**lek. Laura Ziuzia-Januszewska**

# Wybrane czynniki predykcyjne w ocenie prawdopodobieństwa rozpoznania oraz ciężkiego przebiegu zakażenia SARS-CoV-2 z uwzględnieniem zaburzeń węchu

**Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne**

Promotor: Prof. dr hab. n. med. Antoni Krzeski
Promotor pomocniczy: Dr n. med. Paweł Dobrzyński

Klinika Otolaryngologii
Centralny Szpital Kliniczny MSWiA w Warszawie

Obrona rozprawy doktorskiej przed Radą Dyscypliny Nauk Medycznych Warszawskiego Uniwersytetu Medycznego

Warszawa 2022 r.

**SŁOWA KLUCZOWE:**

SARS-CoV-2, COVID-19, warianty SARS-CoV-2, zaburzenia węchu, anosmia, patogeneza, czynniki predykcyjne, ciężkość choroby, przebieg kliniczny, markery stanu zapalnego, wskaźniki laboratoryjne

**KEYWORDS:**

SARS-CoV-2, COVID-19, SARS-CoV-2 variants, olfactory disorders, anosmia, pathogenesis, predictors, disease severity, clinical course, inflammatory markers, laboratory indicators

**WYKAZ PUBLIKACJI STANOWIĄCYCH PRACĘ DOKTORSKĄ**

1. Ziuzia-Januszewska L, Dobrzyński P, Ślączka K, Ciszek J, Krawiec Ł, Wierzba W, Zaczyński A. Simple Disposable Odor Identification Tests for Predicting SARS-CoV-2 Positivity. Int J Environ Res Public Health. 2021 Sep 28;18(19):10185. doi: 10.3390/ijerph181910185.

**Punktacja MEiN: 140** **Impact Factor: 4,614**

2. Ziuzia-Januszewska L, Januszewski M. Pathogenesis of Olfactory Disorders in COVID-19. Brain Sci. 2022 Mar 27;12(4):449. doi: 10.3390/brainsci12040449.

**Punktacja MEiN: 100** **Impact Factor: 3,333**

3. Ziuzia-Januszewska L, Januszewski M, Sosnowska-Nowak J, Janiszewski M, Dobrzyński P, Jakimiuk AA, Jakimiuk AJ. COVID-19 severity and mortality in two pandemic waves in Poland and predictors of poor outcomes of SARS-CoV-2 infection in hospitalized young adults. Viruses 2022, 14 (8), 1700. doi: 10.3390/v14081700.

**Punktacja MEiN: 100** **Impact Factor: 5,818**

4. Januszewski M, Ziuzia-Januszewska L, Jakimiuk AA, Oleksik T, Pokulniewicz M, Wierzba W, Kozlowski K, Jakimiuk AJ. Predictors of COVID-19 severity among pregnant patients. Bosn J Basic Med Sci. 2022 Jun 5. doi: 10.17305/bjbms.2022.7181.

**Punktacja MEiN: 70** **Impact Factor: 3,759**

**Łącznie:**
**Punktacja MEiN: 410; Impact Factor: 17,524**

WARSZAWSKI
UNIWERSYTET
MEDYCZNY

BIBLIOTEKA UCZELNIANA

**Nr referencyjny**
**BIBG/Punktacja/ 408 /2022/KK**

**Warszawa, 24.08.2022**

**Sz. Pani**
**Laura Ziuzia-Januszewska**

ANALIZA BIBLIOMETRYCZNA PUBLIKACJI
PANI LAURY ZIUZIA-JANUSZEWSKIEJ,
WCHODZĄCYCH W SKŁAD CYKLU PUBLIKACJI STANOWIĄCYCH ROZPRAWĘ DOKTORSKĄ

| Lp. | Opis bibliograficzny | Impact Factor | MEiN |
|---|---|---|---|
| Artykuły | | | |
| 1. | **Ziuzia-Januszewska L** [aut. koresp.], Januszewski M, Sosnowska-Nowak J, Janiszewski M, Dobrzyński P, Jakimiuk AA, Jakimiuk AJ. COVID-19 Severity and Mortality in Two Pandemic Waves in Poland and Predictors of Poor Outcomes of SARS-CoV-2 Infection in Hospitalized Young Adults. Viruses. 2022;14(8):1700 [Rodzaj publikacji: praca oryginalna] | 5,818 | 100 |
| 2. | **Ziuzia-Januszewska L** [aut. koresp.], Januszewski M. Pathogenesis of Olfactory Disorders in COVID-19. Brain Sciences. 2022;12(4):449 [Rodzaj publikacji: praca poglądowa] | 3,333 | 100 |
| 3. | Januszewski M, **Ziuzia-Januszewska L**, Jakimiuk AA, Oleksik T, Pokulniewicz M, Wierzba W, Kozlowski K, Jakimiuk AJ. Predictors of COVID-19 severity among pregnant patients. Bosnian Journal of Basic Medical Sciences. 2022 Jun 5. doi: 10.17305/bjbms.2022.7181 [Rodzaj publikacji: praca oryginalna] | 3,759 | 70 |
| 4. | **Ziuzia-Januszewska L** [aut. koresp.], Dobrzyński P, Ślączka K, Ciszek J, Krawiec Ł, Wierzba W, Zaczyński A. Simple Disposable Odor Identification Tests for Predicting SARS-CoV-2 Positivity. International Journal of Environmental Research and Public Health. 2021;18(19):10185 [Rodzaj publikacji: praca oryginalna] | 4,614 | 140 |
| | Łącznie: | **17,524** | **410** |
| Książki | | | |
| 1. | - | | |
| Rozdziały w książkach | | | |
| 1. | - | | |

KIEROWNIK
Oddziału Informacji Naukowej
mgr Anna Aidukiewicz-Tarkowska

ul. Żwirki i Wigury 63
02-091 Warszawa
www.biblioteka.wum.edu.pl

tel.: +48 22 116 60 11
biblioteka@wum.edu.pl

**SPIS TREŚCI**

## I. WYKAZ ZASTOSOWANYCH SKRÓTÓW

**ACE2** (ang. *angiotensin-converting enzyme 2*) – enzym konwertazy angiotensyny 2

**ALP** (ang. *alkaline phosphatase*) – fosfataza alkaliczna

**ALT** (ang. *alanine aminotransferase*) – aminotransferaza alaninowa

**AT III** (ang. *antithrombin III*) – antytrombina III

**AST** (ang. *aspartate aminotransferase*) – aminotrasferaza asparaginianowa

**ARDS** (ang. *acute respiratory distress syndrome*) – zespół ostrej niewydolności oddechowej

**BMI** (ang. *body mass index*) – wskaźnik masy ciała

**CDC** (ang. *Centers for Disease Control and Prevention*) – Centrum Kontroli i Profilaktyki Zachorowań

**CK** (ang. *creatine kinase*) – kinaza kreatynowa

**CK-MB** (ang. *creatine kinase-myocardial band*) – izoenzym sercowy kinazy kreatynowej

**COVID-19** (ang. *coronavirus disease 19*) – choroba koronawirusowa 2019

**CRP** (ang. *C-reactive protein*) – białko C-reaktywne

**CRRT** (ang. *continuous renal replacement therapy*) – ciągła terapia nerkozastępcza

**ECDC** (ang. *European Centre for Disease Prevention and Control*) – Europejskie Centrum ds. Zapobiegania i Kontroli Chorób

**EGFR** (ang. *estimated glomerular filtration rate*) – szacowany wskaźnik przesączania kłębuszkowego

**GGT** (ang. *gamma-glutamyl transferase*) – gamma-glutamylo-transferaza

**hs-TnI** (ang. *high-sensitive troponin I*) – wysokoczuła troponina I

**IL-1** (ang. interleukin-1) – interleukina-1

**IL-6** (ang. interleukin-6) – interleukina-6

**IQR** (ang. interquartile range) – rozstęp międzykwartylowy

**LDH** (ang. *lactate dehydrogenase*) – dehydrogenaza mleczanowa

**NET** (ang. *neutrophil extracellular traps)* – zewnątrzkomórkowe pułapki neutrofilowe

**NLR** (ang. *neutrophil-to-lymphocyte ratio*) – stosunek liczby neutrofilów do liczby limfocytów

**NPV** (ang. *negative predictive* value) – wartość predykcyjna ujemna

**NRP1** (ang. *neuropilin-1*) – neuropilina-1

**NT-proBNP** (ang. *N-terminal-pro-B-type natriuretic peptide*) – N-końcowy propeptyd natriuretyczny typu B

**OBP** (ang. *odorant binding proteins*) – białka wiążące substancje wonne

**OE** (ang. *olfactory epithelium*) – nabłonek węchowy

**OIT, ICU** (ang. *intensive care unit)* – Oddział Intensywnej Terapii

**OSN** (ang. *olfactory sensory neurons*) – neurony węchowe

**OR** (ang. *odds ratio)* – iloraz szans

**PCT** (ang. *procalcitonin*) – prokalcytonina

**PVOD** (ang. *post-viral olfactory dysfunction*) – powirusowe zaburzenia węchu

**PPV** (ang. *positive predictive* value) – wartość predykcyjna dodatnia

**RAS-KKS** (ang. *Renin-Angiotensin-Kallikrein-Kinin systems*) – układy renina-angiotensyna / kalikreina-kinina

**RT-PCR** (ang. *reverse-transcription polymerase chain reaction*) – reakcja łańcuchowa polimerazy z odwrotną transkryptazą

**SARS-CoV-2** (ang. s*evere acute respiratory distress syndrome coronavirus 2*) – koronawirus zespołu ostrej niewydolności oddechowej typu 2

**SDOIT** (ang. *simple disposable odor identification test*) – prosty jednorazowy test identyfikacji zapachów

**$SpO_2$** (ang. *peripheral oxygen saturation*) **-** saturacja krwi obwodowej tlenem

**SUS** (ang. *sustentacular cells*) – komórki podporowe

**TMPRSS2** (ang. *transmembrane serine protease 2*) – transbłonowa proteaza serynowa 2

**TNF-α** (ang. *tumor necrosis factor alpha*) – czynnik martwicy nowotworu-α

**VAS** (ang. *visual analogue scale)* – wizualna skala analogowa

**VBM** (ang. *variants being monitored*) – warianty podlegające monitorowaniu

**VOC** (ang. *Variants of Concern*) – warianty wzbudzające obawę

## II. STRESZCZENIE W JĘZYKU POLSKIM

Choroba koronawirusowa 2019 (COVID-19) jest wywoływana przez koronawirusa zespołu ciężkiej niewydolności oddechowej typu 2 (SARS-CoV-2*)*. Zakażenie tym wirusem może być bezobjawowe lub objawowe, przy czym przebieg choroby jest znacznie zróżnicowany, od łagodnego, poprzez umiarkowany i ciężki, do krytycznego, mogącego prowadzić do zgonu.

Celami pracy były: określenie czynników ryzyka wystąpienia zakażenia SARS-CoV-2, ze szczególnym uwzględnieniem oceny wartości predykcyjnej zaburzeń węchu z zastosowaniem stworzonego na potrzeby tego badania przesiewowego testu węchowego, podsumowanie aktualnej wiedzy na temat patogenezy anosmii w przebiegu COVID-19, a także określenie niekorzystnych czynników rokowniczych, z uwzględnieniem wpływu wariantu SARS-CoV-2, a także roli czynników demograficznych, klinicznych i laboratoryjnych w predykcji ciężkiego przebiegu choroby w populacjach młodych dorosłych oraz kobiet ciężarnych hospitalizowanych z powodu COVID-19.

Do badania częstości występowania zaburzeń węchu u pacjentów z COVID-19 oraz ich wartości predykcyjnej w ocenie prawdopodobieństwa zakażenia SARS-CoV-2 włączono 64 hospitalizowanych pacjentów z COVID-19 oraz 34 zdrowych ochotników, którzy wypełnili ankietę na temat danych demograficznych, wywiadu chorobowego, przebiegu COVID-19 i subiektywnych zaburzeń powonienia, a także zostali poddani psychofizycznej ocenie węchu z wykorzystaniem opracowanego na potrzeby tego badania prostego**,** jednorazowego testu identyfikacji zapachów (SDOIT, ang. *simple disposable odor identification test*). Przydatność diagnostyczną zgłaszanych przez pacjentów zaburzeń węchu oraz wyników testu psychofizycznego oceniono z wykorzystaniem analizy ROC. Ponadto, w celu podsumowania aktualnej wiedzy na temat patogenezy zaburzeń węchu w przebiegu COVID-19, dokonano przeglądu aktualnej literatury przedmiotu. W celu oceny predyktorów ciężkiego przebiegu zakażenia w grupie młodych dorosłych, z uwzględnieniem wpływu wariantu SARS-CoV-2, przeprowadzono jednoośrodkowe, retrospektywne badanie 229 pacjentów w wieku od 18 do 45 lat wymagających leczenia szpitalnego z powodu COVID-19, w tym 75 chorych hospitalizowanych podczas drugiej fali pandemii oraz 154 pacjentów hospitalizowanych podczas trzeciej fali pandemii. W celu oceny czynników rokowniczych, porównano przebieg COVID-19 pomiędzy falami pandemii, a także oceniono wpływ danych demograficznych i klinicznych oraz wyników badań laboratoryjnych na ciężkość przebiegu choroby. Zbudowano również modele predykcyjne dla wystąpienia zgonu oraz konieczności wentylacji mechanicznej i przyjęcia

do oddziału intensywnej terapii w oparciu o metodę wieloczynnikowej regresji logistycznej. W celu oceny czynników rokowniczych w COVID-19 u kobiet ciężarnych przeprowadzono jednoośrodkowe, retrospektywne badanie 52 pacjentek ciężarnych z potwierdzonym zakażeniem SARS-CoV-2 i oceniono wpływ ich danych demograficznych i klinicznych oraz wyników badań laboratoryjnych na ciężkość przebiegu choroby.

Anosmia w przebiegu zakażenia SARS-CoV-2 wynika przede wszystkim z uszkodzenia nabłonka węchowego, do którego wirus ten wykazuje wysokie powinowactwo. Z tego powodu występowanie zaburzeń węchu w przebiegu COVID-19 jest częste i może stanowić dobry predyktor wystąpienia zakażenia. Stwierdzono, że częstość występowania anosmii określana na podstawie subiektywnej oceny węchu jest prawdopodobnie znacznie niedoszacowana, co podkreśla istotną rolę metod psychofizycznych oceny zmysłu powonienia. Zastosowany test psychofizyczny (SDOIT), zwłaszcza w połączeniu z wynikami oceny subiektywnej, może być użyteczny w skriningu w kierunku zakażenia SARS-CoV-2, pozwalając na wczesną izolację chorych i skierowanie ich na dalszą diagnostykę. Nie stwierdzono natomiast związku subiektywnie odczuwanego osłabienia węchu z ciężkością przebiegu COVID-19. Wariant alfa SARS-CoV-2 prawdopodobnie nie powoduje cięższego przebiegu choroby niż warianty wcześniejsze. Do niekorzystnych czynników rokowniczych u młodych dorosłych hospitalizowanych z powodu COVID-19 należą: otyłość i inne choroby współistniejące, nikotynizm w wywiadzie, wyższy odsetek zajęcia miąższu płucnego przez zmiany zapalne stwierdzany w obrazach tomografii komputerowej (TK), niższa saturacja krwi obwodowej tlenem ($SpO_2$), leukocytoza, neutrofilia, limfopenia, większa liczba niedojrzałych granulocytów, wyższy stosunek liczby neutrofilów do liczby limfocytów (NLR), wyższe stężenia: białka C-reaktywnego (CRP), prokalcytoniny (PCT), interleukiny-6 (IL-6), D-Dimeru, dehydrogenazy mleczanowej (LDH), wysokoczułej troponiny I (hs-TnI), izoenzymu sercowego kinazy kreatynowej (CK-MB), mioglobiny, N-końcowego propeptydu natriuretycznego typu B (NT-proBNP), kreatyniny, mocznika i gamma-glutamylo-transferazy (GGT), niższy szacowany wskaźnik przesączania kłębuszkowego (EGFR) oraz niższe stężenia albuminy, wapnia i witaminy D3, a także prawdopodobnie spadek liczby erytrocytów, stężenia hemoglobiny i poziomu hematokrytu oraz wzrost aktywności kinazy kreatynowej (CK). Z kolei u pacjentek ciężarnych do potencjalnych predyktorów ciężkiego przebiegu COVID-19 należą: występowanie chorób współistniejących, takich jak nadciśnienie tętnicze i cukrzyca, wyższy odsetek zajęcia

miąższu płucnego przez zmiany zapalne stwierdzany w obrazach TK, a także szereg nieprawidłowości w badaniach laboratoryjnych, takich jak limfopenia, hipokalcemia, hipoproteinemia, niskie stężenie cholesterolu całkowitego oraz podwyższone stężenia CRP, PCT, IL-6, ferrytyny, LDH, hs-TnI i glukozy. Określenie niekorzystnych czynników rokowniczych w poszczególnych subpopulacjach pacjentów może ułatwić wczesną identyfikację pacjentów z wysokim ryzykiem ciężkiego przebiegu COVID-19, pozwalając na zastosowanie właściwej strategii postępowania w tych przypadkach oraz poprawę rokowania chorych.

## III. STRESZCZENIE W JĘZYKU ANGIELSKIM

### Selected predictive factors in the evaluation of the risk of SARS-CoV-2 positivity and severity of COVID-19, with emphasis on olfactory disorders

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). SARS-CoV-2 infection may be asymptomatic or symptomatic, with the course of the disease varying widely from mild to severe to critical, and can possibly be fatal.

The objectives of the presented studies were to identify the risk factors for SARS-CoV-2 infection, with particular emphasis on the evaluation of the predictive value of olfactory disorders using an olfactory screening test developed for this study, to summarize the current knowledge on the pathogenesis of COVID-19-related anosmia, and to identify the risk factors for severe COVID-19, including the causative SARS-CoV-2 variant, as well as the role of demographic, clinical and laboratory factors in predicting severe course of disease in the populations of young adults and pregnant women hospitalized for COVID-19.

The study of the prevalence of olfactory disorders in COVID-19 patients and their predictive value in assessing the likelihood of SARS-CoV-2 infection included 64 hospitalized COVID-19 patients and 34 healthy volunteers who completed a questionnaire on demographic data, medical history, course of disease and self-reported olfactory disorders, and underwent psychophysical olfactory assessment using a simple disposable odor identification test (SDOIT) developed for this study. The diagnostic utility of self-reported olfactory disorders and psychophysical test results was assessed using ROC analysis. Moreover, in order to summarize the current knowledge on the pathogenesis of olfactory disorders in the course of COVID-19, a review of the current literature on the subject was conducted. To assess predictors of severe disease in young adults, including the impact of the SARS-CoV-2 causative variant, a single-center, retrospective study of 229 patients hospitalized for COVID-19, aged 18 to 45 years, was conducted, including 75 patients hospitalized during the second wave and 154 patients hospitalized during the third wave of the pandemic. In order to assess prognostic factors, the severity of the disease course between two waves of the pandemic was compared, and the impact of demographic, clinical and laboratory factors on the severity of the disease was evaluated. Moreover, models for the prediction of death, the need for mechanical ventilation and admission to

the intensive care unit were also created using multivariate logistic regression. To evaluate prognostic factors in COVID-19 in the population of pregnant women, a single-center, retrospective study of 52 pregnant patients with confirmed SARS-CoV-2 infection was conducted, and the influence of demographic characteristics, clinical data and laboratory abnormalities on disease severity was assessed.

Anosmia in the course of SARS-CoV-2 infection is probably mainly due to the damage to the olfactory epithelium, to which this virus has a high affinity. Therefore, the occurrence of olfactory disorders in the course of COVID-19 is frequent, and may be a good predictor of infection. The results indicate that self-assessment of smell in COVID-19 tends to underestimate the prevalence of anosmia, highlighting the importance of psychophysical methods of evaluating the sense of smell. The newly designed psychophysical test (SDOIT), especially in combination with self-reported symptoms, may be useful in screening for SARS-CoV-2 infection, allowing for early isolation of patients and referral for further diagnostic tests. There was no association of the self-reported olfactory disorders with the severity of COVID-19. The SARS-CoV-2 alpha variant is unlikely to cause a more severe disease course than the previous variants. Risk factors for poor prognosis in hospitalized young adults include obesity, comorbidities, a history of smoking, higher percentage of lung involvement on computed tomography (CT), lower peripheral oxygen saturation ($SpO_2$), leukocytosis, neutrophilia, lymphopenia, higher immature granulocyte count, higher neutrophil-to-lymphocyte ratio (NLR), higher concentrations of C-reactive protein (CRP), procalcitonin (PCT), interleukin-6 (IL-6), D-Dimer, lactate dehydrogenase (LDH), high-sensitive troponin I (hs-TnI), creatine kinase-myocardial band (CK-MB), myoglobin, N-terminal-pro-B-type natriuretic peptide (NT-proBNP), creatinine, urea and gamma-glutamyl transferase (GGT), lower estimated glomerular filtration rate (EGFR), and lower concentrations of albumin, calcium and vitamin D3, as well as possibly a decrease in erythrocyte count, hemoglobin concentration and hematocrit level and an increase in creatine kinase (CK) activity. In pregnant patients, potential predictors of severe COVID-19 include comorbidities such as hypertension and diabetes, a higher percentage of lung involvement on CT, and a number of laboratory abnormalities such as lymphopenia, hypocalcemia, hypoproteinemia, low total cholesterol, and elevated concentrations of CRP, PCT, IL-6, ferritin, LDH, hs-TnI and glucose. Identification of poor prognostic factors in specific subpopulations may allow for early identification of patients at high risk for severe COVID-19, allowing for the application of an appropriate management strategy in these cases and contributing to a better prognosis.

## IV. OPIS W JĘZYKU POLSKIM

### 1. WSTĘP

Choroba koronawirusowa 2019 (COVID-19, ang. *coronavirus disease 2019*), uznana za pandemię przez Światową Organizację Zdrowia (WHO, ang. *World Health Organization*) w dniu 11 marca 2020 roku [1], jest wywoływana przez koronawirusa ciężkiej niewydolności oddechowej typu 2 (SARS-CoV-2, ang. *severe acute respiratory syndrome coronavirus-2)*.

SARS-CoV-2 jest wysoce zakaźnym wirusem RNA, który wnika do komórek poprzez wiązanie jego białka kolca (S, ang. *spike protein*) z receptorem konwertazy angiotensyny 2 (ACE2, ang. *angiotensin-converting enzyme 2*) gospodarza. Interakcja ta wymaga rozszczepienia białka S przez proteazy komórkowe, w tym transbłonową proteazę serynową 2 (TMPRSS2, ang. *transmembrane protease serine 2*) i furynę, co umożliwia fuzję między błoną komórkową i wirusową oraz wejście wirusa do komórki na drodze endocytozy [2]. Wśród alternatywnych mediatorów wnikania SARS-CoV-2 wymienia się receptory i kofaktory, takie jak neuropilina-1 (NRP1) i CD147, oraz aktywatory, takie jak katepsyna [3,4,5]. ACE2 ulega ekspresji na powierzchniach wielu komórek w całym organizmie, w tym w miąższu płucnym, nabłonku oddechowym, nabłonku przewodu pokarmowego, śródbłonku, mięśniach gładkich tętnic, komórkach glejowych, neuronach, komórkach kanalików nerkowych, sercu i tkankach limfoidalnych [6,7]. Podobnie, ekspresję TMPRSS2 stwierdzono w wielu narządach, w tym w obrębie dróg oddechowych i przewodu pokarmowego, w gruczołach ślinowych, wątrobie i nerkach [5]. To powszechne występowanie ACE2 i TMPRSS2 może stanowić wyjaśnienie obserwowanych plejotropowych efektów zakażenia SARS-CoV-2, niemniej jednak głównymi celami wirusa są komórki wykazujące wysoką ekspresję tych białek, a zwłaszcza ich koekspresję, takie jak komórki nabłonka oddechowego i węchowego [3].

Zakażenie SARS-COV-2 może być bezobjawowe lub objawowe, przy czym przebieg choroby jest znacznie zróżnicowany, od łagodnego, poprzez umiarkowany i ciężki, do krytycznego [8]. Podobnie, Polskie Towarzystwo Epidemiologów i Lekarzy Chorób Zakaźnych wyróżnia cztery stadia choroby. Stadium skąpoobjawowemu, odpowiadającemu łagodnej postaci choroby, towarzyszą niespecyficzne objawy, takie jak: gorączka, dreszcze, kaszel, duszność, zmęczenie, bóle mięśniowe, ból głowy, ból gardła, katar, zapalenie spojówek, jadłowstręt, nudności, wymioty, biegunka, ból brzucha oraz utrata węchu (anosmia) i/lub smaku (ageusia), lecz nie występuje tu hipoksja. W stadium

pełnoobjawowym, odpowiadającym chorobie umiarkowanej, obserwuje się dodatkowo kliniczne i radiologiczne cechy zapalenia płuc z saturacją krwi obwodowej tlenem ($SpO_2$) poniżej 94% przy oddychaniu powietrzem atmosferycznym. Ciężki przebieg COVID-19 związany jest z niewydolnością oddechową, wynikającą głównie z tzw. burzy cytokinowej, ze spadkiem $SpO_2$ poniżej 90% przy oddychaniu powietrzem atmosferycznym. W stadium krytycznym dochodzi do rozwoju zespołu ostrej niewydolności oddechowej (ARDS, ang. *acute respiratory distress syndrome*), wstrząsu oraz niewydolności wielonarządowej, wymagających hospitalizacji w oddziale intensywnej terapii (OIT) i zastosowania wentylacji mechanicznej [9].

Warto zauważyć, że opisane powyżej objawy łagodnej postaci choroby mogą być przydatne w identyfikacji pacjentów z wyższym prawdopodobieństwem zakażenia SARS-CoV-2, co może pozwolić na wcześniejsze skierowanie ich na testy diagnostyczne. W metaanalizie oceniającej przydatność poszczególnych objawów w przewidywaniu COVID-19 kaszel charakteryzował się 62,4-procentową czułością, ale niską swoistością (równą 45,4%), gorączka miała czułość równą 37,6% i swoistość równą 75,2%, duszność – czułość równą 23,3% i swoistość równą 75,7%, zaś zmęczenie – czułość równą 40,2% i swoistość równą 73,6%. Co ciekawe, anosmia, ageusia oraz anosmia lub ageusia charakteryzowały się czułością poniżej 50%, ale wysoką swoistością wynoszącą ponad 90% (czułość równa 26,4%, 23,2% i 39,2% oraz swoistość równa 94,2%, 92,6% i 92,1% odpowiednio dla anosmii, ageusii i anosmii lub ageusii) [10], co może być przydatne w testach przesiewowych. Rzeczywiście SARS-CoV-2 wydaje się mieć szczególnie silne powinowactwo do ACE2, ulegającego wysokiej ekspresji w nabłonku oddechowym i węchowym, szacowane na 10–20-krotnie wyższe niż w przypadku wcześniej występującego wirusa SARS-CoV [11], co może wyjaśniać jego istotny wpływ na układ węchowy. Jednakże pomimo uznanego już powszechnie związku zaburzeń węchu z zakażeniem SARS-CoV-2, ich patogeneza wciąż nie została w pełni wyjaśniona.

Percepcja węchowa rozpoczyna się od związania cząsteczek zapachowych z receptorami zlokalizowanymi na dendrytach neuronów węchowych (OSN, ang. *olfactory sensory neurons*) w nabłonku węchowym (OE, ang. *olfactory epithelium*) [12,13]. Nabłonek węchowy stanowi złożoną strukturę, składającą się z wielu typów komórek, takich jak neurony węchowe, komórki podporowe (SUS, ang. *sustentacular cells*), komórki wydzielnicze gruczołów Bowmana oraz komórki podstawne [12]. Komórki podporowe nie tylko stanowią rusztowanie dla neuronów węchowych, lecz pełnią również funkcję ochronną poprzez usuwanie czynników potencjalnie szkodliwych, uczestniczą

w detekcji zapachów poprzez udział w przenoszeniu i usuwaniu zbędnych cząsteczek zapachowych oraz dostarczanie glukozy niezbędnej w wysokoenergetycznym procesie transdukcji sygnałów węchowych, a także uczestniczą w utrzymaniu równowagi wodno-elektrolitowej [12,14]. Komórki podstawne mogą różnicować się w komórki receptorowe w fizjologicznym „obrocie komórkowym" (ang. *turnover*) lub po ich uszkodzeniu. Gruczoły Bowmana wytwarzają wydzielinę śluzową zawierającą wodę, sole mineralne, glikoproteiny mucynowe, enzymy, przeciwciała oraz białka wiążące substancje wonne (OBP, ang. *odorant binding proteins*), odpowiedzialne za transport hydrofobowych cząsteczek zapachowych poprzez śluz do rzęsek komórek węchowych [15]. Aksony neuronów węchowych wnikają do jamy czaszki przez otwory w blaszce sitowej i tworzą synapsy w obrębie opuszki węchowej, skąd informacja węchowa przekazywana jest do wyższych ośrodków mózgu [12,13].

Zaburzenia węchu można podzielić na: 1) przewodzeniowe, spowodowane niedrożnością jam nosa i wynikającym z niej utrudnieniem przenoszenia substancji zapachowej do nabłonka węchowego; 2) odbiorcze (czuciowo-nerwowe), spowodowane uszkodzeniem nabłonka węchowego lub neuronów węchowych oraz 3) ośrodkowe, wynikające z nieprawidłowego przetwarzania informacji węchowych w ośrodkowym układzie nerwowym [13]. W przebiegu ostrych zakażeń górnych dróg oddechowych najczęściej obserwuje się zaburzenia przewodzeniowe wynikające z obrzęku błony śluzowej oraz zwiększonej ilości wydzieliny w jamach nosa, przy czym po ustąpieniu infekcji węch zwykle wraca do normy. W rzadszych przypadkach osłabienie powonienia może utrzymywać się nawet po ustąpieniu infekcji, co świadczyć może o uszkodzeniu typu odbiorczego, określanym jako powirusowe zaburzenia węchu (PVOD, ang. *post-viral olfactory dysfunction*) [16,17]. PVOD stanowi jedną z najczęstszych przyczyn anosmii u osób dorosłych, obserwowaną w około 11–40% przypadków [18]. Wśród wirusów oddechowych mogących powodować tę patologię wymienia się rhinowirusy, wirusy paragrypy, wirus Ebsteina-Barr oraz niektóre koronawirusy [17]. W patogenezie anosmii w przebiegu COVID-19 zaproponowano kilka teorii, w tym zaburzenia przewodzeniowe związane z niedrożnością nosa i katarem, obrzęk śluzówki okolicy węchowej uniemożliwiający dotarcie substancji zapachowej do nabłonka węchowego, uszkodzenie nabłonka węchowego, zakażenie neuronów węchowych oraz opuszki węchowej poprzez neuroinwazję, a także uszkodzenie ośrodkowych szlaków węchowych w wyniku bezpośredniego zakażenia ich komórek bądź ich pośredniego urazu wywołanego hipoksją, uszkodzeniem śródbłonka lub nadmierną reakcją zapalną. Podobnie, uszkodzenie nabłonka

węchowego może być spowodowane bezpośrednią inwazją wirusa do neuronów węchowych, prawdopodobnie pośredniczoną przez receptor neuropiliny-1, lub zakażeniem komórek pozanerwowych prowadzącym do horyzontalnego rozprzestrzeniania się wirusa do komórek receptorowych lub zaburzenia podpory morfologicznej i funkcji neuronów węchowych [4,12,16,19,20,21].

Jak wspomniano powyżej, wysoka częstość anosmii w przebiegu COVID-19 skłoniła badaczy do wysunięcia hipotezy, że nagłe wystąpienie zaburzeń węchu może stanowić potencjalny predyktor zakażenia SARS-CoV-2. Warto jednak zauważyć, że większość wczesnych badań w tym zakresie opierała się na subiektywnej ocenie węchu, co mogło zaniżać rzeczywistą skalę problemu, jako że zaburzenia powonienia nie zawsze są zauważane i zgłaszane przez pacjentów, zwłaszcza przy współistnieniu innych, poważnych objawów, takich jak niewydolność oddechowa [13,22,23]. Znajduje to odzwierciedlenie w wynikach metaanaliz, wskazujących na większą częstość występowania zaburzeń węchu w badaniach psychofizycznych niż w ocenie subiektywnej (odpowiednio 72,1%–77% i 44,5%–53%) [23–25]. Z drugiej strony psychofizyczne metody oceny węchu są czasochłonne, kosztowne i wymagają wystandaryzowanych warunków laboratoryjnych, a zatem są trudne do przeprowadzenia w warunkach pandemii [24]. Co więcej, ryzyko transmisji zakażenia sprawia, że preferowane powinny być jednorazowe zestawy badawcze [26]. Z tych powodów należy dążyć do stworzenia szybkich, prostych, niedrogich, jednorazowych i wiarygodnych testów przesiewowych do wykrywania zakażenia SARS-CoV-2.

Według raportu opublikowanego przez Chińskie Centrum Kontroli i Prewencji Chorób w początkowym okresie pandemii, do 11 lutego 2020 r., na podstawie 44 500 przypadków potwierdzonego zakażenia SARS-CoV-2, łagodny przebieg obserwowano u 81% pacjentów, ciężki przebieg u 14%, zaś przebieg krytyczny u 5%, z ogólnym wskaźnikiem śmiertelności wynoszącym 2,3% [27]. Podobnie, w raporcie amerykańskiego Centrum Kontroli i Profilaktyki Zachorowań (CDC, ang. *Centers for Disease Control and Prevention*) analizującym przypadki zgłoszone pomiędzy styczniem i majem 2020 r., 14% pacjentów wymagało hospitalizacji, 2% zostało przyjętych do oddziału intensywnej terapii, a 5% zmarło [28]. Co istotne, stan pacjentów, u których początkowo nie obserwuje się ciężkiego przebiegu, w ciągu około tygodnia może ulec gwałtownemu pogorszeniu [29].

COVID-19 o ciężkim przebiegu wynika głównie z zaburzeń o podłożu immunologicznym, związanych z nadmierną reakcją zapalną (tak zwaną burzą cytokinową) oraz stanem nadkrzepliwości, co prowadzi do destrukcji zakażonych tkanek,

powstawania mikrozatorów i niewydolności wielonarządowej [30–32]. Wśród czynników demograficznych i klinicznych związanych z ryzykiem ciężkiego przebiegu choroby wymieniane są między innymi: starszy wiek, płeć męska, nikotynizm oraz otyłość i inne choroby współistniejące [33,34]. Z niekorzystnym rokowaniem może być również powiązanych wiele nieprawidłowości w badaniach laboratoryjnych, takich jak: leukocytoza, neutrofilia, limfopenia, trombocytopenia, podwyższone wartości markerów stanu zapalnego, w tym białka C-reaktywnego (CRP, ang. *C-reactive protein*), prokalcytoniny i ferrytyny, oraz cytokin zapalnych, w tym interleukiny-6 (IL-6), a także wzrost stężeń markerów dysfunkcji narządów i układu krzepnięcia, takich jak: dehydrogenaza mleczanowa (LDH, ang. *lactate dehydrogenase*), troponina, N-końcowy propeptyd natriuretyczny typu B (NT-proBNP, ang. *N-terminal-pro-B-type natriuretic peptide*), kreatynina, kinaza keratynowa (CK, ang. *creatine kinase*), enzymy wątrobowe i D-Dimer, oraz wydłużenie czasu protrombinowego i hipoalbuminemia [35–39].

Choć starszy wiek stanowi czynnik ryzyka zarówno zachorowania na COVID-19, jak i gorszego rokowania, ciężki przebieg choroby i zgony obserwuje się również w grupie młodych dorosłych [40–42]. Warto zauważyć, że choć aktywność zawodowa, edukacja i inne sytuacje związane z kontaktami społecznymi stwarzają większe ryzyko zakażenia, wśród osób młodych obserwuje się mniejszą skłonność do przestrzegania środków zapobiegawczych [43]. Co więcej, charakterystyka kliniczna i wyniki badań laboratoryjnych u osób młodych wydają się być inne niż u pacjentów w podeszłym wieku, co może wskazywać na różną patogenezę ciężkiej postaci COVID-19 w tych grupach wiekowych [41,44]. Niemniej w literaturze wciąż jest niewiele danych dotyczących charakterystyki klinicznej i czynników ryzyka ciężkiego przebiegu COVID-19 u młodych dorosłych.

Szczególną grupą chorych są również kobiety ciężarne. Częstość występowania poszczególnych stadiów ciężkości przebiegu choroby u pacjentek w ciąży jest podobna jak u nieciężarnych – u 86% z nich obserwuje się przebieg łagodny, u 9% ciężki, a u 5% krytyczny [45]. Wart uwagi jest jednak fakt, że podczas ciąży fizjologiczne mechanizmy adaptacyjne w obrębie układu oddechowego, stan nadkrzepliwości, mechanizmy immunomodulacyjne zapobiegające odrzuceniu płodu jako „przeszczepu semiallogenicznego" w drugim trymestrze ciąży oraz nasilona odpowiedź zapalna w trzecim trymestrze, a także skłonność do insulinooporności, cukrzycy, nadciśnienia tętniczego i chorób sercowo-naczyniowych, predysponują kobiety ciężarne zakażone SARS-CoV-2 do ciężkiego przebiegu choroby, co może pogarszać rokowanie zarówno

u matki, jak i u płodu [46–48]. Znajduje to odzwierciedlenie w wynikach badań wskazujących na zwiększone prawdopodobieństwo rozwoju ciężkich objawów, w tym zapalenia płuc, a także częstszą konieczność hospitalizacji w oddziale intensywnej terapii i wentylacji mechanicznej oraz większe ryzyko zgonu u kobiet ciężarnych [49,50].

Innym zjawiskiem istotnym w dyskusji na temat predyktorów ciężkiego przebiegu COVID-19 było pojawienie się nowych wariantów SARS-CoV-2 związanych ze zmianami w zakaźności wirusa, symptomatologii oraz skuteczności metod zapobiegawczych, diagnostycznych i terapeutycznych, co przed końcem 2020 roku stworzyło kolejne zagrożenie dla globalnego zdrowia publicznego [51]. Skłoniło to badaczy i organizacje ochrony zdrowia do scharakteryzowania tzw. wariantów wzbudzających obawę (VOC, ang. *Variants of Concern*), do których zaliczono warianty alfa (B.1.1.7) oraz beta (B.1.351), a później również gamma (P.1), delta (B.1.617.2) i omikron (B.1.1.529) [51,52].

Wariant alfa (B.1.1.7), zidentyfikowany po raz pierwszy w Wielkiej Brytanii, a następnie rozprzestrzeniający się na całym świecie, został zdefiniowany przez obecność licznych mutacji, w tym zmian w białku S (N501Y, A570D, D614G, P681H, T716I, S982A, D1118H, del69-70, del144). Częstość jego występowania w Wielkiej Brytanii wzrosła w czasie od listopada do połowy grudnia 2020 r. z mniej niż 5% wszystkich zakażeń SARS-CoV-2 do ponad 60%, powodując gwałtowny wzrost zapadalności oraz hospitalizacji i śmiertelności z powodu COVID-19 [53]. W Polsce, według danych Europejskiego Centrum ds. Zapobiegania i Kontroli Chorób (ECDC, ang. *European Centre for Disease Prevention and Control*), przed końcem 2020 r. wykryto tylko jeden przypadek wariantu B.1.1.7, zaś w styczniu 2021 r. jego częstość wyniosła 9,5% (z ogólną częstością w okresie od 7 września 2020 r. do końca stycznia 2021 r. wynoszącą 6,5%), a następnie stopniowo wzrastała, przekraczając 50% w siódmym tygodniu 2021 r. (tj. pomiędzy 15 a 21 lutego) i osiągając ponad 90% w marcu 2021 r. [54]

W doniesieniach naukowych opisywano większą zakaźność wariantu alfa w porównaniu do wcześniejszych wariantów SARS-CoV-2 [55,56], co przypisywano większej liczbie kopii wirusa i dłuższemu okresowi wykrywalności w wydzielinach z dróg oddechowych, mogącym wynikać z mutacji w obrębie białka S, w tym w domenie wiążącej receptor oraz w pobliżu miejsca cięcia proteolitycznego przez furynę, a zatem wpływających na wnikanie wirusa do komórki [55,57]. Co do ciężkości przebiegu zakażenia wariantem alfa SARS-CoV-2, dane literaturowe są niejednoznaczne, jako że niektóre badania wskazywały na jego cięższy przebieg [58–60], podczas gdy w innych pracach nie zaobserwowano takiej zależności [57]. Podobnie szeroko dyskutowana kwestia

cięższego przebiegu choroby spowodowanej przez ten wariant u osób młodych pozostaje niewyjaśniona [61,62]. Ponadto, choć powszechnie uważa się, że późniejszy wariant delta powoduje cięższy przebieg, wyniki badań są również nierozstrzygające [63–65]. Warto zauważyć, że aktualnie tylko wariant omikron jest wciąż uważany za „krążący obecnie VOC" (ang. *currently circulating VOCs*), podczas gdy wcześniejsze „warianty wzbudzające obawę" są obecnie określane przez WHO jako „poprzednio krążące VOC" (ang. *previously circulating VOCs*) [51], przez CDC jako „warianty podlegające monitorowaniu" (VBM, ang. *variants being monitored*) [52], zaś przez ECDC jako „warianty deeskalowane" (ang. *de-escalated variants*) [66]. Obecnie dominujący wariant omikron wydaje się powodować łagodniejszy przebieg choroby [67,68], wciąż jednak potrzeba więcej danych [69]. Ponadto, stale zmieniający się odsetek osób zaszczepionych dodatkowo utrudnia porównania wariantów SARS-CoV-2. Co więcej, nawet u pacjentów nieszczepionych wpływ na ciężkość choroby coraz częściej mają inne czynniki, takie jak przebyte wcześniej zakażenie SARS-CoV-2. Nie ulega jednak wątpliwości, że w obliczu wciąż pojawiających się nowych wariantów SARS-CoV-2 i kolejnych fal pandemii mogących powodować ponowne przeciążenie systemów opieki zdrowotnej, kwestią kluczową pozostaje umiejętność szybkiej identyfikacji osób zakażonych oraz priorytetyzacji postępowania medycznego, ze szczególnym uwzględnieniem oceny możliwości wystąpienia ciężkiego przebiegu.

## 2. CELE

1. Ocena częstości występowania zaburzeń węchu w COVID-19 oraz ich wartości predykcyjnej w określeniu prawdopodobieństwa rozpoznania zakażenia SARS-CoV-2 z wykorzystaniem stworzonego na potrzeby badania prostego, jednorazowego i wiarygodnego przesiewowego testu węchowego.
2. Podsumowanie aktualnej wiedzy na temat patogenezy zaburzeń węchu w przebiegu COVID-19 na podstawie przeglądu aktualnej literatury.
3. Ocena wpływu wariantu SARS-CoV-2 (wariantu alfa w porównaniu do wariantów wcześniej występujących) na ciężkość przebiegu COVID-19 w populacji młodych dorosłych hospitalizowanych z powodu ciężkiego przebiegu COVID-19.
4. Próba określenia niekorzystnych czynników rokowniczych w populacji młodych dorosłych hospitalizowanych z powodu ciężkiego przebiegu COVID-19.
5. Próba określenia niekorzystnych czynników rokowniczych w populacji kobiet ciężarnych hospitalizowanych z powodu COVID-19.

# 3. MATERIAŁ I METODY

## 3.1. Materiał i metody w ocenie częstości występowania zaburzeń węchu u pacjentów z COVID-19 oraz ich wartości predykcyjnej w ocenie prawdopodobieństwa zakażenia SARS-CoV-2

### 3.1.1. Materiał

Do badania częstości występowania zaburzeń węchu u pacjentów z COVID-19 oraz ich wartości predykcyjnej w ocenie prawdopodobieństwa zakażenia SARS-CoV-2 włączono 64 pacjentów z COVID-19 hospitalizowanych pomiędzy kwietniem i sierpniem 2020 roku w Centralnym Szpitalu Klinicznym Ministerstwa Spraw Wewnętrznych i Administracji (CSK MSWiA) w Warszawie oraz 34 zdrowych ochotników.

Kryteriami włączenia do grupy badanej były: wiek co najmniej 18 lat oraz dodatni wynik testu RT-PCR (reakcji łańcuchowej polimerazy z odwrotną transkryptazą, ang. *reverse-transcription polymerase chain reaction*) przeprowadzonego na materiale uzyskanym z wymazu z części nosowej gardła. Do grupy kontrolnej włączono zdrowych dorosłych ochotników niemających objawów ostrej infekcji górnych dróg oddechowych oraz COVID-19 (innych niż nowo rozpoznane zaburzenia węchu, których nie uwzględniono jako kryterium wyłączenia, by uniknąć błędu wynikającego z niewłaściwego doboru pacjentów). Choć w grupie kontrolnej nie wykonywano badania w kierunku zakażenia SARS-CoV-2 z uwagi na ograniczoną wówczas dostępność testów RT-PCR u osób bez podejrzenia COVID-19, częstość zakażeń od początku pandemii do końca sierpnia 2020 roku w populacji polskiej była niższa niż 0,18% [70]. Uznano zatem, że prawdopodobieństwo zakażenia u bezobjawowych ochotników było bardzo niskie. Kryteria wyłączenia stanowiły: wiek poniżej 18 lat, ciąża, wcześniej występujące zaburzenia węchu, uraz głowy, przewlekłe choroby rynologiczne, w tym przewlekłe zapalenie błony śluzowej nosa i zatok przynosowych, oraz niezdolność do wypełnienia kwestionariusza wskutek zaburzeń neurologicznych i poznawczych, zaburzeń świadomości, hospitalizacji w oddziale intensywnej terapii lub wentylacji mechanicznej w trakcie przeprowadzania badania. Wszyscy pacjenci wyrazili świadomą, pisemną zgodę na udział w badaniu. Badanie zostało zatwierdzone przez Komisję Bioetyczną CSK MSWiA w Warszawie.

### 3.1.2. Metody

#### 3.1.2.1. Wyniki kliniczne

Wszyscy uczestnicy wypełnili ankietę dotyczącą: 1) podstawowych danych demograficznych; 2) wywiadu chorobowego, w tym chorób współistniejących, przewlekle stosowanych leków, nikotynizmu i wcześniej występujących zaburzeń węchu; 3) przebiegu COVID-19, w tym daty wystąpienia pierwszych objawów, objawów ze strony nosa i objawów ogólnych oraz 4) funkcji węchowej, z subiektywną oceną zmysłu powonienia (opisową, jako „węch prawidłowy", „węch osłabiony" lub „brak węchu", a także za pomocą wizualnej skali analogowej, od 0 – „węch prawidłowy" do 10 – „brak węchu") oraz określeniem czasu wystąpienia i utrzymywania się zaburzeń węchu. Wszelkie dane brakujące w formularzach oraz informacje dotyczące przebiegu choroby zostały uzupełnione na podstawie elektronicznej dokumentacji medycznej. Parametry fizjologiczne oceniano co najmniej raz dziennie za pomocą zmodyfikowanej skali wczesnego ostrzegania (MEWS, ang. *modified early warning score* [71]), zaadaptowanej przez szpitalny komitet terapeutyczny poprzez uwzględnienie $SpO_2$ i potrzeby suplementacji tlenu.

#### 3.1.2.2. Ocena psychofizyczna węchu

Równocześnie z kwestionariuszem u wszystkich uczestników badania przeprowadzono psychofizyczną ocenę węchu przy użyciu prostego jednorazowego testu identyfikacji zapachów (SDOIT, ang. *simple disposable odor identification test*), opracowanego na potrzeby tego badania. Dziesięć jednorazowych papierowych pasków testowych o numerach od 1 do 10, spośród których dziewięć zawierało powszechnie znane substancje zapachowe (dostępne komercyjnie olejki eteryczne: cynamonowy, miętowy, cytrynowy, kawowy, goździkowy, różany, anyżowy i kamforowy oraz alkohol stosowany do dezynfekcji), a pozostały jeden stanowił bezwonną kontrolę (wodę dejonizowaną), umieszczono w plastikowych osłonach w celu uniknięcia mieszania się zapachów. Po zdjęciu osłon każdy z zapachów był prezentowany pacjentowi w 30-sekundowych odstępach, aby zapobiec desensytyzacji węchowej. Zapachy użyte w badaniu były tak dobrane, aby znajdowały się wśród nich zarówno zapachy jednomodalne, z nieznaczną stymulacją nerwu trójdzielnego lub niewywołujące tej stymulacji, jak i dwumodalne, z mieszaną stymulacją nerwu węchowego i nerwu trójdzielnego. Dla każdego paska zapachowego pacjenci byli proszeni o wskazanie, czy wykryli zapach, a następnie o jego zidentyfikowanie (przy użyciu testu jednokrotnego wyboru spośród czterech opcji podanych dla każdego paska zapachowego).

Analizie poddano cztery modele SDOIT: 1) SDOIT-10, oceniający liczbę poprawnych odpowiedzi (tj. prawidłowej identyfikacji dziewięciu zapachów oraz prawidłowego zgłoszenia braku wykrycia zapachu w próbce bezwonnej); 2) SDOIT-9, oceniający liczbę prawidłowo zidentyfikowanych spośród wszystkich dziewięciu zapachów; 3) SDOIT-8, oceniający liczbę prawidłowo zidentyfikowanych spośród ośmiu wybranych zapachów (cynamonu, mięty, cytryny, kawy, goździka, anyżu, kamfory i alkoholu) – z wyłączeniem zapachu róży, niewykazującego w analizie wstępnej istotnych różnic pomiędzy grupą badaną a kontrolną; oraz 4) SDOIT-4, oceniający liczbę prawidłowo zidentyfikowanych spośród czterech zapachów wykazujących największe różnice międzygrupowe w analizie wstępnej (cynamonu, mięty, cytryny i alkoholu).

Zaburzenia węchu zdefiniowano jako identyfikację liczby zapachów poniżej 10. percentyla wyników z grupy kontrolnej, tj. podanie co najmniej 2 nieprawidłowych odpowiedzi w SDOIT-10, SDOIT-9 i SDOIT-8 oraz co najmniej 1 nieprawidłowej odpowiedzi w SDOIT-4. W celu jak największego uproszczenia testu i zminimalizowania czasu potrzebnego na jego przeprowadzenie, w badaniu nie uwzględniono innych aspektów oceny węchu, takich jak próg odczucia zapachu czy dyskryminacja zapachów.

### 3.1.2.3. Analiza statystyczna

Statystyka opisowa została wykonana przy użyciu standardowych metod. Zaburzenia węchu zgłaszane w kwestionariuszu porównano przy pomocy testu dokładnego Fishera, zaś wyniki SDOIT porównano przy pomocy testu U Manna-Whitneya. W celu oceny korelacji pomiędzy zaburzeniami węchu zgłaszanymi w kwestionariuszu i wynikami SDOIT zastosowano test dokładny Fishera i test chi-kwadrat. Do oceny korelacji pomiędzy zaburzeniami węchu i danymi klinicznymi pacjentów z grupy badanej zastosowano współczynnik korelacji Spearmana w przypadkach dwóch zmiennych ilościowych, test U Manna-Whitneya przy jednej zmiennej ilościowej i jednej zmiennej jakościowej oraz test dokładny Fishera dla dwóch zmiennych jakościowych. Analiza statystyczna została wykonana przy pomocy oprogramowania R (wersja 3.6.0, R Foundation for Statistical Computing, Wiedeń, Austria). We wszystkich analizach przyjęto poziom istotności równy 0,05. W celu oceny przydatności wybranych klasyfikatorów w przewidywaniu zakażenia SARS-CoV-2 wykreślono krzywe ROC (ang. *receiver operating characteristic*) i obliczono pola pod wykresami krzywych (AUC, ang. *area under the curve*), a następnie oceniono czułość, swoistość, wartość predykcyjną dodatnią (PPV, ang. *positive predictive value*) i wartość predykcyjną ujemną (NPV, ang. *negative predictive value*) predyktorów.

### 3.2. Materiał i metody w podsumowaniu aktualnej wiedzy na temat patogenezy zaburzeń węchu w przebiegu COVID-19

W celu podsumowania aktualnej wiedzy na temat patogenezy zaburzeń węchu w przebiegu COVID-19 przeprowadzono krytyczny, niesystematyczny przegląd aktualnej literatury przedmiotu w oparciu o bazę danych PubMed oraz wyszukiwarkę Google Scholar z użyciem terminów „COVID-19" lub „SARS-CoV-2" oraz „olfactory" lub „smell" lub „anosmia" lub „parosmia" lub "neuroinvasive" lub "neurological". Na podstawie oceny tytułów i streszczeń wybrano wstępnie artykuły, które przeanalizowano następnie w całości i spośród nich wybrano istotne prace. Ponadto, w celu włączenia do pracy dodatkowych publikacji istotnych dla tematu, przeszukano ręcznie piśmiennictwo publikacji zidentyfikowanych przy pomocy powyższej metody. Do przeglądu włączono jedynie artykuły opublikowane w języku angielskim.

### 3.3. Materiał i metody w badaniu wpływu wariantu SARS-CoV-2 (wariantu alfa w porównaniu do wariantów wcześniej występujących) na ciężkość przebiegu COVID-19 oraz potencjalnych demograficznych, klinicznych i laboratoryjnych predyktorów ciężkiego przebiegu choroby w populacji hospitalizowanych młodych dorosłych

#### 3.3.2. Materiał

Jednoośrodkowe, retrospektywne badanie pacjentów w wieku 18–45 lat, hospitalizowanych z powodu COVID-19 podczas drugiej i trzeciej fali pandemii w Polsce, przeprowadzono w Centralnym Szpitalu Klinicznym MSWiA w Warszawie. Drugą falę pandemii zdefiniowano jako okres od 12 września 2020 roku do 27 stycznia 2021 roku, zaś trzecią falę jako okres od 11 lutego 2021 roku do 10 czerwca 2021 roku. Do badania włączono 229 pacjentów z COVID-19 (172 mężczyzn i 57 kobiet), spośród których 75 pacjentów (59 mężczyzn i 16 kobiet) przypisano do drugiej fali, a 154 pacjentów (113 mężczyzn i 41 kobiet) przypisano do trzeciej fali pandemii COVID-19. Mediana wieku w obu grupach wynosiła 40 lat. Kryteria włączenia do badania stanowiły: wiek od 18 lat do 45 lat[1], zakażenie SARS-CoV-2 rozpoznane na podstawie testu RT-PCR lub szybkiego

[1] Choć w literaturze brak konsensusu odnośnie definicji „młodych dorosłych", a górna granica tego przedziału jest zazwyczaj niższa niż w przedstawionym badaniu [72], w wielu badaniach dotyczących zakażenia SARS-CoV-2 przyjęto podobne, rozszerzone kryteria, włączając do tej grupy wiekowej osoby nie starsze niż 45 lat [73, 74, 75]. Za takim ustaleniem kryterium wiekowego przemawiają wyniki badania pacjentów z COVID-19, w którym stwierdzono rzadsze występowanie zgonów oraz konieczności przyjęcia do OIT w grupie osób w wieku 18–45 lat niż w grupie pacjentów starszych niż 45 lat, a przyjęty punkt odcięcia dla wieku potwierdzono w analizie wrażliwości [74].

testu antygenowego przeprowadzonych na materiale uzyskanym z wymazu z części nosowej gardła, ciężki przebieg COVID-19 (tj. spełnienie kryteriów przyjęcia do szpitala dla COVID-19, w tym $SpO_2$ nie wyższej niż 94% przy oddychaniu powietrzem atmosferycznym lub konieczności suplementacji tlenu). Z badania wykluczono kobiety ciężarne oraz pacjentów przyjętych do szpitala z przyczyn innych niż COVID-19 (takich jak uraz, inne ostre schorzenia lub zaostrzenie chorób przewlekłych). Badanie zostało zatwierdzone przez Komisję Bioetyczną CSK MSWiA w Warszawie, ze zniesieniem obowiązku uzyskania świadomej zgody z uwagi na retrospektywny charakter badania i anonimizację danych.

### 3.3.2. Metody

#### 3.3.2.1. Wyniki kliniczne

Dane demograficzne i kliniczne pacjentów (takie jak wiek, płeć, palenie tytoniu, choroby współistniejące, przebieg leczenia, powikłania, $SpO_2$, konieczność przyjęcia do oddziału intensywnej terapii, zastosowania metod wspomagania oddychania, leków wazopresyjnych oraz ciągłej terapii nerkozastępczej) oraz wyniki badań laboratoryjnych i obrazowych uzyskano z elektronicznej dokumentacji medycznej i wprowadzono do bazy danych po anonimizacji. Wyniki badań laboratoryjnych zebrano z dwóch punktów czasowych: przy przyjęciu do szpitala (+/-2 dni) oraz w 7. dobie hospitalizacji (+/-2 dni).

#### 3.3.2.2. Analiza statystyczna

Porównano dane pacjentów hospitalizowanych podczas drugiej i trzeciej fali pandemii COVID-19 w Polsce oraz dane pacjentów zmarłych i pacjentów, którzy przeżyli. Do porównania zmiennych ilościowych zastosowano test U Manna-Whitneya, zaś do porównania zmiennych jakościowych zastosowano test chi-kwadrat i test dokładny Fishera. Korelacje pomiędzy danymi pacjentów a koniecznością wentylacji mechanicznej i przyjęcia do OIT przeprowadzono dla zmiennych ciągłych z zastosowaniem analizy korelacji Spearmana, zaś związki pomiędzy zmiennymi nominalnymi badano przy pomocy testu chi-kwadrat oraz testu dokładnego Fishera. W celu identyfikacji czynników ryzyka zgonu, wentylacji mechanicznej i przyjęcia do OIT przeprowadzono również analizę regresji logistycznej, a w celu oceny zdolności predykcyjnej zmiennych oraz modeli w regresji wieloczynnikowej wyznaczono krzywe ROC. Analiza została wykonana przy użyciu pakietów R (wersja 4.0.4; R foundation for statistical computing, Wiedeń, Austria) oraz Statistica (wersja 13.3; StatSoft, Polska). We wszystkich analizach przyjęto poziom istotności równy 0,05.

## 3.4. Materiał i metody w badaniu predyktorów ciężkiego przebiegu COVID-19 w grupie kobiet ciężarnych

### 3.4.2. Materiał

Jednoośrodkowe badanie retrospektywne 52 ciężarnych pacjentek przyjętych do szpitala pomiędzy 15 maja 2020 r. a 26 kwietnia 2021 r. przeprowadzono w Centralnym Szpitalu Klinicznym MSWiA w Warszawie. Średni wiek ciężarnych (± odchylenie standardowe) wynosił 31,9 lat ± 4,79 lat. Wiek ciążowy wahał się w zakresie od 17 do 37 tygodni. Kryteria włączenia do badania były zbliżone do kryteriów hospitalizacji ciężarnych z COVID-19, tj. gorączka powyżej 39°C pomimo stosowania paracetamolu, częstość oddechów powyżej 30/minutę, $SpO_2$ poniżej 95% przy oddychaniu powietrzem atmosferycznym, konieczność suplementacji tlenu oraz wystąpienie krytycznego przebiegu choroby. Zakażenie SARS-CoV-2 rozpoznawano na podstawie dodatniego wyniku testu RT-PCR wykonanego przed przyjęciem do szpitala. Za kryteria wyłączenia uznano przyjęcie z powodów położniczych lub innych przyczyn niewynikających z zachorowania na COVID-19 (takich jak uraz, inne ostre schorzenia lub zaostrzenie chorób przewlekłych). Badanie zostało zatwierdzone przez Komisję Bioetyczną CSK MSWiA w Warszawie.

### 3.4.2. Metody

#### 3.3.2.1. Wyniki kliniczne

Dane dotyczące charakterystyki pacjentek (takie jak wiek, wskaźnik masy ciała, palenie tytoniu i choroby współistniejące), wieku ciążowego, przebiegu choroby (w tym objawów, parametrów życiowych, konieczności przyjęcia do OIT, metod wspomagania oddychania), a także wyniki badań laboratoryjnych i obrazowych (tomografii komputerowej klatki piersiowej) przy przyjęciu do szpitala uzyskano z elektronicznej dokumentacji medycznej i wprowadzono do bazy danych po ich anonimizacji.

#### 3.3.2.2. Analiza statystyczna

Do porównania zmiennych ilościowych zastosowano test U Manna-Whitneya, zaś do porównania zmiennych jakościowych zastosowano test chi-kwadrat i test dokładny Fishera. Jako że tylko u jednej pacjentki wystąpił przebieg krytyczny, pacjentki o przebiegu ciężkim i krytycznym zostały połączone w jedną grupę. Porównania pomiędzy tak utworzonymi trzema grupami ciężkości przebiegu (przebieg łagodny, umiarkowany i ciężki lub krytyczny) dla zmiennych ilościowych wykonano z zastosowaniem testu

Kruskala-Wallisa z testem post-hoc Dunn, zaś dla zmiennych jakościowych z zastosowaniem testu chi-kwadrat. Korelacje dla zmiennych ciągłych oceniono z zastosowaniem analizy korelacji Spearmana. W celu identyfikacji czynników związanych z wystąpieniem ciężkiego lub krytycznego przebiegu choroby, przeprowadzono jednoczynnikową regresję logistyczną. Analiza została wykonana przy użyciu oprogramowania Statistica (wersja 13.3; StatSoft, Polska). We wszystkich analizach przyjęto poziom istotności równy 0,05.

## 4. PUBLIKACJE WCHODZĄCE W SKŁAD ROZPRAWY DOKTORSKIEJ – KOPIE OPUBLIKOWANYCH PRAC

**Publikacja 1.**

**Tytuł**: Simple Disposable Odor Identification Tests for Predicting SARS-CoV-2 Positivity

**Autorzy**: Ziuzia-Januszewska L, Dobrzyński P, Ślączka K, Ciszek J, Krawiec Ł, Wierzba W, Zaczyński A

**Czasopismo**: International Journal of Environmental Research and Public Health. 2021; 18(19):10185

**doi**: 10.3390/ijerph181910185

International Journal of
*Environmental Research and Public Health*

MDPI

*Article*

# Simple Disposable Odor Identification Tests for Predicting SARS-CoV-2 Positivity

**Laura Ziuzia-Januszewska [1,*], Paweł Dobrzyński [1], Krzysztof Ślączka [1], Jaromir Ciszek [1], Łukasz Krawiec [1], Waldemar Wierzba [2,3] and Artur Zaczyński [3]**

[1] Department of Otolaryngology, Central Clinical Hospital of the Ministry of the Interior and Administration, 02-507 Warsaw, Poland; pawel.dobrzynski@cskmswia.gov.pl (P.D.); krzysztof.slaczka@cskmswia.gov.pl (K.Ś.); jaromir.ciszek@gmail.com (J.C.); lukasz.krawiec@cskmswia.gov.pl (Ł.K.)
[2] UHE Satellite Campus, University of Humanities and Economics, 01-513 Warsaw, Poland; waldemar.wierzba@cskmswia.gov.pl
[3] Central Clinical Hospital of the Ministry of the Interior and Administration, 02-507 Warsaw, Poland; artur.zaczynski@cskmswia.gov.pl
* Correspondence: laura.ziuzia@cskmswia.gov.pl; Tel.: +48-477-221-182

**Abstract:** Olfactory dysfunction (OD) is a common manifestation of COVID-19 and may be useful for screening. Survey-based olfactory evaluation tends to underestimate the prevalence of OD, while psychophysical olfactory testing during a pandemic has the disadvantage of being time consuming, expensive, and requiring standardized laboratory settings. We aimed to develop a quick, simple, affordable, and reliable test to objectively assess the prevalence and diagnostic accuracy of OD in COVID-19. The olfactory function of 64 COVID-19 inpatients and 34 controls was evaluated using a questionnaire and a simple disposable odor identification test (SDOIT) developed for this study. Four SDOIT models were assessed: 10-SDOIT, 9-SDOIT, 8-SDOIT, and 4-SDOIT, with 10, 9, 8 and 4 samples, respectively. We found a high frequency of self-reported OD in COVID-19 patients, with 32.8% and 42.2% reporting current and recent OD, respectively. Different SDOIT models revealed smell impairment in 54.7–64.1% of COVID-19 patients. The combination of either 10-SDOIT results and self-reported OD, or 8-SDOIT results and self-reported OD, were the best predictors of COVID-19, both with an AUC value of 0.87 (0.85 and 0.86 for the age-matched subjects). OD is a common symptom of COVID-19. A combination of self-reported smell deterioration and OD psychophysically evaluated using SDOIT appears to be a good predictor of COVID-19.

**Keywords:** olfactory; anosmia; COVID-19; objective; SDOIT



**Citation:** Ziuzia-Januszewska, L.; Dobrzyński, P.; Ślączka, K.; Ciszek, J.; Krawiec, Ł.; Wierzba, W.; Zaczyński, A. Simple Disposable Odor Identification Tests for Predicting SARS-CoV-2 Positivity. *Int. J. Environ. Res. Public Health* **2021**, *18*, 10185. https://doi.org/10.3390/ijerph181910185

Academic Editor: Paul B. Tchounwou

Received: 17 August 2021
Accepted: 25 September 2021
Published: 28 September 2021



## 1. Introduction

A growing body of evidence shows a high incidence of olfactory dysfunction (OD) in coronavirus disease 2019 (COVID-19), with prevalence ranging from 5 to 98.3% [1–7]. Therefore, it has been hypothesized that new-onset smell impairment could serve as a potential predictor of SARS-CoV-2 infection [8,9].

Most of the previous studies on OD in COVID-19 are survey-based. However, self-assessment of OD tends to underestimate its true prevalence due to recall bias and subjects not being aware of their smell impairment [2,10], especially while experiencing other, severe symptoms such as respiratory distress [11]. Indeed, Moein et al. [2] found that 98% of COVID-19 patients exhibited OD when assessed objectively, compared with only 28% self-reporting smell deterioration. Similarly, in a study by Vaira et al. [12], objective evaluation revealed mild hyposmia in 30.3% of subjectively normosmic patients. Moreover, several meta-analyses showed a higher overall prevalence of OD when using objective compared with subjective assessment methods (72.1–77% vs. 44.5–53%, respectively) [11,13,14].

However, psychophysical olfactory tests are time consuming, expensive, and require standardized laboratory settings, and thus, they are difficult to perform during

a pandemic [13]. Moreover, disposable tests are preferable to reduce the risk of viral contamination [15]. Self-administered home-based objective olfactory tests have been proposed [16–18]. However, odor identification evaluation in these settings may be less reliable as subjects ought not know which odorants are being tested [16], and the olfactory threshold assessment or intensity ratings that are proposed in the literature [16–18] may be more difficult to apply than performing a relatively simple identification test. Nevertheless, these novel methods appear to be valuable alternatives to the standard psychophysical tests.

In the present study, we aimed to psychophysically evaluate the prevalence of OD in 64 hospitalized patients with laboratory-confirmed COVID-19 and to develop a quick, simple, affordable, and reliable test to screen for SARS-CoV-2 infection. Consequently, we propose a simple disposable odor identification test (SDOIT).

## 2. Materials and Methods

### 2.1. Subjects and Settings

This case-control study was conducted between April 2020 and August 2020 at the Central Clinical Hospital of the Ministry of the Interior and Administration in Warsaw, Poland, which has been designated by the Government for the treatment of patients suffering from COVID-19. The inclusion criteria for the case group were adults (≥18 years old) with laboratory-confirmed SARS-CoV-2 infection. COVID-19 was diagnosed by RT-PCR performed on nasopharyngeal samples, utilizing SARS-CoV-2 nucleic acid detection kit (GeneFinder TM COVID-19 Plus RealAmp Kit) in adherence to the protocols supplied by the kit manufacturer. The control group consisted of healthy adult volunteers with no symptoms of COVID-19 (other than recent OD, which was not queried for to avoid selection bias) or upper respiratory tract infection. Although a negative test result for SARS-CoV-2 was not an inclusion criterion for the control group, the total number of individuals diagnosed with COVID-19 from the beginning of the pandemic to the end of August 2020 was lower than 0.18% of the Polish population (67372/38354000) [19], hence the risk of infection in asymptomatic volunteers was considered to be very low.

The exclusion criteria were age below 18 years of age, pregnancy, a history of pre-existing OD, head trauma, rhinosinusitis, or other chronic nasal disease, and inability to complete the questionnaire due to a history of neurocognitive disorders, an altered state of consciousness, or the need for intensive care and / or invasive ventilation at the time of the survey.

### 2.2. Clinical Outcomes

All participants completed a questionnaire regarding:

(1) General demographic data;
(2) Medical history (comorbidities, chronic medication use, tobacco addiction, and pre-existing OD);
(3) COVID-19 course (date of first symptoms, nasal, and general symptoms), and;
(4) Olfactory function—participants rated their sense of smell at its worst since the onset of the disease ("recent OD") as "normal", "decreased", or "none at all", as well as using the visual analogue scale (VAS), from 0 (normal sense of smell) to 10 (no sense of smell).

The onset (in relation to the day of the survey and to other COVID-19 symptoms, expressed as either before, or concurrently, or after) and persistence (complete, or incomplete, or no recovery, or worsening) of OD were evaluated. As none of the subjects reported an incomplete recovery, the olfactory function at the time of the survey ("current OD") for the patients reporting complete recovery was classified as "normal" and "0" on the descriptive scale and VAS, respectively, and for the remaining patients classified as equal to the "recent OD". Any data missing from the forms and information regarding the course of the disease were transcribed from the electronic medical records. The physiological parameters were assessed at least once a day using the modified early warning score (MEWS) [20] adapted

by the hospital therapeutic committee by including oxygen saturation and need for oxygen supplementation.

### 2.3. Psychophysical Evaluation

The psychophysical olfactory evaluation was performed concurrently with the questionnaire on all participants, using the simple disposable odor identification test (SDOIT) that we had developed for the purpose of this study. Ten disposable test paper strips numbered 1 to 10, 9 of which contained well-known pure odorants (commercially available cinnamon, mint, lemon, coffee, clove, rose, anise and camphor essential oils and disinfectant alcohol) and the remaining 1 with an odorless control (deionized water) were each enclosed in plastic covers so the odors did not mix. Upon removal of the covers, each odor was presented to a patient at 30-second intervals to prevent olfactory desensitization. The odorants used in the study were selected to include both unimodal odors, with little or no trigeminal stimulation, and bimodal odors, with mixed stimulation of the olfactory and trigeminal nerve. For each odor strip, patients were asked to indicate whether they detected an odor and if so, to identify the odor (using a forced choice format, with 4 given options per test odorant; see Table 1). The SDOIT test kit is shown in the Supplementary materials (Figure S1). We defined OD in the SDOIT when a patient correctly identified a number of odors lower than the 10th percentile of the results from the control group, as performed by Iravani et al. [16], and as commonly used in other previous olfactory tests [10,16]. This resulted in defining OD as at least 2 incorrect answers in 10-SDOIT, 9-SDOIT and 8-SDOIT and at least 1 incorrect answer in 4-SDOIT. We did not assess other components of olfaction such as odor threshold and odor discrimination, so that we could maximize the simplicity and minimize the time necessary for the assessment.

**Table 1.** Odors and distractors used in the SDOIT.

| Title 1 | Title 2 |
|---|---|
| cinnamon | honey, vanilla, chocolate |
| mint | onion, gasoline, garlic |
| lemon | peach, apple, plum |
| coffee | tobacco, wine, smoke |
| clove | grass, garlic, chocolate |
| rose | green tea, strawberry, cherry |
| anise | peach, rose, mint |
| camphor | gas, caramel, onion |
| alcohol (disinfectant) | gasoline, cucumber, burned rubber |
| odorless sample | rose, garlic, lemon, mint |

### 2.4. Ethical Concerns

This study was approved by the Ethics Committee of the Central Clinical Hospital of the Ministry of the Interior and Administration in Warsaw and was performed in accordance with the ethical standards of the Declaration of Helsinki and its later amendments. Voluntary written informed consent was obtained from all participants. All tests were performed with the highest regard for patients' and examiners' safety using appropriate personal protective equipment.

### 2.5. Statistical Analyses and ROC Analysis of COVID-19 Predictors

Usual descriptive statistics were used, as shown in Tables 2–8. Fisher's exact test was used to compare self-reported OD and the Mann–Whitney U test was used to compare the SDOIT results between cases and controls. To assess the correlation between the self-report olfactory function and SDOIT results, Fisher's exact test and the Chi-square test were used. In cases, the Spearman correlation coefficient was used to test the correlation between clinical features and OD in the case where the studied variables were quantitative, the Mann-Whitney U test to study differences within a qualitative variable and a quantitative variable, Fisher's exact test for two qualitative variables. Statistical analysis was performed

with R software (version 3.6.0, R Foundation for Statistical Computing, Vienna, Austria). A level of $p < 0.05$ was used to determine statistical significance. The receiver operating characteristic (ROC) curves were plotted and the area under the ROC curve (AUC) analysis was performed to assess the utility of 19 selected classifiers (as described in the Results section below) in predicting SARS-CoV-2 positivity. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of these predictors were also evaluated. For psychophysical test we assessed both the models defining OD as SDOIT scores below the cut-off value set at 10th percentile of the results obtained in the control group, and the models defining OD as scores below the optimal cut-off values calculated in ROC analysis. For the ROC analysis, for the combination of SDOIT results (with OD defined in each case as in the description of a given classifier) and the self-reported OD, a variable with three possible values was introduced: (1) subjective normosmia and normal SDOIT result (no OD in SDOIT); (2) subjective normosmia and OD in SDOIT; (3) subjective OD (it was not necessary to add more categories, as only cases reported subjective OD). We did not include other (non-smell-related) COVID-19 symptoms in the analysis because the role of additional symptoms could be overestimated due to selection bias, as the control group consisted of healthy individuals.

**Table 2.** Comparison of demographic data and smoking status of cases and controls.

| Characteristic | | Total (*N* = 98) | COVID-19 Patients (*N* = 64) | Control Patients (*N* = 34) |
|---|---|---|---|---|
| Age, years | mean ± SD | 48.4 ± 18.8 | 52.3 ± 20.9 | 40.9 ± 10.7 |
| | median (IQR) | 47 (32–64) | 55 (33–68.5) | 40.5 (32–49.8) |
| | range | 20–91 | 20–91 | 27–61 |
| Gender, *N* (%) | female | 55 (56.1%) | 29 (45.3%) | 26 (76.5%) |
| | male | 43 (43.9%) | 35 (54.7%) | 8 (23.5%) |
| Smoking history | nonsmoker, *N* (%) | 64 (65.3%) | 35 (54.7%) | 29 (85.3%) |
| | former smoker, *N* (%) | 23 (23.5%) | 21 (32.8%) | 2 (5.9%) |
| | current smoker, *N* (%) | 11 (11.2%) | 8 (12.5%) | 3 (8.8%) |

**Table 3.** Correlations between self-reported OD and clinical characteristics of COVID-19 patients.

| Variable | | Self-Reported OD | | | | | |
|---|---|---|---|---|---|---|---|
| | | Presence of Self-Reported OD at the Time of the Survey | | | Presence of Self-Reported OD at Any Time since the Onset of COVID-19 | | |
| | | Yes (*N* = 21) | No (*N* = 43) | *p*-Value | Yes (*N* = 27) | No (*N* = 37) | *p*-Value |
| Nasal congestion, *N* (%) | yes | 11 (52.4) | 10 (23.3) | 0.041 [1] | 14 (51.9) | 7 (18.9) | 0.012 [1] |
| | no | 10 (47.6) | 33 (76.7) | | 13 (48.1) | 30 (81.1) | |
| Rhinorrhea, *N* (%) | yes | 9 (42.9) | 10 (23.3) | 0.187 [1] | 14 (51.9) | 5 (13.5) | 0.002 [1] |
| | no | 12 (57.1) | 33 (76.7) | | 13 (48.1) | 32 (86.5) | |
| Current smoking, *N* (%) | yes | 3 (14.3) | 5 (11.6) | 1 [2] | 3 (11.1) | 5 (13.5) | 1 [2] |
| | no | 18 (85.7) | 38 (88.4) | | 24 (88.9) | 32 (86.5) | |
| Former or current smoking, *N* (%) | yes | 8 (38.1) | 21 (48.8) | 0.587 [1] | 8 (29.6) | 21 (56.8) | 0.058 [1] |
| | no | 13 (61.9) | 22 (51.2) | | 19 (70.4) | 16 (43.2) | |
| Death, *N* (%) | yes | 2 (9.5) | 6 (14) | 1 [2] | 2 (7.4) | 6 (16.2) | 0.450 [2] |
| | no | 19 (90.5) | 37 (86) | | 25 (92.6) | 31 (83.8) | |
| Need for oxygen therapy, *N* (%) | yes | 7 (33.3) | 21 (48.8) | 0.365 [1] | 7 (25.9) | 21 (56.8) | 0.028 [1] |
| | no | 14 (66.7) | 22 (51.2) | | 20 (74.1) | 16 (43.2) | |
| Need for ICU stay, *N* (%) | yes | 1 (4.8) | 5 (11.6) | 0.654 [2] | 1 (3.7) | 5 (13.5) | 0.388 [2] |
| | no | 20 (95.2) | 38 (88.4) | | 26 (96.3) | 32 (86.5) | |
| Time interval between first positive PCR result and time of the survey, days | Mean ± SD | 5.1 ± 4.2 | 7.6 ± 6.7 | 0.290 [3] | 6.7 ± 4.8 | 7.1 ± 7 | 0.615 [3] |
| | Median (IQR) | 3 (2–8) | 6 (2–12) | | 5 (2.5–12) | 4 (2–12) | |
| Duration of hospitalisation (excluding deceased patients) | N | 19 | 37 | 0.862 [3] | 25 | 31 | 0.060 [3] |
| | Mean ± SD | 19 ± 10.6 | 20.7 ± 14.2 | | 16.6 ± 10.5 | 23 ± 14.3 | |
| | Median (IQR) | 17 (12–23.5) | 18 (10–24) | | 13 (10–19) | 18 (15–28) | |
| MEWS score at the time of the survey | Mean ± SD | 0.9 ± 1.3 | 0.9 ± 1.7 | 0.837 [3] | 0.7 ± 1.1 | 1 ± 1.8 | 0.254 [3] |
| | Median (IQR) | 0 (0–1) | 0 (0–1) | | 0 (0–1) | 1 (0–1) | |
| Avarage MEWS score | Mean ± SD | 0.9 ± 1.5 | 1 ± 1.3 | 0.481 [3] | 0.7 ± 1.4 | 1.1 ± 1.4 | 0.081 [3] |
| | Median (IQR) | 0 (0–1) | 1 (0–1) | | 0 (0–1) | 1 (0–2) | |

[1] Chi-squared test. [2] Fisher test, [3] Mann–Whitney test.

**Table 4.** Correlations between objective OD (according to SDOIT) and quantitative clinical characteristics of COVID-19 patients; Mann–Whitney test.

| Variable | | SDOIT, % of Correct Answers | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 10-SDOIT | | 9-SDOIT | | 8-SDOIT | | 4-SDOIT | |
| | | Mean ± SD | Median (IQR) | Mean ± SD | Median (IQR) | Mean ± SD | Median (IQR) | Mean ± SD | Median (IQR) |
| Nasal congestion | yes | 72.9 ± 30.4 | 90 (50–100) | 72 ± 33.5 | 88.9 (55.6–100) | 71.4± 33.1 | 87.5 (50–100) | 70.2 ± 40 | 100 (25–100) |
| | no | 63 ± 32.3 | 70 (45–90) | 62.5 ± 34.6 | 77.8 (44.4–88.9) | 62.8 ± 34.9 | 75 (37.5–87.5) | 55.8 ± 38.1 | 75 (25–87.5) |
| | *p*-value | 0.188 | | 0.197 | | 0.240 | | 0.081 | |
| Rhinorrhea | yes | 73.7 ± 30.4 | 90 (50–100) | 73.1 ± 32.4 | 88.9 (56–100) | 73.3 ± 32.1 | 87.5 (56.3–100) | 69.7 ± 37.8 | 100 (37.5–100) |
| | no | 63.1 ± 32.2 | 80 (40–90) | 62.5 ± 34.9 | 77.8 (44.4–88.9) | 62.5 ± 35.1 | 75 (37.5–87.5) | 56.7 ± 39.3 | 75 (25–100) |
| | *p*-value | 0.110 | | 0.152 | | 0.179 | | 0.153 | |
| Current smoking | yes | 70 ± 31.2 | 85 (55–90) | 68.1 ± 35.9 | 83.3 (50–91.7) | 68.8 ± 34.7 | 81.3 (56.4–90.6) | 65.6 ± 37.7 | 75 (43.8–100) |
| | no | 65.7 ± 32.1 | 80 (47.5–90) | 65.3 ± 34.4 | 77.8 (44.4–88.9) | 65.2 ± 34.5 | 75 (37.5–90.6) | 59.8 ± 39.5 | 75 (25–100) |
| | *p*-value | 0.806 | | 0.837 | | 0.829 | | 0.745 | |
| Former or current smoking | yes | 63.1 ± 30.7 | 80 (40–90) | 62.8 ± 32.8 | 77.8 (44.4–88.9) | 62.9 ± 32.8 | 75 (37.5–87.5) | 55.2 ± 40.3 | 75 (25–100) |
| | no | 68.9 ± 32.9 | 80 (50–100) | 67.9 ± 35.7 | 77.8 (55.6–100) | 67.9 ± 35.8 | 75 (56.3–100) | 65 ± 38 | 75 (37.5–100) |
| | *p*-value | 0.241 | | 0.259 | | 0.308 | | 0.335 | |
| Death | yes | 51.2 ± 22.3 | 60 (55–60) | 50 ± 21.4 | 55.6 (52.8–58.3) | 51.6 ± 23.6 | 62.5 (46.9–62.5) | 40.6 ± 29.7 | 37.5 (25–50) |
| | no | 68.4 ± 32.5 | 8 (47.5–90) | 67.9 ± 35.3 | 88.9 (44.4–100) | 67.6 ± 35.3 | 87.5 (37.5–100) | 63.4 ± 39.6 | 75 (25–100) |
| | *p*-value | 0.066 | | 0.048 | | 0.047 | | 0.108 | |
| Need for oxygen therapy | yes | 58.9 ± 30.6 | 6 (47.5–80) | 58.3 ± 32.3 | 61.1 (52.8–80.6) | 58.9 ± 32.4 | 62.5 (46.9–78.1) | 49.1 ± 35.7 | 50 (18.8–75) |
| | no | 71.9 ± 32 | 90 (47.5–100) | 71.3 ± 35.2 | 88.9 (44.4–100) | 70.8 ± 35.2 | 87.5 (37.5–100) | 69.4 ± 39.7 | 100 (25–100) |
| | *p*-value | 0.032 | | 0.026 | | 0.035 | | 0.013 | |
| Need for ICU stay | yes | 68.3 ± 16 | 60 (60–67.5) | 66.7 ± 17.2 | 61.1 (55.6–66.7) | 68.8 ± 17.2 | 62.5 (62.5–71.9) | 58.3 ± 34.2 | 50 (31.3–87.5) |
| | no | 66 ± 33.1 | 80 (40–90) | 65.5 ± 35.7 | 77.8 (44.4–88.9) | 65.3 ± 35.7 | 75 (37.5–96.9) | 60.8 ± 39.8 | 75 (25–100) |
| | *p*-value | 0.852 | | 0.700 | | 0.682 | | 0.877 | |

**Table 5.** Correlations between objective OD (according to SDOIT) and qualitative clinical characteristics of COVID-19 patients; Spearman correlation.

| Variable | | SDOIT | | | |
|---|---|---|---|---|---|
| | | 10-SDOIT | 9-SDOIT | 8-SDOIT | 4-SDOIT |
| Time interval between first positive PCR result and time of the survey, days | ρ | 0.19 | 0.18 | 0.19 | 0.1 |
| | *p*-value | 0.123 | 0.163 | 0.141 | 0.447 |
| Duration of hospitalisation (excluding deceased) | ρ | −0.43 | −0.42 | −0.41 | −0.36 |
| | *p*-value | <0.001 | 0.002 | 0.002 | 0.007 |
| MEWS score at the time of the survey | ρ | −0.29 | −0.32 | −0.32 | −0.25 |
| | *p*-value | 0.02 | 0.011 | 0.011 | 0.043 |
| Avarage MEWS score | ρ | −0.29 | −0.3 | −0.29 | −0.24 |
| | *p*-value | 0.02 | 0.016 | 0.018 | 0.054 |

**Table 6.** Comparison of self-reported and objective olfactory function of cases and controls.

| Characteristic | | COVID-19 Patients (*N* = 64) | Control Patients (*N* = 34) | *p*-Value |
|---|---|---|---|---|
| Reported smell at the time of maximum deterioration, *N* (%) | normosmia<br>hyposmia<br>anosmia | 37 (57.8)<br>21 (32.8)<br>6 (9.4) | 34 (100)<br>0 (0)<br>0 (0) | <0.001 [1] |
| Reported smell at the time of the survey, *N* (%) | normosmia<br>hyposmia<br>anosmia | 43 (67.2)<br>18 (28.1)<br>3 (4.7) | 34 (100)<br>0 (0)<br>0 (0) | <0.001 [1] |
| VAS score of smell deterioration (at the time of maximum deterioration) | mean ± SD<br>median (IQR) | 3.4 ± 3.6<br>2 (0–7) | 0 ± 0<br>0 (0–0) | <0.001 [2] |
| VAS score of smell deterioration (at the time of the survey) | mean ± SD<br>median (IQR) | 2.6 ± 3.2<br>2 (0–5) | 0 ± 0<br>0 (0–0) | <0.001 [2] |
| SDOIT—detected odors, *N* | mean ± SD<br>median (IQR) | 7.5 ± 2.7<br>(83.5% ± 29.4%)<br>9 (7–9)<br>(100% (77.8%–100%)) | 9 ± 0<br>(100% ± 0%)<br>9 (9–9)<br>(100% (100%–100%)) | <0.001 [2] |
| 10-SDOIT, correct answers, *N* (%) | mean ± SD<br>median (IQR) | 6.6 ± 3.2<br>(66.3% ± 31.8%)<br>8 (4.8–9)<br>(80% (47.5%–90%)) | 9.6 ± 0.8<br>(95.6% ± 8.2%)<br>10 (9–10)<br>(100% (90%–100%)) | <0.001 [2] |
| 9-SDOIT, correct answers, *N* (%) | mean ± SD<br>median (IQR) | 5.9 ± 3.1<br>(65.6% ± 34.3%)<br>7 (4–8)<br>(77.8% (44.4%–88.9%)) | 8.6 ± 0.7<br>(95.8% ± 7.7%)<br>9 (8–9)<br>(100% (88.9%–100%)) | <0.001 [2] |
| 8-SDOIT, correct answers, *N* (%) | mean ± SD<br>median (IQR) | 5.3 ± 2.7<br>(65.6 % ± 34.3%)<br>6 (3–7.3)<br>(75% (37.5%–90.6%)) | 7.8 ± 0.5<br>(97.1% ± 6.2%)<br>8 (8–8)<br>(100% (100%–100%)) | <0.001 [2] |
| 4-SDOIT, correct answers, *N* (%) | mean ± SD<br>median (IQR) | 2.4 ± 1.6<br>(60.6% ± 39%)<br>3 (1–4)<br>(75% (25%–100%)) | 3.9 ± 0.2<br>(98.5% ± 6%)<br>4 (4–4)<br>(100% (100%–100%)) | <0.001 [2] |

[1] Fisher test, [2] Mann–Whitney test.

