (18.4)	7 (3.4)	84 (28.9)	<0.001
Malaise	488	376 (77.1)	124 (62.0)	252 (87.5)	<0.001
Headache	488	152 (31.2)	66 (33.0)	86 (29.9)	0.461
Dizziness	488	85 (17.4)	15 (7.5)	70 (24.3)	<0.001
Confusion	491	24 (4.9)	5 (2.5)	19 (6.6)	0.036
Myalgia	488	142 (29.1)	61 (30.5)	81 (28.1)	0.570
Sore throat	488	101 (20.7)	50 (25.0)	51 (17.7)	0.051
Coryza	488	73 (15.0)	30 (15.0)	43 (14.9)	0.983
Cough	489	341 (69.7)	126 (63.0)	215 (74.4)	0.007
Shortness of breath	489	198 (40.5)	36 (18.0)	162 (56.1)	<0.001
Chest pain	488	114 (23.4)	32 (16.0)	82 (28.5)	0.001
Palpitations	488	41 (8.4)	13 (6.5)	28 (9.7)	0.207
General deterioration	492	151 (30.7)	37 (18.2)	114 (39.5)	<0.001
Nausea	488	54 (11.1)	18 (9.0)	36 (12.5)	0.225
Vomiting	495	12 (2.4)	4 (2.0)	8 (2.8)	0.769
Diarrhoea	491	62 (12.6)	15 (7.5)	47 (16.2)	0.004
Abdominal pain	488	28 (5.7)	11 (5.5)	17 (5.9)	0.851
Ageusia	487	75 (15.4)	25 (12.5)	50 (17.4)	0.139
Anosmia	487	109 (22.4)	44 (22.0)	65 (22.7)	0.866
Conjunctivitis	495	11 (2.2)	4 (2.0)	7 (2.4)	1.000
Rash/other dermatological manifestation	495	4 (0.8)	1 (0.5)	3 (1.0)	0.646

Mann-Whitney U test, χ^2 test, or Fisher's exact test, as appropriate, were used for calculations.

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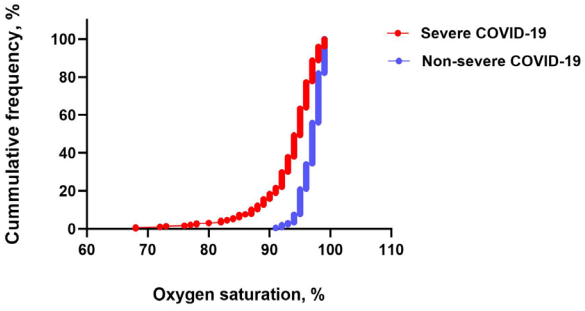

Fig 1. Cumulative distribution of oxygen saturation on admission by COVID-19 severity.

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urea (5.0 vs. 4.3 mmol/L) and creatinine levels (80.0 vs. 74.4 μmol/L). All analysed laboratory findings are detailed and presented in Table 3.

Radiological findings and oxygen therapy

Radiological imaging was performed for the majority of patients during hospitalization (Table 4). Among those who underwent chest x-ray, pulmonary infiltrates were significantly more common in patients with severe disease (90.9% vs. 50.5%). Pleural effusion (9.8% vs. 1.6%) and pulmonary stasis (17.5% vs. 4.3%) were also more frequently detected in the severe COVID-19 group.

Chest CT was performed in a subset of 126 (25.5%) patients. In the severe group, CT findings more frequently revealed peripheral opacities (59.7% vs. 38.8%), parenchymal infiltration (57.1% vs. 36.7%), and pleural effusion (16.9% vs. 4.1%). Although ground-glass opacities and consolidation were more common in severe cases, these differences were not statistically significant (71.4% vs. 55.1% and 29.9% vs.16.3%, respectively).

Table 3. Laboratory characteristics of hospitalized COVID-19 patients on admission by disease course.

