Intensive care for COVID-19. Studies in critically ill patients

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Emma Kooistra

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CHAPTER 1

1

General introduction and outline of this thesis

Coronavirus Disease 2019

When Coronavirus Disease 2019 (COVID-19) emerged in December 2019 in Wuhan, China, few could predict the global impact that it would have. COVID-19 is caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (1, 2), a new variant of the Coronaviridae family that normally causes common cold. SARS-CoV-2 is related to both SARS-CoV-1 which led to the SARS outbreak in 2003 (3) and the Middle East respiratory syndrome-related coronavirus (MERS-CoV) identified in 2012 (4). Symptoms of COVID-19 range from asymptomatic or a mild cold to severe Acute Respiratory Distress Syndrome (ARDS) requiring mechanical ventilation in the Intensive Care Unit (ICU) and high mortality rates (5, 6). During the first months of 2020, the highly contagious SARS-CoV-2 rapidly spread worldwide and the World Health Organization (WHO) declared a pandemic on March 11th, 2020 (7). High infection rates and large numbers of COVID-19 patients in hospital wards and ICUs worldwide resulted in a vast global burden on healthcare. In absence of curative treatments, care for COVID-19 patients initially was only supportive. Therefore, research into the risk factors and pathophysiology of this new disease, as well as options for prevention and effective treatment was a global priority.

Obesity in critically ill COVID-19 patients

Shortly following the emergence of COVID-19, multiple risk factors for infection with SARS-CoV-2 and a severe disease course were reported, including older age, male sex, and comorbidities such as diabetes mellitus type 2, malignant and cardiovascular diseases (8, 9). Moreover, high body mass index (BMI) was identified as an important risk factor for the development of severe COVID-19 as well as mortality, and it received widespread media attention (10-12).

The exact underlying mechanisms of obesity as a risk factor for severe COVID-19 are unknown, but an effect of BMI on the immune system and/or respiratory mechanics has been suggested. In the general population, obesity is also known as an important risk factor for a more severe disease course of several other infectious and non-infectious diseases such as pneumonia, bacterial sepsis, acute kidney injury, and heart failure. Paradoxically, once admitted to the ICU, obesity is associated with a *lower* mortality in these patient categories (13-17), a phenomenon coined *the obesity paradox*. Prior to the conduct of the studies described in this thesis, it was unknown whether immunological differences are present between obese and non-obese critically ill COVID-19 patients and whether or not BMI is associated with outcome once COVID-19 patients are admitted to the ICU.

Furthermore, it was unknown whether BMI is of influence on long-term COVID-19 symptoms of ICU survivors.

Biomarkers and phenotyping in critically ill COVID-19 patients

Inflammatory biomarkers

Multiple early studies reported elevated inflammatory markers in critically ill COVID-19 patients which were associated with impaired outcome (18, 19). Also, severe COVID-19 was widely typified as a so-called *cytokine storm* (20-22). This phenomenon is characterized by excessive systemic levels of pro-inflammatory cytokines (23) as sometimes observed in patients with severe bacterial sepsis (24, 25) or in patients undergoing chimeric antigen receptor (CAR) T-cell therapy (26). However, there was a paucity of studies that actually compared circulating cytokine levels in severe COVID-19 patients to those observed in patients admitted to the ICU for other severe conditions to demonstrate the presence or absence of a *cytokine storm* in this new disease. Also, the value of circulating inflammatory markers, including cytokines, C-reactive protein (CRP) and procalcitonin (PCT) in predicting clinical outcomes or complications such as bacterial secondary infections in critically ill COVID-19 patients was largely unexplored. Finally, after randomized controlled trials showed beneficial effects of the anti-inflammatory drugs dexamethasone (27) and interleukin(IL)-6 receptor antagonists (tocilizumab, sarilumab, discussed in detail below) (28, 29), all severe COVID-19 patients were routinely treated with these drugs. It was however unknown what the effects of the introduction of these therapies are on the kinetics and clinical utility of inflammatory biomarkers.

Fibrotic biomarkers

A complication of severe COVID-19 is the development of pulmonary fibroproliferation (PF), which is related to poor clinical outcomes and long-term respiratory symptoms (30-33). In non-COVID-19 ARDS patients, high concentrations of the biomarker N-terminal pro-peptide of type III procollagen (PIIINP) in broncho-alveolar lavage (BAL) fluid and plasma are associated with the development of PF (34). Previously published studies showed that treatment with corticosteroids is effective in inhibiting fibroproliferative processes and reduces mortality in patients with ARDS of non-COVID-19 origin (34, 35). In COVID-19 patients in the ICU, the incidence of PF, the clinical usability of plasma concentrations of PIIINP, and the clinical course of PF was however unknown. Furthermore, both the efficacy of corticosteroids in treating PF and the influence of the introduction of routine early treatment with dexamethasone and IL-6-receptor antagonists on the incidence and clinical course of PF in critically ill COVID-19 patients remained to be determined. Gaining insight into

these aspects could improve clinical outcomes and prevent long-term respiratory symptoms following severe COVID-19. Additionally, identification of ribonucleic acid (RNA) profiles of critically ill COVID-19 patients who develop pulmonary fibrosis and who do or do not benefit from corticosteroid therapy may help in improving COVID-19 care and clinical outcomes in the ICU.

Clinical phenotyping in severe COVID-19

Besides the identification of appropriate biomarkers or combinations of different biomarkers associated with complications and clinical outcomes, identification of so-called clinical phenotypes in critically ill COVID-19 patients may be of value as well. In patients with sepsis of non-SARS-CoV-2 origin, a previously published study identified four clinical sepsis phenotypes that correlated with host-response patterns and clinical outcomes (36). At the time of performing the studies described in this thesis, it was unclear whether the previously identified sepsis phenotypes (36) are also applicable on COVID-19 patients and if their proportions and associated clinical outcomes are comparable between non-COVID-19 sepsis patients and COVID-19 sepsis patients.

Immunomodulatory treatment in critically ill COVID-19 patients

Inflammation clearly plays a key role in the development of severe disease in patients with COVID-19. As alluded to before, multiple early studies reported increased levels of inflammatory markers related to worse outcome in patients with severe COVID-19 (18, 19, 37-39). Therefore, the effects of anti-inflammatory therapies in severe COVID-19 patients were studied first. Data of the RECOVERY trial showed beneficial effects of dexamethasone treatment on mortality in COVID-19 patients who required supplemental oxygen (27). As a result, hospitalized patients on oxygen are treated with dexamethasone since then. Later, treatment with the IL-6 receptor antagonists tocilizumab and sarilumab was also introduced for severe COVID-19 after randomized trials showed beneficial effects of these drugs (28, 29). However, other anti-inflammatory agents may be of benefit as well. More than two decades ago, a randomized trial investigating the effects of anakinra, a recombinant anti IL-1 receptor antagonist (RA), in patients with bacterial sepsis was published which demonstrated no clinical benefit of this drug (40). However, in a post-hoc analysis performed 19 years after the original study, it was shown that in a small subgroup of patients displaying low platelet counts and liver dysfunction as hallmarks of the hyperinflammatory macrophage activation syndrome (MAS), anakinra treatment significantly reduced mortality (41). Although caution is warranted in the interpretation of post-hoc analyses, the notion that a subgroup of patients that are likely hyperinflamed may show more pronounced benefit from inhibition of the immune response appears plausible. In view of the suspected

hyperinflammatory immune response in COVID-19 patients, it was thought that treatment with anakinra might also demonstrate beneficial effects in COVID-19 patients, but this hypothesis remained to be explored.

Next to the above described overzealous inflammatory response in severe COVID-19 patients, emerging evidence showed that a small subgroup of COVID-19 patients exhibits impaired viral clearance and a higher risk of developing secondary infections, indicating a suppressed immune response, a phenomenon also known as 'immunoparalysis'. Contrary to the use of anti-inflammatory drugs in COVID-19 patients with signs of hyperinflammation, treatment with immunostimulatory drugs such as interferon gamma might be of benefit in a subgroup of COVID-19 patients with signs of immunoparalysis. The use of such therapies in COVID-19 had not yet been described though.

Outline of this thesis

Part I of this thesis focuses on the effects of obesity on immunological parameters, clinical outcomes and long-term symptoms in critically ill COVID-19 patients. In **chapter 2**, innate immune parameters and clinical outcomes in obese and non-obese COVID-19 patients in the ICU are compared. Because it was unknown whether the obesity paradox is present in COVID-19, **chapter 3** describes associations between BMI categories and clinical outcomes in a nationwide cohort of critically ill COVID-19 patients, as well as in large cohorts of critically ill non-SARS-COV-2 viral pneumonia, bacterial pneumonia and multiple trauma patients. **Chapter 4** presents differences between BMI categories in physical, mental and cognitive symptoms at three and twelve months following ICU admission due to severe COVID-19. **Chapter 5** is a review describing the role of obesity in the development and clinical course of severe COVID-19.

Part II describes the clinical usability of various biomarkers in predicting clinical outcomes and complications in severe COVID-19 patients. Also, the presence of previously described sepsis phenotypes in critically ill COVID-19 patients is explored. In **chapter 6**, levels of circulating cytokines of critically ill COVID-19 patients are compared with those in septic shock patients with and without ARDS, out-of-hospital cardiac arrest patients, and multiple trauma patients in the ICU. The influence of the introduction of immunomodulatory therapy as routine COVID-19 care on both the value of C-reactive protein and procalcitonin to detect secondary infections is presented in **chapter 7**. **Chapter 8** describes the influence of early dexamethasone treatment in severe COVID-19 on the incidence and clinical course of pulmonary fibrosis in critically ill COVID-19 patients and differences in RNA profiles were

explored between critically ill COVID-19 patients who developed pulmonary fibrosis and patients who did not. In **chapter 9**, differences in proportions and associated outcomes of previously described sepsis phenotypes are determined between COVID-19 patients (without and with dexamethasone treatment) and non-COVID-19 sepsis patients.

Part III focuses on immunomodulatory treatments in critically ill COVID-19 patients. **Chapter 10** comprises correspondence concerning a study on the use of anakinra in COVID-19. The effects of anakinra treatment on inflammatory parameters and clinical outcomes are described in **chapter 11**. The effects of interferon-gamma therapy in five critically ill COVID-19 patients with impaired cellular immunity are reported in **chapter 12**. Finally, a summary of this thesis and a general discussion and consideration of future perspectives are provided in **chapter 13**.

References

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020;382(8):727-33.
2. Coronaviridae Study Group of the International Committee on Taxonomy of V. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol. 2020;5(4):536-44.
3. Tsang KW, Ho PL, Ooi GC, Yee WK, Wang T, Chan-Yeung M, et al. A cluster of cases of severe acute respiratory syndrome in Hong Kong. N Engl J Med. 2003;348(20):1977-85.
4. de Groot Raoul J, Baker Susan C, Baric Ralph S, Brown Caroline S, Drosten C, Enjuanes L, et al. Commentary: Middle East Respiratory Syndrome Coronavirus (MERS-CoV): Announcement of the Coronavirus Study Group. Journal of Virology. 2013;87(14):7790-2.
5. 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.
6. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review. Jama. 2020;324(8):782-93.
7. WHO Director-General's opening remarks at the media briefing on COVID19. 2020, March 11th.
8. Fang X, Li S, Yu H, Wang P, Zhang Y, Chen Z, et al. Epidemiological, comorbidity factors with severity and prognosis of COVID-19: a systematic review and meta-analysis. Aging (Albany NY). 2020;12(13):12493-503.
9. Zhou Y, Yang Q, Chi J, Dong B, Lv W, Shen L, et al. Comorbidities and the risk of severe or fatal outcomes associated with coronavirus disease 2019: A systematic review and meta-analysis. Int J Infect Dis. 2020;99:47-56.
10. Földi M, Farkas N, Kiss S, Zádori N, Váncsa S, Szakó L, et al. Obesity is a risk factor for developing critical condition in COVID-19 patients: A systematic review and meta-analysis. Obesity Reviews. 2020;21(10):e13095.
11. Healy M. The coronavirus is particularly unkind to those who are obese. Los Angeles Times. April 5, 2020.
12. Boseley S. Obesity increases risk of Covid-19 death by 48%, study finds. The Guardian. August 26, 2020.
13. Schetz M, De Jong A, Deane AM, Druml W, Hemelaar P, Pelosi P, et al. Obesity in the critically ill: a narrative review. Intensive Care Med. 2019;45(6):757-69.
14. Naderi N, Kleine CE, Park C, Hsiung JT, Soohoo M, Tantisattamo E, et al. Obesity Paradox in Advanced Kidney Disease: From Bedside to the Bench. Prog Cardiovasc Dis. 2018;61(2):168-81.
15. Sharma A, Lavie CJ, Borer JS, Vallakati A, Goel S, Lopez-Jimenez F, et al. Meta-analysis of the relation of body mass index to all-cause and cardiovascular mortality and hospitalization in patients with chronic heart failure. Am J Cardiol. 2015;115(10):1428-34.
16. Nie W, Zhang Y, Jee SH, Jung KJ, Li B, Xiu Q. Obesity survival paradox in pneumonia: a meta-analysis. BMC Med. 2014;12:61.

17. Pepper DJ, Sun J, Welsh J, Cui X, Suffredini AF, Eichacker PQ. Increased body mass index and adjusted mortality in ICU patients with sepsis or septic shock: a systematic review and meta-analysis. Crit Care. 2016;20(1):181.
18. Zeng F, Huang Y, Guo Y, Yin M, Chen X, Xiao L, et al. Association of inflammatory markers with the severity of COVID-19: A meta-analysis. Int J Infect Dis. 2020;96:467-74.
19. Hariyanto TI, Japar KV, Kwenandar F, Damay V, Siregar JI, Lugito NPH, et al. Inflammatory and hematologic markers as predictors of severe outcomes in COVID-19 infection: A systematic review and meta-analysis. Am J Emerg Med. 2021;41:110-9.
20. Zhang C, Wu Z, Li JW, Zhao H, Wang GQ. Cytokine release syndrome in severe COVID-19: interleukin-6 receptor antagonist tocilizumab may be the key to reduce mortality. Int J Antimicrob Agents. 2020;55(5):105954.
21. Zhou Y, Fu B, Zheng X, Wang D, Zhao C, Qi Y, et al. Pathogenic T-cells and inflammatory monocytes incite inflammatory storms in severe COVID-19 patients. Natl Sci Rev. 2020;7(6):998-1002.
22. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395(10229):1033-4.
23. Behrens EM, Koretzky GA. Review: Cytokine Storm Syndrome: Looking Toward the Precision Medicine Era. Arthritis & Rheumatology. 2017;69(6):1135-43.
24. Chousterman BG, Swirski FK, Weber GF. Cytokine storm and sepsis disease pathogenesis. Seminars in Immunopathology. 2017;39(5):517-28.
25. Karakike E, Giamarellos-Bourboulis EJ. Macrophage Activation-Like Syndrome: A Distinct Entity Leading to Early Death in Sepsis. Front Immunol. 2019;10:55.
26. Frey NV, Porter DL. Cytokine release syndrome with novel therapeutics for acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program. 2016;2016(1):567-72.
27. Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, et al. Dexamethasone in Hospitalized Patients with Covid-19. N Engl J Med. 2021;384(8):693-704.
28. Gordon AC, Mouncey PR, Al-Beidh F, Rowan KM, Nichol AD, Arabi YM, et al. Interleukin-6 Receptor Antagonists in Critically Ill Patients with Covid-19. N Engl J Med. 2021;384(16):1491-502.
29. RecoveryCollaborativeGroup. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. Lancet (London, England). 2021;397(10285):1637-45.
30. Martin C, Papazian L, Payan M-J, Saux P, Gouin F. Pulmonary Fibrosis Correlates With Outcome in Adult Respiratory Distress Syndrome: A Study in Mechanically Ventilated Patients. Chest. 1995;107(1):196-200.
31. Herridge MS, Cheung AM, Tansey CM, Matte-Martyn A, Diaz-Granados N, Al-Saidi F, et al. One-year outcomes in survivors of the acute respiratory distress syndrome. N Engl J Med. 2003;348(8):683-93.
32. Masclans JR, Roca O, Muñoz X, Pallisa E, Torres F, Rello J, et al. Quality of life, pulmonary function, and tomographic scan abnormalities after ARDS. Chest. 2011;139(6):1340-6.
33. Ichikado K, Muranaka H, Gushima Y, Kotani T, Nader HM, Fujimoto K, et al. Fibroproliferative changes on high-resolution CT in the acute respiratory distress syndrome predict mortality and ventilator dependency: a prospective observational cohort study. BMJ Open. 2012;2(2):e000545.

34. Meduri GU, Tolley EA, Chinn A, Stentz F, Postlethwaite A. Procollagen types I and III aminoterminal propeptide levels during acute respiratory distress syndrome and in response to methylprednisolone treatment. Am J Respir Crit Care Med. 1998;158(5 Pt 1):1432-41.
35. Meduri GU, Golden E, Freire AX, Taylor E, Zaman M, Carson SJ, et al. Methylprednisolone infusion in early severe ARDS: results of a randomized controlled trial. Chest. 2007;131(4):954-63.
36. Seymour CW, Kennedy JN, Wang S, Chang C-CH, Elliott CF, Xu Z, et al. Derivation, Validation, and Potential Treatment Implications of Novel Clinical Phenotypes for Sepsis. JAMA. 2019;321(20):2003-17.
37. Mahat RK, Panda S, Rathore V, Swain S, Yadav L, Sah SP. The dynamics of inflammatory markers in coronavirus disease-2019 (COVID-19) patients: A systematic review and meta-analysis. Clin Epidemiol Glob Health. 2021;11:100727.
38. Tian W, Jiang W, Yao J, Nicholson CJ, Li RH, Sigurslid HH, et al. Predictors of mortality in hospitalized COVID-19 patients: A systematic review and meta-analysis. Journal of Medical Virology. 2020;92(10):1875-83.
39. Khinda J, Janjua NZ, Cheng S, van den Heuvel ER, Bhatti P, Darvishian M. Association between markers of immune response at hospital admission and COVID-19 disease severity and mortality: A meta-analysis and meta-regression. Journal of Medical Virology. 2021;93(2):1078-98.
40. Opal SM, Fisher CJ, Dhainaut J-FA, Vincent J-L, Brase R, Lowry SF, et al. Confirmatory interleukin-1 receptor antagonist trial in severe sepsis: A phase III, randomized, doubleblind, placebo-controlled, multicenter trial. Critical Care Medicine. 1997;25(7).
41. Shakoory B, Carcillo JA, Chatham WW, Amdur RL, Zhao H, Dinarello CA, et al. Interleukin-1 Receptor Blockade Is Associated With Reduced Mortality in Sepsis Patients With Features of Macrophage Activation Syndrome: Reanalysis of a Prior Phase III Trial. Crit Care Med. 2016;44(2):275-81.

Part I

OBESITY IN CRITICALLY ILL COVID-19 PATIENTS

CHAPTER 2

A higher BMI is not associated with a different immune response and disease course in critically ill COVID-19 patients

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Abstract

Background/Objectives

Obesity appears to be an independent risk factor for ICU admission and a severe disease course in COVID-19 patients. An aberrant inflammatory response and impaired respiratory function have been suggested as underlying mechanisms. We investigated whether obesity is associated with differences in inflammatory, respiratory, and clinical outcome parameters in critically ill COVID-19 patients.

Subjects/Methods

Sixty-seven COVID-19 ICU patients were divided into obese (BMI ≥30 kg/m^2, n=18, 72% class I obesity, 28% class II obesity) and non-obese (BMI <30 kg/m^2, n=49) groups. Concentrations of circulating interleukin (IL)-6, IL-8, IL-10, tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), interferon gamma-induced protein (IP)-10, monocyte chemoattractant protein (MCP)-1, and IL-1 receptor antagonist (RA) were determined from ICU admission until 10 days afterwards, and routine laboratory and clinical parameters were collected.

Results

BMI was 32.6 [31.2-34.5] and 26.0 [24.4-27.7] kg/m^2 in the obese and non-obese group, respectively. Apart from temperature, which was significantly lower in obese patients (38.1 [36.9-38.9] vs. 38.7 [38.0 -39.5] °C, p=0.02), there were no between-group differences on ICU admission. Plasma cytokine concentrations declined over time (p<0.05 for all), but no differences between obese and non-obese patients were observed. Also, BMI did not correlate with the cytokine response (IL-6 r=0.09, p=0.61, TNF-α r=0.03, p=0.99, IP-10 r=0.28, p=0.11). The kinetics of clinical inflammatory parameters and respiratory mechanics were also similar in both groups. Finally, no differences in time on ventilator, ICU length of stay or 40-day mortality between obese and non-obese patients were apparent.

Conclusions

In COVID-19 patients requiring mechanical ventilation in the ICU, a higher BMI is not related to a different immunological response, unfavourable respiratory mechanics, or impaired outcome.

Introduction

The Coronavirus Disease 2019 (COVID-19) pandemic currently sweeps across the globe, leading to high morbidity and mortality. This infection, caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), can cause acute respiratory distress syndrome (ARDS) requiring mechanical ventilation in the intensive care unit (ICU). Age, male sex, and multiple comorbidities, including type 2 diabetes, hypertension, and coronary artery disease were identified as risk factors related to severity of COVID-19 disease (1-4). These comorbidities are more prevalent in the obese population than in patients with a normal body weight (5). Also, the prevalence of obesity among COVID-19 patients requiring mechanical ventilation was shown to be higher than expected when compared to controls with non COVID-19-related acute pulmonary diseases, and the need for mechanical ventilation increased with increasing body mass index (BMI) (6, 7). Finally, mortality was shown to be significantly higher in overweight/obese COVID-19 patients compared to normal-weight patients (7). Of interest, obesity itself is also recognized as an independent risk factor of severe pulmonary H1N1 influenza infection and mortality (8).

The underlying mechanisms of obesity as a factor contributing to COVID-19 disease severity are currently poorly understood, but various possible explanations have been proposed. For instance, in obese patients, pulmonary function may be restricted because of decreased functional residual capacity, resulting in lower blood oxygen levels (9). In addition, the angiotensin converting enzyme 2 (ACE2) receptor, which is utilized by SARS-CoV-2 to enter cells, is highly expressed by adipocytes and expression levels were shown to be higher in adipocytes of patients with obesity and type 2 diabetes (10). Therefore, it appears plausible that adipose tissue may serve as a reservoir for the virus, leading to more pronounced and sustained viral shedding, resulting in a perpetual inflammatory response and impaired outcome.

To date, it is not known whether obesity is only a risk factor for COVID-19 susceptibility and for the need of intensive care, or whether it also influences the outcome of the patients once admitted on intensive care units. Previous studies in critically ill COVID-19 patients have yielded ambiguous results, with some reporting associations between lower BMI and mortality (11), whereas others showed increased mortality in (severely) obese patients (12, 13). In the present study, we investigated associations between obesity and the immune response as well as the clinical course of SARS-CoV-2 infection in critically ill patients on the ICU of a tertiary university hospital. Elucidating these mechanisms may pave the way for the development of new treatment strategies for obese COVID-19 patients.

Methods

Study design and participants

In this prospective observational cohort study, all consecutive COVID-19 patients admitted to the ICU in the Radboud university medical centre (Nijmegen, The Netherlands) between March 11th and April 27th were included. COVID-19 was diagnosed by a positive SARS-CoV-2 RT-PCR test in nasopharyngeal and throat swabs and/or by typical chest CT-scan findings. Patients with a pre-existing immunosuppressed status or other comorbidities that strongly influence prognosis were excluded. Patients were divided into an obese (BMI ≥30 kg/m^2) and non-obese (BMI <30 kg/m^2) group, according to the classification of obesity by the World Health Organisation (WHO) (14). Also, individual BMI-values were correlated with circulating cytokine concentrations. The study was carried out in the Netherlands in accordance with the applicable rules concerning the review of research ethics committees and informed consent. All patients or legal representatives were informed about the study details and could decline to participate.

Data collection

Data were collected from the electronic patient files (EPIC, EPIC Systems Corporation, Verona, Wisconsin, USA) and recorded in the good clinical practice (GCP)-compliant data management system Castor (Castor EDC, Amsterdam, the Netherlands). ICU admission day was designated as day 1, and serial data were obtained for 10 consecutive days. Clinical outcomes (time on mechanical ventilation, ICU length of stay (LOS), and mortality) were recorded for 40 consecutive days. Serial values of mean arterial pressure (MAP), body temperature, and leukocyte differentiation as well as circulating levels of C-reactive protein (CRP), procalcitonin (PCT), D-dimer, ferritin, and cytokines (see below) were used to assess the inflammatory response to SARS-CoV-2 infection. Patients were actively cooled when they had a fever of >40°C. In these cases, body temperature was imputed as 40°C. Positive end expiratory pressure (PEEP), tidal volume per kilograms ideal body weight (TV_{IBW}) and PaO_2/FiO_2 ratio were used to assess the level of respiratory support required. Tidal volume was only recorded in patients on volume-controlled mechanical ventilation. Because the vast majority of patients were switched to pressure support ventilation after four days of ICU admission, TV_{IBW} data were analysed until this timepoint. Ideal body weight was calculated based on sex and height, using the formulas provided by the ARDS Network (15).