**Table 7.** Comparison of self-reported and objective olfactory function of the subset of 75% youngest COVID-19 patients and controls.

| Characteristic | | COVID-19 Patients (*N* = 48) | Control Patients (*N* = 34) | *p*-Value |
|---|---|---|---|---|
| Reported smell at the time of maximum deterioration, *N* (%) | normosmia<br>hyposmia<br>anosmia | 28 (58.3)<br>15 (31.2)<br>5 (10.4) | 34 (100)<br>0 (0)<br>0 (0) | <0.001 [1] |
| Reported smell at the time of the survey, *N* (%) | normosmia<br>hyposmia<br>anosmia | 34 (70.8)<br>12 (25)<br>2 (4.2) | 34 (100)<br>0 (0)<br>0 (0) | <0.001 [1] |
| VAS score of smell deterioration (at the time of maximum deterioration) | mean ± SD<br>median [IQR] | 3.4 ± 3.7<br>2 (0–7) | 0 ± 0<br>0 (0–0) | <0.001 [2] |
| VAS score of smell deterioration (at the time of the survey) | mean ± SD<br>median [IQR] | 2.3 ± 3.1<br>0 (0–3) | 0 ± 0<br>0 (0–0) | <0.001 [2] |
| SDOIT-detected odors, *N* | mean ± SD<br>median (IQR) | 7.7 ± 2.5<br>(85.4% ± 28.1%)<br>9 (8–9)<br>(100% (88.9%–100%)) | 9 ± 0<br>(100% ± 0%)<br>9 (9–9)<br>(100% (100%–100%)) | <0.001 [2] |
| 10-SDOIT, correct answers, *N* (%) | mean ± SD<br>median (IQR) | 7.4 ± 3<br>(74.2% ± 29.5%)<br>9 (6–10)<br>(90% (60%–100%)) | 9.56 ± 0.82<br>(95.6% ± 8.2%)<br>10 (9–10)<br>(100% (90%–100%) | <0.001 [2] |
| 9-SDOIT, correct answers, *N* (%) | mean ± SD<br>median (IQR) | 6.6 ± 2.93<br>(72.9% ± 32.6%)<br>8 (5.8–9)<br>(88.9% (63.9%–100%)) | 8.6 ± 0.7<br>(95.8% ± 7.7%)<br>9 (8–9)<br>(100% (88.9%–100%)) | <0.001 [2] |
| 8-SDOIT, correct answers, *N* (%) | mean ± SD<br>median (IQR) | 5.8 ± 2.6<br>(72.7 % ± 32.6%)<br>7 (5–8)<br>(87.5% (62.5%–100%)) | 7.8 ± 0.5<br>(97.1% ± 6.2%)<br>8 (8–8)<br>(100% (100%–100%)) | <0.001 [2] |
| 4-SDOIT, correct answers, *N* (%) | mean ± SD<br>median (IQR) | 2.8 ± 1.5<br>(69.8% ± 36.5%)<br>3 (2–4)<br>(75% (50%–100%)) | 3.9 ± 0.2<br>(98.5% ± 6%)<br>4 (4–4)<br>(100% (100%–100%)) | <0.001 [2] |

[1] Fisher test, [2] Mann–Whitney test.

**Table 8.** Correlation between self-reported olfactory function and objective test results.

| SDOIT Score | | Self-Reported Olfactory Function at the Time of the Survey (Normosmia/OD), *N* (%) | | | VAS Score (Maximum), *N* (%) | | |
|---|---|---|---|---|---|---|---|
| | | Normosmia (*N* = 77) | OD (*N* = 21) | *p*-Value | <5 (*N* = 75) | ≥5 (*N* = 23) | *p*-Value |
| 10-SDOIT | 0–8<br>9–10 | 25 (32.5)<br>52 (67.5) | 16 (76.2)<br>5 (23.8) | <0.001 | 27 (36)<br>48 (64) | 27 (60.9)<br>9 (39.1) | 0.061 |
| 9-SDOIT | 0–7<br>8–9 | 22 (28.6)<br>55 (71.4) | 15 (71.4)<br>6 (28.6) | <0.001 | 24 (32)<br>51 (68) | 13 (56.5)<br>10 (43.5) | 0.049 |
| 8-SDOIT | 0–6<br>7–8 | 6 (7.8)<br>71 (92.2) | 11 (52.4)<br>10 (47.6) | <0.001 | 7 (9.3)<br>68 (90.7) | 10 (43.5)<br>13 (56.5) | <0.001 |
| 4-SDOIT | 0–3<br>4 | 26 (33.8)<br>51 (66.2) | 17 (81)<br>4 (19) | <0.001 | 26 (34.7)<br>49 (65.3) | 17 (73.9)<br>6 (26.1) | 0.002 |

## 3. Results

### *3.1. Clinical Outcomes*

A total 64 cases (29 women and 35 men; mean age, 52.3 ± 20.9 years) and 34 controls (26 women and 8 men; mean age, 40.9 ± 10.7 years) were included in the study. The most prevalent symptoms of COVID-19 (other than OD) were fatigue (70.3%; *n* = 45), cough (39.1%; *n* = 25), fever (37.5%; *n* = 24), headache (35.9%; *n* = 23), and gastrointestinal

complaints (35.4%; $n = 23$). Nasal congestion was reported by 32.8% ($n = 21$), and rhinorrhea by 29.7% ($n = 19$) of those with COVID-19. The demographic and clinical characteristics are summarized in Tables 2–5 and in the Supplementary Materials (Tables S2–S4).

### 3.2. Self-Reported Olfactory Function

The surveys and olfactory evaluations took place 6.9 ± 6.1 days (range 0–24) after the first positive RT-PCR result and 9 ± 7 days (range 1–30) from the reported onset of OD (note that 2 patients did not remember the time of onset of OD). There was no significant correlation between self-reported OD and the time between the positive PCR result and the questionnaire ($p = 0.565$). OD appeared before (14.8%, $n = 4$), simultaneously (11.1%, $n = 3$), or after (74.1%, $n = 20$) the presentation of other COVID-19 symptoms. At the time of the evaluation, 7 patients (25.9%) reported complete recovery of olfactory function, while 20 patients (74.1%) reported no recovery (55.6%; $n = 15$), or worsening of olfactory function (18.5%; $n = 5$).

The frequency of self-reported smell impairment was significantly higher in COVID-19 patients, with 32.8% and 42.2% of patients reporting current and recent OD, respectively, while all the control subjects reported normosmia. The mean VAS score for smell deterioration was significantly higher in the study cases compared with the controls. These results are presented in Table 6.

As there was a difference between cases and controls in terms of age, we have also analyzed self-reported OD and SDOIT scores for the subset of 75% youngest COVID-19 patients ($N = 48$) whose age did not differ significantly from the control group ($p = 0.531$). The inter-group differences remained highly significant, with 29.2% and 41.7% reporting current and recent OD, respectively (Table 7).

### 3.3. Psychophysical Evaluation

The psychophysical evaluation was performed for all the study subjects ($n = 98$). Mean percentages of correct answers (for all 10 samples, including the non-odorant sample), detected odors and identified odors in cases vs. controls were 65.6% vs. 95.8%, 83.5% vs. 100%, and 66.3% vs. 95.6%, respectively (Table 6). For all odors, lower p-values were achieved for identification than for detection. Considering the identification results for each odor separately, we created two additional, shortened psychophysical test models, one with 8 and the other with four selected odorants. Therefore, 4 SDOIT models were included for further analysis:

1. 10-SDOIT, evaluating the number of correct answers (correct identification of nine odors and reporting of no odor detection in an odorless sample);
2. 9-SDOIT evaluating the number of identified odors out of nine odorants;
3. 8-SDOIT evaluating the number of identified odors out of eight odorants (cinnamon, mint, lemon, coffee, clove, anise, camphor, and alcohol)—excluding odorant showing no significant differences between cases and controls (rose), and;
4. 4-SDOIT evaluating the number of identified odors out of four odorants (cinnamon, mint, lemon, and alcohol)—showing the highest intergroup differences (with $p \leq 0.001$).

The results for the individual odor tests are presented in Supplementary materials (Table S1).

In the intergroup comparison, the mean scores in all four models were significantly lower in cases than in controls ($p < 0.001$ for all models, Table 6). Taking the cut-off value at the 10th percentile of the results in controls, we found OD in 59.4% (38/64) vs. 8.8% (3/34), 54.7% (35/64) vs. 5.9% (2/34), 54.7% (35/64) vs. 2.9% (1/34) and 64.1% (41/64) vs. 5.9% (2/34) of cases vs. controls, for 10-SDOIT, 9-SDOIT, 8-SDOIT and 4-SDOIT, respectively. OD was significantly associated with SARS-CoV-2 positivity, with odds ratios (OR) of 15.1, 19.3, 39.8 and 28.5 for 10-SDOIT, 9-SDOIT, 8-SDOIT and 4-SDOIT, respectively.

In the comparison of controls and the subset of 75% youngest COVID-19 patients ($N = 48$) the differences remained highly significant, with mean percentages of correct

answers (for all 10 samples), detected odors and identified odors in 75% youngest patients being 74.2%, 85.4% and 72.9%, respectively (Table 7).

There was a significant correlation between the current self-reported OD and psychophysically assessed OD in all the SDOIT models. (Table 8). Subjectively, normosmic COVID-19 patients showed OD at psychophysical evaluation in 51.2%, 46.5%, 46.5% and 55.8% of cases for 10-SDOIT, 9-SDOIT, 8-SDOIT and 4-SDOIT, respectively.

### 3.4. Correlations between OD and Patient Characteristics

There were no significant gender differences in self-reported olfactory function and psychophysical test results. Mean SDOIT scores were significantly lower among older patients, but there was no correlation between age and maximum VAS score and self-reported OD. Within the COVID-19 group, nasal obstruction was more prevalent in patients reporting OD compared with normosmic subjects (51.9% vs. 18.9%, and 52.4% vs. 23.3% for recent and current OD, respectively) and rhinorrhea was more prevalent in patients reporting recent OD (51.9% vs. 13.5%), but not current OD. No significant correlation was found between nasal symptoms and SDOIT scores. Patients with worse psychophysical test results had higher MEWS scores and were hospitalized longer, and 9-SDOIT and 8-SDOIT scores were significantly lower in patients who later died, while there were no correlations between the length of hospitalization, MEWS score and death with self-reported OD. The need for oxygen supplementation was less frequent in patients reporting recent OD, but more frequent in patients with lower SDOIT scores. The most important correlations between OD and patient characteristics are shown in Tables 3–5. More detailed data regarding these correlations are presented in the Supplementary Materials (Tables S2–S6).

### 3.5. Assessment of COVID-19 Predictors and ROC Analysis

We selected 19 classifiers for predicting SARS-CoV-2 positivity, including self-reported OD (with recent OD found to be a better classifier than current OD and therefore used in further assessments), maximum VAS score (with a cut-off point at <5), 4-SDOIT models and the combination of survey-based and psychophysical olfactory evaluation, as presented in Table 9. Self-reported recent OD achieved sensitivity of 42%, specificity of 100%, PPV of 100%, NPV of 48% and AUC of 0.71, and the maximum VAS score achieved sensitivity of 64%, specificity of 100%, PPV of 100%, NPV of 60% and AUC of 0.82 for predicting SARS-CoV-2 positivity. Our psychophysical evaluation, when defining OD as the score below the 10th percentile of healthy subjects, found 4-SDOIT to be the best classifier, with sensitivity of 64% and specificity of 94%, PPV of 95%, NPV of 63% and AUC of 0.8. However, the optimal cut-off point calculated in the ROC analysis for all SDOIT models was at least one incorrect answer with AUC of at least 0.8 for all models. The combination of SDOIT results and self-reported OD (with any OD, either subjective or objective, indicating COVID-19) improved the diagnostic accuracy. The inclusion of VAS did not improve these classifiers. To minimize the risk of patients suspected of infection eligible for isolation not being detected, classifiers with the highest AUC and the highest sensitivity were selected as the best predictors of COVID-19, combining self-reported OD and OD defined in SDOIT as:

(1) 0-9/10 correct answers in 10-SDOIT (with AUC of 0.87, sensitivity of 91%, specificity of 71%, PPV of 85% and NPV of 80%), and;
(2) 0-7/8 identified odors in 8-SDOIT (with AUC of 0.87, sensitivity of 86%, specificity of 79%, PPV of 89% and NPV of 75%).

**Table 9.** Results of the ROC analysis.

| Classifier | Sensitivity | Specifity | PPV | NPV | AUC |
|---|---|---|---|---|---|
| Self-reported OD at the time maximum deterioration | 0.42 (CI95% 0.3–0.55) | 1 (CI95% 1–1) | 1 (CI95% 1–1) | 0.48 (CI95% 0.43–0.54 | 0.71 (CI95% 0.65–0.77) |
| Maximum VAS | 0.64 (CI95% 0.53–0.75) | 1 (CI95% 1–1) | 1 (CI95% 1–1) | 0.6 (CI95% 0.53–0.68) | 0.82 (CI95% 0.76–0.88) |
| 10-SDOIT (OD $\geq$ 1 incorrect) | 0.8 (CI95% 0.56–0.78) | 0.71 (CI95% 0.62–0.76) | 0.84 (CI95% 0.79–0.86) | 0.65 (CI95% 0.52–0.77) | 0.82 (CI95% 0.74–0.9) |
| 10-SDOIT (OD $\geq$ 1 incorrect 0-9/10) + self-reported OD | 0.91 (CI95% 0.83-0.97) | 0.71 (CI95% 0.56–0.85) | 0.85 (CI95% 0.79–0.92) | 0.8 (CI95% 0.67–0.93) | 0.87 (CI95% 0.8–0.93) |
| 10-SDOIT (OD 0-8/10) + self-reported OD | 0.77 (CI95% 0.66–0.86) | 0.91 (CI95% 0.82–1) | 0.94 (CI95% 0.88–1) | 0.67 (CI95% 0.58–0.78) | 0.86 (CI95% 0.80–0.92) |
| 9-SDOIT (OD $\geq$ 1 incorrect) | 0.77 (CI95% 0.55–0.86) | 0.71 (CI95% 0.59–0.97) | 0.83 (CI95% 0.77–0.97) | 0.62 (CI95% 0.5–0.74) | 0.80 (CI95% 0.73–0.88) |
| 9-SDOIT (OD $\geq$ 1 incorrect 0-8/9) + self-reported OD | 0.88 (CI95% 0.83–0.97) | 0.71 (CI95% 0.56–0.85) | 0.85 (CI955 0.79–0.92) | 0.75 (CI95% 0.68–0.93) | 0.85 (CI95% 0.79–0.92) |
| 9-SDOIT (OD 0-7/9) + self-reported OD | 0.73 (CI95% 0.83–0.97) | 0.94 (CI95% 0.56–0.85) | 0.96 (CI95% 0.79–0.92) | 0.65 (CI95% 0.67–0.93) | 0.85 (CI95% 0.79–0.91) |
| 8-SDOIT (OD $\geq$ 1 incorrect) | 0.75 (CI95% 0.61–0.86) | 0.79 (CI95% 0.68–0.94) | 0.87 (CI95% 0.81–0.96) | 0.63 (CI95% 0.53–0.74) | 0.82 (CI95% 0.75–0.89) |
| 8-SDOIT (OD $\geq$ 1 incorrect 0-7/8) + self-reported OD | 0.86 (CI95% 0.77–0.94) | 0.79 (CI95% 0.65–0.91) | 0.89 (CI95% 0.82–0.95) | 0.75 (CI95% 0.64–0.88) | 0.87 (CI95% 0.81–0.93) |
| 8-SDOIT (OD 0-6/8) + self-reported OD | 0.73 (CI95% 0.62–0.84) | 0.97 (CI95% 0.91–1) | 0.98 (CI95% 0.93–1) | 0.66 (CI95% 0.58–0.76) | 0.86 (CI95% 0.8–0.92) |
| 4-SDOIT (OD $\geq$ 1 incorrect) | 0.64 (CI95% 0.53–0.75) | 0.94 (CI95% 0.85–1) | 0.95 (CI95% 0.89–1) | 0.58 (CI95% 0.51–0.67) | 0.80 (CI95% 0.74–0.87) |
| 4-SDOIT (OD $\geq$ 1 incorrect) + self-reported OD | 0.78 (CI95% 0.67–0.88) | 0.94 (C I95% 0.85–1) | 0.96 (CI95% 0.91–1) | 0.7 (CI95% 0.6–0.8) | 0.87 (CI95% 0.82–0.93) |

The main results are presented in Table 9 and Figure 1. The detailed results of the ROC analysis are presented in Supplementary materials (Table S7).

We have also performed the ROC analysis for controls and the subset of 75% youngest COVID-19 patients to eliminate the potential impact of age. These results are presented in Table 10 and Figure 2. In this analysis, self-reported recent OD achieved sensitivity of 42%, specificity of 100%, PPV of 100%, NPV of 55% and AUC of 0.71, and the maximum VAS score achieved sensitivity of 35%, specificity of 100%, PPV of 100%, NPV of 52% and AUC of 0.68 for predicting SARS-CoV-2 positivity. Similarly to the results of the analysis for the entire cohort, the analysis of age-matched group showed the 4-SDOIT to be the best classifier when defining OD as the score below the 10th percentile of healthy subjects, with sensitivity of 54% and specificity of 94%, PPV of 93%, NPV of 59% and AUC of 0.75, but the optimal cut-off point calculated in the ROC analysis for all SDOIT models was at least one incorrect answer with AUC of 0.76, 0.76, 0.78 and 0.75 for 10-SDOI, 9-SDOIT, 8-SDOI and 4-SDOIT, respectively. The combination of SDOIT results and self-reported OD improved the diagnostic accuracy. Selecting classifiers with the highest AUC and the highest sensitivity, the best predictors of COVID-19 were these combining self-reported OD and OD defined in SDOIT, i.e.,:

(1) 0-9/10 correct answers in 10-SDOIT (with AUC of 0.85, sensitivity of 88%, specificity of 71%, PPV of 81% and NPV of 80%), and;
(2) 0-7/8 identified odors in 8-SDOIT (with AUC of 0.86, sensitivity of 83%, specificity of 79%, PPV of 85% and NPV of 77%).

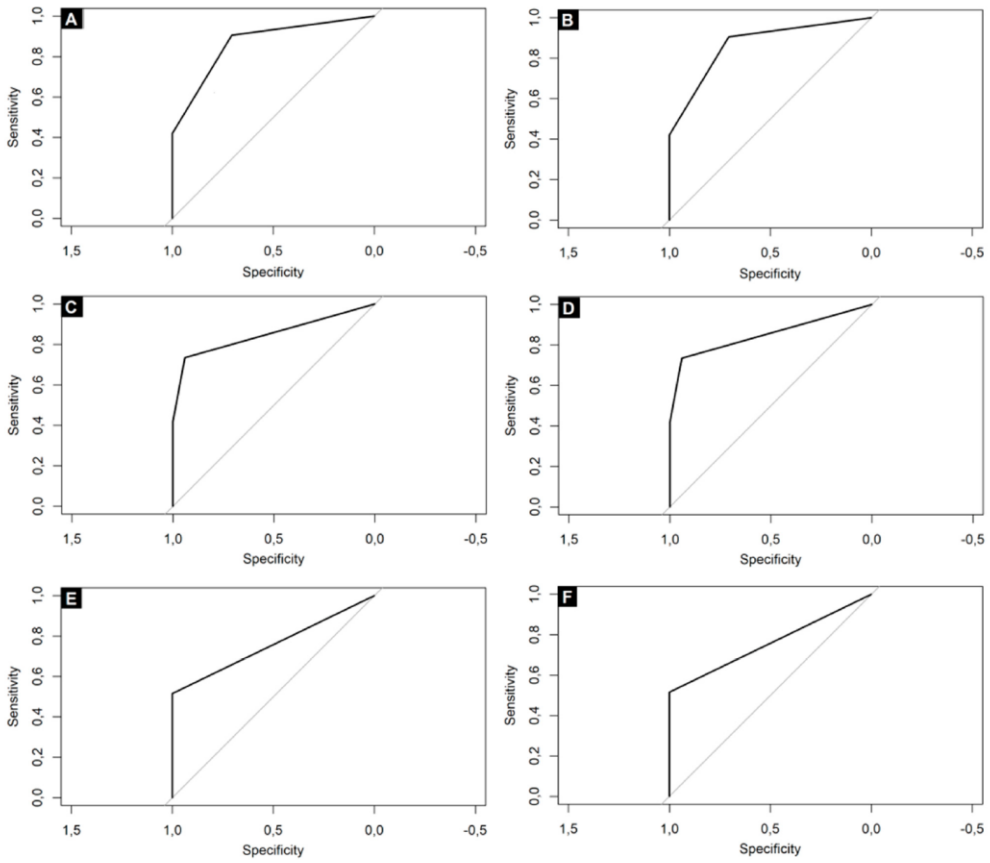


**Figure 1.** ROC curves for the combination of self-reported OD and: (**A**) 10-SDOIT with optimal cut-off value at 95% (OD defined as 0-9/10 correct answers); (**B**) 10-SDOIT with OD defined as 0-8/10 correct answers; (**C**) 9-SDOIT with OD defined as 0-8/9 identified odors; (**D**) 9-SDOIT with OD defined as 0-7/9 identified odors; (**E**) 8-SDOIT with OD defined as 0-7/8 identified odors; (**F**) 8-SDOIT with OD defined as 0-6/8 identified odors.

**Table 10.** Results of the ROC analysis for the subset of 75% youngest COVID-19 patients and controls.

| Classifier | Sensitivity | Specifity | PPV | NPV | AUC |
|---|---|---|---|---|---|
| Self-reported OD at the time of the maximum deterioration | 0.42 (CI95% 0.29–0.56) | 1 (CI95% 1–1) | 1 (CI95% 1–1) | 0.55 (CI95% 0.5–0.62) | 0.71 (CI95% 0.64–0.78) |
| Maximum VAS | 0.35 (CI95% 0.21–0.5) | 1 (CI95% 1–1) | 1 (CI95% 1–1) | 0.52 (CI95% 0.47–0.59) | 0.68 (CI95% 0.61–0.75) |
| 10-SDOIT (OD ≥ 1 incorrect) | 0.73 (CI95% 0.56–0.83) | 0.71 (CI95% 0.56–0.91) | 0.78 (CI95% 0.7–0.9) | 0.65 (CI95% 0.54–0.76) | 0.76 (CI95% 0.67–0.86) |
| 10-SDOIT (OD ≥ 1 incorrect 0-9/10) + self-reported OD | 0.88 (CI95% 0.77–0.96) | 0.71 (CI95% 0.56–0.85) | 0.81 (CI95% 0.73–0.9) | 0.8 (CI95% 0.67–0.93) | 0.85 (CI95% 0.78–0.92) |
| 10-SDOIT (OD 0-8/10) + self-reported OD | 0.71 (CI95% 0.58–0.83) | 0.91 (CI95% 0.79–1) | 0.92 (CI95% 0.83–1) | 0.69 (CI95% 0.6– 0.79) | 0.83 (CI95% 0.75–0.9) |
| 9-SDOIT (OD ≥ 1 incorrect) | 0.71 (CI95% 0.54–0.83) | 0.71 (CI95% 0.56–0.91) | 0.77 (CI95% 0.69–0.9) | 0.63 (CI95% 0.53–0.75) | 0.76 (CI95% 0.66–0.85) |
| 9-SDOIT (OD ≥ 1 incorrect 0-8/9) + self-reported OD | 0.85 (CI95% 0.75–0.96) | 0.71 (CI95% 0.56–0.85) | 0.8 (CI955 0.72–0.9) | 0.77 (CI95% 0.66–0.91) | 0.84 (CI95% 0.77–0.92) |
| 9-SDOIT (OD 0-7/9) + self-reported OD | 0.69 (CI95% 0.54–0.81) | 0.94 (CI95% 0.85–1) | 0.94 (CI95% 0.86–1) | 0.68 (CI95% 0.59–0.78) | 0.83 (CI95% 0.75–0.9) |
| 8-SDOIT (OD ≥ 1 incorrect) | 0.69 (CI95% 0.54–0.81) | 0.79 (CI95% 0.65–0.91) | 0.82 (CI95% 0.73–0.92) | 0.64 (CI95% 0.55–0.76) | 0.78 (CI95% 0.69–0.87) |
| 8-SDOIT (OD ≥ 1 incorrect 0-7/8) + self-reported OD | 0.83 (CI95% 0.73–0.94) | 0.79 (CI95% 0.65–0.91) | 0.85 (CI95% 0.76–0.93) | 0.77 (CI95% 0.66–0.9) | 0.86 (CI95% 0.78–0.93) |
| 8-SDOIT (OD 0-6/8) + self-reported OD | 0.69 (CI95% 0.54–0.81) | 0.97 (CI95% 0.91–1) | 0.97 (CI95% 0.9–1) | 0.69 (CI95% 0.6–0.79) | 0.84 (CI95% 0.77–0.9) |
| 4-SDOIT (OD ≥ 1 incorrect) | 0.54 (CI95% 0.4–0.69) | 0.94 (CI95% 0.85–1) | 0.93 (CI95% 0.83–1) | 0.59 (CI95% 0.52–0.69) | 0.75 (CI95% 0.67–0.83) |
| 4-SDOIT (OD ≥ 1 incorrect) + self-reported OD | 0.73 (CI95% 0.6–0.85) | 0.94 (C I95% 0.85–1) | 0.95 (CI95% 0.87–1) | 0.71 (CI95% 0.62–0.82) | 0.85 (CI95% 0.78–0.92) |

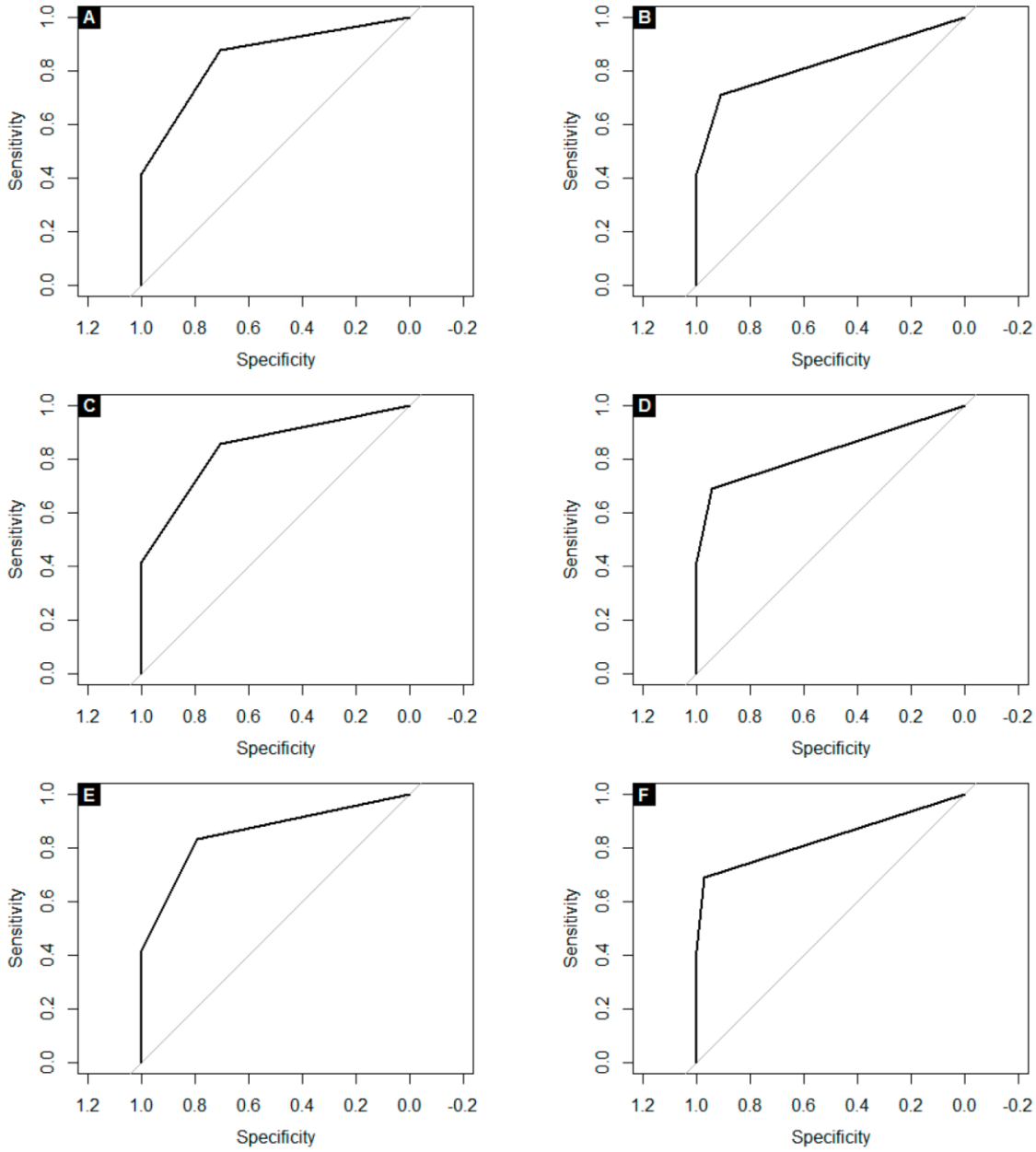


**Figure 2.** ROC curves for the subset of 75% youngest COVID-19 patients and controls for the combination of self-reported OD and: (**A**) 10-SDOIT with optimal cut-off value at 95% (OD defined as 0-9/10 correct answers); (**B**) 10-SDOIT with OD defined as 0-8/10 correct answers; (**C**) 9-SDOIT with OD defined as 0-8/9 identified odors; (**D**) 9-SDOIT with OD defined as 0-7/9 identified odors; (**E**) 8-SDOIT with OD defined as 0-7/8 identified odors; (**F**) 8-SDOIT with OD defined as 0-6/8 identified odors.

## 4. Discussion

We found self-reported recent OD in 42.2% of COVID-19 patients. This is consistent with pooled prevalence estimates for OD reported in meta-analyses, ranging from 35 to 56% [11,13,14,21,22]. Although many previous studies reported a higher prevalence of OD in women [4–6], several authors found no gender differences, especially when using objective tests [2,17]. Likewise, we did not observe any differences in the prevalence of OD

based on sex. This may indicate that previously reported female predominance reflects an increased sensitivity of women in detecting chemosensory dysfunctions or their greater propensity to complete surveys [14,23].

Most of the studies regarding smell impairment in COVID-19 are survey-based, however, studies show that self-assessment of olfactory function tends to underestimate the prevalence of OD [2,10]. Using a SDOIT we found that, although there was a significant correlation between self-reported smell impairment and the psychophysical test results, OD was more frequently revealed by psychophysical evaluation (54.7–64.1% in different SDOIT models), than in the self-reported data (32.8% for current OD). It was noteworthy that in our study, among subjectively normosmic COVID-19 patients, approximately 50% showed OD at psychophysical evaluation. These results are consistent with the findings of the aforementioned studies [2,11–14] and highlight the importance of psychophysical smell assessment.

Many studies have shown anosmia to be associated with the mild course of COVID-19 [5,7,8,14,21,24]. However, other studies either failed to find this relationship [2,17] or reported smell impairment to be associated with severe forms of the disease [16]. We found that longer hospitalization, higher MEWS scores and, for some SDOIT models, death, indicative of severe illness, were associated with worse psychophysical test results, but not with the self-reported OD. Moreover, the need for oxygen supplementation was less frequent in patients reporting OD, but more frequent in subjects with lower SDOIT scores. This confirms a hypothesis that previously reported associations of OD and the mild course of the disease may be due to neglecting smell impairment by patients with severe respiratory distress and should not be considered as a positive prognostic factor [12,23].

Smell impairment usually occurs early in the course of COVID-19 [2,3,12] and may sometimes be the first or even the sole symptom of SARS-CoV-2 infection [7,12]. In our study, OD appeared before (14.8%), simultaneously (11.1%), or after (74.1%) the presentation of other COVID-19 symptoms, which is consistent with a study by Lechien et al. [5], who reported smell dysfunction occurring prior (11.8%), concomitantly (22.8%) and after (65.4%) the appearance of general or ENT manifestations. Moreover, many studies have reported the early recovery [4,6,12,17] of OD in cases of COVID-19. In our study, 25.9% of patients reported complete recovery of olfactory function, while 74.1% reported either no recovery (55.6%) or worsening of olfactory function (18.5%); however, the longest duration of smell impairment at the time of the survey was 30 days. Interestingly, Vaira et al. [17], observed that 80% of patients reporting complete recovery of chemosensitive functions revealed some residual abnormalities in objective testing. In contrast, in our study, almost all the subjectively recovered patients were normosmic upon psychophysical evaluation, with only one subject misidentifying one odor.

The early onset and early recovery of OD argues in favor of a conductive pathomechanism of COVID-19 related anosmia [7]. However, many COVID-19 patients have reported OD in the absence of nasal obstruction and rhinorrhea [3,17]. In our study, although nasal symptoms were significantly more prevalent in subjects reporting OD, they were absent in 40.7% (11/27) of these patients and were not associated with worse SDOIT scores. Hence, rhinitis and nasal congestion do not appear to be the main causative factors in COVID-19 related OD.

Gustatory disorders commonly observed in COVID-19 have been suggested to result from impaired flavour perception due to retronasal olfactory dysfunction [25,26]. However, some studies have shown dysgeusia to be more frequent than OD [17], and expression of ACE2 receptors at high levels has been found in the oral mucosa [27,28], suggesting a distinct pathomechanism [27]. Nevertheless, as true gustatory dysfunction is often difficult to distinguish from OD [3], we chose not to include it in our study.

Smell impairment has been found to be highly associated with SARS-CoV-2 positivity (OR > 10) [8,26], with high specificity (93–99%), but low-to-moderate sensitivity (23–48%) [11,29] and has even been assessed to be the strongest predictor of COVID-19 [8,9]. Similarly, in our study self-reported OD achieved specificity of 100%, sensitivity of 42% and

AUC of 0.71 in predicting SARS-CoV-2 infection, for both the entire, and the age-matched subjects. According to Karni et al. [30], a quantitative smell assessment (1–10 scale) was even more effective, with 66% sensitivity, 97% specificity and 0.81 AUC. Similarly, we found that a maximum reported VAS with a cut-off point of five achieved a higher sensitivity (64%) and AUC (0.82), with specificity of 100%, indicating a better discriminatory ability in predicting COVID-19 compared with binary self-assessment of smell (normosmia vs. OD). However, in the analysis with the 75% youngest patients, the predictive value of VAS score was lower, with the AUC of 0.68. Huart et al. [31], found that an identification score of the extended "Sniffin' Sticks" test battery showed good discrimination between COVID-19 patients and controls, with a 100% sensitivity and 80% specificity. In our study, the SDOIT models also had a good discriminating ability in predicting COVID-19 with AUC of 0.8–0.82, sensitivity of 64–80%, and specificity of 71–94% in the entire cohort and with AUC of 0.75–0.78, sensitivity of 54–85%, and specificity of 71–94% in the age-matched subjects. Moreover, the combination of SDOIT results and self-reported OD resulted in improved diagnostic accuracy with AUC of 0.85–0.87, sensitivity of 78–91%, specificity of 71–94%, PPV of 85–89%, and NPV of 70–80% in the entire cohort and with AUC of 0.84–0.86, sensitivity of 73–88%, and specificity of 71–94% in the age-matched subjects. These findings support the role of OD as the early marker of COVID-19 [9] and an indication for immediate isolation and laboratory testing, or even retesting when the first RT-PCR result is negative [32]. It was noteworthy that the sensitivity (91% and 88% for the entire cohort and the age-matched subjects, respectively) and NPV (80% in both cases) were highest for the 10-SDOIT-based model), while the specificity for the 8-SDOIT-based model (79%) was higher than for the 10-SDOIT-based model (71%). Hence, we suggest than when there is enough time and a satisfactory availability of RT-PCR assay, one should consider the combination of self-reported OD and 10-SDOIT; however, with limited time and resources, the combination of 8-SDOIT and self-reported OD seems to be adequate as a predictor of SARS-CoV-2 positivity and an indication for RT-PCR testing.

Our study had several limitations. First, our sample size was limited, and the results may be influenced by the single institutional nature of the study. To improve sampling, the data were acquired over quite a long period of time. Furthermore, the patients were assessed at different time periods following the onset of infection and some reported having already recovered. However, this may have led to the underestimation of OD prevalence and significance, rather than the opposite. Moreover, we did not assess the recovery pattern of OD. Future follow-up study should be considered. In addition, our test is not yet validated. However, similarly to Calvo-Henriquez et al. [33], we did not aim to validate a new method of olfactory evaluation in general, but rather to create a fast test for predicting COVID-19, hence RT-PCR was used as a gold standard in assessing diagnostic accuracy. Future studies using validated psychophysical olfactory tests are needed to validate SDOIT as a method of olfactory function assessment. It is also worth noting that although our study performed the psychophysical test with the assistance of an examiner, the simplicity of SDOIT and the labeling of samples with numbers (so the patient does not know what odors are presented) would permit it to be conducted remotely. Subjects' answers could then be easily obtained using an online tool, such as Google Forms. This approach would increase the availability of the test as a screening method.

## 5. Conclusions

In conclusion, we present a simple, fast, low-cost, and effective SARS-CoV-2 screening strategy based on combining a survey for new-onset OD with a simple disposable odor identification test (SDOIT), which may be useful in identifying individuals suspected of COVID-19 and eligible for isolation and laboratory-testing when possible. Moreover, given the imperfect sensitivity of RT-PCR, a positive result in the proposed screening method could be an indication for retesting in cases where the initial SARS-CoV-2-RT-PCR result was negative. We suggest that when there is enough time and good availability of RT-PCR assay, one may consider the combination of self-reported OD and 10-SDOIT; however, with

limited time and resources, the combination of self-reported OD and 8-SDOIT appears to be adequate as a predictor of SARS-CoV-2 positivity. This approach could be especially useful in countries with a high number of COVID-19 cases and limited resources to perform RT-PCR for SARS-CoV-2.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/ijerph181910185/s1, Figure S1: SDOIT test kit example: (A) 10 disposable test paper strips numbered 1 to 10 enclosed in plastic covers; (B) Odorants (commercially available cinnamon, mint, lemon, coffee, clove, rose, anise and camphor oil, and deionaised water, numbered 1 to 10) in dropper bottles. Table S1: The intergroup comparison of objective smell test results for all evaluated odors (Fisher's exact test). Table S2: Detailed correlations between self-reported OD and clinical characteristics of COVID-19 patients. Table S3: Detailed correlations between objective OD (according to SDOIT) and quantitative clinical characteristics of COVID-19 patients; Mann-Whitney test. Table S4: Detailed correlations between objective OD (according to SDOIT) and qualitative clinical characteristics of COVID-19 patients; Spearman correlation. Table S5: Correlations between self-reported OD and general characteristics of the entire cohort (cases and controls, $N = 64$). Table S6: Correlations between gender and objective OD (according to SDOIT) of the entire cohort (cases and controls, $N = 64$); Mann-Whitney test. Table S7: Detailed results of the ROC analysis.

**Author Contributions:** Conceptualization, L.Z.-J.; methodology, L.Z.-J.; data curation, L.Z.-J.; formal analysis, L.Z.-J.; investigation, L.Z.-J., K.Ś., J.C., Ł.K.; writing—original draft preparation, L.Z.-J.; writing—review and editing, P.D., W.W., A.Z.; supervision, P.D., W.W., A.Z. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the Central Clinical Hospital of the Ministry of the Interior and Administration in Warsaw (decision number 37/2020, date of approval 3 April 2020).

**Informed Consent Statement:** Written informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** The original, anonymous dataset is available upon request from the corresponding author.

**Acknowledgments:** The authors wish to thank all study participants for their contribution in this study.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Mao, L.; Jin, H.; Wang, M.; Hu, Y.; Chen, S.; He, Q.; Chang, J.; Hong, C.; Zhou, Y.; Wang, D.; et al. Neurologic Manifestations of Hospitalized Patients with Coronavirus Disease 2019 in Wuhan, China. *JAMA Neurol.* **2020**, *77*, 683–690. [CrossRef]
2. Moein, S.T.; Hashemian, S.M.; Mansourafshar, B.; Khorram-Tousi, A.; Tabarsi, P.; Doty, R.L. Smell dysfunction: A biomarker for COVID-19. *Int. Forum Allergy Rhinol.* **2020**, *10*, 944–950. [CrossRef]
3. Kaye, R.; Chang, C.; Kazahaya, K.; Brereton, J.; Denneny, J.C., 3rd. COVID-19 Anosmia Reporting Tool: Initial Findings. *Otolaryngol. Head Neck Surg.* **2020**, *163*, 132–134. [CrossRef]
4. Klopfenstein, T.; Kadiane-Oussou, N.J.; Toko, L.; Royer, P.Y.; Lepiller, Q.; Gendrin, V.; Zayet, S. Features of anosmia in COVID-19. *Med. Mal. Infect.* **2020**, *50*, 436–439. [CrossRef]
5. Lechien, J.R.; Chiesa-Estomba, C.M.; De Siati, D.R.; Horoi, M.; Le Bon, S.D.; Rodriguez, A.; Dequanter, D.; Blecic, S.; El Afia, F.; Distinguin, L.; et al. Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): A multicenter European study. *Eur. Arch. Otorhinolaryngol.* **2020**, *277*, 2251–2261. [CrossRef]
6. Lee, Y.; Min, P.; Lee, S.; Kim, S.W. Prevalence and Duration of Acute Loss of Smell or Taste in COVID-19 Patients. *J. Korean Med. Sci.* **2020**, *35*, e174. [CrossRef]
7. Qiu, C.; Cui, C.; Hautefort, C.; Haehner, A.; Zhao, J.; Yao, Q.; Zeng, H.; Nisenbaum, E.J.; Liu, L.; Zhao, Y.; et al. Olfactory and Gustatory Dysfunction as an Early Identifier of COVID-19 in Adults and Children: An International Multicenter Study. *Otolaryngol. Head Neck Surg.* **2020**, *163*, 714–721. [CrossRef]
8. Yan, C.H.; Faraji, F.; Prajapati, D.P.; Boone, C.E.; DeConde, A.S. Association of chemosensory dysfunction and COVID-19 in patients presenting with influenza-like symptoms. *Int. Forum Allergy Rhinol.* **2020**, *10*, 806–813. [CrossRef]

9. Menni, C.; Valdes, A.M.; Freidin, M.B.; Sudre, C.H.; Nguyen, L.H.; Drew, D.A.; Ganesh, S.; Varsavsky, T.; Cardoso, M.J.; El-Sayed Moustafa, J.S.; et al. Real-time tracking of self-reported symptoms to predict potential COVID-19. *Nat. Med.* **2020**, *26*, 1037–1040. [CrossRef]
10. Hummel, T.; Whitcroft, K.L.; Andrews, P.; Altundag, A.; Cinghi, C.; Costanzo, R.M.; Damm, M.; Frasnelli, J.; Gudziol, H.; Gupta, N.; et al. Position paper on olfactory dysfunction. *Rhinology* **2016**, *56*, 1–30. [CrossRef]
11. Pang, K.W.; Chee, J.; Subramaniam, S.; Ng, C.L. Frequency and Clinical Utility of Olfactory Dysfunction in COVID-19: A Systematic Review and Meta-analysis. *Curr. Allergy Asthma Rep.* **2020**, *20*, 76. [CrossRef] [PubMed]
12. Vaira, L.A.; Hopkins, C.; Salzano, G.; Petrocelli, M.; Melis, A.; Cucurullo, M.; Ferrari, M.; Gagliardini, L.; Pipolo, C.; Deiana, G.; et al. Olfactory and gustatory function impairment in COVID-19 patients: Italian objective multicenter-study. *Head Neck* **2020**, *42*, 1560–1569. [CrossRef] [PubMed]
13. Hannum, M.E.; Ramirez, V.A.; Lipson, S.J.; Herriman, R.D.; Toskala, A.K.; Lin, C.; Joseph, P.V.; Reed, D.R. Objective sensory testing methods reveal a higher prevalence of olfactory loss in COVID-19 positive patients compared to subjective methods: A systematic review and metaanalysis. *Chem. Senses* **2020**, *45*, 865–874. [CrossRef] [PubMed]
14. Saniasiaya, J.; Islam, M.A.; Abdullah, B. Prevalence of Olfactory Dysfunction in Coronavirus Disease 2019 (COVID-19): A Meta-analysis of 27,492 Patients. *Laryngoscope* **2020**, *131*, 865–878. [CrossRef] [PubMed]
15. Marchese-Ragona, R.; Restivo, D.A.; De Corso, E.; Vianello, A.; Nicolai, P.; Ottaviano, G. Loss of smell in COVID-19 patients: A critical review with emphasis on the use of olfactory tests. *Acta Otorhinolaryngol. Ital.* **2020**, *40*, 241–247. [CrossRef] [PubMed]
16. Iravani, B.; Arshamian, A.; Ravia, A.; Mishor, E.; Snitz, K.; Shushan, S.; Roth, Y.; Perl, O.; Honigstein, D.; Weissgross, R.; et al. Relationship between odor intensity estimates and COVID-19 prevalence prediction in a Swedish population. *Chem. Senses* **2020**, *45*, 449–456. [CrossRef]
17. Vaira, L.A.; Deiana, G.; Fois, A.G.; Pirina, P.; Madeddu, G.; De Vito, A.; Babudieri, S.; Petrocelli, M.; Serra, A.; Bussu, F.; et al. Objective evaluation of anosmia and ageusia in COVID-19 patients: Single-center experience on 72 cases. *Head Neck* **2020**, *42*, 1252–1258. [CrossRef]
18. Vaira, L.A.; Salzano, G.; Petrocelli, M.; Deiana, G.; Salzano, F.A.; De Riu, G. Validation of a self-administered olfactory and gustatory test for the remotely evaluation of COVID-19 patients in home quarantine. *Head Neck* **2020**, *42*, 1570–1576. [CrossRef]
19. The Number of Infections in Poland–Historical Data from the Beginning of the Pandemic. Available online: https://arcgis.com/sharing/rest/content/items/b03b454aed9b4154ba50df4ba9e1143b/data? (accessed on 12 June 2021).
20. Subbe, C.P.; Kruger, M.; Rutherford, P.; Gemmel, L. Validation of a modified Early Warning Score in medical admissions. *QJM* **2001**, *94*, 521–526. [CrossRef] [PubMed]
21. von Bartheld, C.S.; Hagen, M.M.; Butowt, R. Prevalence of Chemosensory Dysfunction in COVID-19 Patients: A Systematic Review and Meta-analysis Reveals Significant Ethnic Differences. *ACS Chem. Neurosci.* **2020**, *11*, 2944–2961. [CrossRef] [PubMed]
22. Abdullahi, A.; Candan, S.A.; Abba, M.A.; Bello, A.H.; Alshehri, M.A.; Afamefuna Victor, E.; Umar, N.A.; Kundakci, B. Neurological and Musculoskeletal Features of COVID-19: A Systematic Review and Meta-Analysis. *Front. Neurol.* **2020**, *11*, 687. [CrossRef]
23. Hopkins, C.; Surda, P.; Whitehead, E.; Kumar, B.N. Early recovery following new onset anosmia during the COVID-19 pandemic—An observational cohort study. *J. Otolaryngol. Head Neck Surg.* **2020**, *49*, 26. [CrossRef]
24. Romero-Sánchez, C.M.; Díaz-Maroto, I.; Fernández-Díaz, E.; Sánchez-Larsen, Á.; Layos-Romero, A.; García-García, J.; González, E.; Redondo-Peñas, I.; Perona-Moratalla, A.B.; Del Valle-Pérez, J.A.; et al. Neurologic manifestations in hospitalized patients with COVID-19: The ALBACOVID registry. *Neurology* **2020**, *95*, e1060–e1070. [CrossRef]
25. Le Bon, S.D.; Pisarski, N.; Verbeke, J.; Prunier, L.; Cavelier, G.; Thill, M.P.; Rodriguez, A.; Dequanter, D.; Lechien, J.R.; Le Bon, O.; et al. Psychophysical evaluation of chemosensory functions 5 weeks after olfactory loss due to COVID-19: A prospective cohort study on 72 patients. *Eur. Arch. Otorhinolaryngol.* **2021**, *278*, 101–108. [CrossRef]
26. Lee, D.J.; Lockwood, J.; Das, P.; Wang, R.; Grinspun, E.; Lee, J.M. Self-reported anosmia and dysgeusia as key symptoms of coronavirus disease 2019. *CJEM* **2020**, *22*, 595–602. [CrossRef]
27. Vaira, L.A.; Salzano, G.; Fois, A.G.; Piombino, P.; De Riu, G. Potential pathogenesis of ageusia and anosmia in COVID-19 patients. *Int. Forum Allergy Rhinol.* **2020**, *10*, 1103–1104. [CrossRef]
28. Xu, H.; Zhong, L.; Deng, J.; Peng, J.; Dan, H.; Zeng, X.; Li, T.; Chen, Q. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *Int. J. Oral Sci.* **2020**, *12*, 8. [CrossRef]
29. Wee, L.E.; Chan, Y.; Teo, N.; Cherng, B.; Thien, S.Y.; Wong, H.M.; Wijaya, L.; Toh, S.T.; Tan, T.T. The role of self-reported olfactory and gustatory dysfunction as a screening criterion for suspected COVID-19. *Eur. Arch. Otorhinolaryngol.* **2020**, *277*, 2389–2390. [CrossRef]
30. Karni, N.; Klein, H.; Asseo, K.; Benjamini, Y.; Israel, S.; Nammary, M.; Olshtain-Pops, K.; Nir-Paz, R.; Hershko, A.; Muszkat, M.; et al. Self-rated smell ability enables highly specific predictors of COVID-19 status: A case control study in Israel. *Open Forum Infect. Dis.* **2020**, *8*, ofaa589. [CrossRef]
31. Huart, C.; Philpott, C.; Konstantinidis, I.; Altundag, A.; Whitcroft, K.L.; Trecca, E.; Cassano, M.; Rombaux, P.; Hummel, T. Comparison of COVID-19 and common cold chemosensory dysfunction. *Rhinology* **2020**, *58*, 623–625. [CrossRef]

32. Carignan, A.; Valiquette, L.; Grenier, C.; Musonera, J.B.; Nkengurutse, D.; Marcil-Héguy, A.; Vettese, K.; Marcoux, D.; Valiquette, C.; Xiong, W.T.; et al. Anosmia and dysgeusia associated with SARS-CoV-2 infection: An age-matched case-control study. *CMAJ* **2020**, *192*, E702–E707. [CrossRef] [PubMed]
33. Calvo-Henriquez, C.; Maldonado-Alvarado, B.; Chiesa-Estomba, C.; Rivero-Fernández, I.; Sanz-Rodriguez, M.; Villarreal, I.M.; Rodriguez-Iglesias, M.; Mariño-Sánchez, F.; Rivero-de-Aguilar, A.; Lechien, J.R.; et al. Ethyl alcohol threshold test: A fast, reliable and affordable olfactory Assessment tool for COVID-19 patients. *Eur. Arch. Otorhinolaryngol.* **2020**, *277*, 2783–2792. [CrossRef]

## Supplementary materials

**Table S1.** The intergroup comparison of objective smell test results for all evaluated odors (Fisher's exact test).

| | Cases ($n$ = 64) | Controls ($n$ = 34) | Total ($n$ = 98) | $p$-Value |
|---|---|---|---|---|
| | | **Detection** | | |
| cinnamon | 55 (85.9%) | 34 (100%) | 89 (90.8%) | 0.025 |
| mint | 52 (81.3%) | 34 (100%) | 86 (87.8%) | 0.007 |
| lemon | 52 (81.3%) | 34 (100%) | 86 (87.8%) | 0.007 |
| coffee | 54 (84.4%) | 34 (100%) | 88 (89.8%) | 0.014 |
| clove | 54 (84.4%) | 34 (100%) | 88 (89.8%) | 0.014 |
| rose | 57 (89.1%) | 34 (100%) | 91 (92.9%) | 0.092 |
| anise | 55 (85.9%) | 34 (100%) | 89 (90.8%) | 0.025 |
| camphor | 53 (82.8%) | 34 (100%) | 87 (88.8%) | 0.008 |
| alcohol | 49 (76.6%) | 34 (100%) | 83 (84.7%) | 0.001 |
| | | **Identification** | | |
| cinnamon | 39 (60.9%) | 34 (100%) | 73 (74.5%) | <0.001 |
| mint | 41 (64.1%) | 34 (100%) | 75 (76.5%) | <0.001 |
| lemon | 41 (64.1%) | 33 (97.1%) | 74 (75.5%) | <0.001 |
| coffee | 44 (68.8%) | 31 (91.2%) | 75 (76.5%) | 0.013 |
| clove | 46 (71.9%) | 33 (97.1%) | 79 (80.6%) | 0.002 |
| rose | 42 (65.6%) | 29 (85.3%) | 71 (72.4%) | 0.056 |
| anise | 45 (70.3%) | 33 (97.1%) | 78 (79.6%) | 0.001 |
| camphor | 46 (71.9%) | 33 (97.1%) | 79 (80.6%) | 0.002 |
| alcohol | 34 (53.1%) | 33 (97.1%) | 67 (68.4%) | <0.001 |

**Table S2.** Detailed correlations between self-reported OD and clinical characteristics of COVID-19 patients.

| Variable | | Self-Reported OD | | | | | |
|---|---|---|---|---|---|---|---|
| | | Presence of Self-Reported OD at the Time of the Survey | | | Presence of Self-Reported OD at Any Time since the Onset of COVID-19 | | |
| | | yes ($N$ = 21) | no ($N$ = 43) | $p$-Value | yes ($N$ = 27) | no ($N$ = 37) | $p$-Value |
| Nasal congestion, N (%) | yes | 11 (52.4) | 10 (23.3) | **0.041**[3] | 14 (51.9) | 7 (18.9) | 0.012[3] |
| | no | 10 (47.6) | 33 (76.7) | | 13 (48.1) | 30 (81.1) | |
| Rhinorrhea, N (%) | yes | 9 (42.9) | 10 (23.3) | 0.187[3] | 14 (51.9) | 5 (13.5) | 0.002[3] |
| | no | 12 (57.1) | 33 (76.7) | | 13 (48.1) | 32 (86.5) | |
| Presence of other symptoms at the time of the survey, N (%)[1] | yes | 15 (71.4) | 20 (46.5) | 0.107[3] | 17 (63) | 18 (48.6) | 0.378[3] |
| | no | 6 (28.6) | 23 (53.5) | | 10 (37) | 19 (51.4) | |
| Presence of other symptoms since onset of COVID-19, N (%)[2] | yes | 21 (100) | 34 (79.1) | **0.025**[5] | 27 (100) | 28 (75.7) | 0.008[5] |
| | no | 0 (0) | 9 (20.9) | | 0 (0) | 9 (24.3) | |
| Current smoking, N (%) | yes | 3 (14.3) | 5 (11.6) | 1[5] | 3 (11.1) | 5 (13.5) | 1[5] |
| | no | 18 (85.7) | 38 (88.4) | | 24 (88.9) | 32 (86.5) | |
| Former or current smoking, N (%) | yes | 8 (38.1) | 21 (48.8) | 0.587[3] | 8 (29.6) | 21 (56.8) | 0.058[3] |
| | no | 13 (61.9) | 22 (51.2) | | 19 (70.4) | 16 (43.2) | |
| Death, N (%) | yes | 2 (9.5) | 6 (14) | 1[5] | 2 (7.4) | 6 (16.2) | 0.450[5] |
| | no | 19 (90.5) | 37 (86) | | 25 (92.6) | 31 (83.8) | |
| Need for oxygen therapy, N (%) | yes | 7 (33.3) | 21 (48.8) | 0.365[3] | 7 (25.9) | 21 (56.8) | 0.028[3] |
| | no | 14 (66.7) | 22 (51.2) | | 20 (74.1) | 16 (43.2) | |
| Need for ICU stay, N (%) | yes | 1 (4.8) | 5 (11.6) | 0.654[5] | 1 (3.7) | 5 (13.5) | 0.388[5] |
| | no | 20 (95.2) | 38 (88.4) | | 26 (96.3) | 32 (86.5) | |
| Need for invasive ventilation, N (%) | yes | 1 (4.8) | 4 (9.3) | 1[5] | 1 (3.7) | 4 (10.8) | 0.387[5] |
| | no | 20 (95.2) | 39 (90.7) | | 26 (96.3) | 33 (89.2) | |
| Number of other symptoms at the time of the survey | Mean ± SD | 1.7 ± 1.6 | 1 ± 1.4 | 0.057[4] | 1.4 ± 1.5 | 1.1 ± 1.5 | 0.284[4] |
| | Median (IQR) | 1 (0–3) | 0 (0–2) | | 1 (0–2) | 0 (0–2) | |
| Number of other symptoms since the onset of COVID-19 | Mean ± SD | 4.2 ± 2.2 | 2.9 ± 2.1 | **0.040**[4] | 4.2 ± 1.9 | 2.73 ± 2.2 | 0.013[4] |
| | Median (IQR) | 4 (3–6) | 3 (1–4) | | 4 (3–5) | 3 (1–4) | |
| Time interval between first positive PCR result and time of the survey, days | Mean ± SD | 5.1 ± 4.2 | 7.6 ± 6.7 | 0.290[4] | 6.7 ± 4.8 | 7.1 ± 7 | 0.615[4] |
| | Median (IQR) | 3 (2–8) | 6 (2–12) | | 5 (2.5–12) | 4 (2–12) | |
| Duration of infection (from first positive to first negative PCR result, excluding deceased patients) | N | 19 | 37 | 0.134[4] | 25 | 31 | 0.014[4] |
| | Mean ± SD | 17.47 ± 10.12 | 21.57 ± 13.14 | | 16.48 ± 9.12 | 23.16 ± 13.73 | |
| | Median (IQR) | 13 (10–21.5) | 16 (14–26) | | 12 (11–17) | 18 (14–28.5) | |
| Duration of hospitalisation (excluding deceased patients) | N | 19 | 37 | 0.862[4] | 25 | 31 | 0.060[4] |
| | Mean ± SD | 19 ± 10.64 | 20.68 ± 14.2 | | 16.56 ± 10.5 | 22.97 ± 14.28 | |
| | Median (IQR) | 17 (12–23.5) | 18 (10–24) | | 13 (10–19) | 18 (15–28) | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| MEWS score at the time of the survey | Mean ± SD | 0.86 ± 1.28 | 0.86 ± 1.67 | 0.837[4] | 0.67 ± 1.18 | 1 ± 1.76 | 0.254[4] |
| | Median (IQR) | 0 (0–1) | 0 (0–1) | | 0 (0–1) | 1 (0–1) | |
| Avarage MEWS score | Mean ± SD | 0.86 ± 1.49 | 0.98 ± 1.32 | 0.481[4] | 0.7 ± 1.35 | 1.11 ± 1.37 | 0.081[4] |
| | Median (IQR) | 0 (0–1) | 1 (0–1) | | 0 (0–1) | 1 (0–2) | |

[1] including: fatigue—22 (34.4%), cough—15 (23.4%), fever—2 (3.1%), headache—6 (9.4%), gastrointestinal complaints—14 (21.9%), nasal congestion—5 (7.8%), rhinorhhea—5 (7.8%), chills—2 (3.1%), dyspnea—7 (10.9%). [2] including: fatigue—45 (70.3%), cough—25 (39.1%), fever—24 (37.5%), headache—23 (35.9%), gastrointestinal complaints—23 (35.9%), nasal congestion—21 (32.8%), rhinorhhea—19 (29.7%), chills—18 (28.1%), dyspnea—15 (23.4%). [3] chi-squared test. [4] Mann-Whitney test, [5] Fisher test.

**Table S3.** Detailed correlations between objective OD (according to SDOIT) and quantitative clinical characteristics of COVID-19 patients; Mann-Whitney test.

| Variable | | SDOIT, % of Correct Answers | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 10-SDOIT | | 9-SDOIT | | 8-SDOIT | | 4-SDOIT | |
| | | Mean ± SD | Median (IQR) | Mean ± SD | Median (IQR) | Mean ± SD | Median (IQR) | Mean ± SD | Median (IQR) |
| Nasal congestion | yes | 72.9 ± 30.4 | 90 (50–100) | 72 ± 33.5 | 88.9 (55.6–100) | 71.4± 33.1 | 87.5 (50–100) | 70.2 ± 40 | 100 (25–100) |
| | no | 63 ± 32.3 | 70 (45–90) | 62.5 ± 34.6 | 77.8 (44.4–88.9) | 62.8 ± 34.9 | 75 (37.5–87.5) | 55.8 ± 38.1 | 75 (25–87.5) |
| | *p*-value | 0.188 | | 0.197 | | 0.240 | | 0.081 | |
| Rhinorrhea | yes | 73.7 ± 30.4 | 90 (50–100) | 73.1 ± 32.4 | 88.9 (56–100) | 73.3 ± 32.1 | 87.5 (56.3–100) | 69.7 ± 37.8 | 100 (37.5–100) |
| | no | 63.1 ± 32.2 | 80 (40–90) | 62.5 ± 34.9 | 77.8 (44.4–88.9) | 62.5 ± 35.1 | 75 (37.5–87.5) | 56.7 ± 39.3 | 75 (25–100) |
| | *p*-value | 0.110 | | 0.152 | | 0.179 | | 0.153 | |
| Presence of other symptoms at the time of the survey | yes | 60 ± 31.2 | 60 (40–90) | 59.8 ± 33.9 | 66.7 (38.9–88.9) | 60 ± 33.3 | 62.5 (37.5–87.5) | 55 ± 37.3 | 50 (25–87.5) |
| | no | 73.8 ± 31.3 | 90 (50–100) | 73.2 ± 33.8 | 88.9 (55.6–100) | 72.4 ± 34.8 | 87.5 (50–100) | 67.2 ± 40.7 | 75 (25–100) |
| | *p*-value | **0.032** | | **0.042** | | **0.042** | | 0.139 | |
| Presence of other symptoms since onset of COVID-19 | yes | 66.7 ± 32.4 | 80 (50–90) | 66.1 ± 35.2 | 77.8 (55.6–88.9) | 66.4 ± 34.9 | 75 (50–93.8) | 62.3 ± 39.1 | 75 (25–100) |
| | no | 63.3 ± 29.2 | 50 (40–90) | 63 ± 29.4 | 55.6 (44.4–88.9) | 61.1 ± 31.5 | 62.5 (37.5–87.5) | 50 ± 39.5 | 50 (25–75) |
| | *p*-value | 0.711 | | 0.617 | | 0.576 | | 0.368 | |
| Current smoking | yes | 70 ± 31.2 | 85 (55–90) | 68.1 ± 35.9 | 83.3 (50–91.7) | 68.8 ± 34.7 | 81.3 (56.4–90.6) | 65.6 ± 37.7 | 75 (43.8–100) |
| | no | 65.7 ± 32.1 | 80 (47.5–90) | 65.3 ± 34.4 | 77.8 (44.4–88.9) | 65.2 ± 34.5 | 75 (37.5–90.6) | 59.8 ± 39.5 | 75 (25–100) |
| | *p*-value | 0.806 | | 0.837 | | 0.829 | | 0.745 | |
| Former or current smoking | yes | 63.1 ± 30.7 | 80 (40–90) | 62.8 ± 32.8 | 77.8 (44.4–88.9) | 62.9 ± 32.8 | 75 (37.5–87.5) | 55.2 ± 40.3 | 75 (25–100) |
| | no | 68.9 ± 32.9 | 80 (50–100) | 67.9 ± 35.7 | 77.8 (55.6–100) | 67.9 ± 35.8 | 75 (56.3–100) | 65 ± 38 | 75 (37.5–100) |
| | *p*-value | 0.241 | | 0.259 | | 0.308 | | 0.335 | |
| Death | yes | 51.2 ± 22.3 | 60 (55–60) | 50 ± 21.4 | 55.6 (52.8–58.3) | 51.6 ± 23.6 | 62.5 (46.9–62.5) | 40.6 ± 29.7 | 37.5 (25–50) |
| | no | 68.4 ± 32.5 | 8 (47.5–90) | 67.9 ± 35.3 | 88.9 (44.4–100) | 67.6 ± 35.3 | 87.5 (37.5–100) | 63.4 ± 39.6 | 75 (25–100) |
| | *p*-value | 0.066 | | **0.048** | | **0.047** | | 0.108 | |
| Need for oxygen therapy | yes | 58.9 ± 30.6 | 6 (47.5–80) | 58.3 ± 32.3 | 61.1 (52.8–80.6) | 58.9 ± 32.4 | 62.5 (46.9–78.1) | 49.1 ± 35.7 | 50 (18.8–75) |
| | no | 71.9 ± 32 | 90 (47.5–100) | 71.3 ± 35.2 | 88.9 (44.4–100) | 70.8 ± 35.2 | 87.5 (37.5–100) | 69.4 ± 39.7 | 100 (25–100) |
| | *p*-value | **0.032** | | **0.026** | | **0.035** | | **0.013** | |
| Need for ICU stay | yes | 68.3 ± 16 | 60 (60–67.5) | 66.7 ± 17.2 | 61.1 (55.6–66.7) | 68.8 ± 17.2 | 62.5 (62.5–71.9) | 58.3 ± 34.2 | 50 (31.3–87.5) |
| | no | 66 ± 33.1 | 80 (40–90) | 65.5 ± 35.7 | 77.8 (44.4–88.9) | 65.3 ± 35.7 | 75 (37.5–96.9) | 60.8 ± 39.8 | 75 (25–100) |
| | *p*-value | 0.852 | | 0.700 | | 0.682 | | 0.877 | |
| Need for invasive ventilation | yes | 62 ± 4.5 | 60 (60–60) | 60 ± 6.09 | 55.6 (55.6–66.7) | 62.5 ± 8.8 | 62.5 (62.5–62.5) | 50 ± 30.6 | 50 (25–50) |
| | no | 66.9 ± 33.3 | 80 (40–90) | 66.3 ± 35.9 | 83.3 (44.4–97.2) | 65.9 ± 35.7 | 75 (37.5–100) | 61.6 ± 40.1 | 75 (25–100) |
| | *p*-value | 0.355 | | 0.278 | | 0.292 | | 0.485 | |

**Table S4.** Detailed correlations between objective OD (according to SDOIT) and qualitative clinical characteristics of COVID-19 patients; Spearman correlation.

| Variable | | SDOIT | | | |
|---|---|---|---|---|---|
| | | 10-SDOIT | 9-SDOIT | 8-SDOIT | 4-SDOIT |
| Number of other symptoms at the time of the survey | ϱ | -0.28 | -0.27 | -0.27 | -0.19 |
| | *p*-value | 0.024 | 0.031 | 0.033 | 0.142 |
| Number of other symptoms since the onset of COVID-19 | ϱ | 0.06 | 0.07 | 0.09 | 0.14 |
| | *p*-value | 0.613 | 0.575 | 0.486 | 0.287 |
| Time interval between first positive PCR result and time of the survey, days | ϱ | 0.19 | 0.18 | 0.19 | 0.1 |
| | *p*-value | 0.123 | 0.163 | 0.141 | 0.447 |
| Duration of infection (from first positive to first negative PCR, excluding deceased) | ϱ | -0.15 | -0.14 | -0.14 | -0.11 |
| | *p*-value | 0.265 | 0.294 | 0.319 | 0.422 |
| Duration of hospitalisation (excluding deceased) | ϱ | -0.43 | -0.42 | -0.41 | -0.36 |
| | *p*-value | <0.001 | 0.002 | 0.002 | 0.007 |
| MEWS score at the time of the survey | ϱ | -0.29 | -0.32 | -0.32 | -0.25 |
| | *p*-value | 0.02 | 0.011 | 0.011 | 0.043 |
| Avarage MEWS score | ϱ | -0.29 | -0.3 | -0.29 | -0.24 |
| | *p*-value | 0.02 | 0.016 | 0.018 | 0.054 |

**Table S5.** Correlations between self-reported OD and general characteristics of the entire cohort (cases and controls, $N = 64$).

| Variable | | Self-Reported OD | | | | | |
|---|---|---|---|---|---|---|---|
| | | Presence of Self-Reported OD at the Time of the Survey | | | Presence of Self-Reported OD at Any Time since the Onset of COVID-19 | | |
| | | Yes ($N = 21$) | No ($N = 43$) | *p*-Value | Yes ($N = 27$) | No ($N = 37$) | *p*-Value |
| Age | Mean ± SD | 55.1 ± 21.5 | 46.5 ± 17.7 | 0.395[1] | 47.6 ± 23.7 | 48.6 ± 16.7 | 0.583[1] |
| | Median (IQR) | 57 (34–72) | 45 (32–60) | | 45 (24–69.5) | 47 (34–62.5) | |
| Gender, N (%) | female | 14 (66.7) | 41 (53.2) | 0.842[2] | 15 (55.6) | 40 (56.3) | 1[2] |
| | male | 7 (33.3) | 36 (46.8) | | 12 (44.4) | 31 (43.7) | |
| Current smoking | yes | 3 (14.3) | 5 (11.6) | 1[3] | 3 (11.1) | 5 (13.5) | 1[3] |
| | no | 18 (85.7) | 38 (88.4) | | 24 (88.9) | 32 (86.5) | |
| Current or former smoking | yes | 8 (38.1) | 21 (48.8) | 0.587[1] | 8 (29.6) | 21 (56.8) | 0.058[1] |
| | no | 13 (61.9) | 22 (51.2) | | 19 (70.4) | 15 (43.2) | |

[1]Mann-Whitney test., [2] chi-squared test, [3] Fisher test

**Table S6.** Correlations between gender and objective OD (according to SDOIT) of the entire cohort (cases and controls, $N = 64$); Mann-Whitney test.

| Variable | | SDOIT, % of Correct Answers | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 10-SDOIT | | 9-SDOIT | | 8-SDOIT | | 4-SDOIT | |
| | | Mean ± SD | Median (IQR) | Mean ± SD | Median (IQR) | Mean ± SD | Median (IQR) | Mean ± SD | Median (IQR) |
| Gender | female | 77.9 ± 30.4 | 90 (55–100) | 74.8 ± 32.4 | 77.8 ± 30.6 | 75.3 ± 33.8 | 78.1 ± 30.9 | 73.6 ± 36.8 | 73.8 ± 36.6 |
| | male | 78.4 ± 28.9 | 90 (75–100) | 88.9 (55.6–100) | 88.9 (72.2–100) | 85.7 (71.4–100) | 85.7 (71.4–100) | 100 (50–100) | 100 (50–100) |
| | *p*-value | 0.641 | | 0.676 | | 0.822 | | 0.978 | |

**Table S7.** Detailed results of the ROC analysis.

| Classifier | Sensitivity | Specifity | PPV | NPV | AUC |
|---|---|---|---|---|---|
| Self-reported OD at the time of the survey | 0.33 (CI95% 0.22–0.45) | 1 (CI95% 1–1) | 1 (CI95% 1–1) | 0.44 (CI95% 0.4–0.49) | 0.66 (CI95% 0.61–0.72) |
| Self-reported OD at the time of maximum deterioration | 0.42 (CI95% 0.3–0.55) | 1 (CI95% 1–1) | 1 (CI95% 1–1) | 0.48 (CI95% 0.43–0.54) | 0.71 (CI95% 0.65–0.77) |
| Maximum VAS | 0.64 (CI95% 0.53–0.75) | 1 (CI95% 1–1) | 1 (CI95% 1–1) | 0.6 (CI95% 0.53–0.68) | 0.82 (CI95% 0.76–0.88) |
| 10-SDOIT (cut-off at 95% - OD ≥ 1 incorrect) | 0.8 (CI95% 0.56–0.78) | 0.71 (CI95% 0.62–0.76) | 0.84 (CI95% 0.79–0.86) | 0.65 (CI95% 0.52–0.77) | 0.82 (CI95% 0.74–0.9) |
| 10-SDOIT (OD ≥ 1 incorrect) + self-reported OD | 0.91 (CI95% 0.83–0.97) | 0.71 (CI95% 0.56–0.85) | 0.85 (CI95% 0.79–0.92) | 0.8 (CI95% 0.67–0.93) | 0.87 (CI95% 0.8–0.93) |
| 10-SDOIT (OD ≥2 incorrect) | 0.59 (CI95% 0.47–0.72) | 0.91 (CI95% 0.82–1) | 0.93 (CI95% 0.85–1) | 0.54 (CI95% 0.47–0.63) | 0.75 (CI95% 0.68–0.83) |
| 10-SDOIT (OD OD ≥2 incorrect) + self-reported OD | 0.77 (CI95% 0.66–0.86) | 0.91 (CI95% 0.82–1) | 0.94 (CI95% 0.88–1) | 0.67 (CI95% 0.58–0.78) | 0.86 (CI95% 0.80–0.92) |
| 9-SDOIT (cut-off at 94% - OD ≥ 1 incorrect) | 0.77 (CI95% 0.55–0.86) | 0.71 (CI95% 0.59–0.97) | 0.83 (CI95% 0.77–0.97) | 0.62 (CI95% 0.5–0.74) | 0.80 (CI95% 0.73–0.88) |
| 9-SDOIT (OD ≥ 1 incorrect) + self-reported OD | 0.88 (CI95% 0.83–0.97) | 0.71 (CI95% 0.56–0.85) | 0.85 (CI955 0.79–0.92) | 0.75 (CI95% 0.68–0.93) | 0.85 (CI95% 0.79–0.92) |
| 9-SDOIT (OD ≥2 incorrect) | 0.55 (CI95% 0.42–0.67) | 0.94 (CI95% 0.85–1) | 0.95 (CI95% 0.87–1) | 0.52 (CI95% 0.46–0.6) | 0.74 (CI95% 0.67–0.82) |
| 9-SDOIT (OD OD ≥2 incorrect) + self-reported OD | 0.73 (CI95% 0.83–0.97) | 0.94 (CI95% 0.56–0.85) | 0.96 (CI95% 0.79–0.92) | 0.65 (CI95% 0.67–0.93) | 0.85 (CI95% 0.79–0.91) |
| 8-SDOIT (cut-off at 94% - OD ≥ 1 incorrect) | 0.75 (CI95% 0.61–0.86) | 0.79 (CI95% 0.68–0.94) | 0.87 (CI95% 0.81–0.96) | 0.63 (CI95% 0.53–0.74) | 0.82 (CI95% 0.75–0.89) |
| 8-SDOIT (OD ≥ 1 incorrect) + self-reported OD | 0.86 (CI95% 0.77–0.94) | 0.79 (CI95% 0.65–0.91) | 0.89 (CI95% 0.82–0.95) | 0.75 (CI95% 0.64–0.88) | 0.87 (CI95% 0.81–0.93) |
| 8-SDOIT (OD ≥2 incorrect) | 0.55 (CI95% 0.42–0.67) | 0.97 (CI95% 0.91–1) | 0.97 (CI95% 0.91–1) | 0.53 (CI95% 0.47–0.61) | 0.76 (CI95% 0.69–0.83) |
| 8-SDOIT (OD OD ≥2 incorrect) + self-reported OD | 0.73 (CI95% 0.62–0.84) | 0.97 (CI95% 0.91–1) | 0.98 (CI95% 0.93–1) | 0.66 (CI95% 0.58–0.76) | 0.86 (CI95% 0.8–0.92) |
| 4-SDOIT (cut-off at 88% - ≥ 1 incorrect) | 0.64 (CI95% 0.53–0.75) | 0.94 (CI95% 0.85–1) | 0.95 (CI95% 0.89–1) | 0.58 (CI95% 0.51–0.67) | 0.80 (CI95% 0.74–0.87) |
| 4-SDOIT (OD ≥ 1 incorrect) + self-reported OD | 0.78 (CI95% 0.67–0.88) | 0.94 (C I95% 0.85–1) | 0.96 (CI95% 0.91–1) | 0.7 (CI95% 0.6–0.8) | 0.87 (CI95% 0.82–0.93) |
| 4-SDOIT (OD ≥2 incorrect) | 0.42 (CI95% 0.3–0.53) | 1 (CI95% 1–1) | 1 (CI95% 1–1) | 0.48 (CI95% 0.43–0.53) | 0.72 (CI95% 0.65–0.77) |
| 4-SDOIT (OD ≥2 incorrect) + self-reported OD | 0.64 (CI95% 0.52–0.75) | 1 (CI95% 1–1) | 1 (CI95% 1–1) | 0.6 (CI95% 0.52–0.68) | 0.82 (CI95% 0.76–0.88) |


**Figure S1.** SDOIT test kit example: (**A**) 10 disposable test paper strips numbered 1 to 10 enclosed in plastic covers; (**B**) Odorants (commercially available cinnamon, mint, lemon, coffee, clove, rose, anise and camphor oil, and deionaised water, numbered 1 to 10) in dropper bottles.

**Publikacja 2.**

**Tytuł**: Pathogenesis of Olfactory Disorders in COVID-19

**Autorzy:** Ziuzia-Januszewska L, Januszewski M

**Czasopismo**: Brain Sciences. 2022;12(4):449

**doi**: 10.3390/brainsci12040449

brain sciences

MDPI

*Review*

# Pathogenesis of Olfactory Disorders in COVID-19

**Laura Ziuzia-Januszewska [1,*] and Marcin Januszewski [2]**

[1] Department of Otolaryngology, Central Clinical Hospital, Ministry of Interior and Administration, 02-507 Warsaw, Poland
[2] Department of Obstetrics and Gynecology, Central Clinical Hospital, Ministry of Interior and Administration, 02-507 Warsaw, Poland; lek.med.mjanuszewski@gmail.com
* Correspondence: laura.ziuzia@cskmswia.gov.pl or lauraziuzia@gmail.com; Tel.: +48-477221182

**Abstract:** Since the outbreak of the SARS-CoV-2 pandemic, olfactory disorders have been reported as a frequent symptom of COVID-19; however, its pathogenesis is still debated. The aim of this review is to summarize the current understanding of the pathogenesis of smell impairment in the course of COVID-19 and to highlight potential avenues for future research on this issue. Several theories have been proposed to explain the pathogenesis of COVID-19-related anosmia, including nasal obstruction and rhinorrhea, oedema of the olfactory cleft mucosa, olfactory epithelial damage either within the olfactory receptor cells or the supporting non-neural cells (either direct or immune-mediated), damage to the olfactory bulb, and impairment of the central olfactory pathways. Although the pathogenesis of COVID-19-related anosmia is still not fully elucidated, it appears to be mainly due to sensorineural damage, with infection of the olfactory epithelium support cells via the ACE1 receptor and disruption of the OE caused by immense inflammatory reaction, and possibly with direct olfactory sensory neurons infection mediated by the NRP-1 receptor. Involvement of the higher olfactory pathways and a conductive component of olfactory disorders, as well as genetic factors, may also be considered.

**Keywords:** olfactory disorders; loss of smell; anosmia; COVID-19; SARS-CoV-2; pathogenesis



**Citation:** Ziuzia-Januszewska, L.; Januszewski, M. Pathogenesis of Olfactory Disorders in COVID-19. *Brain Sci.* **2022**, *12*, 449. https://doi.org/10.3390/brainsci12040449

Academic Editor: Tissa Wijeratne

Received: 21 February 2022
Accepted: 23 March 2022
Published: 27 March 2022



## 1. Introduction

Since the outbreak of the SARS-CoV-2 pandemic, smell impairment has been reported as a frequent symptom of COVID-19, with reported prevalence ranging widely in the literature from 5 to 98.3% [1–11]. The association of olfactory disorders (OD) with COVID-19 is now established, but OD pathogenesis is still debated [12–15].

Odor detection begins with the binding of odorant molecules to odor receptors localized on the dendritic cilia of the olfactory sensory neurons (OSNs) in the olfactory epithelium (OE) [16,17]. OSNs axons cross the skull base through the cribriform plate and form synapses within the olfactory bulb (OB) [16,17]. Olfactory information is sent from the OB to higher brain centers [16,17].

The OE is a complex tissue consisting of multiple cell types, including OSNs, sustentacular (SUS) cells, mucus-secreting Bowman's gland cells, microvillar cells, and stem cells including globose and horizontal basal cells. In addition, macrophages and dendritic cells are present in the OE [18–20]. SUS cells act to structurally support OSNs, protect OSNs by phagocyting and/or detoxifying potentially harmful agents, enable odor detection by endocytosis of the olfactory binding protein and odorant complex, supply OSNs with glucose necessary for high energy olfactory transduction cascade, and maintain local fluid and electrolyte balance [17,18,21]. The basal cells can differentiate to replace OSNs during normal turnover or injury [19,20]. Bowman's glands secrete mucus, containing water, salts, mucin glycoproteins, enzymes, antibodies, and odorant binding proteins (OBPs), which transport the hydrophobic odorant molecules through the mucus to the OSNs cilia [20]. Olfactory dysfunctions can be

classified into three types: conductive disorders caused by obstruction of the nasal cavities and subsequent blockage of odorant transmission to the olfactory epithelium (OE), sensorineural loss caused by damage of the OE or olfactory nerve, and central dysfunction resulting from damage to the olfactory processing pathway in the central nervous system (CNS) [16,22].

Loss of the sense of smell due to upper respiratory tract infections (URTI) is primarily considered a conductive loss secondary to rhinorrhea and mucosal edema, and usually normalizes as the infection resolves [23]. However, in some cases, loss of smell may persist after the resolution of UTRI, suggesting a sensorineural disorder known as post-viral olfactory dysfunction (PVOD) [23,24]. PVOD is one of the leading causes of anosmia in adults, accounting for approximately 11–40% of cases [24–26]. According to previous studies, several respiratory viruses can cause PVOD, including rhinovirus, parainfluenza virus, Epstein–Barr virus and some coronaviruses, with previously discovered coronaviruses accounting for 10–15% of cases [24,25,27].

Several theories have been proposed to explain the pathogenesis of COVID-19-related anosmia. These include conductive loss of smell due to nasal obstruction and rhinorrhea, oedema of the olfactory cleft mucosa that prevents odorants from reaching the olfactory epithelium, olfactory epithelial damage, infection of the olfactory nerves and, through retrograde neuroinvasion, the olfactory bulb, and impairment of the central olfactory pathways. due to direct viral invasion or indirect injury caused by hypoxia, endothelial damage, or an abnormal inflammatory response. Damage to the olfactory epithelium may be caused by direct viral invasion of the olfactory sensory neurons (OSN), most likely mediated by the neuropilin-1 receptor, or by infection of the non-neuronal cells of the olfactory epithelium, leading to horizontal viral spread to OSNs or impaired morphological and physiological support of OSNs [13,17,22,23,28–31].

## 2. SARS-CoV-2 Cellular Entry Mechanism

To discuss the pathogenesis of COVID-19-related anosmia, the mechanism of SARS-CoV-2 cellular entry must be elucidated. SARS-CoV-2, similarly to SARS-CoV, utilizes the S1 domain of its spike (S) protein for attaching the virion to the host cell membrane by binding to the host angiotensin-converting enzyme 2 (ACE-2) receptor [32,33]. This interaction requires cleavage and priming of the S protein by cell proteases, including transmembrane protease serine 2 (TMPRSS2) and furin, which allows fusion between the cellular and the viral membranes, and viral entry into the cell via endocytosis [17,32,34,35]. Alternative SARS-CoV-2 entry mediators have also been suggested, including receptors and cofactors such as CD147 and neuropilin-1 (NRP1), and activators such as cathepsin Figure 1 [36–38]. ACE-2 is expressed in multiple cell surfaces throughout the body, including lung parenchyma, respiratory epithelium, gastrointestinal epithelium, endothelium, arterial smooth muscles, neuronal glial cells, neurons, renal tubular cells, heart, and lymphoid tissues [33,39–42]. TMPRSS2 was also found to be expressed in multiple organs, such as in the respiratory tract, salivary glands, gastrointestinal tract, liver, and kidneys [34,43]. This ubiquitous expression of ACE2 and TMPRSS2 may explain the pleiotropic effects of SARS-CoV-2 infection [44]. However, the primary targets of SARS-CoV-2 are the cells manifesting high expression of these proteins, especially those that co-express ACE2 and TMPRSS2, such as respiratory and olfactory epithelial cells [17,38]. It is noteworthy that SARS-CoV-2 appears to have a particularly strong affinity for ACE2, estimated to be 10-to-20-fold higher than for SARS-CoV, which might explain its particular impact on chemosensory systems [29,33,45].

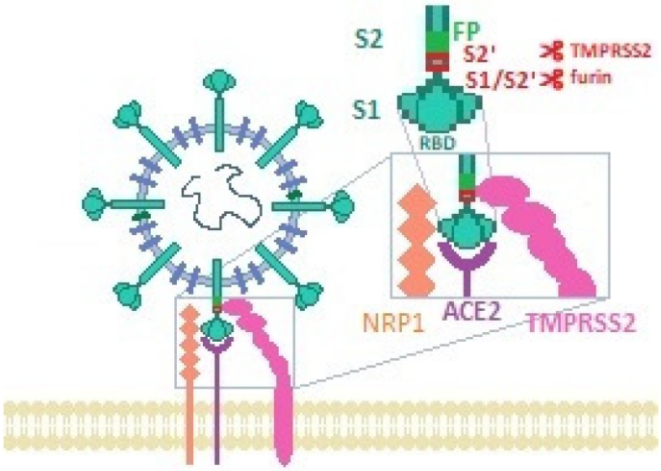


**Figure 1.** SARS-CoV-2 entry. Spike protein domain S1 is pre-activated by the host furin. The receptor binding domain (RBD) of S1 binds to the ACE2 receptor. Cleavage at S2′ site by the type 2 transmembrane protease (TMPRSS2) causes further structural changes in the spike protein and expose the fusion peptide (FP) of S2 that enables the fusion of the viral and host cells. The furin-cleaved S1 fragment of the spike protein may also bind directly to neuropilin-1 (NRP-1).

## 3. The Conductive Pathomechanism of COVID-19-Related Anosmia

Many studies have reported the early onset [2,5,46] and early recovery [4,5,7,8,47,48] of olfactory disorders (OD) in the course of COVID-19. This could argue in favor of a conductive mechanism for OD, related to the inflammation of nasal mucosa [9,47]. However, many COVID-19 patients report OD in the absence of nasal obstruction and rhinorrhea [3,5,6,10,48]. In a study by Chung et al. [47], only 17% of patients with OD reported nasal symptoms, and minimal inflammatory infiltrates were found in nasal biopsy specimens. Moreover, many studies have reported no association between the presence and severity of OD in COVID-19 and nasal symptoms [6,49], and in a study by Lechien et al. [49], some anosmic COVID-19 patients had normal acoustic rhinometry. In addition, Haehner at el. [50], found that self-rated changes in nasal airflow were more pronounced in SARS-CoV-2 negative patients with smell loss compared to SARS-CoV-2 positive patients with OD, while smell deterioration was reported to be more severe by SARS-CoV-2 positive individuals. These findings suggest that rhinitis and nasal mucosa obstruction are not the main factors in the development of COVID-19-related OD.

## 4. Olfactory Cleft Edema

Some imaging studies in anosmic COVID-19 patients show olfactory cleft mucosal edema that may prevent odorants from reaching the olfactory epithelium, even in the absence of nasal congestion [51–53]. However, other reports did not reveal significant olfactory cleft obstruction [54–56], suggesting that it is not the primary mechanism in COVID-19-related anosmia [12,55,56].

## 5. Infection of Higher Olfactory Pathways

Another possible pathomechanism for anosmia is the neural hypothesis, suggesting direct damage to the olfactory nerves, and a retrograde invasion of the olfactory bulb and higher olfactory pathways in the CNS [57,58].

### *5.1. The Neuroinvasive Potential of SARS-CoV-2*

The neuroinvasive potential of SARS-CoV-2 is supported by various neurological manifestations of COVID-19 described in the literature [1,59–61]. In a retrospective case series by Mao et al. [1], 36.4% of 214 SARS-CoV-2-positive patients had neurologic mani-

festations, including dizziness, headache, impaired consciousness, acute cerebrovascular disease, ataxia, seizure, smell or taste impairment, vision impairment, nerve pain, and symptoms of skeletal muscle injury manifestations [1]. In another retrospective study of 814 COVID-19 patients, 57.4% developed neurological symptoms, including myalgias, headache, dizziness, disorders of consciousness, myopathy, dysautonomia, cerebrovascular diseases, seizures, movement disorders, encephalitis, Guillain-Barré syndrome, and optic neuritis [61]. Furthermore, encephalopathy, meningitis, acute transverse myelitis, and coma have also been reported in the literature [59,60]. It has also been hypothesized that viral infection of the respiratory center in the medulla oblongata could cause respiratory failure, which may even occur in the absence of dyspnea [33,41]. Moreover, a study by Bhattacharjeea et al. [62], found that COVID-19 patients have significantly reduced olfactory matching abilities, which the authors considered a sign of cognitive impairment, possibly related to infection in the higher brain centers.