Variable	Total (N = 495)		Non-severe COVID-19 (N = 204)		Severe COVID-19 (N = 291)		p-value
	N	Median (IQR)	N	Median (IQR)	N	Median (IQR)	
WBC, x10^9/L	481	5.6 (4.3–7.1)	193	5.6 (4.4–6.9)	288	5.6 (4.2–7.4)	0.601
Neutrophils, x10^9/L	481	3.8 (2.7–5.2)	193	3.3 (2.4–4.6)	288	4.1 (2.8–5.7)	<0.001
Lymphocytes, x10^9/L	481	1.2 (0.8–1.6)	193	1.4 (1.1–1.8)	288	1.1 (0.8–1.4)	<0.001
Platelets, x10^9/L	481	187 (149–236)	193	190 (160–243)	288	182 (144–232)	0.097
ALP, U/L	392	64.0 (52.0–80.3)	139	65.0 (53.8–81.0)	253	63.0 (51.0–84.5)	0.288
ALT, U/L	443	30.0 (18.0–48.0)	169	24.0 (15.0–37.8)	274	34.0 (21.5–56.0)	<0.001
AST, U/L	430	33.0 (24.0–50.0)	156	24.0 (19.2–33.8)	274	38.0 (29.0–64.3)	<0.001
Ferritin, μg/L	398	358.7 (168.6–775.5)	131	215.0 (97.4–409.2)	267	480.0 (227.3–1,113.7)	<0.001
IL-6, ng/L	401	25.0 (11.0–50.6)	128	9.88 (4.9–22.4)	273	33.40 (16.9–63.0)	<0.001
LDH, U/L	407	269.0 (208.0–348.6)	140	206.4 (179.9–252.7)	267	303.0 (249.5–393.0)	<0.001
D-dimer, μg/L	395	375.0 (215.0–640.0)	126	265.0 (145.0–521.3)	269	425.0 (267.5–695.0)	<0.001
Fibrinogen, g/L	360	5.1 (4.2–6.1)	117	4.4 (3.6–5.5)	243	5.4 (4.6–6.3)	<0.001
CRP, mg/L	479	29.9 (6.8–84.1)	193	6.2 (1.9–21.3)	286	56.0 (25.6–112.0)	<0.001
Lactate, mmol/L	367	1.3 (1.0–1.8)	110	1.1 (0.8–1.5)	257	1.4 (1.0–1.9)	<0.001
Troponin I, ng/L	387	6.6 (3.0–13.0)	124	3.8 (2.0–8.8)	263	8.0 (4.0–16.0)	<0.001
NT-proBNP, ng/L	339	147.2 (71.6–386.0)	109	98.0 (54.6–249.0)	230	170.3 (89.0–429.4)	<0.001
GGT, U/L	391	34.0 (18.0–76.0)	135	21.0 (14.0–58.9)	256	40.5 (22.0–85.1)	<0.001
PT, %	387	90.0 (78.0–104.0)	127	90.0 (77.0–103.0)	260	91.0 (78.0–104.0)	0.630
INR	393	1.04 (0.98–1.11)	128	1.04 (0.99–1.12)	265	1.04 (0.98–1.11)	0.428
Creatinine, μmol/L	467	77.6 (64.0–95.0)	180	74.4 (62.0–87.7)	287	80.0 (65.8–97.4)	0.007
Urea, mmol/L	376	4.7 (3.6–6.4)	125	4.3 (3.3–5.3)	251	5.0 (3.9–6.8)	<0.001

Mann-Whitney U test was used for calculations.

ALP – alkaline phosphatase; ALT – alanine aminotransferase; AST – aspartate aminotransferase; CRP – C-reactive protein; GGT – gamma-glutamyl transferase; INR – international normalized ratio; IL-6 – interleukin 6; IQR – interquartile range; LDH – lactate dehydrogenase; NT-proBNP – N-terminal pro-B-type natriuretic peptide; PT – prothrombin time; WBC – white blood cell.

Reference values: WBC: 4.0–9.8 x10^9/L; neutrophil count: 1.5–6.0 x109/L; lymphocyte count: 1.0–4.0 x10^9/L; platelets: 140–450 x10^9/L; ALP: 40–150 U/L; ALT: ≤40 U/l; AST: ≤40 U/L; ferritin: 25–350 μg/L (for men), 13–232 μg/L (for women); IL-6: 0–7 ng/L; LDH: 125–243 U/L; D-dimer: < 250 μg/L; fibrinogen: 2–4 g/L; CRP: < 5 mg/L; lactate: 0.50–2.20 mmol/L; troponin I: < 19 ng/L; NT-proBNP: for acute heart failure suspicion – < 450 ng/L (for <50 years old); < 900 ng/L (for 50–75 years old); < 1800 ng/L (for >75 years old), for chronic heart failure suspicion – < 125 ng/L; GGT: ≤36 U/L; PT: 70–130%; INR: 0.90–1.19; creatinine: 64–104 μmol/L (for males), 49–90 μmol/L (for females); urea: 2.5–7.5 mmol/L.

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Table 4. Instrumental investigation findings, oxygen therapy, complications, and hospital discharge status of COVID-19 patients by disease course.