Cytokine concentration measurements

A baseline blood sample was obtained within the first 48 hours following ICU admission and serial samples were collected every other day. Ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood was centrifuged (2000g, 10 min, 4 °C), after which plasma was stored at -80 °C until analysis. Concentrations of interleukin (IL)-6, IL-8, IL-10, tumor

necrosis factor (TNF)-α, interferon gamma (IFN-γ), interferon gamma-induced protein (IP)-10, monocyte chemoattractant protein (MCP)-1, and IL-1 receptor antagonist (IL-1RA) were determined in one batch using a Luminex assay (Milliplex, Millipore, Billerica, USA). The lower detection limit was 3.2 pg/mL for all cytokines.

Statistical analysis

Statistical analysis was performed using SPSS 25 (IBM) and Graphpad Prism 8 software (GraphPad Software). Several variables were not measured daily; therefore, depending on the frequency of measurements, data were binned into two or three days using a custom script made in R-studio v3.6.2 (www.r-project.org). Because of the relatively small group size, normality was not assumed and all data are displayed as median with interquartile range [IQR] or geometric mean with 95% confidence interval (CI). Baseline characteristics were analysed using chi-square tests and Mann-Whitney-U tests. Between-group differences over time were analysed using linear mixed effects model analysis on log-transformed data followed by post-hoc analyses using Sidak's multiple comparisons tests in all serially measured variables. Relationships between BMI and cytokines were analysed using Spearman's correlation. Time on mechanical ventilation, ICU LOS, and mortality were analysed using log-rank and chi-square tests. Patients who died in the hospital or those who were still in the ICU and/or receiving mechanical ventilation on day 40 were censored at day 41 for the analysis of time on mechanical ventilation and ICU LOS. For the mortality analysis, patients who were discharged alive from the hospital or were still in the ICU or hospital on day 40 were censored at day 41.

Results

Patient characteristics

All 77 patients with proven COVID-19 consecutively admitted to the ICU of Radboud university medical centre were assessed for study inclusion. Patients with a pre-existing immunosuppressed status were excluded (n=9, see Supplementary Fig. 1), and one patient refused participation. The remaining study population (n=67) was divided in obese (n=18, of which 72% with class I obesity and 28% with class II obesity) and non-obese (n=49) groups (Fig. 1a). BMI of the entire study population was 27.7 [24.9-30.8] kg/m^2 (Fig. 1), with BMI's of 32.6 [31.2-34.5] and 26.0 [24.4-27.7] kg/m^2 in the in the obese and non-obese groups, respectively (p<0.0001, Table 1). All patients were mechanically ventilated. Temperature at ICU admission was 38.1 [36.9-38.9] °C in the obese group and 38.7 [38.0-39.5] °C in the non-obese group (p=0.007). No significant differences in age, sex, APACHE II score, medical history, and other clinical or laboratory parameters between the two groups were observed at ICU admission (Table 1).

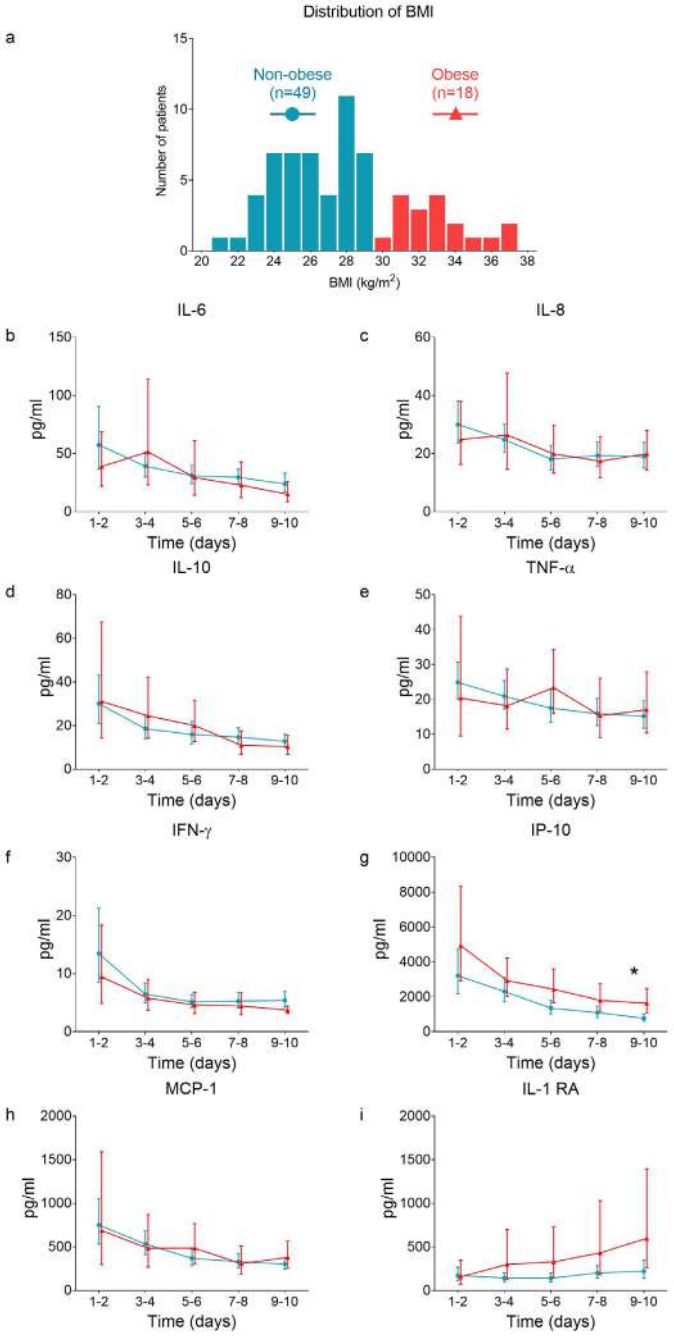

Figure 1. Body mass index distribution and plasma cytokine levels. (a) Histogram depicting body mass index (BMI) frequencies. Kinetics of concentrations of circulating **(b)** interleukin (IL)-6, **(c)** IL-8, **(d)** IL-10, **(e)** tumor necrosis factor alpha (TNF-α), **(f)** interferon gamma (IFN-γ), **(g)** interferon gamma-induced protein (IP)-10, **(h)** monocyte chemoattractant protein (MCP)-1 and **(i)** IL-1 receptor antagonist (RA). Data are presented as geometric mean with 95% confidence interval. * $p<0.05$, calculated using Sidak's post-hoc multiple comparisons tests on individual timepoints.

Table 1. Patient characteristics at the time of ICU admission.

	Non-obese (BMI<30, n=49)	Obese (BMI≥ 30, n=18)	p-value
Characteristics			
Body Mass Index, kg/m^2	26.0 [24.4-27.7]	32.6 [31.2-34.5]	<0.0001
Age, years	65 [59-72]	66.5 [57-72]	0.99
Male	38 (76%)	12 (24%)	0.36
Female	11 (65%)	6 (35%)	0.36
APACHE II	14 [12-19]	17 [9-23]	0.54
SOFA	7 [5-8.5]	4 [4-9]	0.72
Time from first COVID signs to ICU admission, days	12 [8-15.75]	10 [7-13.5]	0.35
Medical history			
- Cardio vascular disease	11 (22%)	7 (39%)	0.18
- Hypertension	23 (47%)	8 (44%)	0.53
- Chronic kidney disease	0 (0%)	1 (6%)	0.10
- Chronic obstructive pulmonary disease	3 (6%)	3 (17%)	0.18
- Diabetes mellitus	11 (22%)	4 (22%)	0.98
- Hematological malignancy	1 (2%)	0 (0%)	0.54
- Metastatic neoplasm	4 (8%)	1 (6%)	0.72
Clinical and laboratory parameters			
- Mean arterial pressure, mmHg	90 [85-94]	84 [78-94]	0.07
- Heart rate, beats/min	86 [77-98]	88 [81-94]	0.98
- Temperature, °Celsius	38.7 [38.0 -39.5]	38.1 [36.9-38.9]	0.02
- Respiratory rate, beats/min	22 [20-25]	22 [20-26]	0.87
- PEEP, cmH2O	12 [10-14]	12 [12-14]	0.15
- Tidal volume$_{IBW}$, ml/kg	6.4 [6.0-6.8]	6.4 [5.9-8.2]	0.78
- PaO2/FiO2 ratio, mmHg	144 [95-180]	144 [107-180]	0.60
- Creatinine, µmol/L	79 [65-101]	97 [58-229]	0.28
- Bilirubin, µmol/L	7.0 [6-12]	6.0 [4-12]	0.71
- Alanine aminotransferase, U/L	38.0 [21.0-56.0]	44.0 [23.5-94.0]	0.38
- Aspartate aminotransferase, U/L	52.0 [37.0-67.0]	55.0 [31.5-63.5]	0.36
- Leukocyte count, x10^9/L	8.2 [6.2-10.8]	8.8 [0.7-10.0]	0.63
- CRP, mg/L	218 [169-291]	245 [136-327]	0.43
- Procalcitonin, µg/L	0.76 [0.39-1.99]	0.53 [0.28-1.56]	0.52
- Ferritin, µg/L	1486 [720-2265]	1129 [724-2404]	0.74
- D-dimer, ng/mL	3330 [1830-10370]	2560 [1545-2560]	0.48

Data are presented as n (%) or median [IQR].

Inflammation and respiration

Plasma concentrations of all measured pro-inflammatory cytokines were highest at ICU admission and decreased over time both in the obese and non-obese groups ($p<0.05$ for all cytokines, Fig. 1b-i). Except for slightly higher IP-10 levels in obese vs. non-obese patients at days 9-10, no between-group differences in any of the measured cytokines on any timepoints

were observed (Fig 1b-i). Furthermore, BMI did not correlate with concentrations of IL-6, TNF-α and IP-10 at ICU admission (r=-0.09 p=0.61, r=0.03 p=0.99 and r=0.28 p=0.11 respectively, Fig. 2) or any other cytokine measured (r-values ranging from -0.11 to 0.06, p-values all >0.50, see Supplementary Fig 2). Despite a trend towards less pronounced leucocytosis in obese patients, no significant differences were present between both groups at any of the timepoints (Fig. 3a). The baseline difference in temperature (i.e. higher in non-obese patients) persisted at later timepoints, but did not attain statistical significance (Fig. 3b). No differences were observed in CRP levels between the obese and non-obese groups (Fig. 3c). Also, no significant differences in plasma concentrations of PCT, ferritin and d-dimer were observed between both groups (Supplementary Fig. 3). Finally, no between-group differences in any of the ventilation parameters were observed (Supplementary Fig. 4).

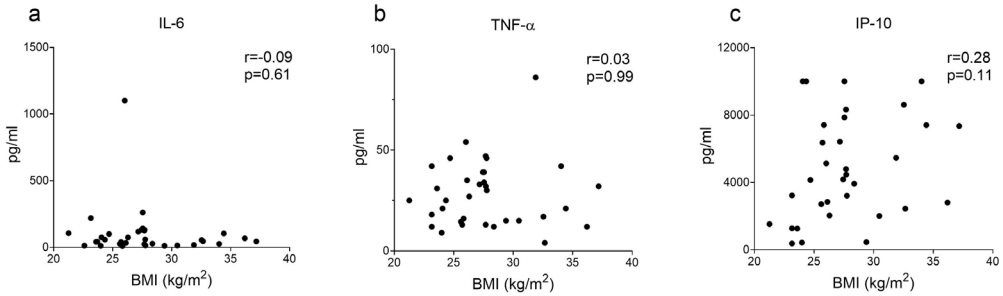

Figure 2. Relationship between BMI and concentrations of circulating cytokines on day of admission to the Intensive Care Unit. (a) Interleukin (IL)-6, **(b)** tumor necrosis factor alpha (TNF-α) and **(c)** interferon gamma-induced protein (IP)-10**.** R- and p-values were calculated using Spearman's correlation. See supplementary figure 2 for the relationships between BMI and the other measured circulating cytokine concentrations.

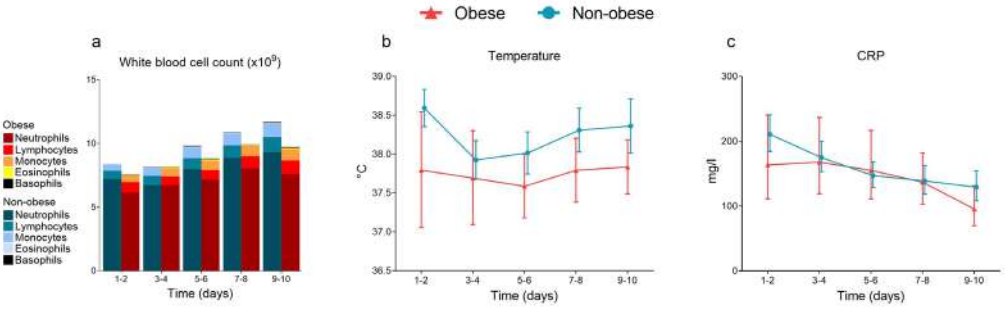

Figure 3. Routine laboratory and clinical inflammatory parameters. (a) White blood cell counts (WBC), **(b)** temperature, **(c)** circulating C-reactive protein (CRP). Data are represented as geometric mean (panel a) or geometric mean with 95% confidence interval (panels b-c). No significant differences on any of the individual timepoints were observed according to Sidak's post-hoc multiple comparisons tests.

Clinical outcomes

On day 40 of ICU admission, 11% (n=2) of the patients of the obese group was still mechanically ventilated and 17% (n=3) was still in the ICU, while 16% (n=8) of patients of the non-obese group were still mechanically ventilated and in the ICU (p=0.60 and p=0.97, respectively). Time on ventilator was 22 [16-40] days in the obese group and 27 [14-40] days in the non-obese group (p=0.41). ICU length of stay was 25 [17-40] and 29 [15-40] days in the obese and non-obese group, respectively (p=0.53). 40-day ICU mortality was 17% (n=3) in the obese group and 24% (n=12) in the non-obese group (p=0.50). The Kaplan-Meier curves for time on mechanical ventilation, ICU LOS and mortality are presented in Figure 4.

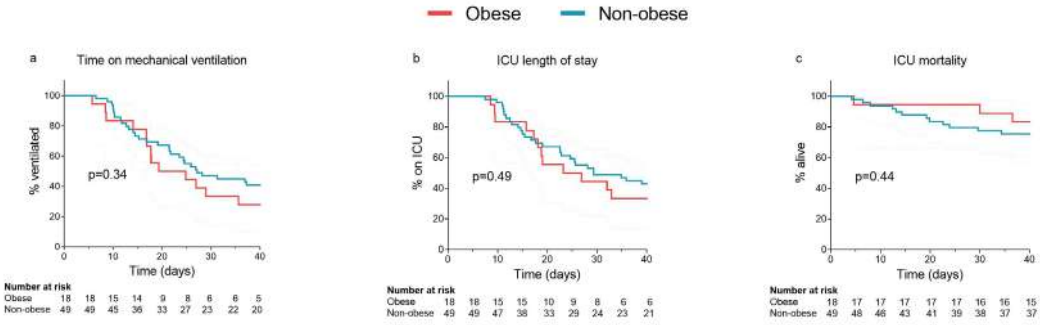

Figure 4. Clinical outcome data. (a) Time on mechanical ventilation **(b)** ICU length-of-stay **(c)** ICU mortality. P-values depicted in the panels were calculated using log-rank tests. Dotted lines indicate 95% confidence intervals.

Discussion

In this observational study in critically ill COVID-19 patients, we observed that, while disease severity and other baseline covariates were similar, temperature at ICU admission was lower in obese compared to non-obese patients. This observation may suggest that the endogenous immune-hypothalamus axis responsible for the induction of fever (IL1-IL6-PGE2 pathway) is less pronounced in obese compared to non-obese patients. However, no significant differences in plasma concentrations of various inflammatory markers and cytokines (most importantly IL-6) were observed between both groups. To gain sensitivity to detect a possible relationship between BMI and the cytokine response we performed correlation analyses that confirmed lack of such a relationship. Also, no significant difference in duration of mechanical ventilation, ICU-length of stay, or mortality was observed between the obese and non-obese group. Taken together, these data indicate that once a COVID-19 patient becomes critically ill, the immune response and clinical course of the disease is not relevantly influenced by the patients' BMI.

Obesity has been identified as a risk factor for severe disease and ICU admission in COVID-19 patients admitted to non-intensive care hospital wards (16, 17). This is not necessarily in contrast with our findings, as obese individuals may be more susceptible to infection, might have a different immune response prior to becoming critically ill, and/or may require more respiratory support (6). Although several studies have assessed associations between BMI and clinical outcomes/mortality in critically ill COVID-19 patients, conflicting findings have been reported (11-13). As such, the relationship between obesity and mortality in these critically ill patients remains unclear. What our study adds pertaining to the role of BMI in critically ill COVID-19 patients are data of inflammatory/immunological markers. Of interest, a paradoxical relationship between obesity and mortality has been described in the general ICU population, as well as in critically ill patients suffering from different infectious diseases, such as pneumonia and bacterial sepsis (18, 19). Overall, obese patients demonstrate a better survival compared to non-obese individuals, even when corrected for several covariates. This phenomenon has been coined the obesity paradox (20). Our study was underpowered to demonstrate statistically significant differences in mortality between obese and non-obese patients, and therefore we are unable to confirm or reject the presence of the obesity paradox in COVID-19 patients.

The present study has several limitations. First, because of the observational nature of the study, no direct link between cause and effect can be deduced. Second, because of the relatively small group of (especially obese) patients, a type 2 error is possible, and our findings should be regarded as indicative, not conclusive. Nevertheless, there were also no nonsignificant trends in inflammatory parameters or correlations between BMI and any of the circulating cytokines, which would be expected in case BMI would play an crucial role in the immune response in critically ill COVID-19 patients. Third, the present study was conducted in a single centre in the Netherlands, where (morbid) obesity may be less widespread compared to other countries. Accordingly, the obese group consisted largely of mildly obese patients and no patients with a BMI above 40 kg/m^2 were present in our cohort. Fourth, because patients in our cohort were all included after ICU admission and received mechanical ventilation, no statements can be made about the relationship between obesity and the risk of ICU admission or requirement of mechanical ventilation. Finally, we focused on innate immunity, so possible differences between obese and non-obese COVID-19 patients in adaptive immune response remain to be explored.

In conclusion, the results of this study indicate that, in critically ill COVID-19 patients requiring mechanical ventilation, the patients' BMI is not related to a different innate immune response, unfavourable respiratory mechanics, or impaired outcome. A larger multi-centre

study with a more expansive BMI distribution is warranted to confirm our findings.

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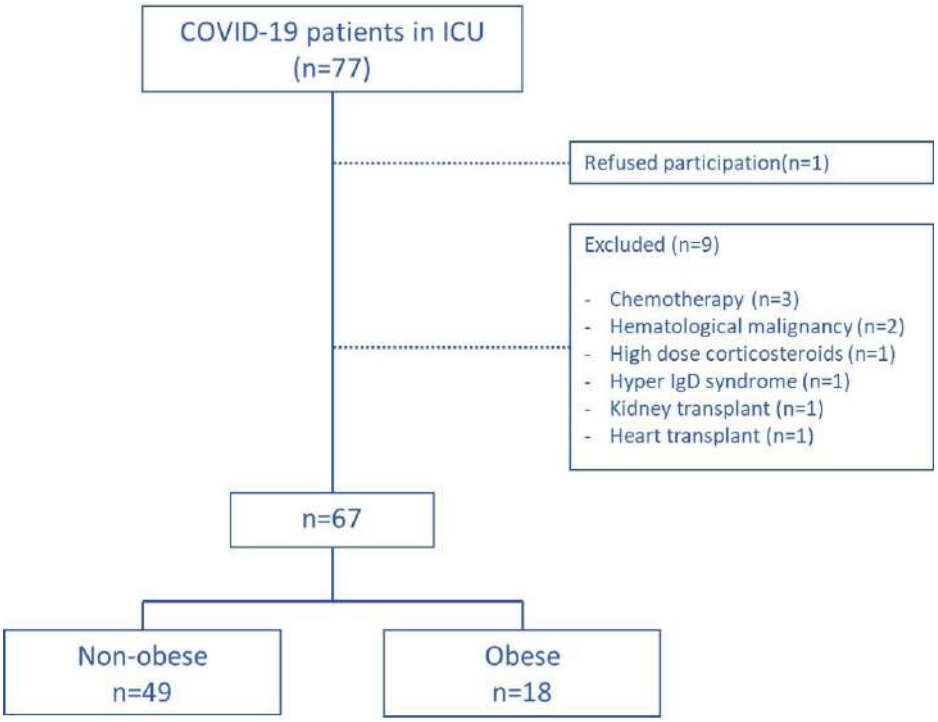
References

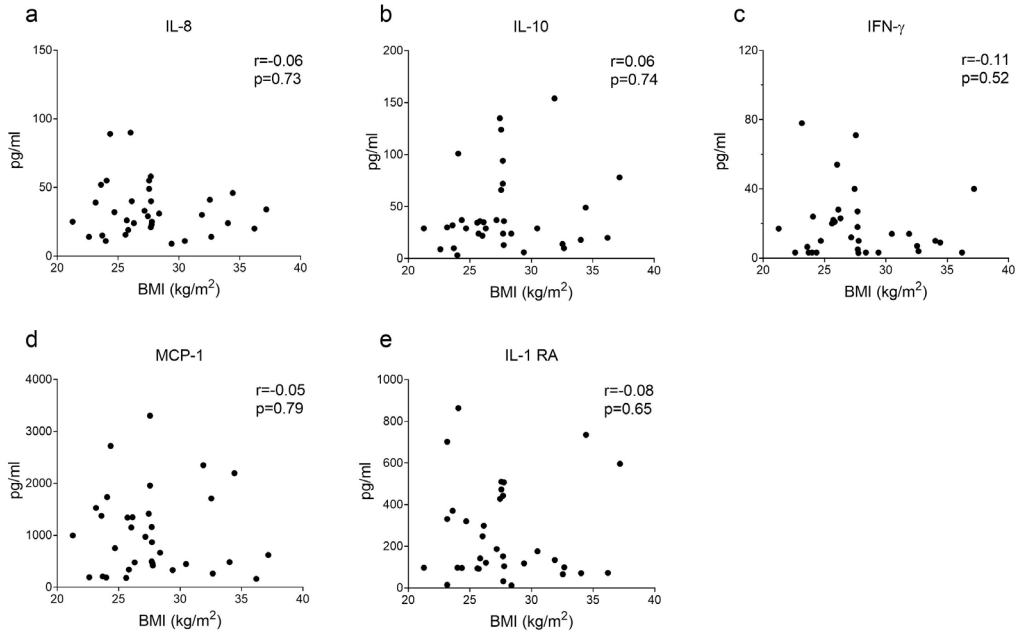
1. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054-62.
2. Li X, Xu S, Yu M, Wang K, Tao Y, Zhou Y, et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. J Allergy Clin Immunol. 2020;146(1):110-8.
3. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med. 2020;46(5):846-8.
4. 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;369:m1966.
5. Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. Circulation. 2006;113(6):898-918.
6. Simonnet A, Chetboun M, Poissy J, Raverdy V, Noulette J, Duhamel A, et al. High prevalence of obesity in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) requiring invasive mechanical ventilation. Obesity (Silver Spring). 2020.
7. Hussain A, Mahawar K, Xia Z, Yang W, El-Hasani S. Obesity and mortality of COVID-19. Meta-analysis. Obes Res Clin Pract. 2020;14(4):295-300.
8. Mertz D, Kim TH, Johnstone J, Lam PP, Science M, Kuster SP, et al. Populations at risk for severe or complicated influenza illness: systematic review and meta-analysis. BMJ. 2013;347:f5061.
9. Littleton SW, Tulaimat A. The effects of obesity on lung volumes and oxygenation. Respir Med. 2017;124:15-20.
10. Kruglikov IL, Scherer PE. The role of adipocytes and adipocyte-like cells in the severity of COVID-19 infections. Obesity (Silver Spring). 2020.
11. Auld SC, Caridi-Scheible M, Blum JM, Robichaux C, Kraft C, Jacob JT, et al. ICU and Ventilator Mortality Among Critically Ill Adults With Coronavirus Disease 2019. Crit Care Med. 2020;48(9):e799-e804.
12. Halasz G, Leoni ML, Villani GQ, Nolli M, Villani M. Obesity, overweight and survival in critically ill patients with SARS-CoV-2 pneumonia: is there an obesity paradox? Preliminary results from Italy. Eur J Prev Cardiol. 2020:2047487320939675.
13. Gupta S, Hayek SS, Wang W, Chan L, Mathews KS, Melamed ML, et al. Factors Associated With Death in Critically Ill Patients With Coronavirus Disease 2019 in the US. JAMA Intern Med. 2020.
14. W.H.O. Obesity: preventing and managing the global epidemic: World Health Organization; 2000.
15. Brower RG, Matthay MA, Morris A, Schoenfeld D, Thompson BT, Wheeler A. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. N Engl J Med. 2000;342(18):1301-8.