However, the neurological symptoms of COVID-19 do not necessarily indicate a direct viral neuroinvasion, as they may also be due to hypoxic brain injury, cerebrovascular injury, or immune mediated damage [59,63,64]. Indeed, severe SARS-CoV-2 pneumonia may lead to systemic hypoxia and hypercapnia, peripheral vasodilatation, and anaerobic metabolism with an accumulation of toxins and subsequent brain damage due to cerebral edema [60,63]. Cerebrovascular injury may result from viral binding to endothelial ACE2 receptors, which in turn leads to increased luminal pressure and intracranial hemorrhage. Moreover, cerebrovascular injury may be due to an abnormal inflammatory response known as the cytokine storm, which involves the overproduction and excessive release of cytokines, including IL-1β, IL-6, CXCL10, and TNFα, and increased activation of T lymphocytes, macrophages, and endothelial cells, leading to a vascular leakage and an overactivation of the complement system and the coagulation cascade, with subsequent disseminated intravascular coagulation, thromboembolism, and multiorgan failure [59,63,64]. Interestingly, IL-6, which has been shown to regulate neuronal and glial cell activity, may play a role in regulating olfactory neuronal activity, and can also directly inhibit olfactory function through activating apoptosis using TNF-α, or affecting signaling by neuropoietin [65]. In a study by Cazzolla et al. [65], OD was correlated with higher levels of IL-6, and improvement of the olfactory function was associated with decreased IL-6 levels [65]. However, Sanli et al. [66], reported significantly lower serum IL-6 levels in COVID-19 patients with OD compared to normosmic subjects, so the role of IL-6 in the pathogenesis of OD remains unclear.

Some studies reported the presence of SARS-CoV-2 particles in post-mortem brain examination [67,68] and SARS-CoV-2 RNA was detected by RT-PCR in cerebrospinal fluid samples [60], supporting the direct brain injury hypothesis, although the brain seems to contain either the least volume of viral particles of all of the sampled tissues, or no particles at all [29,68], and RT-PCR positivity does not necessarily prove the presence of whole viral particles in the cerebrospinal fluid (CSF) [29].

Direct invasion of the CNS could occur either through the hematogenous or the retrograde neuronal route [1]. In the hematogenous pathway, viruses may reach the bloodstream due to increased permeability of local blood vessels and epithelial disruption, and may then enter the brain by one of several ways: invasion of endothelial cells of the blood-brain-barrier (BBB), paracellular transmigration enabled by increased BBB permeability caused by the release of inflammatory mediators, crossing of the blood–CSF barrier in the choroid plexus, or infection of leukocytes capable of passing through the BBB (the "Trojan horse" mechanism) [54,69–71]. However, according to Li et al. [41], the hematogenous route of SARS-CoV spread is unlikely since almost no viral particles were detected in the non-neuronal cells of infected brain areas [41,54], which may also be true for SARS-CoV-2 [72,73].

### 5.2. The Transneuronal Route of CNS Involvement

The olfactory pathway was hypothesized as a potential route for direct viral neuroinvasion. In this mechanism, the virus reaches the brain through the olfactory epithelium by invading peripheral nerve terminals and propagating via axonal transport towards the OB, from where it may spread trans-synaptically using retrograde and anterograde transport to other brain areas [1,59,63,74,75]. Alternatively, the virus may pass from the OE through the olfactory ensheathing cells directly to the CSF surrounding the olfactory nerve bundles and the OB [20,45].

This transneuronal route of CNS involvement is supported by the post-mortem examinations revealing SARS-CoV-2 viral particles and related damage to be more present in the OSNs and the OB than in the brainstem [76,77]. Interestingly, it has also been hypothesized that inflammatory infiltration of the OB with increased INF-I levels may be responsible for the development of anosmia, and at the same time contributes to the arrest of viral spread into the brain [75]. The hypothesis of neuronal damage as the causative mechanism in SARS-CoV-2-related OD is further supported by reports of OB abnormalities in magnetic resonance imaging (MRI), including features of micro bleeding and oedema, observed in anosmic COVID-19 patients [78,79]. Moreover, transient cortical hyperintensity in the right gyrus rectus has been described [78]. Other studies have also shown the reduction in OB volumes, indicative of its atrophy [53,80]. On the other hand, Akkaya et al. [81] found no significant difference in OB volumes between anosmic and normosmic COVID-19 patients. However, they observed an association between OB morphology and OD, with normal, oval or inverted J-shaped OBs (type N) more common in normosmic patients, while shrunken or flattened OBs (type R) and the presence of asymmetric contour lobulation or more than one hyperintense focus on T2 images (type D) were dominant in the anosmia group [81]. In another study, Esposito et al. [82] found no significant difference in OB volume between the previously SARS-CoV-2-infected hyposmic patients and healthy controls; however, diffusion and functional MRI revealed an increase in neural connectivity within the olfactory cortex and functional connectivity of the anterior piriform cortex, indicating a characteristic brain connectivity response in COVID-19-related hyposmia.

Moreover, several animal studies on previous human coronaviruses support the neuronal route of infection [74,83]. HCoV-OC43 was reported to be found in mouse OSNs and OB 3 days after intranasal inoculation and in the brain 4 days post infection [84]. Moreover, in a study using K18-hACE2 transgenic mice expressing human ACE2, Netland et al. [81] demonstrated that intranasal infection with SARS-CoV resulted in neuroinvasion through the OB and rapid viral spread to the brain regions connected to the OB, such as the piriform and infralimbic cortices, the basal ganglia, and the midbrain, with significant neuronal death [54,83]. Similarly, intranasal administration of SARS-CoV-2 in mice with the human ACE2 gene caused a rapid infection of the brain [85]. Additionally, Jiao et al. [86] demonstrated that in rhesus monkeys, SARS-CoV-2 RNA was detectable in the CSF, the olfactory trigone, and the entorhinal area on days 1, 4, and 7 after intranasal inoculation, respectively.

However, it should be noted that the studies in mouse models utilized transgenic mice with human ACE2 that could be ectopically expressed in many cells, including OSNs, and therefore may not be a reliable model of the viral tropism [28,29]. Indeed, several sequencing studies found ACE2 and TMPRSS2 to be co-expressed in non-neuronal cells of the OE rather than in the OSNs [29,38,87]. Moreover, sequencing and immunostaining studies revealed that OB neurons also do not express detectable levels of ACE2 [17,29]. Similarly, no significant expression of ACE2 or TMPRSS2 was found in neurons in the brain [17,29].

Moreover, the higher incidence of anosmia compared with CNS symptoms, as well as the commonly observed early recovery of OD, argue against the central mechanism of SARS-CoV2-related OD [47,58,88,89].

## 6. Damage to the Olfactory Neuroepithelium

Another mechanism for COVID-19-related anosmia may be sensorineural damage, with the disruption of the OE.

Indeed, Vaira et al. [88], reported massive olfactory epithelium disruption in a patient with anosmia persisting for three months after SARS-CoV-2 infection. Similarly, de Melo et al. [13], in a study of the olfactory neuroepithelium of seven COVID-19 patients presenting with acute loss of smell, reported the SARS-CoV2 infection in multiple cell types within the OE, including OSNs, support cells, and immune cells. Interestingly, sampling of the olfactory mucosa of the patients with long-term anosmia revealed the persistence of virus transcripts up to 6 months after initial diagnosis, accompanied by the protracted inflammation [13]. Moreover, the presence of SARS-CoV-2 in OSNs was shown in intranasally infected Syrian hamsters [90].

As mentioned above, several sequencing studies found ACE2 and TMPRSS2 to be co-expressed in non-neuronal cells of the olfactory epithelium, including sustentacular (support) cells, stem cells, and perivascular cells, rather than in the OSNs [17,38,87]. It was therefore hypothesized that SARS-CoV-2 infects high-ACE2-expressing non-neuronal cells of the OE before passing to OSNs [45]. Infection of OSNs may result from horizontal viral spread from the adjacent support cells or from dissemination of the virus within the OE after its tissue architecture is disrupted by inflammatory infiltrates [13]. Of note, in the aforementioned study by Netland et al. [83], SARS-CoV was detected in the OB after approximately 60 h post inoculation, and subsequent transport in the brain only took a further 12–20 h, suggesting that initial replication and accumulation took place within the OE before the neural invasion [6,45,83]. In addition, in a study by de Melo et al. [13], infection of the neuroepithelium was associated with the loss of OSN cilia, which are necessary for odor detection and transduction [13,28], and recovery of olfactory function was observed after the restoration of cilia in the late phase of infection [13]. Furthermore, Zazhytska et al. [91], in a study of SARS-CoV-2-infected hamsters and humans, found no depletion in OSNs, but observed significant and persistent, non-cell autonomous downregulation of the olfactory receptor and their signaling pathway genes, preceded by reorganization in OSNs nuclear architecture, indicating that the virus may alter the physiology of OSNs without their direct infection.

Moreover, as SUS cells play an important role in the structural and functional support of the OSNs, it appears that their infection could cause impaired olfactory function even without direct neuronal invasion, by architectural OE damage and lack of physical support, impaired signaling, ionic imbalance, and initiating an immune response [17,22,28–30]. Indeed, a study by Bryche et al. [28] in a golden Syrian hamster model demonstrated that, after intranasal instillation, SARS-CoV-2 infected SUS cells, with immense infiltration of the immune cells, massive and rapid desquamation of the OE and a significant loss of OSN cilia, but the virus was not detected in the OSNs or OB [28]. This hypothesis is also in agreement with the relatively rapid recovery of most patients, occurring within weeks after infection, which may reflect the regenerative capability of the SUS cells. The infection of OE stem cells, including horizontal basal cells (HBCs), may be responsible for longer lasting OD in some cases [29,30]. A noteworthy study by Torabi et al. [92] of mucosal samples taken from COVID-19 patients found increased levels of proinflammatory cytokine TNF-α, and the activation of an antiviral signaling cascade in the OE is hypothesized in the literature as reducing the expression of odorant receptors in OSNs [89]. Moreover, the involvement of Bowman's gland can deteriorate mucus production, further affecting odor detection [29]. Indeed, as olfactory receptors turn over every 24 h, but do not contain blood and lymphatic vessels or exhibit mitosis, their generation and maturation depend on stem cells activated by growth or transcription factors secreted into nasal mucus from nasal serous glands. SARS-CoV-2 infects these serous glands, and therefore may cause inhibition of stem cell activity and subsequent loss of smell [14].

However, it should be noted that although almost no expression of ACE2 is found in the OSNs, they do express NRP1, which could account for direct OSNs infection [12,13,36].

Cantuti-Castelvetri et al. [36] found abundant expression of NRP1 in almost all cell types of the OE, and study of human autopsies revealed that SARS-CoV-2 infected NRP1-positive cells in the OE and OB. In addition, the same study reported NRP1-mediated transport of virus-sized particles into the CNS after intranasal inoculation in mice [36]. Moreover, the expression of NRP1 has also been found in neuronal progenitor cells [12,36], which could also play a role in the persistent anosmia observed in some COVID-19 patients [12].

Furthermore, although the previously reported rare occurrence of parosmia during recovery was considered to suggest the absence of damage to peripheral sensory neurons [89,93], it should be noted that its prevalence may have been overlooked in early reports, due to the short observation period [94]. In a study by Hopkins et al. [94], the prevalence of parosmia after a median interval of 2.5 months (range 0–6) from the onset of OD was as high as 43.1% [94], which may indicate the presence of disturbed regrowth and the domination of immature neurons in the OE [15]. Moreover, Di Stadio et al. [95] found that 40% of COVID-19 patients with persistent OD reported parosmia, and 16.6% of the parosmic individuals misperceived odors used during olfactory training. The authors hypothesized that SARS-CoV-2-induced inflammation of the neuroepithelium and OB may result in impaired olfactory recovery with aberrant OSNs regeneration and misperception of odors in the neuroepithelium, as well as altered olfactory receptor mapping in the OB. Therefore, stimulation of these inflamed areas during olfactory training could increase both olfactory recovery and parosmia [95].

## 7. Genetic Link to the Pathomechanism of OD

There may be also a genetic link to the pathomechanism of OD in COVID-19. Indeed, in a multi-ancestry genome-wide association study of COVID-19-related self-reported loss of smell or taste, Shelton et al. [96] identified a single associated locus in the vicinity of the UGT2A1 and UGT2A2 genes, encoding uridine diphosphate glucuronosyltransferase (UGT) enzymes. Interestingly, these enzymes are expressed in the OE and in rats UGT2A1 is involved in metabolizing odorants and olfactory signal termination [96–98]. This argues for a possible role of the identified genes in the dysfunction of OE cells and the associated olfactory disorders [96].

## 8. Conclusions

The pathogenesis of COVID-19 related anosmia is still not fully elucidated; however, it appears to be mainly due to sensorineural damage, with infection of the OE support cells via the ACE1 receptor and disruption of the OE caused by immense inflammatory reaction, and possibly with direct OSNs infection mediated by the NRP-1 receptor. Involvement of the higher olfactory pathways and a conductive component of OD, as well as genetic factors, may also be considered.

**Author Contributions:** L.Z.-J. conceived the presented idea and provided substantial contributions to the literature review and writing of the manuscript; L.Z.-J. and M.J. discussed the data and contributed to the revision of the final manuscript. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References


1. Mao, L.; Jin, H.; Wang, M.; Hu, Y.; Chen, S.; He, Q.; Chang, J.; Hong, C.; Zhou, Y.; Wang, D.; et al. Neurologic Manifestations of Hospitalized Patients with Coronavirus Disease 2019 in Wuhan, China. *JAMA Neurol.* **2020**, *77*, 683. [CrossRef] [PubMed]
2. Moein, S.T.; Hashemian, S.M.; Mansourafshar, B.; Khorram-Tousi, A.; Tabarsi, P.; Doty, R.L. Smell dysfunction: A biomarker for COVID-19. *Int. Forum Allergy Rhinol.* **2020**, *10*, 944–950. [CrossRef]
3. Giacomelli, A.; Pezzati, L.; Conti, F.; Bernacchia, D.; Siano, M.; Oreni, L.; Rusconi, S.; Gervasoni, C.; Ridolfo, A.L.; Rizzardini, G.; et al. Self-reported Olfactory and Taste Disorders in Patients With Severe Acute Respiratory Coronavirus 2 Infection: A Cross-sectional Study. *Clin. Infect. Dis.* **2020**, *71*, 889–890. [CrossRef] [PubMed]
4. Klopfenstein, T.; Kadiane-Oussou, N.J.; Toko, L.; Royer, P.-Y.; Lepiller, Q.; Gendrin, V.; Zayet, S. Features of anosmia in COVID-19. *Méd. Mal. Infect.* **2020**, *50*, 436–439. [CrossRef] [PubMed]
5. Lechien, J.R.; Chiesa-Estomba, C.M.; Place, S.; Van Laethem, Y.; Cabaraux, P.; Mat, Q.; Huet, K.; Plzak, J.; Horoi, M.; Hans, S.; et al. Clinical and epidemiological characteristics of 1420 European patients with mild-to-moderate coronavirus disease 2019. *J. Intern. Med.* **2020**, *288*, 335–344. [CrossRef]
6. Lechien, J.R.; Chiesa-Estomba, C.M.; De Siati, D.R.; Horoi, M.; Le Bon, S.D.; Rodriguez, A.; Dequanter, D.; Blecic, S.; El Afia, F.; Distinguin, L.; et al. Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): A multicenter European study. *Eur. Arch. Otorhinolaryngol.* **2020**, *277*, 2251–2261. [CrossRef] [PubMed]
7. Lee, Y.; Min, P.; Lee, S.; Kim, S.-W. Prevalence and Duration of Acute Loss of Smell or Taste in COVID-19 Patients. *J. Korean Med. Sci.* **2020**, *35*, e174. [CrossRef]
8. Paderno, A.; Schreiber, A.; Grammatica, A.; Raffetti, E.; Tomasoni, M.; Gualtieri, T.; Taboni, S.; Zorzi, S.; Lombardi, D.; Deganello, A.; et al. Smell and taste alterations in COVID-19: A cross-sectional analysis of different cohorts. *Int. Forum Allergy Rhinol.* **2020**, *10*, 955–962. [CrossRef]
9. Qiu, C.; Cui, C.; Hautefort, C.; Haehner, A.; Zhao, J.; Yao, Q.; Zeng, H.; Nisenbaum, E.J.; Liu, L.; Zhao, Y.; et al. Olfactory and Gustatory Dysfunction as an Early Identifier of COVID-19 in Adults and Children: An International Multicenter Study. *Otolaryngol. Head. Neck Surg.* **2020**, *163*, 714–721. [CrossRef] [PubMed]
10. Spinato, G.; Fabbris, C.; Polesel, J.; Cazzador, D.; Borsetto, D.; Hopkins, C.; Boscolo-Rizzo, P. Alterations in Smell or Taste in Mildly Symptomatic Outpatients With SARS-CoV-2 Infection. *JAMA* **2020**, *323*, 2089. [CrossRef]
11. Vaira, L.A.; Salzano, G.; Deiana, G.; De Riu, G. Anosmia and Ageusia: Common Findings in COVID-19 Patients. *Laryngoscope* **2020**, *130*, 1787–1787. [CrossRef] [PubMed]
12. Hopkins, C.; Lechien, J.R.; Saussez, S. More that ACE2? NRP1 may play a central role in the underlying pathophysiological mechanism of olfactory dysfunction in COVID-19 and its association with enhanced survival. *Med. Hypotheses* **2021**, *146*, 110406. [CrossRef]
13. de Melo, G.D.; Lazarini, F.; Levallois, S.; Hautefort, C.; Michel, V.; Larrous, F.; Verillaud, B.; Aparicio, C.; Wagner, S.; Gheusi, G.; et al. COVID-19–Related anosmia is associated with viral persistence and inflammation in human olfactory epithelium and brain infection in hamsters. *Sci. Transl. Med.* **2021**, *13*, eabf8396. [CrossRef] [PubMed]
14. Henkin, R.I. How does Covid-19 infection affect smell? *Am. J. Otolaryngol.* **2021**, *42*, 102912. [CrossRef] [PubMed]
15. Saussez, S.; Lechien, J.R.; Hopkins, C. Anosmia: An evolution of our understanding of its importance in COVID-19 and what questions remain to be answered. *Eur. Arch. Otorhinolaryngol.* **2021**, *278*, 2187–2191. [CrossRef]
16. Hummel, T.; Whitcroft, K.L.; Andrews, P.; Altundag, A.; Cinghi, C.; Costanzo, R.M.; Damm, M.; Frasnelli, J.; Gudziol, H.; Gupta, N.; et al. Position paper on olfactory dysfunction. *Rhin* **2017**, *54*, 1–30. [CrossRef] [PubMed]
17. Brann, D.H.; Tsukahara, T.; Weinreb, C.; Lipovsek, M.; Van den Berge, K.; Gong, B.; Chance, R.; Macaulay, I.C.; Chou, H.-J.; Fletcher, R.B.; et al. Non-neuronal expression of SARS-CoV-2 entry genes in the olfactory system suggests mechanisms underlying COVID-19-associated anosmia. *Sci. Adv.* **2020**, *6*, eabc5801. [CrossRef] [PubMed]
18. Liang, F. Sustentacular Cell Enwrapment of Olfactory Receptor Neuronal Dendrites: An Update. *Genes* **2020**, *11*, 493. [CrossRef] [PubMed]
19. Chen, C.; Kachramanoglou, C.; Li, D.; Andrews, P.; Choi, D. Anatomy and Cellular Constituents of the Human Olfactory Mucosa: A Review. *J. Neurol. Surg. B* **2014**, *75*, 293–300. [CrossRef]
20. Glezer, I.; Bruni-Cardoso, A.; Schechtman, D.; Malnic, B. Viral infection and smell loss: The case of COVID-19. *J. Neurochem.* **2021**, *157*, 930–943. [CrossRef]
21. Bilinska, K.; Butowt, R. Anosmia in COVID-19: A Bumpy Road to Establishing a Cellular Mechanism. *ACS Chem. Neurosci.* **2020**, *11*, 2152–2155. [CrossRef] [PubMed]
22. Ueha, R.; Kondo, K.; Kagoya, R.; Shichino, S.; Ueha, S.; Yamasoba, T. ACE2, TMPRSS2, and Furin expression in the nose and olfactory bulb in mice and human. *Rhin* **2020**, *59*, 105–109. [CrossRef] [PubMed]
23. Ramasamy, K.; Saniasiaya, J.; Abdul Gani, N. Olfactory and Gustatory Dysfunctions as a Clinical Manifestation of Coronavirus Disease 2019 in a Malaysian Tertiary Center. *Ann. Otol. Rhinol. Laryngol.* **2021**, *130*, 513–519. [CrossRef] [PubMed]
24. Suzuki, M.; Saito, K.; Min, W.-P.; Vladau, C.; Toida, K.; Itoh, H.; Murakami, S. Identification of Viruses in Patients With Postviral Olfactory Dysfunction. *Laryngoscope* **2007**, *117*, 272–277. [CrossRef]

25. Hopkins, C.; Kumar, N. Loss of Sense of Smell Asmarker of COVID-19 Infection: Joint Statement from the British Rhinological Society and ENT-UK. Published March 21. 2020. Available online: https://www.entuk.org/sites/default/files/files/Loss%20of%20sense%20of%20smell%20as%20marker%20of%20COVID.pdf (accessed on 20 April 2020).
26. Welge-Lüssen, A.; Wolfensberger, M. Olfactory Disorders following Upper Respiratory Tract Infections. In *Advances in Oto-Rhino-Laryngology*; Hummel, T., Welge-Lüssen, A., Eds.; KARGER: Basel, Switzerland, 2006; Volume 63, pp. 125–132. ISBN 978-3-8055-8123-3.
27. Pellegrino, R.; Cooper, K.W.; Di Pizio, A.; Joseph, P.V.; Bhutani, S.; Parma, V. Coronaviruses and the Chemical Senses: Past, Present, and Future. *Chem. Senses* **2020**, *45*, 415–422. [CrossRef]
28. Bryche, B.; St Albin, A.; Murri, S.; Lacôte, S.; Pulido, C.; Ar Gouilh, M.; Lesellier, S.; Servat, A.; Wasniewski, M.; Picard-Meyer, E.; et al. Massive transient damage of the olfactory epithelium associated with infection of sustentacular cells by SARS-CoV-2 in golden Syrian hamsters. *Brain Behav. Immun.* **2020**, *89*, 579–586. [CrossRef] [PubMed]
29. Cooper, K.W.; Brann, D.H.; Farruggia, M.C.; Bhutani, S.; Pellegrino, R.; Tsukahara, T.; Weinreb, C.; Joseph, P.V.; Larson, E.D.; Parma, V.; et al. COVID-19 and the Chemical Senses: Supporting Players Take Center Stage. *Neuron* **2020**, *107*, 219–233. [CrossRef] [PubMed]
30. van Riel, D.; Verdijk, R.; Kuiken, T. The olfactory nerve: A shortcut for influenza and other viral diseases into the central nervous system: The olfactory nerve: A shortcut for viruses into the CNS. *J. Pathol.* **2015**, *235*, 277–287. [CrossRef] [PubMed]
31. Gorzkowski, V.; Bevilacqua, S.; Charmillon, A.; Jankowski, R.; Gallet, P.; Rumeau, C.; Nguyen, D.T. Evolution of Olfactory Disorders in COVID -19 Patients. *Laryngoscope* **2020**, *130*, 2667–2673. [CrossRef] [PubMed]
32. Hoffmann, M.; Kleine-Weber, H.; Schroeder, S.; Krüger, N.; Herrler, T.; Erichsen, S.; Schiergens, T.S.; Herrler, G.; Wu, N.-H.; Nitsche, A.; et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* **2020**, *181*, 271–280.e8. [CrossRef]
33. Baig, A.M.; Khaleeq, A.; Ali, U.; Syeda, H. Evidence of the COVID-19 Virus Targeting the CNS: Tissue Distribution, Host–Virus Interaction, and Proposed Neurotropic Mechanisms. *ACS Chem. Neurosci.* **2020**, *11*, 995–998. [CrossRef] [PubMed]
34. Guadarrama-Ortiz, P.; Choreño-Parra, J.A.; Sánchez-Martínez, C.M.; Pacheco-Sánchez, F.J.; Rodríguez-Nava, A.I.; García-Quintero, G. Neurological Aspects of SARS-CoV-2 Infection: Mechanisms and Manifestations. *Front. Neurol.* **2020**, *11*, 1039. [CrossRef] [PubMed]
35. Gengler, I.; Wang, J.C.; Speth, M.M.; Sedaghat, A.R. Sinonasal pathophysiology of SARS-CoV-2 and COVID-19: A systematic review of the current evidence. *Laryngoscope Investig. Otolaryngol.* **2020**, *5*, 354–359. [CrossRef]
36. Cantuti-Castelvetri, L.; Ojha, R.; Pedro, L.D.; Djannatian, M.; Franz, J.; Kuivanen, S.; van der Meer, F.; Kallio, K.; Kaya, T.; Anastasina, M.; et al. Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. *Science* **2020**, *370*, 856–860. [CrossRef] [PubMed]
37. Davies, J.; Randeva, H.; Chatha, K.; Hall, M.; Spandidos, D.; Karteris, E.; Kyrou, I. Neuropilin-1 as a new potential SARS-CoV-2 infection mediator implicated in the neurologic features and central nervous system involvement of COVID-19. *Mol. Med. Rep.* **2020**, *22*, 4221–4226. [CrossRef] [PubMed]
38. Fodoulian, L.; Tuberosa, J.; Rossier, D.; Boillat, M.; Kan, C.; Pauli, V.; Egervari, K.; Lobrinus, J.A.; Landis, B.N.; Carleton, A.; et al. SARS-CoV-2 Receptors and Entry Genes Are Expressed in the Human Olfactory Neuroepithelium and Brain. *iScience* **2020**, *23*, 101839. [CrossRef] [PubMed]
39. Chen, R.; Wang, K.; Yu, J.; Howard, D.; French, L.; Chen, Z.; Wen, C.; Xu, Z. The Spatial and Cell-Type Distribution of SARS-CoV-2 Receptor ACE2 in the Human and Mouse Brains. *Front. Neurol.* **2021**, *11*, 573095. [CrossRef] [PubMed]
40. Hamming, I.; Timens, W.; Bulthuis, M.; Lely, A.; Navis, G.; van Goor, H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J. Pathol.* **2004**, *203*, 631–637. [CrossRef] [PubMed]
41. Li, Y.; Bai, W.; Hashikawa, T. The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. *J. Med. Virol.* **2020**, *92*, 552–555. [CrossRef]
42. Xu, H.; Zhong, L.; Deng, J.; Peng, J.; Dan, H.; Zeng, X.; Li, T.; Chen, Q. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *Int. J. Oral Sci.* **2020**, *12*, 8. [CrossRef] [PubMed]
43. Vaarala, M.H.; Porvari, K.S.; Kellokumpu, S.; Kyllönen, A.P.; Vihko, P.T. Expression of transmembrane serine protease TMPRSS2 in mouse and human tissues. *J. Pathol.* **2001**, *193*, 134–140. [CrossRef]
44. Bougakov, D.; Podell, K.; Goldberg, E. Multiple Neuroinvasive Pathways in COVID-19. *Mol. Neurobiol.* **2021**, *58*, 564–575. [CrossRef]
45. Butowt, R.; Bilinska, K. SARS-CoV-2: Olfaction, Brain Infection, and the Urgent Need for Clinical Samples Allowing Earlier Virus Detection. *ACS Chem. Neurosci.* **2020**, *11*, 1200–1203. [CrossRef]
46. Vaira, L.A.; Hopkins, C.; Salzano, G.; Petrocelli, M.; Melis, A.; Cucurullo, M.; Ferrari, M.; Gagliardini, L.; Pipolo, C.; Deiana, G.; et al. Olfactory and gustatory function impairment in COVID-19 patients: Italian objective multicenter-study. *Head Neck* **2020**, *42*, 1560–1569. [CrossRef]
47. Chung, T.W.-H.; Sridhar, S.; Zhang, A.J.; Chan, K.-H.; Li, H.-L.; Wong, F.K.-C.; Ng, M.-Y.; Tsang, R.K.-Y.; Lee, A.C.-Y.; Fan, Z.; et al. Olfactory Dysfunction in Coronavirus Disease 2019 Patients: Observational Cohort Study and Systematic Review. *Open Forum Infect. Dis.* **2020**, *7*, ofaa199. [CrossRef] [PubMed]

48. Vaira, L.A.; Deiana, G.; Fois, A.G.; Pirina, P.; Madeddu, G.; De Vito, A.; Babudieri, S.; Petrocelli, M.; Serra, A.; Bussu, F.; et al. Objective evaluation of anosmia and ageusia in COVID-19 patients: Single-center experience on 72 cases. *Head Neck* **2020**, *42*, 1252–1258. [CrossRef]
49. Lechien, J.R.; Cabaraux, P.; Chiesa-Estomba, C.M.; Khalife, M.; Hans, S.; Calvo-Henriquez, C.; Martiny, D.; Journe, F.; Sowerby, L.; Saussez, S. Objective olfactory evaluation of self-reported loss of smell in a case series of 86 COVID-19 patients. *Head Neck* **2020**, *42*, 1583–1590. [CrossRef] [PubMed]
50. Haehner, A.; Draf, J.; Dräger, S.; de With, K.; Hummel, T. Predictive Value of Sudden Olfactory Loss in the Diagnosis of COVID-19. *ORL* **2020**, *82*, 175–180. [CrossRef] [PubMed]
51. Eliezer, M.; Hautefort, C.; Hamel, A.-L.; Verillaud, B.; Herman, P.; Houdart, E.; Eloit, C. Sudden and Complete Olfactory Loss of Function as a Possible Symptom of COVID-19. *JAMA Otolaryngol. Head Neck Surg.* **2020**, *146*, 674. [CrossRef] [PubMed]
52. Altundag, A.; Yıldırım, D.; Tekcan Sanli, D.E.; Cayonu, M.; Kandemirli, S.G.; Sanli, A.N.; Arici Duz, O.; Saatci, O. Olfactory Cleft Measurements and COVID-19–Related Anosmia. *Otolaryngol. Head Neck Surg.* **2021**, *164*, 1337–1344. [CrossRef]
53. Kandemirli, S.G.; Altundag, A.; Yildirim, D.; Tekcan Sanli, D.E.; Saatci, O. Olfactory Bulb MRI and Paranasal Sinus CT Findings in Persistent COVID-19 Anosmia. *Acad. Radiol.* **2021**, *28*, 28–35. [CrossRef] [PubMed]
54. Lin, E.; Lantos, J.E.; Strauss, S.B.; Phillips, C.D.; Campion, T.R.; Navi, B.B.; Parikh, N.S.; Merkler, A.E.; Mir, S.; Zhang, C.; et al. Brain Imaging of Patients with COVID-19: Findings at an Academic Institution during the Height of the Outbreak in New York City. *AJNR Am. J. Neuroradiol.* **2020**, *41*, 2001–2008. [CrossRef] [PubMed]
55. Naeini, A.S.; Karimi-Galougahi, M.; Raad, N.; Ghorbani, J.; Taraghi, A.; Haseli, S.; Mehrparvar, G.; Bakhshayeshkaram, M. Paranasal sinuses computed tomography findings in anosmia of COVID-19. *Am. J. Otolaryngol.* **2020**, *41*, 102636. [CrossRef]
56. Lechien, J.R.; Michel, J.; Radulesco, T.; Chiesa-Estomba, C.M.; Vaira, L.A.; De Riu, G.; Sowerby, L.; Hopkins, C.; Saussez, S. Clinical and Radiological Evaluations of COVID -19 Patients With Anosmia: Preliminary Report. *Laryngoscope* **2020**, *130*, 2526–2531. [CrossRef] [PubMed]
57. Han, A.Y.; Mukdad, L.; Long, J.L.; Lopez, I.A. Anosmia in COVID-19: Mechanisms and Significance. *Chem. Senses* **2020**, *45*, 423–428. [CrossRef] [PubMed]
58. Vaira, L.A.; Salzano, G.; Fois, A.G.; Piombino, P.; De Riu, G. Potential pathogenesis of ageusia and anosmia in COVID-19 patients. *Int. Forum Allergy Rhinol.* **2020**, *10*, 1103–1104. [CrossRef] [PubMed]
59. Ahmad, I.; Rathore, F.A. Neurological manifestations and complications of COVID-19: A literature review. *J. Clin. Neurosci.* **2020**, *77*, 8–12. [CrossRef] [PubMed]
60. Moriguchi, T.; Harii, N.; Goto, J.; Harada, D.; Sugawara, H.; Takamino, J.; Ueno, M.; Sakata, H.; Kondo, K.; Myose, N.; et al. A first case of meningitis/encephalitis associated with SARS-Coronavirus-2. *Int. J. Infect. Dis.* **2020**, *94*, 55–58. [CrossRef] [PubMed]
61. Romero-Sánchez, C.M.; Díaz-Maroto, I.; Fernández-Díaz, E.; Sánchez-Larsen, Á.; Layos-Romero, A.; García-García, J.; González, E.; Redondo-Peñas, I.; Perona-Moratalla, A.B.; Del Valle-Pérez, J.A.; et al. Neurologic manifestations in hospitalized patients with COVID-19: The ALBACOVID registry. *Neurology* **2020**, *95*, e1060–e1070. [CrossRef] [PubMed]
62. Bhattacharjee, A.S.; Joshi, S.V.; Naik, S.; Sangle, S.; Abraham, N.M. Quantitative assessment of olfactory dysfunction accurately detects asymptomatic COVID-19 carriers. *EClinicalMedicine* **2020**, *28*, 100575. [CrossRef]
63. Azim, D.; Nasim, S.; Kumar, S.; Hussain, A.; Patel, S. Neurological Consequences of 2019-nCoV Infection: A Comprehensive Literature Review. *Cureus* **2020**, *12*, e8790. [CrossRef]
64. Moonis, G.; Filippi, C.G.; Kirsch, C.F.E.; Mohan, S.; Stein, E.G.; Hirsch, J.A.; Mahajan, A. The Spectrum of Neuroimaging Findings on CT and MRI in Adults With COVID-19. *Am. J. Roentgenol.* **2021**, *217*, 959–974. [CrossRef] [PubMed]
65. Cazzolla, A.P.; Lovero, R.; Lo Muzio, L.; Testa, N.F.; Schirinzi, A.; Palmieri, G.; Pozzessere, P.; Procacci, V.; Di Comite, M.; Ciavarella, D.; et al. Taste and Smell Disorders in COVID-19 Patients: Role of Interleukin-6. *ACS Chem. Neurosci.* **2020**, *11*, 2774–2781. [CrossRef] [PubMed]
66. Sanli, D.E.T.; Altundag, A.; Kandemirli, S.G.; Yildirim, D.; Sanli, A.N.; Saatci, O.; Kirisoglu, C.E.; Dikensoy, O.; Murrja, E.; Yesil, A.; et al. Relationship between disease severity and serum IL-6 levels in COVID-19 anosmia. *Am. J. Otolaryngol.* **2021**, *42*, 102796. [CrossRef] [PubMed]
67. Paniz-Mondolfi, A.; Bryce, C.; Grimes, Z.; Gordon, R.E.; Reidy, J.; Lednicky, J.; Sordillo, E.M.; Fowkes, M. Central nervous system involvement by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). *J. Med. Virol.* **2020**, *92*, 699–702. [CrossRef] [PubMed]
68. Puelles, V.G.; Lütgehetmann, M.; Lindenmeyer, M.T.; Sperhake, J.P.; Wong, M.N.; Allweiss, L.; Chilla, S.; Heinemann, A.; Wanner, N.; Liu, S.; et al. Multiorgan and Renal Tropism of SARS-CoV-2. *N. Engl. J. Med.* **2020**, *383*, 590–592. [CrossRef] [PubMed]
69. Pouga, L. Encephalitic syndrome and anosmia in COVID-19: Do these clinical presentations really reflect SARS-CoV-2 neurotropism? A theory based on the review of 25 COVID-19 cases. *J. Med. Virol.* **2021**, *93*, 550–558. [CrossRef]
70. Lima, M.; Siokas, V.; Aloizou, A.-M.; Liampas, I.; Mentis, A.-F.A.; Tsouris, Z.; Papadimitriou, A.; Mitsias, P.D.; Tsatsakis, A.; Bogdanos, D.P.; et al. Unraveling the Possible Routes of SARS-COV-2 Invasion into the Central Nervous System. *Curr. Treat. Options Neurol.* **2020**, *22*, 37. [CrossRef]

71. Desforges, M.; Le Coupanec, A.; Brison, É.; Meessen-Pinard, M.; Talbot, P.J. Neuroinvasive and Neurotropic Human Respiratory Coronaviruses: Potential Neurovirulent Agents in Humans. In *Infectious Diseases and Nanomedicine I*; Adhikari, R., Thapa, S., Eds.; Advances in Experimental Medicine and Biology; Springer India: New Delhi, India, 2014; Volume 807, pp. 75–96. ISBN 978-81-322-1776-3.
72. Jacob, F.; Pather, S.R.; Huang, W.-K.; Zhang, F.; Wong, S.Z.H.; Zhou, H.; Cubitt, B.; Fan, W.; Chen, C.Z.; Xu, M.; et al. Human Pluripotent Stem Cell-Derived Neural Cells and Brain Organoids Reveal SARS-CoV-2 Neurotropism Predominates in Choroid Plexus Epithelium. *Cell Stem Cell* **2020**, *27*, 937–950.e9. [CrossRef]
73. Pellegrini, L.; Albecka, A.; Mallery, D.L.; Kellner, M.J.; Paul, D.; Carter, A.P.; James, L.C.; Lancaster, M.A. SARS-CoV-2 Infects the Brain Choroid Plexus and Disrupts the Blood-CSF Barrier in Human Brain Organoids. *Cell Stem Cell* **2020**, *27*, 951–961.e5. [CrossRef]
74. Desforges, M.; Le Coupanec, A.; Dubeau, P.; Bourgouin, A.; Lajoie, L.; Dubé, M.; Talbot, P.J. Human Coronaviruses and Other Respiratory Viruses: Underestimated Opportunistic Pathogens of the Central Nervous System? *Viruses* **2019**, *12*, 14. [CrossRef] [PubMed]
75. Gori, A.; Leone, F.; Loffredo, L.; Cinicola, B.L.; Brindisi, G.; De Castro, G.; Spalice, A.; Duse, M.; Zicari, A.M. COVID-19-Related Anosmia: The Olfactory Pathway Hypothesis and Early Intervention. *Front. Neurol.* **2020**, *11*, 956. [CrossRef] [PubMed]
76. Bulfamante, G.; Chiumello, D.; Canevini, M.P.; Priori, A.; Mazzanti, M.; Centanni, S.; Felisati, G. First ultrastructural autoptic findings of SARS-CoV-2 in olfactory pathways and brainstem. *Minerva Anestesiol.* **2020**, *86*, S0375–S9393. [CrossRef]
77. Menter, T.; Haslbauer, J.D.; Nienhold, R.; Savic, S.; Hopfer, H.; Deigendesch, N.; Frank, S.; Turek, D.; Willi, N.; Pargger, H.; et al. Postmortem examination of COVID-19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings in lungs and other organs suggesting vascular dysfunction. *Histopathology* **2020**, *77*, 198–209. [CrossRef] [PubMed]
78. Politi, L.S.; Salsano, E.; Grimaldi, M. Magnetic Resonance Imaging Alteration of the Brain in a Patient With Coronavirus Disease 2019 (COVID-19) and Anosmia. *JAMA Neurol.* **2020**, *77*, 1028. [CrossRef]
79. Laurendon, T.; Radulesco, T.; Mugnier, J.; Gérault, M.; Chagnaud, C.; El Ahmadi, A.-A.; Varoquaux, A. Bilateral transient olfactory bulb edema during COVID-19–related anosmia. *Neurology* **2020**, *95*, 224–225. [CrossRef] [PubMed]
80. Tsivgoulis, G.; Fragkou, P.C.; Lachanis, S.; Palaiodimou, L.; Lambadiari, V.; Papathanasiou, M.; Sfikakis, P.P.; Voumvourakis, K.I.; Tsiodras, S. Olfactory bulb and mucosa abnormalities in persistent COVID-19-induced anosmia: A magnetic resonance imaging study. *Eur. J. Neurol.* **2021**, *28*, e6–e8. [CrossRef] [PubMed]
81. Akkaya, H.; Kizilog˘lu, A.; Dilek, O.; Belibag˘li, C.; Kaya, Ö.; Yılmaz, C.; Gülek, B. Evaluation of the olfactory bulb volume and morphology in patients with coronavirus disease 2019: Can differences create predisposition to anosmia? *Rev. Assoc. Med. Bras.* **2021**, *67*, 1491–1497. [CrossRef]
82. Esposito, F.; Cirillo, M.; De Micco, R.; Caiazzo, G.; Siciliano, M.; Russo, A.G.; Monari, C.; Coppola, N.; Tedeschi, G.; Tessitore, A. Olfactory loss and brain connectivity after COVID-19. *Hum. Brain Mapp.* **2022**, *43*, 1548–1560. [CrossRef] [PubMed]
83. Netland, J.; Meyerholz, D.K.; Moore, S.; Cassell, M.; Perlman, S. Severe Acute Respiratory Syndrome Coronavirus Infection Causes Neuronal Death in the Absence of Encephalitis in Mice Transgenic for Human ACE2. *J. Virol.* **2008**, *82*, 7264–7275. [CrossRef]
84. Dubé, M.; Le Coupanec, A.; Wong, A.H.M.; Rini, J.M.; Desforges, M.; Talbot, P.J. Axonal Transport Enables Neuron-to-Neuron Propagation of Human Coronavirus OC43. *J. Virol* **2018**, *92*, e00404-18. [CrossRef] [PubMed]
85. Sun, S.-H.; Chen, Q.; Gu, H.-J.; Yang, G.; Wang, Y.-X.; Huang, X.-Y.; Liu, S.-S.; Zhang, N.-N.; Li, X.-F.; Xiong, R.; et al. A Mouse Model of SARS-CoV-2 Infection and Pathogenesis. *Cell Host Microbe* **2020**, *28*, 124–133.e4. [CrossRef] [PubMed]
86. Jiao, L.; Yang, Y.; Yu, W.; Zhao, Y.; Long, H.; Gao, J.; Ding, K.; Ma, C.; Li, J.; Zhao, S.; et al. The olfactory route is a potential way for SARS-CoV-2 to invade the central nervous system of rhesus monkeys. *Sig. Transduct. Target.* **2021**, *6*, 169. [CrossRef] [PubMed]
87. Bilinska, K.; Jakubowska, P.; Von Bartheld, C.S.; Butowt, R. Expression of the SARS-CoV-2 Entry Proteins, ACE2 and TMPRSS2, in Cells of the Olfactory Epithelium: Identification of Cell Types and Trends with Age. *ACS Chem. Neurosci.* **2020**, *11*, 1555–1562. [CrossRef]
88. Vaira, L.A.; Hopkins, C.; Sandison, A.; Manca, A.; Machouchas, N.; Turilli, D.; Lechien, J.R.; Barillari, M.R.; Salzano, G.; Cossu, A.; et al. Olfactory epithelium histopathological findings in long-term coronavirus disease 2019 related anosmia. *J. Laryngol. Otol.* **2020**, *134*, 1123–1127. [CrossRef]
89. Rodriguez, S.; Cao, L.; Rickenbacher, G.T.; Benz, E.G.; Magdamo, C.; Gomez, L.R.; Holbrook, E.H.; Albers, A.D.; Gallagher, R.; Westover, M.B.; et al. Innate immune signaling in the olfactory epithelium reduces odorant receptor levels: Modeling transient smell loss in COVID-19 patients. *medRxiv* **2020**, preprint. [CrossRef]
90. Sia, S.F.; Yan, L.-M.; Chin, A.W.H.; Fung, K.; Choy, K.-T.; Wong, A.Y.L.; Kaewpreedee, P.; Perera, R.A.P.M.; Poon, L.L.M.; Nicholls, J.M.; et al. Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. *Nature* **2020**, *583*, 834–838. [CrossRef]
91. Zazhytska, M.; Kodra, A.; Hoagland, D.A.; Frere, J.; Fullard, J.F.; Shayya, H.; McArthur, N.G.; Moeller, R.; Uhl, S.; Omer, A.D.; et al. Non-cell-autonomous disruption of nuclear architecture as a potential cause of COVID-19-induced anosmia. *Cell* 2022, in press. [CrossRef]

92. Torabi, A.; Mohammadbagheri, E.; Akbari Dilmaghani, N.; Bayat, A.-H.; Fathi, M.; Vakili, K.; Alizadeh, R.; Rezaeimirghaed, O.; Hajiesmaeili, M.; Ramezani, M.; et al. Proinflammatory Cytokines in the Olfactory Mucosa Result in COVID-19 Induced Anosmia. *ACS Chem. Neurosci.* **2020**, *11*, 1909–1913. [CrossRef]
93. Parma, V.; Ohla, K.; Veldhuizen, M.G.; Niv, M.Y.; Kelly, C.E.; Bakke, A.J.; Cooper, K.W.; Bouysset, C.; Pirastu, N.; Dibattista, M.; et al. More Than Smell—COVID-19 Is Associated With Severe Impairment of Smell, Taste, and Chemesthesis. *Chem. Senses* **2020**, *45*, 609–622. [CrossRef]
94. Hopkins, C.; Surda, P.; Vaira, L.A.; Lechien, J.R.; Safarian, M.; Saussez, S.; Kumar, N. Six month follow-up of self-reported loss of smell during the COVID-19 pandemic. *Rhinology* **2020**, *59*, 26–31. [CrossRef] [PubMed]
95. Di Stadio, A.; D'Ascanio, L.; La Mantia, I.; Ralli, M.; Brenner, M.J. Parosmia after COVID-19: Olfactory training, neuroinflammation and distortions of smell. *Eur. Rev. Med. Pharmacol. Sci.* **2022**, *26*, 1–3. [CrossRef] [PubMed]
96. Shelton, J.F.; Shastri, A.J.; Fletez-Brant, K.; The 23andMe COVID-19 Team; Auton, A.; Chubb, A.; Fitch, A.; Kung, A.; Altman, A.; Kill, A.; et al. The UGT2A1/UGT2A2 locus is associated with COVID-19-related loss of smell or taste. *Nat. Genet.* **2022**, *54*, 121–124. [CrossRef]
97. Khan, M.; Yoo, S.-J.; Clijsters, M.; Backaert, W.; Vanstapel, A.; Speleman, K.; Lietaer, C.; Choi, S.; Hether, T.D.; Marcelis, L.; et al. Visualizing in deceased COVID-19 patients how SARS-CoV-2 attacks the respiratory and olfactory mucosae but spares the olfactory bulb. *Cell* **2021**, *184*, 5932–5949.e15. [CrossRef] [PubMed]
98. Lazard, D.; Zupko, K.; Poria, Y.; Net, P.; Lazarovits, J.; Horn, S.; Khen, M.; Lancet, D. Odorant signal termination by olfactory UDP glucuronosyl transferase. *Nature* **1991**, *349*, 790–793. [CrossRef] [PubMed]

**Publikacja 3.**

**Tytuł**: COVID-19 severity and mortality in two pandemic waves in Poland and predictors of poor outcomes of SARS-CoV-2 infection in hospitalized young adults

**Autorzy**: Ziuzia-Januszewska L, Januszewski M, Sosnowska-Nowak J, Janiszewski M, Dobrzyński P, Jakimiuk AA, Jakimiuk AJ

**Czasopismo**: Viruses 2022; 14(8), 1700

**doi**: 10.3390/v14081700

viruses

MDPI

*Article*

# COVID-19 Severity and Mortality in Two Pandemic Waves in Poland and Predictors of Poor Outcomes of SARS-CoV-2 Infection in Hospitalized Young Adults

**Laura Ziuzia-Januszewska [1,*], Marcin Januszewski [2], Joanna Sosnowska-Nowak [1], Mariusz Janiszewski [1], Paweł Dobrzyński [1], Alicja A. Jakimiuk [3] and Artur J. Jakimiuk [2,4]**

[1] Department of Otolaryngology, Central Clinical Hospital of the Ministry of Interior and Administration, 02-507 Warsaw, Poland; joanna.sosnowska@cskmswia.gov.pl (J.S.-N.); mariusz.janiszewski@cskmswia.gov.pl (M.J.); pawel.dobrzynski@cskmswia.gov.pl (P.D.)
[2] Department of Obstetrics and Gynecology, Central Clinical Hospital of the Ministry of Interior and Administration, 02-507 Warsaw, Poland; lek.med.mjanuszewski@gmail.com (M.J.); jakimiuk@yahoo.com (A.J.J.)
[3] Department of Plastic Surgery, Central Clinical Hospital of the Ministry of Interior and Administration, 02-507 Warsaw, Poland; alajakimiuk@hotmail.com
[4] Center for Reproductive Health, Institute of Mother and Child, 01-211 Warsaw, Poland
* Correspondence: lauraziuzia@gmail.com or laura.ziuzia@cskmswia.gov.pl; Tel.: +48-477221182

**Abstract:** SARS-CoV-2 variants pose a significant threat to global public health. However, their influence on disease severity, especially among young adults who may exhibit different clinical characteristics, is debatable. In this retrospective study of 229 young adults hospitalized with COVID-19, we investigated the differences between Poland's second and third waves of the pandemic. To identify potential predictors of severe COVID-19 in young adults, we analyzed patient characteristics and laboratory findings between survivors and non-survivors and we performed logistic regression to assess the risk of death, mechanical ventilation, and intensive care unit treatment. We found no increase in COVID-19 severity comparing the third and second waves of the pandemic, indicating that the alpha variant had no influence on disease severity. In addition, we found that factors, such as obesity, comorbidities, lung involvement, leukocytosis, neutrophilia, lymphopenia, higher IG count, the neutrophil-to-lymphocyte ratio, C-reactive protein, procalcitonin, interleukin-6, D-Dimer, lactate dehydrogenase, high-sensitive troponin I, creatine kinase-myocardial band, myoglobin, N-terminal-pro-B-type natriuretic peptide, creatinine, urea and gamma-glutamyl transferase, lower estimated glomerular filtration rate, albumin, calcium and vitamin D3, possibly a decrease in red blood cell counts, hemoglobin and hematocrit, and an increase in creatine kinase during hospitalization may be associated with poor outcomes of COVID-19.

**Keywords:** COVID-19; SARS-CoV-2; alpha variant; severity; mortality; predictors; young adults



**Citation:** Ziuzia-Januszewska, L.; Januszewski, M.; Sosnowska-Nowak, J.; Janiszewski, M.; Dobrzyński, P.; Jakimiuk, A.A.; Jakimiuk, A.J. COVID-19 Severity and Mortality in Two Pandemic Waves in Poland and Predictors of Poor Outcomes of SARS-CoV-2 Infection in Hospitalized Young Adults. *Viruses* **2022**, *14*, 1700. https://doi.org/10.3390/v14081700

Academic Editor: Yinzhong Shen

Received: 7 July 2022
Accepted: 28 July 2022
Published: 31 July 2022



## 1. Introduction

Coronavirus disease 2019 (COVID-19), declared a pandemic by the World Health Organization (WHO) on 11 March 2020 [1], is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2).

SARS-CoV-2 is a highly infectious RNA virus, which utilizes its spike (S) protein for cellular entry by binding to the host angiotensin-converting enzyme 2 (ACE-2) receptor [2]. This interaction requires the cleavage of the S protein by cell proteases, including transmembrane protease serine 2 (TMPRSS2) [3].

As of 30 June 2022, 544,324,069 cases of COVID-19 had been reported worldwide, causing 6,332,963 deaths [4]. In Poland, despite there being low incidence and mortality rates during the first European wave of the pandemic during the spring of 2020, the second and the third waves were both associated with high case and mortality rates, with the

numbers of daily positive SARS-CoV-2 laboratory test results peaking on 7 November 2020 at 27,875 cases and then again on 1 April 1 2021, at 35,251 cases. [5].

By the end of 2020, the emergence of the new variants of SARS-CoV-2, associated with changes in viral transmissibility, clinical presentation, and/or effectiveness of preventative, diagnostic, and therapeutic measures, posed a further threat to global public health [6]. This has prompted researchers and health organizations to characterize the variants of concern (VOCs), including alpha (B.1.1.7), beta (B.1.351), and later gamma (P.1), delta (B.1.617.2), and omicron (B.1.1.529) variants [6,7]. Notably, only omicron is still considered a currently circulating VOC, while the remaining previous VOCs are now labeled by the WHO as "previously circulating VOCs" [6], by the CDC as "variants being monitored" (VBMs) [7], and by the ECDC as "de-escalated variants" [8].

The alpha variant (B.1.1.7), first identified in the UK and then spreading worldwide, was defined by multiple mutations, including changes in the spike protein (N501Y, A570D, D614G, P681H, T716I, S982A, D1118H, del 69–70, del 144), with case rates increasing from fewer than 5% of all SARS-CoV-2 infections to more than 60% between November and mid-December 2020, causing a sharp increase in COVID-19 incidence, hospitalization, and mortality [9]. In Poland, according to the ECDC report of 15 February 2021, incidence of the alpha variant among all cases sequenced in the preceding weeks was at 9% [8], while according to ECDC data on SARS-CoV-2 variants in the EU/EEA [10] only one case of B.1.1.7 was detected until the end of 2020 (in the week 2020-52), and from the beginning to the end of January 2021 the incidence of alpha variant was 9.5% (52 reported detections of B.1.1.7 variant out of 548 sequences carried out), with the overall incidence in weeks 2020-37 to 2021-04, i.e., from 7 September 2020 to 31 January 2021, of 6.5% (53/821), and then gradually increasing, exceeding 50% in the week 2021-07 (15 February to 21 February 2021) and rapidly rising further to over 90% in March, 2021, with the overall incidence in weeks 2021-06 to 2021-23 (8 February to 13 June 2021) of 92.4% (15,442/16,709) [10]. These data indicate that the third wave of the COVID-19 pandemic in Poland was mainly caused by the alpha variant, while the second wave was caused by the previously known SARS-CoV-2 variants. Hence, one can suspect that the differences between these waves may indicate the differences between the disease courses depending on the causative variants.

Several studies suggested the alpha variant was more transmissible than the previously identified SARS-CoV-2 variants [11,12], and this was hypothesized as resulting from the higher viral load and longer detectability in respiratory secretions, possibly attributable to mutations of the spike protein, including in the receptor-binding domain and adjacent to the furin-cleavage site, and therefore affecting viral cell entry [11,13]. Some reports indicated greater disease severity associated with the alpha variant [14–16], however, others did not find this relationship [13]. Similarly, the widely debated possibility of increased severity among young people remains unclear [17,18]. Interestingly, Kayano et al. [19] reported higher odds of severe illness and death in patients infected with alpha variant compared to preexisting strains, but these results were statistically insignificant among patients aged <40 years and >79 years old with respect to severe cases and among patients aged <50 years with respect to deaths. Although the later delta variant is generally considered to be associated with greater disease severity, data are also inconsistent [20–23]. Nevertheless, the alpha, beta, gamma and delta variants have been de-escalated from being VOCs, as no longer considered to pose significant risk to public health [24]. Currently most predominant omicron variant, still labeled as VOC, appears to cause less severe disease [8,25,26], however, still more data are needed [27].

Although older age is a risk factor for both the incidence and worse prognosis of COVID-19 [28–32], severe disease and death have also been observed among young adults [31,33,34]. Moreover, clinical characteristics and laboratory test results in younger people seem to be different from those in elderly patients [31,34], and this may indicate a different pathogenesis of COVID-19 in these age groups [34]. Furthermore, although work, education, and other social settings put young people at a higher risk of SARS-CoV-2 exposure, this age group appears to be less likely to comply with preventative measures [35].

However, there is limited data available in the literature on the predictors of the severe course of this disease in young adults [31–34].

The primary aim of this study was to investigate the differences between the course of disease in young adult inpatients comparing the second and the third waves of the COVID-19 pandemic in Poland, possibly reflecting differences in the severity of COVID-19 in hospitalized young adults depending on the causative SARS-CoV-2 variant (alpha vs. wild-type variants). The secondary aim was to identify the potential predictors of severe course of COVID-19 in this age group.

## 2. Materials and Methods

### *2.1. Subjects and Settings*

This single-centered, retrospective study of hospitalized young adults with COVID-19 during the second and the third wave of pandemic in Poland was conducted at the Central Clinical Hospital of the Ministry of the Interior and Administration in Warsaw, Poland, which was designated by the government for the treatment of patients suffering from COVID-19.

The inclusion criteria were adults not younger than 18 and not older than 45 years, with laboratory-confirmed SARS-CoV-2 infection, who were admitted due to severe COVID-19 (i.e., meeting hospital admission criteria for COVID-19, with oxygen saturation of 94% or less on room air or the need for oxygen therapy [36]) during the second or third waves of the COVID-19 pandemic in Poland. SARS-CoV-2 infection was diagnosed by either RT-PCR or rapid antigen test performed on nasopharyngeal samples, following the protocols supplied by the testing kits' manufacturers (GeneProof SARS-CoV-2 PCR Kit, GeneProof a.s., Brno, Czech Republic; GeneFinder TM COVID-19 Plus RealAmp Kit; Panbio™ COVID-19 AG Rapid Test Device, Abbott, Abbot Rapid Diagnostics Jena GmbH, Jena, Germany; Bioeasy 2019-nCoV Ag Fluorescence, Shenzhen Bioeasy Biotechnology Co. Ltd., Shenzhen, China), all of which have approximately 100% specificity.

Patients admitted for reasons other than COVID-19 (e.g., trauma, other acute conditions, or serious deterioration in the course of chronic diseases) were excluded from the study. Pregnant women were also excluded due to the possible influence of pregnancy on the severity of the disease [37] and on the results of potential laboratory predictors [38].

In our study, we defined the beginning of a wave as the first day of an at least 14-day period as a continuous increase in the 7-day average number of new cases. The end of a wave was defined as the last day of an at least 14-day period with a continuous decrease in the 7-day average number of new cases, preceding the start of a new wave. Therefore, the second wave was defined as the period from 12 September 2020 to 27 January 2021, and the third wave from 11 February 2021 to 10 June 2021 [5]. It should be noted that, according to the ECDC data on SARS-CoV-2 variants in the EU/EEA, the incidence of the alpha variant in Poland from 7 September 2020 to 31 January 2021 was 6.5%, while in the period from 8 February to 13 June 2021, the alpha variant constituted 92.4% of identified strains [10].

### *2.2. Clinical Outcomes*

Data regarding the patient characteristics (including age, sex, smoking status, and comorbidities), the course of the disease (including the need for Intensive Care Unit (ICU) admission, respiratory support, vasopressors, continuous renal replacement therapy (CRRT), complications, and outcome), and laboratory results, imaging results and blood oxygen saturation ($SpO_2$) were transcribed from electronic medical records and entered into the database after anonymization. The criteria for ICU admission were acute respiratory distress syndrome (ARDS), the need for mechanical ventilation (MV), symptoms of shock or multi-organ failure and impaired consciousness. The laboratory parameters were collected from two time points: at admission to hospital (+/−2 days, "at admission") and on the 7th day of hospitalization (+/−2 days, "7th DOH"). The patients' characteristics in the second and the third waves were compared. Moreover, to assess the role of potential predictors of disease severity, we used in-hospital death as the main outcome measure by comparing

the general and clinical characteristics of the survivors and non-survivors. Additionally, to assess the differences in disease severity, we analyzed the correlations between the patients' characteristics and the risk for MV and ICU treatment, and performed logistic regression to identify factors associated with the risk of death, MV, and ICU treatment.

### *2.3. Ethical Concerns*

The study was approved by the Ethics Committee of the Central Clinical Hospital of the Ministry of the Interior and Administration in Warsaw (decision number 110/2021, date of approval 24 August 2021) with a waiver for written informed consent due to the retrospective nature of the study and the data anonymization. The study was performed in accordance with the ethical standards of the Declaration of Helsinki and its later amendments.

### *2.4. Statistical Analysis*

As most variables were non-normally distributed, continuous variables were reported as median and interquartile range (IQR) and compared with a Mann–Whitney U-test, while categorical variables were presented as the number of patients and percentages and compared with the chi-squared test or Fisher's exact test as appropriate. For some of the quantitative variables both raw data and the groups of ranges were analyzed with the appropriate tests. The correlations between the variables and the need for MV and the ICU treatment were examined using the Spearman correlation analysis for continuous variables, and associations of the nominal variables were investigated with the chi-squared test. These analyses were performed with R software (version 4.0.4; R foundation for statistical computing, Vienna, Austria). A two-sided $p$-value $< 0.05$ was considered statistically significant. As there was no adjustment for multiple comparison, these findings should be considered as exploratory. In cases where outliers were identified, the data were also analyzed after their elimination and replacement with means to assess their impact on the results. If this led to a different result, these data are also presented.

Logistic regression analysis was performed using *Statistica* software (version 13.3; StatSoft, Poland) to determine the association of patients' characteristics and laboratory parameters and the risk of death, ICU admission, and mechanical ventilation. Non-linear data were categorized. Variables with more than 20% of missing values were not considered in this analysis, and in other cases, missing values were imputed using the Weight of Evidence tool. Univariate analysis was first performed, and significant variables obtained on admission that were significant in the univariate analysis were further included in stepwise multivariate logistic regression. ROC (receiver operating characteristic) curve analysis was conducted to assess the predictive ability of covariates and models in multivariate logistic regression. Graphical presentation of data was carried out using *Statistica* software (version 13.3).

## 3. Results

### *3.1. General Characteristics and Comparison of the Second and Third Waves*

Briefly, 229 COVID-19 patients were included in our study (172 men and 57 women), of which 75 patients were attributed to the second wave (59 men and 16 women), and 154 to the third wave (113 men and 41 women) of the COVID-19 pandemic. There was no significant difference regarding gender distribution between the two analyzed waves ($p = 0.480$). The median age in both groups was 40 years (IQR 33.5–42 years in the second wave and 35–43 years in the third wave, range 20–45 years in both waves), with no statistically significant difference between the waves. None of the patients was vaccinated against COVID-19 nor did they have documented previous SARS-CoV-2 infection. The comparison of the general and clinical patients' characteristics in the two analyzed waves is summarized in Tables 1, 2 and S1.

**Table 1.** Comparison of general patients' characteristics between the second and the third wave.

| Parameter | | Total ($n$ = 229) | Second Wave ($n$ = 75) | Third Wave ($n$ = 154) | $p$-Value |
|---|---|---|---|---|---|
| Sex ($n$ = 229) | female<br>male | 57 (24.89%)<br>172 (75.11%) | 16 (21.33%)<br>59 (78.67%) | 41 (26.62%)<br>113 (73.38%) | 0.480 * |
| Age, years ($n$ = 229) | | 40 (34–43) | 40 (33.5–42) | 40 (35–43) | 0.392 ** |
| Weight, kg ($n$ = 160) | | 100 (84–110) | 100 (90–110) | 98 (82–109)<br>(w/o median 97.81) | 0.147 **<br>(w/o 0.178) |
| BMI, kg/m$^2$ ($n$ = 154) | | 30.58 (27.07–34.33) | 31.25 (27.45–33.95)<br>(w/o median 31.02) | 29.74 (29.6–34.34) | 0.316 **<br>(w/o 0.455) |
| Comorbidities ($n$ = 229) | Comorbidities (any of the following) | 93 (40.61%) | 35 (46.67%) | 58 (37.66%) | 0.247 * |
| | Hypertension | 34 (14.85%) | 9 (12%) | 25 (16.23%) | 0.517 * |
| | Asthma | 18 (7.86%) | 8 (10.67%) | 10 (6.49%) | 0.401 * |
| | Chronic arrhythmia | 5 (2.18%) | 2 (2.67%) | 3 (1.95%) | 0.664 *** |
| | Diabetes | 18 (7.86%) | 7 (9.33%) | 11 (7.14%) | 0.752 * |
| | Insulin resistance | 6 (2.62%) | 2 (2.67%) | 4 (2.6%) | 1 *** |
| | Dyslipidemia | 6 (2.62%) | 1 (1.33%) | 5 (3.25%) | 0.667 *** |
| | Hyperthyroidism | 9 (3.93%) | 3 (4%) | 6 (3.9%) | 1 *** |
| | Hashimoto disease | 7 (3.06%) | 2 (2.67%) | 5 (3.25%) | 1 *** |

w/o: without outliers; * Chi-squared with Yates correction; ** Mann–Whitney $U$ test; *** Fisher's exact test. Continuous variables are presented as median (IQR), categorical variables are presented as $n$ (%).

**Table 2.** Comparison of clinical patients' characteristics between the second and the third wave.

| Parameter | | Total ($n$ = 229) | Second Wave ($n$ = 75) | Third Wave ($n$ = 154) | $p$-Value |
|---|---|---|---|---|---|
| Symptoms ($n$ = 229) | Dyspnea | 210 (91.7%) | 69 (92%) | 141 (91.56%) | 1 * |
| | Fever | 195 (85.15%) | 66 (88%) | 129 (83.77%) | 0.517 * |
| | Cough | 195 (85.15%) | 62 (82.67%) | 133 (86.36%) | 0.589 * |
| | Fatigue | 130 (56.77%) | 40 (53.33%) | 90 (58.44%) | 0.555 * |
| | Diarrhea | 41 (17.9%) | 9 (12%) | 32 (20.78%) | 0.149 * |
| | Nausea or vomiting | 27 (11.79%) | 10 (13.33%) | 17 (11.04%) | 0.774 * |
| | Myalgia | 67 (29.26%) | 22 (29.33%) | 45 (29.22%) | 1 * |
| | Sore throat | 18 (7.86%) | 2 (2.67%) | 16 (10.39%) | 0.076 * |
| | Headache | 42 (18.34%) | 11 (14.67%) | 31 (20.13%) | 0.412 * |
| | Smell and/or taste disorders | 43 (18.78%) | 18 (24%) | 25 (16.23%) | 0.218 * |
| | Smell disorders | 37 (16.16%) | 16 (21.33%) | 21 (13.64%) | 0.196 * |
| | Taste disorders | 37 (16.16%) | 15 (20%) | 22 (14.29%) | 0.339 *** |
| | Hemoptysis | 8 (3.49%) | 4 (5.33%) | 4 (2.6%) | 0.444 *** |
| Percentage of lung involvement on CT,% ($n$ = 207) | | 31 (20.5–45) | 30 (20–45) | 33 (25–50) | 0.319 ** |
| Lung involvement on CT $\geq$ 50% ($n$ = 207) | | 51 (24.64%) | 13 (20.31%) | 38 (26.57%) | 0.429 * |
| Death ($n$ = 229) | | 16 (6.99%) | 8 (10.67%) | 8 (5.19%) | 0.212 * |
| Conventional oxygen therapy ($n$ = 229) | | 222 (96.94%) | 73 (97.33%) | 149 (96.75%) | 1 *** |
| HFNO ($n$ = 229) | | 55 (24.02%) | 20 (26.67%) | 35 (22.73%) | 0.624 *** |
| Mechanical ventilation ($n$ = 229) | | 22 (9.61%) | 11 (14.67%) | 11 (7.14%) | 0.115 * |
| ICU admission ($n$ = 229) | | 31 (13.54%) | 15 (20%) | 16 (10.39%) | 0.074 * |
| ICU mortality ($n$ = 31) | | 15 (48.39%) | 8 (53.33%) | 7 (43.75%) | 0.862 * |

* Chi-squared with Yates correction; ** Mann–Whitney $U$ test; *** Fisher's exact test. Continuous variables are presented as median (IQR), categorical variables are presented as $n$ (%).

There were no significant differences between the second and the third wave regarding weight and BMI. It is worth noting that normal BMI (below 25 kg/m$^2$) was found in only 12.34% of patients ($n$ = 19). There were also no significant differences regarding patients' smoking status.

Ninety-three patients (40.61%) had at least one comorbidity, comprising 35 (46.67%) and 58 (37.66%) patients from the second and third waves, respectively, and this difference was not statistically significant. The most common comorbidities were hypertension (14.85%), asthma (7.86%), and diabetes (7.86%). There were no statistically significant differences regarding any of the reported comorbidities between the two waves.

The most commonly reported COVID-19 symptoms were dyspnea (91.7%), fever (85.15%), cough (85.15%), and fatigue (56.77%). Other symptoms included myalgia (29.26%), smell or taste disorders (18.78%), headache (18.34%), diarrhea (17.9%), nausea and/or emesis (11.79%), sore throat (7.86%), and hemoptysis (3.49%). There were no statistically significant differences regarding any of the reported symptoms between the two waves.

The median percentage of lung involvement on computed tomography (CT) was 31%, comprised of 30% in the second wave and 33% in the third wave ($p$ = 0.319). Lung involvement of at least 50% was found in 24.64% of the patients, comprising 20.31% in the second wave and 26.57% in the third wave ($p$ = 0.429).

The median period from the onset of symptoms to hospital admission was eight days (IQR 6–10 days) in the second wave and nine days (IQR 7–11 days) in the third wave ($p$ = 0.074). The median duration of hospitalization among survivors was significantly longer ($p$ = 0.036) in the second wave (11 days, IQR 9–16 days) compared to the third wave (10 days, IQR 8–13.25 days).

Regarding medical treatment, 227 patients (99.1%, including 100% in the second wave and 98.7% in the third wave) received low molecular-weight heparin (LMWH), 222 patients (96.9%, including 98.7% in the second wave and 96.1% in the third wave) received dexamethasone, 166 patients (72.5%, including 74.7% in the second wave and 71.4% in the third wave) received antibiotics, 59 patients (25.8%, including 25.3% in the second wave and 26% in the third wave) received remdesivir, nine patients (3.9%, including 3.9% in the second wave and 4% in the third wave) received tocilizumab, and 20 patients (8.7%, including 9.3% in the second wave and 8.4% in the third wave) received convalescent plasma, with no significant differences between two waves (Table S1).

Due to the study inclusion criteria (patients requiring hospitalization due to severe COVID-19) almost all patients required oxygen therapy (96.94% of all patients, comprising 97.33% and 96.75% of the patients from the second and third waves, respectively). Fifty-five patients (24.02%) required high flow nasal oxygen therapy (HFNO), comprising 20 (26.67%) and 35 (22.73%) patients in the second and third waves, respectively. Mechanical ventilation (MV) was necessary in 9.61% of patients, comprising 14.67% and 7.14% in the second and third waves, respectively. There were no significant differences regarding the need for conventional oxygen therapy, HFNO, MV, the overall length of oxygen therapy, maximum oxygen flow in conventional therapy, and HFNO and FiO2 in HFNO between the two analyzed waves.

Thirty-one patients (13.54%) required ICU admission, comprising 15 patients (20%) from the second wave and 16 patients (10.39%) from the third wave, with no statistically significant difference between two waves ($p$ = 0.074). The median period from the onset of symptoms to ICU admission was 10 days in both groups. There was also no significant difference regarding the need for vasopressors and CRRT. Sixteen patients (6.99%) died, comprising eight (10.67%) patients from the second wave and eight (5.19%) patients from the third wave, with no statistically significant difference between the two waves ($p$ = 0.212). There was no statistically significant difference in ICU mortality between the groups ($p$ = 0.862), with rates of 53.33% and 43.75% for the second and third waves, respectively (Tables 1, 2 and S1).

Leukocyte (white blood cell, WBC), neutrophil and platelet (PLT) counts, neutrophil and immature granulocyte (IG) percentages, the frequency of leukocytosis (defined as WBC

count > 10 × $10^3$/μL) and neutrophilia (defined as neutrophil count > 7 × $10^3$/μL), the neutrophil-to-lymphocyte ratio (NLR), prothrombin time (PT) and INR at admission were higher; and the lymphocyte percentages and activated partial thromboplastin time (APTT) at admission were lower in the second wave compared with the third wave, while these differences were not statistically significant at the 7th DOH. C-reactive protein (CRP) at the 7th DOH was significantly higher in the second wave compared with the third wave. However, this difference was not statistically significant after the removal of the outliers, and there was no significant difference between the two waves regarding CRP levels at admission. Procalcitonin (PCT) was significantly higher, while albumin and myoglobin were significantly lower in the second wave compared with the third wave at the 7th DOH, but not at admission. There were no statistically significant differences between the two waves regarding interleukin-6 (IL-6), ferritin, antithrombin III (AT III), D-Dimer at admission, fibrinogen, lactate dehydrogenase (LDH), calcium and vitamin D3 levels, nor in the levels of cardiac, renal, and liver injury markers. However, D-Dimer at the 7th DOH was significantly higher, and D-Dimer > 500 μg/L FEU at the 7th DOH was significantly more frequent in the second wave than in the third wave. There were no differences in red blood cell (RBC) and lymphocyte counts, the frequency of lymphopenia, thrombocytopenia (defined as PLT count below 150 × $10^3$/μL), and thrombophilia (defined as PLT count above 400 × $10^3$/μL), and hemoglobin and hematocrit levels (Tables 3, 4, S2 and S3).

**Table 3.** Comparison of patients' laboratory parameters (as continuous variables) between the second and the third wave.

| Parameter | Total ($n$ = 229) | Second Wave ($n$ = 75) | Third Wave ($n$ = 154) | $p$-Value * |
|---|---|---|---|---|
| WBC—at admission, ×$10^3$/μL ($n$ = 227) | 6.39 (4.53–8.31) | 7.48 (5.67–11.2) (w/o median 7) | 5.66 (4.32–7.41) | <0.001 |
| WBC—7. DOH, ×$10^3$/μL ($n$ = 187) | 8.72 (6.97–10.61) | 8.99 (7.11–10.37) | 8.68 (6.92–10.64) | 0.699 (w/o 0.691) |
| Neutrophil count—at admission, ×$10^3$/μL ($n$ = 226) | 4.8 (3.17–6.66) | 5.82 (3.93–9.2) (w/o median 5.58) | 3.98 (2.99–5.92) | <0.001 |
| Neutrophil count—7. DOH, ×$10^3$/μL ($n$ = 187) | 5.5 (4.2–7.36) | 5.79 (4.44–7.25) | 5.3 (4.11–7.43) | 0.479 (w/o 0.341) |
| Neutrophil percentage—at admission, % ($n$ = 226) | 77.1 (66.85–83.05) | 79.8 (69.7–85.8) | 75.7 (65.85–81.05) | 0.011 (w/o 0.016) |
| Neutrophil percentage—7. DOH, % ($n$ = 186) | 62.7 (54.3–73.28) | 63.5 (56.8–71.4) | 62.4 (53.9–73.5) | 0.574 |
| Lymphocyte count—at admission, ×$10^3$/μL ($n$ = 226) | 0.94 (0.69–1.24) | 0.93 (0.68–1.25) | 0.94 (0.7–1.24) | 0.700 (w/o 0.690) |
| Lymphocyte count—7. DOH, ×$10^3$/μL ($n$ = 186) | 1.98 (1.31–2.72) | 1.87 (1.3–2.65) | 2.05 (1.35–2.83) (w/o median 2.04) | 0.359 (w/o 0.446) |
| Lymphocyte percentage—at admission, % ($n$ = 226) | 15.45 (10.3–23.23) | 11.8 (8.1–21.35) | 17.2 (12–24.4) (w/o median 17.1) | 0.004 (w/o 0.003) |
| Lymphocyte percentage—7. DOH, % ($n$ = 186) | 23.95 (14.75–32.28) | 21.7 (12.5–30.3) | 24.4 (16.1–33.1) (w/o median 24.3) | 0.172 (w/o 0.198) |
| NLR—at admission ($n$ = 226) | 5.04 (2.85–8.23) | 6.59 (3.31–10.25) (w/o median 6.23) | 4.36 (2.72–6.69) | 0.005 (w/o 0.010) |
| NLR—7. DOH ($n$ = 186) | 2.66 (1.7–4.93) | 2.88 (1.81–6.38) | 2.55 (1.6–4.68) | 0.275 (w/o 0.209) |

**Table 3.** *Cont.*

| Parameter | Total ($n$ = 229) | Second Wave ($n$ = 75) | Third Wave ($n$ = 154) | $p$-Value * |
|---|---|---|---|---|
| IG count—at admission, $\times 10^3/\mu L$ ($n$ = 217) | 0.03 (0.02–0.07) | 0.05 (0.03–0.1) | 0.03 (0.02–0.05) | 0.001 |
| IG count—7. DOH, $\times 10^3/\mu L$ ($n$ = 186) | 0.20 (0.09–0.39) | 0.24 (0.08–0.41) | 0.16 (0.09–0.39) | 0.301 (w/o 0.468) |
| IG percentage—at admission, % ($n$ = 217) | 0.6 (0.4–0.9) | 0.6 (0.5–1.1) | 0.5 (0.4–0.8) | 0.027 (w/o 0.020) |
| IG percentage—7. DOH, % ($n$ = 186) | 2.2 (1.1–4.05) | 2.9 (1.1–4.2) (w/o median2.79) | 2.1 (1.1–3.9) | 0.221 (w/o 0.406) |
| PLT—at admission, $\times 10^3/\mu L$ ($n$ = 227) | 217 (164.5–282.5) | 237 (196–307.5) (w/o median 236) | 203 (157.75–168.5) | 0.009 (w/o 0.011) |
| PLT—7. DOH, $\times 10^3/\mu L$ ($n$ = 187) | 396 (326.5–473.5) | 394 (326.25–460.5) | 400 (339–477) (w/o median 402) | 0.534 (w/o 0.374) |
| CRP—at admission, mg/L ($n$ = 222) | 73.2 (35.8–132.5) | 87.2 (45–144.95) | 63.5 (34.75–121) | 0.074 (w/o 0.118) |
| CRP—7. DOH, mg/L, ($n$ = 187) | 8.8 (4.2–28.5) | 12.7 (5.35–50.7) | 7.55 (3.83–19.9) | 0.049 (w/o 0.077) |
| PCT—at admission, ng/mL ($n$ = 205) | 0.12 (0.07–0.2) | 0.14 (0.08–0.23) | 0.11 (0.07–0.19) | 0.088 (w/o 0.082) |
| PCT—7. DOH ($n$ = 102) | 0.08 (0.05–0.17) | 0.14 (0.06–0.27) | 0.08 (0.05–0.12) | 0.023 |
| D-Dimer—at admission, µg/L FEU ($n$ = 206) | 728 (503–1136.5) | 797.5 (566.5–1195.75) | 710 (480.5–1118.75) | 0.156 |
| D-Dimer—7. DOH, µg/L FEU, $n$ (%) ($n$ = 150) | 867 (539.5–1556.5) | 1399 (823–2763) | 793 (512–1238) | 0.001 |
| PT—at admission, s ($n$ = 198) | 13.2 (12.3–13.8) | 13.4 (12.73–14.68) (w/o 13.35) | 13 (11.98–13.7) | 0.010 w/o 0.018) |
| PT—7. DOH, s ($n$ = 80) | 12.2 (11.7–13.03) | 12.5 (12–13.6) | 12.1 (11.6–12.7) | 0.065 (w/o 0.270) |
| INR—at admission, s ($n$ = 199) | 1.19 (1.1–1.25) | 1.22 (1.15–1.33) | 1.17 (1.08–1.22) | 0.003 (w/o 0.006) |
| INR—7. DOH ($n$ = 80) | 1.11 (1.06–1.18) | 1.12 (1.09–1.22) | 1.1 (1.05–1.16) | 0.082 (w/o 0.060) |
| APTT—at admission, s ($n$ = 196) | 33 (29.78–37.13) | 31.7 (28.9–36.4) | 33.4 (30.45–37.25) | 0.039 (w/o 0.042) |
| APTT—7. DOH, s ($n$ = 65) | 32.1 (28.2–36.6) | 32.5 (28.5–36.4) | 31.95 (27.78–38.7) | 0.944 |
| Albumin—at admission, g/dL ($n$ = 26) | 3.34 (3.18–3.65) | 3.22 (3.02–3.65) | 3.42 (3.27–3.57) | 0.315 |
| Albumin—7. DOH, g/dL ($n$ = 19) | 3.03 (2.74–3.38) | 2.77 (2.65–2.98) | 3.38 (3.11–3.42) | 0.007 |

* Mann–Whitney $U$ test; w/o: without outliers. All variables are presented as median (IQR).

**Table 4.** Comparison of general patients' characteristics between survivors and non-survivors.

| Parameter | | Survivors ($n$ = 213) | Non-Survivors ($n$ = 16) | $p$-Value |
|---|---|---|---|---|
| Sex ($n$ = 229) | female<br>male | 51 (23.94%)<br>162 (76.06%) | 6 (37.5%)<br>10 (62.5%) | 0.236 * |
| Age, years ($n$ = 229) | | 40 (34–43) | 41.5 (39.5–43) | 0.152 ** |
| Weight, kg ($n$ = 160) | | 98 (83–110)<br>(w/o median 97.81) | 105 (98–123.5)<br>(w/o median 104) | 0.026 ** (w/o 0.063) |
| BMI, kg/m$^2$ ($n$ = 154) | | 29.74 (26.46–33.95) | 34.26 (29.59–38.73)<br>(w/o median 33.06) | 0.019 ** (w/o 0.097) |
| BMI, ranges ($n$ = 154) | <25 | 18 (12.95%) | 1 (6.67%) | 0.696 * |
| | 25–29.9 | 52 (37.41%) | 3 (20%) | 0.292 * |
| | 30–34.9 | 41 (29.5%) | 5 (33.33%) | 0.771 * |
| | 35–39.9 | 20 (14.39%) | 3 (20%) | 0.472 * |
| | ≥40 | 8 (5.76%) | 3 (20%) | 0.077 *** |
| Comorbidities ($n$ = 229) | Comorbidities (any of the following) | 82 (38.5%) | 11 (68.75%) | 0.035 * |
| | Hypertension | 30 (14.08%) | 4 (25%) | 0.268 * |
| | Asthma | 17 (7.98%) | 1 (6.25%) | 1 * |
| | Chronic arrhythmia | 3 (1.41%) | 2 (12.5%) | 0.041 *** |
| | Diabetes | 15 (7.04%) | 3 (18.75%) | 0.199 * |
| | Insulin resistance | 4 (1.88%) | 2 (12.5%) | 0.058 *** |
| | Dyslipidemia | 5 (2.35%) | 1 (6.25%) | 0.356 *** |
| | Hypothyroidism | 8 (3.76%) | 1 (6.25%) | 0.485 *** |
| | Hashimoto disease | 7 (3.29%) | 0 (0%) | 1 *** |
| Smoking ($n$ = 164) | Current | 11 (7.14%) | 2 (20%) | 0.182 *** |
| | Current or former | 16 (10.39%) | 4 (40%) | 0.021 * |
| Blood type ($n$ = 62) | A Rh+ | 12 (25%) | 6 (42.86%) | 0.315 * |
| | A Rh− | 3(6.25%) | 0 (0%) | 1 *** |
| | B Rh+ | 11 (22.92%) | 1 (7.14%) | 0.267 * |
| | B Rh− | 2 (4.17%) | 2 (14.29%) | 0.217 *** |
| | AB Rh+ | 2 (4.17%) | 0 (0%) | 1 *** |
| | AB Rh− | 2 (4.17%) | 0 (0%) | 1 *** |
| | 0 Rh+ | 15 (31.25%) | 5 (35.71%) | 0.755 * |
| | 0 Rh− | 1 (2.08%) | 0 (0%) | 1 *** |

w/o: without outliers; * Chi-squared with Yates correction; ** Mann–Whitney $U$ test; *** Fisher's exact test. Continuous variables are presented as median (IQR), categorical variables are presented as $n$ (%).

### *3.2. Factors Associated with Poor Prognosis*

A comparison of the general, clinical, and laboratory characteristics between the survivors and non-survivors is presented in Tables 4, 5, 6, and S4 and Figures 1 and 2.

**Table 5.** Comparison of clinical patients' characteristics between survivors and non-survivors.

| Parameter | Survivors ($n$ = 213) | Non-Survivors ($n$ = 16) | $p$-Value |
|---|---|---|---|
| Percentage of lung involvement on CT, % ($n$ = 207) | 30 (20–44.25) | 70 (40–85) | <0.001 ** |
| Lung involvement on CT ≥ 50% ($n$ = 207) | 42 (21.65%) | 9 (69.23%) | 0.001 * |
| $SpO_2$ at admission, % ($n$ = 190) | 90 (87–92) | 85 (70–89) | 0.004 ** |
| Time from the onset of symptoms to hospital admission, days | 8 (7–11) | 7 (5.5–9) | 0.048 ** |
| Conventional oxygen therapy ($n$ = 229) | 206 (96.71%) | 16 (100%) | 1 *** |
| Maximum oxygen flow (conventional oxygen therapy), l/min ($n$ = 214) | 6.5 (5–15) | 15 (15–15) | <0.001 ** |
| HFNO ($n$ = 229) | 41 (19.25%) | 14 (87.5%) | <0.001 *** |
| Maximum flow—HFNO (l/min; ($n$ = 55) | 60 (55–60) | 60 (60–78.75) | 0.003 ** |
| Maximum FiO2—HFNO,% ($n$ = 55) | 90 (78–95) | 95 (90.5–98.25) | 0.014 ** |
| Mechanical ventilation ($n$ = 229) | 6 (2.82%) | 16 (100%) | <0.001 * |
| Extubation; ($n$ = 22) | 6 (100%) | 0 (0%) | <0.001 *** |
| ECMO ($n$ = 229) | 6 (2.82%) | 2 (12.5%) | 0.100 *** |
| ICU admission ($n$ = 229) | 16 (7.51%) | 15 (93.75%) | <0.001 * |
| Time from the onset of symptoms to ICU admission, days | 10 (8.75–13) | 10 (8.5–13) | 1 ** |
| Vasopressors ($n$ = 229) | 6 (2.82%) | 15 (93.75%) | <0.001 * |
| CRRT ($n$ = 229) | 0 (0%) | 5 (31.25%) | <0.001 *** |

* Chi-squared with Yates correction; ** Mann–Whitney $U$ test; *** Fisher's exact test. Continuous variables are presented as median (IQR), categorical variables are presented as $n$ (%).