Characteristic	Total (N = 495), n (%)	Non-severe COVID-19 (N = 204), n (%)	Severe COVID-19 (N = 291), n (%)	p-value
Chest X-ray	473 (95.6)	187 (91.7)	286 (98.3)	<0.001
Infiltration	354 (75.0)	94 (50.5)	260 (90.9)	<0.001
Pleural effusion	31 (6.6)	3 (1.6)	28 (9.8)	<0.001
Infiltration opacity > 50%	45 (18.4)	0 (0.0)	45 (39.1)	<0.001
Stasis	58 (12.3)	8 (4.3)	50 (17.5)	<0.001
Chest computed tomography	126 (25.5)	49 (24.0)	77 (26.5)	0.539
Peripheral opacity	65 (51.6)	19 (38.8)	46 (59.7)	0.022
Ground-glass opacity	82 (65.1)	27 (55.1)	55 (71.4)	0.061
Consolidation	31 (24.6)	8 (16.3)	23 (29.9)	0.085
Infiltration	62 (49.2)	18 (36.7)	44 (57.1)	0.025
Pleural effusion	15 (11.9)	2 (4.1)	13 (16.9)	0.031
Pneumonia	395 (82.1)	111 (58.4)	284 (97.6)	<0.001
Unilateral	60 (15.2)	31(27.9)	29 (10.2)	<0.0010
Bilateral	335 (84.8)	80 (72.1)	255 (89.8)	
Oxygen therapy	291 (58.8)	0 (0.0)	291 (100.0)	<0.001
Nasal cannula	87 (17.6)	0 (0.0)	87 (29.9)	<0.001
Face mask	218 (44.0)	0 (0.0)	218 (74.9)	<0.001
High-flow therapy	29 (5.9)	0 (0.0)	29 (10.0)	<0.001
Non-invasive ventilation	5 (1.0)	0 (0.0)	5 (1.7)	0.081
Mechanical ventilation	14 (2.8)	0 (0.0)	14 (4.8)	0.001
ECMO	3 (0.6)	0 (0.0)	3 (1.0)	0.272
Complications				
Any complication	114 (25.1)	10 (4.9)	104 (35.7)	<0.001
Acute respiratory distress syndrome	46 (9.3)	0 (0.0)	46 (15.8)	<0.001
Bronchiolitis	1 (0.2)	1 (0.5)	0 (0.0)	0.412
Secondary bacterial pneumonia (confirmed by positive bronchoalveolar lavage culture)	10 (2.0)	0 (0.0)	10 (3.4)	0.007
Other secondary bacterial infection (confirmed by positive culture)	60 (12.1)	5 (2.5)	55 (18.9)	<0.001
Sepsis	18 (3.6)	0 (0.0)	18 (6.2)	<0.001
Acute renal failure	18 (3.6)	1 (0.5)	17 (5.8)	0.002
Heart failure	5 (1.0)	0 (0.0)	5 (1.7)	0.081
Multiple organ dysfunction	14 (2.8)	0 (0.0)	14 (4.8)	0.001
Dermatological complications	3 (0.6)	1 (0.5)	2 (0.7)	1.000
Critical illness myopathy	1 (0.2)	0 (0.0)	1 (0.3)	1.000
Pulmonary embolism	8 (1.6)	0 (0.0)	8 (2.8)	0.024
Other complication	27 (5.5)	2 (1.0)	25 (8.6)	<0.001
Transferred to ICU or HDU	54 (10.9)	0 (0.0)	54 (18.6)	<0.001
Transferred to ICU	49 (9.9)	0 (0.0)	49 (16.8)	<0.001
Transferred to HDU	11 (2.2)	0 (0.0)	11 (3.8)	0.004
Length of stay				
Length of stay in hospital in days, median (IQR)	10.0 (7.0–14.0)	8.0 (4.0–11.0)	12.0 (9.0–16.0)	<0.001
Length of stay in ICU in days, median (IQR)	7.0 (5.0–13.0)	0.0 (0.0–0.0)	7.0 (5.0–13.0)	–
Length of stay in HDU in days, median (IQR)	8.0 (4.0–13.0)	0.0 (0.0–0.0)	8.0 (4.0–13.0)	–

(Continued)

Table 4. (Continued)

Characteristic	Total (N = 495), n (%)	Non-severe COVID-19 (N = 204), n (%)	Severe COVID-19 (N = 291), n (%)	p-value
Length of stay in ICU and HDU in days, median (IQR)	8.0 (5.0–14.0)	0.0 (0.0–0.0)	8.0 (5.0–14.0)	–
Hospital discharge status				
Discharged/Transferred	474 (95.8)	204 (100.0)	270 (92.8)	<0.001
Died during the hospitalization	21 (4.2)	0 (0.0)	21 (7.2)	

Mann-Whitney U test, χ^2 test, or Fisher's exact test, as appropriate, were used for calculations.

ECMO – extracorporeal membrane oxygenation; HDU – high dependency unit; ICU – intensive care unit; IQR – interquartile range.

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A clinical diagnosis of pneumonia was established in nearly all patients with severe COVID-19 (97.6%), compared with 58.4% in non-severe cases, with bilateral pneumonia also significantly more frequent in severe cases (89.8% vs. 72.1%).

The most common oxygen therapy modalities for severe COVID-19 patients included a face mask (74.9%) and a nasal cannula (29.9%). High-flow oxygen therapy was administered to 10.0% of severe patients, and smaller proportions required non-invasive ventilation (1.7%) or mechanical ventilation (4.8%). ECMO was used in 1.0% of severe cases (Table 4).

Complications and hospital discharge status

Severe COVID-19 cases were associated with a significantly higher complication rate. Over one-third (35.7%) of patients in the severe group experienced at least one complication, compared with 4.9% of those in the non-severe group. The most common complication was other (than secondary bacterial pneumonia) secondary bacterial infection confirmed by positive culture. Overall, it developed in 60 (12.1%) patients and was statistically more common in the severe COVID-19 group (18.9% vs. 2.5%). The second most common complication was ARDS, which developed only in 15.8% of severe cases. Other complications more commonly developed in severe COVID-19 patients compared with non-severe patients were sepsis (6.2% vs. 0.0%), acute kidney injury (5.8% vs. 0.5%), secondary bacterial pneumonia (3.4% vs. 0.0%), and pulmonary embolism (2.8% vs. 0.0%). Multiple organ dysfunction syndrome occurred in 4.8% of severe cases. These and additional complications are detailed in Table 4.

Transfer to ICU/HDU was required for 18.6% of patients in the severe group, while none of the non-severe patients needed such care. The median hospital stay was longer in the severe COVID-19 group (12 days) than in non-severe cases (8 days). Among patients transferred to ICU/HDU, the median length of stay in critical care was 8 days (Table 4).

In-hospital mortality occurred exclusively in the severe group, affecting 7.2% of patients. The discharge/transfer rate was correspondingly lower in the severe group (92.8% vs. 100.0%) (Table 4). Overall, 22 (4.4%) patients were transferred to other facilities for continued care.

Predictive accuracy of laboratory parameters for severe COVID-19 disease

The predictive performance of initial laboratory tests for identifying patients at risk of developing severe COVID-19 was assessed using ROC curve analysis. The area under the curve (AUC) values for each parameter are presented in Table 5.