16. Chiappetta S, Sharma AM, Bottino V, Stier C. COVID-19 and the role of chronic inflammation in patients with obesity. Int J Obes (Lond). 2020.
17. Gao F, Zheng KI, Wang XB, Sun QF, Pan KH, Wang TY, et al. Obesity Is a Risk Factor for Greater COVID-19 Severity. Diabetes Care. 2020.
18. Nie W, Zhang Y, Jee SH, Jung KJ, Li B, Xiu Q. Obesity survival paradox in pneumonia: a meta-analysis. BMC Med. 2014;12:61.
19. Pepper DJ, Sun J, Welsh J, Cui X, Suffredini AF, Eichacker PQ. Increased body mass index and adjusted mortality in ICU patients with sepsis or septic shock: a systematic review and meta-analysis. Crit Care. 2016;20(1):181.
20. Schetz M, De Jong A, Deane AM, Druml W, Hemelaar P, Pelosi P, et al. Obesity in the critically ill: a narrative review. Intensive Care Med. 2019;45(6):757-69.

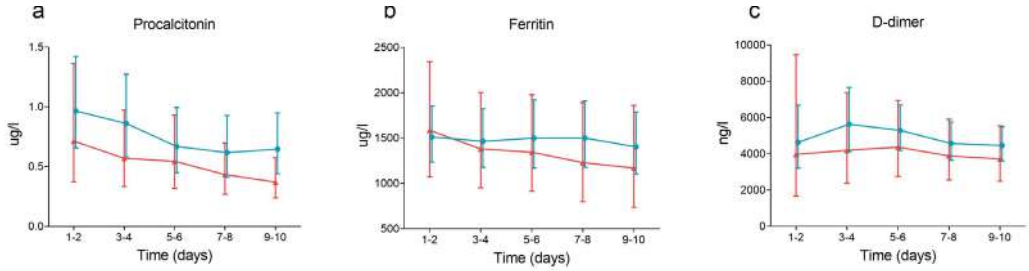
Supplementary figure 1. Study flowchart.



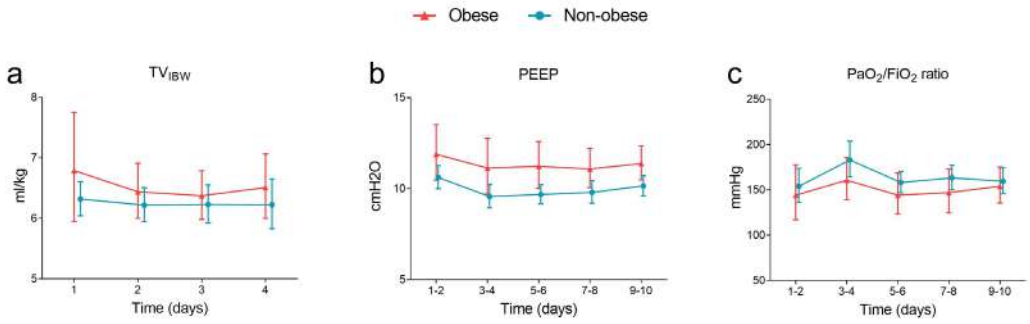
Supplementary figure 2. Relationship between BMI and plasma cytokine levels on day of admission to the Intensive Care Unit.
(a) IL-8, (b) IL-10, (c) interferon gamma (IFN-γ), (d) monocyte chemoattractant protein (MCP)-1 and (e) IL-1 receptor antagonist (RA). R- and p-values were calculated using Spearman correlation.



Supplementary figure 3. Laboratory inflammation parameters.
Plasma levels of **(a)** procalcitonin, **(b)** ferritin, and **(c)** d-dimer over time. Data presented as geometric means with 95% confidence intervals. P-values were calculated using mixed-model analysis (time*group interaction factor). No significant differences on any of the individual timepoints were observed according to Sidak's post-hoc multiple comparisons tests.



Supplementary figure 4. Mechanical ventilation parameters. (a) Tidal volume per kilogram ideal body weight (TVIBW), **(b)** positive end-expiratory pressure (PEEP), **(c)** arterial partial oxygen pressure (PaO2)/ fractional inspired oxygen (FiO2) ratio. Data presented as geometric mean with 95% confidence interval. No significant differences on any of the individual timepoints were observed according to Sidak's post-hoc multiple comparisons tests.



CHAPTER 3

Body mass index and mortality in COVID-19 and other diseases: a cohort study in 35,506 ICU patients

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Abstract

Objective
Obesity is a risk factor for severe COVID-19 and might play a role in its pathophysiology. It is unknown whether BMI is related to clinical outcome following ICU admission, as observed in various other categories of critically ill patients. We investigated the relationship between BMI and in-hospital mortality in critically ill COVID-19 patients and in cohorts of ICU patients with non-SARS-CoV-2 viral pneumonia, bacterial pneumonia, and multiple trauma.

Design
Multicenter observational cohort study.

Setting
Eighty-two Dutch Intensive Care Units participating in the Dutch National Intensive Care Evaluation quality registry.

Patients
35.506 critically ill patients.

Interventions
None.

Measurements and Main Results
Patient characteristics and clinical outcomes were compared between four cohorts (COVID-19, non-SARS-CoV-2 viral pneumonia, bacterial pneumonia, and multiple trauma patients) and between BMI categories within cohorts. Adjusted analyses of the relationship between BMI and in-hospital mortality within each cohort were performed using multivariable logistic regression. COVID-19 patients were more likely male, had a higher BMI, lower PaO_2/FiO_2 ratio, and were more likely mechanically ventilated during the first 24 hours in the ICU compared to the other cohorts. COVID-19 patients had longer ICU and hospital length of stay, and higher in-hospital mortality. Odds ratios for in-hospital mortality for patients with BMI≥35 kg/m^2 compared with normal weight in the COVID-19, non-SARS-CoV-2 viral pneumonia, bacterial pneumonia, and trauma cohorts were 1.15 [0.79-1.67], 0.64 [0.43-0.95], 0.73 [0.61-0.87] and 0.81 [0.57-1.15], respectively.

Conclusions
The obesity paradox, which is the inverse association between BMI and mortality in critically ill patients, is not present in ICU patients with COVID-19-related respiratory failure, in contrast to non-SARS-CoV-2 viral and bacterial respiratory infections.

Introduction

The coronavirus disease 2019 (COVID-19), caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), is characterized by a high variation in disease severity (1). In the most severe cases, patients develop acute respiratory distress syndrome (ARDS) requiring invasive mechanical ventilation, which is associated with high mortality. To date, several risk factors predicting an unfavorable disease course of COVID-19 are known, including higher age, male sex, type 2 diabetes mellitus, hypertension, coronary artery disease, and a higher body mass index (BMI) (2-4). In comparison with obese patients suffering from other acute respiratory pulmonary diseases, obese COVID-19 patients are at higher risk of requiring admission to the intensive care unit (ICU) and invasive mechanical ventilation (5, 6). This BMI-related increased risk for ICU admission is also observed in obese patients with influenza pneumonia, SARS-CoV-1 infections, and the Middle East Respiratory Syndrome (MERS) (7-9).

In the general population, obesity is associated with multiple chronic diseases which are independently associated with increased mortality compared to non-obese patients. Also, in ICU patients, obesity may be a risk factor for developing ARDS and the need for mechanical ventilation (10). In contrast, multiple studies show reduced ICU- and hospital mortality rates in overweight and obese critically ill patients compared to those with a normal BMI (11-13). This observation is known as the *obesity paradox* and several underlying mechanisms have been suggested, including a higher metabolic reserve in obese patients and differences in pulmonary mechanics and immunological aspects between obese and non-obese patients (14). It is currently unclear whether or not the obesity paradox is present in critically ill COVID-19 patients, as fat tissue might play a specific pathophysiological role in this disease, for instance through modulating expression of the angiotensin converting enzyme 2 (ACE2) receptor which facilitates SARS-CoV-2 cell entry (15). Up to now, in smaller cohorts, either no association (16) or an inverse relationship between higher BMI and clinical outcomes after ICU admission (17-19) have been reported.

The aim of this study is to assess associations between BMI and mortality in critically COVID-19 patients using data of 82 Dutch ICUs. Associations between BMI and clinical outcomes of critically ill COVID-19 patients were compared to those of ICU patients with non-SARS-CoV-2 viral pneumonia, bacterial pneumonia, and trauma patients, the latter representing a non-pulmonary critically ill control group.

Materials and Methods

Data collection

Data of all patients admitted to 82 teaching and nonteaching in nonurban and urban hospitals in the Netherlands are collected in the Dutch National Intensive Care Evaluation (NICE) quality registry (http://www.stichting-nice.nl) (20). For this observational multicenter study on prospective collected data, four cohorts were defined: (1) all COVID-19 patients admitted to the ICU from January 2020 until July 2020, (2) all other non-SARS-CoV-2 viral pneumonia patients, (3) all bacterial pneumonia patients, and (4) all multiple trauma patients from June 2015 until June 2020. Data of patients who were re-admitted to the ICU during the same hospitalization period were excluded, so that only data of the first ICU admission were used. Collected data, including patient characteristics (BMI, age, sex), medical history, respiratory function, disease severity scores of the first 24 hours following ICU admission, and clinical outcomes (length of ICU stay and hospital stay as well as in-hospital mortality), were anonymized before use. All four cohorts were divided into BMI categories (<18.5 kg/m^2, 18.5-20 kg/m^2, 20-25 kg/m^2, 25-30 kg/m^2, 30-35 kg/m^2, ≥35 kg/m^2) according to the classification of the World Health Organization (WHO) (21). The primary endpoint of this study was the association between BMI and in-hospital mortality in COVID-19 patients and the other three cohorts, particularly because severe COVID-19 is associated with a prolonged disease course. Also, differences in baseline demographic characteristics and physiological data between the four cohorts and between different BMI categories within each cohort were investigated.

Data collection is completely standardized using strict definitions and subject to data quality checks (22). In accordance to Dutch legislation and compliant with the European GPDR, there is no need to obtain consent when anonymous data is used.

Statistical analyses

Unadjusted analysis of differences in patient characteristics and clinical outcomes between the four cohorts, and between different BMI categories within the cohorts was performed using chi-square tests or Kruskal Wallis tests, followed by post-hoc pairwise chi-square and Wilcoxon tests, respectively. To account for multiple testing, a p-value of <0.001 was considered to indicate statistical significance in all analyses. To assess the association between BMI and in-hospital mortality an etiological approach was used to calculate the adjusted odd ratios and corresponding 95% confidence intervals within each cohort. We identified confounding factors that were not on the causal pathway between BMI and

mortality based on expert opinion (intensivists and data scientist), literature, and availability in the database. The used multivariable logistic regression models included the following confounders: sex, age (categorized in 11 groups), chronic diagnoses (immunological insufficiency, renal insufficiency, chronic respiratory insufficiency, cardiovascular insufficiency, cirrhosis, malignancy, and diabetes mellitus), APACHE III acute physiology score (APS) (categorized in quintiles), need for mechanical ventilation, use of vasoactive medication, and lowest PaO_2/FiO_2-ratio (categorized in quintiles) in the first 24 hours following ICU admission. As one may argue that a chronic diagnosis like diabetes mellitus may be related to both BMI and survival, we also explored whether or not diabetes mellitus was independently associated with mortality. To illustrate the association between BMI and covariate-adjusted in-hospital mortality, the relative mortality risks (RR) according to BMI in all four cohorts is plotted using a smooth plot function and using the BMI category of 18.5-25.0 kg/m^2 as reference.
All statistical analyses were performed using R Studio v1.2.1335 (Rstudio Team (2020), RStudio: Integrated Development for R. RStudio, PBC, Boston, USA).

Results

Patient characteristics
A total of 2635 unique COVID-19 patients, 2940 non-SARS-CoV-2 viral pneumonia patients, 14.250 bacterial pneumonia patients, and 15.681 trauma patients were included. Patient characteristics of the four cohorts are listed in Table 1. In short, compared to all other cohorts, COVID-19 patients were more likely male, had a higher BMI, lower PaO_2/FiO_2 ratio, and were more likely mechanically ventilated during the first 24 hours following ICU admission. Importantly, these differences do not translate into a higher disease severity score, as APACHE III scores of COVID-19 patients were lower compared to non-SARS-CoV-2 viral and bacterial pneumonia patients. The lower APACHE III score in COVID-19 patients admitted to the ICU was mainly driven by younger age and lower prevalence of chronic cardiovascular insufficiency, history of malignancies, immunological insufficiency, COPD, chronic respiratory insufficiency, and chronic renal failure. In contrast, the percentage of patients with diabetes mellitus was higher in all respiratory infection cohorts, including COVID-19, compared to trauma patients.

The distribution of BMI within the four cohorts is shown in Figure 1 and patient characteristics according to BMI categories within the COVID-19 cohort and other three cohorts are listed in Table 2 and Supplemental Content 1-3, respectively. Within the COVID-19 cohort, patients with a higher BMI were younger, less likely male, and more likely to have diabetes

Table 1. Patient characteristics of the four cohorts.

	COVID-19 (n=2635)	non-SARS-CoV-2 viral pneumonia (n=2940)	Bacterial pneumonia (n=14250)	Trauma (n=15681)	p-value
Age, years	65 [56-72]	66 [57-74]*	69 [60-77]*	60 [42-76]*	<0.001
BMI, kg/m^2	27.8 [25.2-31.1]	25.7 [22.6-30.1]*	25.3 [22.4-29.3]*	24.8 [22.6-27.8]*	<0.001
Male sex, n (%)	1899 (72.1)	1484 (50.5)*	8414 (59.0)*	10282 (65.6)*	<0.001
Lowest PaO_2/FiO_2 ratio of the first 24 hours in the ICU, mmHg	118 [84-165]	168 [112-234]*	146 [97-217]*	300 [210-382]*	<0.001
Mechanical ventilation on ICU admission	1272 (48.3)	1555 (52.9)*	5396 (37.9)*	4904 (31.3)*	<0.001
Mechanical ventilation in first 24 hours in the ICU	2064 (78.3)	2043 (69.5)*	7768 (54.5)*	5363 (34.2)*	<0.001
Use of vasoactive medication in first 24 hours in the ICU	1758 (66.7)	1227 (41.7)*	5991 (42.0)*	4409 (28.1)*	<0.001
APACHE III APS	46 [37-57]	48 [37-62]*	53 [40-67]*	33 [24-49]*	<0.001
APACHE III Score	58 [46-71]	62 [49-77]*	68 [54-84]*	45 [31-63]*	<0.001
SAPS II score	37 [29-45]	37 [30-46]	39 [31-49]*	28 [20-39]*	<0.001
Medical history					
Malignancy	60 (2.3)	158 (5.4)*	1116 (7.8)*	201 (1.3)*	<0.001
Immunological insufficiency	195 (7.4)	442 (15.0)*	2326 (16.3)*	364 (2.3)*	<0.001
COPD	217 (8.2)	1382 (47.0)*	5110 (35.9)*	1152 (7.3)	<0.001
Chronic respiratory insufficiency	104 (3.9)	503 (17.1)*	1846 (13.0)*	235 (1.5)*	<0.001
Chronic renal failure	73 (2.8)	188 (6.4)*	1186 (8.3)*	531 (3.4)	<0.001
Chronic cardiovascular insufficiency	32 (1.2)	101 (3.4)*	549 (3.9)*	357 (2.3)	<0.001
Diabetes mellitus	500 (19.0)	589 (20.0)	2998 (21.0)	1450 (9.2)*	<0.001

*Data presented as median [interquartile range] or n (%). P-values calculated using chi-square tests or Kruskal Wallis tests across all four cohorts. * p<0.001 compared with COVID-19 using pairwise chi-square or Wilcoxon tests.*

mellitus. Of interest, higher BMI was neither associated with a lower PaO2/FiO2 ratio or the likelihood to require mechanical ventilation during the first 24 hours following ICU admission (Table 2). A similar pattern concerning age, gender, and diabetes mellitus in relationship to a higher BMI was observed in patients with non-SARS-CoV-2 viral or bacterial pneumonia. In the bacterial pneumonia cohort, a higher BMI was also related to higher likelihood to require mechanical ventilation, both on ICU admission and during the first 24 hours of ICU admission.

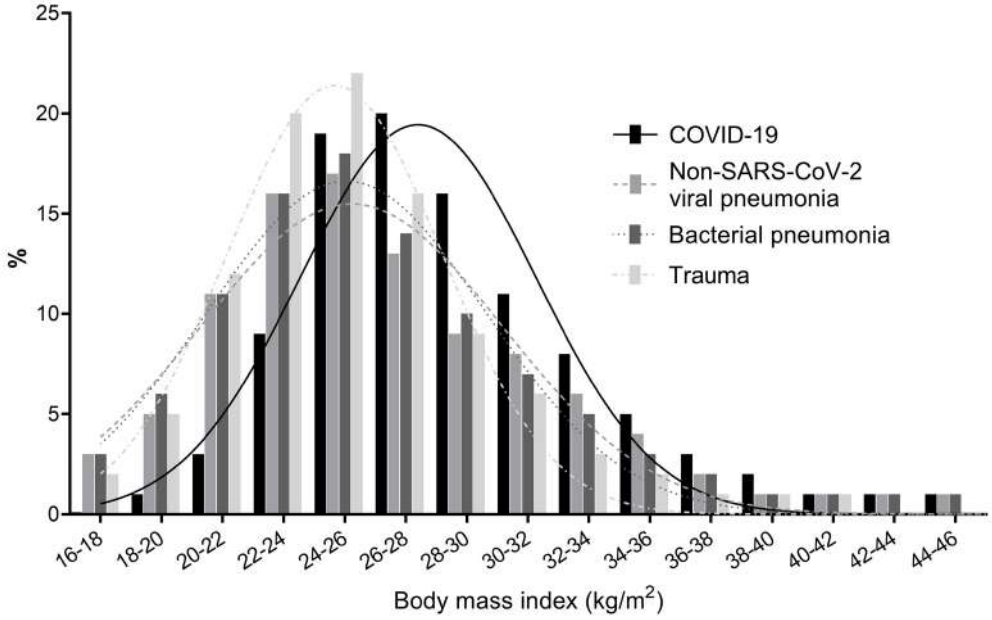


Figure 1. Distribution of BMI for the COVID-19, non-SARS-CoV-2 viral pneumonia, bacterial pneumonia and multiple trauma cohorts. The proportion of patients with overweight and obesity was notably higher in COVID-19 patients compared with the other cohorts.

Table 2. Patient characteristics of BMI categories in the COVID-19 cohort.

	BMI < 18.5 kg/m² (n=10)	BMI 18.5-25 kg/m² (n=592)	BMI 25-30 kg/m² (n=1196)	BMI 30-35 kg/m² (n=565)	BMI ≥35 kg/m² (n=272)	p-value
Age, years	73 [66-75]	67 [58-73]	65 [58-72]	63 [54-71]*	59 [49-67]*	<0.001
Male sex, n (%)	4 (40.0)	444 (75.0)	927 (77.5)	381 (67.4)	143 (52.6)*	<0.001
Lowest PaO_2/FiO_2 ratio of the first 24 hours in the ICU, mmHg	121 [73-190]	126 [89-175]	120 [86-165]	112 [77-155]	106 [76-150]	0.30
Mechanical ventilation on ICU admission	3 (30.0)	286 (48.3)	599 (50.1)	265 (46.9)	119 (43.8)	0.24
Mechanical ventilation in first 24 hours in the ICU	7 (70.0)	453 (76.5)	947 (79.2)	440 (77.9)	217 (79.8)	0.65
Use of vasoactive medication in first 24 hours in the ICU	8 (80.0)	407 (68.8)	802 (67.1)	373 (66.0)	168 (61.8)	0.28
APACHE III APS	57 [43-75]	48 [38-60]	46 [36-56]	46 [37-57]	46 [37-55]	0.15
APACHE III Score	73 [59-86]	61 [49-75]	59 [47-71]	57 [44-71]	56 [43-68]	0.03
SAPS II score	50 [46-58]	37 [30-46]	37 [29-45]	36 [30-45]	34 [27-43]	0.42
Medical history						
Malignancy	0 (0)	23 (3.9)	27 (2.3)	6 (1.1)	4 (1.5)	0.03
Immunological insufficiency	0 (0)	55 (9.3)	77 (6.4)	40 (7.1)	23 (8.5)	0.18
COPD	0 (0)	52 (8.8)	83 (6.9)	53 (9.4)	29 (10.7)	0.13
Chronic respiratory insufficiency	0 (0)	22 (3.7)	40 (3.3)	25 (4.4)	17 (6.2)	0.21
Chronic renal failure	0 (0)	14 (2.4)	31 (2.6)	23 (4.1)	5 (1.8)	0.24
Chronic cardiovascular insufficiency	0 (0)	8 (1.4)	16 (1.3)	6 (1.1)	2 (0.7)	0.86
Diabetes mellitus	0 (0)	86 (14.5)	212 (17.7)	131 (23.2)*	71 (26.1)*	<0.001

*Data presented as median [interquartile range] or n (%). P-values calculated using chi-square tests or Kruskal Wallis tests across all five categories. * p<0.001 compared with BMI 18.5-25 kg/m2 using pairwise chi-square or Wilcoxon tests.*

Clinical outcomes

Outcome parameters of the four cohorts are listed in Table 3. In surviving patients, median ICU-LOS of COVID-19 patients was five-to-six-fold higher compared to the non-SARS-CoV-2 viral and bacterial pneumonia cohorts, and 18-fold higher than that of trauma patients (Table 3). Additionally, median hospital-LOS was approximately threefold higher in COVID-19 survivors compared to the other three cohorts (Table 3). Comparable differences between cohorts were observed in non-survivors. The percentage of readmissions in the COVID-19 cohort was lower, whereas in-hospital mortality (29.2%) was higher compared to all other cohorts (18.5% in non-SARS-CoV-2 viral pneumonia, 21.2% in bacterial pneumonia, and 9.3% in trauma, Table 3).

Table 3. Clinical outcomes of the four cohorts and odds ratios of in-hospital mortality of BMI categories in the multivariable logistic regression model, with BMI 18.5-25 kg/m2 used as reference category

	COVID-19 (n=2635)	non-SARS--CoV-2 viral pneumonia (n=2940)	Bacterial pneumonia (n=14250)	Trauma (n=15681)	p-value
LOS ICU of survived patients, days	18 [10-33]	4 [2-8]*	3 [2-7]*	1 [1-3]*	<0.001
LOS ICU deceased patients, days	10 [5-18]	5 [2-9]*	4 [1-8]*	2 [1-6]*	<0.001
LOS hospital of survived patients, days	31 [19-46]	11 [7-18]*	12 [7-20]*	9 [5-16]*	<0.001
LOS hospital of deceased patients, days	12 [6-19]	7 [3-13]*	7 [3-13]*	5 [2-10]*	<0.001
Number of readmissions	11 (0.4)	48 (1.6)*	1348 (8.6)*	238 (1.5)*	<0.001
28-day mortality, n (%)	660 (25.0)	507 (17.2)*	2858 (20.1)*	1394 (8.9)*	<0.001
In-hospital mortality, n (%)	769 (29.2)	545 (18.5)*	3019 (21.2)*	1456 (9.3)*	<0.001
Odds ratios					
BMI <18.5 kg/m^2	1.92 [0.51-7.13]	1.50 [0.95-2.37]	1.88 [1.57-2.25]	1.23 [0.86-1.78]	
BMI 18.5-25 kg/m^2	1.0 reference	1.0 reference	1.0 reference	1.0 reference	
BMI 25-30 kg/m^2	0.95 [0.75-1.21]	0.78 [0.61-0.99]	0.78 [0.70-0.86]	0.9 [0.78-1.03]	
BMI 30-35 kg/m^2	0.87 [0.65-1.16]	0.76 [0.55-1.04]	0.81 [0.70-0.93]	0.99 [0.79-1.23]	
BMI ≥35 kg/m^2	1.15 [0.79-1.67]	0.64 [0.43-0.95]	0.73 [0.61-0.87]	0.81 [0.57-1.15]	

*Data presented as median [interquartile range] or n (%). P-values calculated using chi-square tests or Kruskal Wallis tests across all four cohorts. * p<0.001 compared with COVID-19 using pairwise chi-square or Wilcoxon tests.Covariates used for the multivariable logistic regression analyses included sex, age, medical history (chronic diagnoses), APACHE III acute physiology score (APS), vasoactive medication and mechanical ventilation and PaO2/FiO2 ratio on ICU admission.*

No statistically significant differences in ICU/hospital-LOS were present between the different BMI categories in both surviving and non-surviving COVID-19 patients (Supplemental Content 4). In the non-SARS-CoV-2 viral pneumonia and bacterial pneumonia cohorts, 28-day mortality was lower in higher BMI categories (Supplemental Content 4). In-hospital mortality was also lower in patients with a higher BMI in the bacterial pneumonia cohort. In both the COVID-19 and trauma cohorts, no differences in 28-day mortality and in-hospital mortality were present between different BMI categories. Survival curves of the different BMI categories in the four cohorts are illustrated in Supplemental Content 5.