**Table 6.** Comparison of patients' laboratory parameters (as continuous variables) between survivors and non-survivors.

| Parameter | Survivors ($n$ = 213) | Non-Survivors ($n$ = 16) | $p$-Value * |
|---|---|---|---|
| **Inflammatory Markers** | | | |
| WBC—at admission, $\times 10^3/\mu L$ ($n$ = 227) | 6.12 (4.5–8.1) | 9.43 (6.95–12.99) | 0.001 |
| WBC—7. DOH, $\times 10^3/\mu L$ ($n$ = 187) | 8.66 (6.84–10.2) | 13.33 (10.1–20.67) | <0.001 |
| Neutrophil count—at admission, $\times 10^3/\mu L$ ($n$ = 226) | 4.46 (3.12–6.39) | 7.69 (5.21–11.58) | 0.001 |
| Neutrophil count—7. DOH, $\times 10^3/\mu L$ ($n$ = 187) | 5.35 (4.11–6.99) | 11.69 (7.73–13.64) | <0.001 |
| Neutrophil percentage—at admission, $n$(%) ($n$ = 226) | 76.9 (66.33–82) | 82.65 (76.88–87.28) | 0.007 |
| Neutrophil percentage—7. DOH,% ($n$ = 186) | 61.9 (53.9–70.3) | 84.5 (74.2–86.2) | <0.001 |
| Lymphocyte count—at admission, $\times 10^3/\mu L$ ($n$ = 226) | 0.93 (0.69–1.24) | 0.97 (0.72–1.25) | 0.896 |
| Lymphocyte count—7. DOH, $\times 10^3/\mu L$ ($n$ = 186) | 2.07 (1.37–2.79) | 0.9 (0.76–1.49) | <0.001 |
| Lymphocyte percentage—at admission,% ($n$ = 226) | 16.7 (10.4–23.95) | 11.35 (7.38–12.3) | 0.001 |
| Lymphocyte percentage—7. DOH, % ($n$ = 186) | 24.4 (17.5–32.8) | 6.9 (6.4–9) | <0.001 |
| NLR—at admission ($n$ = 226) | 4.49 (2.77–7.83) | 7.11 (6.52–11.33) | 0.002 |
| NLR—7. DOH ($n$ = 186) | 2.52 (1.66–4.16) | 11.58 (7.42–13.1) | <0.001 |
| IG count—at admission, $\times 10^3/\mu L$ ($n$ = 217) | 0.03 (0.02–0.06) | 0.09 (0.06–0.14) | <0.001 |
| IG count—7. DOH, $\times 10^3/\mu L$ ($n$ = 186) | 0.18 (0.08–0.37) | 0.64 (0.33–0.76) | 0.001 |
| IG percentage—at admission,% ($n$ = 217) | 0.5 (0.4–0.8) | 1.05 (0.7–1.3) | <0.001 |
| IG percentage—7. DOH,% ($n$ = 186) | 2.2 (1–3.8) | 4.9 (2.6–9.1) | 0.009 |
| CRP—at admission, mg/L ($n$ = 222) | 70.5 (34.65–126.4) | 145.6 (82.11–161.7) | 0.006 |

**Table 6.** *Cont.*

| Parameter | Survivors ($n$ = 213) | Non-Survivors ($n$ = 16) | $p$-Value * |
|---|---|---|---|
| CRP—7. DOH, mg/L, ($n$ = 187) | 7.6 (3.85–18.6) | 91.75 (55.73–127.75) | <0.001 |
| PCT—at admission, ng/mL ($n$ = 205) | 0.11 (0.07–0.18) | 0.39 (0.18–1.22) | <0.001 |
| PCT—7. DOH, ng/mL ($n$ = 102) | 0.07 (0.05–0.13) | 0.61 (0.29–0.74) | <0.001 |
| Ferritin—at admission, ng/mL ($n$ = 61) | 1156 (473–1498) | 2128 (1604–2716.75) | 0.088 |
| Ferritin—7. DOH, ng/mL ($n$ = 31) | 901.5 (430–1343.25) | 1455 (1455–1455) | – |
| IL-6—at admission, ng/mL ($n$ = 116) | 18.5 (6.72–53.78) | 106.4 (69.43–160.75) | 0.015 |
| IL-6—7. DOH, ng/mL ($n$ = 53) | 7.28 (4.01–19.1) | 65.7 (24.35–741.5) | 0.041 |
| AT III—at admission,% ($n$ = 17) | 94.5 (85–100.25) | 95 (80.5–117) | 0.732 |
| AT III—7. DOH,% ($n$ = 10) | 112 (101.5–117.5) | 91 (84.5–95.5) | 0.252 |
| **Coagulation Parameters** | | | |
| PLT—at admission, $\times 10^3/\mu L$ ($n$ = 227) | 217 (161–280.5) | 225 (194–322.75) | 0.253 |
| PLT—7. DOH, $\times 10^3/\mu L$ ($n$ = 187) | 398 (339.25–473.75) | 353 (240–471) | 0.334 |
| D-Dimer—at admission, μg/L FEU ($n$ = 206) | 712 (487–1123) | 991 (728–1593.5) | 0.015 |
| D-Dimer—7. DOH, μg/L FEU ($n$ = 150) | 823 (531–1363.25) | 1810.5 (1346.75–5268) | <0.001 |
| PT—at admission, s ($n$ = 198) | 13.2 (12.3–13.8) | 12.9 (11.98–14) | 0.767 |
| PT—7. DOH, s ($n$ = 80) | 12.2 (11.7–13.1) | 12.5 (12.05–13) | 0.695 |
| INR—at admission ($n$ = 199) | 1.19 (1.11–1.25) | 1.14 (1.07–1.27) | 0.541 |
| INR—7. DOH ($n$ = 80) | 1.11 (1.06–1.19) | 1.12 (1.1–1.17) | 0.883 |
| APTT—at admission, s ($n$ = 196) | 33.25 (30.2–37.35) | 29.25 (27.28–32.45) | 0.012 |
| APTT—7. DOH, s ($n$ = 65) | 32.1 (28.6–35.7) | 34.1 (25.88–46.73) | 0.750 |
| Fibrinogen—at admission, mg/dL ($n$ = 62) | 598 (501.75–737.75) | 531 (392.5–673.75) | 0.255 |
| Fibrinogen—7. DOH, mg/dL ($n$ = 39) | 481.5 (385.5–553.5) | 613 (410–702) | 0.279 |
| RED BLOOD CELL INDICES | | | |
| RBC count—at admission, $\times 10^6/\mu L$ ($n$ = 227) | 4.78 (4.53–5.05) | 4.95 (4.7–5.08) | 0.269 |
| RBC count—7. DOH, $\times 10^6/\mu L$ ($n$ = 187) | 4.77 (4.5–5.06) | 3.85 (3.43–4.12) | <0.001 |
| Hemoglobin—at admission, g/dL ($n$ = 227_ | 14.3 (13.5–15.1) | 14.55 (13.15–15.35) | 0.699 |
| Hemoglobin—7. DOH, g/dL ($n$ = 187) | 14.2 (13.2–15.18) | 11.6 (9.4–12.1) | <0.001 |
| Hematocrit—at admission,% ($n$ = 227) | 41.5 (39.35–44) | 43.45 (39.68–44.13) | 0.292 |
| Hematocrit—7. DOH,% ($n$ = 187) | 41.95 (39.4–44) | 35 (30–36.6) | <0.001 |
| **Non-Specific Tissue Damage and Cardiac Injury Markers** | | | |
| LDH—at admission, U/L ($n$ = 172) | 396 (310–533) | 783 (591–1257) | <0.001 |
| LDH—7. DOH, U/L ($n$ = 46) | 387 (250–442) | 865 (865–865) | – |
| Myoglobin—at admission, ng/mL ($n$ = 15) | 60 (49–118) | 2687.5 (721.75–3087.25) | 0.039 |
| Myoglobin—7. DOH, ng/mL ($n$ = 11) | 28 (23.5–76.5) | 258.5 (124–300) | 0.085 |
| CK—at admission, U/L ($n$ = 142) | 240 (114–422.25) | 426 (97.25–1860.25) | 0.236 |
| CK—7. DOH, U/L ($n$ = 38) | 42.5 (26–101.5) | 203.5 (195–261) | 0.004 |
| CK-MB—at admission, U/L ($n$ = 124) | 18 (15–24) | 23 (18.85.36.5) | 0.022 |
| CK-MB—7. DOH, U/L ($n$ = 27) | 14 (12–30) | 22 (18–30.75) | 0.159 |
| NT-proBNP—at admission, pg/mL ($n$ = 142) | 98 (40.5–175.5) | 322 (92–1068) | 0.012 |

**Table 6.** *Cont.*

| Parameter | Survivors (*n* = 213) | Non-Survivors (*n* = 16) | *p*-Value * |
|---|---|---|---|
| NT-proBNP—7. DOH, pg/mL (*n* = 42) | 111.5 (47.75–207.75) | 429 (205.75–3615.5) | 0.001 |
| hs-TnI—at admission, pg/mL (*n* = 153) | 3.25 (3.2–6.38) | 12.5 (47–23.9) | 0.001 |
| hs-TnI—7. DOH, pg/mL (*n* = 40) | 3.2 (1.2–3.2) | 26.7 (17–165.15) | 0.003 |
| **Renal Injury Markers** | | | |
| Creatinine—at admission, mg/dL (*n* = 222) | 0.9 (0.76–1.03) | 0.99 (0.84–1.27) | 0.036 |
| Creatinine—7. DOH, mg/dL (*n* = 169) | 0.81 (0.72–0.91) | 0.88 (0.55–1.96) | 0.616 |
| EGFR—at admission, mL/min (*n* = 215) | 91 (78–103.5) | 79.5 (52.75–91.75) | 0.011 |
| EGFR—7. DOH, mL/min (*n* = 169) | 102 (89–117.5) | 98 (43.5–139.25) | 0.572 |
| Urea—at admission, mg/dL (*n* = 209) | 27 (21.25–34.75) | 37 (27.85–48.5) | 0.011 |
| Urea—7. DOH, mg/dL (*n* = 124) | 32 (27–37) | 52 (42–81.75) | <0.001 |
| **Liver Injury Markers** | | | |
| ALT—at admission, U/L (*n* = 226) | 47 (33–68) | 49.5 (27–88.75) | 0.758 |
| ALT—7. DOH, U/L (*n* = 150) | 100 (62–154) | 42 (28.5–47.5) | 0.001 |
| AST—at admission, U/L (*n* = 219) | 48 (34.5–70) | 53 (39.75–145.5) | 0.185 |
| AST—7. DOH, U/L (*n* = 148) | 46 (30–71) | 37 (25–44) | 0.149 |
| GGT—at admission, U/L (*n* = 58) | 58 (36.5–137.5) | 136 (71–250.5) | 0.076 |
| GGT—7. DOH, U/L (*n* = 41) | 94 (58–191.75) | 158 (105–184) | 0.690 |
| Total bilirubin—at admission, mg/dL (*n* = 165) | 0.43 (0.31–0.54) | 0.58 (0.37–0.99) | 0.055 |
| Total bilirubin—7. DOH, mg/dL (*n* = 63) | 0.44 (0.31–0.6) | 0.3 (0.25–0.52) | 0.211 |
| **Other Laboratory Parameters** | | | |
| Albumin—at admission, g/dL (*n* = 26) | 3.46 (3.15–3.82) | 3.22 (3.19–3.28) | 0.211 |
| Albumin—7. DOH, g/dL (*n* = 19) | 3.36 (3.06–3.44) | 2.74 (2.67–2.9) | 0.004 |
| Total calcium—at admission, mmol/L (*n* = 42) | 2.16 (2.06–2.24) | 2.05 (2.02–2.09) | 0.049 |
| Total calcium—7. DOH, mmol/L (*n* = 34) | 2.27 (2.2–2.32) | 2.08 (1.99–2.17) | <0.001 |
| Vitamin D3—at admission, ng/mL (*n* = 81) | 27.7 (19.85–33.85) | 18.75 (15.38–25.13) | 0.173 |
| Vitamin D3—7. DOH, ng/mL (*n* = 6) | 22.45 (16.7–35.4) | – | – |

w/o: without outliers; * Mann–Whitney *U* test. All variables are presented as median (IQR).

There were no significant age ($p$ = 0.152) and sex ($p$ = 0.236) differences between the survivors and non-survivors. There were also no differences between these groups regarding the frequencies of any of the blood types. Weight and BMI were higher in the non-survivors compared to survivors. However, these differences were not significant after the removal of the outliers.

Comorbidities were more frequent in the non-survivors compared to survivors (68.75% vs. 38.5%, $p$ = 0.035). Moreover, chronic arrhythmia was significantly more frequent in the non-survivors than in survivors, while there were no statistically significant differences in the frequency of insulin resistance, diabetes, hypertension, asthma, hypothyroidism, or Hashimoto disease. A history of current or former smoking was significantly more frequent among non-survivors than survivors, while there was no significant difference between these groups regarding current smoking status.

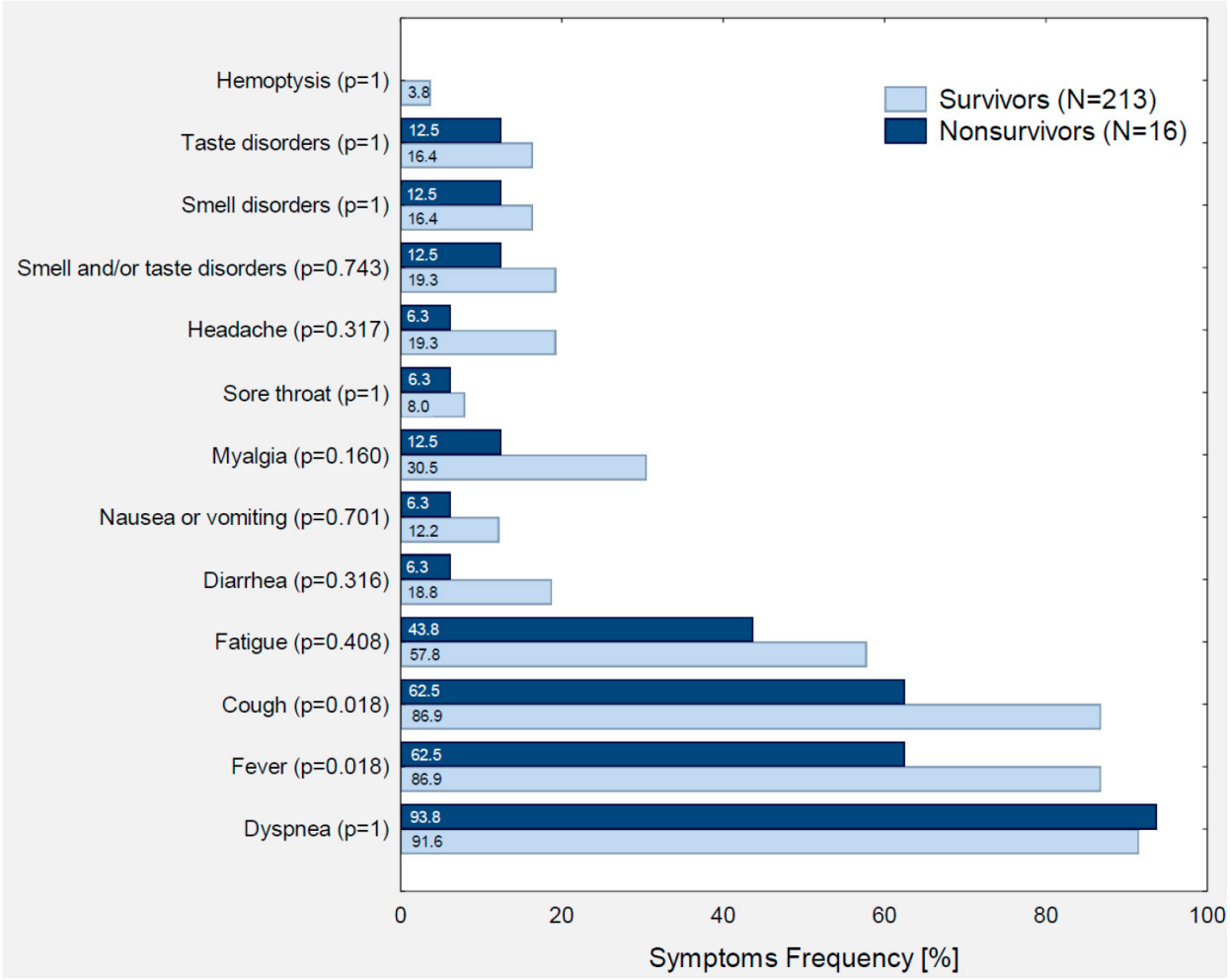


**Figure 1.** Comparison of symptoms frequency between survivors and non-survivors (presented as percentages, with *p*-values for each comparison).

Regarding the reported symptoms, fever and cough were significantly more frequent among the survivors than non-survivors. However, this may be due to the poorer availability of data on baseline symptoms in patients who were at the critical stage of COVID-19 at admission and should be interpreted with caution. There were no significant differences regarding other initial symptoms, such as dyspnea, fatigue, diarrhea, nausea and vomiting, myalgia, sore throat, headache, smell and/or taste disorders, and hemoptysis.

The percentage of lung involvement on CT was significantly higher and lung involvement of at least 50% was more frequent in the non-survivors than in survivors.

$SpO_2$ at admission was significantly lower and the maximum oxygen flow needed in conventional oxygen therapy was significantly higher in the non-survivors than in survivors. Non-survivors required HFNO, MV, ICU admission, vasopressors, and CRRT more frequently than survivors.

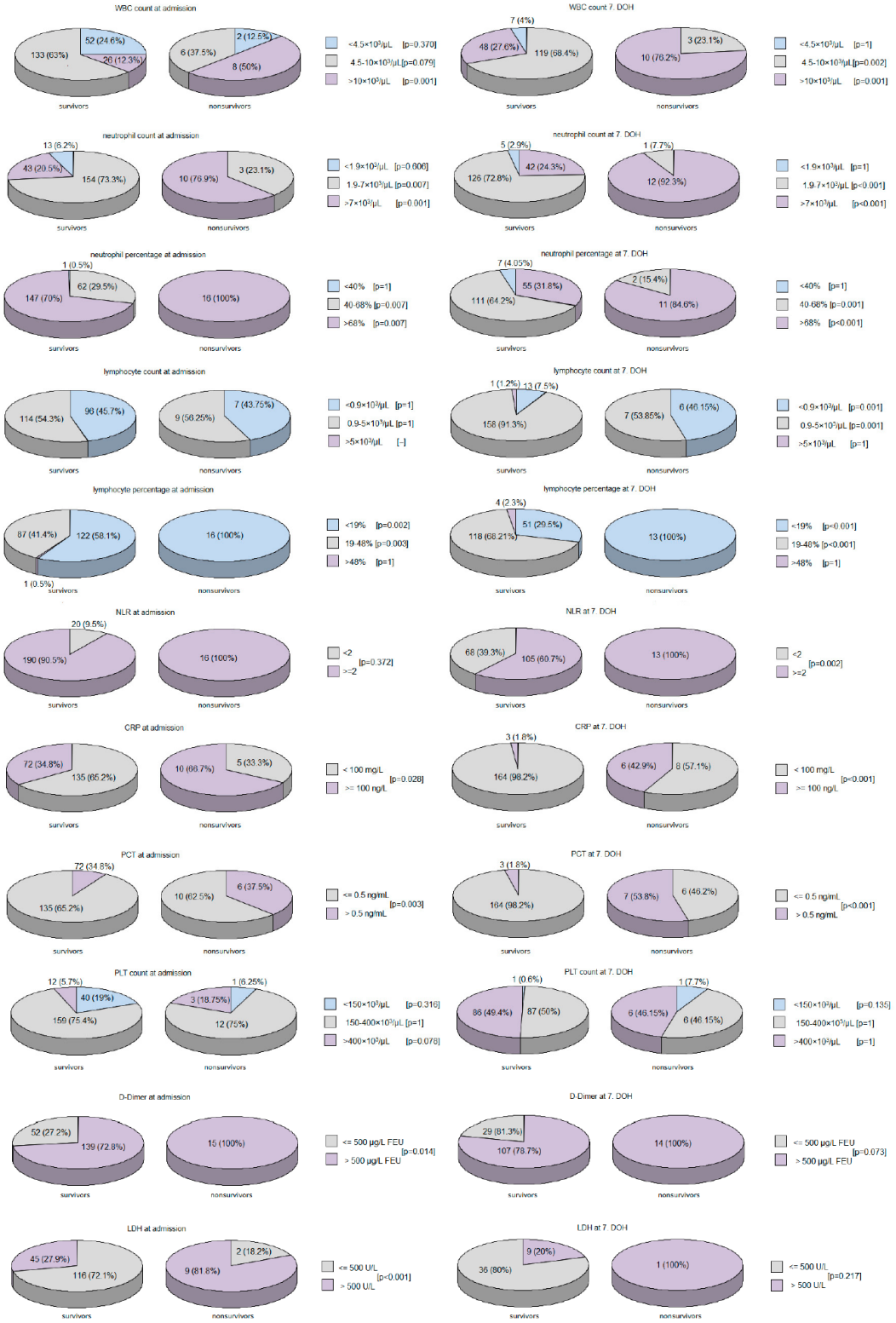


**Figure 2.** Comparison of patients' laboratory parameters (as categorical variables) between survivors and non-survivors. Variables are presented as the number of patients and percentages and compared

with the chi-squared test or Fisher's exact test as appropriate. *p*-values are presented for each comparison. A two-sided *p*-value < 0.05 was considered statistically significant. Leukocytosis and NLR ≥ 2 at 7. DOH, and neutrophilia, lymphopenia, CRP ≥ 100 mg/L and PCT > 0.5 ng/mL at admission and at 7. DOH were found more frequently in non-survivors compared to survivors, indicating a hyperinflammatory reaction. Moreover, a higher prevalence of D-Dimer levels greater than 500 μg/L FEU at admission may indicate hypercoagulability, while a higher prevalence of LDH levels above 500 U/L at admission may reflect more pronounced tissue damage in non-survivors.

WBC count, neutrophil count, neutrophil percentage, NLR, IG count, and IG percentage were significantly higher, leukocytosis and neutrophilia were more frequent, and the lymphocyte percentage was lower in the non-survivors than in survivors, both at admission and at the 7th DOH. The lymphocyte count was significantly lower, and lymphopenia (defined as a lymphocyte count below $0.9 \times 10^3/\mu L$) and NLR of at least 2 were significantly more frequent in the non-survivors than in survivors at the 7th DOH, but not at admission

CRP, PCT, and IL-6 levels were significantly higher, and CRP > 100 mg/L and PCT > 0.5 ng/mL were significantly more frequent in non-survivors compared to survivors at admission and at the 7th DOH. There were no significant differences between non-survivors and survivors regarding ferritin and AT III levels.

D-Dimer at admission and at the 7th DOH was significantly higher and D-Dimer > 500 μg/L FEU at admission was significantly more frequent in non-survivors compared to survivors. APTT at admission, but not at the 7th DOH, was significantly longer in survivors compared to non-survivors, while there were no significant differences between these groups regarding the PLT count, PT, and fibrinogen. LDH levels at admission were significantly higher and LDH > 500 U/L at admission was significantly more frequent in non-survivors than in survivors.

There were no significant differences regarding RBC counts and hematocrit and hemoglobin levels at admission. However, at the 7th DOH these parameters were significantly lower in the non-survivors than in survivors.

High-sensitive troponin I (hs-TnI) and N-terminal-pro-B-type natriuretic peptide (NT-proBNP) levels were significantly higher in non-survivors than in survivors at admission and at the 7th DOH. The creatine kinase-myocardial band (CK-MB) and myoglobin levels were significantly higher in non-survivors than in survivors at admission, while at the 7th DOH these differences were not significant. The creatine kinase (CK) levels at the 7th DOH were significantly higher in non-survivors than in survivors, while this difference was not significant at admission.

Urea levels were significantly higher in non-survivors than in survivors at admission and at the 7th DOH, while creatinine levels were significantly higher, and the estimated glomerular filtration rate (EGFR) was significantly lower in non-survivors than in survivors at admission, but not at the 7th DOH.

Alanine aminotransferase (ALT) levels at the 7th DOH, but not at admission, were significantly higher in survivors compared with non-survivors. However, there were no other statistically significant differences between these groups regarding liver injury markers, such as aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and/or total bilirubin levels.

Albumin concentrations were significantly lower in non-survivors than in survivors at the 7th DOH, while this difference was not significant at admission.

Total calcium concentrations were significantly lower in non-survivors than in survivors at admission and at the 7th DOH, while vitamin D3 levels were significantly lower in non-survivors than in survivors at admission, but not at the 7th DOH.

#### 3.2.1. Correlations between Patients' Characteristics and the Need for MV and ICU Treatment

Correlations between patients' characteristics and the need for MV and ICU treatment are presented in Tables S5–S7.

There was a weak association between weight, BMI, BMI above 40 kg/m$^2$, and comorbidities and the need for MV. There were also weak associations between diabetes and smoking and the need for MV and ICU treatment. There was a positive correlation between the percentage of lung involvement on CT and the maximum oxygen flow in conventional oxygen therapy and a negative correlation between $SpO_2$ at admission, and the need for MV and ICU treatment. The need for HFNO, ICU treatment, vasopressors, and CRRT were associated with the need for MV, and the need for HFNO, MV, vasopressors, and CRRT were associated with the need for ICU treatment.

WBC, neutrophil and IG counts, neutrophil percentage, NLR, CRP, PCT, and IL-6 levels were positively correlated with the need for MV and ICU treatment, and there was an association between leukocytosis and neutrophilia, CRP > 100 mg/L and PCT > 0.5 ng/mL (at admission and at the 7th DOH) and the need for MV and ICU treatment. There was also a weak negative correlation between the lymphocyte count at the 7th DOH and the need for MV and ICU treatment, and an association of lymphopenia at the 7th DOH and the need for MV and ICU treatment. Ferritin levels at admission were positively correlated with the need for MV, while ferritin levels at the 7th DOH—with the need for ICU treatment. RBC counts, hematocrit and hemoglobin levels at the 7th DOH were negatively correlated with the need for MV and ICU treatment.

PLT count and thrombophilia at admission were positively correlated with the need for MV and ICU treatment, and thrombocytopenia at admission with the need for ICU treatment. There was a positive correlation between D-Dimer, and an association of D-Dimer > 500 μg/L FEU (at admission and at the 7th DOH), and the need for MV and ICU treatment.

There was a positive correlation between LDH levels (at admission and at the 7th DOH), and an association of LDH > 500 U/L at admission and the need for MV and ICU treatment. The need for MV and ICU treatment were also correlated positively with the hsTnI and NT-pro-BNP levels at admission and at the 7th DOH, CK-MB and myoglobin levels at admission, and CK levels at the 7th DOH, and there was a positive correlation between CK levels at admission and the need for ICU treatment, but not MV. Urea levels were correlated positively with the need for MV and ICU treatment, and creatinine levels at admission with the need for MV. There was a moderate to strong negative correlation between albumin and calcium concentrations (at admission and at the 7th DOH) and the need for MV and ICU treatment, and between vitamin D3 levels at the 7th DOH and the need for ICU treatment.

#### 3.2.2. Predictors of Death in Logistic Regression

Univariate logistic regression revealed that weight > 100 kg, BMI ≥ 40 kg/m$^2$, comorbidities, diabetes or insulin resistance, chronic arrhythmia, CK-MB > 20 U/L at admission and at the 7th DOH, CK > 190 U/L at the 7th DOH, D-Dimer > 500 μg/L FEU at admission and at the 7th DOH, EGFR < 60 mL/min at admission and at the 7th DOH, GGT > 120 U/L at admission and at the 7th DOH, hematocrit < 40% at the 7th DOH, hemoglobin < 12 g/dL at the 7th DOH, creatinine > 1.2 mg/dL at admission and at the 7th DOH, LDH > 500 U/L at admission, urea > 49 mg/dL at the 7th DOH, NT-proBNP > 190 pg/mL at admission and at the 7th DOH, RBC count < 4.5 × $10^6$/μL at the 7th DOH, hsTnI > 34 pg/mL at the 7th DOH, total calcium < 2.1 mmol/L at admission and at the 7th DOH, NLR ≥ 2 at the 7th DOH, lymphocyte count < 0.9 × $10^3$/μL at the 7th DOH, lymphocyte percentage < 19% at the 7th DOH, neutrophil count > 7 × $10^3$/μL at admission and at the 7th DOH, neutrophil percentage > 68% at the 7th DOH, WBC count > 10 × $10^3$/μL at admission and at the 7th DOH, CRP ≥ 100 mg/L at admission and at the 7th DOH > 100, and PCT > 0.5 ng/mL at admission and at the 7th DOH, were associated with increased risk of death (Tables 7 and 8). Comorbidities, WBC count > 10 ×$10^3$/μL, and PCT > 0.5 ng/mL were associated with death in multivariate analysis for variables obtained at admission (Table 8).

**Table 7.** Univariate logistic regression analysis of selected laboratory parameters at the 7th DOH for the prediction of death, mechanical ventilation, and ICU treatment.

| Variable | Death | | | MV | | | ICU Treatment | | |
|---|---|---|---|---|---|---|---|---|---|
| | OR | 95% CI | *p* | OR | 95% CI | *p* | OR | 95% CI | *p* |
| CK-MB > 20—7. DOH | 26.83 | 7.47–96.23 | <0.001 | 23.09 | 6.67–79.9 | <0.001 | 19.84 | 5.64–69.78 | <0.001 |
| CK > 190—7. DOH | 70 | 15.57–314.86 | <0.001 | 70.96 | 13.88–362.73 | <0.001 | 40.09 | 8.14–197.45 | <0.001 |
| D-Dimer > 500 µg/L FEU—7. DOH | 6.94 | 1.54–31.26 | 0.012 | 10.5 | 2.39–46.05 | 0.002 | 10.54 | 3.1–35.8 | <0.001 |
| EGFR < 60 mL/min—7. DOH | 35.17 | 5.85–211.54 | <0.001 | 60.59 | 6.69–548.62 | <0.001 | 37.89 | 4.26–337.05 | 0.001 |
| GGT > 120 U/L—7. DOH | 6.99 | 2.11–23.14 | 0.001 | 6.09 | 2.02–18.39 | 0.001 | 4.96 | 1.76–14.01 | 0.003 |
| Hematocrit < 40%—7. DOH | 6.81 | 2.26–20.51 | <0.001 | 9.08 | 3.36–24.51 | <0.001 | 5.54 | 2.5–12.29 | <0.001 |
| Hemoglobin < 12 g/dL—7. DOH | 14.21 | 4.64–43.56 | <0.001 | 17.82 | 6.34–50.07 | <0.001 | 11.87 | 4.53–31.1 | <0.001 |
| Creatinine > 1.2 mg/dL—7. DOH | 23.33 | 4.68–116.27 | <0.001 | 30.15 | 5.44–167.16 | <0.001 | 18.85 | 3.48–102.15 | <0.001 |
| Urea > 49—7. DOH | 25.6 | 7.66–85.76 | <0.001 | 179.38 | 34.75–925.99 | <0.001 | 80.71 | 16.92–384.95 | <0.001 |
| NT-proBNP > 190—7. DOH | 15.79 | 4.88–51.09 | <0.001 | 12.57 | 4.2–37.61 | <0.001 | 7.3 | 2.57–20.79 | <0.001 |
| RBC < 4.5 × $10^6$/µL—7. DOH | 8.7 | 2.87–26.36 | <0.001 | 11.86 | 4.35–32.31 | <0.001 | 5.28 | 2.39–11.67 | <0.001 |
| hsTnI > 34 pg/mL—7. DOH | 31.82 | 6.72–150.58 | <0.001 | 38.44 | 7.17–206.09 | <0.001 | 57.46 | 6.78–487.27 | <0.001 |
| Total calcium < 2.1 mmol/L—7. DOH | 31.35 | 7.61–129.1 | <0.001 | 31.73 | 7.44–135.37 | <0.001 | 34.09 | 6.82–170.24 | <0.001 |
| NLR ≥ 2—7. DOH | 4.46 | 1.24–16.09 | 0.023 | 6.91 | 1.98–24.06 | 0.002 | 11.2 | 3.3–38.06 | <0.001 |
| Lymphocyte count < 0.9 × $10^3$/µL—7. DOH | 9.23 | 2.9–29.36 | <0.001 | 7.58 | 2.6–22.11 | <0.001 | 5.91 | 2.16–16.2 | <0.001 |
| Lymphocyte percentage < 19%—7. DOH | 13.76 | 3.77–50.22 | <0.001 | 11.57 | 4.06–33.04 | <0.001 | 13.54 | 5.45–33.67 | <0.001 |
| Neutrophil count > 7 × $10^3$/µL —7. DOH | 12.21 | 3.75–39.79 | <0.001 | 11.86 | 4.35–32.31 | <0.001 | 7.37 | 3.28–16.57 | <0.001 |
| Neutrophil percentage > 68%—7. DOH | 6.32 | 2.1–19 | 0.001 | 5.22 | 2.07–13.14 | <0.001 | 7.14 | 3.14–16.26 | <0.001 |
| WBC count > 10 × $10^3$/µL—7. DOH | 5.73 | 1.98–16.57 | 0.001 | 5.2 | 2.09–12.94 | <0.001 | 3.92 | 1.81–8.66 | <0.001 |
| CRP ≥ 100 mg/L—7. DOH | 42, 95 | 9.15–192.85 | <0.001 | 47.83 | 9.13–250.73 | <0.001 | 28.58 | 5.61–145.54 | <0.001 |
| PCT > 0.5 ng/mL—7. DOH | 54.44 | 12.05–246 | <0.001 | 31.73 | 7.44–135.37 | <0.001 | 34.09 | 6.82–170.29 | <0.001 |

**Table 8.** Univariate and multivariate logistic regression analysis of selected clinical characteristics and laboratory parameters at admission for the prediction of death.

| Variable | Univariate Regression | | | Multivariate Regression | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | *p* | OR | 95% CI | *p* |
| Weight > 100 kg | 6.02 | 2.01–18.09 | 0.001 | | | |
| BMI ≥ 40 kg/m$^2$ | 5.91 | 1.4–27.97 | 0.016 | | | |
| Comorbidities | 3.51 | 1.18–10.48 | 0.024 | 3.96 | 1.21–12.98 | 0.023 |
| Diabetes or insulin resistance | 4.6 | 1.46–14.77 | 0.009 | | | |
| Chronic arrhythmia | 10 | 1.54–64.83 | 0.016 | | | |
| CK-MB > 20—at admission | 3.08 | 1.08–8.73 | 0.035 | | | |
| D-Dimer > 500 µg/L FEU—at admission | 7.99 | 1.03–61.65 | 0.046 | | | |
| EGFR < 60 mL/min—at admission | 9.23 | 2.69–31.67 | <0.001 | | | |
| GGT > 120 U/L—at admission | 7.92 | 2.53–24.77 | <0.001 | | | |
| Creatinine > 1.2 mg/dL—at admission | 4.92 | 1.54–15.74 | 0.007 | | | |
| LDH > 500 U/L—at admission | 4.8 | 1.7–13.6 | 0.003 | | | |
| NT-proBNP > 190 pg/mL—at admission | 3.66 | 1.24–10.81 | 0.019 | | | |
| Total calcium < 2.1 mmol/L—at admission | 8.35 | 2.47–28.24 | <0.001 | | | |
| Neutrophil count > 7 × 10$^3$/µL—at admission | 6.59 | 2.27–19.13 | <0.001 | | | |
| WBC count > 10 × 10$^3$/µL—at admission | 7.19 | 2.47–20.81 | <0.001 | 5.8 | 1.45–16.95 | 0.003 |
| CRP ≥ 100 mg/L—at admission | 3.26 | 1.14–9.34 | 0.027 | | | |
| PCT > 0.5 ng/mL—at admission | 7.39 | 2.38–22.94 | <0.001 | 4.96 | 1.45–16.95 | 0.011 |

#### 3.2.3. Predictors of MV in Logistic Regression

Univariate logistic regression revealed that weight > 100 kg, BMI ≥ 40 kg/m$^2$, comorbidities, diabetes or insulin resistance, chronic arrhythmia, CK-MB > 20 U/L at admission and at the 7th DOH, CK > 190 U/L at admission and at the 7th DOH, D-Dimer > 500 µg/L FEU at admission and at the 7th DOH, EGFR < 60 mL/min at admission and at the 7th DOH, GGT > 120 U/L at admission and at the 7th DOH, hematocrit < 40% at the 7th DOH, hemoglobin < 12 g/dL at the 7th DOH, creatinine > 1.2 mg/dL at admission and at the 7th DOH, LDH > 500 U/L at admission, urea > 49 mg/dL at admission and at the 7th DOH, NT-proBNP > 190 pg/mL at admission and at the 7th DOH, RBC count < 4.5 × 10$^6$/µL at the 7th DOH, hsTnI > 34 pg/mL at admission and at the 7th DOH, total calcium < 2.1 mmol/L at admission and at the 7th DOH, NLR ≥ 2 at the 7th DOH, lymphocyte count < 0.9 × 10$^3$/µL at the 7th DOH, lymphocyte percentage < 19% at the 7th DOH, neutrophil count > 7 × 10$^3$/µL at admission and at the 7th DOH, neutrophil percentage > 68% at the 7th DOH, WBC count > 10 × 10$^3$/µL at admission and at the 7th DOH, CRP > 100 mg/L at admission and at the 7th DOH, and PCT > 0.5 ng/mL at admission and at the 7th DOH, were associated with increased risk of MV (Tables 7 and 9). BMI ≥ 40 kg/m$^2$, LDH > 500 U/L, WBC count > 10 × 10$^3$/µL, and PCT > 0.5 ng/mL, were significantly associated with MV in multivariate analysis for variables obtained at admission (Table 9).

#### 3.2.4. Predictors of ICU Treatment in Logistic Regression

Univariate logistic regression revealed that weight > 100 kg, BMI ≥ 40 kg/m$^2$, diabetes or insulin resistance, CK-MB > 20 U/L at admission and at the 7th DOH, CK > 190 U/L at admission and at the 7th DOH, D-Dimer > 500 µg/L FEU at admission and at the 7th DOH, EGFR < 60 mL/min at admission and at the 7th DOH, GGT > 120 U/L at the 7th DOH, hematocrit < 40% at the 7th DOH, hemoglobin < 12 g/dL at the 7th DOH, creatinine > 1.2 mg/dL at admission and at the 7th DOH, LDH > 500 U/L at admission, urea > 49 mg/dL at the 7th DOH, NT-proBNP > 190 pg/mL at admission and at the 7th DOH, RBC count < 4.5 × 10$^6$/µL at the 7th DOH, hsTnI > 34 pg/mL at admission and at the 7th DOH, total calcium < 2.1 mmol/L at admission and at the 7th DOH, NLR ≥ 2 at the 7th DOH, lymphocyte count < 0.9 × 10$^3$/µL at the 7th DOH, lymphocyte percentage < 19% at admission and at the 7th DOH, neutrophil count > 7 × 10$^3$/µL at

admission and at the 7th DOH, neutrophil percentage > 68% at admission and the 7th DOH, WBC count > 10 × $10^3$/μL at admission and at the 7th DOH, CRP > 100 mg/L at admission and at the 7th DOH, and PCT > 0.5 ng/mL at admission and at the 7th DOH were associated with increased risk of ICU treatment (Tables 7 and 10). D-Dimer > 500 μg/L FEU, LDH > 500 U/L, WBC count > 10 × $10^3$/μL, and PCT > 0.5 ng/mL were significantly associated with ICU treatment in multivariate analysis for variables obtained at admission (Table 10).

**Table 9.** Univariate and multivariate logistic regression analysis of selected clinical characteristics and laboratory parameters at admission for the prediction of mechanical ventilation.

| Variable | Univariate Regression | | | Multivariate Regression | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | *p* | OR | 95% CI | *p* |
| Weight > 100 kg | 4.96 | 1.97–12.47 | <0.001 | | | |
| BMI ≥ 40 kg/$m^2$ | 6.35 | 1.7–23.76 | 0.006 | 6.88 | 1.27–37.45 | 0.026 |
| Comorbidities | 2.84 | 1.14–7.06 | 0.025 | | | |
| Diabetes or insulin resistance | 5.22 | 1.87–14.54 | 0.002 | | | |
| Chronic arrythmia | 6.8 | 1.07–43.13 | 0.042 | | | |
| CK-MB > 20 at admission | 3.48 | 1.4–8.62 | 0.007 | | | |
| CK > 190 at admission | 2.49 | 1.02–6.1 | 0.046 | | | |
| D-Dimer > 500 μg/L FEU—at admission | 11.68 | 1.54–88.62 | 0.017 | | | |
| EGFR < 60—mL/min at admission | 16.33 | 5.17–51.57 | <0.001 | | | |
| GGT > 120 U/L—at admission | 8.53 | 3.03–23.99 | <0.001 | | | |
| Creatinine > 1.2 mg/dL—at admission | 7.31 | 2.65–20.19 | <0.001 | | | |
| LDH > 500 U/L—at admission | 5.85 | 2.34–14.62 | <0.001 | 4.67 | 1.58–13.84 | 0.005 |
| Urea > 49 at admission | 5.24 | 1.63–16.84 | 0.005 | | | |
| NT-proBNP > 190 pg/mL—at admission | 5.8 | 2.28–14.77 | <0.001 | | | |
| hsTnI > 34 pg/mL—at admission | 14.93 | 3.66–60.82 | <0.001 | | | |
| Total calcium < 2.1 mmol/L—at admission | 10.27 | 3.36–31.42 | <0.001 | | | |
| Neutrophil count > 7 × $10^3$/μL—at admission | 7.54 | 2.96–19.22 | <0.001 | | | |
| WBC count > 10 × $10^3$/μL—at admission | 10.09 | 3.91–26.05 | <0.001 | 5.75 | 1.9–17.37 | 0.002 |
| CRP ≥ 100 mg/L—at admission | 5.7 | 2.13–15.22 | <0.001 | | | |
| PCT > 0.5 ng/mL—at admission | 13.54 | 4.87–37.62 | <0.001 | 9.56 | 2.97–30.92 | <0.001 |

**Table 10.** Univariate and multivariate logistic regression analysis of selected clinical characteristics and laboratory parameters at admission for the prediction of ICU treatment.

| Variable | Univariate Regression | | | Multivariate Regression | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | *p* | OR | 95% CI | *p* |
| Weight > 100 kg | 2.57 | 1.19–5.55 | 0.017 | | | |
| BMI ≥ 40 kg/$m^2$ | 4.04 | 1.11–14.73 | 0.034 | | | |
| Diabetes or insulin resistance | 4.99 | 1.96–12.74 | <0.001 | | | |
| CK-MB > 20 at admission | 3.14 | 1.42–6.98 | 0.0049 | | | |
| CK > 190 at admission | 2.9 | 1.33–6.31 | 0.008 | | | |
| D-Dimer > 500 μg/L FEU—at admission | 8.47 | 1.96–36.53 | 0.004 | 5.24 | 1.15–23.84 | 0.032 |
| EGFR < 60 mL/min—at admission | 9.49 | 3.15–28.59 | <0.001 | | | |
| GGT > 120 U/L—at admission | 4.95 | 1.86–13.2 | 0.001 | | | |
| Creatinine > 1.2 mg/dL—at admission | 3.32 | 1.24–8.88 | 0.017 | | | |
| LDH > 500 U/L—at admission | 5.28 | 2.39–11.67 | <0.001 | 3.35 | 1.82–16.17 | 0.002 |
| NT-proBNP > 190 pg/mL—at admission | 5.5 | 2.38–12.67 | <0.001 | | | |
| hsTnI > 34 pg/mL—at admission | 9.33 | 2.35–36.97 | 0.002 | | | |
| Total calcium < 2.1 mmol/L—at admission | 6.13 | 2.09–17.95 | 0.001 | | | |
| Lymphocyte percentage < 19% at admission | 25 | 3.43–186.95 | 0.002 | | | |
| Neutrophil count > 7 × $10^3$/μL—at admission | 7.64 | 3.39–17.2 | <0.001 | | | |
| Neutrophil percentage > 68% at admission | 14.66 | 1.96–109.9 | 0.009 | | | |
| WBC count > 10 × $10^3$/μL—at admission | 6.09 | 2.62–14.17 | <0.001 | 3.69 | 1.38–9.85 | 0.009 |
| CRP ≥ 100 mg/L—at admission | 4.72 | 2.1–10.62 | <0.001 | | | |
| PCT > 0.5 ng/mL—at admission | 9.35 | 3.6–24.29 | <0.001 | 5.42 | 1.82–16.17 | 0.002 |

3.2.5. ROC Analysis

The combined multivariate regression models for predicting death, MV, and ICU treatment had area under the curve (AUCs) values of 0.805, 0.836, and 0.846, respectively. The ROC curves with AUCs of combined models and individual factors are presented in Figure 3.

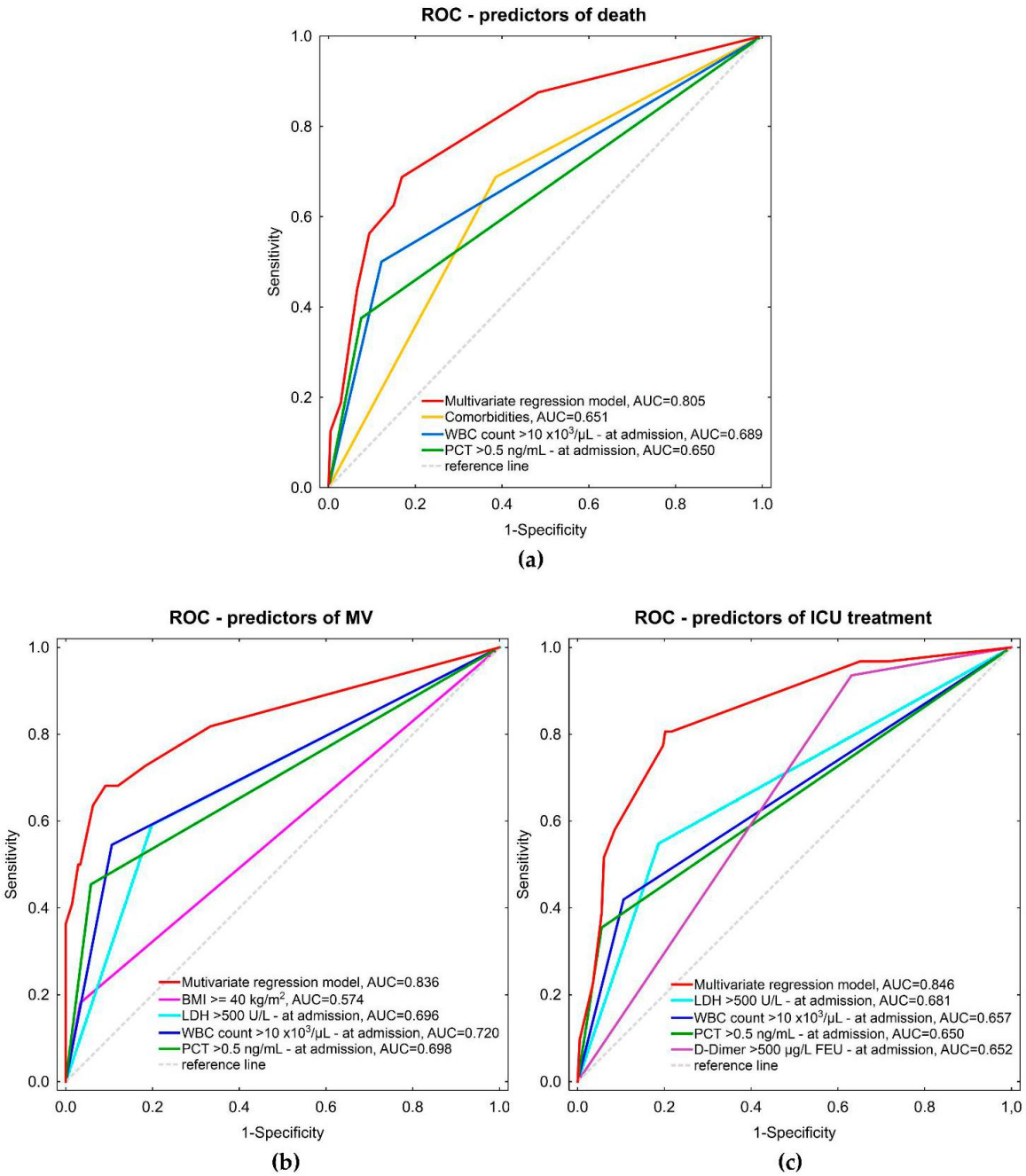


**Figure 3.** The receiver operating characteristic (ROC) curves with an area under the curve (AUCs) of individual factors and combined models in multivariate logistic regression analysis for predicting: (**a**) death; (**b**) MV; and (**c**) ICU treatment. Nominal data (including comorbidities) and categorized clinical and laboratory parameters obtained at admission and significant in univariate regression were

included in this analysis. Comorbidities (AUC = 0.651), WBC count > $10 \times 10^3/\mu L$ (AUC = 0.651), and PCT > 0.5 ng/mL (AUC = 0.650), were associated with death; BMI $\geq$ 40 $kg/m^2$ (AUC = 0.574), LDH > 500 U/L (AUC = 0.696), WBC count > $10 \times 10^3/\mu L$ (AUC = 0.720), and PCT > 0.5 ng/mL (AUC = 0.698) were associated with MV, while D-Dimer > 500 µg/L FEU (AUC = 0.652), LDH > 500 U/L (AUC = 0.681), WBC count > $10 \times 10^3/\mu L$ (AUC = 0.657), and PCT > 0.5 ng/mL (AUC = 0.650), were associated with ICU treatment. The combined multivariate regression models for predicting death, MV and ICU treatment had the AUC values of 0.805, 0.836, and 0.846, respectively.

## 4. Discussion

The SARS-CoV-2 alpha variant has been shown to be more transmissible than the wild-type variants [11,12]. However, its impact on disease severity remains unclear [13–16,18,39].

Challen et al., in a cohort study of 54,906 matched pairs [15], found that the hazard ratio of death within 28 days associated with infection with the alpha variant, compared with the wild-type variants, was 1.64. However, this study only included patients older than 30 years. Moreover, Davies et al. [14] analyzed a dataset of positive SARS-CoV-2 community tests, identifying 4945 deceased patients with known SGTF status, and estimated the hazard of death associated with the alpha variant to be 61% (42–82%) higher than for those with pre-existing variants. Furthermore, Grint et al. [40] found an increased risk of death in SGTF (S gene target failure, indicating the B.1.1.7 variant) compared to non-SGTF cases. However, the age range in the youngest group in an age subgroup analysis was as wide as 0–59 years. Moreover, although an initial analysis of CO-CIN data reported by NERVTAG [39] did not identify increased in-hospital case-fatality rate associated with the alpha variant, several other unpublished analyses summarized in NERVTAG were consistent in reporting increased disease severity in people infected with the alpha variant compared to variants then considered as non-VOCs [39].

On the contrary, a study by Frampton et al. [13] found no association of severe disease and death with B.1.1.7 compared to the non-B.1.1.7 lineage in hospitalized COVID-19 patients. Similarly, Brookman et al. [18] found no evidence of a greater disease severity in children hospitalized during the second wave in England (1 March to 31 May 2020) compared to the first wave (1 November 2020, to 19 January 2021), which prompted them to suggest that there is no appreciably different clinical course of the infection with the B.1.1.7 variant compared to the original variant. It should be noted that these studies showing no increased severity and mortality associated with the alpha variant only included hospitalized patients [13,18,39]. It is therefore also possible that the SARS-CoV-2 alpha variant could have been associated with more severe disease compared to previous variants, increasing the number of patients severe enough to meet hospital admission criteria, but had no impact on the in-hospital outcomes, including mortality [39]. Indeed, Nyberg et al. found that the risk of hospitalization was higher for people infected with the alpha variant compared with wild-type variants, with an adjusted hazard ratio of hospital admission of 1.52 [17]. Moreover, although Martin-Blondel et al. reported a greater severity of the disease associated with the alpha variant in hospitalized patients, this result was statistically significant when defining severe disease as a WHO-scale > 5 or the need of a non-rebreather mask, while the differences in the mortality rate and the need for HFNO, ICU admission and MV or ECMO were not statistically significant [16]. It is also noteworthy that the alpha variant has been de-escalated from being VOC, indicating that it no longer poses a significant risk to public health [7].

In our study, we found no significant differences in disease severity or mortality between the second and third wave patients, which may indicate that in hospitalized severely ill young adults, the SARS-CoV-2 alpha variant did not increase the incidence of critical disease or death. Indeed, we found no significant differences between the two waves regarding mortality and the need for ICU. There were also no differences between the second and third waves regarding the median percentage of lung involvement on CT at admission and the need for MV. Therefore, our results indicate that the alpha variant does

not appear to be associated with worse outcomes in hospitalized young adults than the wild-type variants.

It is noteworthy that we have found no significant differences between the second and third waves regarding sex, BMI, smoking status, and comorbidities. Moreover, in our study WBC, neutrophil and IG counts, IG percentages, NLR and the frequency of leukocytosis and neutrophilia at admission were higher, and the lymphocyte percentage at admission was lower in the second wave compared with the third wave, while there were no significant differences regarding IL-6, CRP, and ferritin levels between these two waves. Furthermore, although there was no significant difference between waves in terms of PCT levels at admission, PCT levels at the 7th DOH were significantly higher in the second wave compared with the third wave. As these markers of hyperinflammation seem to be associated with a worse outcome, as discussed below, these findings further contradict a greater disease severity due to the alpha SARS-CoV-2 variant compared to the wild-type variants. Moreover, high D-Dimer and low albumin levels also appear to be associated with poor prognosis, and we found no significant differences between waves in terms of D-Dimer and albumin levels at admission, while at the 7th DOH D-Dimer levels were even higher and albumin levels lower in the second wave compared with the third wave. In addition, although myoglobin levels at the 7th DOH were significantly lower in the second wave compared with the third wave, there were no differences between waves in other, more cardiac-specific biomarkers. Hence, this finding is not likely to indicate more pronounced myocardial injury in the third wave.

Regarding the delta variant, although it is generally considered to be associated with greater disease severity, data are inconsistent [20–23]. Two studies in the UK showed a greater risk of hospitalization with the delta variant compared to the alpha variant. However, these studies did not report on disease severity or mortality [41,42]. A study from Canada compared then-considered VOC and non-VOC strains, and found increased risk of hospitalization, ICU admission, and death with N501Y-positive variants (alpha, beta, and gamma variants) and even more pronounced for the delta variant [20]. On the other hand, a US study comparing children suffering from COVID-19 during the delta vs. pre-delta eras found no significant difference in hospitalization rates and lower odds of severe disease [22]. Moreover, Kläser et al. [23] found that illness duration was lower in those infected with delta compared to alpha variant, though unchanged in unvaccinated patients, and there was no difference regarding hospitalization.

Omicron variant, currently labeled as VOC, appears to cause less severe disease [8,43], however, more data are still needed [27]. In a study by Lauring et al. [43] the severity was higher for delta than alpha, and lower for omicron than delta variants. Sievers et al. [25] found significantly reduced odds of hospitalization, ICU admission and death in patients infected with omicron compared to delta variant, whereas Van Goethem et al. [26] reported significantly lower risk for severe COVID-19 and ICU admission in hospitalized patients infected with the omicron compared to delta variant, while in-hospital mortality was not significantly different. Wolter et al. [27] found reduced risk of severe disease among patients infected with SGTF (as a proxy for the omicron variant) compared with individuals with earlier delta variant infections, however, the authors did not find the difference in severity between SGTF and non-SGTF infections diagnosed during the same time period, and suggested that immunity, due to previous infection, vaccination, or both, may at least in part account for the reduced severity of omicron compared to delta variant infections. Indeed, continuously changing vaccination status makes these comparisons of SARS-CoV-2 variants even harder. Furthermore, even in non-vaccinated patients the severity of the disease may now be more and more frequently affected by other factors, including prior SARS-CoV-2 infection. Nevertheless, it seems possible that the omicron will share the fate of the previous variants, and be de-escalated.

SARS-CoV-2 infection may be asymptomatic or symptomatic, with the course of the disease varying widely from mild to severe to critical. A report by the Chinese Center for Disease Control and Prevention in the initial period of the pandemic (up to 11 February

2020), based on 44,500 confirmed COVID-19 cases, showed that mild disease was found in 81% of cases, severe disease in 14%, and critical disease (with respiratory failure, septic shock and/or multiple organ failure) in 5%, with an overall case-fatality rate of 2.3% and no deaths among noncritical patients [30]. Similarly, in a CDC report analyzing cases reported between 22 January and 30 May 2020, 14% of patients required hospitalization, 2% were admitted to the ICU, and 5% died [44]. It is noteworthy that initially non-severe COVID-19 patients may progress in approximately a week. In our study, the median time from the onset of symptoms to hospital admission was eight days in the second wave and nine days in the third wave, and the median time from the onset of symptoms to ICU admission was 10 days in both groups. Similarly, in a systematic review by Xie et al. [45], the median time from the onset of the disease to first hospital admission was seven days, dyspnea occurred after 5–8 days, and ARDS after 8–9 days, and the median time to ICU admission was 10.5 days. In young adults, Liu et al. reported a median time from the onset of symptoms to hospital admission of 11 days and 10 days in the survivors and non-survivors, respectively [31], while in a study by Owusu et al. this time period was seven days [46].

Regarding inpatients, in a study of 16,000 adults hospitalized with COVID-19 from March to December 2020, the percentage of patients admitted to the ICU decreased from 37.8% in March to 20.5% in December, and the overall fatality rate was 11.4% [47]. In another study [48], out of 2634 patients hospitalized for COVID-19 between 1 March and 4 April 2020, 14.2% were admitted to the ICU and 21% died.

In the general population, certain demographic and clinical features have been associated with the risk for severe course of COVID-19 including older age [28,29,48–54], male sex [48,52,53], smoking [53–55], obesity [51–53,55], and other comorbidities [44,55] such as diabetes [44,51,54,55], hypertension [30,51], heart conditions [30,52,55], chronic respiratory diseases [30,55], chronic kidney disease [52,55], and cancer [20,53,55]. As already mentioned, data on clinical features and risk factors of severe COVID-19 in young adults are scarce.

Current evidence indicates that older adults are at risk of having more severe disease. In a study by Verity et al. [49] the hospitalization rate was 1.04% in patients aged 20–29, 3.43% in patients aged 30–39, 4.25% in patients aged 40–49, 8.16% in patients aged 50–59, 11.8% in patients aged 60–69, 16.6% in patients aged 70–79, and 18.4% in patients aged 80 or over. Luo et al., after dividing their study population into four age groups, found severe and critical disease, respectively, in none of the children (18 years or younger), 1.5% and 0.8% of young adults (19–44 years), 6.5% and 6.5% of middle-aged adults (45–64 years), and 12.7% and 20.3% of elderly adults (65 years or older) [50]. Age also appears to be associated with increased mortality [28,29,48–50]. In the aforementioned study by Verity et al. [49], the adjusted case-fatality ratio was estimated at 0.06% in patients aged 20–29, 0.15% in patients aged 30–39, 0.3% in patients aged 40–49, 1.25% in patients aged 50–59, 3.99% in patients aged 60–69, 8.61% in patients aged 70–79, and 13.4% in patients aged 80 or over. Williamson et al. [28] found a greater than 20-fold-increased risk of death in patients 80 years and older compared to 50–59-year-olds. For these reasons, many studies of COVID-19 focus either on general epidemiological data or on older populations [29,33,56]. However, COVID-19 may also result in severe disease and death in young adults [33,57–59]. In our study, 13.54% of patients required ICU admission and 7% died, which agrees with other studies of young adults. Indeed, in a study of 395 patients aged 18–35 years hospitalized for COVID-19, 21% required invasive mechanical ventilation and 13.9% died [33]. In a study of SARS-CoV-2-positive 18–45-year-olds, who had presented to emergency departments, 9% died during hospitalization [57]. Richardson et al. [58] found that the 30-day in-hospital mortality in COVID-19 patients aged 18–39 years was 4.9%, while Cunningham et al. [59] found that of the 3222 18–34-year-olds hospitalized for COVID-19, 21% were admitted to the ICU and mortality was 2.7%. Therefore, it is essential to identify the clinical features and risk factors for severe COVID-19 in this age group. Of note, we have not found a significant age difference between survivors and non-survivors, possibly because our study

only involved young adults. Similarly, in a study by Cunningham et al. [59], the odds of MV or death did not vary significantly with age. We believe that studies of young adults are of great importance due to the small influence of other factors, such as comorbidities, on the course of infection in this age group, which may be useful in determining the influence of the causative variant on disease severity.

The male sex of young adults has been related with poor prognosis by some authors [31,35,59], while others have failed to find this relationship [58,60]. In our study, there was no significant difference between the survivors and non-survivors regarding sex. However, it should be noted that we observed a male predominance in both waves, which may indicate that men were more likely to have a disease severe enough to require hospital admission.

Some studies in young adults found no association of smoking and mortality [33,58,61]. Conversely, in our study, a history of current or former smoking, but not current smoking, was significantly more frequent among the non-survivors than survivors, and we have found a weak positive correlation between smoking and the need for MV and ICU treatment. Moreover, according to the CDC, there is evidence that in the general population smoking is associated with a higher risk of severe COVID-19 [55]. Therefore, in our opinion, smoking history should be taken into consideration while assessing the possible risk factors for severe COVID-19 in young adults.

Higher BMI and obesity have been identified as risk factors for poor prognosis in young adults in several studies [33,35,57–59,61]. Although we have also found weight and BMI to be higher in non-survivors compared to survivors, these differences were not significant after the removal of the outliers. However, it should be noted that in our study of COVID-19 patients, all of whom were hospitalized due to severe disease, the median BMI was 30.58 $kg/m^2$ (IQR 27.1–34.3 $kg/m^2$), and normal BMI (below 25 $kg/m^2$) was found in only 12.34%. Moreover, we have also found a positive correlation between BMI and the need for MV. Furthermore, weight > 100 kg and BMI $\geq$ 40 $kg/m^2$ were significant predictors of death, MV and ICU treatment in univariate logistic regression, and BMI $\geq$ 40 $kg/m^2$ was also a significant predictor of MV in multivariate analysis. Therefore, it appears that overweight or obesity should also be considered risk factors for poor prognosis, possibly due to their association with other comorbidities, such as reduced lung volumes and hypercoagulable states [58].

In our study, comorbidities were significantly more frequent in non-survivors compared to survivors, and were associated with the risk of death and MV in univariate logistic regression, and with the risk of death in multivariate analysis. Similarly, Richardson et al. [58] found a Charlson comorbidity index score to be an independent predictor of in-hospital 30-day mortality in young adults hospitalized for COVID-19. We have also found that chronic arrhythmia was significantly more frequent in the non-survivors than in the survivors, while for insulin resistance, diabetes, and hypertension, although their frequency was greater in non-survivors than in survivors, these differences were not significant ($p$ = 0.058, 0.199 and 0.268, respectively). There were no significant differences in the frequency of asthma, hypothyroidism, and Hashimoto disease ($p$ = 1, 0.485 and 1, respectively). In univariate logistic regression, having diabetes or insulin resistance was significantly associated with the risk of death, MV, and ICU treatment, and chronic arrhythmia was significantly associated with the risk of death and MV. Several studies of young adults have identified diabetes as a risk factor for more severe disease [31,33,35,59–61]. Hypertension has been associated with poor prognosis in some studies [31,33,59,61], however, others have not found this association [35,58,60]. Similarly, asthma was predictive of more severe disease in some studies [35,60], but other authors found it was not associated with increased mortality [33,58]. Additionally, cardiac [33,35] and renal [33,35] conditions have been identified as related to poor prognosis in some studies, while no association of thyroid diseases and severe COVID-19 has been found [35,60]. Overall, the association of comorbidities and the outcome of SARS-CoV-2 infection appears to be less pronounced in

young adults than in the general population, as a greater number of comorbidities in elderly patients may lead to a more complex pathogenesis in COVID-19 and its complications [34].

In our study, the percentages of lung involvement and lung involvement of at least 50% on CT were significantly higher in the non-survivors than in survivors. There was also a weak positive correlation between the percentage of lung involvement and the need for MV and ICU treatment. This agreed with a study by Ruch et al. [62] which found that the extent of changes on initial CT was associated with prognosis, with 69.5% of patients who had lung involvement over 50% having developed severe disease, compared to 22.9% of patients with lung involvement no greater than 25%. Similarly, in a study by Annoni et al. [63] the percentage of damaged lung parenchyma volume on CT was correlated with the course of COVID-19, with average infected lung volume significantly higher in the non-survivors.

In our study, $SpO_2$ at admission was significantly lower in non-survivors than in survivors, and there was a weak to moderate negative correlation between $SpO_2$ at admission and the need for MV and ICU treatment. Moreover, we found a more frequent need for HFNO and MV and a higher maximum oxygen flow, in both conventional oxygen therapy and HFNO, in non-survivors than in survivors. This agreed with previous studies among young adults reporting an association of respiratory distress and mortality [31,33,34]. Furthermore, as predicted, we found that ICU admission, vasopressors, and CRRT were more frequent in non-survivors than in survivors.

Current evidence in the general population indicates that a number of laboratory anomalies may be associated with the risk for severe course of COVID-19 and worse outcomes, including elevated WBC [29,54,64–69] and neutrophil counts [64–66,68–70], lymphopenia [29,52,61,64–66,68–70], elevated NLR [64,66], thrombocytopenia [29,64,68–71], increased inflammatory markers, including CRP [52,64–66,68–71], PCT [29,52,66,68–71], and ferritin [29,64–66,68–70], and inflammatory cytokines, such as IL-6 [29,64,65,68–70], as well as organ and coagulation dysfunction markers, including elevated LDH [29,64–66,68–71], troponin and hs-TnI [29,31,52,68,69], NT-proBNP [66,68,70], creatinine [29,52,68–71], CK [29,68,71], liver enzymes [29,65,66,68–71], D-Dimer [29,52,64–71], longer prothrombin time [29,64,65,68,69], and decreased serum albumin levels [29,65,66,68–70]. However, Luo et al. [50] found many laboratory parameters to be significantly different in younger compared to older COVID-19 patients, including higher WBC, lymphocyte and PLT counts, and hemoglobin and albumin levels, and lower levels of CRP, ALT, creatinine, and D-dimer. Similarly, Liu et al. [31] found many significantly different laboratory parameters in younger (defined as younger than 60 years old) compared to older COVID-19 patients, including higher lymphocyte counts and albumin levels, and lower neutrophil counts, NLR, PT, and levels of CRP, PCT, D-dimer, LDH, creatinine, and NT-proBNP. These differences are thought to result from higher incidences of organ dysfunctions and comorbidities, as well as poorer immune responses in older individuals [31,50]. Therefore, the predictors of severe disease and mortality in younger COVID-19 patients seem to differ from those in general population.

SARS-CoV-2 infection causes a host immune response, which in most patients will contribute to viral elimination. However, in some cases, the activation of nucleic acid sensors on lung epithelium and alveolar macrophages triggers the elevated release of cytokine and other proinflammatory mediators, such as IL-1, IL-6, and tumor necrosis factor-α (TNF-α), resulting in the recruitment and infiltration of neutrophils, monocytes, and other leukocytes. Moreover, these cytokines stimulate bone marrow to produce and release immature granulocytes that infiltrate the lungs, further increasing the exuberant inflammatory reaction [72,73]. In addition, recruited neutrophils kill pathogens by producing reactive oxygen species (ROS) and releasing web-like structures consisting of DNA and antimicrobial agents, such as and myeloperoxidase and histones, known as the neutrophil extracellular traps (NETs). However, this may also cause destruction of infected tissue, microthrombosis, and organ damage [73,74]. Furthermore, the release of cytokines, including IL-6, induces the synthesis of acute phase proteins, such as CRP, fibrinogen, and ferritin [72], which, in turn,

may further affect the immune reaction, being able to induce the expression of both pro- and anti-inflammatory mediators [75,76]. Moreover, the exacerbated inflammatory reaction may lead to cytokine-induced lymphocyte apoptosis [74,77,78]. Other possible causes of lymphopenia in COVID-19 include a direct viral infection of ACE2-expressing lymphocytes, destruction of lymphatic organs, and increased lymphocyte consumption in the infected tissues [74,77,78]. For these reasons, an elevated neutrophil to leukocyte ratio (NLR) is also observed in COVID-19 [74]. Indeed, in our study, we found that the WBC, neutrophil, and IG counts, IG percentages, the incidence of leukocytosis, neutrophilia, and lymphopenia, NLR, and IL-6 levels were significantly higher in non-survivors than in survivors. There was also a positive correlation between the WBC, neutrophil an IG count, neutrophil and IG percentages, leukocytosis, neutrophilia, NLR, and IL-6 levels and the need for MV and ICU treatment, and a negative correlation between the lymphocyte percentage and the need for MV and ICU treatment. In addition, CRP was significantly higher and CRP above 100 mg/L was significantly more frequent in non-survivors than in survivors, and there was also a weak to moderate positive correlation between CRP and the need for MV and ICU treatment. Furthermore, univariate logistic regression revealed that NLR $\geq$ 2 and lymphopenia at the 7th DOH, and neutrophilia, leukocytosis, and CRP > 100 mg/L at admission and at the 7th DOH were significantly associated with the risk of death, MV and ICU treatment. In multivariate analysis, CRP > 100 mg/l and leukocytosis at admission were significantly associated with death, MV and ICU treatment. Moreover, although we did not find a significant difference between ferritin levels between non-survivors and survivors, there was a weak positive correlation between ferritin levels at admission and the need for MV, and between ferritin levels at the 7th DOH and the need for ICU treatment.

In accordance with our results, some studies in young adults with COVID-19 found elevated WBC [31,34] and neutrophil counts [31,34], NLR [31] and levels of CRP [31,33,34], and ferritin [34], as well as decreased lymphocyte counts [31,34,60] in deceased cases compared to survivor patients, and elevated neutrophil percentages [79] and CRP [61,79] levels and decreased lymphocyte counts [79] in severe vs. mild patients. However, Altonen et al. [33] found no significant differences in ferritin levels and WBC, neutrophil and lymphocyte counts between survivors and non-survivors, Zhou et al. [79] found lower WBC count and no difference in neutrophil count in severe compared to mild patients, and Maldonado-Cabrera et al. [61] did not observe the increase in NLR in the aggravated COVID-19 patients. Nevertheless, we suggest that the hyperinflammatory reaction may be the reason for the severe course of COVID-19 also in young adults. Furthermore, studies have reported an even stronger influence on poor COVID-19 outcomes of parameters, such as neutrophilia [31] and lymphopenia [60], in young adults than occurring in the elderly, which was attributed to a stronger immune response in young adults with a stronger cytokine storm and hyperinflammatory reaction [31,60].

PCT can be induced directly by bacterial endotoxins and lipopolysaccharides or indirectly through the release of pro-inflammatory cytokines, such as IL-1β, TNF-α and IL-6. However, its synthesis may be inhibited by interferon-γ (INF-γ), with increased in viral infection. Therefore, PCT is not typically elevated in mild SARS-CoV-2-infection, while its increase is observed in severe COVID-19, especially due to a bacterial co-infection [80–82]. Moreover, PCT upregulates the leukocyte surface markers, cytokines, and reactive oxygen species, further aggravating the inflammatory reaction [80]. This is supported by our study, which found that PCT was significantly higher and PCT above 0.5 ng/mL was significantly more frequent in non-survivors compared to survivors, and that there was a positive correlation between PCT, and the need for MV and ICU treatment.

Univariate logistic regression revealed that PCT > 0.5 ng/mL at admission and at the 7th DOH was associated with increased risk of death, MV and ICU treatment, and PCT > 0.5 ng/mL at admission was also associated with increased risk of death, MV and ICU treatment in multivariate analyses. Similarly, other studies of young adult COVID-19 patients have found higher PCT in non-survivors than in survivors [31,33,34].

Apart from the excessive inflammatory reaction, another possible cause of severe course and poor prognosis in COVID-19 may be thrombosis and coagulopathy [61]. Several factors may contribute to coagulation dysfunction in COVID-19, including the cytokine storm leading to an increased production of platelets and fibrinogen, complement activation, vascular dysfunction, Renin-Angiotensin-Kallikrein-Kinin systems (RAS-KKS) imbalance, and excessive intravascular NETs formation by neutrophils [72,73]. This hyperactive coagulation causes an increase in the level of D-Dimer, a fibrin degradation product [73]. In our study, D-Dimer was significantly higher in non-survivors than in survivors, and there was a positive correlation between D-Dimer and the need for MV and ICU treatment. D-Dimer > 500 μg/L FEU at admission was significantly more frequent in non-survivors compared to survivors and there was also an association of D-Dimer > 500 μg/L FEU at admission and at the 7th DOH and the need for MV and ICU treatment. Moreover, univariate logistic regression revealed that D-Dimer > 500 μg/L FEU at admission and at the 7th DOH were associated with increased risk of death, MV and ICU treatment, and D-Dimer > 500 μg/L FEU at admission was also significantly associated with the risk of ICU treatment in multivariate analysis. This accords with the findings of previous studies of young adults with COVID-19, which have found higher D-Dimer levels in deceased vs. alive patients [31,34]. Conversely, other studies have failed to find associations of this marker with mortality [33] and disease severity [61,79]. Some studies have also found elevated PT [31,34] and APTT [31] in non-survivors, while in a study by Lu et al. [34], APTT did not differ between the non-survivors and the survivors, and in a study by Zhou et al. [79] there was no significant difference in PT between mild and severe cases. In our study, APTT at admission, but not at the 7th DOH, was significantly longer in survivors compared to non-survivors, while there were no significant differences between these groups regarding PT. Moreover, although Zhou et al. found higher fibrinogen in severe vs. mild patients [79], we did not observe this relationship. Furthermore, similar to the findings of Liu et al. [31], we did not find differing AT III levels between survivors and non-survivors. It is worth noting that although pro-inflammatory cytokines may increase platelet production, some authors have also found that severe COVID-19 is characterized by thrombocytopenia, which may result from viral infection of bone marrow and decreased platelet production, or increased platelet consumption due to their abnormal activation by immune complexes or excessive thrombosis [83]. Indeed, in a study of 18–50-year-olds, Zhou et al. [79] found decreased PLT counts in patients with severe compared to mild COVID-19. However, we found no significant difference between non-survivors and survivors regarding PLT counts, which was similar to the results of a study among young adults by Lu et al. [34] One possible explanation is that, as mentioned, severe COVID-19 can cause both thrombocytopenia and increased PLT production. Indeed, we have observed a positive correlation between both thrombophilia and thrombocytopenia and the need for ICU treatment.

Some evidence indicates that lower RBC counts and hemoglobin may be associated with a worse outcome [69,84]. However, other authors have not confirmed this relationship [67,68], including in studies of young adults [34,79]. Interestingly, in our study, although there were no significant differences regarding RBC counts, hematocrit, and hemoglobin levels at admission, at the 7th DOH these parameters were significantly lower in non-survivors than in survivors, and there was a negative correlation between RBC counts, hematocrit and hemoglobin levels at the 7th DOH, and the need for MV and ICU treatment. In addition, univariate logistic regression revealed that RBC count < 4.5 $\times 10^6$/μL, hematocrit < 40%, and hemoglobin < 12 g/dL at the 7th DOH were associated with increased risk of death, MV and ICU treatment. This accords with a study by Lanser et al. [85] which found a more distinct decrease in hemoglobin levels in patients with severe COVID-19, and the association of new-onset anemia with a higher risk of ICU admission, which the authors suggested reflects hyperinflammation leading to disease progression.

Another biomarker associated with COVID-19 severity is LDH, an enzyme that is present in all tissues and released into the blood upon tissue damage, including such as viral infection, hypoxia, and inflammation-induced injury [86]. In our study, LDH at admission was significantly higher in non-survivors than in survivors, and there was a positive correlation between LDH, and the need for MV and ICU treatment. In addition, LDH > 500 U/L at admission was associated with increased risk of death, MV and ICU treatment in univariate analyses, and with MV and ICU treatment in multivariate analyses. This accords with another study in young adults [31]. However, other authors [33,34] have failed to find this relationship. The elevation of LDH levels may be due to the multiple organ damage, including renal, myocardial, and liver dysfunction, that has been observed in severe COVID-19 [71]. This multiorgan damage is likely multifactorial and may occur either by direct viral invasion through ACE2 receptors expressed in multiple tissues, including myocardium, renal tubular cells and hepatic tissue, or by indirect injury due to cytokine storm and systemic inflammation, sepsis, hypovolemia, hypoxemia, oxidative stress, microvascular thrombosis, and endothelial damage [61,87–91].

According to current evidence, elevation of cardiac injury biomarkers, such as troponin and hs-TnI, CK-MB, myoglobin and NT-proBNP, is associated with COVID-19 severity and mortality [87,88,92]. Agreeing with this evidence, in our study, hs-TnI, CK-MB, myoglobin, and NT-proBNP were significantly higher in non-survivors than in survivors, and there was a positive correlation between hs-TnI, NT-proBNP, and CK-MB and the need for MV and ICU treatment. Moreover, univariate logistic regression revealed that CK-MB > 20 U/L at admission and at the 7th DOH, NT-proBNP > 190 pg/mL at admission and at the 7th DOH, and hsTnI > 34 pg/mL at the 7th DOH were associated with increased risk of death, MV and ICU treatment, and hsTnI > 34 pg/mL at admission—with increased risk of MV and ICU treatment. These findings are consistent with other studies in young adults that found higher levels of hs-TnI [34], CK-MB [31], myoglobin [31,34], and NT-proBNP [31,34] in deceased vs. alive patients, however, there are other studies that did not find differences in the troponin levels between survivors and non-survivors [33], nor differences in CK-MB levels between severe and mild COVID-19 patients [61,79]. We have also observed a correlation between myoglobin levels, a marker that is less specific to cardiac injury, and the need for MV and ICU treatment. Interestingly, another non-cardiac specific marker, CK, was significantly higher in non-survivors than in survivors, but only at the 7th DOH, and not at admission, and in univariate logistic regression CK > 190 U/L at admission and at the 7th DOH were associated with increased risk of MV and ICU treatment, but only CK > 190 U/L at the 7th DOH was associated with the increased risk of death. This might indicate the progression of muscle damage, including rhabdomyolysis and myocardial injury [71,92], in the course of COVID-19. CK was also found to be higher in severe than in mild young adult cases by Zhou et al. [79], but not by Maldonado-Cabrera et al. [61], and Lu et al. [34] did not find a significant difference in CK levels between deceased and alive patients.

Acute kidney injury has also been found to be a predictor of mortality and severity in COVID-19 patients [89–91], and higher levels of serum creatinine and blood urea nitrogen (BUN) have also been associated with an increase in fatality and severe disease [89,90]. In our study, creatinine and urea levels were significantly higher in the non-survivors compared to the survivors, and EGFR was significantly lower in non-survivors compared to survivors. We also observed a positive correlation between creatinine levels and the need for MV, a positive correlation between urea levels and the need for MV and ICU treatment, and a negative correlation between EGFR and the need for MV. Furthermore, univariate logistic regression revealed that EGFR < 60 mL/min at admission and at the 7th DOH, creatinine > 1.2 mg/dL at admission and at the 7th DOH and urea > 49 mg/dL at the 7th DOH were associated with increased risk of death, MV and ICU treatment, and urea > 49 mg/dL at admission with increased risk of MV. Other studies in young adults have also found higher creatinine [33,34] and urea [31,34] levels in deceased vs. alive

patients. However, creatinine levels did not differ between non-survivors and survivors in a study by Liu et al. [31] nor between mild and severe cases in a study by Zhou et al. [79].

Although COVID-19 has also been hypothesized to cause hepatic injury, data on the association of liver enzyme levels and COVID-19 severity and mortality are inconsistent [93–95]. Moreover, hypertransaminasemia observed in some studies may also be due to myocardial and muscle injury or drug-induced hepatotoxicity [93,96]. In our study, ALT at the 7th DOH was significantly higher in survivors compared with non-survivors, however, no other significant differences between these groups regarding ALT, AST, and total bilirubin levels were found. This is in contrast with a study of COVID-19 young adults by Liu et al. [31] that reported ALT, AST, GGT, and bilirubin to be significantly higher in deceased vs. alive patients. Moreover, Zhou et al. [79] found elevated AST in severe vs. mild patients. However, other studies of young adults have found no difference in ALT and AST levels between survivors and non-survivors [33,34], and Zhou et al. [79] did not observe any difference in bilirubin levels between severe vs. mild patients. Interestingly, although there were no significant differences between survivors and non-survivors regarding GGT level, in univariate logistic regression, we found that GGT > 120 U/L at the 7th DOH was associated with increased risk of death, MV, and ICU admission, and GGT > 120 U/L at admission was associated with increased risk of death and MV.

We found albumin concentrations to be significantly lower in non-survivors than in survivors, and there was a moderate to strong negative correlation between albumin concentration and the need for MV and ICU treatment. This agrees with a meta-analysis by Soetedjo et al., which found that hypoalbuminemia was associated with poor prognosis in COVID-19 patients [97]. Similarly, it agrees with previous studies in young adults, which found lower albumin levels in deceased vs. alive patients [31,34], and in severe vs. mild patients [79]. However, decreased albumin levels may not only result from hepatic dysfunction, but also from prioritizing of acute phase proteins synthesis, cytokine-induced increase in vascular permeability leading to albumin extravascular escape, and excessive renal losses due to kidney injury [93,98]. In addition, as albumin has the ability to reduce tissue-damaging oxidative stress and acts as an anticoagulant due to its ability to bind AT III and inhibit platelet aggregation, hypoalbuminemia may further worsen the prognosis [98]. Hence, the prevalence of liver injury in COVID-19 patients may be overestimated [96].

In meta-analyses, hypocalcemia has been found to be significantly associated with COVID-19 severity and mortality [99,100]. In a study by Yang et al. [101], low calcium and phosphorus levels were more prevalent in severe or critical than in moderate COVID-19 patients. The possible mechanisms for a decrease in serum calcium levels in COVID-19 patients include chronic vitamin D deficiency, especially in older patients, hypoalbuminemia, renal insufficiency, the imbalance of parathyroid hormone caused by proinflammatory cytokines, and elevated levels of unsaturated fatty acids that can bind to calcium [99,100]. It is also important to note that calcium is involved in the immune response [99,101]. In our study, total calcium concentration was significantly lower in the non-survivors than in survivors and there was a negative correlation between calcium concentration and the need for MV and ICU treatment. Moreover, univariate logistic regression revealed that calcium < 2.1 mmol/L at admission and at the 7th DOH were associated with increased risk of death, MV and ICU treatment.

Furthermore, vitamin D, an important regulator of calcium homeostasis, has an immunomodulatory role by influencing the production of antimicrobial peptides, as well as counteracting the cytokine storm by inhibiting the production of pro-inflammatory cytokines and promoting anti-inflammatory cytokines, controlling T-cell mediated responses, and modulating the activity of neutrophils and macrophages [102–104]. Several meta-analyses found vitamin D deficiency to be associated with a higher risk of developing severe disease, while data regarding its impact on mortality are inconsistent [103–106]. In our study, we found vitamin D3 levels at admission to be significantly lower in non-survivors than in survivors. Moreover, there was a negative correlation between vitamin D3 levels at the 7th DOH and the need for ICU treatment.

Our study has several limitations. Firstly, it was a single-center retrospective study with a limited sample size that only included severe, hospitalized patients, which may limit the validity of generalizing its results to the entire young adult population. Therefore, larger, multi-center, prospective studies are needed. Secondly, because this study is an observational and exploratory study in which many statistical tests were performed, our results may be influenced by some false-positive error and confounding factors. Moreover, because of the retrospective nature of this study and the limited resources of the health care system at the time, genotypic results of the causative variant were not available. Hence, conclusions regarding the comparison of the wild-type and alpha variants remain presumptive. However, the prevalence of the alpha variant in Poland during the period defined here as the second wave was low (approximately 6.5%), while in the period defined as the third wave the alpha variant accounted for over 92% of the identified strains [10]. These data strongly support that the analyzed waves correspond well to the causative variant.

Therefore, we believe that our study provides valuable data on the impact of infection with the alpha variant of SARS-CoV-2, compared to with wild-type variants, on the severity of the disease, which, in our opinion, might also be of importance in the discussion of the pathogenicity of the next SARS-CoV-2 variants. Noteworthy, none of the patients was vaccinated nor did they have any previous documented SARS-CoV-2 infection. Moreover, there were no significant differences between the waves in terms of the medical treatment used. Hence, we believe that although the variants studied here are no longer dominant, our results are still relevant, as they may provide valuable information on the mechanisms involved in SARS-CoV-2 infection not affected by these factors, which is difficult to achieve in the studies of later variants. Furthermore, most previous studies have focused on predictors of severe COVID-19 in the general population, with older individuals often predominating among those hospitalized, while risk factors among younger individuals appear to be different. In our study, we propose possible predictors of poor COVID-19 outcomes in hospitalized young adults, which may contribute to the early identification of people in this age group at risk of developing severe disease.

## 5. Conclusions

In hospitalized young adults, the SARS-CoV-2 alpha variant does not appear to cause more severe disease than the wild-type variants. Further studies in this age group can be of great use in establishing the influence of current and potential future VOCs on the disease severity. We suggest that a number of factors, including obesity, comorbidities, current or former smoking, the percentage of lung involvement on CT, lower $SpO_2$, leukocytosis, neutrophilia, lymphopenia, higher IG count, NLR, and higher CRP, PCT, IL-6, D-Dimer, LDH, hs-TnI, CK-MB, myoglobin, NT-proBNP, creatinine, urea and GGT levels, lower EGFR, albumin, calcium and vitamin D3 levels, and possibly a decrease in RBC counts and hemoglobin and hematocrit levels and an increase in CK levels in the course of hospitalization may be associated with poor outcomes of COVID-19. The earlier identification of young, high-risk patients and appropriate intervention may improve outcomes. As severe disease and deaths also occur in young adults, health authorities should emphasize the need for preventative measures and support research on predictors of poor outcomes in this age group.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/v14081700/s1, Table S1: Additional data on general and clinical patients' characteristics between the second and the third wave; Table S2: Additional data on comparison of patients' laboratory parameters (as continuous variables) between the second and the third wave; Table S3: Comparison of patients' laboratory parameters (as categorical variables) between the second and the third wave; Table S4: Additional data on comparison of general patients' characteristics between survivors and non-survivors; Table S5: The correlations between patients' general and clinical characteristics (categorical variables) and the need for mechanical ventilation and ICU treatment; Table S6: The correlations between patients' general and clinical characteristics and laboratory parameters (presented as continuous variables) and the need for mechanical ventilation and ICU treatment; Table S7: The correlations between patients' laboratory parameters (presented as categorical variables) and the need for mechanical ventilation and ICU treatment; Table S8: Univariate logistic regression analysis of COVID-19 symptoms for the prediction of death, mechanical ventilation and ICU treatment.

**Author Contributions:** Conceptualization, L.Z.-J.; methodology, L.Z.-J.; data curation, L.Z.-J., Marcin J., J.S.-N., M.J. (Marcin Januszewski), and A.A.J.; formal analysis, L.Z.-J. and M.J. (Marcin Januszewski); investigation, L.Z.-J., M.J. (Marcin Januszewski), J.S.-N., M.J. (Mariusz Janiszewski) and A.A.J.; writing—original draft preparation, L.Z.-J.; writing—review & editing, L.Z.-J., P.D. and A.J.J.; supervision, P.D. and A.J.J. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Central Clinical Hospital of the Ministry of the Interior and Administration in Warsaw (decision number 110/2021, date of approval 24 August 2021).

**Informed Consent Statement:** Patient consent was waived due to the retrospective nature of the study and data anonymization.

**Data Availability Statement:** The original, anonymous dataset is available upon request from the corresponding author.

**Acknowledgments:** The authors wish to thank all study participants for their contribution in this study.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. WHO. WHO Director-General's Opening Remarks at the Media Briefing on COVID-19—11 March 2020. Available online: https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020 (accessed on 10 February 2022).
2. Zhou, P.; Yang, X.L.; Wang, X.G.; Hu, B.; Zhang, L.; Zhang, W.; Si, H.R.; Zhu, Y.; Li, B.; Huang, C.L.; et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* **2020**, *579*, 270–273. [CrossRef] [PubMed]
3. Hoffmann, M.; Kleine-Weber, H.; Schroeder, S.; Krüger, N.; Herrler, T.; Erichsen, S.; Schiergens, T.S.; Herrler, G.; Wu, N.-H.; Nitsche, A.; et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* **2020**, *181*, 271–280.e8. [CrossRef] [PubMed]
4. WHO. WHO Coronavirus (COVID-19) Dashboard. Available online: https://covid19.who.int/ (accessed on 1 July 2022).
5. Raport Zakażeń Koronawirusem (SARS-CoV-2)—Koronawirus: Informacje i Zalecenia. (Report of coronavirus (SARS-CoV-2) infections—Coronaviruses: Information and recommendations.). Available online: https://www.gov.pl/web/koronawirus/wykaz-zarazen-koronawirusem-sars-cov-2 (accessed on 30 June 2022).
6. WHO. Tracking SARS-CoV-2 Variants. Available online: https://www.who.int/activities/tracking-SARS-CoV-2-variants (accessed on 30 June 2022).
7. Centers for Disease Control and Prevention. SARS-CoV-2 Variant Classifications and Definitions. Available online: https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fcoronavirus%2F2019-ncov%2Fvariants%2Fvariant-info.html (accessed on 30 June 2022).
8. European Centre for Disease Prevention and Control. SARS-CoV-2 Variants of Concern as of 7 July 2022. Available online: https://www.ecdc.europa.eu/en/covid-19/variants-concern (accessed on 7 July 2022).