Among the biomarkers analysed, CRP demonstrated the highest predictive value, with an AUC of 0.84, followed by LDH (AUC = 0.80), IL-6 (AUC = 0.77), AST (AUC = 0.77), and ferritin (AUC = 0.72) (Table 5, Fig 2).

Predictors associated with severe COVID-19 disease course

To identify the key factors associated with severe COVID-19, we performed logistic regression analysis. In univariable analysis, a number of variables showed statistically significant associations with severe disease, including older age, male

Table 5. Predictive accuracy for severe COVID-19 of initial laboratory tests performed on admission.

Variable	AUC (95% CI)	p-value
WBC count, x10^9/l	0.51 (0.46–0.57)	0.601
Neutrophil count, x10^9/l	0.61 (0.56–0.66)	<0.001
Lymphocyte count, x10^9/l	0.31 (0.26–0.36)	<0.001
ALT, U/l	0.65 (0.60–0.70)	<0.001
AST, U/l	0.77 (0.72–0.82)	<0.001
Ferritin, µg/l	0.72 (0.67–0.78)	<0.001
IL-6, ng/l	0.77 (0.72–0.82)	<0.001
LDH, U/l	0.80 (0.76–0.85)	<0.001
D-dimer, µg/l	0.65 (0.59–0.71)	<0.001
CRP, mg/l	0.84 (0.80–0.87)	<0.001
GGT, IU/l	0.64 (0.58–0.70)	<0.001
Creatinine, µmol/l	0.57 (0.52–0.63)	0.007

ROC analysis was used for calculations. Sample sizes for every variable are listed in Table 3.

ALT – alanine aminotransferase; AST – aspartate aminotransferase; AUC – area under the ROC curve; CI – confidence interval; CRP – C-reactive protein; GGT – gamma-glutamyl transferase; IL-6 – interleukin 6; LDH – lactate dehydrogenase; WBC – white blood cell.

Reference values: WBC: 4.0–9.8 x10^9/L; neutrophil count: 1.5–6.0 x10^9/L; lymphocyte count: 1.0–4.0 x10^9/L; ALT: ≤40 U/l; AST: ≤40 U/L; ferritin: 25–350 µg/L (for men), 13–232 µg/L (for women); IL-6: 0–7 ng/L; LDH: 125–243 U/L; D-dimer: < 250 µg/L; CRP: < 5 mg/L; GGT: ≤36 U/L; creatinine: 64–104 µmol/L (for males), 49–90 µmol/L (for females).

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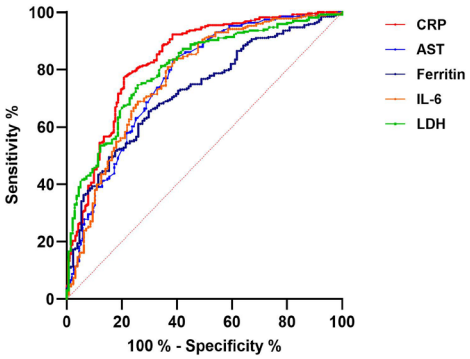

Fig 2. Predictive accuracy of initial laboratory tests on admission for COVID-19 disease severity: ROC curve analysis. AST – aspartate aminotransferase, CRP – C-reactive protein, IL-6 – interleukin 6, LDH – lactate dehydrogenase.

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sex, obesity, arterial hypertension, other cardiovascular disease (not arterial hypertension), COPD, subfebrile fever, febrile fever, chills, tachypnoea, malaise, dizziness, confusion, cough, shortness of breath, chest pain, general deterioration, diarrhoea, lymphocyte count, neutrophil count, ALT, AST, ferritin, IL–6, LDH, D-dimer, fibrinogen, CRP, lactate, creatinine, and urea (S1 Table).

In the multivariable model, six predictors were independently associated with the severe COVID-19 disease course, and five remained significant after internal validation (Fig 3). Older age was associated with an increased odds of severe COVID-19 (OR 1.04 per year, 95% CI 1.00–1.08, p = 0.040), while obesity conferred a 3.55-fold higher odds compared with non-obese patients (OR 3.55, 95% CI 1.35–9.30, p = 0.010). Among symptoms at admission, febrile fever was associated with an increased odds of severe COVID-19 (OR 3.41, 95% CI 1.09–10.73, p = 0.036). Lymphopenia on admission was likewise identified as a significant predictor (OR 3.70, 95% CI 1.37–9.99, p = 0.010). In addition, higher levels of LDH and CRP were independently associated with severe COVID-19 (LDH: OR 1.008, 95% CI 1.00–1.01, p = 0.022; CRP: OR 1.021, 95% CI 1.01–1.04, p = 0.002), corresponding to an 8% increase in odds of severe disease per 10 U/L increase in LDH and a 21% increase per 10 mg/L increase in CRP. The model demonstrated acceptable explanatory power (Cox & Snell $R^2 = 0.362$; Nagelkerke $R^2 = 0.535$). The Hosmer–Lemeshow goodness-of-fit test indicated good calibration ($\chi^2 = 3.43$, df = 8, p = 0.905). Using a probability cut-off of 0.50, the model correctly classified 83.9% of cases. Internal validation using bootstrap resampling confirmed that age, obesity, lymphopenia, LDH, and CRP remained significant predictors of severe COVID-19 (Fig 3).