Multivariable analyses for in-hospital mortality were performed to adjust for differences in baseline patient characteristics between cohorts. For these analyses with BMI as covariate of interest, sex, age, chronic diagnoses, APACHE III acute physiology score (APS), and need for mechanical ventilation, use of vasoactive medication, and lowest PaO_2/FiO_2-ratio in the first 24 hours following ICU were entered as confounders. In these multivariable analyses, BMI remained unrelated to mortality risk in the COVID-19 cohort, while diabetes mellitus was independently associated with a higher in-hospital mortality (Supplemental Content 6). In contrast, following confounder adjustment, a higher BMI was still associated with lower mortality in the non-SARS-CoV-2 viral pneumonia and bacterial pneumonia cohorts compared to the normal BMI category (Table 3). Furthermore, in the bacterial pneumonia cohort, being underweight was associated with higher mortality (Table 3). Similar to the crude analysis, no association between BMI and mortality was found in the adjusted analysis of trauma patients (Table 3). Odds ratios of contribution to mortality for all other covariates are listed in Supplemental Content 6-9. Covariate-adjusted associations between BMI and relative risk of in-hospital mortality in the four cohorts are illustrated in Figure 2. In contrast to the lower RR in the highest BMI in the other cohorts, the RR increases in patients with the highest BMI in the COVID-19 cohort.

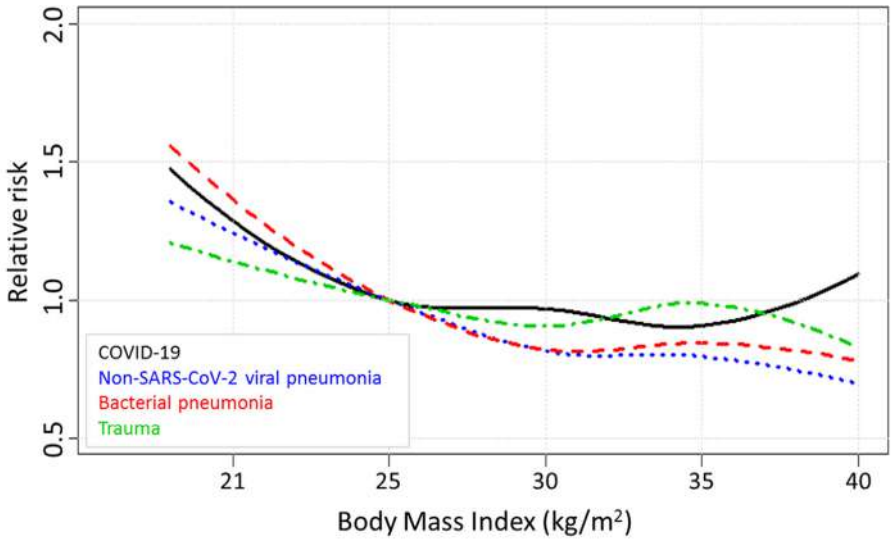


Figure 2. Relative in-hospital mortality risks according to BMI in the four cohorts BMI of 25.0 kg/m^2 was used as reference. Relative risks were adjusted for sex, age, chronic diagnosis, APACHE III acute physiology score (APS) and need for mechanical ventilation, use of vasoactive medication, and lowest PaO2/FiO2-ratio in the first 24 hours following ICU admission. The mortality risk decreases with higher BMI in the non-SARS-CoV-2 viral pneumonia, bacterial pneumonia, and trauma cohorts, but not in COVID-19 patients.

Discussion

In the present study, the association between BMI and in-hospital mortality was investigated in critically ill COVID-19 patients, and compared to non-SARS-CoV-2 viral pneumonia, bacterial pneumonia and trauma patients admitted to the ICU. While COVID-19 patients had a higher BMI compared to the other cohorts, no relationship between BMI and mortality was found in critically ill COVID-19 and trauma patients, while a higher BMI was associated with lower mortality in the other respiratory infection cohorts. Furthermore, being underweight was related to higher mortality in bacterial pneumonia patients.

Previous studies in non-selected patient populations reported increased susceptibility to SARS-CoV-2 in patients with higher BMI (23, 24). Also, obesity has been identified as important risk factors for an unfavorable disease course in patients with COVID-19 requiring admission to the ICU and mechanical ventilation, ultimately translating in an overall higher mortality in overweight patients (3-6). This may have led to the assumption that patients with a high BMI have a worse prognosis at any stage of COVID-19. For critically ill COVID-19 patients, conflicting results have emerged from relatively small studies (16-19). As such, it remained unclear whether obese and/or underweight patients are also at higher risk

for poor clinical outcome once they are in the ICU compared to critically ill COVID-19 patients with a normal weight. This is of particular interest, because previous studies in non-COVID-19 critically ill patients rather demonstrated an inverse relationship between BMI and mortality (11-13), which was coined the obesity paradox. In this large multicenter study including several thousand COVID-19 patients, no increased mortality rates were observed for COVID-19 patients in the higher BMI categories. However, while we confirm the obesity paradox in the non-SARS-CoV-2 viral and bacterial pneumonia patients, we did not observe a lower mortality risk in overweight/obese critically ill COVID-19 patients either. In response to the absence of a protective role of a higher BMI in critically ill COVID-19 patients, one may argue that overweight/obese patients in the ICU are therefore at a relative disadvantage when suffering from COVID-19 compared to other respiratory infections.

Several explanations can be put forward for the discrepancy regarding the relation between BMI and mortality in non-selected COVID-19 patient populations in previous studies and critically ill COVID-19 patients in our study. First, it might be argued that differences in reasons for ICU admission between patients from different BMI categories play a role. Since it is known that obese patients are more likely to develop atelectasis than patients with a normal weight, obese COVID-19 patients may more likely be admitted to the ICU for mechanical respiratory support and possibly also earlier in their disease course, while the reason for ICU admission in patient with normal weight may more often be severe systemic inflammation and/or failure of other organs. If so, this should translate into differences in disease severity on ICU admission, which is the most important prognostic factor for survival. However, this is not supported by our data, as disease severity scores were similar between the different BMI categories and were also included as confounder in the multivariable analyses. Furthermore, we recently demonstrated that inflammatory parameters do not differ between obese and non-obese critically ill COVID-19 patients (25), arguing against BMI-related immunological differences that may explain discrepancies in ICU admission characteristics between BMI-categories. Second, although multiple covariates were included in the multivariable logistic regression analysis, residual confounders might still be present, such as the incidence of smoking, chronic use of immunomodulatory drugs, socioeconomic status and ethnicity.
In keeping with previous reports describing a survival benefit in ICU patients with a higher BMI (11, 12), we confirm a relation between higher BMI and lower mortality in ICU patients suffering from non-SARS-CoV-2 viral pneumonia and bacterial pneumonia. It is tempting to speculate why this obesity paradox is not observed in COVID-19 patients. It has been hypothesized that obese patients display a more anti-inflammatory phenotype (26, 27). As briefly alluded to before, we previously investigated circulating levels of various

inflammatory cytokines, including the anti-inflammatory mediators IL-10 and IL-1RA in critically ill COVID-19 patients, but found no differences between obese and non-obese patients (25). Therefore, this suggested underlying mechanism of the obesity paradox may not be present in critically ill COVID-19 patients but only in ICU patients with other etiologies. Furthermore, it has been postulated that the higher circulating cholesterol and lipid levels in patients with a higher BMI may result in more effective binding of endotoxin, thereby removing an important inflammatory trigger. This may provide an explanation for the survival benefit in obese patients infected with Gram-negative bacteria, which likely constitute a substantial part of our bacterial pneumonia cohort. Furthermore, endotoxin binding may also plays a role in case of translocation of bacteria and/or their products from the gut, which is commonly observed in sepsis patients, also in those not infected with Gram-negative bacteria. Although a recent small study reported the presence of circulating endotoxin in critically ill COVID-19 patients (28), it is unclear whether this is a widespread phenomenon in this disease and does not explain the difference between the COVID-19 and non-SARS-CoV-2 viral pneumonia cohorts in terms of the BMI-mortality relationship. Of interest, adipose tissue, especially visceral fat, may play a pathophysiological role in COVID-19 disease (15). Adipokines such as leptin may enhance pulmonary inflammation and exacerbate respiratory failure (29). Also, the ACE2 expression is higher in adipocytes of people with obesity and diabetes mellitus (30), suggesting that fat tissue may function as a reservoir for the virus. Although its relevance has yet to be elucidated, our findings may be a reflection of this pathophysiological role of adipose tissue.

A strength of this work is that it represents the largest study to date investigating the relation between BMI and mortality in critically ill COVID-19 patients. Furthermore, we included three different relevant comparison groups. This study also has limitations. First, the very small number of underweight COVID-19 patients illustrates that patients with a low BMI are less likely to become critically ill, but hampers assessment of the association between underweight and mortality, which was associated with higher mortality in the bacterial and non-SARS-CoV-2 viral (trend) pneumonia patients. Second, due to the larger sample size of the bacterial pneumonia cohort, the statistical power to detect the presence of an association between BMI and mortality is higher than in in the COVID-19 cohort. However, the obesity paradox was also present in the non-SARS-CoV-2 viral pneumonia cohort, which is similar in size as the COVID-19 cohort. Furthermore, in both the non-SARS-CoV-2 viral and bacterial pneumonia cohorts, the lowest mortality was observed in the group with the highest BMI (>35 kg/m^2), whereas increased odds for mortality were observed for this BMI category in the COVID-19 cohort, although this did not reach statistical significance. Therefore, it seems unlikely that the absence of the obesity paradox in COVID-19 patients is the consequence

of limited statistical power. Third, one may argue that surge capacity issues may have influenced our observations. In the Netherlands, we did not reach conditions in which COVID-19 patients could not be admitted to the ICU if required. However, ICU's did work hard and beyond their normal capacity. As a consequence one would expect a selection of higher BMI COVID-19 patients with less comorbidities and lower age to be admitted to the ICU and this would plausibly translate into a better prognosis of these patients. The opposite was observed: COVID-19 patients with a higher BMI did not have a better outcome, in contrast to non-SARS-CoV-2 ICU patients. Fourth, besides PaO_2/FiO_2 ratio, no statements can be made about the effect of individual clinical parameters on mortality. Nonetheless, the most important clinical parameters are included in APACHE III score, which was used as covariate in the multivariable analyses. Finally, clinical parameters are only recorded during the first 24 hours following ICU admission in the NICE database. Therefore, possible differences between BMI categories and cohorts in the development of complications during ICU stay (e.g. the development of secondary infections or thromboembolic events) could not be assessed. However, such serially collected data would especially be valuable if a relationship between BMI and mortality was apparent in COVID-19 patients, which was not the case. Therefore, it appears unlikely that significant differences in relevant parameters and complications between BMI groups would emerge from a longitudinal dataset.

Conclusion

In conclusion, the obesity paradox, which is the inverse J-shaped association between BMI and mortality in critically ill patients, is not present in critically ill patients with COVID-19-related respiratory failure in contrast to non-SARS-CoV-2 viral and bacterial respiratory infections. Nevertheless, once admitted to the ICU, obese COVID-19 patients also do not have a higher risk for mortality than patients with normal weight. As such, we argue that triage decisions for ICU admission of COVID-19 patients should not be based on BMI. Whether the lack of an association between BMI and mortality in COVID-19 patients is the result of a specific pathophysiological role of (visceral) fat or other factors related to BMI has yet to be elucidated.

Acknowledgements

We would like to thank all Dutch ICUs for their contribution to this study by recording data in the Dutch National Intensive Care Evaluation (NICE) quality registry. We also thank all employees of NICE Research & Support and members of the NICE-COVID-19 Research Consortium for their contribution in data collection of all COVID-19 patients.

References

1. Guan W-j, Ni Z-y, Hu Y, Liang W-h, Ou C-q, He J-x, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. New England Journal of Medicine. 2020;382(18):1708-20.
2. Fang X, Li S, Yu H, Wang P, Zhang Y, Chen Z, et al. Epidemiological, comorbidity factors with severity and prognosis of COVID-19: a systematic review and meta-analysis. Aging (Albany NY). 2020;12(13):12493-503.
3. Zhou Y, Yang Q, Chi J, Dong B, Lv W, Shen L, et al. Comorbidities and the risk of severe or fatal outcomes associated with coronavirus disease 2019: A systematic review and meta-analysis. Int J Infect Dis. 2020;99:47-56.
4. Földi M, Farkas N, Kiss S, Zádori N, Váncsa S, Szakó L, et al. Obesity is a risk factor for developing critical condition in COVID-19 patients: A systematic review and meta-analysis. Obesity Reviews. 2020;21(10):e13095.
5. Simonnet A, Chetboun M, Poissy J, Raverdy V, Noulette J, Duhamel A, et al. High Prevalence of Obesity in Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) Requiring Invasive Mechanical Ventilation. Obesity (Silver Spring). 2020;28(7):1195-9.
6. Hussain A, Mahawar K, Xia Z, Yang W, El-Hasani S. Obesity and mortality of COVID-19. Meta-analysis. Obes Res Clin Pract. 2020;14(4):295-300.
7. Badawi A, Ryoo SG. Prevalence of comorbidities in the Middle East respiratory syndrome coronavirus (MERS-CoV): a systematic review and meta-analysis. Int J Infect Dis. 2016;49:129-33.
8. Kwong JC, Campitelli MA, Rosella LC. Obesity and respiratory hospitalizations during influenza seasons in Ontario, Canada: a cohort study. Clin Infect Dis. 2011;53(5):413-21.
9. Honce R, Schultz-Cherry S. Impact of Obesity on Influenza A Virus Pathogenesis, Immune Response, and Evolution. Front Immunol. 2019;10:1071.
10. Gong MN, Bajwa EK, Thompson BT, Christiani DC. Body mass index is associated with the development of acute respiratory distress syndrome. Thorax. 2010;65(1):44-50.
11. Pickkers P, de Keizer N, Dusseljee J, Weerheijm D, van der Hoeven JG, Peek N. Body mass index is associated with hospital mortality in critically ill patients: an observational cohort study. Crit Care Med. 2013;41(8):1878-83.
12. Hutagalung R, Marques J, Kobylka K, Zeidan M, Kabisch B, Brunkhorst F, et al. The obesity paradox in surgical intensive care unit patients. Intensive Care Med. 2011;37(11):1793-9.
13. Zhi G, Xin W, Ying W, Guohong X, Shuying L. "Obesity Paradox" in Acute Respiratory Distress Syndrome: Asystematic Review and Meta-Analysis. PLoS One. 2016;11(9):e0163677.
14. De Jong A, Wrigge H, Hedenstierna G, Gattinoni L, Chiumello D, Frat JP, et al. How to ventilate obese patients in the ICU. Intensive Care Med. 2020;46(12):2423-35.
15. van Zelst CM, Janssen ML, Pouw N, Birnie E, Castro Cabezas M, Braunstahl GJ. Analyses of abdominal adiposity and metabolic syndrome as risk factors for respiratory distress in COVID-19. BMJ Open Respir Res. 2020;7(1).
16. Biscarini S, Colaneri M, Ludovisi S, Seminari E, Pieri TC, Valsecchi P, et al. The obesity paradox: Analysis from the SMAtteo COvid-19 REgistry (SMACORE) cohort. Nutr Metab Cardiovasc Dis. 2020;30(11):1920-5.

17. Thomson RJ, Hunter J, Dutton J, Schneider J, Khosravi M, Casement A, et al. Clinical characteristics and outcomes of critically ill patients with COVID-19 admitted to an intensive care unit in London: A prospective observational cohort study. PLoS One. 2020;15(12):e0243710.
18. Chand S, Kapoor S, Orsi D, Fazzari MJ, Tanner TG, Umeh GC, et al. COVID-19-Associated Critical Illness-Report of the First 300 Patients Admitted to Intensive Care Units at a New York City Medical Center. J Intensive Care Med. 2020;35(10):963-70.
19. Gupta S, Hayek SS, Wang W, Chan L, Mathews KS, Melamed ML, et al. Factors Associated With Death in Critically Ill Patients With Coronavirus Disease 2019 in the US. JAMA Intern Med. 2020;180(11):1-12.
20. van de Klundert N, Holman R, Dongelmans DA, de Keizer NF. Data Resource Profile: the Dutch National Intensive Care Evaluation (NICE) Registry of Admissions to Adult Intensive Care Units. Int J Epidemiol. 2015;44(6):1850-h.
21. Obesity WHOCo, World Health O. Obesity : preventing and managing the global epidemic : report of a WHO consultation. Geneva: World Health Organization; 2000.
22. Arts D, de Keizer N, Scheffer GJ, de Jonge E. Quality of data collected for severity of illness scores in the Dutch National Intensive Care Evaluation (NICE) registry. Intensive Care Med. 2002;28(5):656-9.
23. de Lusignan S, Dorward J, Correa A, Jones N, Akinyemi O, Amirthalingam G, et al. Risk factors for SARS-CoV-2 among patients in the Oxford Royal College of General Practitioners Research and Surveillance Centre primary care network: a cross-sectional study. Lancet Infect Dis. 2020;20(9):1034-42.
24. Kim SY, Yoo D-M, Min C, Wee JH, Kim J-H, Choi HG. Analysis of Mortality and Morbidity in COVID-19 Patients with Obesity Using Clinical Epidemiological Data from the Korean Center for Disease Control & Prevention. Int J Environ Res Public Health. 2020;17(24):9336.
25. Kooistra EJ, de Nooijer AH, Claassen WJ, Grondman I, Janssen NAF, Netea MG, et al. A higher BMI is not associated with a different immune response and disease course in critically ill COVID-19 patients. International Journal of Obesity. 2021.
26. Fantuzzi G. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol. 2005;115(5):911-9; quiz 20.
27. Zampieri FG, Jacob V, Barbeiro HV, Pinheiro da Silva F, de Souza HP. Influence of Body Mass Index on Inflammatory Profile at Admission in Critically Ill Septic Patients. Int J Inflam. 2015;2015:734857.
28. Sirivongrangson P, Kulvichit W, Payungporn S, Pisitkun T, Chindamporn A, Peerapornratana S, et al. Endotoxemia and circulating bacteriome in severe COVID-19 patients. Intensive Care Med Exp. 2020;8(1):72.
29. van der Voort PHJ, Moser J, Zandstra DF, Muller Kobold AC, Knoester M, Calkhoven CF, et al. Leptin levels in SARS-CoV-2 infection related respiratory failure: A cross-sectional study and a pathophysiological framework on the role of fat tissue. Heliyon. 2020;6(8):e04696.
30. Kruglikov IL, Scherer PE. The Role of Adipocytes and Adipocyte-Like Cells in the Severity of COVID-19 Infections. Obesity. 2020;28(7):1187-90.

Supplemental content 1. Patient characteristics of different BMI categories in the non-SARS-CoV-2 viral pneumonia cohort.

Data presented as median [interquartile range] or n (%). P-values calculated using chi-square tests or Kruskal Wallis tests across all five categories. * p<0.001 compared with BMI 18.5-25 kg/m^2 using pairwise chi-square or Wilcoxon tests.

	BMI < 18.5 kg/m^2 (n=129)	BMI 18.5-25 kg/m^2 (n=1206)	BMI 25-30 kg/m^2 (n=864)	BMI 30-35 kg/m^2 (n=425)	BMI ≥35 kg/m^2 (n=316)	p-value
Age, years	63 [56-71]	66 [58-74]	67 [57-75]	66 [56-74]	61 [53-70]	0.002
Male sex, %	52 (40.3)	619 (51.3)	490 (56.7)	199 (46.8)	124 (39.2)*	<0.001
Lowest PaO_2/FiO_2 ratio of the first 24 hours in the ICU, mmHg	195 [137-264]	176 [114-240]	170 [113-229]	152 [104-219]	155 [107-233]	0.42
Mechanical ventilation on ICU admission	71 (55.0)	623 (51.7)	468 (54.2)	220 (51.8)	173 (54.7)	0.69
Mechanical ventilation in first 24 hours in the ICU	89 (69.0)	837 (69.4)	586 (67.8)	298 (70.1)	233 (73.7)	0.40
Use of vasoactive medication in first 24 hours in the ICU	44 (34.1)	523 (43.4)	369 (42.7)	174 (40.9)	117 (37.0)	0.10
APACHE III APS	52 [40-62]	49 [37-64]	48.5 [37-61]	47 [36-59]	47 [35-61]	0.08
APACHE III Score	65.5 [50-79]	63 [50-79]	62 [50-78]	60 [48-73]	57 [45-76]	0.15
SAPS II score	37 [31-46]	38 [30-47]	38 [30-47]	37 [30-45]	34 [28-44]	0.02
Medical history						
Malignancy	6 (4.7)	66 (5.5)	60 (6.9)	19 (4.5)	7 (2.2)	0.03
Immunological insufficiency	23 (17.8)	185 (15.3)	139 (16.1)	66 (15.5)	29 (9.2)	0.03
COPD	76 (58.9)	572 (47.4)	390 (45.1)	200 (47.1)	144 (45.6)	0.06
Chronic respiratory insufficiency	41 (31.8)*	205 (17.0)	127 (14.7)	71 (16.7)	59 (18.7)	<0.001
Chronic renal failure	8 (6.2)	62 (5.1)	52 (6.0)	31 (7.3)	35 (11.1)	0.01
Chronic cardiovascular insufficiency	3 (2.3)	38 (3.2)	34 (3.9)	17 (4.0)	9 (2.8)	0.71
Diabetes mellitus	10 (7.8)	165 (13.7)	160 (18.5)	131 (30.8)*	123 (38.9)*	<0.001

Supplemental content 2. Patient characteristics of different BMI categories in the bacterial pneumonia cohort. Data presented as median [interquartile range] or n (%). P-values calculated using chi-square tests or Kruskal Wallis tests across all five categories. * p<0.001 compared with BMI 18.5-25 kg/m^2 using pairwise chi-square or Wilcoxon tests.

	BMI < 18.5 kg/m^2 (n=810)	BMI 18.5-25 kg/m^2 (n=6024)	BMI 25-30 kg/m^2 (n=4391)	BMI 30-35 kg/m^2 (n=1815)	BMI ≥35 kg/m^2 (n=1210)	p-value
Age, years	64 [54-72]*	69 [60-77]	70 [62-78]	68 [60-76]	66 [57-73]*	<0.001
Male sex, %	371 (45.8)*	3656 (60.7)	2831 (64.5)	1024 (56.4)	532 (44.0)*	<0.001
Lowest PaO_2/FiO_2 ratio of the first 24 hours in the ICU, mmHg	154 (99-220)	150 (97-223)	144 (97-215)	147 (98-215)	138 (93-204)	0.62
Mechanical ventilation on ICU admission	305 (37.7)	2224 (36.9)	1618 (36.8)	686 (37.8)	563 (46.5)*	<0.001
Mechanical ventilation in first 24 hours in the ICU	420 (51.9)	3248 (53.9)	2320 (52.8)	1005 (55.4)	775 (64.0)*	<0.001
Use of vasoactive medication in first 24 hours in the ICU	316 (39.0)	2597 (43.1)	1822 (41.5)	730 (40.2)	526 (43.5)	0.03
APACHE III APS	55 [41-70]	54 [41-69]	52 [39-66]	51 [39-66]	52 [40-67]	0.05
APACHE III Score	68 [54-84]	69 [55-86]	68 [53-84]	66 [52-83]	65 [52-81]	0.12
SAPS II score	38 [30-48]	39 [31-49]	39 [30-48]	38 [30-48]	38 [30-48]	0.097
Medical history						
Malignancy	61 (7.5)	547 (9.1)	333 (7.6)	130 (7.2)	45 (3.7)*	<0.001
Immunological insufficiency	157 (19.4)	1068 (17.7)	707 (16.1)	249 (13.7)*	145 (12.0)*	<0.001
COPD	362 (44.7)*	2149 (35.7)	1478 (33.7)	644 (35.5)	477 (39.4)	<0.001
Chronic respiratory insufficiency	160 (19.8)*	770 (12.8)	495 (11.3)	208 (11.5)	213 (17.6)*	<0.001
Chronic renal failure	52 (6.4)	452 (7.5)	392 (8.9)	171 (9.4)	119 (9.8)	0.002
Chronic cardiovascular insufficiency	14 (1.7)	207 (3.4)	195 (4.4)	74 (4.1)	59 (4.9)	0.001
Diabetes mellitus	56 (6.9)*	908 (15.1)	931 (21.2)*	610 (33.6)*	493 (40.7)*	<0.001

Supplemental content 3. Patient characteristics of different BMI categories in the trauma cohort. Data presented as median [interquartile range] or n (%). P-values calculated using chi-square tests or Kruskal Wallis tests across all five categories. * p<0.001 compared with BMI 18.5-25 kg/m^2 using pairwise chi-square or Wilcoxon tests.