9. European Centre for Disease Prevention and Control. Risk Assessment: SARS-CoV-2—Increased Circulation of Variants of Concern and Vaccine Rollout in the EU/EEA, 14th Update. 2021. Available online: https://www.ecdc.europa.eu/en/publications-data/covid-19-risk-assessment-variants-vaccine-fourteenth-update-february-2021 (accessed on 10 February 2022).
10. European Centre for Disease Prevention and Control. Data on SARS-CoV-2 Variants in the EU/EEA. Available online: https://www.ecdc.europa.eu/en/publications-data/data-virus-variants-covid-19-eueea (accessed on 30 June 2022).
11. Davies, N.G.; Abbott, S.; Barnard, R.C.; Jarvis, C.I.; Kucharski, A.J.; Munday, J.D.; Pearson, C.A.B.; Russell, T.W.; Tully, D.C.; Washburne, A.D.; et al. Estimated transmissibility and impact of SARS-CoV-2 lineage, B.1.1.7 in England. *Science* **2021**, *372*, eabg3055. [CrossRef] [PubMed]
12. Volz, E.; Mishra, S.; Chand, M.; Barrett, J.C.; Johnson, R.; Geidelberg, L.; Hinsley, W.R.; Laydon, D.J.; Dabrera, G.; O'Toole, Á.; et al. Assessing transmissibility of SARS-CoV-2 lineage, B.1.1.7 in England. *Nature* **2021**, *593*, 266–269. [CrossRef] [PubMed]
13. Frampton, D.; Rampling, T.; Cross, A.; Bailey, H.; Heaney, J.; Byott, M.; Scott, R.; Sconza, R.; Price, J.; Margaritis, M.; et al. Genomic characteristics and clinical effect of the emergent SARS-CoV-2 B.1.1.7 lineage in London, UK: A whole-genome sequencing and hospital-based cohort study. *Lancet Infect. Dis.* **2021**, *21*, 1246–1256. [CrossRef]
14. Davies, N.G.; Jarvis, C.I.; CMMID COVID-19 Working Group; Edmunds, W.J.; Jewell, N.P.; Diaz-Ordaz, K.; Keogh, R.H. Increased mortality in community-tested cases of SARS-CoV-2 lineage, B.1.1.7. *Nature* **2021**, *593*, 270–274. [CrossRef] [PubMed]
15. Challen, R.; Brooks-Pollock, E.; Read, J.M.; Dyson, L.; Tsaneva-Atanasova, K.; Danon, L. Risk of mortality in patients infected with SARS-CoV-2 variant of concern 202012/1: Matched cohort study. *BMJ* **2021**, *372*, n579. [CrossRef] [PubMed]
16. Martin-Blondel, G.; Lescure, F.X.; Assoumou, L.; Charpentier, C.; Chapplain, J.-M.; Perpoint, T.; Grouteau, G.; Cordel, H.; Pialoux, G.; Pacanowski, J.; et al. Increased risk of severe COVID-19 in hospitalized patients with SARS-CoV-2 Alpha variant infection: A multicentre matched cohort study. *BMC Infect. Dis.* **2022**, *22*, 540. [CrossRef]
17. Nyberg, T.; Twohig, K.A.; Harris, R.J.; Seaman, S.R.; Flannagan, J.; Allen, H.; Charlett, A.; De Angelis, D.; Dabrera, G.; Presanis, A.M. Risk of hospital admission for patients with SARS-CoV-2 variant, B.1.1.7: Cohort analysis. *BMJ* **2021**, *373*, n1412. [CrossRef]
18. Brookman, S.; Cook, J.; Zucherman, M.; Broughton, S.; Harman, K.; Gupta, A. Effect of the new SARS-CoV-2 variant, B.1.1.7 on children and young people. *Lancet Child Adolesc. Health* **2021**, *5*, e9–e10. [CrossRef]
19. Kayano, T.; Hayashi, K.; Kobayashi, T.; Nishiura, H. Age-Dependent Risks of COVID-19 Putatively Caused by Variant Alpha in Japan. *Front. Public Health* **2022**, *10*, 837970. [CrossRef] [PubMed]
20. Fisman, D.N.; Tuite, A.R. Evaluation of the relative virulence of novel SARS-CoV-2 variants: A retrospective cohort study in Ontario, Canada. *Can. Med. Assoc. J.* **2021**, *193*, E1619–E1625. [CrossRef] [PubMed]
21. Khan, A.; Ichura, C.; Wang, H.; Rezende, I.; Sahoo, M.K.; Huang, C.; Solis, D.; Sibai, M.; Yamamoto, F.; Nyathi, S.; et al. Characterizing the Severity of SARS-CoV-2 Variants at a Single Pediatric Center. *Front. Med.* **2022**, *9*, 896352. [CrossRef] [PubMed]
22. Martin, B.; DeWitt, P.E.; Russell, S.; Anand, A.; Bradwell, K.R.; Bremer, C.; Gabriel, D.; Girvin, A.T.; Hajagos, J.G.; McMurry, J.A.; et al. Characteristics, Outcomes, and Severity Risk Factors Associated With SARS-CoV-2 Infection Among Children in the US National COVID Cohort Collaborative. *JAMA Netw. Open* **2022**, *5*, e2143151. [CrossRef] [PubMed]
23. Kläser, K.; Molteni, E.; Graham, M.; Canas, L.S.; Österdahl, M.F.; Antonelli, M.; Chen, L.; Deng, J.; Murray, B.; Kerfoot, E.; et al. COVID-19 due to the B.1.617.2 (Delta) variant compared to, B.1.1.7 (Alpha) variant of SARS-CoV-2: A prospective observational cohort study. *Sci. Rep.* **2022**, *12*, 10904. [CrossRef] [PubMed]
24. Centers for Disease Control and Prevention. Coronavirus Disease 2019 (COVID-19). 2020. Available online: https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html (accessed on 12 March 2022).
25. Sievers, C.; Zacher, B.; Ullrich, A.; Huska, M.; Fuchs, S.; Buda, S.; Haas, W.; Diercke, M.; An der Heiden, M.; Kröger, S. SARS-CoV-2 Omicron variants BA.1 and BA.2 both show similarly reduced disease severity of COVID-19 compared to Delta, Germany, 2021 to 2022. *Eurosurveillance* **2022**, *27*, 2200396. [CrossRef]
26. Van Goethem, N.; Chung, P.Y.J.; Meurisse, M.; Vandromme, M.; De Mot, L.; Brondeel, R.; Stouten, V.; Klamer, S.; Cuypers, L.; Braeye, T.; et al. Clinical Severity of SARS-CoV-2 Omicron Variant Compared with Delta among Hospitalized COVID-19 Patients in Belgium during Autumn and Winter Season 2021-2022. *Viruses* **2022**, *14*, 1297. [CrossRef] [PubMed]
27. Wolter, N.; Jassat, W.; Walaza, S.; Welch, R.; Moultrie, H.; Groome, M.; Amoako, D.G.; Everatt, J.; Bhiman, J.N.; Scheepers, C.; et al. Early assessment of the clinical severity of the SARS-CoV-2 omicron variant in South Africa: A data linkage study. *Lancet* **2022**, *399*, 437–446. [CrossRef]
28. Williamson, E.J.; Walker, A.J.; Bhaskaran, K.; Bacon, S.; Bates, C.; Morton, C.E.; Curtis, H.J.; Mehrkar, A.; Evans, D.; Inglesby, P.; et al. Factors associated with COVID-19-related death using OpenSAFELY. *Nature* **2020**, *584*, 430–436. [CrossRef] [PubMed]
29. Zhou, F.; Yu, T.; Du, R.; Fan, G.; Liu, Y.; Liu, Z.; Xiang, J.; Wang, Y.; Song, B.; Gu, X.; et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. *Lancet* **2020**, *395*, 1054–1062. [CrossRef]
30. Wu, Z.; McGoogan, J.M. Characteristics of and Important Lessons from the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases from the Chinese Center for Disease Control and Prevention. *JAMA* **2020**, *323*, 1239. [CrossRef] [PubMed]
31. Liu, Z.; Liu, J.; Ye, L.; Yu, K.; Luo, Z.; Liang, C.; Cao, J.; Wu, X.; Li, S.; Zhu, L.; et al. Predictors of mortality for hospitalized young adults aged less than 60 years old with severe COVID-19: A retrospective study. *J. Thorac. Dis.* **2021**, *13*, 3628–3642. [CrossRef] [PubMed]

32. Bayramoglu, Z.; Cingoz, E.; Comert, R.G.; Gasimli, N.; Kaba, O.; Yanartas, M.S.; Torun, S.H.; Somer, A.; Erturk, S.M.; Tunaci, A. Correlation of laboratory parameters and Chest CT findings in young adults with COVID-19 and comparison of imaging findings with children. *Clin. Imaging* **2021**, *79*, 265–272. [CrossRef] [PubMed]
33. Altonen, B.L.; Arreglado, T.M.; Leroux, O.; Murray-Ramcharan, M.; Engdahl, R. Characteristics, comorbidities and survival analysis of young adults hospitalized with COVID-19 in New York City. *PLoS ONE* **2020**, *15*, e0243343. [CrossRef]
34. Lu, Y.; Huang, Z.; Wang, M.; Tang, K.; Wang, S.; Gao, P.; Xie, J.; Wang, T.; Zhao, J. Clinical characteristics and predictors of mortality in young adults with severe COVID-19: A retrospective observational study. *Ann. Clin. Microbiol. Antimicrob.* **2021**, *20*, 3. [CrossRef] [PubMed]
35. Sandoval, M.; Nguyen, D.T.; Vahidy, F.S.; Graviss, E.A. Risk factors for severity of COVID-19 in hospital patients age 18–29 years. *PLoS ONE* **2021**, *16*, e0255544. [CrossRef] [PubMed]
36. Flisiak, R.; Parczewski, M.; Horban, A.; Jaroszewicz, J.; Kozielewicz, D.; Pawłowska, M.; Piekarska, A.; Simon, K.; Tomasiewicz, K.; Zarębska-Michaluk, D. Management of SARS-CoV-2 infection: Recommendations of the Polish Association of Epidemiologists and Infectiologists. Annex no. 2 as of October 13, 2020. *Pol. Arch. Intern. Med.* **2020**, *130*, 915–918. [CrossRef]
37. Zambrano, L.D.; Ellington, S.; Strid, P.; Galang, R.R.; Oduyebo, T.; Tong, V.T.; Woodworth, K.R.; Nahabedian, J.F.; Azziz-Baumgartner, E.; Gilboa, S.M.; et al. Update: Characteristics of Symptomatic Women of Reproductive Age with Laboratory-Confirmed SARS-CoV-2 Infection by Pregnancy Status—United States, January 22–October 3, 2020. *MMWR Morb. Mortal. Wkly. Rep.* **2020**, *69*, 1641–1647. [CrossRef]
38. Januszewski, M.; Ziuzia-Januszewska, L.; Jakimiuk, A.A.; Wierzba, W.; Gluszko, A.; Zytynska-Daniluk, J.; Jakimiuk, A.J. Is the Course of COVID-19 Different during Pregnancy? A Retrospective Comparative Study. *Int. J. Environ. Res. Public Health* **2021**, *18*, 12011. [CrossRef] [PubMed]
39. GOV.UK. NERVTAG Paper on COVID-19 Variant of Concern, B.1.1.7. Available online: https://www.gov.uk/government/publications/nervtag-paper-on-covid-19-variant-of-concern-b117 (accessed on 11 February 2022).
40. Grint, D.J.; Wing, K.; Williamson, E.; McDonald, H.I.; Bhaskaran, K.; Evans, D.; Evans, S.J.; Walker, A.J.; Hickman, G.; Nightingale, E.; et al. Case fatality risk of the SARS-CoV-2 variant of concern, B.1.1.7 in England, 16 November to 5 February. *Eurosurveillance* **2021**, *26*, 2100256. [CrossRef]
41. Sheikh, A.; McMenamin, J.; Taylor, B.; Robertson, C. SARS-CoV-2 Delta VOC in Scotland: Demographics, risk of hospital admission, and vaccine effectiveness. *Lancet* **2021**, *397*, 2461–2462. [CrossRef]
42. Twohig, K.A.; Nyberg, T.; Zaidi, A.; Thelwall, S.; A Sinnathamby, M.; Aliabadi, S.; Seaman, S.R.; Harris, R.J.; Hope, R.; Lopez-Bernal, J.; et al. Hospital admission and emergency care attendance risk for SARS-CoV-2 delta (B.1.617.2) compared with alpha (B.1.1.7) variants of concern: A cohort study. *Lancet Infect. Dis.* **2022**, *22*, 35–42. [CrossRef]
43. Lauring, A.S.; Tenforde, M.W.; Chappell, J.D.; Gaglani, M.; A Ginde, A.; McNeal, T.; Ghamande, S.; Douin, D.J.; Talbot, H.K.; Casey, J.D.; et al. Clinical severity of, and effectiveness of mRNA vaccines against, COVID-19 from omicron, delta, and alpha SARS-CoV-2 variants in the United States: Prospective observational study. *BMJ* **2022**, *376*, e069761. [CrossRef] [PubMed]
44. Stokes, E.K.; Zambrano, L.D.; Anderson, K.N.; Marder, E.P.; Raz, K.M.; El Burai Felix, S.; Tie, Y.; Fullerton, K.E. Coronavirus Disease 2019 Case Surveillance—United States, January 22–May 30, 2020. *MMWR Morb. Mortal. Wkly. Rep.* **2020**, *69*, 759–765. [CrossRef]
45. Xie, Y.; Wang, Z.; Liao, H.; Marley, G.; Wu, D.; Tang, W. Epidemiologic, clinical, and laboratory findings of the COVID-19 in the current pandemic: Systematic review and meta-analysis. *BMC Infect. Dis.* **2020**, *20*, 640. [CrossRef] [PubMed]
46. Owusu, D.; Kim, L.; O'Halloran, A.; Whitaker, M.; Piasecki, A.M.; Reingold, A.; Alden, N.B.; Maslar, A.; Anderson, E.J.; Ryan, P.A.; et al. Characteristics of Adults Aged 18–49 Years Without Underlying Conditions Hospitalized with Laboratory-Confirmed Coronavirus Disease 2019 in the United States: COVID-NET—March–August 2020. *Clin. Infect. Dis.* **2021**, *72*, e162–e166. [CrossRef]
47. Garg, S.; Patel, K.; Pham, H.; Whitaker, M.M.; O'Halloran, A.; Milucky, M.J.; Anglin, M.O.; Kirley, M.P.D.; Reingold, A.; Kawasaki, M.B.; et al. Clinical Trends Among, U.S. Adults Hospitalized With COVID-19, March to December 2020: A Cross-Sectional Study. *Ann. Intern. Med.* **2021**, *174*, 1409–1419. [CrossRef]
48. Richardson, S.; Hirsch, J.S.; Narasimhan, M.; Crawford, J.M.; McGinn, T.; Davidson, K.W.; the Northwell COVID-19 Research Consortium. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. *JAMA* **2020**, *323*, 2052. [CrossRef] [PubMed]
49. Verity, R.; Okell, L.C.; Dorigatti, I.; Winskill, P.; Whittaker, C.; Imai, N.; Cuomo-Dannenburg, G.; Thompson, H.; Walker, P.G.T.; Fu, H.; et al. Estimates of the severity of coronavirus disease 2019: A model-based analysis. *Lancet Infect. Dis.* **2020**, *20*, 669–677. [CrossRef]
50. Luo, H.; Liu, S.; Wang, Y.; Phillips-Howard, P.A.; Ju, S.; Yang, Y.; Wang, D. Age differences in clinical features and outcomes in patients with COVID-19, Jiangsu, China: A retrospective, multicentre cohort study. *BMJ Open* **2020**, *10*, e039887. [CrossRef]
51. Wolff, D.; Nee, S.; Hickey, N.S.; Marschollek, M. Risk factors for COVID-19 severity and fatality: A structured literature review. *Infection* **2021**, *49*, 15–28. [CrossRef] [PubMed]
52. Petrilli, C.M.; Jones, S.A.; Yang, J.; Rajagopalan, H.; O'Donnell, L.; Chernyak, Y.; Tobin, K.A.; Cerfolio, R.J.; Francois, F.; Horwitz, L.I. Factors associated with hospital admission and critical illness among 5279 people with coronavirus disease 2019 in New York City: Prospective cohort study. *BMJ* **2020**, *369*, m1966. [CrossRef] [PubMed]

53. Booth, A.; Reed, A.B.; Ponzo, S.; Yassaee, A.; Aral, M.; Plans, D.; Labrique, A.; Mohan, D. Population risk factors for severe disease and mortality in COVID-19: A global systematic review and meta-analysis. *PLoS ONE* **2021**, *16*, e0247461. [CrossRef] [PubMed]
54. Hu, L.; Chen, S.; Fu, Y.; Gao, Z.; Long, H.; Ren, H.-W.; Zuo, Y.; Wang, J.; Li, H.; Xu, Q.-B.; et al. Risk Factors Associated with Clinical Outcomes in 323 Coronavirus Disease 2019 (COVID-19) Hospitalized Patients in Wuhan, China. *Clin. Infect. Dis.* **2020**, *71*, 2089–2098. [CrossRef] [PubMed]
55. Centers for Disease Control and Prevention. Healthcare Workers. 2020. Available online: https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-care/underlyingconditions.html (accessed on 14 February 2022).
56. Yang, X.; Yu, Y.; Xu, J.; Shu, H.; Xia, J.; Liu, H.; Wu, Y.; Zhang, L.; Yu, Z.; Fang, M.; et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: A single-centered, retrospective, observational study. *Lancet Respir. Med.* **2020**, *8*, 475–481. [CrossRef]
57. Steinberg, E.; Wright, E.; Kushner, B. In Young Adults with COVID-19, Obesity Is Associated with Adverse Outcomes. *West. J. Emerg. Med.* **2020**, *21*, 752–755. [CrossRef]
58. Richardson, S.; Gitlin, J.; Kozel, Z.; Levy, S.; Rahman, H.; Hirsch, J.S.; McGinn, T.; Diefenbach, M.A. In-Hospital 30-Day Survival Among Young Adults with Coronavirus Disease 2019: A Cohort Study. *Open Forum Infect. Dis.* **2021**, *8*, ofab233. [CrossRef]
59. Cunningham, J.W.; Vaduganathan, M.; Claggett, B.L.; Jering, K.S.; Bhatt, A.S.; Rosenthal, N.; Solomon, S.D. Clinical Outcomes in Young US Adults Hospitalized With COVID-19. *JAMA Intern. Med.* **2021**, *181*, 379. [CrossRef]
60. Panda, S.; Roy, S.; Garg, R.K.; Hui, G.; Gorard, J.; Bhutada, M.; Sun, Y.; Bhatnagar, S.; Mohan, A.; Dar, L.; et al. COVID-19 disease in hospitalized young adults in India and China: Evaluation of risk factors predicting progression across two major ethnic groups. *J. Med. Virol.* **2022**, *94*, 272–278. [CrossRef]
61. Maldonado-Cabrera, A.; Angulo-Molina, A.; Haque, U.; Velazquez, C.; Álvarez-Villaseñor, A.S.; Santacruz-Gómez, K.J.; Gallego-Hernández, A.L. Acute Inflammatory Mediators in Young Adult Patients with COVID-19 in Mexico. *Pathogens* **2021**, *10*, 1056. [CrossRef]
62. Ruch, Y.; Kaeuffer, C.; Ohana, M.; Labani, A.; Fabacher, T.; Bilbault, P.; Kepka, S.; Solis, M.; Greigert, V.; Lefebvre, N.; et al. CT lung lesions as predictors of early death or ICU admission in COVID-19 patients. *Clin. Microbiol. Infect.* **2020**, *26*, 1417.e5–1417.e8. [CrossRef] [PubMed]
63. Annoni, A.D.; Conte, E.; Mancini, M.E.; Gigante, C.; Agalbato, C.; Formenti, A.; Muscogiuri, G.; Mushtaq, S.; Guglielmo, M.; Baggiano, A.; et al. Quantitative Evaluation of COVID-19 Pneumonia Lung Extension by Specific Software and Correlation with Patient Clinical Outcome. *Diagnostics* **2021**, *11*, 265. [CrossRef] [PubMed]
64. Liao, D.; Zhou, F.; Luo, L.; Xu, M.; Wang, H.; Xia, J.; Gao, Y.; Cai, L.; Wang, Z.; Yin, P.; et al. Haematological characteristics and risk factors in the classification and prognosis evaluation of COVID-19: A retrospective cohort study. *Lancet Haematol.* **2020**, *7*, e671–e678. [CrossRef]
65. Wu, C.; Chen, X.; Cai, Y.; Xia, J.; Zhou, X.; Xu, S.; Huang, H.; Zhang, L.; Zhou, X.; Du, C.; et al. Risk Factors Associated with Acute Respiratory Distress Syndrome and Death in Patients with Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Intern. Med.* **2020**, *180*, 934. [CrossRef] [PubMed]
66. Liu, J.; Tu, C.; Zhu, M.; Wang, J.; Yang, C.; Liu, W.; Xiong, B. The clinical course and prognostic factors of severe COVID-19 in Wuhan, China: A retrospective case-control study. *Medicine* **2021**, *100*, e23996. [CrossRef]
67. Alsagaby, S.A.; Aljouie, A.; Alshammari, T.H.; Alshammari, T.H.; Mir, S.A.; Alhumaydhi, F.A.; Al Abdulmonem, W.; Alshaalan, H.; Alomaish, H.; Daghistani, R.; et al. Haematological and radiological-based prognostic markers of COVID-19. *J. Infect. Public Health* **2021**, *14*, 1650–1657. [CrossRef] [PubMed]
68. Shi, C.; Wang, L.; Ye, J.; Gu, Z.; Wang, S.; Xia, J.; Xie, Y.; Li, Q.; Xu, R.; Lin, N. Predictors of mortality in patients with coronavirus disease 2019: A systematic review and meta-analysis. *BMC Infect. Dis.* **2021**, *21*, 663. [CrossRef] [PubMed]
69. Mesas, A.E.; Cavero-Redondo, I.; Álvarez-Bueno, C.; Cabrera, M.A.S.; De Andrade, S.M.; Sequí-Dominguez, I.; Martínez-Vizcaíno, V. Predictors of in-hospital COVID-19 mortality: A comprehensive systematic review and meta-analysis exploring differences by age, sex and health conditions. *PLoS ONE* **2020**, *15*, e0241742. [CrossRef]
70. Li, X.; Xu, S.; Yu, M.; Wang, K.; Tao, Y.; Zhou, Y.; Shi, J.; Zhou, M.; Wu, B.; Yang, Z.; et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. *J. Allergy Clin. Immunol.* **2020**, *146*, 110–118. [CrossRef]
71. Malik, P.; Patel, U.; Mehta, D.; Patel, N.; Kelkar, R.; Akrmah, M.; Gabrilove, J.L.; Sacks, H. Biomarkers and outcomes of COVID-19 hospitalisations: Systematic review and meta-analysis. *BMJ Evid.-Based Med.* **2021**, *26*, 107–108. [CrossRef]
72. Polidoro, R.B.; Hagan, R.S.; de Santis Santiago, R.; Schmidt, N.W. Overview: Systemic Inflammatory Response Derived from Lung Injury Caused by SARS-CoV-2 Infection Explains Severe Outcomes in COVID-19. *Front. Immunol.* **2020**, *11*, 1626. [CrossRef] [PubMed]
73. Barnes, B.J.; Adrover, J.M.; Baxter-Stoltzfus, A.; Borczuk, A.; Cools-Lartigue, J.; Crawford, J.M.; Daßler-Plenker, J.; Guerci, P.; Huynh, C.; Knight, J.S.; et al. Targeting potential drivers of COVID-19: Neutrophil extracellular traps. *J. Exp. Med.* **2020**, *217*, e20200652. [CrossRef] [PubMed]
74. Feng, X.; Li, S.; Sun, Q.; Zhu, J.; Chen, B.; Xiong, M.; Cao, G. Immune-Inflammatory Parameters in COVID-19 Cases: A Systematic Review and Meta-Analysis. *Front. Med.* **2020**, *7*, 301. [CrossRef] [PubMed]
75. Luo, X.; Zhou, W.; Yan, X.; Guo, T.; Wang, B.; Xia, H.; Ye, L.; Xiong, J.; Jiang, Z.; Liu, Y.; et al. Prognostic Value of C-Reactive Protein in Patients with Coronavirus 2019. *Clin. Infect. Dis.* **2020**, *71*, 2174–2179. [CrossRef] [PubMed]

76. Gómez-Pastora, J.; Weigand, M.; Kim, J.; Wu, X.; Strayer, J.; Palmer, A.F.; Zborowski, M.; Yazer, M.; Chalmers, J.J. Hyperferritinemia in critically ill COVID-19 patients—Is ferritin the product of inflammation or a pathogenic mediator? *Clin. Chim. Acta* **2020**, *509*, 249–251. [CrossRef] [PubMed]
77. Xu, J.-B.; Xu, C.; Zhang, R.-B.; Wu, M.; Pan, C.-K.; Li, X.-J.; Wang, Q.; Zeng, F.-F.; Zhu, S. Associations of procalcitonin, C-reaction protein and neutrophil-to-lymphocyte ratio with mortality in hospitalized COVID-19 patients in China. *Sci. Rep.* **2020**, *10*, 15058. [CrossRef] [PubMed]
78. Asan, A.; Üstündağ, Y.; Koca, N.; Şimşek, A.; Sayan, H.E.; Parildar, H.; Cilo, B.D.; Huysal, K. Do initial hematologic indices predict the severity of COVID-19 patients? *Turk. J. Med. Sci.* **2021**, *51*, 39–44. [CrossRef]
79. Zhou, C.; Huang, Z.; Tan, W.; Li, X.; Yin, W.; Xiao, Y.; Tao, Z.; Geng, S.; Hu, Y. Predictive factors of severe coronavirus disease 2019 in previously healthy young adults: A single-center, retrospective study. *Respir. Res.* **2020**, *21*, 157. [CrossRef]
80. Shen, Y.; Cheng, C.; Zheng, X.; Jin, Y.; Duan, G.; Chen, M.; Chen, S. Elevated Procalcitonin Is Positively Associated with the Severity of COVID-19: A Meta-Analysis Based on 10 Cohort Studies. *Medicina* **2021**, *57*, 594. [CrossRef]
81. Lippi, G.; Plebani, M. Procalcitonin in patients with severe coronavirus disease 2019 (COVID-19): A meta-analysis. *Clin. Chim. Acta* **2020**, *505*, 190–191. [CrossRef] [PubMed]
82. Zare, M.E.; Wang, Y.; Nasir Kansestani, A.; Almasi, A.; Zhang, J. Procalcitonin Has Good Accuracy for Prognosis of Critical Condition and Mortality in COVID-19: A Diagnostic Test Accuracy Systematic Review and Meta-analysis. *Iran. J. Allergy Asthma Immunol.* **2020**, *19*, 557–569. [CrossRef] [PubMed]
83. Soraya, G.V.; Ulhaq, Z.S. Crucial laboratory parameters in COVID-19 diagnosis and prognosis: An updated meta-analysis. *Med. Clín.* **2020**, *155*, 143–151. [CrossRef]
84. Taneri, P.E.; Gómez-Ochoa, S.A.; Llanaj, E.; Raguindin, P.F.; Rojas, L.Z.; Roa-Díaz, Z.M.; Salvador, D., Jr.; Groothof, D.; Minder, B.; Kopp-Heim, D.; et al. Anemia and iron metabolism in COVID-19: A systematic review and meta-analysis. *Eur. J. Epidemiol.* **2020**, *35*, 763–773. [CrossRef]
85. Lanser, L.; Burkert, F.R.; Bellmann-Weiler, R.; Schroll, A.; Wildner, S.; Fritsche, G.; Weiss, G. Dynamics in Anemia Development and Dysregulation of Iron Homeostasis in Hospitalized Patients with COVID-19. *Metabolites* **2021**, *11*, 653. [CrossRef] [PubMed]
86. Yan, H.; Liang, X.; Du, J.; He, Z.; Wang, Y.; Lyu, M.; Yue, L.; Zhang, F.; Xue, Z.; Xu, L.; et al. Proteomic and metabolomic investigation of serum lactate dehydrogenase elevation in COVID-19 patients. *Proteomics* **2021**, *21*, 2100002. [CrossRef] [PubMed]
87. Wungu, C.D.K.; Khaerunnisa, S.; Putri, E.A.C.; Hidayati, H.B.; Qurnianingsih, E.; Lukitasari, L.; Humairah, I.; Soetjipto. Meta-analysis of cardiac markers for predictive factors on severity and mortality of COVID-19. *Int. J. Infect. Dis.* **2021**, *105*, 551–559. [CrossRef] [PubMed]
88. Ogungbe, O.; Kumbe, B.; Fadodun, O.A.; Latha, T.; Meyer, D.; Asala, A.F.; Davidson, P.M.; Himmelfarb, C.R.D.; Post, W.S.; Commodore-Mensah, Y. Subclinical myocardial injury, coagulopathy, and inflammation in COVID-19: A meta-analysis of 41,013 hospitalized patients. *IJC Heart Vasc.* **2022**, *40*, 100950. [CrossRef] [PubMed]
89. Ouyang, L.; Gong, Y.; Zhu, Y.; Gong, J. Association of acute kidney injury with the severity and mortality of SARS-CoV-2 infection: A meta-analysis. *Am. J. Emerg. Med.* **2021**, *43*, 149–157. [CrossRef] [PubMed]
90. Shao, M.; Li, X.; Liu, F.; Tian, T.; Luo, J.; Yang, Y. Acute kidney injury is associated with severe infection and fatality in patients with COVID-19: A systematic review and meta-analysis of 40 studies and 24,527 patients. *Pharmacol. Res.* **2020**, *161*, 105107. [CrossRef]
91. Hansrivijit, P.; Qian, C.; Boonpheng, B.; Thongprayoon, C.; Vallabhajosyula, S.; Cheungpasitporn, W.; Ghahramani, N. Incidence of acute kidney injury and its association with mortality in patients with COVID-19: A meta-analysis. *J. Investig. Med.* **2020**, *68*, 1261–1270. [CrossRef]
92. Zhu, Z.; Wang, M.; Lin, W.; Cai, Q.; Zhang, L.; Chen, D.; Liu, F.; Xiong, X.; Chu, J.; Peng, J.; et al. Cardiac biomarkers, cardiac injury, and comorbidities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis. *Immun. Inflamm. Dis.* **2021**, *9*, 1071–1100. [CrossRef] [PubMed]
93. Kumar, M.P.; Mishra, S.; Jha, D.K.; Shukla, J.; Choudhury, A.; Mohindra, R.; Mandavdhare, H.S.; Dutta, U.; Sharma, V. Coronavirus disease (COVID-19) and the liver: A comprehensive systematic review and meta-analysis. *Hepatol. Int.* **2020**, *14*, 711–722. [CrossRef] [PubMed]
94. Wu, Z.; Yang, D. A meta-analysis of the impact of COVID-19 on liver dysfunction. *Eur. J. Med. Res.* **2020**, *25*, 54. [CrossRef] [PubMed]
95. Bzeizi, K.; Abdulla, M.; Mohammed, N.; Alqamish, J.; Jamshidi, N.; Broering, D. Effect of COVID-19 on liver abnormalities: A systematic review and meta-analysis. *Sci. Rep.* **2021**, *11*, 10599. [CrossRef] [PubMed]
96. Li, G.; Yang, Y.; Gao, D.; Xu, Y.; Gu, J.; Liu, P. Is liver involvement overestimated in COVID-19 patients? A meta-analysis. *Int. J. Med. Sci.* **2021**, *18*, 1285–1296. [CrossRef]
97. Soetedjo, N.N.M.; Iryaningrum, M.R.; Damara, F.A.; Permadhi, I.; Sutanto, L.B.; Hartono, H.; Rasyid, H. Prognostic properties of hypoalbuminemia in COVID-19 patients: A systematic review and diagnostic meta-analysis. *Clin. Nutr. ESPEN* **2021**, *45*, 120–126. [CrossRef] [PubMed]
98. Kheir, M.; Saleem, F.; Wang, C.; Mann, A.; Chua, J. Higher albumin levels on admission predict better prognosis in patients with confirmed COVID-19. *PLoS ONE* **2021**, *16*, e0248358. [CrossRef] [PubMed]

99. Alemzadeh, E.; Alemzadeh, E.; Ziaee, M.; Abedi, A.; Salehiniya, H. The effect of low serum calcium level on the severity and mortality of COVID patients: A systematic review and meta-analysis. *Immun. Inflamm. Dis.* **2021**, *9*, 1219–1228. [CrossRef] [PubMed]
100. Martha, J.W.; Wibowo, A.; Pranata, R. Hypocalcemia is associated with severe COVID-19: A systematic review and meta-analysis. *Diabetes Metab. Syndr. Clin. Res. Rev.* **2021**, *15*, 337–342. [CrossRef] [PubMed]
101. Yang, C.; Ma, X.; Wu, J.; Han, J.; Zheng, Z.; Duan, H.; Liu, Q.; Wu, C.; Dong, Y.; Dong, L. Low serum calcium and phosphorus and their clinical performance in detecting COVID-19 patients. *J. Med. Virol.* **2021**, *93*, 1639–1651. [CrossRef]
102. Menshawey, E.; Menshawey, R.; Nabeh, O.A. Shedding light on vitamin D: The shared mechanistic and pathophysiological role between hypovitaminosis D and COVID-19 risk factors and complications. *Inflammopharmacology* **2021**, *29*, 1017–1031. [CrossRef] [PubMed]
103. Chiodini, I.; Gatti, D.; Soranna, D.; Merlotti, D.; Mingiano, C.; Fassio, A.; Adami, G.; Falchetti, A.; Eller-Vainicher, C.; Rossini, M.; et al. Vitamin D Status and SARS-CoV-2 Infection and COVID-19 Clinical Outcomes. *Front. Public Health* **2021**, *9*, 736665. [CrossRef] [PubMed]
104. Kaya, M.O.; Pamukçu, E.; Yakar, B. The role of vitamin D deficiency on the COVID-19: A systematic review and meta-analysis of observational studies. *Epidemiol. Health* **2021**, *43*, e2021074. [CrossRef] [PubMed]
105. Dissanayake, H.A.; de Silva, N.L.; Sumanatilleke, M.; de Silva, S.D.N.; Gamage, K.K.K.; Dematapitiya, C.; Kuruppu, D.C.; Ranasinghe, P.; Pathmanathan, S.; Katulanda, P. Prognostic and Therapeutic Role of Vitamin D in COVID-19: Systematic Review and Meta-analysis. *J. Clin. Endocrinol. Metab.* **2021**, *107*, 1484–1502. [CrossRef] [PubMed]
106. Ghasemian, R.; Shamshirian, A.; Heydari, K.; Malekan, M.; Alizadeh-Navaei, R.; Ebrahimzadeh, M.A.; Warkiani, M.E.; Jafarpour, H.; Bazaz, S.R.; Shahmirzadi, A.R.; et al. The role of vitamin D in the age of COVID-19: A systematic review and meta-analysis. *Int. J. Clin. Pract.* **2021**, *75*, e14675. [CrossRef] [PubMed]

## Supplementary Materials

**Table S1.** Additional data on general and clinical patients' characteristics between the second and the third wave.

| Parameter | | Total (N=229) | Second wave (N=75) | Third wave (N=154) | p-value |
|---|---|---|---|---|---|
| BMI, ranges (N=154) | 18-24.9 kg/m² | 19 (12.34%) | 5 (10.2%) | 14 (13.33%) | 0.774* |
| | 25-29.9 kg/m² | 55 (35.71%) | 16 (32.65%) | 39 (37.14%) | 0.718* |
| | 30-34.9 kg/m² | 46 (29.87%) | 16 (32.65%) | 30 (28.57%) | 0.744* |
| | 35-39.9 kg/m² | 23 (14.94%) | 6 (12.24%) | 17 (16.19%) | 0.691* |
| | ≥ 40 kg/m² | 11 (7.14%) | 6 (12.24%) | 5 (4.76%) | 0.105*** |
| Smoking (N=164) | Current | 13 (7.93%) | 3 (5.26%) | 10 (9.35%) | 0.545*** |
| | Current or former | 20 (12.2%) | 6 (10.53%) | 14 (13.08%) | 0.821* |
| Blood type (N=62) | A Rh+ | 18 (29.03%) | 11 (30.56%) | 7 (26.92%) | 0.978* |
| | A Rh- | 3 (4.84%) | 2 (5.56%) | 1 (3.85%) | 1*** |
| | B Rh+ | 12 (19.35%) | 8 (22.22%) | 4 (15.38%) | 0.729* |
| | B Rh- | 4 (6.45%) | 3 (8.33%) | 1 (3.85%) | 0.633*** |
| | AB Rh+ | 2 (3.23%) | 1 (2.78%) | 1 (3.85%) | 1*** |
| | AB Rh- | 2 (3.23%) | 1 (2.78%) | 1 (3.85%) | 1*** |
| | 0 Rh+ | 20 (32.26%) | 9 (25%) | 11 (42.31%) | 0.245* |
| | 0 Rh- | 1 (1.61%) | 1 (2.78%) | 0 (0%) | 1*** |
| Treatment | Steroids | 222 (96.9%) | 74 (98.7%) | 148 (96.1%) | 0.431*** |
| | Remdesivir | 59 (25.8%) | 19 (25.3%) | 40 (26%) | 0.955* |
| | Tocilizumab | 9 (3.9%) | 3 (4%) | 6 (3.9%) | 1*** |
| | Antibiotics | 166 (72.5%) | 56 (74.7%) | 110 (71.4%) | 0.721* |
| | Convalescent plasma | 20 (8.7%) | 7 (9.3%) | 13 (8.4%) | 0.980* |
| | LMWH | 227 (99.1%) | 75 (100%) | 152 (98.7%) | 0.815* |
| SpO2 at admission,% (N=190) | | 90 (87-92) | 89 (85.5-92) | 90 (87-92) | 0.641** |
| Time from the onset of symptoms to hospital admission, days (N=229) | | 8 (7-11) | 8 (6-10) | 9 (7-11) | 0.074** |
| Duration of hospitalization (excluding deceased), days (N=212) | | 10.5 (8-14) | 11 (9-16) | 10 (8-13.25) | 0.036** |
| Time from hospital admission to death (N=16) | | 15 (8.75-27.25) | 20 (14.5-24.75) | 10 (7.25-30.75) | 0.329** |
| Conventional oxygen therapy (N=229) | | 222 (96.94%) | 73 (97.33%) | 149 (96.75%) | 1*** |
| Maximum flow – HFNO, l/min (N=55) | | 60 (57.5-60) | 60 (55-61.25) | 60 (60-60) | 0.814** |
| Maximum FiO2 – HFNO, % (N=55) | | 90 (83.5-95) | 94.5 (87.5-95.25) | 90 (83.5-95) | 0.277** |
| Extubation (N=22) | | 6 (27.27%) | 3 (27.27%) | 3 (27.27%) | 1*** |
| Duration of oxygen therapy (conventional/HFNO/invasive ventilation), days (N=206) | | | 8 (5-12) | 6 (4-10) | 0.814** |
| ECMO (N=229) | | 8 (3.49%) | 4 (5.33%) | 4 (2.6%) | 0.444*** |
| Length of ICU stay (excluding deceased), days (N=16) | | 9 (6-18.25) | 9 (6-25.5) | 9 (6-16) | 1** |
| Time from the onset of symptoms to ICU admission, days (N=31) | | 10 (8.5-13) | 10 (8-13) | 10 (9-13) | 0.524** |
| Vasopressors (N=229) | | 21 (9.17%) | 11 (14.67%) | 10 (6.49%) | 0.077* |
| CRRT (N=229) | | 5 (2.18%) | 3 (4%) | 2 (1.3%) | 0.334** |

w/o – without outliers; * - Chi-squared with Yates correction; ** - Mann-Whitney U test; *** - Fisher's exact test. Continuous variables are presented as median (IQR), categorical variables are presented as N (%).
LMWH – Low molecular-weight heparin, CRRT - continuous renal replacement therapy.

**Table S2.** Additional data on comparison of patients' laboratory parameters (as continuous variables) between the second and the third wave.

| Parameter | Total (N=229) | Second wave (N=75) | Third wave (N=154) | p-value* |
|---|---|---|---|---|
| RBC count - at admission, $\times 10^6/\mu L$ (N=227) | 4.79 (4.54-5.05) | 4.78 (4.46-5.06) (w/o median 4.8) | 4.79 (4.59-5.05) | 0.761 (w/o 0.835) |
| RBC count - 7. DOH, $\times 10^6/\mu L$ (N=187) | 4.72 (4.45-5.04) | 4.62 (4.37-4.99) (w/o median 4.67) | 4.75 (4.5-5.09) | 0.246 (w/o 0.321) |
| Hemoglobin - at admission, g/dL (N=227) | 14.3 (13.45-15.1) | 14.3 (13.35-15.25) | 14.35 (13.6-15.03) | 0.818 (w/o 0.813) |
| Hemoglobin – 7. DOH, g/dL (N=187) | 14 (13-15) | 13.95 (12.8-14.8) | 14.1 (13.2-15.2) | 0.239 |
| Hematocrit - at admission, % (N=227) | 41.6 (39.35-44.05) | 41.2 (39.2-44) | 41.7 (39.68-44.03) | 0.375 (w/o 0.417) |
| Hematocrit – 7. DOH, % (N=187) | 41.7 (38.5-44.6) | 41.25 (37.3-44.25) | 41.9 (39.4-44.6) | 0.094 (w/o 0.128) |
| Ferritin - at admission, ng/mL (N=61) | 1160 (487-1562) | 1160 (725.5-1386) | 1159 (470.5-1567.25) | 0.802 (w/o 0.947) |
| Ferritin – 7. DOH, ng/mL (N=31) | 951 (438-1394) | 920 (585.75-1484.5) | 951 (438-1323.5) | 0.740 |
| IL-6 - at admission, ng/mL (N=116) | 20.75 (7.2-56.6) | 18.8 (8.22-45) | 20.8 (5.56-65.2) | 0.622 |
| IL-6 – 7. DOH, ng/mL (N=53) | 9.1 (4.23-21.8) | 9.19 (6.11-22.2) | 8.45 (2.34-21.05) | 0.426 |
| AT III - at admission, % (N=17) | 95 (85-101) | 93 (76.5-97) | 102.5 (95-128) | 0.159 |
| AT III – 7. DOH, % (N=10) | 93 (90.25-108) | 91 (82-94.75) | 109 (93.75-123) | 0.238 |
| Fibrinogen - at admission, mg/dL (N=62) | 598 (467-728.25) | 646 (510-773) | 585 (466-683) | 0.278 (w/o 0.191) |
| Fibrinogen – 7. DOH, mg/dL (N=39) | 486 (386-578) | 532.5 (385.5-619.75) | 477 (410-557) | 0.548 |
| LDH - at admission, U/L (N=172) | 410 (313-554) | 380 (292.25-530.75) | 419.5 (324.75-575) | 0.178 |
| LDH – 7. DOH, U/L (N=46) | 389.5 (256.75-447.25) | 397 (353-529.75) | 368.5 (243.5-434.5) | 0.181 |
| hs-TnI - at admission, pg/mL (N=153) | 3.4 (3.2-7.9) | 3.9 (2.55-7.35) | 3.2 (3.2-7.83) | 0.667 |
| hs-TnI – 7. DOH, pg/mL (N=40) | 3.2 (2.28-22.1) | 2.45 (1.08-23.1) | 3.2 (3.2-13.25) | 0.244 |
| CK - at admission, U/L (N=142) | 250.5 (114-457) | 207.5 (88.5-465.75) | 256.5 (134-452.25) | 0.268 |
| CK – 7. DOH, U/L (N=38) | 69.5 (35.25-197) | 83.5 (25.75-225) | 67.5 (36.75-129.25) | 0.745 |
| CK-MB - at admission, U/L (N=124) | 19 (15-25) | 19 (15-22) | 18 (15-27) | 0.998 |
| CK-MB – 7. DOH, U/L (N=27) | 18 (13.5-30.5) | 20 (13.5-30) | 15 (14-36) | 0.865 |
| NT-proBNP - at admission, pg/mL (N=142) | 101.5 (43-192.75) | 116.5 (49-234.75) | 100.6 (40.75-178.25) | 0.524 |
| NT-proBNP – 7. DOH, pg/mL (N=42) | 147 (57.75-264.25) | 208.5 (66.5-272) | 119 (65.25-218.25) | 0.247 |
| Myoglobin - at admission, ng/mL (N=15) | 118 (54.5-1459) | 60 (48-218) | 1459 (430.75-2579.5) | 0.078 |
| Myoglobin – 7. DOH, ng/mL (N=11) | 150 (37-277) | 125 (28-248) | 811 (578-1044) | 0.034 |
| Creatinine - at admission, mg/dL (N=222) | 0.9 (0.78-1.04) | 0.92 (0.79-1.06) | 0.89 (0.77-1.03) | 0.301 (w/o 0.170) |
| Creatinine – 7. DOH, mg/dL (N=169) | 0.82 (0.71-0.91) | 0.83 (0.73-0.91) | 0.81 (0.71-0.92) | 0.634 (w/o 0.840) |
| EGFR - at admission, mL/min (N=215) | 90 (77-103) | 88 (77-102) | 91 (77.25-103) (w/o median 90.16) | 0.489 (w/o 0.594) |
| EGFR – 7. DOH, mL/min (N=169) | 101 (89-118) | 100.5 (88.5-122.5) (w/o median 101.5) | 102 (89-115) | 0.815 (w/o 0.854) |
| Urea - at admission, mg/dL (N=209) | 28 (22-36) | 29.5 (23.75-37) | 26 (21.35) | 0.174 (w/o 0.107) |
| Urea – 7. DOH, mg/dL (N=124) | 33 (28-40) | 35.5 (28.5-52.5) (w/o median 35.29) | 32 (27.25-39.75) | 0.170 (w/o 0.164) |
| ALT - at admission, U/L (N=226) | 47 (32.25-68.75) | 45 (34-62.5) | 48 (32-72.5) | 0.506 (w/o 0.574) |
| ALT – 7. DOH, U/L (N=150) | 95 (55-68.75) | 118 (55-174) | 90 (55-140) | 0.224 |
| AST - at admission, U/L (N=219) | 48 (35-71.4) | 45 (33.5-73) | 49.5 (36.78-72.25) | 0.142 (w/o 0.155) |
| AST – 7. DOH, U/L (N=148) | 45 (30-69.25) | 54 (36-71) | 41 (29-64.5) | 0.181 |
| GGT - at admission, U/L (N=58) | 68 (43-152) | 92 (46-120.75) | 62 (40-152) | 0.972 |
| GGT – 7. DOH, U/L (N=41) | 112 (58-192) | 160 (57-208) | 93.5 (58-160) | 0.408 |
| Total bilirubin - at admission, mg/dL (N=165) | 0.43 (0.32-0.57) | 0.41 (0.32-0.54) | 0.44 (0.33-0.58) | 0.440 |
| Total bilirubin – 7. DOH, mg/dL (N=63) | 0.42 (0.3-0.6) | 0.41 (0.28-0.59) | 0.43 (0.3-0.6) | 0.605 |
| Total calcium – at admission, mmol/L (N=42) | 2.14 (2.04-2.23) | 2.13 (2.07-2.19) | 2.14 (2.04-2.23) | 0.959 |
| Total calcium - 7. DOH, mmol/L (N=34) | 2.23 (2.1-2.28) | 2.18 (2.08-2.28) | 2.23 (2.1-2.28) | 0.743 |
| Vitamin D3 - at admission, ng/mL (N=81) | 26.9 (19.5-33.8) | 26.8 (19.75-31.53) | 27.7 (19.5-39.2) | 0.797 (w/o 0.894) |
| Vitamin D3 – 7. DOH, ng/mL (N=6) | 22.45 (16.7-35.4) | - | 22.45 (16.7-35.4) | - |

*- Mann-Whitney U test; w/o – without outliers. All variables are presented as median (IQR).

**Table S3.** Comparison of patients' laboratory parameters (as categorical variables) between the second and the third wave.

| Parameter | | Total (N=229) | Second wave (N=75) | Third wave (N=154) | p-value |
|---|---|---|---|---|---|
| WBC ranges - at admission (N=227) | < 4.5 ×10³/μL | 54 (23.79%) | 12 (16%) | 42 (27.63%) | 0.077* |
| | 4.5-10 ×10³/μL | 139 (61.23%) | 42 (56%) | 97 (63.82%) | 0.321* |
| | >10 ×10³/μL | 34 (14.98%) | 21 (28%) | 13 (8.55%) | <0.001* |
| WBC ranges – 7. DOH (N=187) | < 4.5 ×10³/μL | 7 (3.74%) | 4 (6.45%) | 3 (2.4%) | 0.223** |
| | 4.5-10 ×10³/μL | 122 (65.24%) | 37 (59.68%) | 85 (68%) | 0.336* |
| | >10 ×10³/μL | 58 (31.02%) | 21 (33.87%) | 37 (29.6%) | 0.670* |
| Neutrophil count ranges - at admission (N=226) | < 1.9 ×10³/μL | 13 (5.75%) | 2 (2.67%) | 11 (7.28%) | 0.229** |
| | 1.9-7 ×10³/μL | 160 (70.8%) | 45 (60%) | 115 (76.16%) | 0.018* |
| | >7 ×10³/μL | 53 (23.45%) | 28 (37.33%) | 25 (16.56%) | 0.001* |
| Neutrophil count ranges - 7. DOH (N=187) | < 1.9 ×10³/μL | 5 (2.69%) | 3 (4.92%) | 2 (1.6%) | 0.333** |
| | 1.9-7 ×10³/μL | 127 (68.28%) | 40 (65.57%) | 87 (69.6%) | 0.600* |
| | >7 ×10³/μL | 54 (29.03%) | 18 (29.51%) | 36 (28.8%) | 1* |
| Neutrophil percentage ranges - at admission (N=226) | < 40% | 1 (0.44%) | 0 (0%) | 1 (0.66%) | 1** |
| | 40-68% | 62 (27.43%) | 16 (21.33%) | 46 (30.46%) | 0.197* |
| | >68% | 163 (72.12%) | 59 (78.67%) | 104 (68.87%) | 0.165* |
| Neutrophil percentage ranges - 7. DOH (N=186) | < 40% | 7 (3.76%) | 2 (3.28%) | 5 (4%) | 1** |
| | 40-68% | 113 (60.75%) | 39 (63.93%) | 74 (59.2%) | 0.645* |
| | >68% | 66 (35.48%) | 20 (32.79%) | 46 (36.8%) | 0.709* |
| Lymphocyte count ranges - at admission (N=226) | < 0.9 ×10³/μL | 103 (45.58%) | 34 (45.33%) | 69 (45.7%) | 1* |
| | 0.9-5 ×10³/μL | 123 (54.42%) | 41 (54.67%) | 82 (54.3%) | 1* |
| | >5 ×10³/μL | 0 | 0 | 0 | - |
| Lymphocyte count ranges - 7. DOH, N(%); (N=186) | < 0.9 ×10³/μL | 19 (10.22%) | 8 (13.11%) | 11 (8.8%) | 0.513* |
| | 0.9-5 ×10³/μL | 165 (88.71%) | 53 (86.89%) | 112 (89.6%) | 0.762* |
| | >5 ×10³/μL | 2 (1.08%) | 0 (0%) | 2 (1.6%) | 1** |
| Lymphocyte percentage ranges - at admission (N=226) | < 19% | 138 (61.06%) | 51 (68%) | 87 (57.62%) | 0.173* |
| | 19-48% | 87 (38.5%) | 23 (30.67%) | 64 (42.38%) | 0.119* |
| | >48% | 1 (0.44%) | 1 (1.33%) | 0 (0%) | 0.332** |
| Lymphocyte percentage ranges- 7. DOH (N=186) | < 19% | 64 (34.41%) | 22 (36.07%) | 42 (33.6%) | 0.867* |
| | 19-48% | 118 (63.44%) | 37 (60.66%) | 81 (64.8%) | 0.697* |
| | >48% | 4 (2.15%) | 2 (3.28%) | 2 (1.6%) | 0.599** |
| PLT ranges- at admission (N=227) | < 150 ×10³/μL | 41 (18.06%) | 11 (14.67%) | 30 (19.74%) | 0.453* |
| | 150-400 ×10³/μL | 171 (75.33%) | 58 (77.33%) | 113 (74.34%) | 0.743* |
| | >400 ×10³/μL | 15 (6.61%) | 6 (8%) | 9 (5.92%) | 0.757* |
| PLT ranges – 7. DOH (N=187) | < 150 ×10³/μL | 2 (1.07%) | 1 (1.61%) | 1 (0.8%) | 1** |
| | 150-400 ×10³/μL | 93 (49.73%) | 31 (50%) | 62 (49.6%) | 1* |
| | >400 ×10³/μL | 92 (49.2%) | 30 (48.39%) | 62 (49.6%) | 0.999* |
| NLR ≥2 - at admission (N=226) | | 206 (91.15%) | 69 (92%) | 137 (90.73%) | 0.946* |
| NLR ≥2- 7. DOH (N=186) | | 118 (63.44%) | 43 (70.49%) | 75 (60%) | 0.218* |
| CRP ≥100 mg/L - at admission (N=222) | | 82 (36.94%) | 33 (44%) | 49 (33.33%) | 0.158* |
| CRP ≥100 mg/L - 7. DOH (N=187) | | 9 (4.97%) | 6 (10.17%) | 3 (2.46%) | 0.060** |
| PCT > 0.5 ng/mL – at admission; (N=205) | | 22 (10.73%) | 10 (14.08%) | 12 (8.96%) | 0.372* |
| PCT > 0.5 ng/mL – 7. DOH; (N=102) | | 10 (9.8%) | 5 (14.71%) | 5 (7.35%) | 0.295** |
| D-Dimer > 500 μg/L FEU - at admission; (N=206) | | 154 (74.76%) | 47 (83.93%) | 107 (71.33%) | 0.095* |
| D-Dimer > 500 μg/L FEU – 7. DOH (N=150) | | 121 (80.67%) | 34 (97.14%) | 87 (75.65%) | 0.003** |
| LDH >500 U/L – at admission (N=172) | | 54 (31.4%) | 17 (28.33%) | 37 (33.04%) | 0.645* |
| LDH >500 U/L – 7. DOH (N=46) | | 10 (21.74%) | 4 (28.57%) | 6 (18.75%) | 0.723** |

* - Chi-squared with Yates correction; ** - Fisher's exact test. All variables are presented as N (%).

**Table S4.** Additional data on comparison of general patients' characteristics between survivors and non-survivors.

| Parameter | | Survivors (N=213) | Non-survivors (N=16) | p-value |
|---|---|---|---|---|
| BMI, ranges (N=154) | <25 | 18 (12.95%) | 1 (6.67%) | 0.696* |
| | 25-29.9 | 52 (37.41%) | 3 (20%) | 0.292* |
| | 30-34.9 | 41 (29.5%) | 5 (33.33%) | 0.771* |
| | 35-39.9 | 20 (14.39%) | 3 (20%) | 0.472* |
| | ≥ 40 | 8 (5.76%) | 3 (20%) | 0.077*** |
| Blood type (N=62) | A Rh+ | 12 (25%) | 6 (42.86%) | 0.315* |
| | A Rh- | 3(6.25%) | 0 (0%) | 1*** |
| | B Rh+ | 11 (22.92%) | 1 (7.14%) | 0.267* |
| | B Rh- | 2 (4.17%) | 2 (14.29%) | 0.217*** |
| | AB Rh+ | 2 (4.17%) | 0 (0%) | 1*** |
| | AB Rh- | 2 (4.17%) | 0 (0%) | 1*** |
| | 0 Rh+ | 15 (31.25%) | 5 (35.71%) | 0.755* |
| | 0 Rh- | 1 (2.08%) | 0 (0%) | 1*** |

* - Chi-squared with Yates correction; ** - Mann-Whitney U test; *** - Fisher's exact test. Continuous variables are presented as median (IQR), categorical variables are presented as N (%).

**Table S5.** The correlations between patients' general and clinical characteristics (categorical variables) and the need for mechanical ventilation and ICU treatment.

| Parameter | | Mechanical ventilation | | | | ICU treatment | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | no | yes | phi | p | no | yes | phi | p |
| Sex (N=229) | female | 51 (24.64%) | 6 (27.27%) | 0.02 | 0.990* | 50 (25.25%) | 7 (22.58%) | 0.02 | 0.923* |
| | male | 156 (75.36%) | 16 (72.73%) | | | 148 (74.75%) | 24 (77.42%) | | |
| BMI, ranges (N=154) | 18-24.9 kg/m² | 18 (13.53%) | 1 (4.76%) | 0.09 | 0.474** | 18 (14.17%) | 1 (3.7%) | 0.12 | 0.199** |
| | 25-29.9 kg/m² | 50 (37.59%) | 5 (23.81%) | 0.1 | 0.327** | 47 (37.01%) | 8 (29.63%) | 0.06 | 0.613* |
| | 30-34.9 kg/m² | 40 (30.08%) | 6 (28.57%) | 0.01 | 1* | 38 (29.92%) | 8 (29.63%) | 0 | 1* |
| | 35-39.9 kg/m² | 18 (13.53%) | 5 (23.81%) | 0.1 | 0.318** | 17 (13.39%) | 6 (22.22%) | 0.09 | 0.244** |
| | ≥ 40 kg/m² | 7 (5.26%) | 4 (19.05%) | 0.18 | 0.045** | 7 (5.51%) | 4 (14.81%) | 0.14 | 0.103** |
| Comorbidities (N=229) | Comorbidities (any of the following) | 79 (38.16%) | 14 (63.64%) | 0.15 | 0.037* | 76 (38.38%) | 17 (54.84%) | 0.11 | 0.124* |
| | Hypertension | 30 (14.49%) | 4 (18.18%) | 0.03 | 0.751** | 28 (14.14%) | 6 (19.35%) | 0.05 | 0.423** |
| | Asthma | 17 (8.21%) | 1 (4.55%) | 0.04 | 1** | 15 (7.58%) | 3 (9.68%) | 0.03 | 0.718** |
| | Chronic arrhythmia | 3 (1.45%) | 2 (9.09%) | 0.15 | 0.074** | 4 (2.02%) | 1 (3.23%) | 0.03 | 0.520** |
| | Diabetes | 13 (6.28%) | 5 (22.73%) | 0.18 | 0.019** | 11 (5.56%) | 7 (22.58%) | 0.22 | 0.005** |
| | Insulin resistance | 4 (1.93%) | 2 (.09%) | 0.13 | 0.104** | 4 (2.02%) | 2 (6.45%) | 0.09 | 0.188** |
| | Dyslipidemia | 5 (2.42%) | 1 (4.55%) | 0.04 | 0.458** | 5 (2.53%) | 1 (3.23%) | 0.02 | 0.587** |
| | Hypothyroidism | 8 (3.86%) | 1 (4.55%) | 0.01 | 0.604** | 7 (3.54%) | 2 (6.45%) | 0.05 | 0.350** |
| | Hashimoto disease | 7 (3.38%) | 0 (0%) | 0.06 | 1** | 6 (3.03%) | 1 (3.23%) | 0 | 1** |
| Smoking (N=164) | Current | 10 (6.58%) | 3 (25%) | 0.18 | 0.057** | 9 (6.12%) | 4 (23.53%) | 0.2 | 0.032** |
| | Current or former | 15 (9.87%) | 5 (41.67%) | 0.25 | 0.007** | 14 (9.52%) | 6 (35.29%) | 0.24 | 0.008** |
| Symptoms (N=229) | Dyspnea | 189 (91.3%) | 21 (95.45%) | 0.04 | 1** | 181 (91.41) | 29 (93.55%) | 0.03 | 1 |
| | Fever | 182 (87.92%) | 13 (59.09%) | 0.24 | 0.002** | 174 (87.88%) | 21 (67.74%) | 0.19 | 0.011** |
| | Cough | 180 (86.96%) | 15 (68.18%) | 0.16 | 0.028** | 173 (87.37%) | 22 (70.97%) | 0.16 | 0.027** |
| | Fatigue | 121 (58.45%) | 9 (40.91%) | 0.1 | 0.176* | 116 (58.59%) | 14 (45.16%) | 0.09 | 0.227* |
| | Diarrhea | 40 (19.32%) | 1 (4.55%) | 0.11 | 0.139** | 40 (20.2%) | 1 (3.23%) | 0.15 | 0.041* |
| | Nausea or vomiting | 25 (12.08%) | 2 (9.09%) | 0.03 | 1** | 26 (13.13%) | 1 (3.23%) | 0.11 | 0.140** |
| | Myalgia | 64 (30.92%) | 3 (13.64%) | 0.11 | 0.148** | 61 (30.81%) | 6 (19.35%) | 0.09 | 0.275* |
| | Sore throat | 16 (7.73%) | 2 (.09%) | 0.01 | 0.686** | 16 (8.08%) | 2 (6.45%) | 0.02 | 1** |
| | Headache | 41 (19.81%) | 1 (4.55%) | 0.12 | 0.088** | 39 (19.7%) | 3 (9.68%) | 0.09 | 0.275* |
| | Smell and/or taste disorders | 40 (19.32%) | 3 (13.64%) | 0.04 | 0.774** | 38 (19.19%) | 5 (16.13%) | 0.03 | 0.874* |
| | Smell disorders | 34 (16.43%) | 3 (13.64%) | 0.02 | 1** | 32 (16.16%) | 5 (16.13%) | 0 | 1* |
| | Taste disorders | 34 (16.43%) | 3 (13.64%) | 0.02 | 1** | 32 (16.16%) | 5 (16.13%) | 0 | 1* |
| | Hemoptysis | 7 (3.38%) | 1 (4.55%) | 0.02 | 0.560** | 6 (3.03%) | 2 (6.45%) | 0.06 | 0.296* |
| Lung involvement on CT ≥ 50% (%) (N=207) | | 38 (20.11%) | 13 (72.22%) | 0.34 | <0.001** | 35 (19.13%) | 16 (66.67%) | 0.35 | <0.001* |
| SpO2 at admission <90% (N=190) | | 77 (44%) | 11 (73.33%) | 0.16 | 0.055* | 74 (43.53%) | 14 (70%) | 0.16 | 0.045* |
| Conventional oxygen therapy (N=229) | | 200 (96.62%) | 22 (100%) | 0.06 | 1** | 191 (96.46%) | 31 (100%) | 0.07 | 0.598** |
| HFNO (N=229) | | 36 (17.39%) | 19 (86.36%) | 0.48 | <0.001* | 29(14.65%) | 26 (83.87%) | 0.55 | <0.001* |
| Mechanical ventilation (N=229) | | - | - | - | - | 1 (0.51%) | 21 (67.74%) | 0.78 | <0.001** |
| ECMO (N=229) | | 5 (2.42%) | 3 (13.64%) | 0.18 | 0.032** | 6 (3.03%) | 2 (6.45%) | 0.06 | 0.296** |
| ICU admission (N=229) | | 10 (4.83%) | 21 (95.45%) | 0.78 | <0.001** | - | - | - | - |
| Vasopressors (N=229) | | 0 (0%) | 21 (95.45%) | 0.97 | <0.001** | 1 (0.51%) | 20 (64.52%) | 0.76 | <0.001** |
| CRRT (N=229) | | 0 (0%) | 5 (22.73%) | 0.46 | <0.001** | 0 (0%) | 5 (16.13%) | 0.38 | <0.001** |

* chi-squared test with Yates' correction; ** Fisher's exact test; All variables are presented as N (%).

**Table S6.** The correlations between patients' general and clinical characteristics and laboratory parameters (presented as continuous variables) and the need for mechanical ventilation and ICU treatment.

| Parameter | Mechanical ventilation | | ICU treatment | |
|---|---|---|---|---|
| | rho | p-value* | rho | p-value* |
| Age (N=229) | 0.12 | 0.066 | 0.04 | 0.592 |
| Weight (N=160) | 0.19 (w/o 0.17) | 0.016 (w/o 0.033) | 0.14 (w/o 0.12) | 0.076 (w/o 0.120) |
| BMI (N=154) | 0.21 (w/o 0.17) | 0.009 (w/o 0.040) | 0.19 (w/o 0.16) | 0.017 (w/o 0.054) |
| Percentage of lung involvement on CT (N=207) | 0.35 | <0.001 | 0.36 | <0.001 |
| SpO2 at admission (N=190) | 0.25 (w/o 0.15) | <0.001 (w/o 0.034) | -0.2 (w/o 0.16) | <0.001 (w/o 0.026) |
| WBC - at admission (N=227) | 0.26 | <0.001 | 0.28 | <0.001 |
| WBC - 7. DOH (N=187) | 0.32 (w/o 0.28) | <0.001 | 0.25 (w/o 0.22) | <0.001 (w/o 0.003) |
| Neutrophil count - at admission (N=226) | 0.27 | <0.001 | 0.3 | <0.001 |
| Neutrophil count - 7. DOH (N=186) | 0.39 (w/o 0.38) | <0.001 | 0.36 | <0.001 |
| Neutrophil percentage - at admission (N=226) | 0.2 | 0.002 (w/o 0.003) | 0.27 | <0.001 |
| Neutrophil percentage - 7. DOH (N=186) | 0.34 | <0.001 | 0.39 | <0.001 |
| Lymphocyte count - at admission (N=226) | 0.01 (w/o 0.02) | 0.898 (w/o 0.821) | 0.08 (w/o 0.07) | 0.237 (w/o 0.276) |
| Lymphocyte count - 7. DOH (N=186) | -0.25 | <0.001 (w/o 0.001) | -0.33 | <0.001 |
| Lymphocyte percentage - at admission (N=226) | -0.24 (w/o 0.23) | <0.001 | -0.31 | <0.001 |
| Lymphocyte percentage - 7. DOH (N=186) | -0.43 | <0.001 | -0.46 | <0.001 |
| NLR - at admission (N=226) | 0.23 | <0.001 (w/o 0.001) | 0.3 (w/o 0.31) | <0.001 |
| NLR - 7. DOH (N=186) | 0.42 (w/o 0.39) | <0.001 | 0.45 (w/o 0.43) | <0.001 |
| IG count - at admission (N=217) | 0.32 (w/o 0.30) | <0.001 | 0.35 (w/o 0.32) | <0.001 |
| IG count - 7. DOH (N=186) | 0.29 (w/o 0.21) | <0.001 (w/o 0.005) | 0.20 (w/o 0.13) | 0.007 (w/o 0.068) |
| IG percentage – at admission (N=217) | 0.33 (w/o 0.29) | <0.001 | 0.37 (w/o 0.33) | <0.001 |
| IG percentage – 7. DOH (N=186) | 0.24 (w/o 0.18) | <0.001 (w/o 0.016) | 0.16 (w/o 0.11) | 0.028 (w/o 0.137) |
| RBC count - at admission (N=227) | 0.06 | 0.363 (w/o 0.400) | 0.05 | 0.420 (w/o 0.473) |
| RBC count - 7. DOH (N=187) | -0.41 (w/o -0.42) | <0.001 | -0.34 | <0.001 |
| hemoglobin - at admission to hospital (N=227) | 0.01 | 0.830 (w/o 0.923) | -0.01 (w/o -0.02) | 0.929 (w/o 0.818) |
| hemoglobin - 7. DOH (N=187) | -0.42 | <0.001 | -0.33 | <0.001 |
| hematocrit - at admission (N=227) | 0.05 | 0.435 (w/o 0.472) | 0.04 | 0.537 (w/o 0.592) |
| hematocrit - 7. DOH (N=187) | -0.36 (w/o -0.37) | <0.001 | -0.29 (w/o -0.30) | <0.001 |
| PLT - at admission to hospital (N=227) | 0.14 (w/o 0.15) | 0.038 (w/o 0.025) | 0.19 (w/o 0.17) | 0.003 (w/o 0.012) |
| PLT - 7. DOH (N=187) | -0.04 (w/o -0.01) | 0.573 (w/o 0.911) | -0.03 (w/o 0) | 0.683 (w/o 0.975) |
| CRP - at admission (N=222) | 0.29 (w/o 0.3) | <0.001 | 0.28 (w/o 0.27) | <0.001 |
| CRP - 7. DOH (N=181) | 0.48 (w/o 0.43) | <0.001 | 0.51 (w/o 0.48) | <0.001 |
| Ferritin - at admission (N=61) | 0.29 (w/o 0.3) | 0.025 (w/o 0.018) | 0.22 (w/o 0.24) | 0.093 (w/o 0.068) |
| Ferritin - 7. DOH (N=31) | 0.12 | 0.529 | 0.38 | 0.037 |
| IL-6 - at admission (N=116) | 0.18 | 0.049 | 0.11 | 0.236 |
| IL-6 - 7. DOH (N=53) | 0.33 | 0.015 | 0.43 | 0.001 |
| AT III - at admission (N=17) | 0.16 | 0.542 | 0.12 | 0.650 |
| AT III - 7. DOH (N=10) | -0.29 | 0.413 | -0.29 | 0.413 |
| PCT - at admission (N=205) | 0.38 (w/o 0.039) | <0.001 | 0.37 (w/o 0.39) | <0.001 |
| PCT - 7. DOH (N=102) | 0.56 | <0.001 | 0.61 | <0.001 |
| D-Dimer - at admission (N=206) | 0.24 | <0.001 (w/o 0.001) | 0.27 | <0.001 |
| D-Dimer - 7. DOH (N=150) | 0.36 | <0.001 | 0.36 | <0.001 |
| PT - at admission (N=198) | 0.03 (w/o -0.02) | 0.672 (w/o 0.812) | 0.12 (w/o 0.11) | 0.093 (w/o 0.126) |
| PT - 7. DOH (N=80) | 0.17 (w/o 0.18) | 0.139 (w/o 0.113) | 0.26 (w/o 0.28) | 0.020 (w/o 0.013) |
| INR - at admission (N=199) | 0.02 (w/o -0.03) | 0.768 (w/o 0.780) | 0.12 (w/o 0.07) | 0.092 (w/o 0.469) |
| INR - 7. DOH (N=80) | 0.15 (w/o 0.16) | 0.183 (w/o 0.150) | 0.25 (w/o 0.26) | 0.028 (w/o 0.109) |
| APTT - at admission (N=196) | -0.13 (w/o -0.12) | 0.077 (w/o 0.101) | -0.12 (w/o -0.11) | 0.091 (w/o 0.128) |
| APTT - 7. DOH (N=65) | 0.09 | 0.454 | 0.17 | 0.167 |
| Fibrinogen - at admission (N=62) | -0.08 (w/o 0.04) | 0.538 (w/o 0.751) | -0.07 (w/o -0.04) | 0.574 (w/o 0.740) |
| Fibrinogen - 7. DOH (N=39) | 0.15 | 0.355 | 0.14 | 0.402 |
| LDH - at admission (N=172) | 0.37 | <0.001 | 0.35 | <0.001 |

| | | | | |
|---|---|---|---|---|
| LDH - 7. DOH (N=46) | 0.36 | 0.014 | 0.41 | 0.005 |
| hs-TnI - at admission (N=153) | 0.39 | <0.001 | 0.33 | <0.001 |
| hs-TnI - 7. DOH (N=40) | 0.55 | <0.001 | 0.56 | <0.001 |
| CK - at admission (N=142) | 0.14 | 0.088 | 0.17 | 0.046 |
| CK - 7. DOH (N=38) | 0.55 | <0.001 | 0.43 | 0.007 |
| CK-MB - at admission (N=124) | 0.22 | 0.014 | 0.21 | 0.017 |
| CK-MB - 7. DOH (N=27) | 0.35 | 0.074 | 0.26 | 0.186 |
| NT-proBNP - at admission (N=142) | 0.29 | <0.001 | 0.32 | <0.001 |
| NT-proBNP - 7. DOH (N=42) | 0.48 | 0.001 | 0.39 | 0.010 |
| Myoglobin - at admission (N=15) | 0.69 | 0.004 | 0.39 | 0.147 |
| Myoglobin - 7. DOH (N=11) | 0.60 | 0.053 | 0.60 | 0.053 |
| Creatinine - at admission (N=222) | 0.22 (w/o 0.18) | 0.001 (w/o 0.006) | 0.11 (w/o 0.11) | 0.090 (w/o 0.114) |
| Creatinine - 7. DOH (N=169) | 0.03 (w/o -0.05) | 0.695 (w/o 0.485) | 0 ( w/o -0.07) | 0.959 (w/o 0.355) |
| EGFR - at admission to hospital (N=215) | -0.24 | <0.001 | -0.1 | 0.132 |
| EGFR - 7. DOH (N=169) | -0.02 (w/o 0.05) | 0.835 (w/o 0.488) | 0.04 (w/o 0.1) | 0.598 (w/o 0.204) |
| Urea - at admission (N=209) | 0.28 (w/o 0.24) | <0.001 | 0.26 (w/o 0.25) | <0.001 |
| Urea - 7. DOH (N=124) | 0.53 (w/o 0.47) | <0.001 | 0.56 (w/o 0.52) | <0.001 |
| ALT - at admission (N=226) | 0 (w/o -0.01) | 0.978 (w/o 0.836) | 0.01 (w/o 0.02) | 0.839 (w/o 0.779) |
| ALT - 7. DOH (N=150) | -0.3 | <0.001 | -0.24 | 0.003 |
| AST - at admission (N=219) | 0.13 | 0.057 | 0.09 | 0.203 |
| AST - 7. DOH (N=148) | -0.13 | 0.124 | -0.07 | 0.365 |
| GGT - at admission (N=58) | 0.24 | 0.064 | 0.21 | 0.113 |
| GGT - 7. DOH (N=41) | -0.03 | 0.858 | 0.01 | 0.931 |
| Total bilirubin - at admission (N=165) | 0.13 | 0.089 | 0.16 | 0.039 |
| Total bilirubin - 7. DOH (N=63) | -0.20 | 0.113 | -0.06 | 0.625 |
| Albumin – at admission (N=26) | -0.42 | 0.032 | -0.44 | 0.025 |
| Total calcium – at admission (N=42) | -0.41 | 0.007 | -0.33 | 0.036 |
| Total calcium - 7. DOH (N=34) | -0.51 | 0.002 | -0.49 | 0.004 |
| Vitamin D3 - at admission (N=81) | -0.08 (w/o -0.13) | 0.502 (w/o 0.250) | -0.02 (w/o -0.07) | 0.887 (w/o 0.559) |
| Vitamin D3 - 7. DOH (N=6) | - | - | -0.83 | 0.042 |
| Time from the onset of symptoms to hospital admission (N=229) | -0.14 | 0.034 | -0.10 | 0.441 |
| Maximum flow - HFNO (N=55) | 0.27 | 0.046 | 0.42 | 0.002 |
| Maximum FiO2 – HFNO (N=55) | 0.29 | 0.030 | 0.32 | 0.019 |
| Time from the onset of symptoms to ICU admission (N=31) | -0.21 | 0.257 | - | - |

w/o – without outliers. * - Spearman's correlation.

**Table S7.** The correlations between patients' laboratory parameters (presented as categorical variables) and the need for mechanical ventilation and ICU treatment.

| Parameter | | Mechanical ventilation | | | | ICU treatment | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | no | yes | phi | p | no | yes | phi | p |
| WBC - at admission, ranges (N=227) | <4.5 ×10³/μL | 51 (24.88%) | 3 (13.64%) | 0.08 | 0.361* | 50 (25.1%) | 4 (12.9%) | 0.1 | 0.192* |
| | 4.5-10 ×10³/μL | 132 (64.39%) | 7 (31.82%) | 0.2 | 0.006* | 125 (63.78%) | 14 (45.16%) | 0.13 | 0.075* |
| | >10 ×10³/μL | 22 (10.73%) | 12 (54.55%) | 0.36 | <0.001** | 21 (10.71%) | 13 (41.94%) | 0.3 | <0.001** |
| WBC – 7. DOH, ranges (N=187) | <4.5 ×10³/μL | 7 (4.17%) | 0 (0%) | 0.07 | 1** | 7 (4.43%) | 0 (0%) | 0.08 | 0.598** |
| | 4.5-10 ×10³/μL | 116 (69.05%) | 6 (31.58%) | 0.24 | 0.003* | 109 (68.99%) | 13 (44.83%) | 0.18 | 0.021* |
| | >10 ×10³/μL | 45 (26.79%) | 13 (68.42%) | 0.27 | 0.001* | 42 (26.58%) | 16 (55.17%) | 0.22 | 0.004* |
| Neutrophil count - at admission, ranges (N=226) | <1.9 ×10³/μL | 13 (6.37%) | 0 (0%) | 0.08 | 0.622** | 13 (6.67%) | 0 (0%) | 0.1 | 0.224** |
| | 1.9-7 ×10³/μL | 152 (74.51%) | 8 (36.36%) | 0.25 | <0.001* | 148 (75.9%) | 12 (38.71%) | 0.28 | <0.001* |
| | >7 ×10³/μL | 39 (19.12%) | 14 (63.64%) | 0.31 | <0.001* | 34 (17.44%) | 19 (61.29%) | 0.36 | <0.001* |
| Neutrophil count - 7. DOH, ranges (N=186) | <1.9 ×10³/μL | 5 (2.99%) | 0 (0%) | 0.06 | 1** | 5 (3.18%) | 0 (0%) | 0.07 | 1** |
| | 1.9-7 ×10³/μL | 124 (74.25%) | 3 (15.79%) | 0.38 | <0.001* | 117 (74.53%) | 10 (34.48%) | 0.31 | <0.001* |
| | >7 ×10³/μL | 38 (22.75%) | 16 (84.21%) | 0.41 | <0.001* | 35 (22.29%) | 19 (65.52%) | 0.35 | <0.001* |
| Neutrophil percentage - at admission, ranges (N=226) | <40% | 1 (0.49%) | 0 (0%) | 0.02 | 1** | 1 (0.51%) | 0 (0%) | 0.03 | 1** |
| | 40-68% | 62 (30.39%) | 0 (0%) | 0.2 | 0.005* | 61 (31.28%) | 1 (3.23%) | 0.22 | 0.002* |
| | >68% | 141 (69.12%) | 22 (100%) | 0.2 | 0.005* | 133 (68.21%) | 30 (96.77%) | 0.22 | 0.002* |
| Neutrophil percentage - 7. DOH, ranges (N=186) | <40% | 7 (4.19%) | 0 (0%) | 0.07 | 1** | 7 (4.46%) | 0 (0%) | 0.08 | 0.598** |
| | 40-68% | 108 (64.67%) | 5 (26.32%) | 0.24 | 0.003* | 105 (66.88%) | 8 (27.59%) | 0.29 | <0.001* |
| | >68% | 52 (31.14%) | 14 (73.68%) | 0.27 | 0.001* | 45 (28.66%) | 21 (72.41%) | 0.33 | <0.001* |
| Lymphocyte count - at admission, ranges (N=226) | <0.9 ×10³/μL | 94 (46.08%) | 9 (40.91%) | 0.03 | 0.812* | 87 (44.62%0 | 16 (51.61%) | 0.05 | 0.594* |
| | 0.9-5 ×10³/μL | 110 (53.92%) | 13 (59.09%) | 0.03 | 0.812* | 108 (55.38%) | 15 (48.39%) | -0.05 | 0.594* |
| | >5 ×10³/μL | 0 (0%) | 0 (0%) | - | - | 0 (0%) | 0 (0%) | - | - |
| Lymphocyte count - 7. DOH, ranges (N=186) | <0.9 ×10³/μL | 12 (7.19%) | 7 (36.84%) | 0.2 | 0.001** | 11 (7.01%) | 8 (27.59%) | 0.25 | 0.038** |
| | 0.9-5 ×10³/μL | 153 (91.62%) | 12 (63.16%) | 0.27 | 0.002** | 144 (91.72%) | 21 (72.41%) | 0.22 | 0.007** |
| | >5 ×10³/μL | 2 (1.2%) | 0 (0%) | 0.04 | 1** | 2 (1.27%) | 0 (0%) | 0.04 | 1** |
| Lymphocyte percentage - at admission, ranges (N=226) | <19% | 116 (56.86%) | 22 (100%) | 0.26 | <0.001* | 108 (55.38%) | 30 (96.77%) | 0.29 | <0.001* |
| | 19-48% | 87 (42.65%) | 0 (0%) | 0.26 | <0.001* | 86 (44.1%) | 1 (3.23%) | 0.29 | <0.001* |
| | >48% | 1 (0.49%) | 0 (0%) | 0.02 | 1** | 1 (0.51%) | 0 (0%) | 0.03 | 1** |
| Lymphocyte percentage - 7. DOH, ranges (N=186) | <19% | 47 (28.14%) | 17 (89.47%) | 0.39 | <0.001* | 40 (25.48%) | 24 (82.76%) | 0.44 | <0.001* |
| | 19-48% | 116 (69.46%) | 2 (10.53%) | 0.37 | <0.001* | 113 (71.97%) | 5 (17.24%) | 0.41 | <0.001* |
| | >48% | 4 (2.4%) | 0 (0%) | 0.05 | 1** | 4 (2.55%) | 0 (0%) | 0.06 | 1** |
| NLR ≥2 - at admission (N=226) | | 184 (90.2%) | 22 (100%) | 0.1 | 0.231** | 176 (90.26%) | 30 (96.77%) | 0.08 | 0.324** |
| NLR ≥2- 7. DOH (N=186) | | 99 (59.28%) | 19 (100%) | 0.26 | 0.001* | 90 (57.32%) | 28 (96.55%) | 0.30 | <0.001* |
| PLT - at admission, ranges (N=227) | <150 ×10³/μL | 40 (19.51%) | 1 (4.55%) | 0.12 | 0.139** | 41 (20.92%) | 0 (0%) | 0.19 | 0.010* |
| | 150-400 ×10³/μL | 155 (75.61%) | 16 (72.73%) | 0.02 | 0.970* | 147 (75%) | 24(77.42%) | 0.02 | 0.947* |
| | >400 ×10³/μL | 10 (4.88%) | 5 (22.73%) | 0.21 | 0.008** | 8 (4.08%) | 7 (22.58%) | 0.26 | 0.001** |
| PLT – 7. DOH, ranges (N=187) | <150 ×10³/μL | 1 (0.6%) | 1 (5.26%) | 0.14 | 0.193** | 1 (0.63%) | 1 (3.45%) | 0.1 | 0.287** |
| | 150-400 ×10³/μL | 85 (50.6%) | 8 (42.11%) | 0.05 | 0.646* | 80 (50.63%) | 13 (44.83%) | 0.04 | 0.709* |
| | >400 ×10³/μL | 82 (48.81%) | 10 (52.63%) | 0.02 | 0.941* | 77 (48.73%) | 15 (51.72%) | 0.02 | 0.925* |
| CRP>100 mg/L – at admission (N=222) | | 66 (32.84%) | 16 (76.19%) | 0.26 | <0.001 | 61 (31.94%) | 21 (67.75%) | 0.26 | <0.001 |
| CRP>100 mg/L – 7. DOH (N=181) | | 2 (1.24%) | 7 (35%) | 0.49 | <0.001 | 2 (1.32%) | 7 (24.14%) | 0.39 | <0.001 |
| PCT > 0.5 ng/mL – at admission (N=205) | | 12 (6.56%) | 10 (45.45%) | 0.39 | <0.001** | 11 (6.32%) | 11 (35.48%) | 0.34 | <0.001** |
| PCT > 0.5 ng/mL – 7. DOH (N=102) | | 3 (3.57%) | 7 (38.89%) | 0.45 | <0.001** | 2 (2.63%) | 8 (30.77%) | 0.41 | <0.001** |
| D-Dimer > 500 μg/L FEU - at admission; (N=206) | | 133 (71.89%) | 21 (100%) | 0.20 | 0.003** | 125 (71.02%) | 29 (96.67%) | 0.21 | 0.001** |
| D-Dimer > 500 μg/L FEU – 7. DOH; (N=150) | | 101 (77.69%) | 20 (13.33%) | 0.19 | 0.014** | 93 (76.23%) | 28 (100%) | 0.23 | 0.002** |
| LDH >500 U/L – at admission (N=172) | | 41 (26.11%) | 13 (86.67%) | 0.37 | <0.001** | 37 (24.5%) | 17 (80.95%) | 0.40 | <0.001** |
| LDH >500 U/L – 7. DOH (N=46) | | 8 (19.05%) | 2 (50%) | 0.21 | 0.201** | 6 (15.79%) | 4 (50%) | 0.31 | 0.055** |

* chi-squared test with Yates' correction; ** Fisher's exact test; All variables are presented as N (%).

**Table S8**. Univariate logistic regression analysis of COVID-19 symptoms for the prediction of death, mechanical ventilation and ICU treatment.

| Symptom | death | | | MV | | | ICU treatment | | |
|---|---|---|---|---|---|---|---|---|---|
| | OR | 95%CI | p | OR | 95%CI | p | OR | 95%CI | p |
| Dyspnoea | 1.39 | 0.17-11.1 | 0.202 | 2 | 0.25-15.75 | 0.510 | 1.36 | 0.3-6.21 | 0.690 |
| Fever | 0.25 | 0.09-0.75 | 0.013 | 0.2 | 0.08-0.51 | <0.001 | 0.29 | 0.12-0.69 | 0.005 |
| Cough | 0.25 | 0.09-0.75 | 0.013 | 0.32 | 0.12-0.86 | 0.024 | 0.35 | 0.15-0.85 | 0.021 |
| Fatigue | 0.57 | 0.2-1.59 | 0.281 | 0.49 | 0.2-1.2 | 0.120 | 0.58 | 0.27-1.25 | 0.164 |
| Dairrhoea | 0.29 | 0.04-2.25 | 0.235 | 0.2 | 0.03-1.53 | 0.200 | 0.13 | 0.02-1 | 0.049 |
| Nausea or vomiting | 0.48 | 0.06-3.78 | 0.486 | 0.73 | 0.16-3.3 | 0.681 | 0.22 | 0.03-1.69 | 0.145 |
| Myalgia | 0.33 | 0.07-1.47 | 0.145 | 0.35 | 0.1-1.24 | 0.103 | 0.54 | 0.21-1.38 | 0.198 |
| Sore throat | 0.77 | 0.1-6.18 | 0.805 | 1.19 | 0.26-5.57 | 0.822 | 0.78 | 0.17-3.59 | 0.755 |
| Headache | 0.28 | 0.04-2.18 | 0.224 | 0.19 | 0.03-1.48 | 0.113 | 0.44 | 0.13-1.51 | 0.191 |
| Smell or taste disorders | 0.6 | 0.13-2.74 | 0.509 | 0.66 | 0.19-2.34 | 0.519 | 1 | 0.36-2.79 | 0.996 |
| Smell disorders | 0.72 | 0.16-3.34 | 0.681 | 0.8 | 0.23-2.87 | 0.736 | 1 | 0.36-2.79 | 0.996 |
| Taste disorders | 0.72 | 0.16-3.34 | 0.681 | 0.8 | 0.23-2.87 | 0.736 | 0.81 | 0.29-2.25 | 0.685 |

**Publikacja 4.**

**Tytuł**: Predictors of COVID-19 severity among pregnant patients

**Autorzy**: Januszewski M, Ziuzia-Januszewska L, Jakimiuk AA, Oleksik T, Pokulniewicz M, Wierzba W, Kozlowski K, Jakimiuk AJ

**Czasopismo**: Bosnian Journal of Basic Medical Sciences. 2022

**doi**: 10.17305/bjbms.2022.7181

# Predictors of COVID-19 severity among pregnant patients

**Marcin Januszewski[1], Laura Ziuzia-Januszewska[2], Alicja A. Jakimiuk[3], Tomasz Oleksik[1], Marek Pokulniewicz[1], Waldemar Wierzba[1,4], Krzysztof Kozłowski[5], Artur J. Jakimiuk[1,6*]**

## ABSTRACT

Coronavirus disease 2019 (COVID-19) was declared a pandemic and has spread around the globe, unsparingly affecting vulnerable populations. Effective prevention measures for pregnant women, who are particularly affected, include early identification of those patients at risk of developing in-hospital complications, and the continuous improvement of maternal-fetal treatment strategies to ensure the efficient use of health resources. The objective of our retrospective study was to determine which patient biomarkers on hospital admission correlate with disease severity as measured by disease course classification, the need for oxygen supplementation and higher demand for oxygen, the need for mechanical ventilation, intensive care unit admission, and length of hospital stay. Analysis of 52 PCR SARS-CoV-2 positive pregnant women revealed that the median date of hospital admission was the 30$^{th}$ gestational week, with dyspnea, cough, and fever as the leading symptoms. The presence of diabetes and hypertension predisposed pregnant women to the severe course of illness. Lung involvement shown by CT scans on admission correlated with the greater clinical severity. The main laboratory predictors of disease progression were lymphocytopenia, hypocalcemia, low total cholesterol, low total protein levels, and high serum levels of C-reactive protein, ferritin, interleukin-6, glucose, lactate dehydrogenase, procalcitonin, and troponin I. Further, research with a larger cohort of pregnant women is needed to determine the utility of these results for everyday practice.

KEYWORDS: COVID-19; pregnancy; SARS-CoV-2; clinical course; predictors; disease severity; lymphocytopenia; hypocalcemia; low total protein; inflammation biomarkers

## INTRODUCTION

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is widespread and globally claims more victims with each passing month [1]. As more alarming data about the characteristics of new SARS-CoV-2 variants emerge and as we witness the natural evolution and increased infection rates of COVID-19, it is increasingly important to be able to prioritize critical care services in situations, where the number of patients may be overwhelming. Prenatal care, which is particularly affected, deserves special attention and continuous improvement of its treatment strategies.