Variation in COVID-19 manifestation across pandemic waves

A total of 337 patients were hospitalized during the Alpha variant wave, 96 during the Beta variant wave, and 62 during the Delta variant wave. The median age of patients did not differ significantly between waves: Alpha 56 years (IQR 43–68), Beta 56.5 years (IQR 47–61), and Delta 50.5 years (IQR 41–60.25) (p = 0.142). The proportion of male patients was 49.3% in the Alpha wave, 62.5% in the Beta wave, and 58.1% in the Delta wave.

Severe COVID-19 disease was diagnosed in 43.6% of patients in wave 1, 93.8% in wave 2, and 87.1% in wave 3. Severe disease was statistically significantly (p-values <0.001) more common in wave 2 and wave 3 as compared with wave 1.

Compared with wave 1, patients in waves 2 and 3 more frequently experienced chills, malaise, dizziness, coryza, cough, shortness of breath, palpitations, general deterioration, and nausea. Additionally, wave 2 patients more often presented with febrile fever and tachypnoea, while wave 3 patients more commonly reported headache, chest pain, vomiting, diarrhoea, ageusia, anosmia, and conjunctivitis. Compared with wave 2, patients in wave 3 more frequently had chills, headache, myalgia, cough, palpitations, anosmia, and conjunctivitis, and less frequently – febrile fever (S2 Table).

Compared with wave 1 patients, initial laboratory test results of patients in waves 2 and 3 showed significantly higher neutrophil counts and lower lymphocyte counts, more pronounced elevations in inflammatory markers such as CRP,

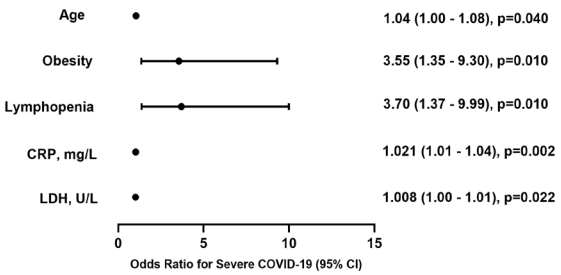

Fig 3. Significant predictors associated with severe COVID-19 disease course, multivariable logistic regression model after internal validation. CI – confidence interval; CRP – C-reactive protein; LDH – lactate dehydrogenase.

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ferritin, IL-6, and fibrinogen, as well as higher levels of biomarkers indicating tissue injury or organ dysfunction, including LDH, D-dimer, ALT, AST, GGT, NT-proBNP, and lactate (S3 Table).

Chest imaging findings revealed that patients in waves 2 and 3 had significantly higher rates of radiologically confirmed pneumonia, especially bilateral, compared to wave 1 ($p < 0.001$). Infiltrates involving more than 50% of the lung fields on chest X-ray were more frequently observed in waves 2 and 3, while ground-glass opacities on chest CT were markedly more common in wave 3 compared to wave 1 (S4 Table).

Patients in waves 2 and 3 more often required oxygen therapy, particularly via face masks, compared with those in wave 1 ($p < 0.001$). The need for high-flow oxygen therapy was significantly higher in wave 3 than in wave 1 (0.6% vs 14.5%, $p < 0.001$), while there were no differences between waves 2 and 3 on these parameters (S4 Table).

The incidence of complications – including ARDS, secondary bacterial infections, and PATE – was more frequent in the later waves. ARDS occurred significantly more often in wave 3 than in wave 1 (17.7% vs. 8.9%) and wave 2 (17.7% vs. 5.2%). Non-pulmonary secondary bacterial infections were also more frequent in wave 2 compared with wave 1 (20.8% vs. 9.5%) (S4 Table). Patients were less frequently transferred to ICU/HDU in wave 1 compared with wave 2 (7.7% vs. 15.6%) and wave 3 (7.7% vs. 21.0%), and there were no differences between wave 2 and 3. There were no significant differences in length of stay in the hospital and the result of hospitalization between waves (S4 Table).

Discussion

In this observational study, we evaluated the demographic, clinical, laboratory, radiological characteristics, and risk factors associated with a severe COVID-19 disease course among hospitalized adult patients during the first three pandemic waves in a tertiary care centre in Vilnius, Lithuania. As one of the few studies from the Baltic region, our findings contribute to a more geographically diverse understanding of COVID-19 heterogeneous pathogenesis and help address the global imbalance in COVID-19 data representation. We identified older age, obesity, lymphopenia, and higher concentrations of CRP and LDH to be independent risk factors for developing a severe COVID-19 disease course described as the need for oxygen therapy. These findings align with a large body of global research demonstrating that age-related and adiposity-driven immune dysregulation, inflammation, the presence of multiple comorbid conditions, and end-organ injury are central to the pathophysiology, progression, and poor outcomes of COVID-19 [3,5,6,20].