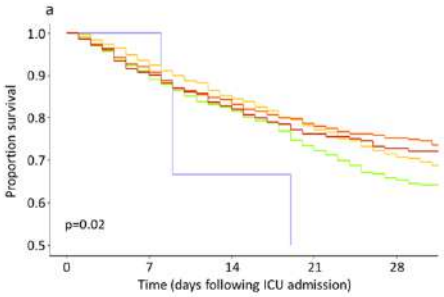
	BMI < 18.5 kg/m^2 (n=461)	BMI 18.5-25 kg/m^2 (n=7797)	BMI 25-30 kg/m^2 (n=5201)	BMI 30-35 kg/m^2 (n=1541)	BMI ≥35 kg/m^2 (n=681)	p-value
Age, years	59 [26-80]	57 [34-76]	62 [48-77]*	63 [50-74]*	60 [48-72]*	<0.001
Male sex, %	169 (36.7)*	5042 (64.7)	3630 (69.8)*	1052 (68.3)	389 (57.1)	<0.001
Lowest PaO_2/FiO_2 ratio of the first 24 hours in the ICU, mmHg	352 [252-450]	317 [226-404]	290 [203-364]	258 [182-342]	250 [174-324]	0.02
Mechanical ventilation on ICU admission	124 (26.9)	2528 (32.4)	1644 (31.6)	440 (28.6)	168 (24.7)*	<0.001
Mechanical ventilation in first 24 hours in the ICU	133 (28.9)	2744 (35.2)	1790 (34.4)	492 (31.9)	204 (30.0)	0.001
Use of vasoactive medication in first 24 hours in the ICU	126 (27.3)	2216 (28.4)	1473 (28.3)	415 (26.9)	179 (26.3)	0.59
APACHE III APS	36 [26-48]	34 [24-50]	33 [23-48]	32 [23-47]	31 [22-46]	0.30
APACHE III Score	48 [34-64]	45 [31-64]	46 [32-63]	44 [32-62]	42 [30-59]	0.10
SAPS II score	28 [20-37]	28 [20-39]	28 [20-39]	27 [20-37]	25.5 [17-36]*	<0.001
Medical history						
Malignancy	6 (1.3)	94 (1.2)	66 (1.3)	25 (1.6)	10 (1.5)	0.75
Immunological insufficiency	18 (3.9)	159 (2.0)	133 (2.6)	40 (2.6)	14 (2.1)	0.04
COPD	48 (10.4)	512 (6.6)	329 (6.3)	178 (11.6)*	85 (12.5)*	<0.001
Chronic respiratory insufficiency	11 (2.4)	82 (1.1)	65 (1.2)	42 (2.7)*	35 (5.1)*	<0.001
Chronic renal failure	20 (4.3)	237 (3.0)	160 (3.1)	70 (4.5)	44 (6.5)*	<0.001
Chronic cardiovascular insufficiency	14 (3.0)	184 (2.4)	95 (1.8)	44 (2.9)	20 (2.9)	0.05
Diabetes mellitus	19 (4.1)	462 (5.9)	543 (10.4)*	236 (15.3)*	190 (27.9)*	<0.001

Supplemental content 4. Clinical outcomes of different BMI categories in all four cohorts. Data presented as median [interquartile range] or n (%). P-values calculated using chi-square tests or Kruskal Wallis tests across all five categories. * p<0.001 compared with BMI 18.5-25 kg/m^2 using pairwise chi-square or Wilcoxon tests.

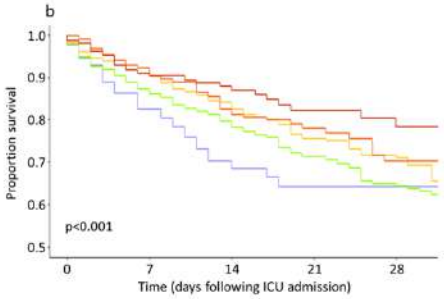
	BMI < 18.5 kg/m^2	BMI 18.5-25 kg/m^2	BMI 25-30 kg/m^2	BMI 30-35 kg/m^2	BMI ≥35 kg/m^2	p-value
COVID-19	**n=10**	**n=592**	**n=1196**	**n=565**	**n=272**	
Number of readmissions	0 (0)	0 (0)	6 (0.5)	4 (0.7)	1 (0.4)	0.35
LOS ICU of survived patients, days	13 [8-41]	16 [8-31]	17 [10-32]	18 [10-31]	19 [12-32]	0.39
LOS ICU deceased patients, days	9 [9-19]	11 [5-21]	14 [7-23]	10 [5-20]	9 [4-15]	0.63
LOS hospital of survived patients, days	18 [18-46]	28 [18-47]	30 [19-46]	31 [19-46]	31 [20-46]	0.34
LOS hospital of deceased patients, days	9 [9-19]	14 [7-23]	16 [9-25]	12 [6-22]	11 [6-18]	0.96
28-day mortality, n (%)	5 (50.0)	170 (28.7)	295 (24.7)	124 (21.9)	66 (24.3)	0.03
In-hospital mortality, n (%)	5 (50.0)	194 (32.8)	353 (29.5)	147 (26.0)	70 (25.7)	0.04
non-SARS-CoV-2 viral pneumonia	**n=129**	**n=1206**	**n=864**	**n=425**	**n=316**	
Number of readmissions	2 (1.5)	22 (1.8)	13 (1.5)	9 (2.1)	2 (0.6)	0.59
LOS ICU of survived patients, days	4 [2-9]	4 [2-8]	4 [2-8]	4 [2-9]	4 [2-9]	0.64
LOS ICU deceased patients, days	4 [2-9]	5 [2-10]	6 [2-14]	6 [3-13]	4 [2-11]	0.47
LOS hospital of survived patients, days	12 [8-22]	11 [7-18]	10 [7-17]	11 [7-18]	11 [7-18]	0.31
LOS hospital of deceased patients, days	6 [4-11]	7 [3-14]	9 [3-17]	8 [4-15]	5 [3-12]	0.74
28-day mortality, n (%)	34 (26.4)	237 (19.7)	134 (15.5)	64 (15.1)	38 (12.0)	<0.001
In-hospital mortality, n (%)	34 (26.4)	250 (20.7)	152 (17.6)	69 (16.2)	40 (12.7)	0.002
Bacterial pneumonia	**n=810**	**n=6024**	**n=4391**	**n=1815**	**n=1210**	
Number of readmissions	69 (7.8)	585 (8.9)	439 (9.1)	175 (8.8)	80 (6.2)	0.02
LOS ICU of survived patients, days	3 [2-6]	3 [2-7]	3 [2-7]	3 [2-7]	4 [2-7]	0.52
LOS ICU deceased patients, days	3 [1-6]	4 [1-8]	4 [2-9]	4 [2-9]	5 [2-12]	0.40
LOS hospital of survived patients, days	12 [7-19]	12 [8-20]	11 [7-19]	11 [7-18]	12 [7-19]	0.86
LOS hospital of deceased patients, days	7 [4-12]	7 [3-14]	9 [4-16]	8 [3-14]	7 [3-16]	0.44
28-day mortality, n (%)	228 (28.1)*	1331 (22.1)	799 (18.2)*	322 (17.7)*	178 (14.7)*	<0.001
In-hospital mortality, n (%)	236 (29.1)*	1401 (23.3)	850 (19.4)*	340 (18.7)*	192 (15.9)*	<0.001

Trauma	**n=461**	**n=7797**	**n=5201**	**n=1541**	**n=681**	
Number of readmissions	6 (1.3)	108 (1.4)	82 (1.6)	28 (1.8)	14 (2.0)	0.50
LOS ICU of survived patients, days	1 [1-2]	1 [1-3]	1 [1-3]	1 [1-3]	1 [1-3]	0.98
LOS ICU deceased patients, days	2 [1-3]	2 [1-5]	3 [1-7]	3 [1-9]	3 [1-15]	0.48
LOS hospital of survived patients, days	8 [5-15]	8 [5-16]	9 [5-16]	10 [5-17]	9 [5-17]	0.20
LOS hospital of deceased patients, days	5 [2-10]	5 [2-10]	5 [2-11]	6 [3-13]	7 [4-15]	0.85
28-day mortality, n (%)	45 (9.8)	701 (9.0)	458 (8.8)	143 (9.3)	47 (6.9)	0.37
In-hospital mortality, n (%)	48 (10.4)	726 (9.3)	482 (9.3)	150 (9.7)	50 (7.3)	0.40

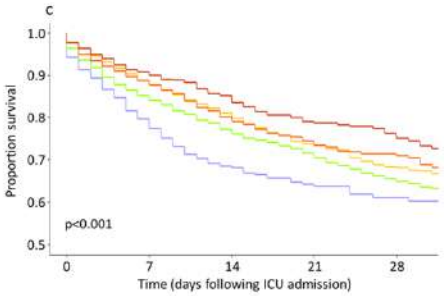
Supplemental content 5. Twenty-eight-day survival curves of different BMI categories in (a) COVID-19 patients, (b) non-SARS-CoV-2 viral pneumonia patients, (c) bacterial pneumonia patients and (d) trauma patients. P-values were calculated using log-rank tests.



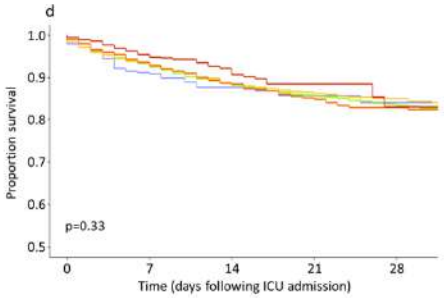
BMI (kg/m²)	Number at risk				
<18.5	10	9	6	3	2
18.5-25	592	519	410	295	210
25-30	1196	1093	892	672	493
30-35	565	501	411	310	243
≥35	272	240	195	151	113



BMI (kg/m²)	Number at risk				
<18.5	129	82	42	22	8
18.5-25	1206	757	377	180	110
25-30	864	555	267	139	93
30-35	425	285	1334	77	46
≥35	316	201	100	51	36



BMI (kg/m²)	Number at risk				
<18.5	810	489	226	120	78
18.5-25	6024	3915	1977	1021	574
25-30	4391	2867	1350	744	426
30-35	1815	1178	534	296	155
≥35	1210	828	421	216	135



BMI (kg/m²)	Number at risk				
<18.5	461	228	107	68	46
18.5-25	7797	4134	1986	1157	732
25-30	5201	2866	1392	837	523
30-35	1541	907	437	257	159
≥35	681	370	189	120	69

Supplemental content 6. Estimated odds ratios of in-hospital mortality of the multivariate regression model in the COVID-19 cohort. Covariates used for this analyses included sex, age, medical history (chronic diagnoses), APACHE III acute physiology score (APS), vasoactive medication and mechanical ventilation and PaO2/FiO2 ratio in the first 24 hours following ICU admission.

Variable	Odds ratio [95% CI]
BMI <18.5 kg/m^2	1.92 [0.51-7.13]
BMI 25-30 kg/m^2	0.95 [0.75-1.21]
BMI 30-35 kg/m^2	0.87 [0.65-1.16]
BMI ≥35 kg/m^2	1.15 [0.79-1.67]
Female	**1.47 [1.18-1.83]**
Age 40-45 years	0.79 [0.22-2.80]
Age 45-50 years	1.21 [0.49-3.02]
Age 50-55 years	0.78 [0.32-1.88]
Age 55-60 years	1.85 [0.84-4.08]
Age 60-65 years	**2.61 [1.20-5.69]**
Age 65-70 years	**4.46 [2.07-9.61]**
Age 70-75 years	**5.45 [2.54-11.69]**
Age 75-80 years	**8.66 [3.98-18.85]**
Age 80-85 years	**14.79 [5.79-37.79]**
Age ≥85 years	**20.73 [3.62-118.54]**
Immunological insufficiency	1.13 [0.79-1.63]
Renal insufficiency	**1.98 [1.16-3.38]**
Chronic respiratory insufficiency	**1.54 [1.17-2.04]**
Cardiovasculair insufficiency	1.77 [0.81-3.87]
Cirrhosis	1.47 [0.09-24.33]
Malignancy	**2.53 [1.38-4.62]**
Diabetes mellitus	**1.29 [1.02-1.63]**
APACHE IV APS 24-34	1.23 [0.69-2.2]
APACHE IV APS 34-45	1.37 [0.80-2.37]
APACHE IV APS 45-63	1.64 [0.96-2.82]
APACHE IV APS ≥63	**3.87 [2.21-6.78]**
Mechanical ventilation during first 24 hours in the ICU	**1.48 [1.09-2.02]**
Use of vasoactive medication	1.17 [0.90-1.51]
PaO_2/FiO_2 ratio 155-226 mmHg	0.89 [0.69-1.15]
PaO_2/FiO_2 ratio 226-296 mmHg	0.65 [0.42-1.00]
PaO_2/FiO_2 ratio 296-371 mmHg	0.65 [0.34-1.25]
PaO_2/FiO_2 ratio ≥371 mmHg	0.89 [0.43-1.83]
Missing PaO_2/FiO_2 ratio	1.02 [0.74-1.42]

Supplemental content 7. Estimated odds ratios of in-hospital mortality of the multivariate regression model in the non-SARS-CoV-2 viral pneumonia cohort. Covariates used for this analyses included BMI, sex, age, medical history (chronic diagnoses), APACHE III acute physiology score (APS), vasoactive medication and mechanical ventilation and PaO2/FiO2 ratio in the first 24 hours following ICU admission.

Variable	Odds ratio [95% CI]
BMI <18.5 kg/m^2	1.50 (0.95-2.37)
BMI 25-30 kg/m²	**0.78 (0.61-0.99)**
BMI 30-35 kg/m^2	0.76 (0.55-1.04)
BMI ≥35 kg/m^2	**0.64 (0.43-0.95)**
Female	0.84 (0.69-1.04)
Age 40-45 years	0.67 (0.12-3.71)
Age 45-50 years	**3.31 (1.14-9.63)**
Age 50-55 years	**3.35 (1.20-9.33)**
Age 55-60 years	**4.69 (1.79-12.29)**
Age 60-65 years	**5.69 (2.21-14.65)**
Age 65-70 years	**6.57 (2.56-16.84)**
Age 70-75 years	**8.67 (3.40-22.16)**
Age 75-80 years	**12.64 (4.93-32.40)**
Age 80-85 years	**11.18 (4.29-29.17)**
Age ≥85 years	**15.54 (5.76-41.9)**
Immunological insufficiency	**1.47 (1.12-1.95)**
Renal insufficiency	1.14 (0.78-1.68)
Chronic respiratory insufficiency	**1.63 (1.30-2.04)**
Cardiovasculair insufficiency	**2.42 (1.52-3.86)**
Cirrhosis	1.58 (0.32-7.75)
Malignancy	**2.28 (1.52-3.42)**
Diabetes mellitus	0.90 (0.70-1.16)
APACHE IV APS 24-34	1.97 (0.84-4.59)
APACHE IV APS 34-45	2.11 (0.93-4.79)
APACHE IV APS 45-63	**3.05 (1.36-6.84)**
APACHE IV APS ≥63	**6.20 (2.73-14.05)**
Mechanical ventilation during first 24 hours in the ICU	**1.85 (1.42-2.41)**
Use of vasoactive medication	1.11 (0.89-1.38)
PaO_2/FiO_2 ratio 155-226 mmHg	**0.71 (0.55-0.93)**
PaO_2/FiO_2 ratio 226-296 mmHg	0.72 (0.52-1.00)
PaO_2/FiO_2 ratio 296-371 mmHg	**0.60 (0.37-0.97)**
PaO_2/FiO_2 ratio ≥371 mmHg	**0.43 (0.21-0.89)**
Missing PaO_2/FiO_2 ratio	0.76 (0.54-1.07)

Supplemental content 8. Estimated odds ratios of 28-day mortality of the multivariate regression model in the bacterial pneumonia cohort. Covariates used for this analyses included sex, age, medical history (chronic diagnoses), APACHE III acute physiology score (APS), vasoactive medication and mechanical ventilation and PaO2/FiO2 ratio on ICU admission.

Variable	Odds ratio [95% CI]
BMI <18.5 kg/m^2	**1.88 (1.57-2.25)**
BMI 25-30 kg/m^2	**0.78 (0.70-0.86)**
BMI 30-35 kg/m^2	**0.81 (0.70-0.93)**
BMI ≥35 kg/m^2	**0.73 (0.61-0.87)**
Female	1.06 (0.97-1.16)
Age 40-45 years	1.49 (0.87-2.57)
Age 45-50 years	**1.81 (1.15-2.85)**
Age 50-55 years	**2.22 (1.48-3.33)**
Age 55-60 years	**2.32 (1.59-3.41)**
Age 60-65 years	**2.88 (1.99-4.15)**
Age 65-70 years	**3.85 (2.69-5.52)**
Age 70-75 years	**5.07 (3.55-7.23)**
Age 75-80 years	**6.80 (4.76-9.71)**
Age 80-85 years	**8.67 (6.05-12.41)**
Age ≥85 years	**12.43 (8.56-18.06)**
Immunological insufficiency	**1.28 (1.14-1.45)**
Renal insufficiency	1.14 (0.98-1.32)
Chronic respiratory insufficiency	**1.09 (1.00-1.20)**
Cardiovasculair insufficiency	**1.74 (1.43-2.13)**
Cirrhosis	**2.77 (1.99-3.86)**
Malignancy	**2.19 (1.88-2.55)**
Diabetes mellitus	0.93 (0.83-1.03)
APACHE IV APS 24-34	1.12 (0.77-1.63)
APACHE IV APS 34-45	**1.56 (1.11-2.19)**
APACHE IV APS 45-63	**2.37 (1.70-3.30)**
APACHE IV APS ≥63	**5.14 (3.68-7.16)**
Mechanical ventilation during first 24 hours in the ICU	**1.71 (1.55-1.89)**
Use of vasoactive medication	**1.11 (1.01-1.22)**
PaO_2/FiO_2 ratio 155-226 mmHg	**0.75 (0.67-0.84)**
PaO_2/FiO_2 ratio 226-296 mmHg	**0.56 (0.47-0.66)**
PaO_2/FiO_2 ratio 296-371 mmHg	**0.62 (0.49-0.77)**
PaO_2/FiO_2 ratio ≥371 mmHg	**0.64 (0.46-0.89)**
Missing PaO_2/FiO_2 ratio	0.97 (0.85-1.11)

Supplemental content 9. Estimated odds ratios of 28-day mortality of the multivariate regression model in the multiple trauma cohort. Covariates used for this analyses included sex, age, medical history (chronic diagnoses), APACHE III acute physiology score (APS), vasoactive medication and mechanical ventilation and PaO2/FiO2 ratio in the first 24 hours following ICU admission.

Variable	Odds ratio [95% CI]
BMI <18.5 kg/m^2	1.23 (0.86-1.78)
BMI 25-30 kg/m^2	0.90 (0.78-1.03)
BMI 30-35 kg/m^2	0.99 (0.79-1.23)
BMI ≥35 kg/m^2	0.81 (0.57-1.15)
Female	**1.45 (1.26-1.66)**
Age 40-45 years	1.41 (0.94-2.11)
Age 45-50 years	**1.56 (1.10-2.23)**
Age 50-55 years	**2.30 (1.66-3.19)**
Age 55-60 years	**1.85 (1.33-2.56)**
Age 60-65 years	**2.65 (1.95-3.60)**
Age 65-70 years	**3.76 (2.82-5.01)**
Age 70-75 years	**5.40 (4.13-7.08)**
Age 75-80 years	**6.56 (5.00-8.61)**
Age 80-85 years	**9.44 (7.20-12.37)**
Age ≥85 years	**12.44 (9.54-16.21)**
Immunological insufficiency	1.08 (0.74-1.57)
Renal insufficiency	1.00 (0.76-1.30)
Chronic respiratory insufficiency	**1.26 (1.03-1.54)**
Cardiovasculair insufficiency	**1.38 (1.00-1.89)**
Cirrhosis	**2.44 (1.30-4.58)**
Malignancy	1.52 (0.96-2.41)
Diabetes mellitus	1.11 (0.91-1.35)
APACHE IV APS 24-34	**1.77 (1.22-2.57)**
APACHE IV APS 34-45	**2.87 (2.01-4.09)**
APACHE IV APS 45-63	**5.57 (3.93-7.91)**
APACHE IV APS ≥63	**27.53 (19.43-39.02)**
Mechanical ventilation during first 24 hours in the ICU	**1.90 (1.59-2.26)**
Use of vasoactive medication	**1.97 (1.72-2.26)**
PaO_2/FiO_2 ratio 155-226 mmHg	**0.76 (0.60-0.98)**
PaO_2/FiO_2 ratio 226-296 mmHg	0.80 (0.63-1.01)
PaO_2/FiO_2 ratio 296-371 mmHg	**0.63 (0.50-0.81)**
PaO_2/FiO_2 ratio ≥371 mmHg	**0.64 (0.50-0.82)**
Missing PaO_2/FiO_2 ratio	0.83 (0.67-1.03)

CHAPTER 4

Long-term impairments are most pronounced in critically ill COVID-19 patients with severe obesity

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Introduction

While obesity is an important risk factor for the development of severe COVID-19 (1), once admitted to the intensive care unit (ICU), no clear relationship between body mass index (BMI) and short-term outcomes is present (2). Many patients experience long-term symptoms following a ICU admission with COVID-19 (3, 4), but it is unknown whether the prevalence rates of long-term symptoms differ between different BMI groups. Therefore, this study aimed to examine the differences between BMI categories in the occurrence of physical, mental and cognitive symptoms 3 and 12 months following ICU treatment in critically ill patients with COVID-19.

Methods

In this prospective multicenter cohort study, patients with COVID-19 admitted to ICUs in 11 Dutch hospitals between March 1, 2020 and July 1, 2020 were included after obtaining informed consent (5). Patients received three questionnaires regarding their health status: pre-ICU (baseline; answered in retrospect as soon possible after ICU admission), 3 and 12 months post-ICU treatment, which could be completed by the patient or a proxy. Patients who completed the baseline and 12-months follow-up questionnaires without missing data on BMI were included in the analyses. Four hospitals were not able to provide 3-months data. Primary outcomes were occurrence of physical (fatigue and new physical problems), mental (symptoms of anxiety, depression, and post-traumatic stress disorder [PTSD]) and cognitive symptoms. Patients were categorized into BMI categories according to the World Health Organization (WHO) definition: normal weight (18.5-25.0 kg/m^2), overweight (25.0-30.0 kg/m^2), obese class I (30.0-35.0 kg/m^2) and obese class II/III (≥35.0 kg/m^2) (6). Differences in patient characteristics between BMI categories were tested using $\chi 2$ or Kruskal-Wallis tests followed by post-hoc pairwise $\chi 2$ or Dunn's multiple comparisons tests, respectively. Differences in symptom occurrence rates between BMI categories were tested using multivariable logistic regression analysis including the following covariables: age, sex, severity of illness (APACHE-IV), and length of stay (LOS) in ICU. Additionally, the presence of symptoms pre-ICU was included as covariable for the 3- and 12-month analysis as well. Furthermore, differences in change in outcome scores between baseline and both moments of follow-up were tested using linear regression analysis (available for fatigue, anxiety, depression and cognitive impairment), including the same covariates as in the logistic regression analysis. IBM SPSS version 25 was used for the statistical analysis.

Results

A total of 302 patients participated of whom 239 patients were included because they completed the 12-months follow-up. Included patients had similar baseline characteristics compared to non-responders. Patients with obesity class II/III were younger, less likely male and had a lower APACHE-IV score and shorter LOS-ICU compared to normal weight patients (Table 1). No statistically significant differences between BMI categories in baseline physical, mental, or cognitive symptoms were present (Figure 1).

There is a significant interplay between the BMI categories and the incidence of physical and mental symptoms at both 3- and 12-months. This was most pronounced for the class II/III category at 3-months for symptoms of fatigue, anxiety, and PTSD (Figure 1).

At 12-months post-ICU, patients in the obesity class II/III category were still significantly more likely to experience symptoms of fatigue, physical symptoms, and symptoms of anxiety, depression and PTSD compared to the other BMI categories (Figure 1). Cognitive symptoms were similar between BMI categories.

Additionally, patients in obesity class II/III experience a greater deterioration in outcome scores compared to the other BMI categories for fatigue, anxiety and cognitive impairment at 3- and 12-month follow-up (Figure 1).

Table 1. Demographic patient characteristics.

BMI category*	Normal weight 18.5-25 kg/m² (n=69)	Overweight 25-30 kg/m² (n=108)	Obesity Class I 30-35 kg/m² (n=41)	Obesity Class II/III ≥35 kg/m² (n=21)	p-value (overall)
Patient characteristics					
Age, median [IQR], y	65 [59-70]	62 [55-69]	58 [52-65][a]	57 [53-61][a]	<0.001
Sex, male, n (%)	52 (75)	84 (78)	27 (66)	9 (43)[a b]	0.01
BMI, median [IQR], kg/m²	23.9 [22.9-24.6]	27.3 [26.3-28.4][a]	32.6 [30.8-32.7][a b]	37.0 [36.4-39.1][a b]	<0.001
APACHE-IV, median [IQR]	59 [49-68]	59 [49-68]	54 [43-68]	50 [42-58][a]	0.02
LOS ICU, median [IQR], days	27 [13-39]	18 [11-31]	18 [12-28]	12 [9-20][a]	0.004
LOS hospital, median [IQR],-days	39 [25-54]	29 [20-44]	27 [18-43]	24 [15-35][a]	0.006

** There were no patients with a BMI <18.5 kg/m²*
ap<0.05 compared to BMI 18.5-25 kg/m²
b p<0.05 compared to BMI 25-30 kg/m²
IQR: interquartile range. BMI: Body Mass Index. APACHE-IV: Acute Physiology and Chronic Health Evaluation IV. LOS: length of stay. ICU: intensive care unit. CFS: Clinical frailty Scale. COVID-19: coronavirus disease 2019. CIS: Checklist individual Strength. HADS-A/HADS-D: Hospital Anxiety and Depression Scale. PTSD: Post-traumatic stress disorder. CFQ: Cognitive Failure Questionnaire.