The distribution of disease severity in pregnant women is similar to the distribution seen in non-pregnant populations, with 86% of pregnant women manifesting mild disease, 9% severe, and 5% critical [2].

SARS-CoV-2 affects nearly every organ system [3-7], as well as affecting the mental health of both infected and non-infected pregnant women [8]. Leading symptoms include fever (88.7%), cough (67.8%), fatigue (38%), and the over-production of mucus (33.7%) [4]. Severe COVID-19 is characterized by the development of acute respiratory distress syndrome (ARDS), hypotensive shock, and multiorgan failure and requires the patient's admission to intensive care unit (ICU) and mechanical ventilation [3-5].

Severe disease risk factors include comorbidities, advanced age, male sex, obesity, and genetic predispositions [3,4,5,9]. Severe COVID-19 is mainly an immune-mediated disorder triggered by the SARS-CoV-2 infection promoting excessive inflammation and hypercoagulable states [10].

During pregnancy, physiological adaptations of the respiratory tract, immunomodulation, hypercoagulability, processes that increase insulin resistance, and the development of hypertension, predispose SARS-CoV-2-infected women

[1]Department of Obstetrics and Gynecology, Central Clinical Hospital of the Ministry of the Interior and Administration, Warsaw, Poland,
[2]Department of Otolaryngology, Central Clinical Hospital of the Ministry of Interior and Administration, Warsaw, Poland,
[3]Department of Plastic Surgery, Central Clinical Hospital of the Ministry of the Interior and Administration, Warsaw, Poland,
[4]University of Humanities and Economics in Lodz, Satellite Campus in Warsaw, Warsaw, Poland,
[5]Department of Constitutional Law, Jagiellonian University in Krakow, Krakow, Poland,
[6]Center for Reproductive Health, Institute of Mother and Child, Warsaw, Poland

*Corresponding author: Artur J. Jakimiuk, Center for Reproductive Health, Institute of Mother and Child, Warsaw, Poland. E-mail: jakimiuk@yahoo.com

DOI: https://doi.org/10.17305/bjbms.2022.7181

Submitted: 12 March 2022/Accepted: 14 May 2022/
Published online: 05 June 2022

Conflicts of interest: Authors declare no conflicts of interest.

Funding: Authors received no specific funding for this work.

toward a severe course of illness, leading to maternal and fetal mortality and morbidity [10-14].

Data emerging from meta-analyses in the literature show that pregnant women may have an increased risk of developing severe symptoms and a higher risk of pneumonia, ICU admission, the requirement for invasive ventilation and extracorporeal membrane oxygenation (ECMO), and death [15-17].

Moreover, serious adverse outcomes have been observed among pregnant women with previous coronavirus infections, namely, severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) [18], influenza [19,20], and respiratory syncytial virus [21].

There is currently no prognostic biomarker available to identify pregnant patients who are at imminent risk of a severe course of COVID-19, with all associated maternal and fetal complications, and who require immediate medical attention.

The objective of our study was to determine to determine, in which patient characteristics and laboratory results on hospital admission correlate with disease severity as measured by disease course classification, the need for oxygen supplementation and higher demand, the need for mechanical ventilation, ICU admission, and length of hospital stay.

## MATERIALS AND METHODS

### Study population

This retrospective single-center study was undertaken in the Department of Obstetrics and Gynecology, at the Central Clinical Hospital of the Ministry of the Interior and Administration in Warsaw, Poland. The study group comprised 52 pregnant women with COVID-19 who had been admitted for treatment between 15 May 2020 and 26 April 2021.

Inclusion criteria, similar to admission indications for pregnant women with COVID-19, were temperature >39°C despite the use of acetaminophen, respiratory rate >30/min, $SpO_2$ <95% measured at time of admission without oxygen supplementation, patient requiring oxygen, and critical disease. COVID-19 was confirmed using a PCR test prior to admission.

Exclusion criteria were that the patient was admitted to hospital for obstetric and/or other non-COVID-19-related reasons.

### Clinical course of the disease

According to the guidelines of the Polish Association of Epidemiologists and Infectiologists, patients were divided into four cohorts based on the severity of their symptoms and test results, corresponding to the relative course of their illness: mild, moderate, severe, and critical [22].

Mild cases were characteristically clinically stable with mild upper respiratory tract symptoms. Moderate cases included clinical indicators as well as lung involvement shown on imaging. Patients in the severe cohort demonstrated respiratory failure and peripheral $SpO_2$ <90%. Those in the critical cohort were characterized by ARDS, hypotensive shock, multiorgan failure, and loss of consciousness [22].

### Study procedures

On admission, all women underwent complete blood biochemistry and urine tests, a coagulation profile, and in cases where moderate, severe, or critical forms of COVID-19 were suspected, a CT chest scan (without contrast) was performed.

We analyzed the following data: age of patient, body mass index (BMI), gestational age, initial vital signs and symptoms, pre-existing comorbidities such as diabetes mellitus, hypertension, hypothyroidism, asthma, and any history of smoking.

### Ethical statement

The research project was approved by the local Bioethics Committee (Decision Number104/2021).

### Statistical analysis

We used Statistica 13.3 (StatSoft Poland) for our data analysis. Mean values and standard deviations were used to describe the study groups. In case of skewed distributions, the median was calculated as a measure of central tendency, and the scatter of data was shown in relation to the 25$^{th}$ and 75$^{th}$ percentiles. Qualitative variables were presented as percentages. Spearman's rank correlation was used to assess correlation. In case of qualitative variables, the Chi-square test was used to compare the frequencies of the studied characteristics. Differences were considered statistically significant at $p < 0.05$. Logistic regression was performed to analyze the association of patient characteristics and laboratory parameters and the risk of severe-to-critical disease. Non-linear data were categorized. Variables with more than 20% of their values missing were not considered in this analysis, in other cases, missing values were analyzed as a separate category. Comparisons between the four disease severity groups were performed using the Kruskal–Wallis test followed by pair-wise comparison using Dunn's *post hoc* test for continuous variables, and Pearson's chi-square test for categorical variables. As there was only one patient with a critical course of the disease, the severe and critical groups were combined in the analysis as a new grouping, severe-to-critical disease.

## RESULTS

Gestational age ranged from 17 to 37 weeks. Four patients were at 17-22 weeks of gestation, 14 were at 24-28, 17 were at 29-33, 15 were at 34-36, and 2 patients were at >37 weeks of gestation. The mean age of the patients was 31.9 ± 4.79 years. Mean BMI at admission was 28.36 (9.88) kg/m$^2$. None of the patients reported a history of smoking. Symptoms on admission were: dyspnea (n = 48, 92.31%), cough (n = 47, 90.38%), fever (n = 33, 63.46%), fatigue and muscle aches (n = 22, 42.31%), smell and taste disorders (n = 14, 26.92%), headache (n = 12, 23.08%), sore throat (n = 6, 11.54%), and nasal discharge (n = 5, 9.62%). Coexisting diseases were diabetes (n = 9, 17.65%), hypertension (n = 5, 10.00%), hypothyroidism (n = 18, 35.29%), and asthma (n = 2, 3.85%). Mild, moderate, severe, and critical COVID-19 accounted for n = 9 (17.31%), n = 25 (48.08%), n = 17 (32.69%), and n = 1 (1.92%) cases, respectively. The main outcomes measured were length of hospitalization (median = 8 [range = 2-23] days), the need for oxygen supplementation (n = 42, 80.77%), median oxygen flow rate (median = 4 [range = 0-15]), requirement for high-flow therapy (n = 9, 17.31%), and the need for ICU admission (n = 2, 3.85%). There were no cases of tracheal intubation, mechanical ventilation, or ECMO. The median lung involvement seen by CT imaging was 20% (IQR = 11), ranging from 1% to 60%. The most common abnormalities shown in the laboratory results that were elevated C-reactive protein (CRP) (94.23%), elevated D-dimer (90.63%), elevated interleukin 6 (IL-6) (88.46%), elevated fibrinogen (88%), hypoproteinemia (66.67%), decreased vitamin D (62.22%), elevated lactate dehydrogenase (LDH) (56%), hyperglycemia (48.78%), anemia (48.08%), elevated alkaline phosphatase (ALP) (46.15%), elevated aspartate aminotransferase (AST) (40.38%), lymphopenia (38.46%), neutrophilia (30.77%), elevated alanine transaminase (ALT) (30%), and elevated bile acids (35.71%). Data regarding patients' characteristics, clinical course parameters, and laboratory abnormalities are presented in Table 1.

**TABLE 1.** Clinical characteristics of 52 pregnant COVID-19 patients

| Variable | Cases |
|---|---|
| Age, years (n=52) | |
| Mean±SD | 31.9±4.79 |
| Hbd, weeks (n=52) | |
| Median (IQR) | 30 (7) |
| Hbd, ranges, n (%); (n=52) | |
| ≤26 | 12 (23.08) |
| 27-30 | 15 (28.85) |
| 31-33 | 8 (15.38) |
| 34-36 | 15 (28.85) |
| ≥37 | 2 (3.85) |
| Weight, kg (n=43) | |
| Median (IQR) | 77 (26) |
| Body mass index, kg/m$^2$ (n=43) | |
| Median (IQR) | 28.36 (9.88) |
| Preeclampsia, n (%) | 1 (1.96%) |
| Hypothyroidism, n (%) | 18 (35.29%) |
| Hypertension, n (%) | 5 (10.00%) |
| Diabetes mellitus, n (%) | 9 (17.65%) |
| Severity, n (%) | |
| 1 - Mild ilness | 9 (17.31%) |
| 2 - Moderate illness | 25 (48.08%) |
| 3 - Severe illness | 17 (32.69%) |
| 4 - Critical illness | 1 (1.92%) |
| Length of hospitalisation, days (n=52) | |
| Median (IQR) | 8 (6) |
| Percentage of lung involvement on CT, % (n=34) | |
| Median (IQR) | 20 (11) |
| Time from the onset of COVID-19 symptoms, days (n=51) | |
| Median (IQR) | 7 (6) |
| Oxygen flow, l/min (n=52) | |
| Median (IQR) | 4 (4.5) |
| Need for oxygen supplementation, n (%) | 42 (80.77%) |
| Need for invasive ventilation, n (%) | 0 (0.00%) |
| Need for high-flow oxygen therapy, n (%) | 9 (17.31%) |
| Need for ICU admission, n (%) | 2 (3.85%) |
| Hemoglobin (g/dl) (n=52) | |
| Median (IQR) | 12 (1.1) |
| Leukocytes, ×10$^3$/μl (n=52) | |
| Median (IQR) | 7.7 (3.23) |
| Lymphocytes, ×10$^3$/μl (n=52) | |
| Median (IQR) | 0.97 (0.32) |
| Neutrophils, ×10$^3$/μl (n=52) | |
| Median (IQR) | 5.8 (3.11) |
| Platelets (×10$^3$/μl) | |
| Median (IQR) | 184.5 (58.5) |
| ALP, U/L (n=42) | |
| Median (IQR) | 117 (63) |
| AST, U/l (n=52) | |
| Median (IQR) | 28 (18.5) |
| APTT, s (n=49) | |
| Median (IQR) | 33.7 (5.5) |
| ALT, U/l (n=50) | |
| Median (IQR) | 24 (24) |
| Bilirubin, mg/dl (n=49) | |
| Median (IQR) | 0.46 (0.51) |
| NT pro-BNP, pg/ml (n=44) | |
| Median (IQR) | 29.5 (39) |

*(Contd...)*

### Main predictors of severe course of illness

Diabetes as a comorbidity was correlated with the need for high-flow oxygen therapy and higher oxygen flow. Hypertension was correlated with oxygen flow demand during hospitalization. The percentage of lung involvement correlated with four of the six main outcomes: the severity of the course of the COVID-19, the oxygen flow (l/min), the need for high-flow oxygen therapy, and the need for ICU admission (Table 2).

Lymphocytopenia, low levels of serum calcium, total cholesterol and total protein levels, high levels of serum

**TABLE 1.** (*Continued*)

| Variable | Cases |
|---|---|
| Calcium, mmol/l (n=43) | |
| Mean±SD | 2.16±0.09 |
| Total cholesterol, mg/dl | |
| Mean±SD | 188.51±56.09 |
| CK, U/l (n=50) | |
| Median (IQR) | 61.5 (69) |
| CRP, mg/l (n=52) | |
| Median (IQR) | 46.95 (52.4) |
| D-dimer, µg/l FEU (n=32) | |
| Median (IQR) | 1178 (515.5) |
| Ferritin, ng/ml (n=50) | |
| Median (IQR) | 83.5 (109) |
| Fibrinogen, mg/dl (n=50) | |
| Median (IQR) | 553 (225) |
| GGTP, U/L (n=50) | |
| Median (IQR) | 16 (20) |
| Glucose, mg/dl (n=41) | |
| Median (IQR) | 92 (17) |
| Creatinine, mg/dl (n=50) | |
| Median (IQR) | 0.52 (0.17) |
| Lactate dehydrogenase, U/l (n=50) | |
| Median (IQR) | 226.5 (91) |
| Magnesium, mg/dl (n=46) | |
| Median (IQR) | 1.74 (0.16) |
| Lactates, mmol/l (n=42) | |
| Median (IQR) | 1.17 (0.71) |
| Procalcitonin, ng/ml (n=51) | |
| Median (IQR) | 0.14 (0.19) |
| INR (n=51) | |
| Median (IQR) | 1 (0.08) |
| Total protein, g/dl (n=51) | |
| Median (IQR) | 6.13 (0.6) |
| Urea, mg/dl (n=52) | |
| Median (IQR) | 11 (5) |
| Uric acid, mg/dl (n=50) | |
| Median (IQR) | 4.2 (1.7) |
| Vitamin D3 25(OH), ng/ml (n=45) | |
| Mean±SD | 27.15±10.82 |
| Interleukin 6, pg/ml (n=52) | |
| Median (IQR) | 26.5 (31.6) |
| High-sensitive troponin I, ng/ml (n=52) | |
| Median (IQR) | 2.75 (2.15) |

ICU: Intensive care unit; BMI: Body mass index; ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; APPT: Activated partial thromboplastin time; ALT: Alanine aminotransferase; CK: Creatinine kinase; CRP: C-reactive protein; GGTP: Gamma-glutamyl transpeptidase; LDH: Lactate dehydrogenase; INR: Internationalized normalized ratio; IL-6: Interleukin 6; Hs-TnI: High-sensitivity troponin I

CRP, ferritin, IL-6 glucose, LDH, procalcitonin (PCT), and high-sensitivity (hs) troponin I predicted a severe course of illness as measured by disease course classification, the need for oxygen supplementation, higher demand for oxygen supplementation, length of hospital stay, the need for mechanical ventilation, and ICU admission. The results are presented in Table 3. Patients' characteristics and laboratory markers compared across four severity categories are presented in Table 4. Univariate logistic regression revealed that diabetes

**TABLE 2.** Correlation of comorbidities and COVID-19 severity

| Variable | Need for high-flow oxygen therapy | | | Need for invasive ventilation | | | Need for ICU admission | | | Course severity of COVID-19 (1-4) | | | | | Time from the onset of COVID-19 symptoms (days) | Length of hospitalization (days) | Oxygen flow (l/min) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No | Yes | *p*-value* | No | Yes | *p*-value* | No | Yes | *p*-value* | 1 | 2 | 3 | 4 | *p*-value* | *p*-value** | *p*-value** | *p*-value** |
| Hypothyroidism (n=51) | | | | | | | | | | | | | | | | | |
| No | 28 (84.85%) | 5 (15.5%) | 0.530 | 33 (100%) | 0 (0%) | 1 | 31 (93.94%) | 2 (6.06%) | 0.28 | 5 (15.15%) | 17 (51.52%) | 10 (30.3%) | 1 (3.03%) | 0.794 | 0.17 | 0.792 | 0.730 |
| Yes | 14 (77.78%) | 4 (22.22%) | | 18 (100%) | 0 (0%) | | 18 (100%) | 0 (0%) | | 4 (22.22%) | 8 (44.44%) | 6 (33.33%) | 0 (0%) | | | | |
| Hypertension (n=50) | | | | | | | | | | | | | | | | | |
| No | 37 (82.22%) | 8 (17.78%) | 0.9 | 45 (100%) | 0 (0%) | 1 | 43 (95.56%) | 2 (4.44%) | 0.63 | 8 (17.78%) | 23 (51.11%) | 13 (28.89%) | 1 (2.22%) | 0.936 | 0.24 | 0.83 | <0.001 |
| Yes | 4 (80%) | 1 (20%) | | 5 (100%) | 0 (0%) | | 5 (100%) | 0 (0%) | | 1 (20%) | 2 (40%) | 2 (40%) | 0 (0%) | | | | |
| Asthma (n=52) | | | | | | | | | | | | | | | | | |
| No | 41 (82%) | 9 (18%) | 0.509 | 50 (100%) | 0 (0%) | 1 | 48 (96%) | 2 (4%) | 0.773 | 8 (16%) | 25 (50%) | 16 (32%) | 1 (2%) | 0.473 | 1 | 0.980 | 0.54 |
| Yes | 2 (100%) | 0 (0%) | | 2 (100%) | 0 (0%) | | 2 (100%) | 0 (0%) | | 1 (50%) | 0 (0%) | 1 (50%) | 0 (0%) | | | | |
| Diabetes (n=51) | | | | | | | | | | | | | | | | | |
| No | 37 (88%) | 5 (12%) | 0.020 | 42 (100%) | 0 (0%) | 1 | 41 (98%) | 1 (2%) | 0.221 | 8 (19.05%) | 24 (57.14%) | 9 (21.43%) | 1 (2.38%) | 0.011 | 0.602 | 0.373 | 0.03 |
| Yes | 5 (56%) | 4 (44%) | | 9 (100%) | 0 (0%) | | 8 (89%) | 1 (11%) | | 1 (11.11%) | 1 (11.11%) | 7 (77.78%) | 0 (0%) | | | | |

*Pearson's Chi-square test, **U-Mann–Whitney test-corrected for continuity

**TABLE 3.** Correlations of patient`s general and clinical characteristics and COVID-19 outcomes

| Variable | Need for oxygen supplementation | | | Need for high-flow oxygen therapy | | | Need for ICU admission | | | Length of hospitalization (days) | | | Oxygen flow (l/min) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Yes | No | *p*-value* | Yes | No | *p*-value* | Yes | No | *p*-value* | n | *p* | *p*-value** | n | *p* | *p*-value** |
| Weight | 35 | 8 | 0.302 | 36 | 7 | 0.236 | 42 | 1 | <0.001 | 43 | 0.123 | 0.433 | 43 | 0.252 | 0.103 |
| Body mass index | 35 | 8 | 0.302 | 36 | 7 | 0.104 | 42 | 1 | <0.001 | 43 | 0.131 | 0.403 | 43 | 0.283 | 0.066 |
| Age | 42 | 10 | 0.918 | 43 | 9 | 0.475 | 50 | 2 | 0.73 | 52 | 0.026 | 0.856 | 52 | –0.135 | 0.341 |
| Length of hospitalization (days) | 42 | 10 | <0.001 | 43 | 9 | <0.001 | 50 | 2 | 0.183 | | | | 52 | 0.65671 | <0.001 |
| Percentage of lung involvement on CT (%) | 31 | 3 | 0.237 | 27 | 7 | 0.013 | 33 | 1 | <0.001 | 34 | 0.321 | 0.064 | 34 | 0.445 | 0.008 |
| Hemoglobin | 42 | 10 | 0.591 | 43 | 9 | 0.195 | 50 | 2 | 0.386 | 52 | –0.253 | 0.07 | 52 | –0.163 | 0.248 |
| Leukocytes | 42 | 10 | 0.758 | 43 | 9 | 0.52 | 50 | 2 | 0.41 | 52 | –0.252 | 0.0717 | 52 | –0.055 | 0.7 |
| Lymphocytes | 42 | 10 | 0.021 | 43 | 9 | 0.058 | 50 | 2 | 0.573 | 52 | –0.3 | 0.031 | 52 | –0.471 | <0.001 |
| Neutrophils | 42 | 10 | 0.623 | 43 | 9 | 0.924 | 50 | 2 | 0.317 | 52 | –0.144 | 0.309 | 52 | 0.077 | 0.585 |
| Platelets | 42 | 10 | 0.265 | 43 | 9 | 0.431 | 50 | 2 | 0.045 | 52 | –0.2 | 0.164 | 52 | –0.14 | 0.323 |
| ALP | 34 | 8 | 0.912 | 35 | 7 | 0.947 | 40 | 2 | 0.794 | 42 | –0.082 | 0.604 | 42 | –0.123 | 0.438 |
| AST | 42 | 10 | 0.706 | 43 | 9 | 0.46 | 50 | 2 | 0.905 | 52 | 0.415 | 0.002 | 52 | 0.153 | 0.28 |
| APTT | 39 | 10 | 0.064 | 40 | 9 | 0.97 | 47 | 2 | 0.034 | 49 | 0.109 | 0.456 | 49 | 0.2 | 0.168 |
| ALT | 40 | 10 | 0.409 | 41 | 9 | 0.318 | 48 | 2 | 0.864 | 50 | 0.233 | 0.103 | 50 | 0.075 | 0.607 |
| Bilirubin | 39 | 10 | 0.171 | 40 | 9 | 0.352 | 47 | 2 | 0.463 | 49 | 0.042 | 0.777 | 49 | –0.006 | 0.965 |
| NT pro-BNP | 35 | 9 | 0.819 | 38 | 6 | 0.514 | 43 | 1 | <0.001 | 44 | 0.074 | 0.633 | 44 | 0.029 | 0.851 |
| Calcium | 34 | 9 | 0.22 | 36 | 7 | 0.176 | 41 | 2 | 0.199 | 43 | –0.421 | 0.005 | 43 | –0.491 | <0.001 |
| Total cholesterol | 32 | 9 | 0.004 | 35 | 6 | 0.268 | 40 | 1 | <0.001 | 41 | –0.401 | 0.009 | 41 | –0.543 | <0.001 |
| CK | 40 | 10 | 0.641 | 41 | 9 | 0.901 | 48 | 2 | 0.32 | 50 | 0.065 | 0.653 | 50 | 0.097 | 0.502 |
| CRP | 42 | 10 | <0.001 | 43 | 9 | 0.111 | 50 | 2 | 0.633 | 52 | 0.28 | 0.044 | 52 | 0.511 | <0.001 |
| D-dimer | 31 | 1 | <0.001 | 23 | 9 | 0.869 | 30 | 2 | 0.847 | 32 | –0.154 | 0.399 | 32 | –0.101 | 0.583 |
| Ferritin | 40 | 10 | 0.056 | 41 | 9 | 0.054 | 48 | 2 | 0.216 | 50 | 0.423 | 0.002 | 50 | 0.328 | 0.02 |
| Fibrinogen | 40 | 10 | 0.641 | 41 | 9 | 0.71 | 48 | 2 | 0.392 | 50 | –0.249 | 0.082 | 50 | –0.041 | 0.778 |
| Gamma-glutamyl transpeptidase | 40 | 10 | 0.074 | 41 | 9 | 0.44 | 48 | 2 | 0.826 | 50 | 0.206 | 0.151 | 50 | 0.168 | 0.242 |
| Glucose | 32 | 9 | 0.012 | 36 | 5 | 0.003 | 41 | 0 | <0.001 | 41 | 0.388 | 0.012 | 41 | 0.518 | <0.001 |
| Creatinine | 40 | 10 | 0.765 | 41 | 9 | 0.502 | 48 | 2 | 0.276 | 50 | –0.192 | 0.182 | 50 | –0.136 | 0.347 |
| Lactate dehydrogenase | 40 | 10 | 0.022 | 41 | 9 | 0.0244 | 48 | 2 | 0.367 | 50 | 0.426 | 0.002 | 50 | 0.374 | 0.007 |
| Magnesium | 37 | 9 | 0.311 | 37 | 9 | 0.683 | 44 | 2 | 0.816 | 46 | 0.187 | 0.214 | 46 | 0.127 | 0.402 |
| Lactates | 33 | 9 | 0.092 | 37 | 5 | 0.52 | 42 | 0 | <0.001 | 42 | 0.276 | 0.077 | 42 | 0.283 | 0.07 |
| Procalcitonin | 41 | 10 | 0.001 | 42 | 9 | 0.005 | 49 | 2 | 0.056 | 51 | 0.509 | <0.001 | 51 | 0.573 | <0.001 |
| PT | 41 | 10 | 0.419 | 42 | 9 | 0.118 | 49 | 2 | 0.48 | 51 | 0.1 | 0.495 | 51 | 0.305 | 0.029 |
| Total protein | 41 | 10 | 0.019 | 42 | 9 | 0.069 | 49 | 2 | 0.245 | 51 | –0.406 | 0.003 | 51 | –0.485 | <0.001 |
| Urea | 42 | 10 | 0.149 | 43 | 9 | 0.417 | 50 | 2 | 0.018 | 52 | 0.046 | 0.744 | 52 | –0.114 | 0.42 |
| Uric acid | 40 | 10 | 0.711 | 42 | 8 | 0.631 | 49 | 1 | <0.001 | 50 | –0.036 | 0.804 | 50 | –0.129 | 0.372 |
| Vitamin D3 25(OH) | 35 | 10 | 0.697 | 39 | 6 | 0.987 | 45 | 0 | <0.001 | 45 | 0.035 | 0.818 | 45 | 0.052 | 0.734 |
| Troponin I | 42 | 10 | 0.365 | 43 | 9 | 0.156 | 50 | 2 | 0.386 | 52 | 0.111 | 0.435 | 52 | 0.285 | 0.041 |
| Interleukin 6 | 42 | 10 | <0.001 | 43 | 9 | 0.018 | 50 | 2 | 1 | 52 | 0.422 | 0.002 | 52 | 0.618 | <0.001 |
| Time from the onset of COVID-19 symptoms (days) | 41 | 10 | 0.055 | 43 | 8 | 0.55 | 49 | 2 | 0.98 | 51 | 0.257 | 0.069 | 51 | 0.218 | 0.124 |
| Oxygen flow (l/min) | 42 | 10 | <0.001 | 43 | 9 | <0.001 | 50 | 2 | 0.030 | 52 | 0.658 | <0.001 | | | |
| High-flow oxygen therapy – flow (l/min) | 9 | 0 | <0.001 | 0 | 9 | <0.001 | 7 | 2 | 0.889 | 9 | 0.008 | 0.983 | 9 | | |
| High-flow oxygen therapy – $FiO_2$ | 9 | 0 | <0.001 | 0 | 9 | <0.001 | 7 | 2 | 0.667 | 9 | –0.068 | 0.862 | 9 | | |

*U-Mann–Whitney test corrected for continuity; **Spearman test. ICU: Intensive care unit; BMI: Body mass index; ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; APPT: Activated partial thromboplastin time; ALT: Alanine aminotransferase; CK: Creatinine kinase; CRP: C-reactive protein; GGTP: Gamma-glutamyl transpeptidase; LDH: Lactate dehydrogenase; PT: Prothrombin time; IL-6: Interleukin 6

(odds ratio [OR] 10.18, 95% CI 1.83-56.54; $p$ = 0.008), gestational age < 32 weeks (OR 5, 95% CI 1.36-18.43; $p$ = 0.016), lung involvement on CT imaging > 20% (OR 5.8, 95% CI 1.54-21.81, $p$ = 0.009), lymphocyte count < 1($x10^3/\mu l$) (OR 27.43, 95% CI 3.26-231.58; $p$ = 0.002), calcium level ≤ 2.15 (mmol/l) (OR 5.56, 95% CI 1.61-19.22; $p$ = 0.007), CRP > 75(mg/l) (OR 9.11, 95% CI 2.38-34.85; $p$ = 0.001), IL-6 > 60 (pg/ml) (OR 16.5, 95% CI 1.8-151.58; $p$ = 0.013), procalcitonin > 0.2(ng/ml) (OR 5.11, 95% CI 1.49-17.56; $p$ = 0.010), LDH > 270 (U/l) (OR 3.73, 95% CI 1.04-13.45; $p$ = 0.044), total cholesterol ≤ 180 (mg/dl) (OR

**TABLE 4.** Association of patients' characteristics and COVID-19 severity

| Variable | Course severity of COVID-19 (1-4) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3-4 | H | *p*-value |
| Age, years | 32 (31-35) | 33 (29-33) | 31 (29-35) | 0.406 | 0.816 |
| Gestational age, weeks | 33 (26-35) | 32 (29-34) | 28 (26-31) | 4.433 | 0.109 |
| Weight, kg | 72.5 (61.5-86.5) | 76 (71-84) | 92.5 (68-102) | 3.099 | 0.212 |
| Body mass index, kg/m² | 25.72 (22.72-31.72) | 27.43 (25.65-30.08) | 33.71 (26.17-37.64) | 3.69 | 0.158 |
| Percentage of lung involvement on CT, % | - | 16.5 (10-20) | 25 (19.5-41) | 0 | 1 ($p^{2/3-4}$=0.028) |
| Hemoglobin, g/dl | 12 (11.9-12.4) | 12 (11.6-12.6) | 11.65 (11-12.1) | 2.727 | 0.256 |
| Leukocytes, ×10³/μL | 7.7 (5.4-8.95) | 7.75 (6.59-9.34) | 7.73 (5.21-8.88) | 0.586 | 0.746 |
| Lymphocytes, ×10³/μL | 1.12 (0.88-1.32) | 1.1 (0.92-1.1) | 0.85 (0.8-0.93) | 10.702 | 0.005 ($p^{2/3-4}$=0.006) |
| Neutrophils, ×10³/μL | 5.53 (4.19-6.97) | 5.78 (5.1-7.81) | 6.09 (3.98-7.5) | 0.883 | 0.643 |
| Platelets, ×10³/μL | 188 (179-192) | 191 (165-249) | 176.5 (145-200) | 1.324 | 0.515 |
| Alkaline phosphatase, U/l | 112 (95-165) | 137 (95-158) | 94 (89-126) | 3.145 | 0.207 |
| Aspartate aminotransferase, U/l | 26 (19-28) | 31 (22-39) | 26 (19-54) | 1.908 | 0.385 |
| Activated partial thromboplastin time, s | 31.5 (31.2-35.6) | 34.1 (31.7-35.8) | 34.35 (32-39.45) | 1.352 | 0.507 |
| Alanine aminotransferase, U/l | 30 (19-31) | 22 (12.5-40.5) | 26 (13-59) | 0.236 | 0.889 |
| Bilirubin, mg/dl | 0.55 (0.21-0.81) | 0.38 (0.3-0.78) | 0.53 (0.26-0.71) | 0.017 | 0.991 |
| NT pro-BNP, pg/ml | 31 (23-39.5) | 29 (16-48) | 44 (16-128) | 0.277 | 0.871 |
| Calcium, mmol/l | 2.22 (2.16-2.29) | 2.19 (2.12-2.22) | 2.11 (2.07-2.14) | 9.875 | 0.007 ($p^{1/3-4}$=0.020; $p^{2/3-4}$=0.027) |
| Total cholesterol, mgdl | 221 (203-262.5) | 196 (180-206) | 160.5 (122-194) | 11.051 | 0.004 ($p^{1/3-4}$=0.003) |
| Creatine kinase, U/l | 62 (21-78) | 60 (27-95.5) | 56 (32-99) | 0.061 | 0.970 |
| C-reactive protein, mg/l | 18.4 (11.4-40.8) | 48 (38.3-66.3) | 86.15 (36.9-126.5) | 12.980 | 0.002 ($p^{1/2}$=0.045; $p^{1/3-4}$<0.001) |
| D-dimer, μg/l FEU | 1721 (1046-2396) | 1186.5 (1029-1396) | 1088.5 (828-1386) | 1.288 | 0.525 |
| Ferritin, ng/ml | 59 (37-72) | 84 (50.5-146.5) | 130 (75-188) | 6.761 | 0.034 ($p^{1/3-4}$=0.028) |
| Fibrinogen, mg/dl | 410 (392-596) | 590.5 (457.5-663) | 519 (467-683) | 2.41 | 0.299 |
| Gamma-glutamyl transpeptidase, U/l | 10 (6-16) | 19 (11.5-29.5) | 17 (9-33) | 3.223 | 0.200 |
| Glucose, mg/dl | 84 (80-92) | 90 (86-97) | 101.5 (94-113) | 7.627 | 0.022 ($p^{1/3-4}$=0.027) |
| Creatinine, mg/dl | 0.51 (0.49-0.65) | 0.52 (0.47-0.61) | 0.5 (0.41-0.6) | 1.174 | 0.556 |
| lactate dehydrogenase, U/l | 203 (189-210) | 235 (195.5-267.5) | 245 (198-289) | 4.039 | 0.132 |
| Magnesium, mg/dl | 1.69 (1.67-1.76) | 1.74 (1.64-1.8) | 1.77 (1.67-1.98) | 1.451 | 0.484 |
| Lactates, mmol/l | 1 (0.82-1.31) | 1.17 (0.95-1.68) | 1.33 (0.95-1.86) | 2.183 | 0.336 |
| Procalcitonin, ng/ml | 0.07 (0.05-0.08) | 0.14 (0.09-0.22) | 0.24 (0.14-0.5) | 16.626 | <0.001 ($p^{1/2}$=0.026; $p^{1/3-4}$<0.001) |
| Prothrombin time, s | 1.01 (0.96-1.04) | 0.98 (0.97-1.01) | 1.05 (1-1.11) | 7.013 | 0.030 ($p^{2/3-4}$=0.025) |
| Total protein, g/dl | 6.43 (6.13-6.66) | 6.34 (6.04-6.47) | 5.84 (5.65-6.1) | 13.283 | 0.001 ($p^{1/3-4}$=0.011; $p^{1/34}$=0.003) |
| Urea, mg/dl | 12 (11-14) | 11 (9-14) | 11 (8-14) | 1.040 | 0.595 |
| Uric acid, mg/dl | 4.3 (3-4.6) | 4.2 (3.6-5.7) | 3.8 (2.7-4.75) | 3.696 | 0.158 |
| Vitamin D3 25(OH), ng/ml | 25.2 (23-33.6) | 29.1 (16-34.6) | 26 (19.1-30) | 0.907 | 0.635 |
| Troponin I, ng/ml | 1.4 (1.1-2) | 2.8 (0.8-3.2) | 3.2 (1.6-6.8) | 6.624 | 0.036 |
| Interleukin 6, pg/ml | 15.2 (5.92-17) | 28 (14.6-36.9) | 46.55 (22.9-65.7) | 12.509 | 0.002 ($p^{1/3-4}$=0.001) |

Data are presented as median (interquartile ranges). *P* values were calculated using the Kruskal–Wallis test (df=2 for all variables) followed by Dunn's *post hoc* test. $p^{1/2}$ values indicate the comparison between course severity 1 (mild disease) and 2 (moderate disease); $p^{1/3-4}$ values indicate the comparison between course severity 1 (mild disease) and 3-4 (severe or critical disease); $p^{2/3-4}$ values indicate the comparison between course severity 2 (moderate disease) and 3-4 (severe or critical disease). ICU: Intensive care unit; BMI: Body mass index; ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; APPT: Activated partial thromboplastin time; ALT: Alanine aminotransferase; CK: Creatinine kinase; CRP: C-reactive protein; GGTP: Gamma-glutamyl transpeptidase; LDH: Lactate dehydrogenase; PT: Prothrombin time; IL-6: Interleukin 6

5.65, 95% CI 1.53-20.93; $p$ = 0.010), total protein level ≤ 6.3 (g/dl) (OR 9, 95% CI 1.79-45.34; $p$ = 0.008), hs-troponin I > 6 (ng/ml) (OR 12.69, 95% CI 1.35-119.34), and glucose > 99 (mg/dl) (OR 6, 95% CI 1.48-24.27; $p$ = 0.012) were associated with increased risk of severe-to-critical COVID-19.

## DISCUSSION

In our study, the median date of the pregnant women's hospital admission was the 30th gestational week (range = 17-37th week). Because COVID-19 is an immune-mediated intracellular viral infection, it may pose a threat during pregnancy due to the special immunological adaptations that improve a pregnant woman's tolerance to the fetal semi-allograft late in the second trimester and the increased inflammatory response in the third trimester [10,14,23-25]. In addition, hypertension, diabetes, and cardiovascular diseases that develop during the third trimester may predispose pregnant women to the severe course of illness. Therefore, we advise vaccination in the second trimester for maternal and fetal benefits [26].

We found that median lung involvement was 20%, with a range of 1-60%, and as lung involvement at the time of admission correlated with 4 of the 6 main outcomes – the severity

of the course of the COVID-19 disease, oxygen flow (l/min), the need for high-flow oxygen therapy, and the need for ICU admission – lung involvement may be considered as a predictor of disease aggravation. Based on the pathophysiology of COVID-19 progression, it seems like a truism to claim that the greater lung involvement is, the greater the severity of the disease, which is corroborated in our findings. However, the previous studies in pregnant women report contrasting results: One indicating greater lung involvement among pregnant women, and another reporting that lung involvement was similar in both pregnant and non-pregnant subjects [27,28].

Several previous studies have attempted to determine which laboratory parameters correlate with disease severity among pregnant women with COVID-19. Those studies found that subjects' laboratory results largely mirrored those in the adult non-pregnant population, especially regarding lymphopenia and inflammation parameters.

Severe COVID-19 is associated with higher levels of inflammatory markers than in mild disease. Therefore, tracking these markers may permit early identification of patients at risk of disease progression. Likewise, a link between increased cardiac markers and disease aggravation with a few potential pathomechanisms is well established in the literature [29]. COVID-19 can cause direct or indirect heart injury: cardiomyocyte viral infection, cytokine-mediated systemic inflammation, supply-demand mismatch, and micro- and macrovascular thrombosis [29-31].

Lymphopenia has been identified as the most distinctive predictive parameter [32-34]. In a study by Lombardi et al., lymphocyte values at admission correlated with the oxygen need. CRP levels were found to be the inflammatory biomarker that better mirrored the course of the disease than D-dimer or ferritin levels, which were not reliable predictors of a poor outcome [32]. The retrospective study of 217 pregnant women with COVID-19 by Bozkurt et al. showed that elevated LDH, CRP, IL-6, and ferritin levels coupled with low albumin levels on hospital admission were predictive parameters for a more severe course of illness, and that elevated serum levels of blood urea nitrogen and creatine were the most predictive parameters for ICU admission [35]. Data support that the host's immune system overreaction (cytokine storm syndrome or cytokine release syndrome) may play an important role in the pathogenesis of SARS [36]. SARS-CoV infection may lead to hyper-induction of the immune system, causing increased levels of cytokines, e.g., IL-6 and chemokines, all of which have been observed in SARS patients. However, there are also contradicting results [37]. Data have shown that IL-6 levels are also significantly higher in COVID-19 patients with severe disease compared with those with a non-severe condition. Therefore, IL-6 is a prognostic marker for serious COVID-19 cases in pregnant [38] and non-pregnant cohorts [39-41].

Our study identified a positive correlation between exact glucose values at admission and poorer patient outcomes. This observation suggests that the elevated blood sugar levels we observed may be the result of physiological stress triggered by the disease. COVID-19 disrupts glucose regulation, rendering poor glycemic control, and necessitating particularly careful management in patients with diabetes [42,43]. Indeed, prior work has shown that even in cases of well-controlled pre-existing diabetes, hyperglycemia was commonly observed in acutely ill hospitalized patients and linked to adverse outcomes [44,45]. It seems that COVID-19 may lead to high blood glucose levels in patients with normal glycemic status by modulating immune and inflammatory responses, directly affecting morbidity and mortality [46-48]. In a study by Charoenngam et al. in patients without a history of diabetes, hyperglycemia on the day of admission was shown to have a statistically significant association with mortality, ICU admission, intubation, acute kidney injury, and severe sepsis/septic shock, after adjusting for potential confounders. Therefore, it could be a strong indicator of a high inflammatory burden, leading to a higher risk of severe COVID-19 [49]. Thereby, we recommend that clinicians pay more attention to the blood glucose status of pregnant women with COVID-19, even those who may not have been diagnosed with diabetes prior to admission.

In our study, calcium serum levels were negatively correlated with three measured clinical outcomes: the length of hospitalization (days), the severity of the course of COVID-19 (1-4), and oxygen flow (l/min). These findings were consistent with previously published reports which have showed that low serum calcium levels are associated with disease severity and a poor prognosis for patients with COVID-19 [50-53]. In a study by Zhang et al. low serum calcium levels were the most predictive feature of COVID-19 diagnosis of all models tested [54]. The cause of hypocalcemia in COVID-19 patients is not clear. It is commonly found in the laboratory results in patients diagnosed with viral infections and pneumonia [55], and several mechanisms may be suggested. Firstly, the pro-inflammatory cytokines in COVID-19 patients inhibit parathyroid hormone (PTH) secretion, and the resulting impaired response to PTH causes an imbalance of calcium levels [56]. According to the previous studies, levels of the disease progression indicators CRP, PCT, IL-6, and D-dimer are found to be significantly higher in COVID-19 patients with hypocalcemia. When this is coupled with calcium serum levels which are negatively correlated with these indicators, it means that these patients may have

a greater inflammatory response [50,51,53]. Secondly, the occurrence of hypocalcemia may be associated with calcium inflow due to hypoxemic tissue damage. Another theory is that modification of calcium levels is crucial for the survival and replication of the SARS-CoV-2, since calcium is used in virus structure formation, entry, gene expression, virion maturation, and release [57]. Pregnancy also often leads to vitamin D deficiency resulting in hypocalcemia due to impaired intestinal absorption and thus an inadequate intake of calcium [58]. Finally, calcium is predominantly bound to albumin in plasma, and a decrease in serum albumin or total protein levels, mainly occurring in the third trimester, will cause hypocalcemia [59].

Our work showed that low total protein serum levels are a predictive factor for both a longer time to clinical improvement and a greater severity of disease, and as such this predictor can provide useful information for clinicians caring for pregnant women with COVID-19. Several mechanisms were proposed, including anti-oxidative and anti-inflammatory values of albumin [60,61], downregulation of albumin and prealbumin caused by the cytokine storm [62], and dysregulation of the immune system triggered by low protein serum levels [63]. The previous studies have indicated that serum albumin [64-66], prealbumin [62], and total protein levels are poor prognosis parameters among non-pregnant cohorts [63]. As mentioned earlier, total protein and albumin levels decrease because of the physiological processes of pregnancy during the third trimester. This condition poses a major threat to pregnant SARS-CoV-2 infected women as outlined in a study by Bozkurt et al. [35].

Our study showed that on admission, disrupted total cholesterol levels correlate with a greater severity of disease and with five out of the six main outcomes: the length of hospitalization (days), the severity of the course of COVID-19 (1-4), oxygen flow (l/min), the need for oxygen supplementation, and the need for ICU admission. The role of cholesterol in immunity is well established in numerous observational studies. In addition, dynamic changes in lipid levels caused by SARS-CoV-2 might be explained by several hypotheses. Firstly, the production of apolipoproteins and lipoproteins might be impaired by liver damage [67] and cytokine activity [68], and secondly, capillary leakage may occur, relocating them to extravascular compartments [69]. A study by Wei et al. demonstrated that patients with COVID-19 develop hypolipidemia during early stages of the disease and that abnormalities in lipid metabolism progressively became worse in association with the severity of the disease [70]. Lower levels of total cholesterol, low-density lipoprotein, and high-density lipoprotein (HDL) were linked to higher mortality rates and poorer prognoses in patients with COVID-19 [70-73]. Moreover, high CRP/HDL-C ratios were established as an independent predictor of in-hospital mortality [74]. However, none of these previously published studies involved pregnant women.

The main limitations of our investigation include its single-center nature, as well as its small and homogeneous cohort of patients. Moreover, we evaluated markers on admission, and not their response to disease progression and treatment.

## CONCLUSION

Pregnant women with COVID-19 were hospitalized during their second or third trimester, with dyspnea, cough, and fever as leading symptoms. Concomitant conditions, including diabetes and hypertension, modified the course of illness. CT chest scan at initial presentation may enable medical services required by pregnant patients infected with SARS-CoV-2 to be prioritized. Lymphocytopenia, hypocalcemia, low total cholesterol, low total protein levels, and high serum levels of CRP, ferritin, IL-6, procalcitonin, hs-troponin I, LDH, and glucose measured on hospital admission are good predictors of disease severity and may lead to early identification of patients at risk for developing complications, thereby improving optimization and prevention efforts in this cohort. Further, research with a larger patient sample and risk models is needed to provide useful information for effective health resource management during the COVID-19 pandemic.

## REFERENCES

[1] WHO Coronavirus (COVID-19) Dashboard. Available from: https://www.covid19.who.int [Last accessed on 2022 Apr 18].
[2] Breslin N, Baptiste C, Gyamfi-Bannerman C, Miller R, Martinez R, Bernstein K, et al. Coronavirus disease 2019 infection among asymptomatic and symptomatic pregnant women: Two weeks of confirmed presentations to an affiliated pair of New York City hospitals. Am J Obstet Gynecol MFM 2020;2(2):100118. http://doi.org/10.1016/j.ajogmf.2020.100118
[3] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395(10223):497-506. http://doi.org/10.1016/S0140-6736(20)30183-5
[4] Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. China medical treatment expert group for covid-19. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020;382(18):1708-20. http://doi.org/10.1056/NEJMoa2002032
[5] Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 2020;323(11):1061-9. http://doi.org/10.1001/jama.2020.1585
[6] Grant MC, Geoghegan L, Arbyn M, Mohammed Z, McGuinness L, Clarke EL, et al. The prevalence of symptoms in 24,410 adults infected by the novel coronavirus (SARS-CoV-2; COVID-19): A systematic review and meta-analysis of 148 studies from 9 countries. PLoS One 2020;15(6):e0234765. http://doi.org/10.1371/journal.pone.0234765
[7] Rogers JP, Chesney E, Oliver D, Pollak TA, McGuire P, Fusar-Poli P,

et al. Psychiatric and neuropsychiatric presentations associated with severe coronavirus infections: A systematic review and meta-analysis with comparison to the COVID-19 pandemic. Lancet Psychiatry 2020;7(7):611-27.
http://doi.org/10.1016/S2215-0366(20)30203-0

[8] Nowacka U, Kozlowski S, Januszewski M, Sierdzinski J, Jakimiuk A, Issat T. COVID-19 pandemic-related anxiety in pregnant women. Int J Environ Res Public Health 2021;18(14):7221.
http://doi.org/10.3390/ijerph18147221

[9] Kotsev SV, Miteva D, Krayselska S, Shopova M, Pishmisheva-Peleva M, Stanilova SA, et al. Hypotheses and facts for genetic factors related to severe COVID-19. World J Virol 2021;10(4):137-55.
http://doi.org/10.5501/wjv.v10.i4.137

[10] Ferrer-Oliveras R, Mendoza M, Capote S, Pratcorona L, Esteve-Valverde E, Cabero-Roura L, et al. Immunological and physiopathological approach of COVID-19 in pregnancy. Arch Gynecol Obstet 2021;304(1):39-57.
http://doi.org/10.1007/s00404-021-06061-3

[11] Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W, et al. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: A retrospective review of medical records. Lancet 2020;395(10226):809-15.
http://doi.org/10.1016/S0140-6736(20)30360-3

[12] Liu H, Wang LL, Zhao SJ, Kwak-Kim J, Mor G, Liao AH. Why are pregnant women susceptible to COVID-19? An immunological viewpoint. J Reprod Immunol 2020;139:103122.
http://doi.org/10.1016/j.jri.2020.103122

[13] Rasmussen SA, Jamieson DJ, Uyeki TM. Effects of influenza on pregnant women and infants. Am J Obstet Gynecol 2012;207(3):S3-8.
http://doi.org/10.1016/j.ajog.2012.06.068

[14] Mor G, Aldo P, Alvero AB. The unique immunological and microbial aspects of pregnancy. Nat Rev Immunol 2017;17(8):469-82.
http://doi.org/10.1038/nri.2017.64

[15] Allotey J, Stallings E, Bonet M, Yap M, Chatterjee S, Kew T, et al. for PregCOV-19 living systematic review consortium. Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: Living systematic review and meta-analysis. BMJ 2020;370:m3320.
http://doi.org/10.1136/bmj.m3320

[16] Ellington S, Strid P, Tong VT, Woodworth K, Galang RR, Zambrano LD, et al. Characteristics of women of reproductive age with laboratory-confirmed SARS-CoV-2 infection by pregnancy status-United States, January 22-June 7, 2020. MMWR Morb Mortal Wkly Rep 2020;69(25):769-75.
http://doi.org/10.15585/mmwr.mm6925a1

[17] Zambrano LD, Ellington S, Strid P, Galang RR, Oduyebo T, Tong VT, et al. CDC COVID-19 response pregnancy and infant linked outcomes team. Update: Characteristics of symptomatic women of reproductive age with laboratory-confirmed SARS-CoV-2 infection by pregnancy status-United States, January 22-October 3, 2020. MMWR Morb Mortal Wkly Rep 2020;69(44):1641-7.
http://doi.org/10.15585/mmwr.mm6944e3

[18] Schwartz DA, Graham AL. Potential maternal and infant outcomes from (Wuhan) coronavirus 2019-nCoV infecting pregnant women: Lessons from SARS, MERS, and other human coronavirus infections. Viruses 2020;12(2):194.
http://doi.org/10.3390/v12020194

[19] Siston AM, Rasmussen SA, Honein MA, Fry AM, Seib K, Callaghan WM, et al., Pandemic H1N1 Influenza in Pregnancy Working Group. Pandemic 2009 influenza A(H1N1) virus illness among pregnant women in the United States. JAMA 2010;303(15):1517-25.
http://doi.org/10.1001/jama.2010.479

[20] Jamieson DJ, Honein MA, Rasmussen SA, Williams JL, Swerdlow DL, Biggerstaff MS, et al., Novel Influenza A (H1N1) Pregnancy Working Group. H1N1 2009 influenza virus infection during pregnancy in the USA. Lancet 2009;374(9688):451-8.
http://doi.org/10.1016/S0140-6736(09)61304-0

[21] Hause AM, Panagiotakopoulos L, Weintraub ES, Sy LS, Glenn SC. Adverse outcomes in pregnant women hospitalized with respiratory syncytial virus infection: A case series. Clin Infect Dis 2021;72(1):138-40.
https://doi.org/10.1093/cid/ciaa668

[22] Flisiak R, Horban A, Jaroszewicz J, Kozielewicz D, Pawłowska M, Parczewski M, et al. Management of SARS-CoV-2 infection: Recommendations of the Polish association of epidemiologists and infectiologists as of March 31, 2020. Pol Arch Intern Med 2020;130(4):352-7.
https://doi.org/10.20452/pamw.15270

[23] Figueiro-Filho EA, Yudin M, Farine D. COVID-19 during pregnancy: An overview of maternal characteristics, clinical symptoms, maternal and neonatal outcomes of 10,996 cases described in 15 countries. J Perinat Med 2020;48(9):900-11.
https://doi.org/10.1515/jpm-2020-0364

[24] Littauer EQ, Esser ES, Antao OQ, Vassilieva EV, Compans RW, Skountzou I. H1N1 influenza virus infection results in adverse pregnancy outcomes by disrupting tissue-specific hormonal regulation. PLoS Pathog 2017;13(11):e1006757.
https://doi.org/10.1371/journal.ppat.1006757

[25] Kumpel BM, Manoussaka MS. Placental immunology and maternal alloimmune responses. Vox Sang 2012;102(1):2-12.
https://doi.org/10.1111/j.1423-0410.2011.01533.x

[26] Zdanowski W, Waśniewski T. Evaluation of SARS-CoV-2 spike protein antibody titers in cord blood after COVID-19 vaccination during pregnancy in polish healthcare workers: Preliminary results. Vaccines (Basel) 2021;9(6):675.
https://doi.org/10.3390/vaccines9060675

[27] Abedzadeh-Kalahroudi M, Sehat M, Vahedpour Z, Talebian P, Haghighi A. Clinical and obstetric characteristics of pregnant women with Covid-19: A case series study on 26 patients. Taiwan J Obstet Gynecol 2021;60(3):458-62.
https://doi.org/10.1016/j.tjog.2021.03.012

[28] Liu F, Liu H, Li J, Hou L, Lan W, Wang D. Clinico-Radiological Features and Outcomes in Pregnant Women with COVID-19: Compared with Age-Matched Non-Pregnant Women. Available from: https://www.ssrn.com/abstract=3556647
http://doi.org/10.2139/ssrn.3556647

[29] Wungu CD, Khaerunnisa S, Putri EA, Hidayati HB, Qurnianingsih E, Lukitasari L, et al. Meta-analysis of cardiac markers for predictive factors on severity and mortality of COVID-19. Int J Infect Dis 2021;105:551-9.
http://doi.org/10.1016/j.ijid.2021.03.008

[30] Sattar Y, Ullah W, Rauf H, Virk HU, Yadav S, Chowdhury M, et al. COVID-19 cardiovascular epidemiology, cellular pathogenesis, clinical manifestations and management. Int J Cardiol Heart Vasc 2020;29:100589.
http://doi.org/10.1016/j.ijcha.2020.100589

[31] Lang JP, Wang X, Moura FA, Siddiqi HK, Morrow DA, Bohula EA. A current review of COVID-19 for the cardiovascular specialist. Am Heart J 2020;226:29-44.
https://doi.org/10.1016/j.ahj.2020.04.025

[32] Lombardi A, Duiella S, Piani LL, Comelli A, Ceriotti F, Oggioni M, et al. Inflammatory biomarkers in pregnant women with COVID-19: A retrospective cohort study. Sci Rep 2021;11(1):13350.
https://doi.org/10.1038/s41598-021-92885-7

[33] Scott R, Hewitt H, Mallet C, Herd L, Shibley C, Bolger S, et al. Recognition and Treatment of Severe COVID-19 in Pregnancy: Lessons from a Cohort of 69 Infected Women and an Evidence-based Guideline. United States: Authorea; 2020.
https://doi.org/10.22541/au.160616173.35255142/v1.

[34] Andrikopoulou M, Madden N, Wen T, Aubey JJ, Aziz A, Baptiste CD, et al. Symptoms and critical illness among obstetric patients with coronavirus disease 2019 (COVID-19) infection. Obstet Gynecol 2020;136(2):291-9.
https://doi.org/10.1097/AOG.0000000000003996.

[35] Bozkurt F, Çoşkun Ö, Yeleç S, Bekçibaşi M, Asena M, Bağli I.

Comparison of the clinical and laboratory findings in COVID-19 positive pregnants without comorbidity. Turk J Med Sci 2021 Jul 8. doi: 10.3906/sag-2105-116. Epub ahead of print
https://doi.org/10.3906/sag-2105-116
[36] Song P, Li W, Xie J, Hou Y, You C. Cytokine storm induced by SARS-CoV-2. Clin Chim Acta 2020;509:280-7.
https://doi.org/10.1016/j.cca.2020.06.017
[37] Zhu Z, Cai T, Fan L, Lou K, Hua X, Huang Z, et al. Clinical value of immune-inflammatory parameters to assess the severity of coronavirus disease 2019. Int J Infect Dis 2020;95:332-9.
https://doi.org/10.1016/j.ijid.2020.04.041
[38] Tanacan A, Yazihan N, Erol SA, Anuk AT, Yucel Yetiskin FD, Biriken D, et al. The impact of COVID-19 infection on the cytokine profile of pregnant women: A prospective case-control study. Cytokine 2021;140:155431.
https://doi.org/10.1016/j.cyto.2021.155431
[39] Chen X, Zhao B, Qu Y, Chen Y, Xiong J. Detectable serum SARS-CoV-2 viral load [RNAaemia] is closely correlated with drastically elevated interleukin 6 [IL-6] level in critically ill COVID-19 patients. Clin Infect Dis 2020;71:1937-42.
[40] Ulhaq ZS, Soraya GV. Interleukin-6 as a potential biomarker of COVID-19 progression. Med Mal Infect 2020;50:382-3.
https://doi.org/10.1016/j.medmal.2020.04.002
[41] Aziz M, Fatima R, Assaly R. Elevated interleukin-6 and severe COVID-19: A meta-analysis. J Med Virol 2020;92(11):2283-5.
https://doi.org/10.1002/jmv.25948
[42] Gianchandani R, Esfandiari NH, Ang L, Iyengar J, Knotts S, Choksi P, et al. Managing hyperglycemia in the COVID-19 inflammatory storm. Diabetes 2020;69(10):2048-53.
https://doi.org/10.2337/dbi20-0022
[43] Feldman EL, Savelieff MG, Hayek SS, Pennathur S, Kretzler M, Pop-Busui R. COVID-19 and diabetes: A collision and collusion of two diseases. Diabetes 2020;69(12):2549-65.
https://doi.org/10.2337/dbi20-0032
[44] Corathers SD, Falciglia M. The role of hyperglycemia in acute illness: Supporting evidence and its limitations. Nutrition 2011;27(3):276-81.
https://doi.org/10.1016/j.nut.2010.07.013
[45] McCowen KC, Malhotra A, Bistrian BR. Stress-induced hyperglycemia. Crit Care Clin 2001;17(1):107-24.
https://doi.org/10.1016/s0749-0704(05)70154-8
[46] Miftode E, Miftode L, Coman I, Prepeliuc C, Obreja M, Stămăteanu O, et al. Diabetes mellitus-a risk factor for unfavourable outcome in COVID-19 patients-the experience of an infectious diseases regional hospital. Healthcare (Basel) 2021;9(7):788.
https://doi.org/10.3390/healthcare9070788
[47] Zhang Y, Li H, Zhang J, Cao Y, Zhao X, Yu N, et al. The clinical characteristics and outcomes of patients with diabetes and secondary hyperglycaemia with coronavirus disease 2019: A single-centre, retrospective, observational study in Wuhan. Diabetes Obes Metab 2020;22(8):1443-54.
https://doi.org/10.1111/dom.14086
[48] Wang S, Ma P, Zhang S, Song S, Wang Z, Ma Y, et al. Fasting blood glucose at admission is an independent predictor for 28-day mortality in patients with COVID-19 without previous diagnosis of diabetes: A multi-centre retrospective study. Diabetologia 2020;63(10):2102-11.
https://doi.org/10.1007/s00125-020-05209-1
[49] Charoenngam N, Alexanian SM, Apovian CM, Holick MF. Association between hyperglycemia at hospital presentation and hospital outcomes in COVID-19 patients with and without type 2 diabetes: A retrospective cohort study of hospitalized inner-city COVID-19 patients. Nutrients 2021;13(7):2199.
https://doi.org/10.3390/nu13072199
[50] Liu J, Han P, Wu J, Gong J, Tian D. Prevalence and predictive value of hypocalcemia in severe COVID-19 patients. J Infect Public Health 2020;13(9):1224-8.
https://doi.org/10.1016/j.jiph.2020.05.029
[51] Sun JK, Zhang WH, Zou L, Liu Y, Li JJ, Kan XH, et al. Serum calcium as a biomarker of clinical severity and prognosis in patients with coronavirus disease 2019. Aging (Albany NY). 2020;12(12):11287-95.
https://doi.org/10.18632/aging.103526
[52] Cappellini F, Brivio R, Casati M, Cavallero A, Contro E, Brambilla P. Low levels of total and ionized calcium in blood of COVID-19 patients. Clin Chem Lab Med 2020;58(9):e171-3.
https://doi.org/10.1515/cclm-2020-0611
[53] Qi X, Kong H, Ding W, Wu C, Ji N, Huang M, et al. Abnormal coagulation function of patients with COVID-19 is significantly related to hypocalcemia and severe inflammation. Front Med (Lausanne) 2021;8:638194.
https://doi.org/10.3389/fmed.2021.638194
[54] Zhang J, Jun T, Frank J, Nirenberg S, Kovatch P, Huang KL. Prediction of individual COVID-19 diagnosis using baseline demographics and lab data. Sci Rep 2021;11(1):13913.
https://doi.org/10.1038/s41598-021-93126-7
[55] Sankaran RT, Mattana J, Pollack S, Bhat P, Ahuja T, Patel A, et al. Laboratory abnormalities in patients with bacterial pneumonia. Chest 1997;111(3):595-600.
https://doi.org/10.1378/chest.111.3.595
[56] Fong J, Khan A. Hypocalcemia: Updates in diagnosis and management for primary care. Can Fam Physician 2012;58(2):158-62.
[57] Wang JM, Liu W, Chen X, McRae MP, McDevitt JT, Fenyö D. Predictive modeling of morbidity and mortality in patients hospitalized with COVID-19 and its clinical implications: Algorithm development and interpretation. J Med Internet Res 2021;23(7):e29514.
https://doi.org/10.2196/29514
[58] Holick MF. Vitamin D deficiency. N Engl J Med 2007;357(3):266-81.
https://doi.org/10.1056/NEJMra070553
[59] Elango R, Ball RO. Protein and amino acid requirements during pregnancy. Adv Nutr 2016;7(4):839S-44.
https://doi.org/10.3945/an.115.011817
[60] Artigas A, Wernerman J, Arroyo V, Vincent JL, Levy M. Role of albumin in diseases associated with severe systemic inflammation: Pathophysiologic and clinical evidence in sepsis and in decompensated cirrhosis. J Crit Care 2016;33:62-70.
https://doi.org/10.1016/j.jcrc.2015.12.019
[61] Rozga J, Piątek T, Małkowski P. Human albumin: Old, new, and emerging applications. Ann Transplant 2013;18:205-17.
https://doi.org/10.12659/AOT.889188
[62] Akbar MR, Pranata R, Wibowo A, Lim MA, Sihite TA, Martha JW, et al. The association between serum prealbumin and poor outcome in COVID-19-systematic review and meta-analysis. Eur Rev Med Pharmacol Sci 2021;25(10):3879-85.
https://doi.org/10.26355/eurrev_202105_25955
[63] Mei Q, Wang AY, Bryant A, Yang Y, Li M, Wang F, et al. Development and validation of prognostic model for predicting mortality of COVID-19 patients in Wuhan, China. Sci Rep 2020;10(1):22451.
https://doi.org/10.1038/s41598-020-78870-6
[64] Acharya R, Poudel D, Patel A, Schultz E, Bourgeois M, Paswan R, et al. Low serum albumin and the risk of hospitalization in COVID-19 infection: A retrospective case-control study. PLoS One 2021;16(4):e0250906.
https://doi.org/10.1371/journal.pone.0250906
[65] Kheir M, Saleem F, Wang C, Mann A, Chua J. Higher albumin levels on admission predict better prognosis in patients with confirmed COVID-19. PLoS One 2021;16(3):e0248358.
https://doi.org/10.1371/journal.pone.0248358
[66] Xu Y, Yang H, Wang J, Li X, Xue C, Niu C, et al. Serum albumin levels are a predictor of COVID-19 patient prognosis: Evidence from a single cohort in Chongqing, China. Int J Gen Med 2021;14:2785-97.
https://doi.org/10.2147/IJGM.S312521
[67] Poynard T, Deckmyn O, Rudler M, Peta V, Ngo Y, Vautier M, et al. Performance of serum apolipoprotein-A1 as a sentinel of Covid-19. PLoS One 2020;15(11):e0242306.

https://doi.org/10.1371/journal.pone.0242306
[68] Ettinger WH, Varma VK, Sorci-Thomas M, Parks JS, Sigmon RC, Smith TK, et al. Cytokines decrease apolipoprotein accumulation in medium from Hep G2 cells. Arterioscler Thromb 1994;14(1):8-13. https://doi.org/10.1161/01.atv.14.1.8
[69] van Leeuwen HJ, Heezius EC, Dallinga GM, van Strijp JA, Verhoef J, van Kessel KP. Lipoprotein metabolism in patients with severe sepsis. Crit Care Med 2003;31(5):1359-66. https://doi.org/10.1097/01.CCM.0000059724.08290.51
[70] Wei X, Zeng W, Su J, Wan H, Yu X, Cao X, et al. Hypolipidemia is associated with the severity of COVID-19. J Clin Lipidol 2020;14(3):297-304. https://doi.org/10.1016/j.jacl.2020.04.008
[71] Yue J, Xu H, Zhou Y, Liu W, Han X, Mao Q, et al. Dyslipidemia is related to mortality in critical patients with coronavirus disease 2019: A retrospective study. Front Endocrinol 2021;12:611526. https://doi.org/10.3389/fendo.2021.611526
[72] Zhao M, Luo Z, He H, Shen B, Liang J, Zhang J, et al. Decreased low-density lipoprotein cholesterol level indicates poor prognosis of severe and critical COVID-19 Patients: A retrospective, single-center study. Front Med (Lausanne) 2021;8:585851. https://doi.org/10.3389/fmed.2021.585851
[73] Lassale C, Hamer M, Hernáez Á, Gale CR, Batty GD. Association of pre-pandemic high-density lipoprotein cholesterol with risk of COVID-19 hospitalisation and death: The UK Biobank cohort study. Prev Med Rep 2021;23:101461. https://doi.org/10.1101/2021.01.20.21250152
[74] Li Y, Zhang Y, Lu R, Dai M, Shen M, Zhang J, et al. Lipid metabolism changes in patients with severe COVID-19. Clin Chim Acta. 2021;517:66-73. https://doi.org/10.1016/j.cca.2021.02.011

## Related articles published in BJBMS

1. Clinical features and major bleeding predictors for 161 fatal cases of COVID-19: A retrospective observational study
   Gokhan Alici et al., BJBMS, 2021
2. Comparison of confirmed and probable COVID-19 patients in the intensive care unit during the normalization period
   Fatma İrem Yeşiler et al., BJBMS, 2021
3. The value of lymphocyte count in determining the severity of COVID-19 and estimating the time for nucleic acid test results to turn negative
   Yuanchao Li et al., BJBMS, 2021
4. Sustained seroprevalence of SARS-CoV-2 antibodies one year after infection: one of the first COVID-19 cluster cases in Bosnia and Herzegovina
   Božo Šušak et al., BJBMS, 2020

# 5. PODSUMOWANIE

## 5.1. Wyniki oceny częstości występowania zaburzeń węchu u pacjentów z COVID-19 oraz ich wartości predykcyjnej w ocenie prawdopodobieństwa zakażenia SARS-CoV-2

Do badania włączono 64 hospitalizowanych pacjentów z COVID-19 oraz 34 zdrowych ochotników. Najczęstszymi objawami COVID-19 (innymi niż zaburzenia węchu) były: zmęczenie (70,3%), kaszel (39,1%), gorączka (37,5%), bóle głowy (35,9%) oraz dolegliwości żołądkowo-jelitowe (35,4%). Upośledzenie drożności nosa występowało u 32,8%, a katar u 29,7% pacjentów z COVID-19. Zaburzenia węchu wystąpiły wcześniej niż inne objawy u 14,8% chorych, jednocześnie z innymi objawami u 11,1%, zaś później niż inne objawy u 74,1%. W czasie badania 25,9% pacjentów zgłaszało całkowity powrót węchu, 55,6% podawało brak poprawy, zaś 18,5% zgłaszało nasilenie zaburzeń powonienia. Częstość odczuwanych subiektywnie zaburzeń węchu była istotnie wyższa u pacjentów z COVID-19 niż w grupie kontrolnej, przy czym niedawne wystąpienie zaburzeń węchu i osłabienie powonienia występujące w chwili badania zgłaszało odpowiednio 32,8% i 42,2% pacjentów zakażonych SARS-CoV-2, podczas gdy wszystkie osoby w grupie kontrolnej zgłaszały normosmię. Średnia punktacja w wizualnej skali analogowej (VAS, ang. *visual analogue scale*) była również wyższa w grupie badanej niż w grupie kontrolnej. Jako że grupy badana i kontrolna różniły się pod względem wieku, przeanalizowano dodatkowo występowanie zaburzeń węchu dla podgrupy 75% najmłodszych pacjentów z COVID-19, w której wiek nie był istotnie różny niż w grupie kontrolnej. Różnice w ocenie zmysłu powonienia pomiędzy tymi grupami pozostały wysoce istotne statystycznie, przy czym w podgrupie 75% najmłodszych pacjentów niedawne wystąpienie zaburzeń węchu i osłabienie powonienia występujące w chwili badania zgłaszało odpowiednio 29,2% i 41,7% badanych.

U wszystkich osób biorących udział w badaniu dokonano psychofizycznej oceny węchu. Średni odsetek prawidłowych odpowiedzi (dla wszystkich 10 próbek, w tym próby bezzapachowej), prawidłowo wykrytych zapachów oraz prawidłowo zidentyfikowanych zapachów w grupie badanej w porównaniu do grupy kontrolnej wynosił odpowiednio 65,6% vs. 95,8%, 83,5% vs. 100% i 66,3% vs. 95,6%. W porównaniu grupy kontrolnej z podgrupą 75% najmłodszych pacjentów z COVID-19 różnice pozostały wysoce istotne statystycznie, przy czym w tej podgrupie średni odsetek prawidłowych odpowiedzi wyniósł 74,2%, średni odsetek prawidłowo wykrytych zapachów – 85,4%, zaś prawidłowo zidentyfikowanych zapachów – 72,9%.

Dla wszystkich substancji zapachowych niższe wartości p dla różnic pomiędzy

grupami stwierdzano dla identyfikacji niż dla rozpoznawania zapachów. Uwzględniając wyniki rozpoznawania każdej spośród badanych substancji wonnych, stworzono dwa dodatkowe, skrócone modele testu psychofizycznego, jeden z ośmioma, a drugi z czterema wybranymi zapachami, jak opisano powyżej. Średnia punktacja uzyskana w teście psychofizycznym była znacznie niższa w grupie badanej niż w grupie kontrolnej ($p < 0,001$) dla wszystkich ocenianych modeli. Przy przyjęciu punktu odcięcia dla rozpoznania zaburzeń węchu jako wartości poniżej 10. percentyla wyników uzyskanych w grupie kontrolnej, osłabienie powonienia w modelach SDOIT-10, SDOIT-9, SDOIT-8 i SDOIT-4 w grupie badanej vs. w grupie kontrolnej stwierdzono odpowiednio u 59,4% vs. 8,8%, 54,7% vs. 5,9%, 54,7% vs. 2,9% i 64,1% vs. 5,9% pacjentów. Zaobserwowano istotną korelację pomiędzy zaburzeniami węchu stwierdzanymi na podstawie każdego z czterech modeli testu psychofizycznego a subiektywnym osłabieniem powonienia zgłaszanym przez pacjentów w chwili badania. Jednocześnie należy podkreślić, że wyniki ankiety wskazywały na niższą częstość osłabienia powonienia niż testy psychofizyczne, a zatem częstość zaburzeń węchu w ocenie subiektywnej jest prawdopodobnie niedoszacowana, co wskazuje na istotną rolę metod psychofizycznych w ocenie zmysłu węchu w COVID-19. Co więcej, wśród osób oceniających subiektywnie swój węch jako prawidłowy, w modelach SDOIT-10, SDOIT-9, SDOIT-8 i SDOIT-4 testu psychofizycznego stwierdzono zaburzenia powonienia odpowiednio w 51,2%, 46,5%, 46,5% i 55,8% przypadków. Nie zaobserwowano istotnych różnic pomiędzy mężczyznami i kobietami odnośnie zarówno subiektywnych, jak i stwierdzanych w testach psychofizycznych zaburzeń węchu.

W grupie badanej upośledzenie drożności nosa występowało częściej u chorych z subiektywnymi zaburzeniami węchu niż u pacjentów z normosmią, zaś częstość kataru była istotnie wyższa u pacjentów zgłaszających niedawne wystąpienie zaburzeń węchu niż u osób z normosmią, podczas gdy w przypadku osłabienia powonienia występującego w chwili badania różnica ta nie była istotna statystycznie. Nie stwierdzono istotnych korelacji pomiędzy objawami ze strony nosa a wynikami SDOIT.

Pacjenci z gorszymi wynikami psychofizycznych testów węchowych mieli wyższą punktację w skali MEWS i byli dłużej hospitalizowani, a wyniki modeli SDOIT-9 oraz SDOIT-8 były istotnie gorsze u pacjentów, którzy później zmarli, nie stwierdzono zaś korelacji pomiędzy długością hospitalizacji, punktacją MEWS oraz częstością zgonów a zgłaszanym przez pacjentów subiektywnym osłabieniem powonienia. Konieczność suplementacji tlenu obserwowano rzadziej u pacjentów zgłaszających niedawne

wystąpienie zaburzeń węchu, ale częściej u pacjentów z niższymi wynikami SDOIT.

Stwierdzenie zaburzeń węchu na podstawie testu psychofizycznego dla każdego z czterech modeli SDOIT było istotnie związane z pozytywnym wynikiem badania RT-PCR w kierunku zakażenia SARS-CoV-2 (z OR dla modeli SDOIT-10, SDOIT-9, SDOIT-8 i SDOIT-4 wynoszącymi odpowiednio 15,1, 19,3, 39,8 i 28,5). W analizie ROC niedawne wystąpienie subiektywnych zaburzeń węchu w predykcji zakażenia SARS-CoV-2 miało czułość równą 42%, swoistość równą 100%, PPV równą 100% oraz NPV równą 48%, z AUC wynoszącym 0,71. W testach psychofizycznych, przy zdefiniowaniu zaburzeń węchu jako wartości poniżej 10. percentyla wyników uzyskanych w grupie kontrolnej, najlepszym predyktorem okazał się model SDOIT-4, z czułością równą 64%, swoistością równą 94%, PPV równą 95%, NPV równą 63% oraz AUC wynoszącym 0,8. Jednakże dla wszystkich modeli SDOIT optymalnym punktem odcięcia dla rozpoznania zaburzeń węchu w analizie ROC, wyznaczonym z wykorzystaniem metody stycznej, było udzielenie co najmniej jednej nieprawidłowej odpowiedzi, z AUC $\geq$ 0,8 dla wszystkich modeli. Jeszcze wyższą skuteczność diagnostyczną osiągnięto dla klasyfikatorów łączących wyniki SDOIT i subiektywnej oceny węchu (przy czym za predyktor COVID-19 uznano tu wystąpienie zaburzeń węchu w co najmniej jednej metodzie, to jest w ocenie subiektywnej i/lub w teście psychofizycznym). W celu zminimalizowania ryzyka niewykrycia COVID-19, co mogłoby prowadzić do braku izolacji osób faktycznie zakażonych, jako najlepsze predyktory wybrano ostatecznie dwa klasyfikatory z największym AUC i najwyższą czułością, łączące zgłaszanie przez pacjenta niedawnego wystąpienia subiektywnych zaburzeń węchu i/lub:

1. udzielenie co najmniej jednej nieprawidłowej odpowiedzi w modelu SDOIT-10 (z AUC równym 0,87, czułością równą 91%, swoistością równą 71%, PPV równą 85% i NPV równą 80%) albo
2. nieprawidłowe zidentyfikowanie co najmniej jednego zapachu w modelu SDOIT-8 (z AUC równym 0,87, czułością równą 86%, swoistością równą 79%, PPV równą 89% i NPV równą 75%).

Ponadto w celu wyeliminowania wpływu wieku pacjentów na wyniki, analizę ROC wykonano też dla porównania grupy kontrolnej i podzbioru 75% najmłodszych pacjentów z grupy badanej. Analiza grup dopasowanych pod względem wieku również wykazała, że przy zdefiniowaniu zaburzeń węchu w SDOIT jako wartości poniżej 10. percentyla wyników uzyskanych w grupie kontrolnej najlepszym predyktorem wystąpienia zakażenia SARS-CoV-2 był model SDOIT-4 (z czułością równą 54%, swoistością równą 94%, PPV

równą 93%, NPV równą 59% oraz AUC równą 0,75), jednak przy wyznaczeniu optymalnego punktu odcięcia metodą stycznej, najlepszą definicją zaburzeń węchu we wszystkich modelach SDOIT okazało się być podanie co najmniej jednej nieprawidłowej odpowiedzi, z AUC ≥ 0,75 dla wszystkich modeli. Podobnie jak w przypadku całej próby, tu również modele łączące wyniki SDOIT i subiektywną ocenę powonienia pozwalały osiągnąć wyższą skuteczność diagnostyczną, a jako najlepsze predyktory wybrano ostatecznie dwa klasyfikatory z największym AUC i najwyższą czułością, łączące zgłaszanie przez pacjenta niedawnego wystąpienia subiektywnych zaburzeń węchu i/lub:

1. udzielenie co najmniej jednej nieprawidłowej odpowiedzi w modelu SDOIT-10 (z AUC równym 0,85, czułością równą 88%, swoistością równą 71%, PPV równą 81% i NPV równą 80%) albo
2. nieprawidłowe zidentyfikowanie co najmniej jednego zapachu w modelu SDOIT-8 (z AUC równym 0,86, czułością równą 83%, swoistością równą 79%, PPV równą 85% i NPV równą 77%).

Ograniczeniami przedstawionego badania są jego jednoośrodkowy charakter oraz ograniczona wielkość próby. Co więcej, ocena węchu odbywała się w różnym odstępie czasu od rozpoznania zakażenia, a część pacjentów zgłaszała w chwili badania subiektywną poprawę węchu. Fakty te mogłyby jednak prowadzić raczej do niedoszacowania niż do przeszacowania częstości zaburzeń węchu w COVID-19, a tym samym ich znaczenia diagnostycznego. Ponadto nie oceniano rokowania odnośnie poprawy węchu. Z tego powodu wskazane jest przeprowadzenie dalszych badań z dłuższym okresem obserwacji. Co więcej, zastosowany test psychofizyczny (SDOIT) nie został zwalidowany, a zaburzenia węchu oceniano w nim na podstawie identyfikacji zapachów, bez oceny ich dyskryminacji czy progu ich odczuwania. Należy jednak zauważyć, że celem badania nie była walidacja nowego testu do oceny funkcji zmysłu węchu, a stworzenie szybkiego testu przesiewowego dla predykcji wystąpienia zakażenia SARS-CoV-2. Z tego powodu jako złoty standard w ocenie przydatności diagnostycznej zastosowano test RT-PCR, a w projekcie uwzględniono rozpoznawanie zapachów jako najprostszy do przeprowadzenia i jednocześnie wiarygodny element badania zmysłu powonienia. Niemniej jednak w przyszłości przydatna byłaby walidacja SDOIT jako testu diagnostycznego w ocenie funkcji węchu. Warto jednocześnie podkreślić, że choć w prezentowanym badaniu test psychofizyczny wykonywano w asyście badacza, prostota SDOIT umożliwia również jego przeprowadzenie zdalne, co mogłoby dodatkowo zwiększyć jego dostępność jako metody przesiewowej.

## 5.2. Wyniki podsumowania aktualnej wiedzy na temat patogenezy zaburzeń węchu w przebiegu COVID-19

Choć opisywane w literaturze wczesny początek oraz wczesne ustępowanie zaburzeń węchu w przebiegu COVID-19 mogłyby przemawiać za przewodzeniowym patomechanizmem anosmii, u wielu pacjentów zakażonych SARS-CoV-2 osłabienie powonienia występuje pomimo braku upośledzenia drożności nosa czy kataru, co wskazuje, iż nie są one głównymi czynnikami w rozwoju zaburzeń węchu w przebiegu tej choroby [76,77,78]. Podobnie w literaturze opisywano występowanie w badaniach obrazowych obrzęku śluzówki okolicy węchowej, który mógłby utrudniać dotarcie substancji zapachowych do nabłonka węchowego nawet przy zachowanej drożności nosa [79,80], jednak w innych pracach nie potwierdzono tych doniesień [81,82], co przemawia przeciwko jego zasadniczej roli w patogenezie osłabienia powonienia.

Innym możliwym patomechanizmem anosmii jest uszkodzenie struktur nerwowych, w tym neuronów węchowych, opuszki węchowej oraz wyższych ośrodków węchowych [83,84]. Zdolność SARS-CoV-2 do neuroinwazji potwierdzają szeroko opisywane w literaturze objawy neurologiczne COVID-19 [85]. Bezpośrednie zajęcie ośrodkowego układu nerwowego może odbywać się na drodze krwionośnej bądź drogą szlaków nerwowych [84]. W pierwszym przypadku wirus może wnikać do krążenia przekraczając barierę krew-mózg poprzez zakażenie komórek śródbłonka w jej obrębie, przenikanie przez przestrzeń zewnątrzkomórkową wskutek zwiększonej przepuszczalności tej bariery, wywołanej uwolnieniem mediatorów zapalnych, przenikanie w obrębie splotów naczyniówkowych lub zakażenie leukocytów zdolnych do przechodzenia przez tę barierę (w tzw. mechanizmie „konia trojańskiego”) [86,87]. Z kolei w mechanizmie neuroinwazji przez drogę węchową wirus dociera do mózgu wskutek wnikania przez nabłonek węchowy i zajęcia obwodowych zakończeń nerwowych, a następnie propagacji drogą transportu aksonalnego poprzez neurony węchowe w kierunku opuszki węchowej, skąd może rozprzestrzeniać się trans-synaptycznie do innych obszarów mózgu [84,85,88]. Alternatywnie wirus może przenikać z nabłonka węchowego bezpośrednio do płynu mózgowo-rdzeniowego otaczającego pęczki nerwowe i opuszkę węchową [15]. Za neuroinwazją poprzez drogę węchową przemawia stwierdzana w badaniach pośmiertnych większa liczba cząstek wirusa SARS-CoV-2 oraz bardziej nasilone uszkodzenia w obrębie neuronów węchowych i opuszki węchowej niż w pniu mózgu. Ponadto w badaniach rezonansu magnetycznego u pacjentów z COVID-19 opisywano nieprawidłowości

w obrębie opuszki węchowej, w tym cechy mikrokrwotoków, obrzęk, zmniejszenie objętości opuszki wskazujące na jej atrofię lub inne zmiany jej morfologii [89–93]. Co więcej, w badaniach SARS-CoV-2 oraz wcześniej występujących ludzkich koronawirusów na modelach zwierzęcych, wykazano zakażenie mózgowia po donosowej aplikacji wirusa [94–96]. Należy jednak zauważyć, że badania na modelu mysim prowadzone są na zwierzętach transgenicznych z ludzkim receptorem ACE2 mogącym ulegać ektopowej ekspresji w wielu komórkach, w tym w neuronach węchowych, przez co model ten może nie być w pełni wiarygodny w ocenie tropizmu wirusa [20,97]. Rzeczywiście, w badaniach z wykorzystaniem sekwencjonowania stwierdzono koekspresję ACE2 i TMPRSS2 w komórkach pozanerwowych nabłonka węchowego, nie zaś w neuronach węchowych, opuszce węchowej czy mózgu [3,20,98]. Ponadto objawy neurologiczne COVID-19 niekoniecznie wskazują na bezpośrednią neuroinwazję, ale mogą wynikać również z uszkodzenia mózgu spowodowanego hipoksją, zaburzeniami naczyniowymi lub nadmierną reakcją immunologiczną [99]. Co więcej, choć w niektórych badaniach stwierdzono obecność cząstek wirusowych w preparatach pośmiertnych mózgu [100] oraz RNA SARS-CoV2 w płynie mózgowo-rdzeniowym [47], liczba kopii wirusa w mózgu wydaje się najmniejsza spośród ocenianych tkanek, a w części prac w ogóle nie stwierdza się jego obecności, zaś dodatni wynik RT-PCR niekoniecznie świadczy o obecności całych cząstek wirusowych w płynie mózgowo-rdzeniowym [20]. Przeciwko zasadniczej roli uszkodzenia ośrodkowego układu nerwowego w patomechanizmie anosmii przemawia również znacznie większa częstość występowania upośledzenia powonienia niż innych objawów neurologicznych oraz powszechnie obserwowane szybkie ustępowanie zaburzeń węchu [76,78].

Innym prawdopodobnym mechanizmem powstawania anosmii w przebiegu COVID-19 jest uszkodzenie czuciowo-nerwowe, z zaburzeniem struktury nabłonka węchowego. Rzeczywiście, zakażenie SARS-CoV-2 stwierdza się w wielu typach komórek nabłonka węchowego, w tym w obrębie neuronów węchowych, komórek podporowych oraz komórek układu immunologicznego [101]. Jak wspomniano, koekspresję ACE2 i TMPRSS2 obserwuje się w przeważającej mierze w obrębie pozanerwowych komórek nabłonka węchowego, takich jak komórki podporowe, podstawne oraz okołonaczyniowe, nie zaś w neuronach węchowych [3,12,98]. Zakażenie neuronów jest zatem prawdopodobnie poprzedzone infekcją komórek pozanerwowych, która może następnie prowadzić do horyzontalnego rozprzestrzeniania się wirusa do neuronów węchowych z przylegających komórek podporowych, a także do zniszczenia

struktury nabłonka węchowego przez naciek zapalny, ułatwiającego szerzenie się wirusa [101]. Co więcej, zakażenie komórek pozanerwowych może zaburzać funkcję neuronów węchowych nawet bez ich bezpośredniego zakażenia poprzez uszkodzenie architektoniki nabłonka węchowego i brak prawidłowej funkcji podporowej, a także zaburzenie szlaków sygnałowych, zaburzenia jonowe oraz nadmierną odpowiedź immunologiczną [12,19,20,97]. Hipotezę tę potwierdza stosunkowo szybkie ustępowanie zaburzeń węchu, trwające zwykle do kilku tygodni po zachorowaniu, co może odzwierciedlać zdolności regeneracyjne komórek podporowych, podczas gdy za dłużej trwające zaburzenia odpowiadać może uszkodzenie komórek podstawnych [20].

Należy jednak zauważyć, że choć neurony węchowe niemal nie wykazują ekspresji receptora ACE2, obserwuje się w nich ekspresję receptora NRP1, który może być odpowiedzialny za bezpośrednie zakażenie tych komórek [4,101,102]. Co więcej, ekspresję receptora NRP1 stwierdzono również w obrębie nerwowych komórek progenitorowych, co mogłoby odpowiadać za obserwowane w części przypadków utrzymywanie się anosmii po przechorowaniu COVID-19 [4,102]. Ponadto opisywane wcześniej rzadkie występowanie parosmii po przebyciu COVID-19 prawdopodobnie wynikało z początkowego niedoszacowania jej częstości, podczas gdy obecnie ocenia się ją na około 40% [103,104]. Jedna z hipotez odnośnie mechanizmu powstawania parosmii mówi, że zapalenie *neuroepithelium* i opuszki węchowej wywołane zakażeniem SARS-CoV-2 może powodować nieprawidłową regenerację neuronów węchowych, prowadzącą do zaburzenia odbierania bodźców zapachowych w nabłonku węchowym i nieprawidłowego mapowania receptorów w obrębie opuszki węchowej [104].

Możliwe jest również genetyczne podłoże zaburzeń węchu w COVID-19, z prawdopodobną rolą genów *UGT2A1* i *UGT2A2*, kodujących enzym difosfo-glukuronozylotransferazę urydyny (UGT) i ulegających ekspresji w nabłonku węchowym, które mogą odgrywać rolę w metabolizowaniu substancji zapachowych oraz terminacji węchowych szlaków sygnałowych [105,106].

### 5.3. Wyniki badania wpływu wariantu SARS-CoV-2 (wariantu alfa w porównaniu do wariantów wcześniej występujących) na ciężkość przebiegu COVID-19 oraz potencjalnych demograficznych, klinicznych i laboratoryjnych predyktorów ciężkiego przebiegu choroby w populacji hospitalizowanych młodych dorosłych.

Do badania włączono 229 pacjentów z COVID-19, w tym 75 pacjentów hospitalizowanych podczas drugiej fali oraz 154 pacjentów hospitalizowanych podczas trzeciej fali pandemii. Najczęściej zgłaszanymi objawami COVID-19 były: duszność (91,7%), gorączka (85,2%), kaszel (85,2%) i osłabienie (56,8%). Wśród innych objawów występowały: bóle mięśniowe (29,3%), zaburzenia węchu i/lub smaku (18,8%), ból głowy (18,3%), biegunka (17,9%), nudności i/lub wymioty (11,8%), ból gardła (97,9%) oraz krwioplucie (3,5%). Nie stwierdzono istotnych różnic pomiędzy falami pandemii w zakresie zgłaszanych objawów, płci, wskaźnika masy ciała (BMI, ang. *body mass index*), palenia tytoniu i chorób współistniejących.