The predictive model construction, which includes many patient-specific disease characteristics, laboratory test results, and imaging modality specifics, has not been a new endeavour since the start of the pandemic. Several other clinical studies, even using machine learning techniques, have also aimed to identify key factors associated with COVID-19 severity and outcomes. However, their accuracy has been repeatedly questioned due to many limitations [21–23]. Numerous studies' predictive models have identified demographic and clinical factors associated with worse outcomes, including older age [24,25], male sex [26,27], obesity [28–34], and the presence of underlying comorbidities such as cardiovascular disease, diabetes mellitus, chronic lung disease (especially COPD) [35–41], immunosuppression [3–6,42], and neuropsychiatric comorbidities [37]. In our study, older age and obesity were the only demographic or comorbidity factors that independently increased the odds of developing a severe COVID-19 disease course. The observed association between older age and severe COVID-19 is consistent with previous evidence showing that advanced age is a major risk factor for severe disease and mortality, likely due to age-related immune dysregulation, including immunosenescence and chronic low-grade inflammation [43–46]. In addition, in a meta-analysis performed by Foldi et al. in 2020, obesity was identified as a significant risk factor for ICU admission (OR=1.21, CI: 1.002–1.46) and for the need of invasive mechanical ventilation (OR=2.05, CI: 1.16–3.64) [30]. Even though in our study other demographic factors and comorbidities, such as male sex, COPD, arterial hypertension, and other cardiovascular diseases, were more prevalent among the severe COVID-19 cases as compared to non-severe cases, they were not statistically significant predictors of severe COVID-19 disease course. Smoking is another factor frequently identified as a risk factor for severe COVID-19 disease [34,37]. In our study, the number of current smokers within the COVID-19 severity subgroups was too small to allow for meaningful statistical analysis.

Therefore, this variable was not included in the analysis, and we could not assess its relevance to the development of the severe COVID-19 disease course in our research.

Our results showed that systemic and lower respiratory symptoms were more commonly associated with severe COVID-19, while upper respiratory symptoms may be linked to milder illness. In the multivariable logistic regression analysis, febrile fever initially emerged as the only clinical variable associated with severe COVID-19. However, after internal validation of the model, this association was no longer statistically significant. These findings suggest that clinical symptoms at admission may have limited value as independent predictors of severe COVID-19. This observation is consistent with previous large-scale studies showing that commonly reported symptoms, such as fever, cough, or headache, do not independently predict COVID-19 severity (ICU admission or critical illness) after adjustment for confounding factors, highlighting the greater prognostic relevance of demographic characteristics and laboratory biomarkers [47–53]. Although dyspnoea has been identified as the symptom most consistently associated with severe outcomes in prior studies [47–53], it remains unclear whether symptoms such as shortness of breath should be considered independent predictors of severe COVID-19 or rather manifestations of ongoing clinical deterioration. Furthermore, the longer delay in hospital presentation among severe cases also suggested that early recognition and timely care could play a role in preventing disease progression. The finding that patients with severe COVID-19 were admitted to the hospital later after symptoms' onset than those with non-severe illness (7 days vs. 4 days, respectively) raises an important question: whether more severe symptoms appear later in the course of the disease or whether the patients seek medical help later in the disease course [54–57]. There was a possibility that some patients with milder symptoms at first might not be admitted to the hospital immediately but would return later when their condition worsened. We did not evaluate the fact of revisits to the emergency department in our study; therefore, we could not answer this question. This possibility emphasizes how crucial it is to recognize early clinical features, relevant aspects of patient history, or laboratory-based warning signs, which might point to a higher risk of developing severe disease. Better tools for the early identification of high-risk patients could ensure they get the necessary care at the right time.

This study reinforces the clinical utility of widely available laboratory tests for early risk stratification in COVID-19. Our research results demonstrated that CRP, LDH, IL-6, AST, and ferritin, performed on admission, demonstrated good accuracy for predicting severe COVID-19 cases. Multivariable analysis confirmed lymphopenia, elevated levels of CRP and LDH, together with age and obesity, to be predictors for a severe COVID-19 course. An integrated approach that combines biomarkers of inflammation, activation of the coagulation cascade, organ injury, and immune suppression with simple clinical observations is consistent with recent initiatives to create straightforward, scalable assessment instruments for the early detection of at-risk patients, particularly in limited-resource or high-burden settings [7,49]. The associations of severe COVID-19 with CRP, IL-6, LDH, AST, and ALT reinforce the prevailing notion that systemic inflammation and hepatic involvement are crucial factors in the initial phases of illness progression. LDH, in particular, has been widely recognized as a robust marker of tissue damage and hypoxia in severe viral pneumonias. In COVID-19 patients, higher LDH levels were related to increased odds of developing severe disease [58], higher mortality, longer invasive mechanical ventilation [59], and ARDS development [60]. Similarly, increased CRP and IL-6 levels were found in severe COVID-19 cases, indicating an infectious-inflammatory response [61].

A lower lymphocyte count was one of the main haematological differences observed in patients with severe COVID-19 at the time of hospitalization, reflecting the commonly reported lymphopenia in advanced cases. Lymphopenia also remained an independent risk factor for severe COVID-19 disease course, increasing the odds 3.70 times (95% CI: 1.37–9.99). Analogous results were published in a meta-analysis of 13 case series, which found that lymphopenia increases the risk of severe COVID-19 disease 3-fold [62]. While this marker may not be sensitive enough for broad screening, it may serve as a valuable rule-in criterion that could help to confirm the likelihood of severe disease when present. Additional studies support this finding, showing that patients with lower lymphocyte counts were more likely to be admitted to the ICU and had longer hospital stays [63]. Ongoing lymphopenia has also been linked to a higher risk

of developing ARDS [64], and when combined with T-cell suppression, it has been associated with more severe illness and worse clinical outcomes [20].

In addition to clinical history, imaging features like CT bilateral involvement, non-ground-glass opacities have emerged as strong predictors of disease severity and mortality [57]. When discussing chest CT findings, we found out that infiltration, peripheral opacity, and pleural effusion were significantly more common in severe COVID-19 cases. As chest CT was performed in only one-fourth of our cohort, we did not include CT findings in the final regression model and could not assess them as independent predictors.