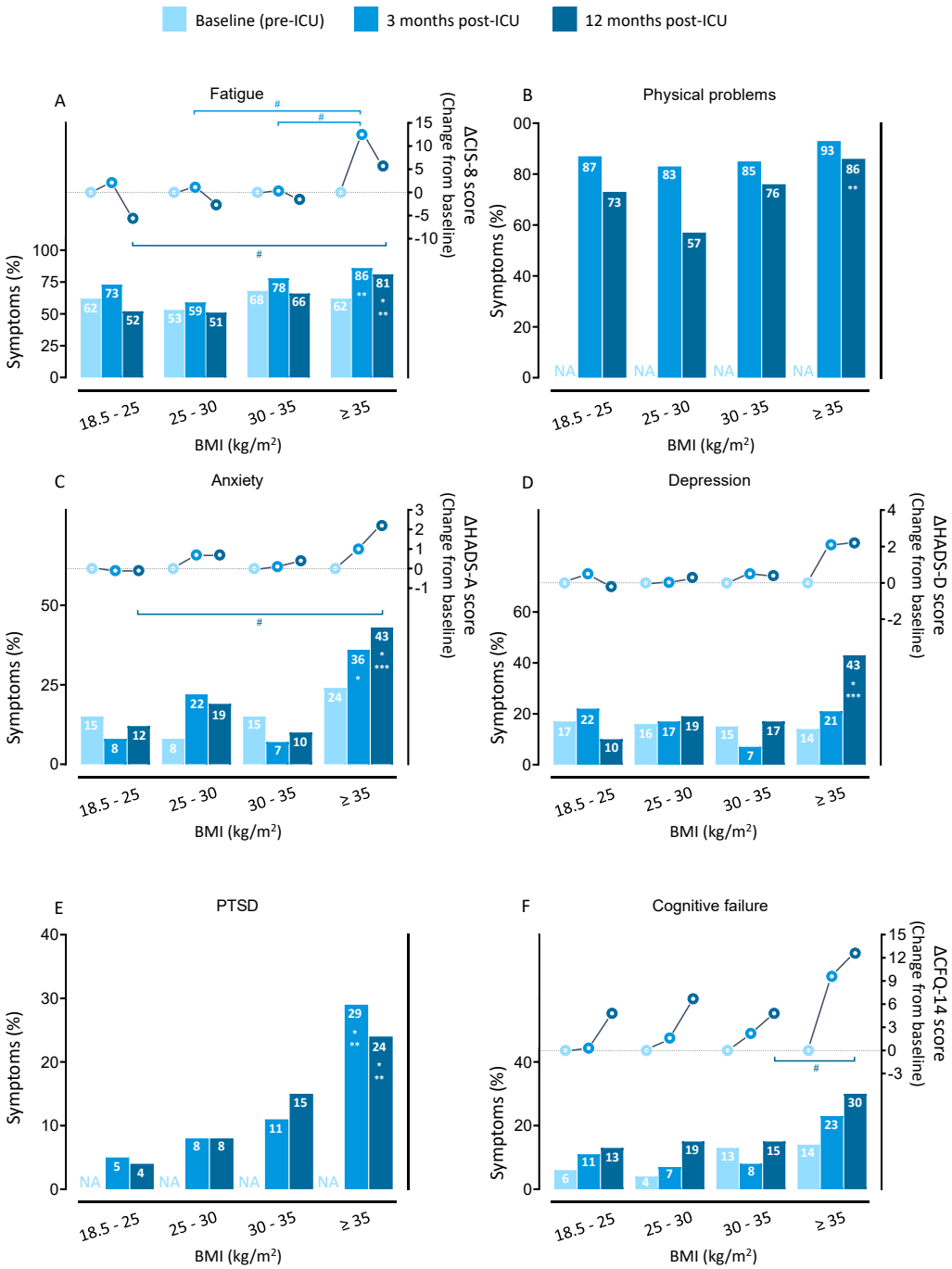
Baseline (pre-ICU)
3 months post-ICU
12 months post-ICU
A
Fatigue
Symptoms (%)
ΔCIS-8 score (Change from baseline)
B
Physical problems
Symptoms (%)
C
Anxiety
Symptoms (%)
ΔHADS-A score (Change from baseline)
D
Depression
Symptoms (%)
ΔHADS-D score (Change from baseline)
E
PTSD
Symptoms (%)
F
Cognitive failure
Symptoms (%)
ΔCFQ-14 score (Change from baseline)
18.5 - 25
25 - 30
30 - 35
≥ 35
BMI (kg/m²)

Figure 1. *Prevalence of symptoms (left y axis) and absolute change scores (right y axis) between baseline (light blue dots) and 3- months (azure blue dots) and 12- months (dark blue dots) follow-up of A) fatigue, B) physical problems C) anxiety, D) depression E) post-traumatic stress disorder (PTSD) and F) cognitive impairment at baseline (pre-ICU), and 3- and 12-months post-ICU in different Body Mass Index (BMI) categories in patients with COVID-19. NA: occurrence rates of PTSD as a result of ICU treatment and new physical problems as a result of ICU treatment are only available post-ICU. aFatigue was defined by a score of ≥27 on the Checklist individual Strength – fatigue subscale (CIS-8), ranging from 8-56. bPhysical problems were defined as at least one new or worsened problem (e.g. weakened condition, muscle weakness, dyspnea, abdominal problems). c+dAnxiety and depression symptoms were defined by a score of ≥8 on the Hospital Anxiety and Depression Scale (HADS) Anxiety and Depression subscales, both ranging from 0-21. eSymptoms of Post-traumatic stress disorder (PTSD) were defined by a mean score of ≥1.75 on the Impact Event Scale (IES)-6, ranging from 0-4. fCognitive impairment was defined as a score of ≥43 on the abbreviated Cognitive Failure Questionnaire (CFQ-14), ranging from 0-100.*
** p<0.05 compared to BMI 18.5-25 kg/m²*
*** p<0.05 compared to BMI 25-30 kg/m²*
**** p<0.05 compared to BMI 30-35 kg/m²*
 # Change in outcome score between baseline and follow-up is statistically significant between groups (p<0.05)

Discussion

Severely obese ICU survivors with COVID-19 experience more long-term physical and mental symptoms compared to patients in lower BMI categories, whereas no significant differences were present prior to ICU admission. In contrast with the absence of an association of BMI and ICU mortality (2), these long-term symptoms may be directly related to BMI. The long-term impact of COVID-19 may be more pronounced in obese patients or they may have limited ability to rehabilitate following their hospital stay. Therefore, future research should also focus on the role of BMI in long-term symptoms in non-COVID-19 ICU survivors as well. However, regardless the underlying causes, it implicates that long-term follow-up is of explicit importance in obese COVID-19 ICU patients.

References

1. Raeisi T, Mozaffari H, Sepehri N, Darand M, Razi B, Garousi N, Alizadeh M, Alizadeh S. The negative impact of obesity on the occurrence and prognosis of the 2019 novel coronavirus (COVID-19) disease: a systematic review and meta-analysis. Eat Weight Disord 2021: 1-19.
2. Kooistra EJ, Brinkman S, van der Voort PHJ, de Keizer NF, Dongelmans DA, Kox M, Pickkers P. Body Mass Index and Mortality in Coronavirus Disease 2019 and Other Diseases: A Cohort Study in 35,506 ICU Patients. Critical care medicine 2022; 50: e1-e10.
3. Morin L, Savale L, Pham T, Colle R, Figueiredo S, Harrois A, Gasnier M, Lecoq AL, Meyrignac O, Noel N, Baudry E, Bellin MF, Beurnier A, Choucha W, Corruble E, Dortet L, Hardy-Leger I, Radiguer F, Sportouch S, Verny C, Wyplosz B, Zaidan M, Becquemont L, Montani D, Monnet X. Four-Month Clinical Status of a Cohort of Patients After Hospitalization for COVID-19. Jama 2021; 325: 1525-1534.
4. Heesakkers H, van der Hoeven JG, Corsten S, Janssen I, Ewalds E, Simons KS, Westerhof B, Rettig TCD, Jacobs C, van Santen S, Slooter AJC, van der Woude MCE, van den Boogaard M, Zegers M. Clinical Outcomes Among Patients With 1-Year Survival Following Intensive Care Unit Treatment for COVID-19. JAMA 2022.
5. Geense W, Zegers M, Vermeulen H, van den Boogaard M, van der Hoeven J. MONITOR-IC study, a mixed methods prospective multicentre controlled cohort study assessing 5-year outcomes of ICU survivors and related healthcare costs: a study protocol. BMJ Open 2017; 7: e018006.
6. Obesity WCo. World Health Organization: Obesity: Preventing and Managing the Global Epidemic: Report of a WHO Consultation. Geneva, Switzerland, World Health Organization. 2000.

CHAPTER 5

How COVID-19 will shape the future of critical care for obese patients: A narrative review

5

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Abstract

Since the end of 2019, the SARS-CoV-2 pandemic has infected nearly 520 million people and caused over six million deaths. Obesity was quickly considered a risk factor for severe COVID-19. With nearly two years of hindsight, the pathophysiology and relationship between obesity and COVID-19 are becoming clearer. Obesity has always affected ICU patient management. First, during the initial phase of the pandemic, the number of ICU patients with obesity doubled, with up to 40% of the patients admitted having obesity, providing a snapshot of the future epidemiology in the ICU. Second, for patients in the ICU, obesity was a risk factor for prolonged ICU length of stay, severe respiratory failure with an increased risk of mechanical ventilation and complications such as acute renal injury. Third, obesity has also challenged the "obesity paradox" regarding ICU mortality as previously described in critically ill obese patients. Post-COVID-19 syndrome, described as a new clinical entity, has also particularly impacted these patients, leading to impaired long-term quality of life. There are real issues in the management of these patients, and clinicians and other health care professionals should be trained to recognize the severity of COVID-19 infection and post-COVID-19 symptoms and perform close clinical monitoring of obese patients, even after the acute phase of infection and until complete recovery. In this review, we focus on the central role of obesity in critically ill patients during this pandemic by highlighting its specificities, updating the potential immunological mechanisms regarding COVID-19 severity and identifying areas for future investigations.

Background

Severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2) was the third pathogenic coronavirus to emerge during the last two decades, emerging after SARS coronavirus 1 in 2002 and Middle East respiratory syndrome coronavirus in 2012. From the Chinese epicentre, the coronavirus disease 2019 (COVID-19) pandemic spread rapidly and is now evolving in successive waves of varying intensity. Over 520 million cases have been confirmed, resulting in more than six million deaths across six continents as reported by the World Health Organization (1). COVID-19 symptoms have varying severity, ranging from an initial asymptomatic stage, "flu-like syndrome" with anosmia or mild symptoms with active viral replication to more severe pulmonary symptoms leading to acute respiratory failure with systemic inflammation and multisystem organ failure (2). This pandemic has led to important research efforts to identify the risk factors for developing severe COVID-19 and mortality. During the first wave, the initial data pointed to elderly individuals, who were particularly vulnerable, such as those with chronic cardiovascular, respiratory, or kidney diseases and diabetes mellitus (3). Obesity, defined as a body mass index (BMI) > 30 kg/m^2, quickly emerged as a strong risk factor for severe COVID-19, intensive care unit (ICU) admission and increased length of stay in the hospital (4–8). Over the last several months, many other studies have shown an association between obesity and acute respiratory distress syndrome (ARDS), which occurs in 40% of patients with pneumonia and 60–80% of those requiring intensive care (9,10), increasing the risk of mechanical ventilation (MV), morbidity and mortality (11–17). Indeed, before this pandemic, there was no reported increased hospital mortality in obese patients with moderate or severe ARDS (18); lower mortality has even been suggested, supporting a possible "obesity paradox" (19,20). However, during this pandemic and in the general population, obesity has been shown to be an independent risk factor for high mortality. In critically ill patients with COVID-19-related respiratory failure, obesity was not a risk factor for death, challenging this previously described paradox in these patients (21). Moreover, during the first wave, many studies reported that disease severity increases with BMI, such as acute kidney injury (AKI), with an initial association between obesity and mortality in COVID-19 patients in the ICU (22). Obesity was also described as a risk factor for post-COVID-19 syndrome (23), which is a new clinical entity. Thus, obesity is always a risk factor for COVID-19 patient management. After more than two years of caring for these patients, what is the true impact of obesity on critically ill patients? Therefore, it is of paramount importance to better understand the complex relationship between obesity and COVID-19. The molecular mechanisms enhancing viral entry and spread, the existing metabolic overload, the dysregulation of host cell homeostasis, adiposopathy and SARS-CoV-2 viral adipotrophy are different underlying mechanisms

that contribute to exacerbating the pathological pathways and severe adverse outcomes related to COVID-19 in obese patients in comparison with nonobese patients (15). While some studies have suggested a specific immunological signature with a veritable cytokine storm (24,25), particularly in obese patients who are already in a hyperinflammatory state, others have disproved this pathway (26). In this review, we provide a broad overview of the strong interaction between COVID-19 and obesity in critically ill patients to highlight the role of obesity and its potential immunological mechanisms in COVID-19 severity, ARDS, coinfections, and AKI and to identify areas for future investigations.

At the outset of the pandemic

Obesity in the intensive care unit: prevalence and consequences

Obesity is a major public health issue associated with an increased risk of many diseases, such as dyslipidaemia, hypertension, cardiac and renal diseases, and type 2 diabetes mellitus, and has become a pandemic, affecting high-, middle- and low-income countries (27). Since 1975, its prevalence has tripled to affect between 603 and 640 million adults worldwide (27,28). As a growing portion of the general population, obese patients account for a significant portion of ICU admissions. In the pre-COVID-19 era, 15 to 20% of patients admitted to the ICU were obese (29–31).

The COVID-19 pandemic has led to a sharp increase in the prevalence of obesity in the ICU, which has doubled (up to 40% of ICU-admitted patients) (21,32–35). Obesity is associated with a severe disease course with an increased risk of ICU admission (4–8). Among patients < 60 years of age, BMIs of 30 to 34 kg/m^2 and >35 kg/m^2 are associated with an increased risk of ICU admission of 1.8 times and 3.6 times, respectively, compared with nonobese patients (36). Moreover, the prevalence of obesity is increased in mechanically ventilated COVID-19 patients, with a more than 7-fold higher risk of being intubated and ventilated for those with a BMI >35 than for those with a BMI <25 kg/m^2 (11). As expected, a prolonged duration of MV and ICU length of stay have been reported in obese COVID-19 patients (5,6,32). As obesity has broad effects on pulmonary physiology, endothelial function, glucose and lipid metabolism, adipose tissue biology, and immune system function, and as emerging data suggest parallel changes in several molecular and signalling pathways that are dysregulated in both SARS-CoV-2 infection and obesity (2), it is tempting to associate obesity with an increased susceptibility to SARS-CoV-2 infection (12) and especially to severe forms of COVID-19 (5,13,14) with their associated increased rates of hospital and ICU admission (11) and their related higher morbidity and mortality (15,16). A recent meta-analysis of 75 studies and 399,461 patients showed that obesity was associated with an increased risk of COVID-19 disease by 46% (odds ratio (OR) = 1.46; 95% CI 1.30-1.65)

and increased rates of COVID-19-related hospitalization by 113% (OR = 2.13; 95% CI 1.74-2.60), ICU admission by 74% (OR = 1.74; 95% CI 1.46-2.08) and mortality by 48% (OR = 1.48; 95% CI 1.22-1.80) (17).

There is a critical need to better understand the consequences of obesity in COVID-19 patients, especially regarding ICU care and needs. Indeed, this situation is most likely a snapshot of the future epidemiology in the ICU, as the global prevalence of obesity is expected to reach 20% worldwide in 2025 (27) and approximately 30% in Europe (37).

Immunology in critically ill COVID-19 patients with obesity

Obesity (or excess ectopic fat) may influence the effect of SARS-CoV-2 infection through multiple pathways, such as underlying impairments in cardiovascular, respiratory, metabolic, and thrombotic diseases, reduced physiological reserve and reduced ability to cope with COVID-19, as well as by affecting the ability of the immune system to deal with this infection. During the first phase of the COVID-19 pandemic, increased values of circulating inflammatory markers, such as C-reactive protein (CRP) levels, procalcitonin levels, the neutrophil-to-lymphocyte ratio, D-dimer levels, ferritin levels and interleukin (IL)-6, IL-8, and IL-10 levels, have been shown to be associated with impaired clinical outcomes (24,38–44). Initially, a 'cytokine storm' was suspected in COVID-19 patients, representing an uncontrolled systemic hyperinflammatory state characterized by an overproduction of circulating cytokines (24,25). However, when compared with inflammatory parameters in other critically ill sepsis patients with or without ARDS, the increase in inflammatory parameters in COVID-19 patients was significantly less pronounced (45–49), and their circulating cytokine levels were similar to cardiac arrest and trauma patients admitted to the ICU (45), conditions that are not known to cause a cytokine storm. This indicates that COVID-19-related ARDS was not characterized by a systemic cytokine storm. Furthermore, the concept of an overproduction of distinct cytokine profiles following SARS-CoV-2 infection, although not as a unique cytokine storm, has emphasized the importance of cytokine combinations in assessing severity and mortality (42,43). Nevertheless, approximately two-thirds of the patients who died from COVID-19 showed haemophagocytes with erythrophagocytosis in their bone marrow at autopsy, and these patients had increased inflammatory parameters 2-5 days prior to death compared to patients without haemophagocytes (50). This observation suggested that a subgroup of patients may suffer from macrophage activation syndrome, a syndrome characterized by a highly stimulated but ineffective immune response related to the activation and uncontrolled proliferation of T-lymphocytes and well-differentiated macrophages. This syndrome is a recognized entity in sepsis patients, and the resultant haemophagocytosis and cytokine overproduction account for the impaired

prognosis of these patients (51). Of interest, in a subgroup of non-COVID-19 sepsis patients with clinical features of macrophage activation syndrome, immunosuppressive therapy (e.g., IL-1 receptor blockade) appeared to exert therapeutic efficacy (52), while this was not the case in the total group of unselected sepsis patients (53). Importantly, in COVID-19 patients, cytokine concentrations measured in endotracheal aspiration are 10-10,000 times higher than those in serum, indicative of the alveolar compartmentalization of inflammatory biomarkers (54,55). Based on these observations, it can be concluded that a systemic cytokine storm is not typical in COVID-19 patients, while respiratory hyperinflammation is.

At the same time, there are several reasons why obese individuals may have a dysregulated or amplified immune response, linked both to greater viral exposure and the possibility that excess adipose tissue potentiates the immune response. Adipose tissue is an endocrine organ that produces hormones (adipokines) and pro- and anti-inflammatory factors (56–58). In obese patients, the secretion of these factors may be dysregulated, resulting in a chronic low-grade inflammatory state that is characterized by elevated levels of CRP and cytokines (59,60). The subsequent effects in both the innate and adaptive immune response may be related to an increased risk of bacterial and viral infections (58,61,62). In addition, a hyperinflammatory state is also associated with the development of several comorbidities, e.g., insulin resistance leading to type 2 diabetes mellitus (63,64) (Figure 1). Furthermore, it is plausible that adipose tissue operates as a viral reservoir in obese patients infected with SARS-CoV-2 since the expression of angiotensin-converting enzyme-2 (ACE-2), which is used by SARS-CoV-2 to enter cells, is higher in adipose tissue than in lung tissue (60,65,66). Consequently, more pronounced and sustained viral shedding leading to a perpetual inflammatory response and an impaired outcome might appear plausible (Figure 1). However, the clinical consequences of this remain unclear. In a cohort study, similar kinetics of circulating cytokines were observed between obese and nonobese critically ill COVID-19 patients from ICU admission onwards, indicating that no relevant differences in the innate immune response were present (26). Additionally, correlation analyses between BMI and circulating cytokines have confirmed the lack of a relationship between BMI and inflammation in critically ill COVID-19 patients (26,67). Furthermore, similar levels of inflammatory markers, such as CRP, have been observed across different BMI categories at ICU admission, also suggesting that BMI is not a significant driver of the inflammatory response and subsequent clinical course in COVID-19 patients (26,67,68).

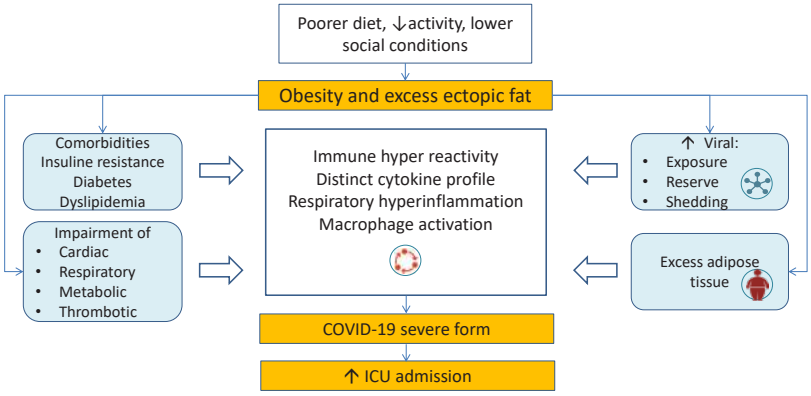

Figure 1. Relationship between obesity and COVID-19.

While these observational data from COVID-19 patients emerged, results from pharmacological intervention trials have become available. Large randomized controlled studies have shown that the administration of immunosuppressive drugs, such as corticosteroids (dexamethasone) (69) and IL-6 receptor inhibitors (tocilizumab, sarilumab) (70), results in survival benefits in severely ill COVID-19 patients. The therapeutic efficacy of these immunosuppressive therapies strongly suggests that inflammation plays a key role in the outcomes of COVID-19 patients. This is supported by the results of post hoc analyses, which showed that the benefit from dexamethasone (71,72) and tocilizumab (73) treatment appears to be most pronounced in patients with signs of hyperinflammation. In contrast, a direct interaction between BMI and the therapeutic efficacy of immunosuppressive treatments has not been reported. Hence, while both obesity and inflammation play a key role in the development of severe COVID-19, there is no indication that a link between obesity and a dysregulated immune response is present in these patients. Therefore, the higher risk of developing severe COVID-19 in obese patients is more likely to be explained by other mechanisms.

During the management of the pandemic

Acute respiratory distress syndrome in critically ill obese COVID-19 patients

As mentioned above, obesity is considered a risk factor for acquiring a severe form of SARS-CoV-2-associated ARDS (74). A recent study suggested that metabolic syndrome is associated with increased risks of ARDS and death in COVID-19 patients (75). Moreover, the risk for invasive MV gradually increases with BMI, reaching nearly 90% in patients with a BMI > 35 kg/m^2 (74).

The pathophysiology of COVID-19-induced ARDS appears similar to that of usual ARDS (76). Lung histopathology is also comparable to that of ARDS from other causes (77): acute-phase diffuse alveolar damage was reported in 88% of patients, which is comparable with both H1N1- (90%) and SARS-related ARDS (98%) (77), with pulmonary microthrombi reported in 57% of COVID-19 and 58% of SARS patients, compared with only 24% of H1N1 influenza patients (77). However, ARDS from COVID-19 is more often moderate or severe, compared with ARDS from other causes (76,78), with similar outcomes according to the severity of ARDS. In the specific population of patients with obesity, both in COVID-19 and non-COVID-19 ARDS patients, the negative effects of thoracic wall weight and abdominal fat mass on pulmonary compliance, which leads to decreased functional residual capacity and arterial oxygenation that is at least partly related to increased atelectasis formation (enhancing intrapulmonary shunt), must be taken into account (79,80).

Noninvasive oxygenation strategies used in COVID-19 patients include noninvasive ventilation (NIV), high-flow nasal oxygen (HFNO) and standard oxygen. During the different waves of the pandemic, HFNO and NIV were increasingly used (81). HFNO is associated with a decreased rate of invasive MV, without a difference in mortality (82–84). In these studies, the median BMI was elevated (approximately 29 kg/m^2), indicating a high rate of obese patients. A recent study assessed an initial strategy of continuous positive airway pressure (85) with a significant reduction in the risk of tracheal intubation or mortality in comparison with conventional oxygen therapy, but without a significant difference between an initial strategy of HFNO and conventional oxygen therapy (85). There was also no interaction between oxygenation methods and obesity, indicating similar results in patients with and without obesity. Thus, the initial oxygenation strategy (Figure 2) might be associated with NIV and HFNO in patients with obesity. For patients under HFNO, it has been shown that awake prone positioning of patients was associated with less treatment failure and the need for intubation (86). The mean BMI was almost 30 kg/m^2, indicating a potential beneficial effect in patients with obesity, even if no specific analysis has been performed (86). The awake prone position with helmet continuous positive airway pressure was also associated with a reduction in the work of breathing and an improvement in oxygenation in a study including a sample of patients in which 25% had a BMI ≥ 31 kg/m^2 (87).