Mediana zajęcia miąższu płucnego przez zmiany zapalne w badaniu tomografii komputerowej (TK) wynosiła 31%, w tym 30% w drugiej fali i 33% w trzeciej fali pandemii. Mediana czasu od wystąpienia objawów do przyjęcia do szpitala wynosiła w drugiej fali 8 dni (IQR 6–10 dni), zaś w trzeciej fali 9 dni (IQR 7–11 dni). Jako że kryterium włączenia do badania była hospitalizacja spowodowana ciężkim przebiegiem COVID-19, niemal wszyscy pacjenci (96,9%) wymagali suplementacji tlenu. Tlenoterapia wysokoprzepływowa (HFNO, ang. *high flow nasal oxygen therapy*) była zastosowana u 24% chorych, wentylacja mechaniczna była konieczna u 9,6%, 13,5% pacjentów wymagało przyjęcia do OIT, a 7% zmarło. Śmiertelność w OIT wyniosła 48,4%. Nie stwierdzono istotnych różnic pomiędzy badanymi falami pandemii pod względem odsetka zajętego miąższu płucnego, konieczności suplementacji tlenu, HFNO, wentylacji mechanicznej, przyjęcia do OIT oraz śmiertelności. Wyniki te wskazują, że u młodych dorosłych hospitalizowanych z powodu ciężkiego przebiegu COVID-19 wariant alfa SARS-CoV-2 nie zwiększa ryzyka krytycznego przebiegu choroby i zgonu. Co więcej, w drugiej fali obserwowano większą liczbę leukocytów, neutrofilów i niedojrzałych granulocytów, większy odsetek niedojrzałych granulocytów, wyższy stosunek liczby neutrofilów do liczby limfocytów (NLR, ang. *neutrophil-to-lymphocyte ratio*) oraz większą częstość leukocytozy i neutrofilii przy przyjęciu do szpitala, zaś odsetek limfocytów przy przyjęciu do szpitala był niższy niż u pacjentów z trzeciej fali. Nie stwierdzono natomiast istotnych różnic pomiędzy falami w zakresie stężenia interleukiny-6

(IL-6), CRP i ferrytyny. Ponadto, choć nie zaobserwowano istotnych różnic w stężeniu PCT przy przyjęciu do szpitala, jej stężenie w 7. dniu hospitalizacji było istotnie wyższe u pacjentów z drugiej fali niż w fali trzeciej. Jako że powyższe wskaźniki nadmiernej reakcji zapalnej są prawdopodobnie związane z cięższym przebiegiem choroby, jak opisano poniżej, obserwacje te również przemawiają przeciwko cięższemu przebiegowi zakażenia wariantem alfa SARS-CoV-2 w porównaniu z wariantami wcześniejszymi. Co więcej, choć nie zaobserwowano istotnych różnic w zakresie stężeń D-Dimeru i albuminy przy przyjęciu do szpitala, w 7. dniu hospitalizacji stężenie D-Dimeru było wyższe, a albuminy niższe w drugiej fali w porównaniu z falą trzecią, przy czym wysokie stężenie D-Dimeru i niskie stężenie albuminy są również prawdopodobnie związane z gorszym rokowaniem. Ponadto, choć stężenie mioglobiny w 7. dniu hospitalizacji było niższe w drugiej niż w trzeciej fali pandemii, to nie zaobserwowano istotnych różnic pomiędzy falami w zakresie innych, bardziej swoistych markerów uszkodzenia mięśnia sercowego. Nie stwierdzono również istotnych różnic pomiędzy falami w zakresie stężeń antytrombiny III (AT III), fibrynogenu, LDH, wapnia, witaminy D3 oraz markerów uszkodzenia nerek i wątroby.

Porównując pacjentów, którzy przeżyli, z pacjentami, którzy zmarli w przebiegu hospitalizacji, nie stwierdzono istotnych różnic w zakresie wieku, płci czy grupy krwi. Choć masa ciała i BMI były wyższe u pacjentów, którzy zmarli, różnice te nie były istotne po wyeliminowaniu z analizy obserwacji odstających. Należy jednak zauważyć, że w tym badaniu, do którego włączono jedynie pacjentów hospitalizowanych z powodu ciężkiego przebiegu COVID-19, mediana BMI wynosiła 30,6 kg/m$^2$ (IQR 27,1–34,3 kg/m$^2$), a jedynie u 12,3% chorych obserwowano jego prawidłowe wartości (18–25 kg/m$^2$). Co więcej, stwierdzono dodatnią korelację pomiędzy BMI a koniecznością wentylacji mechanicznej. Ponadto, w jednoczynnikowej analizie regresji logistycznej masa ciała > 100 kg oraz BMI ≥ 40 kg/m$^2$ stanowiły istotne predyktory zgonu, konieczności wentylacji mechanicznej i przyjęcia do OIT, a BMI ≥ 40 kg/m$^2$ stanowił również istotny predyktor konieczności wentylacji mechanicznej w analizie wieloczynnikowej (OR = 6,9). Wydaje się zatem, że nadwaga i otyłość powinny być brane pod uwagę jako predyktory gorszego rokowania, prawdopodobnie z powodu ich związku z innymi chorobami współistniejącymi, zmniejszoną objętością płuc oraz skłonnością do nadkrzepliwości.

Pacjenci, którzy później zmarli, istotnie częściej zgłaszali w wywiadzie palenie tytoniu (w przeszłości lub obecnie), zaobserwowano również słabą dodatnią korelację pomiędzy nikotynizmem w wywiadzie a koniecznością wentylacji mechanicznej

i przyjęcia do OIT, co wskazuje, że palenie tytoniu również należy rozważać jako potencjalny czynnik ryzyka ciężkiego przebiegu COVID-19 u młodych dorosłych. U pacjentów zmarłych istotnie częściej obserwowano również występowanie chorób współistniejących (68,75% w porównaniu do 38,5% pacjentów, którzy przeżyli), a ich obecność była istotnie związana z ryzykiem zgonu i wentylacji mechanicznej w jednoczynnikowej analizie regresji logistycznej, a także z ryzykiem zgonu w modelu wieloczynnikowym (OR = 4). U pacjentów, którzy zmarli, istotnie częściej obserwowano przewlekłe zaburzenia rytmu serca, nie stwierdzono zaś istotnych różnic w częstości insulinooporności, cukrzycy, nadciśnienia tętniczego, astmy, niedoczynności tarczycy oraz choroby Hashimoto. W regresji logistycznej, w analizie jednoczynnikowej, obecność cukrzycy lub insulinooporności w wywiadzie była istotnie związana z ryzykiem zgonu, wentylacji mechanicznej i przyjęcia do OIT, zaś przewlekłe zaburzenia rytmu serca – z ryzykiem zgonu i wentylacji mechanicznej. Ogólnie jednak związek chorób współistniejących z rokowaniem w przebiegu zakażenia SARS-CoV-2 wydaje się być słabiej wyrażony niż w populacji ogólnej, jako że większa liczba chorób współistniejących u osób starszych może prowadzić do bardziej złożonych patomechanizmów w przebiegu COVID-19 i jej powikłań [44].

Odsetek zajęcia płuc przez zmiany zapalne stwierdzany w TK był istotnie wyższy, a zajęcie ponad 50% miąższu płucnego było istotnie częstsze u pacjentów, którzy zmarli. Stwierdzono również słabą dodatnią korelację pomiędzy odsetkiem zajęcia płuc a koniecznością wentylacji mechanicznej i przyjęcia do OIT. $SpO_2$ przy przyjęciu do szpitala była istotnie niższa u pacjentów, którzy zmarli. Zaobserwowano również ujemną korelację pomiędzy $SpO_2$ przy przyjęciu do szpitala a koniecznością wentylacji mechanicznej i hospitalizacji w OIT. Pacjenci, którzy zmarli, częściej wymagali HFNO, wentylacji mechanicznej, przyjęcia do OIT, wlewu amin presyjnych oraz ciągłej terapii nerkozastępczej (CRRT, ang. *continuous renal replacement therapy*).

Zakażenie SARS-CoV-2 wywołuje odpowiedź immunologiczną gospodarza, która u większości pacjentów prowadzi do eliminacji wirusa. Jednakże w niektórych przypadkach wywołana zakażeniem aktywacja makrofagów pęcherzykowych powoduje zwiększone uwalnianie cytokin, takich jak IL-1, IL-6 i czynnik martwicy nowotworu-α (TNF-α, ang. *tumor necrosis factor alpha*), oraz innych mediatorów prozapalnych, co określane jest mianem „burzy cytokinowej". Prowadzi to do rekrutacji i naciekania neutrofilów, monocytów i innych leukocytów. Cytokiny stymulują też szpik kostny do produkcji i uwalniania niedojrzałych granulocytów, które naciekają płuca, dodatkowo

nasilając nadmierną reakcję zapalną [9,30,32]. Ponadto, uwalnianie cytokin, w tym IL-6, indukuje syntezę białek ostrej fazy, takich jak CRP, fibrynogen i ferrytyna [32], co z kolei może dalej modulować reakcję immunologiczną, zwiększając ekspresję mediatorów zarówno prozapalnych, jak i przeciwzapalnych [107,108]. Nasilona reakcja zapalna może również prowadzić do indukowanej cytokinami apoptozy limfocytów [31,109,110]. Inne możliwe przyczyny limfopenii w COVID-19 obejmują bezpośrednie zakażenie wirusowe limfocytów, wykazujących ekspresję ACE2, uszkodzenie narządów limfatycznych oraz zwiększone zużycie limfocytów w zakażonych tkankach [31,109,110]. Z tych powodów w COVID-19 obserwuje się również podwyższony stosunek liczby neutrofilów do liczby limfocytów (NLR) [31]. Potwierdzają to wyniki przedstawionego badania, w którym liczba leukocytów, neutrofilów oraz niedojrzałych granulocytów, odsetek niedojrzałych granulocytów oraz częstość leukocytozy, neutrofilii, limfopenii, a także NLR i stężenie IL-6, były istotnie wyższe u pacjentów, którzy zmarli. Co więcej, u pacjentów tych mediana stężenia CRP była istotnie wyższa i istotnie częściej obserwowano stężenie CRP powyżej 100 mg/l. Zaobserwowano również dodatnią korelację pomiędzy liczbą leukocytów, neutrofilów oraz niedojrzałych granulocytów, odsetkiem neutrofilów i niedojrzałych granulocytów, częstością leukocytozy i neutrofilii, NLR oraz stężeniem IL-6 i CRP, a koniecznością wentylacji mechanicznej i przyjęcia do OIT, oraz ujemną korelację pomiędzy odsetkiem limfocytów a koniecznością wentylacji mechanicznej i przyjęcia do OIT. W jednoczynnikowej analizie regresji logistycznej NLR $\geq$ 2 i limfopenia w 7. dniu hospitalizacji, a także neutrofilia, leukocytoza i stężenie CRP > 100 mg/l przy przyjęciu do szpitala i w 7. dniu hospitalizacji, były istotnie związane z ryzykiem zgonu, wentylacji mechanicznej i przyjęcia do OIT, a w analizie wieloczynnikowej leukocytoza przy przyjęciu do szpitala stanowiła istotny predyktor zgonu, konieczności wentylacji mechanicznej i przyjęcia do OIT (z OR równymi odpowiednio 5,8, 5,75 i 3,7). Co więcej, choć nie stwierdzono istotnych różnic w zakresie stężenia ferrytyny pomiędzy pacjentami, którzy zmarli, a tymi, którzy przeżyli, zaobserwowano słabą pozytywną korelację pomiędzy stężeniem ferrytyny przy przyjęciu do szpitala a koniecznością wentylacji mechanicznej oraz pomiędzy stężeniem ferrytyny w 7. dniu hospitalizacji a koniecznością przyjęcia do OIT.

Prokalcytonina może być indukowana bezpośrednio przez endotoksyny i lipopolisacharydy bakteryjne lub pośrednio poprzez uwalnianie cytokin prozapalnych, takich jak IL-1β, TNF-α i IL-6. Z drugiej strony jej synteza może być hamowana przez interferon-γ (INF-γ), którego stężenie wzrasta podczas infekcji wirusowych. Z tego

powodu stężenie PCT nie jest typowo podwyższone podczas zakażenia SARS-CoV-2 o łagodnym przebiegu, wzrasta zaś podczas ciężkiej postaci COVID-19, zwłaszcza wskutek koinfekcji bakteryjnych [111–113]. Potwierdza to zaobserwowane w obecnym badaniu wyższe stężenie PCT i częstsze występowanie stężenia PCT > 0,5 ng/ml u pacjentów, którzy zmarli, a także dodatnia korelacja pomiędzy stężeniem PCT a koniecznością wentylacji mechanicznej i przyjęcia do OIT. Co więcej, zarówno w jednoczynnikowej, jak i wieloczynnikowej analizie regresji logistycznej, stężenie PCT przy przyjęciu do szpitala > 0,5 ng/ml było istotnie związane z ryzykiem zgonu, konieczności wentylacji mechanicznej i przyjęcia do OIT (z OR w analizie wieloczynnikowej równymi odpowiednio 5, 9,6 i 5,4).

Choć nie zaobserwowano istotnych różnic w zakresie liczby erytrocytów, poziomu hematokrytu oraz stężenia hemoglobiny przy przyjęciu do szpitala, parametry te w 7. dniu hospitalizacji były istotnie niższe u pacjentów, którzy zmarli, a także zaobserwowano ich ujemną korelację z koniecznością wentylacji mechanicznej i przyjęcia do OIT. Co więcej, w jednoczynnikowej analizie regresji logistycznej liczba erytrocytów < 4.5 $\times 10^6/\mu l$, poziom hematokrytu < 40% oraz stężenie hemoglobiny < 12 g/dl w 7. dniu hospitalizacji były istotnie związane z ryzykiem zgonu, wentylacji mechanicznej i przyjęcia do OIT. Zaobserwowany rozwój niedokrwistości w przebiegu COVID-19 może również odzwierciedlać nadmierną reakcję zapalną prowadzącą do progresji choroby [114].

Kolejnymi przyczynami ciężkiego przebiegu i niekorzystnego rokowania w COVID-19 są prawdopodobnie zakrzepica i koagulopatia. Do zaburzeń układu krzepnięcia w przebiegu zakażenia SARS-CoV-2 mogą przyczyniać się takie czynniki, jak: burza cytokinowa prowadząca do zwiększonej produkcji płytek krwi i fibrynogenu, aktywacja dopełniacza, dysfunkcja naczyń, zaburzenia równowagi układów renina-angiotensyna / kalikreina-kinina (RAS-KKS, ang. *Renin-Angiotensin-Kallikrein-Kinin systems*) oraz nadmierne wewnątrznaczyniowe wytwarzanie przez neutrofile tzw. zewnątrzkomórkowych pułapek neutrofilowych (NET, ang. *neutrophil extracellular traps)* [30,32]. Stan nadkrzepliwości prowadzi do wzrostu stężenia D-Dimeru, będącego produktem degradacji fibryny [30]. Potwierdzają to wyniki przeprowadzonego badania, w którym stwierdzono istotnie wyższe stężenie D-Dimeru i częstsze występowanie stężenia D-Dimeru > 500 μg/l przy przyjęciu do szpitala u osób, które zmarły, a także dodatni związek pomiędzy stężeniem D-Dimeru > 500 μg/l a koniecznością wentylacji mechanicznej i przyjęcia do OIT. Co więcej, jednoczynnikowa analiza regresji logistycznej wykazała, że stężenie D-Dimeru > 500 μg/l było związane z ryzykiem zgonu, wentylacji

mechanicznej i przyjęcia do OIT, a w analizie wieloczynnikowej stężenie D-Dimeru przy przyjęciu do szpitala > 500 μg/l było istotnie związane z ryzykiem przyjęcia do OIT (OR = 5,2). Nie stwierdzono natomiast istotnych różnic pomiędzy pacjentami, którzy zmarli a tymi, którzy przeżyli, w zakresie liczby płytek krwi. Wynika to prawdopodobnie z faktu, że w ciężkim przebiegu COVID-19 może występować zarówno trombocytopenia, wywołana zakażeniem szpiku, jak i zwiększona produkcja płytek krwi indukowana przez cytokiny prozapalne [115]. Rzeczywiście, w przedstawionym badaniu zarówno trombocytopenia, jak i trombofilia, wykazywały dodatnią korelację z koniecznością przyjęcia do OIT.

Kolejnym biomarkerem związanym z ciężkością przebiegu COVID-19 jest LDH, enzym obecny we wszystkich tkankach i uwalniany do krwi podczas ich uszkodzenia, które może być wywołane infekcją wirusową, hipoksją oraz nadmierną reakcją zapalną [116]. Potwierdzają to wyniki przedstawionej pracy, w której u pacjentów, którzy zmarli, zaobserwowano wyższą aktywność LDH przy przyjęciu do szpitala, a także stwierdzono dodatnią korelację między aktywnością LDH a koniecznością wentylacji mechanicznej i przyjęcia do OIT. Co więcej, jednoczynnikowa analiza regresji logistycznej wykazała, że aktywność LDH > 500 U/l była związana z ryzykiem zgonu, wentylacji mechanicznej i przyjęcia do OIT, a w analizie wieloczynnikowej aktywność LDH przy przyjęciu do szpitala > 500 U/l była istotnie związana z ryzykiem wentylacji mechanicznej i przyjęcia do OIT (z OR równym odpowiednio 4,7 i 3,4). Zwiększona aktywność LDH może wynikać z niewydolności wielonarządowej obserwowanej w ciężkich postaciach COVID-19, w tym z dysfunkcji nerek, mięśnia sercowego oraz wątroby.

Doniesienia naukowe wskazują, że podwyższone stężenia markerów uszkodzenia mięśnia sercowego, takich jak wysokoczuła troponina I (hs-TnI, ang. *high-sensitive troponin I*), izoenzym sercowy kinazy kreatynowej (CK-MB, ang. *creatine kinase-myocardial band*), mioglobina i N-końcowy propeptyd natriuretyczny typu B (NT-proBNP, ang. *N-terminal-pro-B-type natriuretic peptide*), związane są z większą ciężkością COVID-19 i z wyższą śmiertelnością [117]. Rzeczywiście, stężenia hs-TnI, CK-MB oraz NT-proBNP były istotnie wyższe u pacjentów, którzy zmarli, a także obserwowano ich dodatnią korelację z koniecznością wentylacji mechanicznej i przyjęcia do OIT. Co więcej, jednoczynnikowa analiza regresji logistycznej wykazała, że stężenia CK-MB > 20 U/l i NT-proBNP > 190 pg/ml przy przyjęciu do szpitala oraz w 7. dniu hospitalizacji, a także stężenie hsTnI > 34 pg/ml w 7. dniu hospitalizacji były związane z wyższym ryzykiem zgonu, wentylacji mechanicznej i przyjęcia do OIT, a stężenie hsTnI

> 34 pg/ml przy przyjęciu do szpitala – z wyższym ryzykiem wentylacji mechanicznej i przyjęcia do OIT. Zaobserwowano również dodatnią korelację pomiędzy stężeniem mioglobiny, będącej markerem mniej swoistym dla uszkodzenia mięśnia sercowego, a ryzykiem wentylacji mechanicznej i przyjęcia do OIT. Co ciekawe, aktywność CK, innego markera nieswoistego dla uszkodzenia kardiomiocytów, była istotnie wyższa u pacjentów zmarłych jedynie w pomiarach dokonanych w 7. dniu hospitalizacji, ale nie przy przyjęciu do szpitala. Jednoczynnikowa analiza regresji logistycznej wykazała z kolei, że aktywność CK > 190 U/l, zarówno przy przyjęciu do szpitala, jak i w 7. dniu hospitalizacji, związana była ze zwiększonym ryzykiem wentylacji mechanicznej i przyjęcia do OIT, jednak predyktorem zgonu była jedynie aktywność CK > 190 U/l w 7. dniu hospitalizacji. Może to oznaczać, że wzrost aktywności CK odzwierciedla progresję choroby.

W literaturze istnieją również doniesienia wskazujące, że predyktorem ciężkiego przebiegu COVID-19 oraz związanych z nim zgonów jest ostre uszkodzenie nerek [118]. Potwierdzają to wyniki przedstawianego badania, w którym stwierdzono wyższe stężenia kreatyniny i mocznika oraz niższy szacowany wskaźnik przesączania kłębuszkowego (EGFR, ang. *estimated glomerular filtration rate*) u osób, które zmarły. Zaobserwowano również dodatnią korelację pomiędzy stężeniem kreatyniny a koniecznością wentylacji mechanicznej i pomiędzy stężeniem mocznika a koniecznością wentylacji mechanicznej i przyjęcia do OIT, a także ujemną korelację pomiędzy EGFR a koniecznością wentylacji mechanicznej. Co więcej, w jednoczynnikowej analizie regresji logistycznej stwierdzono, że EGFR < 60 ml/min oraz kreatynina >1,2 mg/ml przy przyjęciu do szpitala i w 7. dniu hospitalizacji, a także stężenie mocznika > 49 mg/dl w 7. dniu hospitalizacji były istotnie związane z ryzykiem zgonu, wentylacji mechanicznej i przyjęcia do OIT, zaś stężenie mocznika > 49 mg/ml przy przyjęciu do szpitala – z ryzykiem wentylacji mechanicznej.

Pomimo doniesień literaturowych, iż COVID-19 powoduje również uszkodzenie wątroby, dane odnośnie związku aktywności enzymów wątrobowych z ciężkością przebiegu choroby i związaną z nią śmiertelnością pozostają niespójne [119–121]. Co więcej, obserwowana w niektórych badaniach hipertransaminazemia może wynikać również z uszkodzenia mięśni i kardiomiocytów lub hepatotoksyczności leków stosowanych w COVID-19 [122]. W przedstawionym badaniu nie zaobserwowano istotnych różnic w zakresie ALT, aminotrasferazy asparaginianowej (AST, ang. *aspartate aminotransferase*) oraz bilirubiny całkowitej przy przyjęciu do szpitala pomiędzy osobami, które przeżyły, a tymi, które zmarły. Co ciekawe, choć nie stwierdzono różnic pomiędzy

pacjentami, którzy przeżyli i tymi, którzy zmarli, w zakresie aktywności gamma–glutamylo-transferazy (GGT, ang. *gamma-glutamyl transferase*), w jednoczynnikowej analizie regresji logistycznej aktywność GGT > 120 U/l była związana ze zwiększonym ryzykiem zgonu oraz wentylacji mechanicznej. Stężenie albuminy w 7. dniu hospitalizacji było istotnie niższe u pacjentów, którzy zmarli. Zaobserwowano również ujemną korelację pomiędzy stężeniem albuminy a koniecznością wentylacji mechanicznej i przyjęcia do OIT. Hipoalbuminemia w przebiegu COVID-19 może wynikać jednak nie tylko z dysfunkcji wątroby, ale też z priorytetyzacji syntezy białek ostrej fazy, indukowanej przez cytokiny zwiększonej przepuszczalności naczyń, prowadzącej do ucieczki albumin do przestrzeni zewnątrznaczyniowej, oraz zwiększonej utraty albuminy z moczem, spowodowanej uszkodzeniem nerek [119,123].

Stężenie wapnia całkowitego było istotnie niższe u pacjentów, którzy zmarli. Stwierdzono również ujemną korelację pomiędzy stężeniem wapnia a koniecznością wentylacji mechanicznej i przyjęcia do OIT. Co więcej, jednoczynnikowa analiza regresji logistycznej wykazała, że stężenie wapnia niższe niż 2,1 mmol/l związane było z ryzykiem zgonu, wentylacji mechanicznej i przyjęcia do OIT. Wyniki te są zgodne z doniesieniami naukowymi, jako że w metaanalizach wykazano związek hipokalcemii ze zwiększoną ciężkością choroby i śmiertelnością w przebiegu COVID-19, prawdopodobnie ze względu na rolę wapnia w odpowiedzi immunologicznej [124,125]. Ponadto witamina D, będąca istotnym regulatorem homeostazy wapnia, wykazuje działanie immunomodulacyjne poprzez wpływ na produkcję peptydów antybakteryjnych, hamowanie produkcji cytokin prozapalnych i promowanie cytokin przeciwzapalnych, kontrolę odpowiedzi immunologicznej zależnej od komórek T oraz modulację aktywności neutrofilów i makrofagów [126–128]. Co ciekawe, w prezentowanym badaniu stężenie witaminy D3 przy przyjęciu do szpitala było istotnie niższe u pacjentów, którzy zmarli, a także zaobserwowano ujemną korelację pomiędzy stężeniem witaminy D3 w 7. dniu hospitalizacji a koniecznością przyjęcia do OIT.

W analizie ROC choroby współistniejące (AUC = 0,65), liczba leukocytów > $10\times10^3$/μl (AUC = 0,65) oraz PCT > 0,5 ng/ml (AUC = 0,65) stanowiły predyktory zgonu; BMI ≥ 40 kg/m$^2$ (AUC = 0,57), aktywność LDH > 500 U/l (AUC = 0,7), liczba leukocytów > $10\times10^3$/μl (AUC = 0,72) oraz PCT > 0,5 ng/ml (AUC = 0,7) – predyktory konieczności wentylacji mechanicznej, zaś stężenie D-Dimeru > 500 μg/l FEU (AUC = 0,65), aktywność LDH > 500 U/l (AUC = 0,68), liczba leukocytów > $10\times10^3$/μl (AUC = 0,66) oraz PCT > 0,5 ng/ml (AUC = 0,65) – predyktory przyjęcia do OIT. Całkowite AUC

wieloczynnikowych modeli regresji logistycznej dla predykcji zgonu, wentylacji mechanicznej i przyjęcia do OIT wynosiły odpowiednio 0,81, 0,84 i 0,85.

Przedstawione badanie ma pewne ograniczenia. Po pierwsze, było to badanie jednoośrodkowe o stosunkowo niewielkiej liczebności próby, do którego włączono jedynie pacjentów hospitalizowanych z powodu ciężkiego przebiegu COVID-19, co może ograniczać możliwość generalizacji jego wyników na całą populację młodych dorosłych. Z tego powodu konieczne są większe, wieloośrodkowe badania prospektywne. Po drugie, jako że było to badanie obserwacyjne i eksploracyjne, z dużą liczbą przeprowadzonych testów statystycznych, może ono być obarczone ryzykiem wyników fałszywie dodatnich oraz wpływem innych czynników zakłócających. Ponadto, z uwagi na retrospektywną naturę badania i ograniczone zasoby systemu opieki zdrowotnej w czasie jego prowadzenia, nie wykonywano badań genetycznych w kierunku określenia wariantu SARS-CoV-2. Należy jednak zauważyć, że w Polsce częstość występowania wariantu alfa SARS-CoV-2 w okresie zdefiniowanym w dysertacji jako druga fala pandemii była niska (około 6,5%), podczas gdy w okresie zdefiniowanym jako trzecia fala wariant alfa stanowił ponad 92% identyfikowanych szczepów wirusa [54], co wskazuje, że analizowane fale dobrze korespondują z wariantami sprawczymi SARS-CoV-2. Co więcej, żaden z pacjentów nie był wcześniej zaszczepiony przeciwko SARS-CoV-2 ani nie miał w wywiadzie udokumentowanego przebytego zakażenia tym wirusem. Nie zaobserwowano również istotnych różnic w zakresie stosowanej terapii pomiędzy badanymi falami. Z tych powodów, choć omawiane tu warianty SARS-CoV-2 nie są już dominujące, wyniki badania mogą wciąż być istotne, jako że dostarczają one cennych informacji na temat mechanizmów związanych z zakażeniem SARS-CoV-2 bez zakłócającego wpływu wymienionych wyżej czynników, co jest niezmiernie trudne do osiągnięcia w badaniach wariantów późniejszych. Warto również zauważyć, że większość wcześniejszych badań koncentrowała się na predyktorach ciężkiego przebiegu COVID-19 w populacji ogólnej, przy czym często w badanych próbach pacjentów hospitalizowanych przeważały osoby starsze, podczas gdy czynniki ryzyka u młodych dorosłych wydają się być inne, a znalezienie predyktorów niekorzystnego rokowania w tej grupie wiekowej może przyczynić się do wcześniejszej i lepszej identyfikacji osób o podwyższonych ryzyku ciężkiego przebiegu choroby.

## 5.4. Wyniki badania predyktorów ciężkiego przebiegu COVID-19 w grupie kobiet ciężarnych.

Do badania włączono 52 kobiety ciężarne. Średni BMI pacjentek przy przyjęciu do szpitala wynosił 28,4 kg/m$^2$. Do objawów COVID-19 obserwowanych przy przyjęciu do szpitala należały: duszność (92,3%), kaszel (90,4%), gorączka (63,5%), osłabienie i bóle mięśniowe (42,3%), zaburzenia węchu i/lub smaku (26,9%), ból głowy (23,1%), ból gardła (11,5%) oraz katar (9,6%). Najczęściej obserwowanymi chorobami współistniejącymi były: niedoczynność tarczycy (35,3%), cukrzyca (17,7%), nadciśnienie tętnicze (10%) i astma (3,9%). Łagodny, umiarkowany, ciężki i krytyczny przebieg COVID-19 obserwowano odpowiednio u 17,3%, 48,1%, 32,7% i 1,9% chorych. Mediana czasu trwania hospitalizacji wyniosła 8 dni. Konwencjonalna tlenoterapia (przez wąsy tlenowe albo maskę tlenową) była konieczna u 80,8% pacjentek, 17,3% z nich wymagało tlenoterapii wysokoprzepływowej (HFNO), zaś w 3,9% przypadków konieczne było przyjęcie do OIT. Mediana odsetka zajęcia miąższu płucnego przez zmiany zapalne wynosiła 20% (przy zakresie 1-60%). Najczęściej obserwowanymi nieprawidłowościami w badaniach laboratoryjnych były: podwyższone stężenia CRP (94,2%), D-Dimeru (90,6%), IL-6 (88,5%) i fibrynogenu (88%), hipoproteinemia (66,7%), obniżone stężenie witaminy D3 (62,2%), podwyższona aktywność LDH (56%), hiperglikemia (48,4%), niedokrwistość (48,1%), podwyższona aktywność fosfatazy alkalicznej (ALP, ang. *alkaline phosphatase*; 46,2%), podwyższona aktywność AST (40,4%), limfopenia (38,5%), podwyższone stężenie kwasów żółciowych (35,7%), neutrofilia (30,8%) oraz podwyższona aktywność ALT (30%). Zaobserwowano związek pomiędzy występowaniem nadciśnienia tętniczego a koniecznością zastosowania wyższych przepływów tlenu oraz pomiędzy występowaniem cukrzycy a koniecznością stosowania wyższych przepływów tlenu i HFNO. Odsetek zajętego przez zmiany zapalne miąższu płucnego w TK wykazywał dodatnią korelację ze stopniem ciężkości przebiegu COVID-19, maksymalnym przepływem tlenu w tlenoterapii, a także koniecznością zastosowania HFNO oraz przyjęcia do OIT. Limfopenia, niższe stężenia wapnia, cholesterolu całkowitego i białka całkowitego, a także wyższe stężenia CRP, ferrytyny, IL-6, glukozy, LDH, PCT i hs-TnI, były predyktorami ciężkiego przebiegu COVID-19, wpływając na stopień ciężkości choroby, konieczność suplementacji tlenu i zastosowania wyższych jego przepływów, długość hospitalizacji, a także konieczność zastosowania wentylacji mechanicznej i przyjęcia do OIT. W jednoczynnikowej analizie regresji logistycznej czynnikami ryzyka wystąpienia ciężkiego lub krytycznego przebiegu COVID-19 były: cukrzyca (OR = 10,2),

wiek ciążowy poniżej 32 tygodni (OR = 5), odsetek zajętego przez zmiany zapalne miąższu płucnego > 20% (OR = 5,8), liczba limfocytów < $1\times10^3$/μl (OR = 27,4), oraz poziomy CRP > 75 mg/l (OR = 9,1), IL-6 > 60 pg/ml (OR = 16,5), PCT > 0,2 ng/ml (OR = 5,1), LDH > 270 U/l (OR = 3,7), hsTnI > 6 ng/ml (OR = 12,7), wapnia całkowitego ≤ 2,15 mmol/l (OR = 5,6), cholesterolu całkowitego ≤ 180 mg/dl (OR = 5,7), białka całkowitego ≤ 6,3 g/dl (OR = 9) oraz glukozy > 99 mg/dl (OR = 6). Wskaźniki te mogą stanowić zatem predyktory ciężkiego przebiegu COVID-19, pozwalając na wczesną identyfikację pacjentek obarczonych wysokim ryzykiem progresji choroby. Rzeczywiście, jak wspomniano wyżej, podwyższone markery stanu zapalnego i limfopenia często towarzyszą burzy cytokinowej, powszechnie obserwowanej w ciężkiej postaci COVID-19. Nieprawidłowa odpowiedź immunologiczna może również wpływać na złą kontrolę glikemii, co powinno skłaniać klinicystów do zwracania szczególnej uwagi na poziom glukozy we krwi podczas ciąży, zarówno u kobiet z cukrzycą w wywiadzie, jak i u pacjentek wcześniej zdrowych [129–132]. Na występowanie hipoproteinemii może mieć wpływ zarówno spadek wytwarzania albuminy i prealbuminy wywołany przez burzę cytokinową [133], jak i wzrost zapotrzebowania na białko w okresie ciąży [134]. Zmiany w gospodarce lipidowej mogą z kolei wynikać z upośledzonej produkcji apolipoprotein i lipoprotein wskutek uszkodzenia wątroby oraz aktywności cytokin, a także z tzw. „przecieku naczyniowego” (ang. *capillary leak*), powodującego ucieczkę lipidów do kompartmentów pozanaczyniowych [135,136].

Główne ograniczenia przedstawionego badania stanowią jego jednoośrodkowy charakter oraz niewielka, dość jednorodna grupa pacjentek. Z tego powodu konieczne są dalsze badania nad predyktorami ciężkiego przebiegu COVID-19 u ciężarnych.

## 6. WNIOSKI

1. Na podstawie wyników stworzonego na potrzeby tego badania prostego, jednorazowego psychofizycznego testu węchowego wykazano, że w przebiegu COVID-19 często obserwuje się zaburzenia węchu, a ich wystąpienie jest związane z wysokim prawdopodobieństwem rozpoznania zakażenia SARS-CoV-2, przez co objaw ten stanowi dobry predyktor COVID-19, przy czym:
    a. jako że subiektywne występowanie anosmii lub hiposmii zgłaszało 32%-44% chorych, podczas gdy w teście psychofizycznym stwierdzono je u 55%-64% pacjentów, ocena subiektywna prowadzi prawdopodobnie do niedoszacowania częstości występowania zaburzeń węchu, co wskazuje na istotną rolę metod psychofizycznych oceny zmysłu powonienia w przebiegu COVID-19;
    b. subiektywne osłabienie węchu nie ma związku z ciężkością przebiegu COVID-19, natomiast w przypadku testów psychofizycznych, choć ich gorsze wyniki były związane z pewnymi wykładnikami ciężkiego przebiegu choroby, konieczne są większe, wieloośrodkowe, prospektywne badania;
    c. wykazano, że subiektywne zaburzenia węchu mają wysoką swoistość, ale niską lub umiarkowaną czułość w predykcji wystąpienia zakażenia SARS-CoV-2, podczas gdy metody psychofizyczne charakteryzuje zarówno wysoka swoistość, jak i czułość, a połączenie wyników testu psychofizycznego z informacjami uzyskanymi z wywiadu pozwala na dalsze zwiększenie ich dokładności diagnostycznej w ocenie prawdopodobieństwa zakażenia SARS-CoV-2. Umożliwia to stworzenie niedrogiego i efektywnego skriningu w kierunku COVID-19, opartego na połączeniu ankiety oceniającej niedawne wystąpienie zaburzeń węchu i wyników prostego, jednorazowego testu identyfikacji zapachów, pozwalając na odpowiednio wczesne skierowanie pacjentów ze stwierdzonym w ten sposób wysokim prawdopodobieństwem zakażenia SARS-CoV-2 na izolację i, w miarę możliwości, odpowiednie badania laboratoryjne. Może mieć to szczególne znaczenie w krajach o wysokim odsetku zachorowań i ograniczonych zasobach, powodujących zmniejszenie możliwości powszechnego wykonywania testów RT-PCR.
2. Na podstawie przeglądu literatury stwierdzono, że w patogenezie anosmii w przebiegu COVID-19 decydującą rolę pełni prawdopodobnie uszkodzenie

czuciowo-nerwowe, które wynika z zakażenia poprzez receptor dla ACE2 komórek podporowych nabłonka węchowego, mających istotne znaczenie w prawidłowym funkcjonowaniu neuronów węchowych, a także prawdopodobnie z bezpośredniego zakażenia neuronów węchowych poprzez receptor dla NRP1. Choć obserwowane zwykle w COVID-19 wczesny początek i wczesne ustępowanie zaburzeń węchu mogłyby sugerować ich przewodzeniowy charakter, brak objawów zapalenia błony śluzowej nosa u większości pacjentów przeczy dominującej roli niedrożności nosa w powstawaniu anosmii związanej z zakażeniem SARS-CoV-2. Innymi czynnikami mogącymi mieć udział w patogenezie związanych z COVID-19 zaburzeń węchu mogą być zajęcie wyższych odcinków drogi węchowej oraz czynniki genetyczne.

3. W grupie młodych dorosłych hospitalizowanych z powodu ciężkiego przebiegu COVID-19 wariant alfa SARS-CoV-2 nie wiązał się z gorszym rokowaniem i cięższym przebiegiem choroby niż warianty wcześniejsze.
4. W grupie młodych dorosłych hospitalizowanych z powodu ciężkiego przebiegu COVID-19 z niekorzystnym rokowaniem związanych może być wiele czynników, takich jak: otyłość, obecność chorób współistniejących, nikotynizm w wywiadzie, wyższy odsetek zajęcia przez zmiany zapalne miąższu płucnego w obrazach tomografii komputerowej, niższa saturacja krwi obwodowej tlenem ($SpO_2$), leukocytoza, neutrofilia, limfopenia, większa liczba niedojrzałych granulocytów, wyższy stosunek liczby neutrofilów do liczby limfocytów (NLR), wyższe stężenia CRP, PCT, IL-6, D-Dimeru, LDH, hs-TnI, CK-MB, mioglobiny, NT-proBNP, kreatyniny, mocznika i GGT, niższy EGFR oraz niższe stężenia albuminy, wapnia i witaminy D3, a także spadek liczby erytrocytów, stężenia hemoglobiny i poziomu hematokrytu oraz wzrost aktywności CK w przebiegu choroby. Określenie tych czynników rokowniczych może pozwalać na wczesną identyfikację pacjentów z grupy wysokiego ryzyka progresji COVID-19, pozwalając na zastosowanie odpowiednich strategii postępowania. Jako że ciężki przebieg choroby, a nawet zgony, mogą występować również w populacji młodych dorosłych, instytucje opieki zdrowotnej powinny podkreślać potrzebę stosowania środków zapobiegawczych i wspierać dalsze badania nad niekorzystnymi czynnikami rokowniczymi w tej grupie wiekowej.
5. W grupie pacjentek ciężarnych wymagających hospitalizacji z powodu COVID-19 niekorzystnymi czynnikami rokowniczymi są: występowanie chorób

współistniejących, takich jak nadciśnienie tętnicze i cukrzyca, wyższy odsetek zajęcia przez zmiany zapalne miąższu płucnego w obrazach tomografii komputerowej, a także szereg nieprawidłowości w badaniach laboratoryjnych, takich jak limfopenia, hipokalcemia, hipoproteinemia, niskie stężenie cholesterolu całkowitego oraz podwyższone stężenia CRP, PCT, IL-6, ferrytyny, LDH, hs-TnI i glukozy. Określenie takich czynników może pozwolić na identyfikację kobiet ciężarnych będących w grupie wysokiego ryzyka ciężkiego przebiegu choroby, a przez to na wcześniejsze wdrożenie właściwego postępowania u tych pacjentek i poprawę rokowania zarówno u matki, jak i u płodu.

## V. OPIS W JĘZYKU ANGIELSKIM

### 1. INTRODUCTION

Coronavirus disease 2019 (COVID-19), declared a pandemic by the World Health Organization (WHO) on March 11, 2020 [1], is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2).

SARS-CoV-2 is a highly infectious RNA virus, which utilizes its spike (S) protein for cellular entry by binding to the host angiotensin-converting enzyme 2 (ACE2) receptor. This interaction requires the cleavage of the S protein by cell proteases, including transmembrane protease serine 2 (TMPRSS2) and furin, which allows fusion between the cellular and the viral membranes, and viral entry into the cell via endocytosis [2]. Alternative SARS-CoV-2 entry mediators include receptors and cofactors such as CD147 and neuropilin-1 (NRP1), and activators such as cathepsin [3,4,5]. ACE2 is expressed in multiple cell surfaces throughout the body, including lung parenchyma, respiratory epithelium, gastrointestinal epithelium, endothelium, arterial smooth muscles, neuronal glial cells, neurons, renal tubular cells, heart, and lymphoid tissues [6,7]. TMPRSS2 was also found to be expressed in multiple organs, such as in the respiratory tract, salivary glands, gastrointestinal tract, liver, and kidneys [5]. This ubiquitous expression of ACE2 and TMPRSS2 may explain the pleiotropic effects of SARS-CoV-2 infection. However, the primary targets of SARS-CoV-2 are the cells manifesting high expression of these proteins, especially those that co-express ACE2 and TMPRSS2, such as respiratory and olfactory epithelial cells [3].

SARS-CoV-2 infection may be asymptomatic or symptomatic, with the course of the disease varying widely from mild to moderate to severe to critical [8]. Similarly, Polish Association of Epidemiologists and Infectiologists distinguishes four stages of the disease. The oligosymptomatic stage, corresponding to the mild form of the disease, is characterised by non-specific symptoms such as fever, chills, cough, dyspnoea, fatigue, myalgia, headache, sore throat, rhinorrhea, conjunctivitis, anorexia, nausea, vomiting, diarrhea, abdominal pain and loss of smell (anosmia) and / or taste (ageusia), but without hypoxia. In the fully symptomatic stage, corresponding to moderate disease, the clinical and radiological features of pneumonia with peripheral oxygen saturation ($SpO_2$) below 94% on room air are also observed. Severe COVID-19 is characterized by respiratory failure, mostly due to cytokine storm, while in critical disease acute respiratory distress

syndrome (ARDS), hypotensive shock and multi-organ failure develop, requiring admission to an intensive care unit (ICU) and the use of mechanical ventilation [9].

Of note, the aforementioned symptoms of mild COVID-19 may be useful in identifying patients at increased risk of SARS-CoV-2 infection, which may be important for earlier referral for diagnostic testing. In a meta-analysis evaluating the utility of symptoms in predicting COVID-19, cough had a sensitivity of 62.4%, but low specificity (45.4%), fever had a sensitivity of 37.6% and a specificity of 75.2%, dyspnoea had a sensitivity of 23.3 % and a specificity of 75.7%, and fatigue had a sensitivity of 40.2 % and a specificity of 73.6%. Interestingly, anosmia, ageusia, and anosmia or ageusia were characterized by sensitivities below 50% but high specificities of over 90% (a sensitivity of 26.4%, 23.2% and 39.2%, and a specificity of 94.2%, 92.6% and of 92.1% for anosmia, ageusia, and anosmia or ageusia, respectively) [10], which may be particularly useful in screening. Indeed, SARS-CoV-2 appears to have a particularly strong affinity for ACE2, highly expressed in the olfactory epithelium, estimated to be 10-to-20-fold higher than for SARS-CoV [11], which might explain its particular impact on chemosensory systems. However, although the association of olfactory disorders (OD) with COVID-19 is now established, the pathogenesis of OD is still debated.

Odor detection begins with the binding of odorant molecules to odor receptors localized on the dendritic cilia of the olfactory sensory neurons (OSNs) in the olfactory epithelium (OE) [12,13]. The OE is a complex tissue consisting of multiple cell types, including OSNs, sustentacular (SUS) cells, mucus-secreting Bowman's gland cells, and basal cells [12]. SUS cells act to structurally support OSNs, protect OSNs by detoxifying potentially harmful agents, enable odor detection by participating in the transfer and removal of unnecessary odor molecules, supply OSNs with glucose necessary for high energy olfactory transduction cascade, and maintain local fluid and electrolyte balance [12,14]. The basal cells can differentiate to replace OSNs during normal turnover or injury. Bowman's glands secrete mucus, containing water, salts, mucin glycoproteins, enzymes, antibodies, and odorant binding proteins (OBP), which transport the hydrophobic odorant molecules through the mucus to the OSN cilia [15]. OSN axons cross the skull base through the cribriform plate and form synapses within the olfactory bulb (OB), from where olfactory information is transmitted to higher brain centers [12,13].

Olfactory dysfunctions can be classified into three types: 1) conductive disorders caused by obstruction of the nasal cavities and subsequent blockage of odorant transmission to the OE, 2) sensorineural loss caused by damage of the OE or olfactory

nerves, and 3) central dysfunction resulting from damage to the olfactory processing pathway in the central nervous system [13]. Loss of the sense of smell due to upper respiratory tract infections is primarily considered a conductive loss secondary to rhinorrhea and mucosal edema, and usually normalizes as the infection resolves. However, in some cases, loss of smell may persist after the resolution of upper respiratory tract infections, suggesting a sensorineural disorder known as post-viral olfactory dysfunction (PVOD) [16,17]. PVOD is one of the leading causes of anosmia in adults, accounting for approximately 11–40% of cases [18]. Several respiratory viruses can cause PVOD, including rhinovirus, parainfluenza virus, Epstein–Barr virus and some coronaviruses [17].

Regarding the pathogenesis of COVID-19-related anosmia, several theories have been proposed. These include conductive loss of smell due to nasal obstruction and rhinorrhea, oedema of the olfactory cleft mucosa that prevents odorants from reaching the olfactory epithelium, olfactory epithelial damage, infection of the olfactory nerves and, through neuroinvasion, the olfactory bulb, and impairment of the central olfactory pathways due to direct viral invasion or indirect injury caused by hypoxia, endothelial damage, or an abnormal inflammatory response. Similarly, damage to the olfactory epithelium may be caused by direct viral invasion of the OSNs, most likely mediated by the NRP1 receptor, or by infection of the non-neuronal cells of the olfactory epithelium, leading to horizontal viral spread to OSNs or impaired morphological and physiological support of OSNs [4,12,16,19,20,21].

As mentioned above, high incidence of OD in COVID-19 prompted researchers to hypothesize that new-onset smell impairment could serve as a potential predictor of SARS-CoV-2 infection. Notably, most of the early studies on COVID-19-related anosmia were based on self-assessment of OD, which tends to underestimate its true prevalence due to recall bias and subjects not being aware of their smell impairment, especially while experiencing other, severe symptoms such as respiratory distress [13,22,23]. Indeed, several meta-analyses showed a higher overall prevalence of OD when using objective compared with subjective assessment methods (72.1%–77% vs. 44.5%–53%, respectively) [23-25]. However, psychophysical olfactory tests are time consuming, expensive, and require standardized laboratory settings, and thus, they are difficult to perform during a pandemic [24]. Moreover, disposable tests are preferable to reduce the risk of viral contamination [26]. For these reasons, efforts should be made to develop quick, simple, affordable, disposable and reliable tests for screening for SARS-CoV-2 infection.

A report by the Chinese Center for Disease Control and Prevention in the initial period of the pandemic, up to 11 February 2020, based on 44,500 confirmed COVID-19 cases, showed that mild disease was found in 81% of cases, severe disease in 14% and critical disease in 5%, with an overall case-fatality rate of 2.3% [27]. Similarly, in a Centers for Disease Control and Prevention (CDC) report analyzing cases reported between January and May 2020, 14% of patients required hospitalization, 2% were admitted to the ICU, and 5% died [28]. It is noteworthy that initially non-severe COVID-19 patients may progress in approximately a week [29].

Severe COVID-19 is mainly an immune-mediated disorder triggered by the SARS-CoV-2 infection, promoting an exuberant inflammatory reaction (known as the cytokine storm) and hypercoagulability, and causing destruction of infected tissues, microthrombosis, and organ damage [30-32]. Certain demographic and clinical features have been associated with the risk for severe course of COVID-19 including older age, male sex, smoking, obesity, and other comorbidities [33,34]. Moreover, a number of laboratory anomalies may be associated with the risk for severe course of COVID-19 and worse outcomes, including leukocytosis, neutrophilia, lymphopenia, thrombocytopenia, increased inflammatory markers, including C-reactive protein (CRP), procalcitonin, and ferritin, and inflammatory cytokines, such as interleukin-6 (IL-6), as well as organ and coagulation dysfunction markers, including elevated lactate dehydrogenase (LDH), troponin, N-terminal-pro-B-type natriuretic peptide (NT-proBNP), creatinine, creatine kinase (CK), liver enzymes, D-Dimer, longer prothrombin time, and hypoalbuminemia [35-39].

Although older age is a risk factor for both the incidence and worse prognosis of COVID-19, severe disease and death have also been observed among young adults [40-42]. Notably, although work, education, and other social settings put young people at a higher risk of SARS-CoV-2 exposure, this age group appears to be less likely to comply with preventative measures [43]. Moreover, clinical characteristics and laboratory test results in younger people seem to be different from those in elderly patients, and this may indicate a different pathogenesis of COVID-19 in these age groups [40,43]. However, data on clinical features and risk factors of severe COVID-19 in young adults are scarce.

Another particular group of patients are pregnant women. The distribution of disease severity in pregnant women is similar to the distribution seen in non-pregnant populations, with 86% of pregnant women manifesting mild disease, 9% severe, and 5% critical [44]. However, it should be noted that physiological adaptations of the respiratory tract,

hypercoagulability, immunomodulation that improve a pregnant woman's tolerance to the fetal semi-allograft in the second trimester and the increased inflammatory response in the third trimester, as well as the susceptibility to insulin resistance, diabetes, hypertension and cardiovascular diseases, predispose SARS-CoV-2-infected pregnant women toward a severe course of illness, which may cause worse maternal and fetal outcomes [46-48]. Indeed, data emerging from literature suggest that pregnant women may have an increased risk of developing severe symptoms and a higher risk of pneumonia, the need for ICU admission and mechanical ventilation, and death [49,50].

Another phenomenon relevant to the discussion on predictors of COVID-19 severity was the emergence of the new variants of SARS-CoV-2, associated with changes in viral transmissibility, clinical presentation, and / or effectiveness of preventative, diagnostic, and therapeutic measures, which by the end of 2020 posed a further threat to global public health [51]. This has prompted researchers and health organizations to characterize the Variants of Concern (VOCs), including alpha (B.1.1.7), beta (B.1.351), and later gamma (P.1), delta (B.1.617.2) and omicron (B.1.1.529) variants [51,52].

The alpha variant (B.1.1.7), first identified in the UK and then spreading worldwide, was defined by multiple mutations, including changes in the spike protein (N501Y, A570D, D614G, P681H, T716I, S982A, D1118H, del69-70, del144), with case rates in the UK increasing from fewer than 5% of all SARS-CoV-2 infections to more than 60% between November and mid-December 2020, causing a sharp increase in COVID-19 incidence, hospitalization, and mortality [53]. In Poland, according to European Centre for Disease Prevention and Control (ECDC) Data on SARS-CoV-2 variants in the EU/EEA only one case of B.1.1.7 was detected until the end of 2020, and from the beginning to the end of January 2021 the incidence of alpha variant was 9.5 % (with the overall incidence from September 7, 2020 to January 31, 2021, of 6.5%), and then gradually increasing, exceeding 50% in the week 2021-07 (February 15 to February 21, 2021) and rapidly rising further to over 90% in March, 2021 [54].

Several studies suggested the alpha variant was more transmissible than the previously identified SARS-CoV-2 variants [55,56], and this was hypothesized as resulting from the higher viral load and longer detectability in respiratory secretions, possibly attributable to mutations of the spike protein, including in the receptor-binding domain and adjacent to the furin-cleavage site, and therefore affecting viral cell entry [55,57]. Some reports indicated greater disease severity associated with the alpha variant [58-60], however, others did not find this relationship [57]. Similarly, the widely debated possibility

of increased severity of the infection with this variant among young people remains unclear [61,62]. Moreover, although the later delta variant is generally considered to be associated with greater disease severity, data are also inconsistent [63-65]. Notably, only omicron is still considered a currently circulating VOC, while the remaining previous VOCs are now labeled by WHO as “previously circulating VOCs” [51], by CDC as "variants being monitored" (VBMs) [52], and by ECDC as “de-escalated variants” [66]. Currently most predominant omicron variant appears to cause less severe disease [67,68], however, still more data are needed [69]. Moreover, continuously changing vaccination status makes these comparisons of SARS-CoV-2 variants even harder. Furthermore, even in non-vaccinated patients the severity of the disease may now be more and more frequently affected by other factors, including prior SARS-CoV-2 infection. Nevertheless, with newly emerging SARS-CoV-2 variants and as we witness natural evolution and subsequent waves of pandemics, that may overburden health systems once again, the ability to quickly identify infected individuals and to prioritize medical management, with particular focus on the possibility of critical course, remains a key issue.

## 2. AIMS

1. To evaluate the prevalence of olfactory disorders in COVID-19 and their predictive value in assessing the probability of SARS-CoV-2 infection using a simple, disposable and reliable olfactory screening test created for this study.
2. To summarize the current understanding of the pathogenesis of olfactory disorders in the course of COVID-19 based on critical review of current literature.
3. To investigate the impact of the causative SARS-CoV-2 variant (alpha vs. previous variants) on COVID-19 severity in young adults hospitalized due to severe COVID-19.
4. To identify the potential adverse prognostic factors in young adults hospitalized due to severe COVID-19.
5. To identify the potential adverse prognostic factors in pregnant women hospitalized for COVID-19.

# 3. MATERIAL AND METHODS

## 3.1. Material and methods to evaluate the prevalence of olfactory disorders in COVID-19 patients and their predictive value in assessing the probability of SARS-CoV-2 infection

### 3.1.1. Material

The study of the prevalence of olfactory disorders in COVID-19 and their predictive value in assessing the probability of SARS-CoV-2 infection included 64 COVID-19 patients hospitalized between April and August 2020 at the Central Clinical Hospital of the Ministry of the Interior and Administration in Warsaw and 34 healthy volunteers.

The inclusion criteria for the case group were adults (≥18 years old) with SARS-CoV-2 infection diagnosed by RT-PCR (reverse-transcription polymerase chain reaction) performed on nasopharyngeal samples. The control group consisted of healthy adult volunteers with no symptoms of upper respiratory tract infection or COVID-19 (other than recent OD, which was not queried for to avoid selection bias). Although a negative test result for SARS-CoV-2 was not an inclusion criterion for the control group, the total number of individuals diagnosed with COVID-19 from the beginning of the pandemic to the end of August 2020 was lower than 0.18% of the Polish population [70], hence the risk of infection in asymptomatic volunteers was considered to be very low. The exclusion criteria were age below 18 years of age, pregnancy, a history of pre-existing OD, head trauma, rhinosinusitis, or other chronic nasal disease, and inability to complete the questionnaire due to a history of neurocognitive disorders, an altered state of consciousness, or the need for intensive care and / or invasive ventilation at the time of the survey. Voluntary written informed consent was obtained from all participants. The study was approved by the Ethics Committee of the Central Clinical Hospital of the Ministry of the Interior and Administration in Warsaw.

### 3.1.2. Methods

#### 3.1.2.1. Clinical Outcomes

All participants completed a questionnaire regarding: 1) general demographic data, 2) medical history: comorbidities, chronic medication use, tobacco addiction, and pre-existing OD, 3) the course of COVID-19: date of first symptoms, nasal and general

symptoms, and 4) olfactory function: subjective assessment of sense of smell (descriptive – as “normal”, “decreased”, or “none at all” – and using the visual analogue scale, from 0 - normal sense of smell to 10 - no sense of smell); the onset and persistence of OD. Any data missing from the forms and information regarding the course of the disease were transcribed from the electronic medical records. The physiological parameters were assessed at least once a day using the modified early warning score (MEWS) [71] adapted by the hospital therapeutic committee by including $SpO_2$ and need for oxygen supplementation.

#### 3.1.2.2. Psychophysical Evaluation

The psychophysical olfactory evaluation was performed concurrently with the questionnaire on all participants, using the simple disposable odor identification test (SDOIT) developed for the purpose of this study. Ten disposable test paper strips numbered 1 to 10, nine of which contained well-known pure odorants (commercially available cinnamon, mint, lemon, coffee, clove, rose, anise and camphor essential oils and disinfectant alcohol) and the remaining one with an odorless control (deionized water) were each enclosed in plastic covers so the odors did not mix. Upon removal of the covers, each odor was presented to a patient at 30-second intervals to prevent olfactory desensitization. The odorants used in the study were selected to include both unimodal odors, with little or no trigeminal stimulation, and bimodal odors, with mixed stimulation of the olfactory and trigeminal nerve. For each odor strip, patients were asked to indicate whether they detected an odor and if so, to identify the odor (using a forced choice format, with 4 given options per test odorant).

Four SDOIT models were analyzed: 1) SDOIT-10, evaluating the number of correct answers (correct identification of nine odors and reporting of no odor detection in an odorless sample); 2) SDOIT-9 evaluating the number of identified odors out of nine odorants; 3) SDOIT-8 evaluating the number of identified odors out of eight chosen odorants (cinnamon, mint, lemon, coffee, clove, anise, camphor, and alcohol)—excluding odorant showing no significant differences between cases and controls in initial analysis (rose); and 4) SDOIT-4 evaluating the number of identified odors out of four odorants showing the highest intergroup differences in initial analysis (cinnamon, mint, lemon, and alcohol).

OD was defined as identifying a number of odors below the 10th percentile of the results from the control group, i.e. at least 2 incorrect answers in SDOIT-10, SDOIT-9 and

SDOIT-8 and at least 1 incorrect answer in SDOIT-4. Other components of olfaction such as odor threshold and odor discrimination were not assessed in order to maximize the simplicity and minimize the time necessary for the assessment.

#### 3.1.2.3. Statistical Analysis

Usual descriptive statistics were used. Fisher's exact test was used to compare self-reported OD and the Mann–Whitney U test was used to compare the SDOIT results between cases and controls. To assess the correlation between the self-report olfactory function and SDOIT results, Fisher's exact test and the Chi-square test were used. In cases, the Spearman correlation coefficient was used to test the correlation between clinical features and OD in the case where the studied variables were quantitative, the Mann-Whitney U test to study differences between qualitative and quantitative variables, and the Fisher's exact test for two qualitative variables. Statistical analysis was performed with R software (version 3.6.0, R Foundation for Statistical Computing, Vienna, Austria). A level of $p < 0.05$ was used to determine statistical significance. The receiver operating characteristic (ROC) curves were plotted and the area under the ROC curve (AUC) analysis was performed to assess the utility of selected classifiers in predicting SARS-CoV-2 positivity. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of these predictors were evaluated.

### 3.2. Material and Methods for summarizing current knowledge on the pathogenesis of olfactory disorders in the course of COVID-19

In order to summarize the current knowledge on the pathogenesis of olfactory disorders in the course of COVID-19, a critical, non-systematic review of the current literature was carried out. During the review, PubMed database and Google Scholar were searched using the terms „COVID-19" or „SARS-CoV-2" and „olfactory" or „smell" or „anosmia" or „parosmia" or "neuroinvasive" or "neurological". Articles pre-selected based on the evaluation of titles and abstracts were then analyzed in full to select relevant papers. The reference lists of these studies were hand-searched to include additional publications relevant to the topic in the review. Only articles published in English were included in the review.

## 3.3. Material and Methods in the study of the differences between the course of COVID-19 in hospitalized young adults depending on the causative SARS-CoV-2 variant (alpha vs. previous variants) and demographic, clinical and laboratory predictors of severe disease course in hospitalized young adults

### 3.3.1. Material

The single-center, retrospective study of hospitalized young adults, aged 18 to 45 years, hospitalized for COVID-19 during the second and the third wave of pandemic in Poland was conducted at the Central Clinical Hospital of the Ministry of the Interior and Administration in Warsaw. The second wave was defined as the period from 12 September 2020 to 27 January 2021, and the third wave from 11 February 2021 to 10 June 2021. 229 COVID-19 patients (172 men and 57 women) were included in this study, of which 75 patients (59 men and 16 women) were attributed to the second wave, and 154 (113 men and 41 women) to the third wave of the COVID-19 pandemic. The median age in both groups was 40 years. The inclusion criteria were adults not younger than 18 and not older than 45 years[2], with SARS-CoV-2 infection diagnosed by either RT-PCR or rapid antigen test performed on nasopharyngeal samples, who were admitted due to severe COVID-19 (i.e., meeting hospital admission criteria for COVID-19, with oxygen saturation of 94% or less on room air or the need for oxygen therapy). Pregnant women and patients admitted for reasons other than COVID-19 (e.g., trauma, other acute conditions, or serious deterioration in the course of chronic diseases) were excluded from the study. The study was approved by the Ethics Committee of the Central Clinical Hospital of the Ministry of the Interior and Administration in Warsaw with a waiver for written informed consent due to the retrospective nature of the study and the data anonymization.

### 3.3.2. Methods

#### 3.3.2.1. Clinical Outcomes

Data regarding the patient demographic and clinical characteristics (including age, sex, smoking status, comorbidities, the course of the disease, complications, $SpO_2$, the need for Intensive Care Unit (ICU) admission, respiratory support, vasopressors,

[2] Although there is no consensus in the literature on the definition of "young adults," and the upper limit of this age range is usually lower [72], a number of studies on SARS-CoV-2 infection have adopted similar broader criteria, including those aged 45 and under [73,74,75]. Such an age criterion is supported by the study of COVID-19 patients, which found a lower incidence of death and the need for ICU admission in individuals aged 18-45 years than in the group of patients older than 45 years, and this cut-off point for age was confirmed in the sensitivity analysis [74].

continuous renal replacement therapy), as well as laboratory and imaging results, were transcribed from electronic medical records and entered into the database after anonymization. The laboratory parameters were collected from two time points: at admission to hospital (+/−2 days) and on the 7th day of hospitalization (+/−2 days).

#### 3.3.2.2. Statistical Analysis

The patients' characteristics were compared between the second and the third waves of COVID-19 pandemic in Poland and between survivors and non-survivors. Continuous variables were compared with a Mann–Whitney U test, while categorical variables were compared with the chi-squared test or Fisher's exact test as appropriate. The correlations between the patients' characteristics and the risk for MV and ICU treatment were analyzed using the Spearman correlation analysis for continuous variables, and associations of the nominal variables were investigated with the chi-squared test. To identify factors associated with the risk of death, MV, and ICU treatment logistic regression was also performed, and ROC curve analysis was conducted to assess the predictive ability of covariates and models in multivariate logistic regression. Analyses were performed with R software (version 4.0.4; R foundation for statistical computing, Vienna, Austria) and Statistica software (version 13.3; StatSoft, Poland). A level of $p < 0.05$ was used to determine statistical significance.

## 3.4. Material and Methods in the study of the potential predictors of severe COVID-19 in pregnant women.

### 3.4.2. Material

The single-center, retrospective study was conducted at the Central Clinical Hospital of the Ministry of the Interior and Administration in Warsaw. The study group comprised 52 pregnant women with COVID-19 admitted to hospital between 15 May 2020 and 26 April 2021. The mean age of the patients was 31.9 ± 4.79 years. Gestational age ranged from 17 to 37 weeks. Inclusion criteria were similar to admission criteria for pregnant women with COVID-19, including temperature >39°C despite the use of acetaminophen, respiratory rate >30/min, $SpO_2$ <95% on room air, the need for oxygen therapy, and critical disease. SARS-COV-2 infection was confirmed using RT-PCR test prior to admission. Patients admitted for obstetric and/or other non-COVID-19-related reasons (such as trauma, other acute conditions, or serious deterioration in the course of

chronic diseases) were excluded from the study. The study was approved by the Ethics Committee of the Central Clinical Hospital of the Ministry of the Interior and Administration in Warsaw.

### 3.4.2. Methods

#### 3.4.2.1. Clinical Outcomes

Data regarding the patient characteristics (including age, body mass index, smoking status, and comorbidities), gestational age, the course of the disease (including symptoms, vital signs, the need for ICU admission, and respiratory support), as well as laboratory and imaging (chest computed tomography) results at admission were transcribed from electronic medical records and entered into the database after anonymization.

#### 3.4.2.2. Statistical Analysis

Continuous variables were compared with a Mann–Whitney U test, while categorical variables were compared with the chi-squared test or Fisher's exact test as appropriate. As there was only one patient with a critical course of the disease, the severe and critical groups were combined in the analysis. Comparisons between these three disease severity groups (mild, moderate and severe-to-critical) were performed using the Kruskal–Wallis test followed by Dunn's post hoc tests for continuous variables, and chi-square test for categorical variables. The correlations of continuous variables were assessed using the Spearman analysis. Univariate logistic regression was performed to identify factors associated with severe-to-critical disease. Analyses were performed with Statistica software (version 13.3; StatSoft, Poland). A level of $p < 0.05$ was used to determine statistical significance.

## 4. SUMMARY

### 4.1 Results of the evaluation of the prevalence of olfactory disorders in COVID-19 patients and their predictive value in assessing the probability of SARS-CoV-2 infection

A total 64 cases and 34 controls were included in the study. The most prevalent symptoms of COVID-19 (other than OD) were fatigue (70.3%), cough (39.1%), fever (37.5%), headache (35.9%), and gastrointestinal complaints (35.4%). Nasal congestion was reported by 32.8% and rhinorrhea by 29.7% of those with COVID-19. OD appeared before (14.8%), simultaneously (11.1%), or after (74.1%) the presentation of other COVID-19 symptoms. At the time of the evaluation, 25.9% of patients reported complete recovery of olfactory function, 55.6% reported no recovery, and 18.5% reported worsening. The frequency of self-reported smell impairment was significantly higher in COVID-19 patients, with 32.8% and 42.2% of patients reporting current and recent OD, respectively, while all the control subjects reported normosmia. The mean VAS score for smell deterioration was significantly higher in cases compared with controls. As there was a difference between cases and controls in terms of age, self-reported OD and psychophysical test scores were also analyzed for the subset of 75% youngest COVID-19 patients whose age did not differ significantly from the control group. The inter-group differences remained highly significant, with 29.2% and 41.7% of 75% youngest COVID-19 patients reporting current and recent OD, respectively.

The psychophysical evaluation was performed for all the study subjects. Mean percentages of correct answers (for all 10 samples, including the non-odorant sample), detected odors and identified odors in cases vs. controls were 65.6% vs. 95.8%, 83.5% vs. 100%, and 66.3% vs. 95.6%, respectively. In the comparison of controls and the subset of 75% youngest COVID-19 patients the differences remained highly significant, with mean percentages of correct answers, detected odors and identified odors in 75% youngest patients being 74.2%, 85.4% and 72.9%, respectively.

For all odors, lower p-values were achieved for identification than for detection. Considering the identification results for each odor separately, two additional, shortened psychophysical test models were created, one with eight and the other with four selected odorants, as described above. In the intergroup comparison, the mean scores in all four models were significantly lower in cases than in controls ($p < 0.001$ for all models). Taking the cut-off value at the 10th percentile of the results in controls, we found OD in 59.4% vs. 8.8%, 54.7% vs. 5.9%, 54.7% vs. 2.9% and 64.1% vs. 5.9% of cases vs. controls, for

SDOIT-10, SDOIT-9, SDOIT-8 and SDOIT-4, respectively. There was a significant correlation between the current self-reported OD and psychophysically assessed OD in all the SDOIT models. Notably, the results of the questionnaire indicated a lower prevalence of olfactory impairment than psychophysical tests, which indicates that self-assessment of olfactory function in COVID-19 tends to underestimate the prevalence of anosmia, highlighting the importance of psychophysical smell assessment in COVID-19 patients. Moreover, subjectively normosmic COVID-19 patients showed OD at psychophysical evaluation in 51.2%, 46.5%, 46.5% and 55.8% of cases for SDOIT-10, SDOIT-9, SDOIT-8 and SDOIT-4, respectively. There were no significant gender differences in self-reported olfactory function and psychophysical test results.

Within the COVID-19 group, nasal obstruction was more prevalent in patients reporting OD compared with normosmic subjects and rhinorrhea was more prevalent in patients reporting recent OD, but not current OD. No significant correlation was found between nasal symptoms and SDOIT scores.

Patients with worse psychophysical test results had higher MEWS scores and were hospitalized longer, and SDOIT-9 and SDOIT-8 scores were significantly lower in patients who later died, while there were no correlations between the length of hospitalization, MEWS score and death with self-reported OD. The need for oxygen supplementation was less frequent in patients reporting recent OD, but more frequent in patients with lower SDOIT scores.

OD was significantly associated with SARS-CoV-2 positivity in all SDOIT models, with odds ratios (OR) of 15.1, 19.3, 39.8 and 28.5 for SDOIT-10, SDOIT-9, SDOIT-8 and SDOIT-4, respectively. In the ROC analysis, self-reported recent OD achieved sensitivity of 42%, specificity of 100%, PPV of 100%, NPV of 48% and AUC of 0.71 for predicting SARS-CoV-2 positivity. Psychophysical evaluation, when defining OD as the score below the 10th percentile of healthy subjects, found SDOIT-4 to be the best classifier, with sensitivity of 64% and specificity of 94%, PPV of 95%, NPV of 63% and AUC of 0.8. However, the optimal cut-off point calculated in the ROC analysis for all SDOIT models was at least one incorrect answer with AUC of at least 0.8 for all models. The combination of SDOIT results and self-reported OD (with any OD, either subjective or assessed based on psychophysical test, indicating COVID-19) further improved the diagnostic accuracy. To minimize the risk of patients suspected of infection eligible for isolation not being detected, classifiers with the highest AUC and the highest sensitivity were selected as the best predictors of COVID-19, combining self-reported OD and/or OD defined in SDOIT

as:

1. at least one incorrect answer in SDOIT-10 (with AUC of 0.87, sensitivity of 91%, specificity of 71%, PPV of 85% and NPV of 80%); or
2. at least one misidentified odor in SDOIT-8 (with AUC of 0.87, sensitivity of 86%, specificity of 79%, PPV of 89% and NPV of 75%).

ROC analysis for controls and the subset of 75% youngest COVID-19 patients was also performed to eliminate the potential impact of age. Similarly to the results of the analysis for the entire cohort, the analysis of age-matched group showed the SDOIT-4 to be the best classifier when defining OD as the score below the 10th percentile of healthy subjects, with sensitivity of 54% and specificity of 94%, PPV of 93%, NPV of 59% and AUC of 0.75, but the optimal cut-off point calculated in the ROC analysis for all SDOIT models was at least one incorrect answer with AUC of at least 0.75. The combination of SDOIT results and self-reported OD improved the diagnostic accuracy. The best predictors of COVID-19, with the highest AUC and sensitivity, were these combining self-reported OD and/or OD defined in SDOIT as:

1. at least one incorrect answer in SDOIT-10 (with AUC of 0.85, sensitivity of 88%, specificity of 71%, PPV of 81% and NPV of 80%); or
2. at least one misidentified odor in SDOIT-8 (with AUC of 0.86, sensitivity of 83%, specificity of 79%, PPV of 85% and NPV of 77%).

The limitations of this study were its single-center nature and limited sample size. Furthermore, the sense of smell were assessed at different time periods following the onset of infection and some patients reported having already recovered. However, this may have led to the underestimation of OD prevalence and significance, rather than the opposite. Moreover, the recovery pattern of OD was not assessed. Future follow-up study should be considered. In addition, SDOIT is not yet validated and only odor identification without evaluating discrimination or detection threshold were assessed. However, the aim was not to validate a new method of olfactory evaluation in general, but rather to create a fast test for predicting COVID-19, hence RT-PCR was used as a gold standard in assessing diagnostic accuracy and identification was chosen as the simplest and most accurate element of olfactory testing. Nevertheless, future studies are needed to validate this Simple Disposable Odor Identification Test as a method of olfactory function assessment. It is also worth noting that although this study performed the psychophysical test with the assistance of an examiner, its simplicity would permit it to be conducted remotely. This approach would increase the availability of the test as a screening method.

### 4.2 Results of the summary of the current knowledge on the pathogenesis of olfactory disorders in the course of COVID-19

Although the early onset and early recovery of OD in the course of COVID-19 reported in some studies could argue in favor of a conductive mechanism for anosmia, many COVID-19 patients report OD in the absence of nasal obstruction and rhinorrhea, suggesting that they are not the main factors in the development of COVID-19-related OD [76,77,78]. Similarly, some imaging studies in anosmic COVID-19 patients show olfactory cleft mucosal edema that may prevent odorants from reaching the olfactory epithelium, even in the absence of nasal congestion [79,80], however, other reports did not confirm this finding [81,82], suggesting that it is not the primary mechanism of anosmia.

Another possible pathomechanism of anosmia is the neural hypothesis, suggesting damage to the olfactory nerves, the olfactory bulb and higher olfactory brain centers [83,84]. The neuroinvasive potential of SARS-CoV-2 is supported by various neurological manifestations of COVID-19 described in the literature [85]. Direct invasion of the central nervous system could occur either through the hematogenous or the neuronal route [84]. In the hematogenous pathway, viruses may reach the bloodstream due to invasion of endothelial cells of the blood-brain-barrier (BBB), paracellular transmigration enabled by increased BBB permeability caused by the release of inflammatory mediators, crossing of the blood-cerebrospinal fluid barrier in the choroid plexus, or infection of leukocytes capable of passing through the BBB (known as the “Trojan horse” mechanism) [86,87]. Another potential route for direct viral neuroinvasion is the neuronal route through the olfactory pathway. In this mechanism, the virus reaches the brain through the olfactory epithelium by invading peripheral nerve terminals and propagating via axonal transport in OSNs towards the OB, from where it may spread trans-synaptically to other brain areas [84,85,88]. Alternatively, the virus may pass from the OE through the olfactory ensheathing cells directly to the cerebrospinal fluid surrounding the olfactory nerve bundles and the OB [15]. This transneuronal route of CNS involvement is supported by the post-mortem examinations revealing SARS-CoV-2 viral particles and related damage to be more present in the OSNs and the OB than in the brainstem. Moreover, magnetic resonance imaging studies reported OB abnormalities, including features of micro bleeding and oedema, the reduction in OB volumes, indicative of its atrophy, or changes in OB morphology, observed in COVID-19 patients [89-93]. Furthermore, several animal studies on SARS-CoV-2 and previous human coronaviruses have shown infection of the brain after intranasal inoculation of the virus [94-96]. However, it should be noted that the

studies in mouse models utilized transgenic mice with human ACE2 that could be ectopically expressed in many cells, including OSNs, and therefore may not be a reliable model of the viral tropism [20,97]. Indeed, several sequencing studies found ACE2 and TMPRSS2 to be co-expressed in non-neuronal cells of the OE rather than in the OSNs and the neurons in the OB and the brain [3,20,98]. Moreover, the neurological symptoms of COVID-19 do not necessarily indicate a direct viral neuroinvasion, as they may also be due to hypoxic brain injury, cerebrovascular injury, or immune mediated damage [99]. In addition, although some studies reported the presence of SARS-CoV-2 particles in post-mortem brain examination [100] and SARS-CoV-2 RNA was detected by RT-PCR in cerebrospinal fluid samples [47], the brain seems to contain either the least volume of viral particles of all of the sampled tissues, or no particles at all, and RT-PCR positivity does not necessarily prove the presence of whole viral particles in the cerebrospinal fluid [20]. Furthermore, the higher incidence of anosmia compared with CNS symptoms, as well as the commonly observed early recovery of OD, argue against the central mechanism of SARS-CoV2-related OD [76,78].

Another mechanism for COVID-19-related anosmia may be sensorineural damage, with the disruption of the OE. Indeed, in anosmic COVID-19 patients, SARS-CoV2 infection was found in multiple cell types within the OE, including OSNs, support cells, and immune cells [101]. As already mentioned, ACE2 and TMPRSS2 are co-expressed in non-neuronal cells of the olfactory epithelium, including sustentacular cells, stem cells, and perivascular cells, rather than in the OSNs [3,12,98]. Therefore, it was hypothesized that SARS-CoV-2 infects high-ACE2-expressing non-neuronal cells of the OE before passing to OSNs. Infection of OSNs may result from horizontal viral spread from the adjacent support cells or from dissemination of the virus within the OE after its tissue architecture is disrupted by inflammatory infiltrates [101]. Furthermore, infection of non-neuronal cells within the OE may alter the physiology of OSNs without their direct invasion, by architectural OE damage and lack of physical support, impaired signaling, ionic imbalance, and initiating an excessive immune response [12,19,20,97]. This hypothesis is also in agreement with the relatively rapid recovery of most patients, occurring within weeks after infection, which may reflect the regenerative capability of the SUS cells. The infection of OE stem cells may be responsible for longer lasting OD in some cases [20].

However, it should be noted that although almost no expression of ACE2 is found in the OSNs, they do express NRP1, which could account for direct OSNs infection [4,101,102]. Moreover, the expression of NRP1 has also been found in neuronal progenitor

cells, which could play a role in the persistent anosmia observed in some COVID-19 patients [4,102]. Furthermore, although the previously reported rare occurrence of parosmia during recovery was considered to suggest the absence of damage to peripheral sensory neurons, its prevalence may have been overlooked in early reports, while in later studies it was estimated to be about 40% [103,104]. It has been hypothesized that SARS-CoV-2-induced inflammation of the neuroepithelium and OB may result in impaired olfactory recovery with aberrant OSNs regeneration and misperception of odors in the neuroepithelium, as well as altered olfactory receptor mapping in the OB [104].

Moreover, there may be a genetic link to the pathomechanism of OD in COVID-19, with the possible role of the *UGT2A1* and *UGT2A2* genes, encoding uridine diphosphate glucuronosyltransferase (UGT) enzymes, which are expressed in the OE, and may be involved in metabolizing odorants and olfactory signal termination [105,106].

### 4.3. Results of the study of the differences between the course of the disease in hospitalized young adults depending on the causative SARS-CoV-2 variant (alpha vs. previous variants) and demographic, clinical and laboratory predictors of severe course of COVID-19 in hospitalized young adults

229 COVID-19 patients were included, of which 75 patients were attributed to the second wave , and 154 to the third wave of the COVID-19 pandemic. The most commonly reported COVID-19 symptoms were dyspnea (91.7%), fever (85.2%), cough (85.2%), and fatigue (56.8%). Other symptoms included myalgia (29.3%), smell or taste disorders (18.8%), headache (18.3%), diarrhea (17.9%), nausea and/or emesis (11.8%), sore throat (7.9%), and hemoptysis (3.5%). There were no statistically significant differences between the waves regarding any of the reported symptoms, sex, body mass index (BMI), smoking status, and comorbidities.

The median percentage of lung involvement on computed tomography (CT) was 31%, comprised of 30% in the second wave and 33% in the third wave. The median period from the onset of symptoms to hospital admission was eight days (IQR 6–10 days) in the second wave and nine days (IQR 7–11 days) in the third wave. Due to the study inclusion criteria (patients requiring hospitalization due to severe COVID-19) almost all patients (96.9%) required oxygen therapy, while 24% required high flow nasal oxygen therapy (HFNO). Mechanical ventilation (MV) was necessary in 9.6% of patients, 13.5% of patients required ICU admission, and 7% died. The ICU mortality was 48.4%. There were no significant differences between the two waves regarding the median percentage of lung

involvement on CT at admission, the need for conventional oxygen therapy, HFNO, MV, and ICU treatment, and mortality. These results indicate that the alpha variant does not appear to be associated with worse outcomes in hospitalized young adults than the wild-type variants. Moreover, leukocyte (white blood cell, WBC), neutrophil and immature granulocyte (IG) counts, IG percentages, the neutrophil-to-lymphocyte ratio (NLR) and the frequency of leukocytosis and neutrophilia at admission were higher, and the lymphocyte percentage at admission was lower in the second wave compared with the third wave, while there were no significant differences regarding interleukin-6 (IL-6), C-reactive protein (CRP), and ferritin levels at admission between these two waves. Furthermore, although there was no significant difference between waves in terms of procalcitonin (PCT) levels at admission, PCT levels at the 7th day of hospitalization were significantly higher in the second wave compared with the third wave. As these markers of hyperinflammation seem to be associated with a worse outcome, as discussed below, these findings further contradict a greater disease severity due to the alpha SARS-CoV-2 variant compared to the wild-type variants. Moreover, high D-Dimer and low albumin levels also appear to be associated with poor prognosis, and we found no significant differences between waves in terms of D-Dimer and albumin levels at admission, while at the 7th day of hospitalization D-Dimer levels were even higher and albumin levels lower in the second wave compared with the third wave. In addition, although myoglobin levels at the 7th day of hospitalization were significantly lower in the second wave compared with the third wave, there were no differences between waves in other, more cardiac-specific biomarkers. There were also no statistically significant differences between the two waves regarding antithrombin III (AT III), fibrinogen, lactate dehydrogenase (LDH), calcium and vitamin D3 levels, nor in the levels of renal and liver injury markers.

There were no significant age and sex differences between the survivors and non-survivors. There were also no differences between these groups regarding the frequencies of any of the blood types. Although weight and BMI were higher in non-survivors compared to survivors, these differences were not significant after the removal of the outliers. However, it should be noted that in this study of COVID-19 patients, all of whom were hospitalized due to severe disease, the median BMI was 30.6 $kg/m^2$ (IQR 27.1‑34.3 $kg/m^2$), and normal BMI (18-25 $kg/m^2$) was found in only 12.3%. Moreover, there was a positive correlation between BMI and the need for MV. Furthermore, weight > 100 kg and BMI $\geq$ 40 $kg/m^2$ were significant predictors of death, MV and ICU treatment in univariate logistic regression, and BMI $\geq$ 40 $kg/m^2$ was also a significant predictor of MV in

multivariate analysis (OR = 6.9). Therefore, it appears that overweight or obesity should also be considered risk factors for poor prognosis, possibly due to their association with other comorbidities, reduced lung volumes and hypercoagulable states.

A history of current or former smoking was significantly more frequent among the non-survivors than survivors, and there was a weak positive correlation between smoking and the need for MV and ICU treatment. Therefore, smoking history should be taken into consideration while assessing the possible risk factors for severe COVID-19 in young adults. Comorbidities were also significantly more frequent in the non-survivors compared to survivors (68.75% vs. 38.5%), and were associated with the risk of death and MV in univariate logistic regression, and with the risk of death in multivariate analysis (OR = 4). Chronic arrhythmia was significantly more frequent in the non-survivors than in survivors, while there were no statistically significant differences in the frequency of insulin resistance, diabetes, hypertension, asthma, hypothyroidism, or Hashimoto disease. In univariate logistic regression, having diabetes or insulin resistance was significantly associated with the risk of death, MV, and ICU treatment, and chronic arrhythmia was significantly associated with the risk of death and MV. Overall, the association of comorbidities and the outcome of SARS-CoV-2 infection appears to be less pronounced in young adults than in the general population, as a greater number of comorbidities in elderly patients may lead to a more complex pathogenesis in COVID-19 and its complications [44].

The percentage of lung involvement on CT was significantly higher and lung involvement of at least 50% was more frequent in the non-survivors than in survivors. There was also a weak positive correlation between the percentage of lung involvement and the need for MV and ICU treatment. $SpO_2$ at admission was significantly lower in non-survivors than in survivors, and there was a negative correlation between $SpO_2$ at admission and the need for MV and ICU treatment. Moreover, non-survivors required HFNO, MV, ICU admission, vasopressors, and continuous renal replacement therapy (CRRT) more frequently than survivors.

SARS-CoV-2 infection causes a host immune response, which in most patients will contribute to viral elimination. However, in some cases, the activation of alveolar macrophages triggers the elevated release of cytokines, such as IL-1, IL-6, and tumor necrosis factor-α (TNF-α), and other proinflammatory mediators, known as a "cytokine storm”. This results in the recruitment and infiltration of neutrophils, monocytes, and other leukocytes. Moreover, these cytokines stimulate bone marrow to produce and release

immature granulocytes that infiltrate the lungs, further increasing the exuberant inflammatory reaction [9,30,32]. Furthermore, the release of cytokines, including IL-6, induces the synthesis of acute phase proteins, such as CRP, fibrinogen, and ferritin [32], which, in turn, may further affect the immune reaction, being able to induce the expression of both pro- and anti-inflammatory mediators [107,108]. The exacerbated inflammatory reaction may also lead to cytokine-induced lymphocyte apoptosis [31,109,110]. Other possible causes of lymphopenia in COVID-19 include a direct viral infection of ACE2-expressing lymphocytes, destruction of lymphatic organs, and increased lymphocyte consumption in the infected tissues [31,109,110]. For these reasons, an elevated neutrophil-to-lymphocyte ratio (NLR) is also observed in COVID-19 [31]. Indeed, WBC, neutrophil, and IG counts, IG percentages, the incidence of leukocytosis, neutrophilia, and lymphopenia, NLR, and IL-6 levels were significantly higher in non-survivors than in survivors. In addition, CRP was significantly higher and CRP above 100 mg/L was significantly more frequent in non-survivors than in survivors. There was also a positive correlation between the WBC, neutrophil and IG count, neutrophil and IG percentages, leukocytosis, neutrophilia, NLR, and IL-6 and CRP levels and the need for MV and ICU treatment, as well as a negative correlation between the lymphocyte percentage and the need for MV and ICU treatment. Furthermore, univariate logistic regression revealed that NLR $\geq$ 2 and lymphopenia at the 7th day of hospitalization, as well as neutrophilia, leukocytosis, and CRP > 100 mg/L at admission and at the 7th day of hospitalization were significantly associated with the risk of death, MV and ICU treatment. In multivariate analysis, leukocytosis at admission was significantly associated with death, MV and ICU treatment (with OR of 5.8, 5.5 and 3.7, respectively). Moreover, although there was no significant difference between ferritin levels between non-survivors and survivors, there was a weak positive correlation between ferritin levels at admission and the need for MV, and between ferritin levels at the 7th DOH and the need for ICU treatment.

PCT can be induced directly by bacterial endotoxins and lipopolysaccharides or indirectly through the release of pro-inflammatory cytokines, such as IL-1β, TNF-α and IL-6. However, its synthesis may be inhibited by interferon-γ (INF-γ), increased in viral infection. Therefore, PCT is not typically elevated in mild SARS-CoV-2-infection, while its increase is observed in severe COVID-19, especially due to a bacterial co-infection [111-113]. Indeed, PCT was significantly higher and PCT above 0.5 ng/mL was significantly more frequent in non-survivors compared to survivors, and there was a positive correlation between PCT, and the need for MV and ICU treatment. Moreover,

univariate and multivariate logistic regression revealed that PCT > 0.5 ng/mL at admission was associated with increased risk of death, MV and ICU treatment (with OR in multivariate regression of 5, 9.6 and 5.4, respectively).

Interestingly, although there were no significant differences regarding RBC counts, hematocrit, and hemoglobin levels at admission, at the 7th day of hospitalization these parameters were significantly lower in non-survivors than in survivors, and there was a negative correlation between RBC counts, hematocrit and hemoglobin levels and the need for MV and ICU treatment. In addition, univariate logistic regression revealed that RBC count < 4.5 $\times 10^6/\mu L$, hematocrit < 40%, and hemoglobin < 12 g/dL at the 7th day of hospitalization were associated with increased risk of death, MV and ICU treatment. This new-onset anemia may also reflect hyperinflammation leading to disease progression [114].

Another possible causes of severe course and poor prognosis in COVID-19 may be thrombosis and coagulopathy. Several factors may contribute to coagulation dysfunction in COVID-19, including the cytokine storm leading to an increased production of platelets and fibrinogen, complement activation, vascular dysfunction, Renin-Angiotensin-Kallikrein-Kinin systems (RAS-KKS) imbalance, and excessive intravascular neutrophil extracellular traps (NETs) formation by neutrophils [30,32]. This hyperactive coagulation causes an increase in the level of D-Dimer, a fibrin degradation product [30]. Indeed, D-Dimer was significantly higher and D-Dimer > 500 μg/L FEU at admission was significantly more frequent in non-survivors than in survivors, and there was an association between D-Dimer and D-Dimer > 500 μg/L FEU, and the need for MV and ICU treatment. Furthermore, univariate logistic regression revealed that D-Dimer > 500 μg/L FEU was associated with increased risk of death, MV and ICU treatment, and D-Dimer > 500 μg/L FEU at admission was also significantly associated with the risk of ICU treatment in multivariate analysis (OR = 5.2). No significant difference between non-survivors and survivors regarding PLT counts were found. One possible explanation is that severe COVID-19 can cause both thrombocytopenia, due to viral infection of bone marrow, and increased PLT production, induced by the pro-inflammatory cytokines [115]. Indeed, we observed a positive correlation between both thrombophilia and thrombocytopenia and the need for ICU treatment.