A comparison of regional data from Central and Eastern Europe – an area still underrepresented in global COVID-19 research – adds important context and reinforces the key findings of our study. To contextualize our findings within our region, we reviewed Polish studies alongside a large Estonian population-based cohort. Though methodologically diverse, these regional datasets provide valuable insights into COVID-19 severity and its clinical predictors. A large retrospective cohort study from Estonia, using nationwide e-health records, analysed 66,295 individuals with confirmed COVID-19 and 254,958 population controls [65]. It found that male sex, older age, and comorbidity burden were strongly associated with disease severity, with renal disease, previous myocardial infarction, and obesity being the top contributors to critical illness. Predictors of mortality also included renal, liver, cerebrovascular disease, or cancer. Although this registry-based study did not assess clinical symptoms, its large-scale, anonymized dataset provides rare, population-level insights from the Baltic region. In Poland, a large single-centre cohort of 5,191 hospitalized patients found that diabetes was associated with higher rates of ICU admission and mortality [66]. Independent predictors of poor outcomes included chronic kidney disease, heart failure, elevated CRP, D-dimer, and hyperglycaemia, especially in patients with preexisting metabolic disorders. This aligns with the Estonian study's emphasis on renal and cardiometabolic comorbidities but adds detail by including laboratory-based predictors. Another Polish study emphasized baseline comorbidities, such as COPD, hypertension, and diabetes, as predictors of severe progression, reinforcing the broader pattern observed across all datasets [67]. Together, these findings from Central and Eastern European cohorts complement our results by showing consistent risk profiles. Despite differences in sample size and scope, this underscores the added value of including clinical symptoms and laboratory biomarkers to enhance risk stratification in hospital settings.

Our previous study on COVID-19 in-hospital mortality, based on a larger depersonalized cohort from the same institution, identified several overlapping and distinct predictors of adverse outcomes [68]. Specifically, in-hospital mortality was significantly associated with age, obesity, congestive heart failure, COPD, previous stroke, and elevated concentrations of urea (> 7.01 mmol/L), LDH (> 452.5 U/L), CRP (> 92.68 mg/L), IL-6 (> 69.55, ng/L), troponin I (> 18.95 ng/L), and ALT to AST ratio (> 1.49). Notably, older age, obesity, CRP, and LDH were significantly associated with both severe disease progression and mortality, underlining their central role in COVID-19 pathophysiology.

Although our analysis did not include data on specific SARS-CoV-2 variants, placing the findings within the context of Lithuania's COVID-19 wave timeline provides valuable insights into wave-specific variations in disease presentation. Lithuania's initial wave (spring 2020) was driven by the wild-type virus. During the study period, COVID-19 in Lithuania progressed through three distinct waves, dominated by the Alpha (until September 30, 2020), Beta (from October 1, 2020, to July 1, 2021), and Delta (until December 31, 2021) variants. Following our study period, Omicron and its sublineages (BA.1, BA.2, BA.4/BA.5) took hold in early 2022, marked by extremely high case numbers but consistently lower case severity and fewer ICU admissions compared to Delta. Our findings correspond with global observations and show that severe COVID-19 rates were significantly higher in wave 2 (93.8%) and wave 3 (87.1%) compared with wave 1 (43.6%) in Lithuania. Throughout the pandemic, each successive wave often brought shifts in hospital admission criteria and treatment recommendations as clinicians adapted to the virulence of circulating viral variants and incorporated accumulated knowledge. Notably, later waves dominated by Beta and Delta variants tended to involve more selective hospitalization, often reserving inpatient care for only the most severe cases, which likely amplified the observed severity rates despite advances in treatment and prevention protocols [69–72]. Also, the Alpha and Delta waves were characterized by higher

hospitalization and ICU admission rates, reflecting increased disease severity [71,73–75]. In contrast, the Omicron waves had reduced case severity, although the number of infections skyrocketed [76–78]. Therefore, while our results showed increased severity in later waves, this likely reflects a combination of biological factors – such as the greater virulence of the Delta variant – and health-care related factors, including stricter hospital admission driven by resource constraints and updated clinical guidelines.

Interestingly, although only a small proportion of the cohort was vaccinated, we observed a higher prevalence of vaccinated individuals in the severe disease group. In Lithuania, COVID-19 vaccination began on December 27, 2020, initially targeting healthcare workers. On March 26, 2021, eligibility expanded to include individuals with chronic illnesses and those aged 65 and older – groups inherently at greater risk of developing severe disease. Mass vaccination of the general population only commenced on May 31, 2021. As our study period largely overlapped with these early phases of the rollout, most vaccinated patients in our cohort likely belonged to high-risk groups predisposed to poorer outcomes. Similar patterns have been reported in other retrospective inpatient studies, where the protective effects of vaccination are confounded by the underlying vulnerability of early vaccine recipients, as well as by a lack of adjustment for timing, dose number, and immune response status [79]. Due to the small number of vaccinated individuals in our cohort, consistent with the limited vaccine availability during the early rollout phase, we could not perform detailed subgroup analyses or reliably assess the impact of vaccination on disease severity.