The timing of intubation has been shown to be a crucial point during the COVID-19 disease course in both patients with and without obesity (Figure 2). Unexpectedly, a recent meta-analysis did not reveal any association between mortality and the timing of intubation (88). However, clinical practices have evolved during the COVID-19 pandemic with an initial

strategy of very early intubation and increasingly late intubation during the last waves. After intubation, protective ventilation should be used, with a tidal volume set according to predicted body weight (80). Soon after the establishment of MV, COVID-19 patients followed ARDS physiology, with compliance reduction related to the degree of hypoxemia, characteristics of respiratory mechanics and potential for recruitment (89). It is likely that recruitability is even higher in obese patients, who are more prone to atelectasis, and that the required positive end-expiratory pressure (PEEP) level is consequently higher (79,80,90). The assessment of the respiratory mechanics of COVID-19 ARDS patients, guided by transpulmonary pressure monitoring, might be helpful to set PEEP (91). Prone positioning could be used even more in patients with obesity than in patients without obesity (92), allowing more recruitment in the dorsal region than derecruitment in the ventral region and more homogeneously distributed lung inflation along the dorsoventral axis of the lung with a decrease in ventilation perfusion inequalities (80). During the COVID-19 pandemic, the prone position has been widely adopted in mechanically ventilated patients with respiratory failure (93). In a multicentre, national cohort study, the prone position was applied in 61% of the patients with a median BMI of 28 kg/m^2 (25% of the patients had a BMI > 31 kg/m^2) (93). In cases of failure of prone positioning, extra corporeal membrane oxygenation (ECMO) can be used for obese patients (94), and was used in approximately eight percent of patients in a large cohort study (35). Despite a high mortality rate (35,95,96) in patients with ARDS and ECMO, obesity was shown to be an independent factor associated with improved survival at 90 days in one report (97) but not in another (96).

A recent post hoc analysis of a randomized controlled trial suggested that NIV combined with HFNO after extubation was superior to HFNO alone in preventing reintubation within seven days after extubation (98) (Figure 2). An ongoing multicentre randomized controlled trial is assessing the use of NIV compared to oxygen (standard oxygen or HFNO) to prevent reintubation in the specific population of patients with obesity (99), including both COVID-19 patients and patients with other causes of ARDS.

To summarize, COVID-19 is an ARDS with a histopathology and prognosis that is comparable to ARDS from other causes. The ventilatory characteristics and treatments of COVID-19 patients (prone position, PEEP, neuromuscular blocking agents, ECMO (100)) are the same as those that are used in moderate or severe ARDS patients (101). Thromboprophylaxis seems to be more advised even if the level of proof is low (102,103). Prone positioning (awake or under invasive MV) is probably the treatment of choice in COVID-19 ARDS patients with obesity (Figure 2).

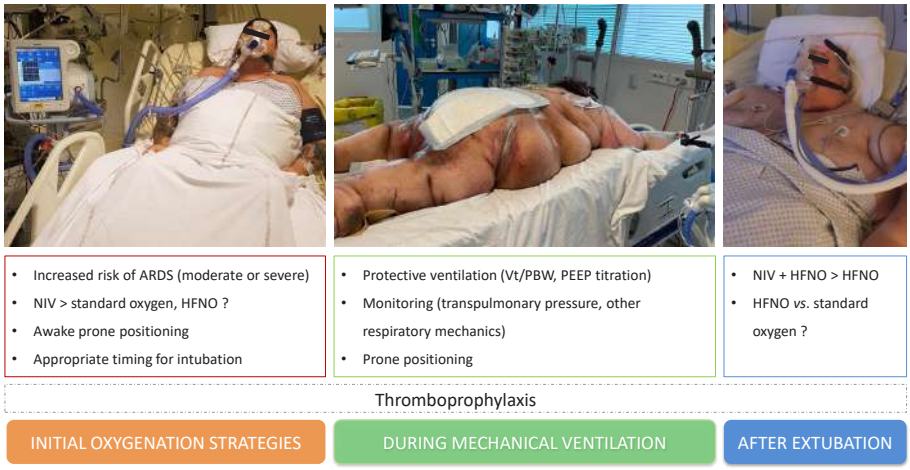

Figure 2. Acute respiratory distress syndrome in COVID-19 patients with obesity.
ARDS: acute respiratory distress syndrome; HFNO: high-flow nasal oxygen; NIV: noninvasive ventilation; PBW: predicted body weight; PEEP: positive end-expiratory pressure; Vt: tidal volume.

Coinfection in COVID-19 obese patients

In a national survey in England, secondary infections were shown to be uncommon in COVID-19 patients, with a rate of one percent of all patients (104). In a critically ill Italian COVID-19 cohort, Grasselli *et al.* included 774 patients from eight hospitals and reported that hospital-acquired infection (HAI) was observed in 46% of them; ventilator-associated pneumonia (VAP) was the most common HAI, reported in half of all patients, followed by bloodstream infections (24%) and catheter-related bloodstream infections (10%) (105). Obesity is a risk factor for infections, including community-acquired, postoperative or other nosocomial infections (106). However, in the Grasselli *et al.* study (105), obesity was not reported as a risk factor for HAI during COVID-19 infection, with a hazard ratio of 1.00 (95% CI 0.99-1.02).

During the COVID-19 pandemic, no study has specifically evaluated the relationship between BMI and the incidence of VAP. In a prospective cohort study carried out in 138 hospitals, a VAP diagnosis was reported in 58% of intubated patients (35), and obesity was associated with a higher 90-day mortality rate, but the relationship between BMI and VAP was not assessed (35). In a multicentre retrospective European cohort study performed in 36 ICUs (coVAPid cohort), an incidence of VAP of 36% was reported (107), which was significantly higher than that in the influenza pneumonia and nonviral groups (22% and 17%, respectively). Despite a higher BMI in the SARS-CoV-2 patient group than in the two

other groups, BMI did not differ between patients with or without a VAP diagnosis (107).

The increased risk of catheter-related bloodstream infections with BMI is well described in the literature, including in ICU patients (108–110). However, data regarding these outcomes in COVID-19 patients are still lacking as for urinary tract infections. Thus, further studies are needed to assess the relationship between obesity and HAI.

Acute kidney injury in critically ill obese COVID-19 patients

COVID-19-related AKI is a heterogeneous renal syndrome (Figure 3). Renal biopsy studies showed that a) most patients had varying degrees of tubular injury (acute tubular necrosis); b) a minority of patients had glomerular injury; and c) many patients had underlying renal disease (hypertensive nephrosclerosis, diabetic nephropathy) (111,112). The pathogenesis of COVID-19 AKI is multifactorial. The SARS-CoV-2 virus has direct effects on the kidney (113), and causes systemic inflammatory and immune dysregulation effects, endothelial injury, microvascular emboli and activation of the renin-angiotensin system (Figure 3). AKI may also be caused by multiorgan failure due to viral infection or its treatment (114) (Figure 3).

During the first COVID-19 wave in 2020, AKI affected almost half of the critically ill patients with SARS-CoV-2 infection. More than 20% of patients had severe AKI (stage 3), stretching hospital resources (115). The risk for AKI is increased in COVID-19 patients in the ICU, who have a higher demand for renal replacement therapy (RRT) than the general ICU population (116) or patients with influenza pneumonia (117). Critically ill COVID-19 patients with AKI present a longer hospital stay, a higher mortality, an increased need for MV (> 3-fold) (118) and a reduced recovery of renal function (119). Additionally, AKI has been found to be an independent risk factor for all-cause in-hospital mortality in COVID-19 patients (120). The clinical features differed between the first and second COVID-19 waves in New York (121). Patients with a younger age, fewer comorbidities, early hospital admission and fewer adverse outcomes (AKI, MV and in-hospital mortality) characterized the second wave.

Compared to nonobese patients, obese patients who developed COVID-19 were more likely to develop AKI requiring RRT (122–124). In a multicentre American cohort study, 21% of patients developed AKI and treated with RRT within 14 days of ICU admission (125); this was confirmed by another European cohort reporting that 28% of the patients were treated RRT (35). A retrospective analysis including 4,587 hospitalized COVID-19 patients reported a J-curve association between BMI and AKI (22). The authors found that class III obese COVID-19 patients exhibited a significantly higher prevalence and mortality rate of AKI (22). These data were corroborated by further studies (16,123,124,126,127). Increased BMI, starting at a BMI of 25 kg/m^2, was associated with a greater risk of AKI requiring RRT

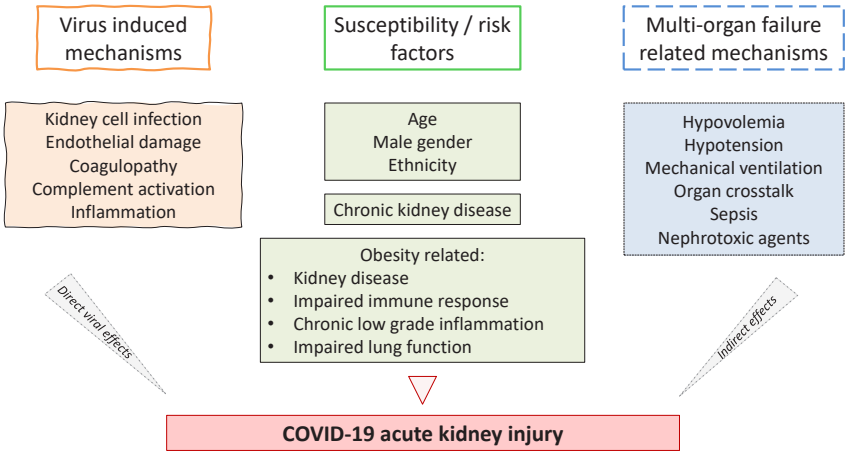

Figure 3. Pathogenesis of acute kidney injury in critically ill obese patients with COVID-19.
Adapted from Nadim et al. (114).

in another cohort study, but it was not associated with increased mortality (67), which was confirmed in another multicentre cohort study (125). Further conflicting data came from a retrospective single centre study that found that obesity did not affect AKI rates in critically ill obese COVID-19 patients (128). The mechanisms underlying the link between obesity and AKI in COVID-19 patients are poorly understood. These patients may be more vulnerable to AKI by virtue of the increased risk of obesity-associated chronic kidney disease, enhanced inflammatory responses, higher expression of the binding sites for SARS-CoV-2 (ACE-2) and higher prevalence of ARDS with possibly high PEEP (Figure 3). The true association between obesity and mortality in COVID-19 patients with AKI in the ICU remains unclear (129). Large cohort studies are needed to clarify the existence of an obesity paradox for critically ill obese COVID-19 patients with AKI.

The adverse impact of obesity after ICU care

Mortality in COVID-19 patients with obesity

In the general population, obesity is a risk factor for the development of severe infectious and noninfectious diseases, resulting in an increased risk of early mortality (130–132). In contrast, obesity has been reported to represent a protective factor in patients with both chronic and acute diseases once admitted to the ICU, such as end-stage kidney injury (133), heart failure (134), severe pneumonia (20,135,136) and sepsis (137,138), and these patients did not appear to have an impaired prognosis compared to normal weight patients (29,30).

Furthermore, lower mortality for obese patients with ARDS was suggested in a meta-analysis of five studies, suggesting a possible inverse association between BMI and mortality (19). In another meta-analysis of 24 studies including 9,187,248 patients with ARDS, obesity was associated with an increased risk of ARDS but a lower risk of mortality, providing evidence of this paradox (139). This phenomenon, the paradoxical J-shaped association between BMI and mortality, is called *"the obesity paradox"*.

Since the emergence of the COVID-19 pandemic, obesity has also been shown to be associated with high mortality rates in COVID-19 patients (4,7,8,140). In an unselected population, a recent meta-analysis of 28 studies showed that obese COVID-19 patients had a high mortality risk with a nonlinear relationship, shown as a J-shaped curve, between BMI and mortality, indicating that both underweight and obese COVID-19 patients had a higher mortality risk than patients with normal weight (141), as confirmed by two other studies (142,143) (Table 1). In a multicentre cohort study performed with 2,215 critically ill patients, higher BMI (≥ 40 kg/m^2) was independently associated with death (144). However, BMI was no longer associated with impaired outcomes once patients were admitted to the ICU (21). Following adjustments for other prognostic factors, such as age, disease severity, preexisting comorbidities, the need for MV and use of vasoactive medications, obese patients had similar in-hospital mortality compared to normal weight patients in a large cohort trial of 2,635 critically ill COVID-19 patients (21). In line with this observation, other studies and meta-analyses have reported an increased risk of ICU admission and the need for ECMO, MV and prone positioning in obese patients with COVID-19, but no significant differences in mortality were observed among critically ill COVID-19 patients in different BMI categories (26,32,145,146) (Table 1). Intriguingly, while the obesity paradox was not observed in critically ill patients with COVID-19-related respiratory failure, severely obese (BMI >35 kg/m^2) critically ill patients with non-COVID-19 respiratory infections had a significantly reduced OR for in-hospital mortality for viral and bacterial respiratory infections using normal weight patients as a reference (21). The reason for this discrepancy remains unclear. Possible bias with a statistical artefact has been suggested (147). Additionally, the underlying mechanism of the obesity paradox may not be present in critically ill COVID-19 patients but only in ICU patients with other aetiologies. Although the exact mechanisms for the obesity paradox are unknown in non-COVID-19 patients, immunological and mechanical respiratory differences between obese and nonobese patients have been suggested, as well as adipose tissue functioning as a fuel source in critically ill obese patients. In COVID-19 patients, there is no clear interplay between BMI and the immune response (26). The observation that obesity is positively associated with the need for MV may suggest that mechanical respiratory reasons account for early ICU

Table 1. Main results of studies and meta-analyses on mortality in COVID-19 patients in the general population and critically ill patients.

Population	Authors	Characteristics of population	Results
General	Yang *et al.* (142)	50 studies; 18,260,378 patients	Obesity increased the risk of mortality by 114% (OR 1.65, 95% CI 1.21-2.25)
General	Zhao *et al.* (143)	11 studies; 9,787 patients	Morbid obesity increased the risk of mortality by 376% (OR 3.76, 95% CI 2.67-5.28)
General	Huang *et al.* (141)	28 studies; 112,682 patients	Obesity increased the risk of mortality (pooled RR 1.33, 95% CI 1.15–1.53)
General	Popkin *et al.* (17)	75 studies; 399,461 patients	Obesity increased the risk of mortality by 48% (OR = 1.48, 95% CI, 1.22–1.80)
General	Zhang *et al.* (146)	9 studies; 20,597 patients	Obese patients had similar likelihoods of death from COVID-19 to nonobese patients (OR 0.96, 95% CI 0.74-1.25)
ICU	Kooistra *et al.* (21)	1 study; 2,635 patients	Similar in-hospital mortality compared to normal weight patients (OR 1.15, 95% CI 0.79-1.67)
ICU	Kooistra *et al.* (26)	1 study; 77 patients	ICU mortality was 17% in the obese group vs. 24% in the nonobese group (p=0.05)
ICU	Chetboun *et al.* (32)	1 study; 1,461 patients	ICU mortality only increased in obese class III patients (HR 1.68, 95% CI 1.06-2.64)
ICU	Gupta *et al.* (144)	1 study; 2,215 patients	Factors independently associated with death included higher BMI (³40 $kg.m^2$)
ICU	Abumayyaleh *et al.* (145)	1 study; 3,635 patients	Obesity did not impact the mortality rate (HR 1.15, 95% CI 0.89-1.48)

BMI: body mass index; HR: hazard ratio; OR: odds ratio

admission in obese patients. It is unclear how mechanical respiratory factors would account for the obesity paradox in non-COVID-19 patients, but apparently, these factors do not play an important role in critically ill COVID-19 patients. Since the obesity paradox is not present in COVID-19 patients, one may argue that obese critically ill COVID-19 patients are at a relative disadvantage.

Long-term post-COVID-19 symptoms in obese patients

Long COVID is now recognized as a new clinical entity post SARS-CoV-2 infection that is associated with symptoms persisting for several weeks after acute infection (148–151).

Initially, no consensus on the definition and classification was accepted (148–154), and the persistence of post-COVID-19 symptoms can vary for more than three weeks (152,153), six weeks (149,150,154) or five to 24 weeks (149–151,154) after the onset of illness. Currently, the World Health Organization defines post-COVID-19 syndrome as usually occurring three months from acute infection and lasting longer than two months, with no probable alternative diagnosis (23)**.** Recent guidelines have been published for the management of the long-term effects of COVID-19 (155,156). The incidence ranges from 10% to 35% in nonsevere patients (157) and up to 80% in hospitalized and critically ill patients (158–161).

Post-COVID-19 syndrome may encompass a plethora of symptoms (148–152,159,162–164), the most common of which include fatigue (149,153,157,159,160,163–167), dyspnoea and irregular pulmonary patterns (149,153,159,162,164–166,168), chest pain (149,160,164,166), gastrointestinal symptoms (160), olfactory and gustatory dysfunction (149,164), sleep and mental disorders (149,160,164,169) and irregular cardiac patterns (162). The pathogenesis of post-COVID-19 syndrome remains largely unknown and potentially multifactorial, with several implicated mechanisms (148,150). SARS-CoV-2 immune dysregulation with a persistent inflammatory state with cytokine production (170) and unresolved inflammation, called multisystem inflammatory syndrome in adults, has a key role in the pathogenesis of several symptoms (148,167,170–175). Other pathogenetic mechanisms, such as viral persistence (176), immune-mediated vascular dysfunction and endotheliopathy (177,178), thromboembolism (177), nervous system dysfunction, long-term tissue damage (148), autonomic dysfunction (167) and gut microbiome disruption (148), have been suggested.

Post-COVID-19 syndrome risk factors, including female sex (160,164,179), a prior psychiatric disorder (160,164,179), older age (164), more than five symptoms during the first week of illness (148,180), the presence of comorbidities and initial acute COVID-19 severity (148,164,180), have been described. Obesity was also an independent risk factor highlighting the major role of metabolic diseases as determinants of long-term patient outcomes after SARS-CoV-2 infection (164,179,181–183). In the most recent studies, obesity was associated with several long-term post-COVID symptoms without association with a specific symptom (182). Since obesity is a multifactorial chronic metabolic disease, it is possible that these patients have a plethora of post-COVID-19 symptoms to a greater extent than those without obesity. It is also possible that the multisystemic changes, i.e., hormonal, metabolic, proinflammatory or immune changes, associated with obesity lead to the development of more, but not specific, post-COVID-19 symptoms.

Further research is needed to improve the understanding of the physiopathology and the underlying mechanisms, clinical spectrum, predictive biomarkers and prognosis of post-COVID-19 syndrome.

Conclusions

Since the very beginning of 2020, we have dealt with a new virus specifically affecting patients with obesity. An increased prevalence of obese patients presenting with severe forms of COVID-19 who are admitted to the ICU has been reported. The link between obesity and ICU mortality and the presence of the so-called obesity paradox has also been questioned. In addition, we are just beginning to understand the pathophysiological mechanisms or pathways that impact obese patients either more or less severely during mild, moderate or severe COVID-19 infection and the long-term implications of post-COVID-19 syndrome on the health of COVID-19 obese patients. Obesity is always a clear risk factor in the management of viral infection. Thus, we need to still explore the outcomes of COVID-19 infection in obese patients to better understand the link between obesity and mortality in ICU patients. Clinicians and other health care professionals should be trained to recognize the severity of COVID-19 and post-COVID-19 symptoms and perform close clinical monitoring of obese patients, even after the acute phase of infection and until recovery. Several metabolic dysfunctions could be reversed by weight reduction, and some of these molecular pathways are so critical that targeting them could have immense therapeutic potential.

References

1. WHO Coronavirus (COVID-19) Dashboard [Internet]. [cité 18 févr 2022]. Disponible sur: https://covid19.who.int
2. O'Rourke RW, Lumeng CN. Pathways to Severe COVID-19 for People with Obesity. Obes Silver Spring Md. avr 2021;29(4):645-53.
3. Williamson EJ, Walker AJ, Bhaskaran K, Bacon S, Bates C, Morton CE, et al. Factors associated with COVID-19-related death using OpenSAFELY. Nature. août 2020;584(7821):430-6.
4. Kristensen NM, Gribsholt SB, Andersen AL, Richelsen B, Bruun JM. Obesity augments the disease burden in COVID-19: Updated data from an umbrella review. Clin Obes. 8 févr 2022;e12508.
5. Raeisi T, Mozaffari H, Sepehri N, Darand M, Razi B, Garousi N, et al. The negative impact of obesity on the occurrence and prognosis of the 2019 novel coronavirus (COVID-19) disease: a systematic review and meta-analysis. Eat Weight Disord EWD. avr 2022;27(3):893-911.
6. Huang Y, Lu Y, Huang YM, Wang M, Ling W, Sui Y, et al. Obesity in patients with COVID-19: a systematic review and meta-analysis. Metabolism. déc 2020;113:154378.
7. Jayawardena R, Jeyakumar DT, Misra A, Hills AP, Ranasinghe P. Obesity: A potential risk factor for infection and mortality in the current COVID-19 epidemic. Diabetes Metab Syndr. déc 2020;14(6):2199-203.
8. Zhou Y, Yang Q, Chi J, Dong B, Lv W, Shen L, et al. Comorbidities and the risk of severe or fatal outcomes associated with coronavirus disease 2019: A systematic review and meta-analysis. Int J Infect Dis IJID Off Publ Int Soc Infect Dis. oct 2020;99:47-56.
9. 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 Lond Engl. 15 2020;395(10223):497-506.
10. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, 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. 1 juill 2020;180(7):934-43.
11. Simonnet A, Chetboun M, Poissy J, Raverdy V, Noulette J, Duhamel A, et al. High Prevalence of Obesity in Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) Requiring Invasive Mechanical Ventilation. Obesity. juill 2020;28(7):1195-9.
12. de Lusignan S, Dorward J, Correa A, Jones N, Akinyemi O, Amirthalingam G, et al. Risk factors for SARS-CoV-2 among patients in the Oxford Royal College of General Practitioners Research and Surveillance Centre primary care network: a cross-sectional study. Lancet Infect Dis. sept 2020;20(9):1034-42.
13. Docherty AB, Harrison EM, Green CA, Hardwick HE, Pius R, Norman L, et al. Features of 20 133 UK patients in hospital with covid-19 using the ISARIC WHO Clinical Characterisation Protocol: prospective observational cohort study. BMJ. 22 mai 2020;m1985.
14. Chiumello D, Pozzi T, Storti E, Caccioppola A, Pontiroli AE, Coppola S. Body mass index and acute respiratory distress severity in patients with and without SARS-CoV-2 infection. Br J Anaesth. oct 2020;125(4):e376-7.
15. Sudhakar M, Winfred SB, Meiyazhagan G, Venkatachalam DP. Mechanisms contributing to adverse outcomes of COVID-19 in obesity. Mol Cell Biochem [Internet]. 27 janv 2022 [cité 20 mars 2022];1-39. Disponible sur: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8793096/

16. Hendren NS, de Lemos JA, Ayers C, Das SR, Rao A, Carter S, et al. Association of Body Mass Index and Age With Morbidity and Mortality in Patients Hospitalized With COVID-19: Results From the American Heart Association COVID-19 Cardiovascular Disease Registry. Circulation. 12 janv 2021;143(2):135-44.
17. Popkin BM, Du S, Green WD, Beck MA, Algaith T, Herbst CH, et al. Individuals with obesity and COVID-19: A global perspective on the epidemiology and biological relationships. Obes Rev Off J Int Assoc Study Obes. nov 2020;21(11):e13128.
18. Tlayjeh H, Arabi YM, Ferguson ND, Zhou Q, Lamontagne F, Arroliga A, et al. Body Mass Index and Mortality in Subjects With ARDS: Post-hoc Analysis of the OSCILLATE Trial. Respir Care. sept 2019;64(9):1042-8.
19. Ni YN, Luo J, Yu H, Wang YW, Hu YH, Liu D, et al. Can body mass index predict clinical outcomes for patients with acute lung injury/acute respiratory distress syndrome? A meta-analysis. Crit Care [Internet]. 22 févr 2017 [cité 11 mai 2022];21:36. Disponible sur: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5320793/
20. Zhi G, Xin W, Ying W, Guohong X, Shuying L. "Obesity Paradox" in Acute Respiratory Distress Syndrome: Asystematic Review and Meta-Analysis. Zhao YY, éditeur. PLOS ONE. 29 sept 2016;11(9):e0163677.
21. Kooistra EJ, Brinkman S, van der Voort PHJ, de Keizer NF, Dongelmans DA, Kox M, et al. Body Mass Index and Mortality in Coronavirus Disease 2019 and Other Diseases: A Cohort Study in 35,506 ICU Patients. Crit Care Med. janv 2022;50(1):e1-10.
22. So M, Takahashi M, Miyamoto Y, Ishisaka Y, Iwagami M, Tsugawa Y, et al. The effect of obesity on in-hospital mortality among patients with COVID-19 receiving corticosteroids. Diabetes Metab Syndr. janv 2022;16(1):102373.
23. A clinical case definition of post COVID-19 condition by a Delphi consensus, 6 October 2021 [Internet]. [cité 28 avr 2022]. Disponible sur: https://www.who.int/publications-detail-redirect/WHO-2019-nCoV-Post_COVID-19_condition-Clinical_case_definition-2021.1
24. Pedersen SF, Ho YC. SARS-CoV-2: a storm is raging. J Clin Invest. 1 mai 2020;130(5):2202-5.
25. Behrens EM, Koretzky GA. Review: Cytokine Storm Syndrome: Looking Toward the Precision Medicine Era. Arthritis Rheumatol Hoboken NJ. juin 2017;69(6):1135-43.
26. Kooistra EJ, de Nooijer AH, Claassen WJ, Grondman I, Janssen NAF, Netea MG, et al. A higher BMI is not associated with a different immune response and disease course in critically ill COVID-19 patients. Int J Obes 2005 [Internet]. 25 janv 2021 [cité 4 nov 2021];1-8. Disponible sur: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7829495/
27. Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants. The Lancet. avr 2016;387(10026):1377-96.
28. GBD 2015 Obesity Collaborators, Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, et al. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. N Engl J Med. 6 juill 2017;377(1):13-27.
29. Pickkers P, de Keizer N, Dusseljee J, Weerheijm D, van der Hoeven JG, Peek N. Body Mass Index Is Associated With Hospital Mortality in Critically Ill Patients: An Observational Cohort Study. Crit Care Med. août 2013;41(8):1878-83.