Another biomarker associated with COVID-19 severity is LDH, an enzyme that is present in all tissues and released into the blood upon tissue damage, including such as viral infection, hypoxia, and inflammation-induced injury [116]. Indeed, LDH at admission

was significantly higher in non-survivors than in survivors, and there was a positive correlation between LDH, and the need for MV and ICU treatment. In addition, LDH > 500 U/L at admission was associated with increased risk of death, MV and ICU treatment in univariate analyses, and with MV and ICU treatment in multivariate analyses (OR of 4.7 and 3.4, respectively). The elevation of LDH levels may be due to the multiple organ damage, including renal, myocardial, and liver dysfunction, that has been observed in severe COVID-19.

According to current evidence, elevation of cardiac injury biomarkers, such as high-sensitive troponin I (hs-TnI), creatine kinase-myocardial band (CK-MB), myoglobin, and N-terminal-pro-B-type natriuretic peptide (NT-proBNP), is associated with COVID-19 severity and mortality [117]. Indeed, hs-TnI, CK-MB, NT-proBNP levels were significantly higher in non-survivors than in survivors, and there was a positive correlation between hs-TnI, NT-proBNP, and CK-MB and the need for MV and ICU treatment. Moreover, univariate logistic regression revealed that CK-MB > 20 U/L at admission and at the 7th day of hospitalization, NT-proBNP > 190 pg/mL at admission and at the 7th day of hospitalization, and hsTnI > 34 pg/mL at the 7th day of hospitalization were associated with increased risk of death, MV and ICU treatment, and hsTnI > 34 pg/mL at admission – with increased risk of MV and ICU treatment. We have also observed a correlation between myoglobin levels, a marker that is less specific to cardiac injury, and the need for MV and ICU treatment. Interestingly, another non-cardiac specific marker, CK, was significantly higher in non-survivors than in survivors, but only at the 7th day of hospitalization, and not at admission, and in univariate logistic regression CK > 190 U/L at admission and at the 7th day of hospitalization were associated with increased risk of MV and ICU treatment, but only CK > 190 U/L at the 7th day of hospitalization was associated with the increased risk of death. This might indicate that the increase in CK activity reflects disease progression.

Acute kidney injury has also been found to be a predictor of mortality and severity in COVID-19 patients [118]. Indeed, creatinine and urea levels were significantly higher in the non-survivors compared to the survivors, and EGFR was significantly lower in non-survivors compared to survivors. There was also a positive correlation between creatinine levels and the need for MV, a positive correlation between urea levels and the need for MV and ICU treatment, and a negative correlation between EGFR and the need for MV. Furthermore, univariate logistic regression revealed that EGFR < 60 mL/min at admission and at the 7th day of hospitalization, creatinine > 1.2 mg/dL at admission and at the 7th

day of hospitalization and urea > 49 mg/dL at the 7th day of hospitalization were associated with increased risk of death, MV and ICU treatment, and urea > 49 mg/dL at admission with increased risk of MV.

Although COVID-19 has also been hypothesized to cause hepatic injury, data on the association of liver enzyme levels and COVID-19 severity and mortality are inconsistent [119-121]. Moreover, hypertransaminasemia observed in some studies may also be due to myocardial and muscle injury or drug-induced hepatotoxicity [122]. In this study, no significant differences between survivors and non-survivors were found regarding ALT, AST, and total bilirubin levels at admission. Interestingly, although there were no significant differences between survivors and non-survivors regarding GGT level, in univariate logistic regression GGT > 120 U/L was associated with increased risk of death and MV. Albumin concentrations at the 7th day of hospitalization were significantly lower in non-survivors than in survivors, and there was a negative correlation between albumin concentration and the need for MV and ICU treatment. However, decreased albumin levels may not only result from hepatic dysfunction, but also from prioritizing of acute phase proteins synthesis, cytokine-induced increase in vascular permeability leading to albumin extravascular escape, and excessive renal losses due to kidney injury [119,123].

Total calcium concentration was significantly lower in the non-survivors than in survivors and there was a negative correlation between calcium concentration and the need for MV and ICU treatment. Moreover, univariate logistic regression revealed that calcium < 2.1 mmol/L at admission and at the 7th day of hospitalization were associated with increased risk of death, MV and ICU treatment. This is in agreement with the results of meta-analyses showing hypocalcemia to be significantly associated with COVID-19 severity and mortality, possibly due to its role in the immune response [124,125]. Furthermore, vitamin D, an important regulator of calcium homeostasis, has an immunomodulatory role by influencing the production of antimicrobial peptides, as well as counteracting the cytokine storm by inhibiting the production of pro-inflammatory cytokines and promoting anti-inflammatory cytokines, controlling T-cell mediated responses, and modulating the activity of neutrophils and macrophages [126-128]. Interestingly, vitamin D3 levels at admission were significantly lower in non-survivors than in survivors and there was a negative correlation between vitamin D3 levels at the 7th day of hospitalization and the need for ICU treatment.

In ROC analysis, comorbidities (AUC = 0.65), WBC count > $10\times10^3/\mu L$ (AUC = 0.65), and PCT > 0.5 ng/mL (AUC = 0.65), were associated with death; BMI ≥ 40 $kg/m^2$

(AUC = 0.57), LDH > 500 U/L (AUC = 0.7), WBC count > $10\times10^3$/μL (AUC = 0.72), and PCT > 0.5 ng/mL (AUC = 0.7) were associated with MV, while D-Dimer > 500 μg/L FEU (AUC = 0.65), LDH > 500 U/L (AUC = 0.68), WBC count > $10\times10^3$/μL (AUC = 0.66), and PCT > 0.5 ng/mL (AUC = 0.65), were associated with ICU treatment. The combined multivariate regression models for predicting death, MV and ICU treatment had the area under the curve (AUC) values of 0.81, 0.84, and 0.85, respectively.

This study had several limitations. Firstly, it was a single-center retrospective study with a limited sample size that only included severe, hospitalized patients, which may limit the validity of generalizing its results to the entire young adult population. Therefore, larger, multi-center, prospective studies are needed. Secondly, because this study is an observational and exploratory study in which many statistical tests were performed, results may be influenced by some false-positive error and confounding factors. Moreover, because of the retrospective nature of this study and the limited resources of the health care system at the time, genotypic results of the causative variant were not available. However, the prevalence of the SARS-CoV-2 alpha variant in Poland during the period defined here as the second wave of pandemic was low (approximately 6.5%), while in the period defined as the third wave the alpha variant accounted for over 92% of the identified strains [54]. These data strongly support that the analyzed waves correspond well to the causative variants. Noteworthy, none of the patients was vaccinated nor did they have any previous documented SARS-CoV-2 infection. Moreover, there were no significant differences between the waves in terms of the medical treatment used. Hence, although the variants studied here are no longer dominant, these results may still be relevant, as they may provide valuable information on the mechanisms involved in SARS-CoV-2 infection not affected by these factors, which is difficult to achieve in the studies of later variants. Furthermore, most previous studies have focused on predictors of severe COVID-19 in the general population, with older individuals often predominating among those hospitalized, while risk factors among younger individuals appear to be different, and finding the predictors of poor COVID-19 outcomes in this age group may contribute to the early identification of individuals at risk of developing severe disease.

### 4.4. Results of the study of the potential predictors of severe COVID-19 in pregnant women

The study included 52 pregnant patients. Mean BMI on admission was 28.4 kg/m$^2$. Symptoms on admission were: dyspnea (92.3%), cough (90.4%), fever (63.5%), fatigue and myalgia (42.3%), smell and taste disorders (26.9%), headache (23.1%), sore throat (11.5%), and nasal discharge (9.6%). Most common comorbidities were hypothyroidism (35.3%), diabetes (17.7%), hypertension (10%), and asthma (3.9%). Mild, moderate, severe, and critical COVID-19 accounted for 17.3%, 48.1%, 32.7%, and 1.9% of cases, respectively. Median length of hospitalization was 8 days. Conventional oxygen therapy (either through an oxygen nasal cannula or an oxygen mask) was necessary in 80.8% of patients, 17.3% of patients needed HFNO and 3.9% needed ICU admission. None of the patients required mechanical ventilation. The median lung involvement on CT was 20% (with a range of 1%-60%). The most common laboratory abnormalities were elevated CRP (94.2%), D-dimer (90.6%), IL-6 (88.5%) and fibrinogen (88%), hypoproteinemia (66.7%), decreased vitamin D (62.2%), elevated LDH (56%), hyperglycemia (48.8%), anemia (48.1%), elevated alkaline phosphatase (ALP) (46.2%), elevated AST (40.4%), lymphopenia (38.5%), elevated bile acids (35.7%), neutrophilia (30.8%), and elevated alanine transaminase (ALT; 30%). Hypertension was correlated with higher oxygen flow, while diabetes was correlated with higher oxygen flow and the need for HFNO. The percentage of lung involvement on CT was correlated with the severity of COVID-19, the oxygen flow, and the need for HFNO and ICU admission. Lymphocytopenia, low levels of serum calcium, total cholesterol and total protein levels, as well as high levels of serum CRP, ferritin, IL-6, glucose, LDH, PCT, and hs-TnI predicted a severe course of illness as measured by disease severity, the need for oxygen supplementation and higher oxygen flow, length of hospital stay, and the need for mechanical ventilation and ICU admission. Univariate logistic regression revealed that diabetes (OR = 10.2), gestational age < 32 weeks (OR = 5), lung involvement on CT > 20% (OR = 5.8), lymphocyte count < $1\times10^3$/μL (OR = 27.4), CRP > 75 mg/L (OR = 9.1), IL-6 > 60 pg/mL (OR = 16.5), PCT > 0.2 ng/mL (OR = 5.1), LDH > 270 U/L (OR = 3.7), hs-TnI > 6 ng/mL (OR = 12.7), calcium level ≤ 2.15 mmol/L (OR = 5.6), total cholesterol ≤ 180 mg/dL (OR = 5.7), total protein level ≤ 6.3 g/dL (OR = 9), and glucose > 99 mg/dL (OR = 6) were associated with increased risk of severe-to-critical COVID-19. Therefore, these indicators may serve as the predictors of severe disease, enabling an early identification of patients at risk of disease progression. Indeed, as already mentioned, higher inflammatory markers levels and

lymphopenia are observed in cytokine storm, commonly seen in severe COVID-19. Moreover, COVID-19 may cause poor glycemic control by modulating an immune response. This should prompt clinicians to pay special attention to glycemic levels during pregnancy, both in patients with a history of diabetes and in previously healthy women [129-132]. Hypoproteinemia may be caused by both the decrease in albumin and prealbumin production induced by the cytokine storm [133], and the physiological increase in protein requirements during pregnancy [134]. Changes in lipid levels in COVID-19 may result from impaired production of apolipoproteins and lipoproteins due to liver damage and cytokine activity, as well as capillary leak causing lipid relocation to extravascular compartments [135,136].

The main limitations of this study include its single-center nature, as well as its small and homogeneous cohort of patients. Therefore, further research is needed to identify potential predictors of severe COVID-19 in pregnancy.

## 5. CONCLUSIONS

1. Based on the results of the simple, disposable psychophysical olfactory test, created for the purpose of the study, it was shown that olfactory disorders are common in COVID-19 and seem to be highly associated with SARS-CoV-2 positivity, making this symptom a good predictor of COVID-19, whereas:
    a. as anosmia or hyposmia was self-reported by 32%-44% of patients, while it was found in 55%-64% of patients in the psychophysical test, self-assessment of olfactory function tends to underestimate the prevalence of OD, which highlights the importance of psychophysical smell assessment in COVID-19 patients;
    b. self-reported anosmia does not seem to be related to the severity of COVID-19, while for psychophysical tests, although their worse results were associated with some factors indicative of severe disease, larger, multi-center, prospective studies are needed;
    c. self-reported OD was observed to have high specificity, but low-to-moderate sensitivity in predicting SARS-COV-2 infection, while psychophysical evaluation appears to have both high specificity and sensitivity. Moreover, the combination of psychophysical tests results and self-reported OD results in improved diagnostic accuracy in predicting SARS-CoV-2 infection. This enables the development of a fast, affordable, and effective SARS-CoV-2 screening strategy based on combining a survey for new-onset OD with a simple disposable odor identification test, allowing for early identification of the individuals suspected of COVID-19 and eligible for isolation and laboratory-testing when possible. This approach could be especially useful in countries with a high number of COVID-19 cases and limited resources to perform RT-PCR for SARS-CoV-2.
2. In a review of the literature, it was found that the pathogenesis of COVID-19 related anosmia appears to be mainly due to sensorineural damage, with infection of the OE support cells via the ACE2 receptor, affecting the function of the olfactory neurons, and possibly with direct OSN infection mediated by the NRP1 receptor. Although early onset and early recovery of anosmia observed in most COVID-19 patients could argue in favor of conductive mechanism of OD, most patients do not present nasal symptoms, suggesting that is not the main factors in

the development of OD in SARS-CoV-2 infection. Involvement of the higher olfactory pathways and genetic factors may also be considered.

3. In young adults hospitalized for severe COVID-19, the SARS-CoV-2 alpha variant was not associated with a worse prognosis and a more severe course of the disease than the previous variants.
4. In young adults hospitalized for severe COVID-19, a number of factors, including obesity, comorbidities, smoking, the percentage of lung involvement on CT, lower $SpO_2$, leukocytosis, neutrophilia, lymphopenia, higher IG count, NLR, and higher CRP, PCT, IL-6, D-Dimer, LDH, hs-TnI, CK-MB, myoglobin, NT-proBNP, creatinine, urea and GGT levels, lower EGFR, albumin, calcium and vitamin D3 levels, and possibly a decrease in RBC counts and hemoglobin and hematocrit levels and an increase in CK levels in the course of hospitalization may be associated with poor outcomes of COVID-19. The identification of these factors may allow for earlier identification of high-risk patients and appropriate intervention. As severe disease and deaths also occur in young adults, health authorities should emphasize the need for preventative measures and support research on predictors of poor outcomes in this age group.
5. In pregnant patients hospitalized due to COVID-19, comorbidities, such as hypertension and diabetes, higher lung involvement on CT and a number of laboratory parameters measured at hospital admission, including lymphopenia, hypocalcemia, hypoproteinemia, low total cholesterol, as well as higher CRP, PCT, IL-6, ferritin, LDH, hs-TnI and glucose levels, appear to be adverse prognostic factors in the course of COVID-19. The identification of such factors may help identify pregnant patients at risk of severe disease, allowing for the earlier implementation of appropriate management in these patients and improvement of both maternal and fetal outcomes.

# VII. BIBLIOGRAFIA


1. World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020. Dostępny na stronie internetowej: https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020 (data dostępu: 16.08.2022)
2. Gengler I, Wang JC, Speth MM, Sedaghat AR. Sinonasal pathophysiology of SARS-CoV-2 and COVID-19: A systematic review of the current evidence. Laryngoscope Investig Otolaryngol. 2020;5(3):354-359. doi: 10.1002/lio2.384
3. Fodoulian L, Tuberosa J, Rossier D, Boillat M, Kan C, Pauli V, et al. SARS-CoV-2 Receptors and Entry Genes Are Expressed in the Human Olfactory Neuroepithelium and Brain. iScience. 2020;23(12):101839. doi: 10.1016/j.isci.2020.101839
4. Cantuti-Castelvetri L, Ojha R, Pedro LD, Djannatian M, Franz J, Kuivanen S, et al. Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. Science. 2020;370(6518):856-860. doi: 10.1126/science.abd2985
5. Guadarrama-Ortiz P, Choreño-Parra JA, Sánchez-Martínez CM, Pacheco-Sánchez FJ, Rodríguez-Nava AI, García-Quintero G. Neurological Aspects of SARS-CoV-2 Infection: Mechanisms and Manifestations. Front Neurol. 2020;11:1039. doi:10.3389/fneur.2020.01039
6. Hamming I, Timens W, Bulthuis M, Lely A, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol. 2004;203(2):631-637. doi:10.1002/path.1570
7. Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci. 2020;12(1):8. doi: 10.1038/s41368-020-0074-x
8. National Institutes of Health. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. Dostępne na stronie internetowej: https://files.covid19treatmentguidelines.nih.gov/guidelines/covid19treatmentguidelines.pdf (data dostępu: 16.08.2022)
9. Flisiak R, Horban A, Jaroszewicz J, Kozielewicz D, Mastalerz-Migas A, Owczuk R, et al. Zalecenia postępowania w zakażeniach SARS-CoV-2 Polskiego Towarzystwa Epidemiologów i Lekarzy Chorób Zakaźnych, na dzień 26 kwietnia 2021. Dostępne na stronie internetowej: http://www.pteilchz.org.pl/wp-content/uploads/2021/04/REKOMENDACJE-pl-w-C19-2021-26-04-2021b.pdf (data dostępu: 16.08.2022)
10. Struyf T, Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Leeflang MM, et al; Cochrane COVID-19 Diagnostic Test Accuracy Group. Signs and symptoms to determine if a patient presenting in primary care or hospital outpatient settings has COVID-19. Cochrane Database Syst Rev. 2022;5(5):CD013665. doi: 10.1002/14651858.CD013665.pub3
11. Baig AM, Khaleeq A, Ali U, Syeda H. Evidence of the COVID-19 Virus Targeting the CNS: Tissue Distribution, Host–Virus Interaction, and Proposed Neurotropic Mechanisms. ACS Chem Neurosci. 2020;11(7):995-998. doi:10.1021/acschemneuro.0c00122
12. Brann DH, Tsukahara T, Weinreb C, Lipovsek M, Van den Berge K, Gong B, et al. Non-neuronal expression of SARS-CoV-2 entry genes in the olfactory system suggests mechanisms underlying COVID-19-associated anosmia. Sci Adv. 2020;6(31):eabc5801. doi: 10.1126/sciadv.abc5801
13. Hummel T, Whitcroft KL, Andrews P, Altundag A, Cinghi C, Costanzo RM, et al. Position paper on olfactory dysfunction. Rhinology. 2016;56(1):1-30. doi: 10.4193/Rhino16.248
14. Bilinska K, Butowt R. Anosmia in COVID-19: A Bumpy Road to Establishing a Cellular Mechanism. ACS Chem Neurosci. 2020;11(15):2152-2155. doi:10.1021/acschemneuro.0c00406
15. Glezer I, Bruni-Cardoso A, Schechtman D, Malnic B. Viral infection and smell loss: The case of COVID-19. J Neurochem. 2021;157(4):930-943. doi:10.1111/jnc.15197
16. Ramasamy K, Saniasiaya J, Abdul Gani N. Olfactory and Gustatory Dysfunctions as a Clinical Manifestation of Coronavirus Disease 2019 in a Malaysian Tertiary Center. Ann Otol Rhinol Laryngol. 2021;130(5):513-519. doi:10.1177/0003489420963165
17. Suzuki M, Saito K, Min WP, Vladau C, Toida K, Itoh H, Murakami S. Identification of viruses in patients with postviral olfactory dysfunction. Laryngoscope. 2007;117(2):272-277. doi: 10.1097/01.mlg.0000249922.37381.1e
18. Welge-Lüssen A, Wolfensberger M. Olfactory Disorders following Upper Respiratory Tract Infections. In: Hummel T, Welge-Lüssen A, eds. Advances in Oto-Rhino-Laryngology. Vol 63. KARGER; 2006:125-132. doi:10.1159/000093758
19. Ueha R, Kondo K, Kagoya R, Shichino S, Shichino S, Yamasoba T. ACE2, TMPRSS2, and Furin expression in the nose and olfactory bulb in mice and humans. Rhinology. 2021;59(1):105-109. doi: 10.4193/Rhin20.324
20. Cooper KW, Brann DH, Farruggia MC, Bhutani S, Pellegrino R, Tsukahara T, et al. COVID-19 and

the Chemical Senses: Supporting Players Take Center Stage. Neuron. 2020;107(2):219-233. doi: 10.1016/j.neuron.2020.06.032
21. Gorzkowski V, Bevilacqua S, Charmillon A, Jankowski R, Gallet P, Rumeau C, Nguyen DT. Evolution of Olfactory Disorders in COVID-19 Patients. Laryngoscope. 2020;130(11):2667-2673. doi: 10.1002/lary.28957
22. Moein ST, Hashemian SM, Mansourafshar B, Khorram-Tousi A, Tabarsi P, Doty RL. Smell dysfunction: a biomarker for COVID-19. Int Forum Allergy Rhinol. 2020;10(8):944-950. doi: 10.1002/alr.22587
23. Pang KW, Chee J, Subramaniam S, Ng CL. Frequency and Clinical Utility of Olfactory Dysfunction in COVID-19: a Systematic Review and Meta-analysis. Curr Allergy Asthma Rep. 2020;20(12):76. doi: 10.1007/s11882-020-00972-y
24. Hannum ME, Ramirez VA, Lipson SJ, Herriman RD, Toskala AK, Lin C, et al. Objective Sensory Testing Methods Reveal a Higher Prevalence of Olfactory Loss in COVID-19-Positive Patients Compared to Subjective Methods: A Systematic Review and Meta-Analysis. Chem Senses. 2020;45(9):865-874. doi: 10.1093/chemse/bjaa064
25. Saniasiaya J, Islam MA, Abdullah B. Prevalence of Olfactory Dysfunction in Coronavirus Disease 2019 (COVID-19): A Meta-analysis of 27,492 Patients. The Laryngoscope. 2021;131(4):865-878. doi: 10.1002/lary.29286
26. Marchese-Ragona R, Restivo DA, De Corso E, Vianello A, Nicolai P, Ottaviano G. Loss of smell in COVID-19 patients: a critical review with emphasis on the use of olfactory tests. Acta Otorhinolaryngol Ital. 2020;40(4):241-247. doi:10.14639/0392-100X-N0862
27. Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. JAMA. 2020;323(13):1239. doi:10.1001/jama.2020.2648
28. Stokes EK, Zambrano LD, Anderson KN, Marder EP, Raz KM, El Burai Felix S, et al. Coronavirus Disease 2019 Case Surveillance - United States, January 22-May 30, 2020. MMWR Morb Mortal Wkly Rep. 2020;69(24):759-765. doi: 10.15585/mmwr.mm6924e2
29. Xie Y, Wang Z, Liao H, Marley G, Wu D, Tang W. Epidemiologic, clinical, and laboratory findings of the COVID-19 in the current pandemic: systematic review and meta-analysis. BMC Infect Dis. 2020;20(1):640. doi:10.1186/s12879-020-05371-2
30. Barnes BJ, Adrover JM, Baxter-Stoltzfus A, Borczuk A, Cools-Lartigue J, Crawford JM, et al. Targeting potential drivers of COVID-19: Neutrophil extracellular traps. J Exp Med. 2020;217(6):e20200652. doi: 10.1084/jem.20200652
31. Feng X, Li S, Sun Q, Zhu J, Chen B, Xiong M, Cao G. Immune-Inflammatory Parameters in COVID-19 Cases: A Systematic Review and Meta-Analysis. Front Med (Lausanne). 2020;7:301. doi: 10.3389/fmed.2020.00301
32. Polidoro RB, Hagan RS, de Santis Santiago R, Schmidt NW. Overview: Systemic Inflammatory Response Derived From Lung Injury Caused by SARS-CoV-2 Infection Explains Severe Outcomes in COVID-19. Front Immunol. 2020;11:1626. doi:10.3389/fimmu.2020.01626
33. Booth A, Reed AB, Ponzo S, Yassaee A, Aral M, Plans D, et al. Population risk factors for severe disease and mortality in COVID-19: A global systematic review and meta-analysis. PLoS One. 2021;16(3):e0247461. doi: 10.1371/journal.pone.0247461
34. Petrilli CM, Jones SA, Yang J, Rajagopalan H, O'Donnell L, Chernyak Y, et al. Factors associated with hospital admission and critical illness among 5279 people with coronavirus disease 2019 in New York City: prospective cohort study. BMJ. 2020 May 22;369:m1966. doi: 10.1136/bmj.m1966
35. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054-1062. doi: 10.1016/S0140-6736(20)30566-3
36. Liu J, Tu C, Zhu M, Wang J, Yang C, Liu W, Xiong B. The clinical course and prognostic factors of severe COVID-19 in Wuhan, China: A retrospective case-control study. Medicine (Baltimore). 2021;100(8):e23996. doi: 10.1097/MD.0000000000023996
37. Shi C, Wang L, Ye J, Gu Z, Wang S, Xia J, et al. Predictors of mortality in patients with coronavirus disease 2019: a systematic review and meta-analysis. BMC Infect Dis. 2021;21(1):663. doi: 10.1186/s12879-021-06369-0
38. Mesas AE, Cavero-Redondo I, Álvarez-Bueno C, Sarriá Cabrera MA, Maffei de Andrade S, Sequí-Dominguez I, et al. Predictors of in-hospital COVID-19 mortality: A comprehensive systematic review and meta-analysis exploring differences by age, sex and health conditions. PLoS One. 2020;15(11):e0241742. doi: 10.1371/journal.pone.0241742
39. Altonen BL, Arreglado TM, Leroux O, Murray-Ramcharan M, Engdahl R. Characteristics, comorbidities and survival analysis of young adults hospitalized with COVID-19 in New York City. Tan W, ed. PLoS ONE. 2020;15(12):e0243343. doi:10.1371/journal.pone.0243343

40. Liu Z, Liu J, Ye L, Yu K, Luo Z, Liang C, et al. Predictors of mortality for hospitalized young adults aged less than 60 years old with severe COVID-19: a retrospective study. J Thorac Dis. 2021;13(6):3628-3642. doi: 10.21037/jtd-21-120
41. Williamson EJ, Walker AJ, Bhaskaran K, Bacon S, Bates C, Morton CE, et al. Factors associated with COVID-19-related death using OpenSAFELY. Nature. 2020;584(7821):430-436. doi: 10.1038/s41586-020-2521-4
42. Sandoval M, Nguyen DT, Vahidy FS, Graviss EA. Risk factors for severity of COVID-19 in hospital patients age 18–29 years. Zivkovic AR, ed. PLoS ONE. 2021;16(7):e0255544. doi: 10.1371/journal.pone.0255544
43. Lu Y, Huang Z, Wang M, Tang K, Wang S, Gao P, et al. Clinical characteristics and predictors of mortality in young adults with severe COVID-19: a retrospective observational study. Ann Clin Microbiol Antimicrob. 2021;20(1):3. doi: 10.1186/s12941-020-00412-9
44. Breslin N, Baptiste C, Gyamfi-Bannerman C, Miller R, Martinez R, Bernstein K, et al. Coronavirus disease 2019 infection among asymptomatic and symptomatic pregnant women: two weeks of confirmed presentations to an affiliated pair of New York City hospitals. Am J Obstet Gynecol MFM. 2020;2(2):100118. doi: 10.1016/j.ajogmf.2020.100118
45. Ferrer-Oliveras R, Mendoza M, Capote S, Pratcorona L, Esteve-Valverde E, Cabero-Roura L, et al. Immunological and physiopathological approach of COVID-19 in pregnancy. Arch Gynecol Obstet. 2021;304(1):39-57. doi: 10.1007/s00404-021-06061-3
46. Mor G, Aldo P, Alvero AB. The unique immunological and microbial aspects of pregnancy. Nat Rev Immunol. 2017;17(8):469-482. doi:10.1038/nri.2017.64
47. Rasmussen SA, Jamieson DJ, Uyeki TM. Effects of influenza on pregnant women and infants. American Journal of Obstetrics and Gynecology. 2012;207(3):S3-S8. doi:10.1016/j.ajog.2012.06.068
48. Allotey J, Stallings E, Bonet M, Yap M, Chatterjee S, Kew T, et al.; for PregCOV-19 Living Systematic Review Consortium. Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: living systematic review and meta-analysis. BMJ. 2020;370:m3320. doi: 10.1136/bmj.m3320
49. Zambrano LD, Ellington S, Strid P, Galang RR, Oduyebo T, Tong VT, et al. CDC COVID-19 Response Pregnancy and Infant Linked Outcomes Team. Update: Characteristics of Symptomatic Women of Reproductive Age with Laboratory-Confirmed SARS-CoV-2 Infection by Pregnancy Status - United States, January 22-October 3, 2020. MMWR Morb Mortal Wkly Rep. 2020;69(44):1641-1647. doi: 10.15585/mmwr.mm6944e3
50. World Health Organization. Tracking SARS-CoV-2 variants. Dostępne na stronie internetowej: https://www.who.int/activities/tracking-SARS-CoV-2-variants (data dostępu: 17.08.2022)
51. Centers for Disease Control and Prevention. Coronavirus Disease 2019 (COVID-19). Dostępne na stronie internetowej: https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html (data dostępu: 17.08.2022)
52. European Centre for Disease Prevention and Control. Risk assessment: SARS-CoV-2 - increased circulation of variants of concern and vaccine rollout in the EU/EEA, 14th update. Dostępne na stronie internetowej: https://www.ecdc.europa.eu/en/publications-data/covid-19-risk-assessment-variants-vaccine-fourteenth-update-february-2021 (data dostępu: 17.08.2022)
53. European Centre for Disease Prevention and Control. Data on SARS-CoV-2 variants in the EU/EEA. Dostepne na stronie internetowej: https://www.ecdc.europa.eu/en/publications-data/data-virus-variants-covid-19-eueea (data dostępu: 17.08.2022)
54. Davies NG, Abbott S, Barnard RC, Jarvis CI, Kucharski AJ, Munday JD, et al. CMMID COVID-19 Working Group; COVID-19 Genomics UK (COG-UK) Consortium. Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. Science. 2021;372(6538):eabg3055. doi: 10.1126/science.abg3055
55. Volz E, Mishra S, Chand M, Barrett JC, Johnson R, Geidelberg L, et al. COVID-19 Genomics UK (COG-UK) consortium. Assessing transmissibility of SARS-CoV-2 lineage B.1.1.7 in England. Nature. 2021;593(7858):266-269. doi: 10.1038/s41586-021-03470-x.
56. Frampton D, Rampling T, Cross A, Bailey H, Heaney J, Byott M, et al. Genomic characteristics and clinical effect of the emergent SARS-CoV-2 B.1.1.7 lineage in London, UK: a whole-genome sequencing and hospital-based cohort study. Lancet Infect Dis. 2021;21(9):1246-1256. doi: 10.1016/S1473-3099(21)00170-5
57. Davies NG, Jarvis CI; CMMID COVID-19 Working Group, Edmunds WJ, Jewell NP, Diaz-Ordaz K, Keogh RH. Increased mortality in community-tested cases of SARS-CoV-2 lineage B.1.1.7. Nature. 2021 May;593(7858):270-274. doi: 10.1038/s41586-021-03426-1
58. Martin-Blondel G, Lescure FX, Assoumou L, Charpentier C, Chapplain JM, Perpoint T, et al. CoCliCo Study Group. Increased risk of severe COVID-19 in hospitalized patients with SARS-CoV-2 Alpha variant infection: a multicentre matched cohort study. BMC Infect Dis. 2022;22(1):540. doi:

10.1186/s12879-022-07508-x
59. Nyberg T, Twohig KA, Harris RJ, Seaman SR, Flannagan J, Allen H, et al. Risk of hospital admission for patients with SARS-CoV-2 variant B.1.1.7: cohort analysis. BMJ. 2021;373:n1412. doi: 10.1136/bmj.n1412
60. Brookman S, Cook J, Zucherman M, Broughton S, Harman K, Gupta A. Effect of the new SARS-CoV-2 variant B.1.1.7 on children and young people. The Lancet Child & Adolescent Health. 2021;5(4):e9-e10. doi:10.1016/S2352-4642(21)00030-4
61. Fisman DN, Tuite AR. Evaluation of the relative virulence of novel SARS-CoV-2 variants: a retrospective cohort study in Ontario, Canada. CMAJ. 2021;193(42):E1619-E1625. doi: 10.1503/cmaj.211248
62. Martin B, DeWitt PE, Russell S, Anand A, Bradwell KR, Bremer C, et al. Characteristics, Outcomes, and Severity Risk Factors Associated With SARS-CoV-2 Infection Among Children in the US National COVID Cohort Collaborative. JAMA Netw Open. 2022;5(2):e2143151. doi: 10.1001/jamanetworkopen.2021.43151
63. Kläser K, Molteni E, Graham M, Canas LS, Österdahl MF, Antonelli M, et al.; COVID-19 Genomics U. K. (COG-UK) Consortium. COVID-19 due to the B.1.617.2 (Delta) variant compared to B.1.1.7 (Alpha) variant of SARS-CoV-2: a prospective observational cohort study. Sci Rep. 2022;12(1):10904. doi: 10.1038/s41598-022-14016-0
64. European Centre for Disease Prevention and Control. SARS-CoV-2 variants of concern as of 11 August 2022. Dostępne na stronie internetowej: https://www.ecdc.europa.eu/en/covid-19/variants-concern (data dostępu: 17.08.2022)
65. Van Goethem N, Chung PYJ, Meurisse M, Vandromme M, De Mot L, Brondeel R. Clinical Severity of SARS-CoV-2 Omicron Variant Compared with Delta among Hospitalized COVID-19 Patients in Belgium during Autumn and Winter Season 2021-2022. Viruses. 2022;14(6):1297. doi: 10.3390/v14061297
66. Sievers C, Zacher B, Ullrich A, Huska M, Fuchs S, Buda S, et al. SARS-CoV-2 Omicron variants BA.1 and BA.2 both show similarly reduced disease severity of COVID-19 compared to Delta, Germany, 2021 to 2022. Euro Surveill. 2022;27(22):2200396. doi: 10.2807/1560-7917.ES.2022.27.22.2200396
67. Wolter N, Jassat W, Walaza S, Welch R, Moultrie H, Groome M, et al. Early assessment of the clinical severity of the SARS-CoV-2 omicron variant in South Africa: a data linkage study. Lancet. 2022;399(10323):437-446. doi: 10.1016/S0140-6736(22)00017-4
68. Serwis Rzeczypospolitej Polskiej. Koronawirus - informacje i zalecenia. Pliki archiwalne - dane historyczne. Dostępne na stronie internetowej: https://www.gov.pl/web/koronawirus/pliki-archiwalne-dane-historyczne (data dostępu: 17.08.2022)
69. Subbe CP. Validation of a modified Early Warning Score in medical admissions. *QJM*. 2001;94(10):521-526. doi:10.1093/qjmed/94.10.521
70. Society for Adolescent Health and Medicine. Young Adult Health and Well-Being: A Position Statement of the Society for Adolescent Health and Medicine. J Adolesc Health. 2017;60(6):758-759. doi: 10.1016/j.jadohealth.2017.03.021
71. Donisi V, Gajofatto A, Mazzi MA, Gobbin F, Busch IM, Ghellere A, et al. Insights for Fostering Resilience in Young Adults With Multiple Sclerosis in the Aftermath of the COVID-19 Emergency: An Italian Survey. Front Psychiatry. 2021;11:588275. doi: 10.3389/fpsyt.2020.588275
72. Trimaille A, Ribeyrolles S, Fauvel C, Chaumont C, Weizman O, Pommier T, et al. The Critical Covid-France Investigators. Cardiovascular Characteristics and Outcomes of Young Patients with COVID-19. J Cardiovasc Dev Dis. 2021;8(12):165. doi: 10.3390/jcdd8120165
73. Steinberg E, Wright E, Kushner B. In Young Adults with COVID-19, Obesity Is Associated with Adverse Outcomes. West J Emerg Med. 2020;21(4):752-755. doi: 10.5811/westjem.2020.5.47972
74. Lechien JR, Chiesa-Estomba CM, Place S, Van Laethem Y, Cabaraux P, Mat Q, et al. COVID-19 Task Force of YO-IFOS. Clinical and epidemiological characteristics of 1420 European patients with mild-to-moderate coronavirus disease 2019. J Intern Med. 2020;288(3):335-344. doi: 10.1111/joim.13089
75. Vaira LA, Deiana G, Fois AG, Pirina P, Madeddu G, De Vito A, et al. Objective evaluation of anosmia and ageusia in COVID-19 patients: Single-center experience on 72 cases. Head Neck. 2020;42(6):1252-1258. doi: 10.1002/hed.26204
76. Eliezer M, Hautefort C, Hamel AL, Verillaud B, Herman P, Houdart E, Eloit C. Sudden and Complete Olfactory Loss of Function as a Possible Symptom of COVID-19. JAMA Otolaryngol Head Neck Surg. 2020;146(7):674-675. doi: 10.1001/jamaoto.2020.0832
77. Altundag A, Yıldırım D, Tekcan Sanli DE, Cayonu M, Kandemirli SG, Sanli AN, et al. Olfactory Cleft Measurements and COVID-19-Related Anosmia. Otolaryngol Head Neck Surg. 2021;164(6):1337-1344. doi: 10.1177/0194599820965920

78. Naeini AS, Karimi-Galougahi M, Raad N, Ghorbani J, Taraghi A, Haseli S, et al. Paranasal sinuses computed tomography findings in anosmia of COVID-19. Am J Otolaryngol. 2020;41(6):102636. doi: 10.1016/j.amjoto.2020.102636
79. Han AY, Mukdad L, Long JL, Lopez IA. Anosmia in COVID-19: Mechanisms and Significance. Chemical Senses. 2020;45(6):423-428. doi:10.1093/chemse/bjaa040
80. Mao L, Jin H, Wang M, Hu Y, Chen S, He Q, et al. Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China. JAMA Neurol. 2020;77(6):683-690. doi: 10.1001/jamaneurol.2020.1127
81. Ahmad I, Rathore FA. Neurological manifestations and complications of COVID-19: A literature review. J Clin Neurosci. 2020;77:8-12. doi: 10.1016/j.jocn.2020.05.017
82. Pouga L. Encephalitic syndrome and anosmia in COVID-19: Do these clinical presentations really reflect SARS-CoV-2 neurotropism? A theory based on the review of 25 COVID-19 cases. J Med Virol. 2021;93(1):550-558. doi: 10.1002/jmv.26309
83. Lima M, Siokas V, Aloizou AM, Liampas I, Mentis AA, Tsouris Z, et al. Unraveling the Possible Routes of SARS-COV-2 Invasion into the Central Nervous System. Curr Treat Options Neurol. 2020;22(11):37. doi: 10.1007/s11940-020-00647-z
84. Gori A, Leone F, Loffredo L, Cinicola BL, Brindisi G, De Castro G, et al. COVID-19-Related Anosmia: The Olfactory Pathway Hypothesis and Early Intervention. Front Neurol. 2020;11:956. doi: 10.3389/fneur.2020.00956
85. Kandemirli SG, Altundag A, Yildirim D, Tekcan Sanli DE, Saatci O. Olfactory Bulb MRI and Paranasal Sinus CT Findings in Persistent COVID-19 Anosmia. Acad Radiol. 2021;28(1):28-35. doi: 10.1016/j.acra.2020.10.006
86. Bulfamante G, Chiumello D, Canevini MP, Priori A, Mazzanti M, Centanni S, et al. First ultrastructural autoptic findings of SARS -Cov-2 in olfactory pathways and brainstem. Minerva Anestesiol. 2020;86(6):678-679. doi: 10.23736/S0375-9393.20.14772-2
87. Menter T, Haslbauer JD, Nienhold R, Savic S, Hopfer H, Deigendesch N, et al. Postmortem examination of COVID-19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings in lungs and other organs suggesting vascular dysfunction. Histopathology. 2020;77(2):198-209. doi: 10.1111/his.14134
88. Laurendon T, Radulesco T, Mugnier J, Gérault M, Chagnaud C, El Ahmadi AA, Varoquaux A. Bilateral transient olfactory bulb edema during COVID-19-related anosmia. Neurology. 2020;95(5):224-225. doi: 10.1212/WNL.0000000000009850
89. Aragão MFVV, Leal MC, Cartaxo Filho OQ, Fonseca TM, Valença MM. Anosmia in COVID-19 Associated with Injury to the Olfactory Bulbs Evident on MRI. AJNR Am J Neuroradiol. 2020;41(9):1703-1706. doi: 10.3174/ajnr.A6675
90. Netland J, Meyerholz DK, Moore S, Cassell M, Perlman S. Severe Acute Respiratory Syndrome Coronavirus Infection Causes Neuronal Death in the Absence of Encephalitis in Mice Transgenic for Human ACE2. J Virol. 2008;82(15):7264-7275. doi:10.1128/JVI.00737-08
91. Dubé M, Le Coupanec A, Wong AHM, Rini JM, Desforges M, Talbot PJ. Axonal Transport Enables Neuron-to-Neuron Propagation of Human Coronavirus OC43. Diamond MS, ed. J Virol. 2018;92(17):e00404-18. doi:10.1128/JVI.00404-18
92. Sun SH, Chen Q, Gu HJ, Yang G, Wang YX, Huang XY, et al. A Mouse Model of SARS-CoV-2 Infection and Pathogenesis. Cell Host Microbe. 2020;28(1):124-133.e4. doi: 10.1016/j.chom.2020.05.020
93. Bryche B, St Albin A, Murri S, Lacôte S, Pulido C, Ar Gouilh M, et al. Massive transient damage of the olfactory epithelium associated with infection of sustentacular cells by SARS-CoV-2 in golden Syrian hamsters. Brain Behav Immun. 2020;89:579-586. doi: 10.1016/j.bbi.2020.06.032
94. Bilinska K, Jakubowska P, Von Bartheld CS, Butowt R. Expression of the SARS-CoV-2 Entry Proteins, ACE2 and TMPRSS2, in Cells of the Olfactory Epithelium: Identification of Cell Types and Trends with Age. ACS Chem Neurosci. 2020;11(11):1555-1562. doi:10.1021/acschemneuro.0c00210
95. Azim D, Nasim S, Kumar S, Hussain A, Patel S. Neurological Consequences of 2019-nCoV Infection: A Comprehensive Literature Review. Cureus. 2020;12(6):e8790. doi: 10.7759/cureus.8790
96. Paniz-Mondolfi A, Bryce C, Grimes Z, Gordon RE, Reidy J, Lednicky J, et al. Central nervous system involvement by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). J Med Virol. 2020;92(7):699-702. doi: 10.1002/jmv.25915
97. de Melo GD, Lazarini F, Levallois S, Hautefort C, Michel V, Larrous F, et al. COVID-19-related anosmia is associated with viral persistence and inflammation in human olfactory epithelium and brain infection in hamsters. Sci Transl Med. 2021;13(596):eabf8396. doi: 10.1126/scitranslmed.abf8396
98. Hopkins C, Lechien JR, Saussez S. More that ACE2? NRP1 may play a central role in the underlying pathophysiological mechanism of olfactory dysfunction in COVID-19 and its association with

enhanced survival. Med Hypotheses. 2021;146:110406. doi: 10.1016/j.mehy.2020.110406
99. Hopkins C, Surda P, Vaira LA, Lechien JR, Safarian M, Saussez S, Kumar N. Six month follow-up of self-reported loss of smell during the COVID-19 pandemic. Rhinology. 2021;59(1):26-31. doi: 10.4193/Rhin20.544
100. Di Stadio A, D'Ascanio L, La Mantia I, Ralli M, Brenner MJ. Parosmia after COVID-19: olfactory training, neuroinflammation and distortions of smell. European Review for Medical and Pharmacological Sciences. 2022;26(1):1-3. doi:10.26355/eurrev_202201_27739
101. Shelton JF, Shastri AJ, Fletez-Brant K; 23andMe COVID-19 Team; Aslibekyan S, Auton A. The UGT2A1/UGT2A2 locus is associated with COVID-19-related loss of smell or taste. Nat Genet. 2022 Feb;54(2):121-124. doi: 10.1038/s41588-021-00986-w
102. Lazard D, Zupko K, Poria Y, Nef P, Lazarovits J, Horn S, et al. Odorant signal termination by olfactory UDP glucuronosyl transferase. Nature. 1991;349(6312):790-793. doi: 10.1038/349790a0
103. Luo X, Zhou W, Yan X, Guo T, Wang B, Xia H, et al. Prognostic Value of C-Reactive Protein in Patients With Coronavirus 2019. Clin Infect Dis. 2020;71(16):2174-2179. doi: 10.1093/cid/ciaa641
104. Gómez-Pastora J, Weigand M, Kim J, Wu X, Strayer J, Palmer AF, et al. Hyperferritinemia in critically ill COVID-19 patients - Is ferritin the product of inflammation or a pathogenic mediator? Clin Chim Acta. 2020;509:249-251. doi: 10.1016/j.cca.2020.06.033
105. Xu JB, Xu C, Zhang RB, Wu M, Pan CK, Li XJ, et al. Associations of procalcitonin, C-reaction protein and neutrophil-to-lymphocyte ratio with mortality in hospitalized COVID-19 patients in China. Sci Rep. 2020;10(1):15058. doi: 10.1038/s41598-020-72164-7
106. Asan A, Üstündağ Y, Koca N, Şimşek A, Sayan HE, Parildar H, et al. Do initial hematologic indices predict the severity of COVID-19 patients? Turk J Med Sci. 2021;51(1):39-44. doi: 10.3906/sag-2007-97
107. Zare ME, Wang Y, Nasir Kansestani A, Almasi A, Zhang J. Procalcitonin Has Good Accuracy for Prognosis of Critical Condition and Mortality in COVID-19: A Diagnostic Test Accuracy Systematic Review and Meta-analysis. Iran J Allergy Asthma Immunol. 2020;19(6):557-569. doi: 10.18502/ijaai.v19i6.4926
108. Lippi G, Plebani M. Procalcitonin in patients with severe coronavirus disease 2019 (COVID-19): A meta-analysis. Clinica Chimica Acta. 2020;505:190-191. doi:10.1016/j.cca.2020.03.004
109. Shen Y, Cheng C, Zheng X, Jin Y, Duan G, Chen M, et al. Elevated Procalcitonin Is Positively Associated with the Severity of COVID-19: A Meta-Analysis Based on 10 Cohort Studies. Medicina (Kaunas). 2021;57(6):594. doi: 10.3390/medicina57060594
110. Lanser L, Burkert FR, Bellmann-Weiler R, Schroll A, Wildner S, Fritsche G, et al. Dynamics in Anemia Development and Dysregulation of Iron Homeostasis in Hospitalized Patients with COVID-19. Metabolites. 2021;11(10):653. doi: 10.3390/metabo11100653
111. Soraya GV, Ulhaq ZS. Crucial laboratory parameters in COVID-19 diagnosis and prognosis: An updated meta-analysis. Medicina Clínica. 2020;155(4):143-151. doi:10.1016/j.medcli.2020.05.017
112. Yan H, Liang X, Du J, He Z, Wang Y, Lyu M, et al. Proteomic and metabolomic investigation of serum lactate dehydrogenase elevation in COVID-19 patients. Proteomics. 2021;21(15):e2100002. doi: 10.1002/pmic.202100002
113. Zhu Z, Wang M, Lin W, Cai Q, Zhang L, Chen D, et al. Cardiac biomarkers, cardiac injury, and comorbidities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis. Immun Inflamm Dis. 2021;9(4):1071-1100. doi: 10.1002/iid3.471
114. Ouyang L, Gong Y, Zhu Y, Gong J. Association of acute kidney injury with the severity and mortality of SARS-CoV-2 infection: A meta-analysis. The American Journal of Emergency Medicine. 2021;43:149-157. doi:10.1016/j.ajem.2020.08.089
115. Kumar MP, Mishra S, Jha DK, Shukla J, Choudhury A, Mohindra R, et al. Coronavirus disease (COVID-19) and the liver: a comprehensive systematic review and meta-analysis. Hepatol Int. 2020;14(5):711-722. doi: 10.1007/s12072-020-10071-9
116. Wu ZH, Yang DL. A meta-analysis of the impact of COVID-19 on liver dysfunction. Eur J Med Res. 2020;25(1):54. doi: 10.1186/s40001-020-00454-x
117. Bzeizi K, Abdulla M, Mohammed N, Alqamish J, Jamshidi N, Broering D. Effect of COVID-19 on liver abnormalities: a systematic review and meta-analysis. Sci Rep. 2021;11(1):10599. doi:10.1038/s41598-021-89513-9
118. Li G, Yang Y, Gao D, Xu Y, Gu J, Liu P. Is liver involvement overestimated in COVID-19 patients? A meta-analysis. Int J Med Sci. 2021;18(5):1285-1296. doi:10.7150/ijms.51174
119. Kheir M, Saleem F, Wang C, Mann A, Chua J. Higher albumin levels on admission predict better prognosis in patients with confirmed COVID-19. PLoS One. 2021;16(3):e0248358. doi: 10.1371/journal.pone.0248358
120. Alemzadeh E, Alemzadeh E, Ziaee M, Abedi A, Salehiniya H. The effect of low serum calcium level

on the severity and mortality of Covid patients: A systematic review and meta-analysis. Immun Inflamm Dis. 2021;9(4):1219-1228. doi:10.1002/iid3.528
121. Martha JW, Wibowo A, Pranata R. Hypocalcemia is associated with severe COVID-19: A systematic review and meta-analysis. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2021;15(1):337-342. doi:10.1016/j.dsx.2021.01.003
122. Menshawey E, Menshawey R, Nabeh OA. Shedding light on vitamin D: the shared mechanistic and pathophysiological role between hypovitaminosis D and COVID-19 risk factors and complications. Inflammopharmacol. 2021;29(4):1017-1031. doi:10.1007/s10787-021-00835-6
123. Chiodini I, Gatti D, Soranna D, Merlotti D, Mingiano C, Fassio A, et al. Vitamin D Status and SARS-CoV-2 Infection and COVID-19 Clinical Outcomes. Front Public Health. 2021;9:736665. doi: 10.3389/fpubh.2021.736665
124. Kaya MO, Pamukçu E, Yakar B. The role of vitamin D deficiency on the Covid-19: A systematic review and meta-analysis of observational studies. Epidemiol Health. 2021:e2021074. doi:10.4178/epih.e2021074
125. Gianchandani R, Esfandiari NH, Ang L, Iyengar J, Knotts S, Choksi P, et al. Managing Hyperglycemia in the COVID-19 Inflammatory Storm. Diabetes. 2020;69(10):2048-2053. doi: 10.2337/dbi20-0022
126. Feldman EL, Savelieff MG, Hayek SS, Pennathur S, Kretzler M, Pop-Busui R. COVID-19 and Diabetes: A Collision and Collusion of Two Diseases. Diabetes. 2020;69(12):2549-2565. doi: 10.2337/dbi20-0032
127. Miftode E, Miftode L, Coman I, Prepeliuc C, Obreja M, Stămăteanu O, et al. Diabetes Mellitus-A Risk Factor for Unfavourable Outcome in COVID-19 Patients-The Experience of an Infectious Diseases Regional Hospital. Healthcare (Basel). 2021;9(7):788. doi: 10.3390/healthcare9070788
128. Wang S, Ma P, Zhang S, Song S, Wang Z, Ma Y, et al. Fasting blood glucose at admission is an independent predictor for 28-day mortality in patients with COVID-19 without previous diagnosis of diabetes: a multi-centre retrospective study. Diabetologia. 2020;63(10):2102-2111. doi: 10.1007/s00125-020-05209-1
129. Akbar MR, Pranata R, Wibowo A, Lim MA, Sihite TA, Martha JW. The association between serum prealbumin and poor outcome in COVID-19 – Systematic review and meta-analysis. European Review for Medical and Pharmacological Sciences. 2021;25(10):3879-3885. doi: 10.26355/eurrev_202105_25955
130. Elango R, Ball RO. Protein and Amino Acid Requirements during Pregnancy. Adv Nutr. 2016;7(4):839S-844S. doi:10.3945/an.115.011817
131. Poynard T, Deckmyn O, Rudler M, Peta V, Ngo Y, Vautier M, et al. Performance of serum apolipoprotein-A1 as a sentinel of Covid-19. PLoS One. 2020;15(11):e0242306. doi: 10.1371/journal.pone.0242306
132. van Leeuwen HJ, Heezius ECJM, Dallinga GM, van Strijp JAG, Verhoef J, van Kessel KPM. Lipoprotein metabolism in patients with severe sepsis: Critical Care Medicine. 2003;31(5):1359-1366. doi: 10.1097/01.CCM.0000059724.08290.51

## VIII. OPINIE KOMISJI BIOETYCZNEJ

### 1. Ocena częstości występowania zaburzeń węchu u pacjentów z COVID-19 oraz ich wartości predykcyjnej w ocenie prawdopodobieństwa zakażenia SARS-CoV-2.

**Komisja Etyki i Nadzoru nad Badaniami na Ludziach i Zwierzętach**
**przy Centralnym Szpitalu Klinicznym MSWiA w Warszawie**
ul.Wołoska 137, 02-507 Warszawa
email: komisja.etyki@cskmswia.pl tel. +48-22-5081681 fax. +48-22-5081881
Przewodniczący Komisji: prof. dr hab.n.med. Robert J. Gil

**DECYZJA NR 37/2020**
z dn. 03.04.2020r.

Komisja Etyki i Nadzoru nad Badaniami na Ludziach i Zwierzętach przy CSK MSWiA w Warszawie na posiedzeniu w dniu **03 kwietnia 2020 r.**

**- zapoznała się z projektem nowego badania pt.**

***„Zaburzenia węchu w COVID-19(zakażenie wirusem SARS CoV-2)"***

którego badaczem jest lek. Laura Ziuzia- Januszewska Kliniki Otolaryngologiczna Centralnego Szpitala Klinicznego MSWiA w Warszawie.

**dotyczących: - wniosek o wyrażenie opinii o projekcie eksperymentu medycznego przez Komisję Bioetyczną CSK MSWiA w Warszawie.**

Po zapoznaniu się z całością dokumentacji* zgodnie z zasadami ICH-GCP Komisji Bioetycznej CSK MSWiA w Warszawie

- wyraziła zgodę na przeprowadzenie badania zgodnie z przedstawionym protokołem

Warszawa, 03.04.2020 r.

Przewodniczący Komisji Etyki

Prof. dr hab. n. med. Robert Gil

**Komisja Etyki i Nadzoru nad Badaniami na Ludziach i Zwierzętach**
**przy Centralnym Szpitalu Klinicznym MSWiA w Warszawie**
ul.Wołoska 137, 02-507 Warszawa
email: komisja.etyki@cskmswia.pl tel. +48-22-5081681 fax. +48-22-5081881
Przewodniczący Komisji: prof. dr hab.n.med. Robert J. Gil

***Do Komisji Etycznej wpłynęły następujące dokumenty:**

1. Wniosek
2. Protokół
3. Zgoda Dyrektora na przeprowadzenie badania

*Załącznik nr 1 do Decyzji nr 37/2020 z dn. 03.04.2020r.*

# SKŁAD KOMISJI ETYCZNEJ PODEJMUJĄCEJ DECYZJĘ

| | | |
|---|---|---|
| **Prof. dr hab. n. med. Robert Gil** - Przewodniczący | Kardiolog | CSK MSWiA Klinika Kardiologii Inwazyjnej |
| **Ks. Dariusz Cempura** - z-ca przewodniczącego | Ksiądz | Parafia św. Jana Chrzciciela w Cegłowie |
| **Prof. dr hab. n. med. Edward Franek** | Endokrynolog | CSK MSWiA Klinika Chorób Wewnętrznych Endokrynologii i Diabetologii |
| **Prof. dr hab. n. med. Michał Powolny** | Ginekolog-położnik | CSK MSWiA Klinika Ginekologii i Położnictwa |
| **Prof. dr hab. n.med. Piotr Andziak** | Chirurg | CSK MSWiA Klinika Chirurgii Ogólnej i Naczyniowej |
| **Prof. dr hab. n. med. Maria Barcikowska** | Neurolog | CSK MSWiA Klinika Neurologii |

| | | |
|---|---|---|
| **Prof. dr hab. n. med. Andrzej Rydzewski** | Nefrolog | CSK MSWiA<br>Klinika Chorób Wewnętrznych, Nefrologii i Transplantologii |
| **Prof. dr hab. n.med. Grażyna Rydzewska - Wyszkowska** | Specjalista Chorób Wewnętrznych | CSK MSWiA<br>Klinika Chorób Wewnętrznych I Gastroenterologii |
| **Mgr farm. Irena Szoszkiewicz** | Farmaceuta | CSK MSWiA<br>Apteka Szpitalna |
| **Mec. Jarosław Kocznur** | Prawnik | |
| **Dr n. med. Jolanta Kołakowska** | Kardiolog | CSK MSWiA<br>Oddział Rehabilitacji Kardiologicznej |
| **Dr hab. n. o zdr. Adam Fronczak** | Farmakolog kliniczny | |
| **Bożenna Majewska** | Pielęgniarka | |

**2. Badanie wpływu wariantu SARS-CoV-2 (wariantu alfa w porównaniu do wariantów wcześniej występujących) na ciężkość przebiegu COVID-19 oraz identyfikacji demograficznych, klinicznych i laboratoryjnych predyktorów ciężkiego przebiegu choroby w populacji hospitalizowanych młodych dorosłych.**

**Komisja Etyki i Nadzoru nad Badaniami na Ludziach i Zwierzętach przy Centralnym Szpitalu Klinicznym MSWiA w Warszawie**
ul.Wołoska 137, 02-507 Warszawa
email: komisja.etyki@cskmswia.pl tel. +48-47-7221681
Przewodniczący Komisji: prof. dr hab.n.med. Robert J. Gil

## DECYZJA NR 110/2021

z dn. 24.08.2021r.

Komisja Etyki i Nadzoru nad Badaniami na Ludziach i Zwierzętach przy CSK MSWiA w Warszawie na posiedzeniu w dniu **24.08.2021r.**

**- zapoznała się z projektem nowego badania klinicznego:**

**Tytuł badania:** „Przebieg COVID-19 u młodych dorosłych podczas drugiej i trzeciej fali pandemii COVID-19 w Polsce".

**Ośrodek badawczy:** Klinika Otolaryngologii CSK MSWiA

**Główny badacz:** lek. Laura Ziuzia-Januszewska

**Badanie własne.**

**dotyczący: - wniosek o wyrażenie zgody na przeprowadzenie badania.**

Po zapoznaniu się z całością dokumentacji* zgodnie z zasadami ICH-GCP Komisja Etyki

- wyraziła zgodę na przeprowadzenie badania.

W ramach niniejszego zezwolenia badania mogą być prowadzone w trakcie ważnej polisy ubezpieczeniowej

Warszawa, 01.09.2021 r.

Przewodniczący Komisji Etyki

PRZEWODNICZĄCY Komisji Etyki i Nadzoru nad Badaniami na Ludziach i Zwierzętach przy Centralnym Szpitalu Klinicznym MSWiA prof. dr hab. n. med. Robert Gil

Prof. dr hab. n. med. Robert Gil

**Komisja Etyki i Nadzoru nad Badaniami na Ludziach i Zwierzętach**
**przy Centralnym Szpitalu Klinicznym MSWiA w Warszawie**
ul.Wołoska 137, 02-507 Warszawa
email: komisja.etyki@cskmswia.pl tel. +48-47-7221681
Przewodniczący Komisji: prof. dr hab.n.med. Robert J. Gil

***Do Komisji Etycznej wpłynęły następujące dokumenty:**

1. Wniosek do dyrektora CSK MSWiA o wyrażenie zgody na przeprowadzenie badania własnego – zgoda.
2. Wniosek Komisji Bioetycznej przy CSK MSWiA w Warszawie o wyrażenie opinii dotyczącej przeprowadzenie badania własnego .
3. Protokół badania.

Zał. 1

**Skład Komisji Etyki i Nadzoru nad Badaniami na Ludziach i Zwierzętach**

Przewodniczący:

prof. dr hab. n. med. Robert Gil

Z-ca przewodniczącego:

ks. Dariusz Cempura

Członkowie:

prof. dr hab. n. med. Edward Franek
prof. dr hab. n. med. Michał Powolny
prof. dr hab. n. med. Piotr Andziak
dr n. med. Małgorzata Dorobek
prof. dr hab. n. med. Andrzej Rydzewski
prof. dr hab. n. med. Grażyna Rydzewska- Wyszkowska
dr n. med. Jolanta Kołakowska
dr hab. n. o zdr. Adam Fronczak
dr n. med. Zbigniew Król
dr hab. n. med. Adam Sybilski
mgr farm. Irena Szoszkiewicz
mec. Jarosław Kocznur
piel. lic. Bożenna Majewska

Komisja Etyki i Nadzoru nad Badaniami na Ludziach i Zwierzętach przy Centralnym Szpitalu Klinicznym MSWiA w Warszawie 02-507 Warszawa, ul. Wołoska 137

Zał. 2

**Uprzejmie informuję, że dnia 24.08.2021r. następujące osoby wzięły udział w posiedzeniu Komisji Etyki i Nadzoru nad Badaniami na Ludziach i Zwierzętach przy CSK MSWiA w Warszawie:**

1. Prof. dr hab. n. med. Robert GIL .....
2. Prof. dr hab. n med. Piotr ANDZIAK .....
3. Ks. Dariusz CEMPURA .....
4. Prof. dr hab. n med. Edward FRANEK .....
5. Prof. dr hab. n med. Michał POWOLNY .....
6. Prof. dr hab. n med. Grażyna RYDZEWSKA-WYSZKOWSKA .....
7. Prof. dr hab. n med. Andrzej RYDZEWSKI .....
8. Dr. n. med. Małgorzata DOROBEK .....
9. Dr. n. med. Jolanta KOŁAKOWSKA .....
10. Dr. hab. n. o zdr. Adam FRONCZAK .....
11. Dr. n med. Zbigniew KRÓL .....
12. Dr. hab. n. med. Adam SYBILSKI .....
13. Mec. Jarosław KOCZNUR .....
14. Mgr. Farmacji Irena SZOSZKIEWICZ .....
15. Piel. Bożenna MAJEWSKA ..... 11.05.

PRZEWODNICZĄCY Komisji Etyki i Nadzoru nad Badaniami na Ludziach i Zwierzętach przy Centralnym Szpitalu Klinicznym MSWiA w Warszawie prof. dr hab. n. med. Robert Gil

## 3. Badanie predyktorów ciężkiego przebiegu COVID-19 w grupie kobiet ciężarnych.

Badanie przeprowadzone zostało w ramach większego projektu oceniającego różne aspekty zakażenia SARS-COV-2 u kobiet ciężarnych, przy czym w protokole badania uwzględniono również określenie potencjalnych predyktorów ciężkiego przebiegu COVID-19 u kobiet ciężarnych.

**Komisja Etyki i Nadzoru nad Badaniami na Ludziach i Zwierzętach**
**przy Centralnym Szpitalu Klinicznym MSWiA w Warszawie**
ul.Wołoska 137, 02-507 Warszawa
email: komisja.etyki@cskmswia.pl tel. +48-47-7221681
Przewodniczący Komisji: prof. dr hab.n.med. Robert J. Gil

**DECYZJA NR 104/2021**
z dn. 24.08.2021r.

Komisja Etyki i Nadzoru nad Badaniami na Ludziach i Zwierzętach przy CSK MSWiA w Warszawie na posiedzeniu w dniu **24.08.2021r.**

**- zapoznała się z projektem nowego badania klinicznego:**

**Tytuł badania:** „Porównanie przebiegu hospitalizacji kobiet ciężarnych z COVID-19 do kobiet nie ciężarnych i przebiegu porodu kobiet SARS CoV-2 dodatnich i ujemnych".

**Ośrodek badawczy:** Klinika Położnictwa, Chorób Kobiecych i Ginekologii Onkologicznej CSK MSWiA

**Główny badacz:** prof. dr hab. n. med. Artur Jakimiuk

**Badanie własne.**

**dotyczący: - wniosek o wyrażenie zgody na przeprowadzenie badania.**

Po zapoznaniu się z całością dokumentacji* zgodnie z zasadami ICH-GCP Komisja Etyki

- wyraziła zgodę na przeprowadzenie badania.

W ramach niniejszego zezwolenia badania mogą być prowadzone w trakcie ważnej polisy ubezpieczeniowej

Warszawa, 01.09.2021 r.

PRZEWODNICZĄCY
Komisji Etyki i Nadzoru nad Badaniami
na Ludziach i Zwierzętach
przy Centralnym Szpitalu Klinicznym MSWiA
Warszawa, ul. Wołoska 137
prof. dr hab. n. med. Robert Gil

Przewodniczący Komisji Etyki

Prof. dr hab. n. med. Robert Gil

**Komisja Etyki i Nadzoru nad Badaniami na Ludziach i Zwierzętach**
**przy Centralnym Szpitalu Klinicznym MSWiA w Warszawie**
ul.Wołoska 137, 02-507 Warszawa
email: komisja.etyki@cskmswia.pl tel. +48-47-7221681
Przewodniczący Komisji: prof. dr hab.n.med. Robert J. Gil

***Do Komisji Etycznej wpłynęły następujące dokumenty:**

1. Wniosek do dyrektora CSK MSWiA o wyrażenie zgody na przeprowadzenie badania własnego – zgoda.
2. Wniosek Komisji Bioetycznej przy CSK MSWiA w Warszawie o wyrażenie opinii dotyczącej przeprowadzenie badania własnego .
3. Protokół badania.

Komisja Etyki i Nadzoru nad Badaniami
na Ludziach i Zwierzętach
przy Centralnym Szpitalu Klinicznym MSWiA
w Warszawie
02-507 Warszawa, ul. Wołoska 137

Zał. 1

**Skład Komisji Etyki i Nadzoru nad Badaniami na Ludziach i Zwierzętach**

Przewodniczący:

prof. dr hab. n. med. Robert Gil

Z-ca przewodniczącego:

ks. Dariusz Cempura

Członkowie:

prof. dr hab. n. med. Edward Franek
prof. dr hab. n. med. Michał Powolny
prof. dr hab. n. med. Piotr Andziak
dr n. med. Małgorzata Dorobek
prof. dr hab. n. med. Andrzej Rydzewski
prof. dr hab. n. med. Grażyna Rydzewska- Wyszkowska
dr n. med. Jolanta Kołakowska
dr hab. n. o zdr. Adam Fronczak
dr n. med. Zbigniew Król
dr hab. n. med. Adam Sybilski
mgr farm. Irena Szoszkiewicz
mec. Jarosław Kocznur
piel. lic. Bożenna Majewska

PRZEWODNICZĄCY
Komisji Etyki i Nadzoru nad Badaniami
na Ludziach i Zwierzętach
przy Centralnym Szpitalu Klinicznym MSWiA
Warszawa, ul. Wołoska 137
prof. dr hab. n. med. Robert Gil

Zał. 2

**Uprzejmie informuję, że dnia 24.08.2021r. następujące osoby wzięły udział w posiedzeniu Komisji Etyki i Nadzoru nad Badaniami na Ludziach i Zwierzętach przy CSK MSWiA w Warszawie:**

1. Prof. dr hab. n. med. Robert GIL
2. Prof. dr hab. n med. Piotr ANDZIAK
3. Ks. Dariusz CEMPURA
4. Prof. dr hab. n med. Edward FRANEK
5. Prof. dr hab. n med. Michał POWOLNY
6. Prof. dr hab. n med. Grażyna RYDZEWSKA-WYSZKOWSKA
7. Prof. dr hab. n med. Andrzej RYDZEWSKI
8. Dr. n. med. Małgorzata DOROBEK
9. Dr. n. med. Jolanta KOŁAKOWSKA
10. Dr. hab. n. o zdr. Adam FRONCZAK
11. Dr. n med. Zbigniew KRÓL
12. Dr. hab. n. med. Adam SYBILSKI
13. Mec. Jarosław KOCZNUR
14. Mgr. Farmacji Irena SZOSZKIEWICZ
15. Piel. Bożenna MAJEWSKA

11 05.

## IX. OŚWIADCZENIA WSPÓŁAUTORÓW PUBLIKACJI OKREŚLAJĄCE INDYWIDUALNY WKŁAD KAŻDEGO Z NICH W ICH POWSTANIE

Ad. Publikacja 1.: Ziuzia-Januszewska L, Dobrzyński P, Ślączka K, Ciszek J, Krawiec Ł, Wierzba W, Zaczyński A. Simple Disposable Odor Identification Tests for Predicting SARS-CoV-2 Positivity. Int J Environ Res Public Health. 2021;18(19):10185. doi: 10.3390/ijerph181910185.

Warszawa, 24.08.2022 r.

lek. Laura Ziuzia-Januszewska

**OŚWIADCZENIE**

Jako współautor pracy pt. „Simple Disposable Odor Identification Tests for Predicting SARS-CoV-2 Positivity" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

opracowanie koncepcji i zaprojektowanie badania, rekrutację pacjentów do badania, przeprowadzanie testów węchowych oraz badań ankietowych, stworzenie bazy danych, analizę bazy danych, interpretację wyników, stworzenie treści artykułu oraz akceptację ostatecznej wersji artykułu.

Mój udział procentowy w przygotowaniu publikacji określam jako 86 %.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część mojej rozprawy doktorskiej.

Laura Ziuzia-Januszewska

(podpis oświadczającego)

Warszawa, 24.08.2022 r.

Dr n. med. Paweł Dobrzyński

## OŚWIADCZENIE

Jako współautor pracy pt. „Simple Disposable Odor Identification Tests for Predicting SARS-CoV-2 Positivity" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

nadzór merytoryczny oraz akceptacja ostatecznej wersji artykułu.

Mój udział procentowy w przygotowaniu publikacji określam jako 3 %.

Wkład lek. Laury Ziuzia-Januszewskiej w powstawanie publikacji określam jako 86 %,

obejmował on: opracowanie koncepcji i zaprojektowanie badania, rekrutację pacjentów do badania, przeprowadzanie testów węchowych oraz badań ankietowych, stworzenie bazy danych, analizę bazy danych, interpretację wyników, stworzenie treści artykułu oraz akceptację ostatecznej wersji artykułu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Laury Ziuzia-Januszewskiej

.....

(podpis oświadczającego)

Warszawa, 24.08.2022 r.

lek. Krzysztof Ślączka

## OŚWIADCZENIE

Jako współautor pracy pt. „Simple Disposable Odor Identification Tests for Predicting SARS-CoV-2 Positivity" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

przeprowadzanie testów węchowych oraz badań ankietowych.

Mój udział procentowy w przygotowaniu publikacji określam jako 3 %.

Wkład lek. Laury Ziuzia-Januszewskiej w powstawanie publikacji określam jako 86 %,

obejmował on: opracowanie koncepcji i zaprojektowanie badania, rekrutację pacjentów do badania, przeprowadzanie testów węchowych oraz badań ankietowych, stworzenie bazy danych, analizę bazy danych, interpretację wyników, stworzenie treści artykułu oraz akceptację ostatecznej wersji artykułu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Laury Ziuzia-Januszewskiej

.....

(podpis oświadczającego)

Warszawa, 24.08.2022 r.

lek. Jaromir Ciszek

## OŚWIADCZENIE

Jako współautor pracy pt. „Simple Disposable Odor Identification Tests for Predicting SARS-CoV-2 Positivity" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

przeprowadzanie testów węchowych oraz badań ankietowych.

Mój udział procentowy w przygotowaniu publikacji określam jako 2 %.

Wkład lek. Laury Ziuzia-Januszewskiej w powstawanie publikacji określam jako 86 %,

obejmował on: opracowanie koncepcji i zaprojektowanie badania, rekrutację pacjentów do badania, przeprowadzanie testów węchowych oraz badań ankietowych, stworzenie bazy danych, analizę bazy danych, interpretację wyników, stworzenie treści artykułu oraz akceptację ostatecznej wersji artykułu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Laury Ziuzia-Januszewskiej

Jaromir Ciszek

.....

(podpis oświadczającego)

Warszawa, 24.08.2022 r.

lek. Łukasz Krawiec

**OŚWIADCZENIE**

Jako współautor pracy pt. „Simple Disposable Odor Identification Tests for Predicting SARS-CoV-2 Positivity" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
przeprowadzanie testów węchowych oraz badań ankietowych.
Mój udział procentowy w przygotowaniu publikacji określam jako 2 %.

Wkład lek. Laury Ziuzia-Januszewskiej w powstawanie publikacji określam jako 86 %,

obejmował on: opracowanie koncepcji i zaprojektowanie badania, rekrutację pacjentów do badania, przeprowadzanie testów węchowych oraz badań ankietowych, stworzenie bazy danych, analizę bazy danych, interpretację wyników, stworzenie treści artykułu oraz akceptację ostatecznej wersji artykułu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Laury Ziuzia-Januszewskiej

Łukasz Krawiec

(podpis oświadczającego)

Warszawa, 24.08.2022 r.

Prof. dr hab. n. med. Waldemar Wierzba

## OŚWIADCZENIE

Jako współautor pracy pt. „Simple Disposable Odor Identification Tests for Predicting SARS-CoV-2 Positivity" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
nadzór merytoryczny oraz akceptacja ostatecznej wersji artykułu.
Mój udział procentowy w przygotowaniu publikacji określam jako 2 %.

Wkład lek. Laury Ziuzia-Januszewskiej w powstawanie publikacji określam jako 86 %,

obejmował on: opracowanie koncepcji i zaprojektowanie badania, rekrutację pacjentów do badania, przeprowadzanie testów węchowych oraz badań ankietowych, stworzenie bazy danych, analizę bazy danych, interpretację wyników, stworzenie treści artykułu oraz akceptację ostatecznej wersji artykułu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Laury Ziuzia-Januszewskiej

.....

(podpis oświadczającego)

Warszawa, 24.08.2022 r.

Dr n. med. Artur Zaczyński

## OŚWIADCZENIE

Jako współautor pracy pt. „Simple Disposable Odor Identification Tests for Predicting SARS-CoV-2 Positivity" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
nadzór merytoryczny oraz akceptacja ostatecznej wersji artykułu.
Mój udział procentowy w przygotowaniu publikacji określam jako 2 %.

Wkład lek. Laury Ziuzia-Januszewskiej w powstawanie publikacji określam jako 86 %,

obejmował on: opracowanie koncepcji i zaprojektowanie badania, rekrutację pacjentów do badania, przeprowadzanie testów węchowych oraz badań ankietowych, stworzenie bazy danych, analizę bazy danych, interpretację wyników, stworzenie treści artykułu oraz akceptację ostatecznej wersji artykułu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Laury Ziuzia-Januszewskiej

ZASTĘPCA DYREKTORA
ds. MEDYCZNYCH
Centralnego Szpitala Klinicznego MSWiA
w Warszawie

dr n. med. Artur Zaczyński
(podpis oświadczającego)

Ad. Publikacja 2: Ziuzia-Januszewska L, Januszewski M. Pathogenesis of Olfactory Disorders in COVID-19. Brain Sci. 2022;12(4):449. doi: 10.3390/brainsci12040449.

Warszawa, 24.08.2022 r.

lek. Laura Ziuzia-Januszewska

**OŚWIADCZENIE**

Jako współautor pracy pt. „Pathogenesis of Olfactory Disorders in COVID-19" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
opracowanie koncepcji pracy, zebranie i interpretację piśmiennictwa, stworzenie treści artykułu oraz akceptacja ostatecznej wersji artykułu.
Mój udział procentowy w przygotowaniu publikacji określam jako 92 %.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część mojej rozprawy doktorskiej

Laura Ziuzia-Januszewska
.....
(podpis oświadczającego)

Warszawa, 24.08.2022 r.

dr n. med. Marcin Januszewski

**OŚWIADCZENIE**

Jako współautor pracy pt. „Pathogenesis of Olfactory Disorders in COVID-19" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
udział w interpretacji piśmiennictwa oraz akceptacja ostatecznej wersji artykułu.
Mój udział procentowy w przygotowaniu publikacji określam jako 8 %.

Wkład lek. Laury Ziuzia-Januszewskiej w powstawanie publikacji określam jako 92 %,

obejmował on: opracowanie koncepcji pracy, zebranie i interpretację piśmiennictwa, stworzenie treści artykułu oraz akceptację ostatecznej wersji artykułu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Laury Ziuzia-Januszewskiej

.....
(podpis oświadczającego)

Ad. Publikacja 3: Ziuzia-Januszewska L, Januszewski M, Sosnowska-Nowak J, Janiszewski M, Dobrzyński P, Jakimiuk AA, Jakimiuk AJ. COVID-19 severity and mortality in two pandemic waves in Poland and predictors of poor outcomes of SARS-CoV-2 infection in hospitalized young adults. Viruses. 2022;14(8), 1700; https://doi.org/10.3390/v14081700

Warszawa, 24.08.2022 r.

lek. Laura Ziuzia-Januszewska

**OŚWIADCZENIE**

Jako współautor pracy pt. „COVID-19 severity and mortality in two pandemic waves in Poland and predictors of poor outcomes of SARS-CoV-2 infection in hospitalized young adults" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
opracowanie koncepcji i zaprojektowanie badania, rekrutacja pacjentów do badania, tworzenie bazy danych, analiza bazy danych, interpretacja wyników, stworzenie treści artykułu oraz akceptacja ostatecznej wersji artykułu.

Mój udział procentowy w przygotowaniu publikacji określam jako 85 %.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część mojej rozprawy doktorskiej.

Laura Ziuzia-Januszewska

(podpis oświadczającego)

Warszawa, 24.08.2022 r.

dr n. med. Marcin Januszewski

**OŚWIADCZENIE**

Jako współautor pracy pt. „COVID-19 severity and mortality in two pandemic waves in Poland and predictors of poor outcomes of SARS-CoV-2 infection in hospitalized young adults" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
rekrutacja pacjentów do badania, tworzenie bazy danych oraz analiza bazy danych.

Mój udział procentowy w przygotowaniu publikacji określam jako 4 %.

Wkład lek. Laury Ziuzia-Januszewskiej w powstawanie publikacji określam jako 85 %,

obejmował on: opracowanie koncepcji i zaprojektowanie badania, rekrutację pacjentów do badania, tworzenie bazy danych, analizę bazy danych, interpretację wyników, stworzenie treści artykułu oraz akceptację ostatecznej wersji artykułu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Laury Ziuzia-Januszewskiej.

.....
(podpis oświadczającego)

Warszawa, 24.08.2022 r.

lek. Joanna Sosnowska-Nowak

**OŚWIADCZENIE**

Jako współautor pracy pt. „COVID-19 severity and mortality in two pandemic waves in Poland and predictors of poor outcomes of SARS-CoV-2 infection in hospitalized young adults" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
rekrutacja pacjentów do badania oraz tworzenie bazy danych.

Mój udział procentowy w przygotowaniu publikacji określam jako 3 %.

Wkład lek. Laury Ziuzia-Januszewskiej w powstawanie publikacji określam jako 85 %,

obejmował on: opracowanie koncepcji i zaprojektowanie badania, rekrutację pacjentów do badania, tworzenie bazy danych, analizę bazy danych, interpretację wyników, stworzenie treści artykułu oraz akceptację ostatecznej wersji artykułu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Laury Ziuzia-Januszewskiej.

.....
(podpis oświadczającego)

Warszawa, 24.08.2022 r.

lek. Mariusz Janiszewski

## OŚWIADCZENIE

Jako współautor pracy pt. „COVID-19 severity and mortality in two pandemic waves in Poland and predictors of poor outcomes of SARS-CoV-2 infection in hospitalized young adults" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
rekrutacja pacjentów do badania.

Mój udział procentowy w przygotowaniu publikacji określam jako 2 %.

Wkład lek. Laury Ziuzia-Januszewskiej w powstawanie publikacji określam jako 85 %,

obejmował on: opracowanie koncepcji i zaprojektowanie badania, rekrutację pacjentów do badania, tworzenie bazy danych, analizę bazy danych, interpretację wyników, stworzenie treści artykułu oraz akceptację ostatecznej wersji artykułu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Laury Ziuzia-Januszewskiej.

.....

(podpis oświadczającego)

Warszawa, 24.08.2022 r.

dr n. med. Paweł Dobrzyński

# OŚWIADCZENIE

Jako współautor pracy pt. „COVID-19 severity and mortality in two pandemic waves in Poland and predictors of poor outcomes of SARS-CoV-2 infection in hospitalized young adults" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
nadzór merytoryczny oraz akceptacja ostatecznej wersji artykułu.

Mój udział procentowy w przygotowaniu publikacji określam jako 2 %.

Wkład lek. Laury Ziuzia-Januszewskiej w powstawanie publikacji określam jako 85 %,

obejmował on: opracowanie koncepcji i zaprojektowanie badania, rekrutację pacjentów do badania, tworzenie bazy danych, analizę bazy danych, interpretację wyników, stworzenie treści artykułu oraz akceptację ostatecznej wersji artykułu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Laury Ziuzia-Januszewskiej.

.....

(podpis oświadczającego)

Warszawa, 24.08.2022 r.

lek. Alicja Jakimiuk

**OŚWIADCZENIE**

Jako współautor pracy pt. „COVID-19 severity and mortality in two pandemic waves in Poland and predictors of poor outcomes of SARS-CoV-2 infection in hospitalized young adults" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
rekrutacja pacjentów do badania.

Mój udział procentowy w przygotowaniu publikacji określam jako 2 %.

Wkład lek. Laury Ziuzia-Januszewskiej w powstawanie publikacji określam jako 85 %,

obejmował on: opracowanie koncepcji i zaprojektowanie badania, rekrutację pacjentów do badania, tworzenie bazy danych, analizę bazy danych, interpretację wyników, stworzenie treści artykułu oraz akceptację ostatecznej wersji artykułu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Laury Ziuzia-Januszewskiej.

Alicja Jakimiuk
.....
(podpis oświadczającego)

Warszawa, 24.08.2022 r.

Prof. dr hab. n. med. Artur Jakimiuk

# OŚWIADCZENIE

Jako współautor pracy pt. „COVID-19 severity and mortality in two pandemic waves in Poland and predictors of poor outcomes of SARS-CoV-2 infection in hospitalized young adults" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
nadzór merytoryczny oraz akceptacja ostatecznej wersji artykułu.

Mój udział procentowy w przygotowaniu publikacji określam jako 2 %.

Wkład lek. Laury Ziuzia-Januszewskiej w powstawanie publikacji określam jako 85 %,

obejmował on: opracowanie koncepcji i zaprojektowanie badania, rekrutację pacjentów do badania, tworzenie bazy danych, analizę bazy danych, interpretację wyników, stworzenie treści artykułu oraz akceptację ostatecznej wersji artykułu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Laury Ziuzia-Januszewskiej.

.....

(podpis oświadczającego)

Ad. Publikacja 4: Januszewski M, Ziuzia-Januszewska L, Jakimiuk AA, Oleksik T, Pokulniewicz M, Wierzba W, Kozlowski K, Jakimiuk AJ. Predictors of COVID-19 severity among pregnant patients. Bosn J Basic Med Sci. 2022. doi: 10.17305/bjbms.2022.7181

Warszawa, 24.08.2022 r.

lek. Laura Ziuzia-Januszewska

**OŚWIADCZENIE**

Jako współautor pracy pt. „Predictors of COVID-19 severity among pregnant patients" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
tworzenie bazy danych, analiza bazy danych, interpretacja wyników, tworzenie treści artykułu oraz akceptacja ostatecznej wersji artykułu.

Mój udział procentowy w przygotowaniu publikacji określam jako 35 %.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część mojej rozprawy doktorskiej.

Laura Ziuzia-Januszewska
.....
(podpis oświadczającego)

Warszawa, 24.08.2022 r.

dr n. med. Marcin Januszewski

**OŚWIADCZENIE**

Jako współautor pracy pt. „Predictors of COVID-19 severity among pregnant patients" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
opracowanie koncepcji i zaprojektowanie badania, rekrutacja pacjentek do badania, tworzenie bazy danych, analiza bazy danych, interpretacja wyników, stworzenie treści artykułu oraz akceptacja ostatecznej wersji artykułu.

Mój udział procentowy w przygotowaniu publikacji określam jako 45 %.

Wkład lek. Laury Ziuzia-Januszewskiej w powstawanie publikacji określam jako 35 %,

obejmował on: tworzenie bazy danych, analizę bazy danych, interpretację wyników, tworzenie treści artykułu oraz akceptację ostatecznej wersji artykułu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Laury Ziuzia-Januszewskiej.

.....
(podpis oświadczającego)

Warszawa, 24.08.2022 r.

lek. Alicja Jakimiuk

## OŚWIADCZENIE

Jako współautor pracy pt. „Predictors of COVID-19 severity among pregnant patients" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
tworzenie bazy danych.

Mój udział procentowy w przygotowaniu publikacji określam jako 3 %.

Wkład lek. Laury Ziuzia-Januszewskiej w powstawanie publikacji określam jako 35 %,

obejmował on: tworzenie bazy danych, analizę bazy danych, interpretację wyników, tworzenie treści artykułu oraz akceptację ostatecznej wersji artykułu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Laury Ziuzia-Januszewskiej.

Alicja Jakimiuk

.....

(podpis oświadczającego)

Warszawa, 24.08.2022 r.

lek. Tomasz Oleksik

## OŚWIADCZENIE

Jako współautor pracy pt. „Predictors of COVID-19 severity among pregnant patients" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
rekrutacja pacjentek do badania.

Mój udział procentowy w przygotowaniu publikacji określam jako 3 %.

Wkład lek. Laury Ziuzia-Januszewskiej w powstawanie publikacji określam jako 35 %,

obejmował on: tworzenie bazy danych, analizę bazy danych, interpretację wyników, tworzenie treści artykułu oraz akceptację ostatecznej wersji artykułu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Laury Ziuzia-Januszewskiej.

Tomasz Oleksik
LEKARZ
Specjalista położnictwa i ginekologii

.....

(podpis oświadczającego)

Warszawa, 24.08.2022 r.

lek. Marek Pokulniewicz

# OŚWIADCZENIE

Jako współautor pracy pt. „Predictors of COVID-19 severity among pregnant patients" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
rekrutacja pacjentek do badania.

Mój udział procentowy w przygotowaniu publikacji określam jako 3 %.

Wkład lek. Laury Ziuzia-Januszewskiej w powstawanie publikacji określam jako 35 %,

obejmował on: tworzenie bazy danych, analizę bazy danych, interpretację wyników, tworzenie treści artykułu oraz akceptację ostatecznej wersji artykułu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Laury Ziuzia-Januszewskiej.

Marek Pokulniewicz
lekarz
specjalista położnictwa i ginekologii
2609471

.....
(podpis oświadczającego)

Warszawa, 24.08.2022 r.

Prof. dr hab. n. med. Waldemar Wierzba

## OŚWIADCZENIE

Jako współautor pracy pt. „Predictors of COVID-19 severity among pregnant patients" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
nadzór merytoryczny oraz akceptacja ostatecznej wersji artykułu.

Mój udział procentowy w przygotowaniu publikacji określam jako 3 %.

Wkład lek. Laury Ziuzia-Januszewskiej w powstawanie publikacji określam jako 35 %,

obejmował on: tworzenie bazy danych, analizę bazy danych, interpretację wyników, tworzenie treści artykułu oraz akceptację ostatecznej wersji artykułu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Laury Ziuzia-Januszewskiej.

.....

(podpis oświadczającego)

Warszawa, 24.08.2022 r.

Krzysztof Kozłowski

**OŚWIADCZENIE**

Jako współautor pracy pt. „Predictors of COVID-19 severity among pregnant patients" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
nadzór merytoryczny oraz akceptacja ostatecznej wersji artykułu.

Mój udział procentowy w przygotowaniu publikacji określam jako 3 %.

Wkład lek. Laury Ziuzia-Januszewskiej w powstawanie publikacji określam jako 35 %,

obejmował on: tworzenie bazy danych, analizę bazy danych, interpretację wyników, tworzenie treści artykułu oraz akceptację ostatecznej wersji artykułu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Laury Ziuzia-Januszewskiej.

Krzysztof Kozłowski

(podpis oświadczającego)

Warszawa, 24.08.2022 r.

Prof dr hab. n. med. Artur Jakimiuk

## OŚWIADCZENIE

Jako współautor pracy pt. „Predictors of COVID-19 severity among pregnant patients" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
nadzór merytoryczny oraz akceptacja ostatecznej wersji artykułu.

Mój udział procentowy w przygotowaniu publikacji określam jako 5 %.

Wkład lek. Laury Ziuzia-Januszewskiej w powstawanie publikacji określam jako 35 %,

obejmował on: tworzenie bazy danych, analizę bazy danych, interpretację wyników, tworzenie treści artykułu oraz akceptację ostatecznej wersji artykułu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Laury Ziuzia-Januszewskiej.

.....

(podpis oświadczającego)