Altogether, our study underlines the multifactorial nature of severe COVID-19. The combination of older age, obesity, immune suppression, and organ dysfunction biomarkers defines a recognizable profile of patients at the highest risk for developing severe disease, which could support triage decisions and early treatment escalation in hospital settings. Furthermore, by contributing data from Lithuania, this study helps expand global understanding of COVID-19, particularly within the context of Central and Eastern Europe. These populations have been underrepresented in global research datasets, despite experiencing substantial burdens during the pandemic. Differences in healthcare infrastructure, vaccination strategies, and population characteristics may limit the applicability of risk prediction models developed in Western Europe, Asia, or North America to the Baltic region. Our findings thus offer important insights for regional decision-making and health system preparedness, while contributing to broader efforts toward international validation of risk prediction models.

Strengths and limitations

A major strength of our work is the inclusion of comprehensive variables, including symptoms, laboratory analytes, and imaging, from a single centre with standardized protocols, which helps to minimize interinstitutional variability.

However, several limitations ought to be acknowledged. First, the study was conducted at a single tertiary care centre, which may limit the generalizability of the findings to other healthcare settings with differing patient populations, clinical practices, and resource availability. As a tertiary hospital with strict admission criteria, our centre may have treated a higher proportion of patients with more severe disease, particularly during the later pandemic waves.

The partially retrospective design may have introduced information bias, particularly due to undocumented comorbidities and subjective clinical symptoms. Smoking status was not systematically recorded for retrospectively included patients, which resulted in a substantial proportion of missing smoking data and limited our ability to reliably assess its association with COVID-19 severity. Although BMI data were available for a large proportion of the cohort, it was not complete for all participants. To ensure transparency and minimize misinterpretation, both obesity and malnutrition were assessed using all available documentation, including BMI values and diagnostic codes. Nonetheless, some degree of underreporting or misclassification may still be present.

Several laboratory variables had relatively high rates of missing data. Laboratory testing upon admission was performed according to clinical indications; therefore, more analytes were tested more frequently in patients whose condition was more severe at the time of admission. As a result, some comparisons involved smaller subgroups, and some

predictors that showed significance in univariable logistic regression could not be included in the final multivariable model, potentially affecting the comprehensiveness of the analysis.

The study extended across several pandemic waves, during which clinical practice and hospital admission strategies evolved. In later waves, hospital admission was generally reserved for patients with more severe disease. This may have influenced the distribution of clinical characteristics and outcomes across pandemic waves. Finally, due to the absence of SARS-CoV-2 sequencing data, we could not analyse outcomes by specific viral variants. Instead, COVID-19 waves were classified based on their timing relative to known variant prevalence in Lithuania, which may limit the precision of wave-based comparisons.

Conclusions

Our results contribute to the knowledge that various chronic comorbidities and increased levels of biomarkers of inflammation and tissue injury influence the risk of developing a severe COVID-19 disease course. We found that older age, obesity, lymphopenia, and higher CRP and LDH levels were associated with severe COVID-19 disease course. Combining relevant patient history with routinely available laboratory parameters provides a practical risk stratification approach to help clinicians identify high-risk patients early and intervene quickly and efficiently. This approach may not only improve individual outcomes but also ease the strain on healthcare systems, both during the COVID-19 pandemic and in future pandemics, particularly in regions where such research and data remain limited.

Supporting information

S1 Table. Predictors of severe COVID-19, univariable logistic regression analysis. ALT – alanine aminotransferase; AST – aspartate aminotransferase; CI – confidence interval; COPD – chronic obstructive pulmonary disease; CRP – C-reactive protein; IL-6 – interleukin 6; IQR – interquartile range; LDH – lactate dehydrogenase; NEWS – National Early Warning Score; NT-proBNP – N-terminal pro-B-type natriuretic peptide; OR – odds ratio; SpO_2 – oxygen saturation.
(PDF)

S2 Table. Clinical symptoms (as reported by patients) of COVID-19 on admission by pandemic waves.
(PDF)

S3 Table. Laboratory characteristics of hospitalized COVID-19 patients on admission by pandemic waves. ALT – alanine aminotransferase; ALP – alkaline phosphatase; AST – aspartate aminotransferase; CRP – C-reactive protein; GGT – gamma-glutamyl transferase; IL-6 – interleukin 6; INR – international normalized ratio; IQR – interquartile range; LDH – lactate dehydrogenase; NT-proBNP – N-terminal pro-B-type natriuretic peptide; PT – prothrombin time; WBC – white blood cell.
(PDF)

S4 Table. Instrumental investigation findings, oxygen therapy, complications, and results of hospitalization of COVID-19 patients by pandemic waves. ECMO – extracorporeal membrane oxygenation; HDU – high dependency unit; ICU – intensive care unit; IQR – interquartile range.
(PDF)

S5 Data. Data file.
(XLSX)

Author contributions

Conceptualization: Giedre Gefenaite, Aukse Mickiene, Ligita Jancoriene.

Data curation: Ieva Kubiliute, Jurgita Urboniene.

Formal analysis: Ieva Kubiliute, Jurgita Urboniene.

Funding acquisition: Aukse Mickiene.

Investigation: Ieva Kubiliute, Birute Zablockiene.

Methodology: Ieva Kubiliute, Jurgita Urboniene.

Project administration: Aukse Mickiene.

Supervision: Aukse Mickiene, Ligita Jancoriene.

Visualization: Ieva Kubiliute, Jurgita Urboniene.

Writing – original draft: Ieva Kubiliute, Edgaras Zaboras, Jurgita Urboniene.

Writing – review & editing: Ieva Kubiliute, Fausta Majauskaite, Birute Zablockiene, Giedre Gefenaite, Aukse Mickiene, Ligita Jancoriene.

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