30. Sakr Y, Alhussami I, Nanchal R, Wunderink RG, Pellis T, Wittebole X, et al. Being Overweight Is Associated With Greater Survival in ICU Patients: Results From the Intensive Care Over Nations Audit*. Crit Care Med. déc 2015;43(12):2623-32.
31. Decruyenaere A, Steen J, Colpaert K, Benoit DD, Decruyenaere J, Vansteelandt S. The obesity paradox in critically ill patients: a causal learning approach to a casual finding. Crit Care. déc 2020;24(1):485.
32. Chetboun M, Raverdy V, Labreuche J, Simonnet A, Wallet F, Caussy C, et al. BMI and pneumonia outcomes in critically ill COVID-19 patients: An international multicenter study. Obesity. sept 2021;29(9):1477-86.
33. Sjögren L, Stenberg E, Thuccani M, Martikainen J, Rylander C, Wallenius V, et al. Impact of obesity on intensive care outcomes in patients with COVID-19 in Sweden—A cohort study. Zivkovic AR, éditeur. PLOS ONE. 13 oct 2021;16(10):e0257891.
34. Ñamendys-Silva SA. Patients With Coronavirus Disease 2019 Requiring Invasive Mechanical Ventilation in Mexico in the First, Second, and Exponential Growth Phase of the Third Wave of the Coronavirus Disease 2019 Pandemic. Crit Care Explor. 5 oct 2021;3(10):e556.
35. COVID-ICU Group on behalf of the REVA Network and the COVID-ICU Investigators. Clinical characteristics and day-90 outcomes of 4244 critically ill adults with COVID-19: a prospective cohort study. Intensive Care Med. janv 2021;47(1):60-73.
36. Lighter J, Phillips M, Hochman S, Sterling S, Johnson D, Francois F, et al. Obesity in Patients Younger Than 60 Years Is a Risk Factor for COVID-19 Hospital Admission. Clin Infect Dis Off Publ Infect Dis Soc Am. 28 juill 2020;71(15):896-7.
37. Pineda E, Sanchez-Romero LM, Brown M, Jaccard A, Jewell J, Galea G, et al. Forecasting Future Trends in Obesity across Europe: The Value of Improving Surveillance. Obes Facts. nov 2018;11(5):360-71.
38. Zeng F, Huang Y, Guo Y, Yin M, Chen X, Xiao L, et al. Association of inflammatory markers with the severity of COVID-19: A meta-analysis. Int J Infect Dis IJID Off Publ Int Soc Infect Dis. juill 2020;96:467-74.
39. Mahat RK, Panda S, Rathore V, Swain S, Yadav L, Sah SP. The dynamics of inflammatory markers in coronavirus disease-2019 (COVID-19) patients: A systematic review and meta-analysis. Clin Epidemiol Glob Health. sept 2021;11:100727.
40. Hariyanto TI, Japar KV, Kwenandar F, Damay V, Siregar JI, Lugito NPH, et al. Inflammatory and hematologic markers as predictors of severe outcomes in COVID-19 infection: A systematic review and meta-analysis. Am J Emerg Med. mars 2021;41:110-9.
41. Gubernatorova EO, Gorshkova EA, Polinova AI, Drutskaya MS. IL-6: Relevance for immunopathology of SARS-CoV-2. Cytokine Growth Factor Rev. juin 2020;53:13-24.
42. Dorgham K, Quentric P, Gökkaya M, Marot S, Parizot C, Sauce D, et al. Distinct cytokine profiles associated with COVID-19 severity and mortality. J Allergy Clin Immunol. juin 2021;147(6):2098-107.
43. Del Valle DM, Kim-Schulze S, Huang HH, Beckmann ND, Nirenberg S, Wang B, et al. An inflammatory cytokine signature predicts COVID-19 severity and survival. Nat Med. oct 2020;26(10):1636-43.
44. Herold T, Jurinovic V, Arnreich C, Lipworth BJ, Hellmuth JC, von Bergwelt-Baildon M, et al. Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. J Allergy Clin Immunol. juill 2020;146(1):128-136.e4.

45. Kox M, Waalders NJB, Kooistra EJ, Gerretsen J, Pickkers P. Cytokine Levels in Critically Ill Patients With COVID-19 and Other Conditions. JAMA [Internet]. 20 oct 2020 [cité 3 déc 2021];324(15):1565-7. Disponible sur: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7489366/
46. Wilson JG, Simpson LJ, Ferreira AM, Rustagi A, Roque J, Asuni A, et al. Cytokine profile in plasma of severe COVID-19 does not differ from ARDS and sepsis. JCI Insight. 3 sept 2020;5(17):140289.
47. Wu M, Zou ZY, Chen YH, Wang CL, Feng YW, Liu ZF. Severe COVID-19-associated sepsis is different from classical sepsis induced by pulmonary infection with carbapenem-resistant klebsiella pneumonia (CrKP). Chin J Traumatol Zhonghua Chuang Shang Za Zhi. janv 2022;25(1):17-24.
48. Dong X, Wang C, Liu X, Gao W, Bai X, Li Z. Lessons Learned Comparing Immune System Alterations of Bacterial Sepsis and SARS-CoV-2 Sepsis. Front Immunol. 2020;11:598404.
49. Loftus TJ, Ungaro R, Dirain M, Efron PA, Mazer MB, Remy KE, et al. Overlapping but Disparate Inflammatory and Immunosuppressive Responses to SARS-CoV-2 and Bacterial Sepsis: An Immunological Time Course Analysis. Front Immunol. 2021;12:792448.
50. Swoboda J, Wittschieber D, Sanft J, Kleemann S, Elschner S, Ihle H, et al. Bone marrow haemophagocytosis indicates severe infection with severe acute respiratory syndrome coronavirus 2. Histopathology. avr 2021;78(5):727-37.
51. Kyriazopoulou E, Leventogiannis K, Norrby-Teglund A, Dimopoulos G, Pantazi A, Orfanos SE, et al. Macrophage activation-like syndrome: an immunological entity associated with rapid progression to death in sepsis. BMC Med. 18 sept 2017;15(1):172.
52. Shakoory B, Carcillo JA, Chatham WW, Amdur RL, Zhao H, Dinarello CA, et al. Interleukin-1 Receptor Blockade Is Associated With Reduced Mortality in Sepsis Patients With Features of Macrophage Activation Syndrome: Reanalysis of a Prior Phase III Trial. Crit Care Med. févr 2016;44(2):275-81.
53. Opal SM, Fisher CJ, Dhainaut JF, Vincent JL, Brase R, Lowry SF, et al. Confirmatory interleukin-1 receptor antagonist trial in severe sepsis: a phase III, randomized, double-blind, placebo-controlled, multicenter trial. The Interleukin-1 Receptor Antagonist Sepsis Investigator Group. Crit Care Med. juill 1997;25(7):1115-24.
54. Jouan Y, Baranek T, Si-Tahar M, Paget C, Guillon A. Lung compartmentalization of inflammatory biomarkers in COVID-19-related ARDS. Crit Care Lond Engl. 24 mars 2021;25(1):120.
55. Bendib I, Beldi-Ferchiou A, Schlemmer F, Surenaud M, Maitre B, Plonquet A, et al. Alveolar compartmentalization of inflammatory and immune cell biomarkers in pneumonia-related ARDS. Crit Care Lond Engl. 9 janv 2021;25(1):23.
56. Muscogiuri G, Pugliese G, Laudisio D, Castellucci B, Barrea L, Savastano S, et al. The impact of obesity on immune response to infection: Plausible mechanisms and outcomes. Obes Rev Off J Int Assoc Study Obes. juin 2021;22(6):e13216.
57. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol. févr 2011;11(2):85-97.
58. Fantuzzi G. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol. mai 2005;115(5):911-9; quiz 920.

59. McGuire TR, Brusnahan SK, Bilek LD, Jackson JD, Kessinger MA, Berger AM, et al. Inflammation associated with obesity: relationship with blood and bone marrow endothelial cells. Obes Silver Spring Md. nov 2011;19(11):2130-6.
60. Maurya R, Sebastian P, Namdeo M, Devender M, Gertler A. COVID-19 Severity in Obesity: Leptin and Inflammatory Cytokine Interplay in the Link Between High Morbidity and Mortality. Front Immunol. 2021;12:649359.
61. Huttunen R, Karppelin M, Syrjänen J. Obesity and nosocomial infections. J Hosp Infect. sept 2013;85(1):8-16.
62. Serrano PE, Khuder SA, Fath JJ. Obesity as a risk factor for nosocomial infections in trauma patients. J Am Coll Surg. juill 2010;211(1):61-7.
63. Wang X, Bao W, Liu J, Ouyang YY, Wang D, Rong S, et al. Inflammatory markers and risk of type 2 diabetes: a systematic review and meta-analysis. Diabetes Care. janv 2013;36(1):166-75.
64. Esser N, Legrand-Poels S, Piette J, Scheen AJ, Paquot N. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. Diabetes Res Clin Pract. août 2014;105(2):141-50.
65. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 27 nov 2003;426(6965):450-4.
66. Al-Benna S. Association of high level gene expression of ACE2 in adipose tissue with mortality of COVID-19 infection in obese patients. Obes Med. sept 2020;19:100283.
67. Friedman AN, Guirguis J, Kapoor R, Gupta S, Leaf DE, Timsina LR, et al. Obesity, inflammatory and thrombotic markers, and major clinical outcomes in critically ill patients with COVID-19 in the US. Obes Silver Spring Md. oct 2021;29(10):1719-30.
68. Wolf M, Alladina J, Navarrete-Welton A, Shoults B, Brait K, Ziehr D, et al. Obesity and Critical Illness in COVID-19: Respiratory Pathophysiology. Obes Silver Spring Md. mai 2021;29(5):870-8.
69. RECOVERY Collaborative Group, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, et al. Dexamethasone in Hospitalized Patients with Covid-19. N Engl J Med. 25 févr 2021;384(8):693-704.
70. REMAP-CAP Investigators, Gordon AC, Mouncey PR, Al-Beidh F, Rowan KM, Nichol AD, et al. Interleukin-6 Receptor Antagonists in Critically Ill Patients with Covid-19. N Engl J Med. 22 avr 2021;384(16):1491-502.
71. Sinha P, Furfaro D, Cummings MJ, Abrams D, Delucchi K, Maddali MV, et al. Latent Class Analysis Reveals COVID-19-related Acute Respiratory Distress Syndrome Subgroups with Differential Responses to Corticosteroids. Am J Respir Crit Care Med. 1 déc 2021;204(11):1274-85.
72. Chen H, Xie J, Su N, Wang J, Sun Q, Li S, et al. Corticosteroid Therapy Is Associated With Improved Outcome in Critically Ill Patients With COVID-19 With Hyperinflammatory Phenotype. Chest. mai 2021;159(5):1793-802.
73. Galván-Román JM, Rodríguez-García SC, Roy-Vallejo E, Marcos-Jiménez A, Sánchez-Alonso S, Fernández-Díaz C, et al. IL-6 serum levels predict severity and response to tocilizumab in COVID-19: An observational study. J Allergy Clin Immunol. janv 2021;147(1):72-80.e8.
74. Simonnet A, Chetboun M, Poissy J, Raverdy V, Noulette J, Duhamel A, et al. High Prevalence of Obesity in Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) Requiring Invasive Mechanical Ventilation. Obes Silver Spring Md. juill 2020;28(7):1195-9.

75. Denson JL, Gillet AS, Zu Y, Brown M, Pham T, Yoshida Y, et al. Metabolic Syndrome and Acute Respiratory Distress Syndrome in Hospitalized Patients With COVID-19. JAMA Netw Open. 1 déc 2021;4(12):e2140568.
76. Grasselli G, Tonetti T, Protti A, Langer T, Girardis M, Bellani G, et al. Pathophysiology of COVID-19-associated acute respiratory distress syndrome: a multicentre prospective observational study. Lancet Respir Med. déc 2020;8(12):1201-8.
77. Hariri LP, North CM, Shih AR, Israel RA, Maley JH, Villalba JA, et al. Lung Histopathology in Coronavirus Disease 2019 as Compared With Severe Acute Respiratory Sydrome and H1N1 Influenza: A Systematic Review. Chest. janv 2021;159(1):73-84.
78. Ferrando C, Suarez-Sipmann F, Mellado-Artigas R, Hernández M, Gea A, Arruti E, et al. Clinical features, ventilatory management, and outcome of ARDS caused by COVID-19 are similar to other causes of ARDS. Intensive Care Med. déc 2020;46(12):2200-11.
79. De Jong A, Chanques G, Jaber S. Mechanical ventilation in obese ICU patients: from intubation to extubation. Crit Care Lond Engl. 21 mars 2017;21(1):63.
80. De Jong A, Wrigge H, Hedenstierna G, Gattinoni L, Chiumello D, Frat JP, et al. How to ventilate obese patients in the ICU. Intensive Care Med. déc 2020;46(12):2423-35.
81. Garcia MA, Johnson SW, Sisson EK, Sheldrick CR, Kumar VK, Boman K, et al. Variation in Use of High-Flow Nasal Cannula and Noninvasive Ventilation Among Patients With COVID-19. Respir Care. 7 juin 2022;respcare.09672.
82. Mellado-Artigas R, Ferreyro BL, Angriman F, Hernández-Sanz M, Arruti E, Torres A, et al. High-flow nasal oxygen in patients with COVID-19-associated acute respiratory failure. Crit Care Lond Engl. 11 févr 2021;25(1):58.
83. Bonnet N, Martin O, Boubaya M, Levy V, Ebstein N, Karoubi P, et al. High flow nasal oxygen therapy to avoid invasive mechanical ventilation in SARS-CoV-2 pneumonia: a retrospective study. Ann Intensive Care. 27 févr 2021;11(1):37.
84. Demoule A, Vieillard Baron A, Darmon M, Beurton A, Géri G, Voiriot G, et al. High-Flow Nasal Cannula in Critically III Patients with Severe COVID-19. Am J Respir Crit Care Med. 1 oct 2020;202(7):1039-42.
85. Perkins GD, Ji C, Connolly BA, Couper K, Lall R, Baillie JK, et al. Effect of Noninvasive Respiratory Strategies on Intubation or Mortality Among Patients With Acute Hypoxemic Respiratory Failure and COVID-19: The RECOVERY-RS Randomized Clinical Trial. JAMA. 8 févr 2022;327(6):546-58.
86. Ehrmann S, Li J, Ibarra-Estrada M, Perez Y, Pavlov I, McNicholas B, et al. Awake prone positioning for COVID-19 acute hypoxaemic respiratory failure: a randomised, controlled, multinational, open-label meta-trial. Lancet Respir Med. déc 2021;9(12):1387-95.
87. Chiumello D, Chiodaroli E, Coppola S, Cappio Borlino S, Granata C, Pitimada M, et al. Awake prone position reduces work of breathing in patients with COVID-19 ARDS supported by CPAP. Ann Intensive Care. 20 déc 2021;11(1):179.
88. Papoutsi E, Giannakoulis VG, Xourgia E, Routsi C, Kotanidou A, Siempos II. Effect of timing of intubation on clinical outcomes of critically ill patients with COVID-19: a systematic review and meta-analysis of non-randomized cohort studies. Crit Care Lond Engl. 25 mars 2021;25(1):121.
89. Grieco DL, Bongiovanni F, Chen L, Menga LS, Cutuli SL, Pintaudi G, et al. Respiratory physiology of COVID-19-induced respiratory failure compared to ARDS of other etiologies. Crit Care Lond Engl. 28 août 2020;24(1):529.

90. De Santis Santiago R, Teggia Droghi M, Fumagalli J, Marrazzo F, Florio G, Grassi LG, et al. High Pleural Pressure Prevents Alveolar Overdistension and Hemodynamic Collapse in ARDS with Class III Obesity. Am J Respir Crit Care Med. 2 sept 2020;
91. Mezidi M, Daviet F, Chabert P, Hraiech S, Bitker L, Forel JM, et al. Transpulmonary pressures in obese and non-obese COVID-19 ARDS. Ann Intensive Care. 1 oct 2020;10(1):129.
92. De Jong A, Molinari N, Sebbane M, Prades A, Futier E, Jung B, et al. Feasibility and effectiveness of prone position in morbidly obese patients with ARDS: a case-control clinical study. Chest. juin 2013;143(6):1554-61.
93. Langer T, Brioni M, Guzzardella A, Carlesso E, Cabrini L, Castelli G, et al. Prone position in intubated, mechanically ventilated patients with COVID-19: a multi-centric study of more than 1000 patients. Crit Care Lond Engl. 6 avr 2021;25(1):128.
94. Mongero LB, Stammers AH, Tesdahl EA, Petersen C, Patel K, Jacobs JP. The Use of Extracorporeal Membrane Oxygenation in COVID-19 Patients with Severe Cardiorespiratory Failure: The Influence of Obesity on Outcomes. J Extra Corpor Technol. déc 2021;53(4):293-8.
95. Schmidt M, Hajage D, Lebreton G, Monsel A, Voiriot G, Levy D, et al. Extracorporeal membrane oxygenation for severe acute respiratory distress syndrome associated with COVID-19: a retrospective cohort study. Lancet Respir Med. nov 2020;8(11):1121-31.
96. Nesseler N, Fadel G, Mansour A, Para M, Falcoz PE, Mongardon N, et al. Extracorporeal Membrane Oxygenation for Respiratory Failure Related to COVID-19: A Nationwide Cohort Study. Anesthesiology. 1 mai 2022;136(5):732-48.
97. Daviet F, Guilloux P, Hraiech S, Tonon D, Velly L, Bourenne J, et al. Impact of obesity on survival in COVID-19 ARDS patients receiving ECMO: results from an ambispective observational cohort. Ann Intensive Care. 15 nov 2021;11(1):157.
98. Thille AW, Coudroy R, Nay MA, Gacouin A, Decavèle M, Sonneville R, et al. Beneficial Effects of Noninvasive Ventilation after Extubation in Obese or Overweight Patients: A Post Hoc Analysis of a Randomized Clinical Trial. Am J Respir Crit Care Med. 15 févr 2022;205(4):440-9.
99. De Jong A, Huguet H, Molinari N, Jaber S. Non-invasive ventilation versus oxygen therapy after extubation in patients with obesity in intensive care units: the multicentre randomised EXTUB-OBESE study protocol. BMJ Open. 19 janv 2022;12(1):e052712.
100. van Paassen J, Vos JS, Hoekstra EM, Neumann KMI, Boot PC, Arbous SM. Corticosteroid use in COVID-19 patients: a systematic review and meta-analysis on clinical outcomes. Crit Care Lond Engl. 14 déc 2020;24(1):696.
101. Ferguson ND, Pham T, Gong MN. How severe COVID-19 infection is changing ARDS management. Intensive Care Med. déc 2020;46(12):2184-6.
102. REMAP-CAP Investigators, ACTIV-4a Investigators, ATTACC Investigators, Goligher EC, Bradbury CA, McVerry BJ, et al. Therapeutic Anticoagulation with Heparin in Critically Ill Patients with Covid-19. N Engl J Med. 26 août 2021;385(9):777-89.
103. COVID-19 rapid guideline: reducing the risk of venous thromboembolism in over 16s with COVID-19 [Internet]. London: National Institute for Health and Care Excellence (NICE); 2020 [cité 10 mai 2022]. (National Institute for Health and Care Excellence: Clinical Guidelines). Disponible sur: http://www.ncbi.nlm.nih.gov/books/NBK566720/
104. Gerver SM, Guy R, Wilson K, Thelwall S, Nsonwu O, Rooney G, et al. National surveillance of bacterial and fungal coinfection and secondary infection in COVID-19 patients in England: lessons from the first wave. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. nov 2021;27(11):1658-65.

105. Grasselli G, Scaravilli V, Mangioni D, Scudeller L, Alagna L, Bartoletti M, et al. Hospital-Acquired Infections in Critically Ill Patients With COVID-19. Chest. août 2021;160(2):454-65.
106. Falagas ME, Kompoti M. Obesity and infection. Lancet Infect Dis. juill 2006;6(7):438-46.
107. Rouzé A, Martin-Loeches I, Povoa P, Makris D, Artigas A, Bouchereau M, et al. Relationship between SARS-CoV-2 infection and the incidence of ventilator-associated lower respiratory tract infections: a European multicenter cohort study. Intensive Care Med. févr 2021;47(2):188-98.
108. Dossett LA, Dageforde LA, Swenson BR, Metzger R, Bonatti H, Sawyer RG, et al. Obesity and site-specific nosocomial infection risk in the intensive care unit. Surg Infect. avr 2009;10(2):137-42.
109. Timsit JF, Ruppé E, Barbier F, Tabah A, Bassetti M. Bloodstream infections in critically ill patients: an expert statement. Intensive Care Med. févr 2020;46(2):266-84.
110. Buetti N, Souweine B, Mermel L, Mimoz O, Ruckly S, Loiodice A, et al. Obesity and risk of catheter-related infections in the ICU. A post hoc analysis of four large randomized controlled trials. Intensive Care Med. avr 2021;47(4):435-43.
111. Sharma P, Uppal NN, Wanchoo R, Shah HH, Yang Y, Parikh R, et al. COVID-19-Associated Kidney Injury: A Case Series of Kidney Biopsy Findings. J Am Soc Nephrol JASN. sept 2020;31(9):1948-58.
112. Volbeda M, Jou-Valencia D, van den Heuvel MC, Knoester M, Zwiers PJ, Pillay J, et al. Comparison of renal histopathology and gene expression profiles between severe COVID-19 and bacterial sepsis in critically ill patients. Crit Care Lond Engl. 10 juin 2021;25(1):202.
113. Jansen J, Reimer KC, Nagai JS, Varghese FS, Overheul GJ, de Beer M, et al. SARS-CoV-2 infects the human kidney and drives fibrosis in kidney organoids. Cell Stem Cell. 3 févr 2022;29(2):217-231.e8.
114. Nadim MK, Forni LG, Mehta RL, Connor MJ, Liu KD, Ostermann M, et al. COVID-19-associated acute kidney injury: consensus report of the 25th Acute Disease Quality Initiative (ADQI) Workgroup. Nat Rev Nephrol. déc 2020;16(12):747-64.
115. Silver SA, Beaubien-Souligny W, Shah PS, Harel S, Blum D, Kishibe T, et al. The Prevalence of Acute Kidney Injury in Patients Hospitalized With COVID-19 Infection: A Systematic Review and Meta-analysis. Kidney Med. févr 2021;3(1):83-98.e1.
116. Moledina DG, Simonov M, Yamamoto Y, Alausa J, Arora T, Biswas A, et al. The Association of COVID-19 With Acute Kidney Injury Independent of Severity of Illness: A Multicenter Cohort Study. Am J Kidney Dis Off J Natl Kidney Found. avr 2021;77(4):490-499.e1.
117. Birkelo BC, Parr SK, Perkins AM, Greevy RA, Hung AM, Shah SC, et al. Comparison of COVID-19 versus influenza on the incidence, features, and recovery from acute kidney injury in hospitalized United States Veterans. Kidney Int. oct 2021;100(4):894-905.
118. Kolhe NV, Fluck RJ, Selby NM, Taal MW. Acute kidney injury associated with COVID-19: A retrospective cohort study. PLoS Med [Internet]. 30 oct 2020 [cité 26 avr 2022];17(10):e1003406. Disponible sur: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7598516/
119. Chan L, Chaudhary K, Saha A, Chauhan K, Vaid A, Zhao S, et al. AKI in Hospitalized Patients with COVID-19. J Am Soc Nephrol JASN. janv 2021;32(1):151-60.
120. Wan YI, Bien Z, Apea VJ, Orkin CM, Dhairyawan R, Kirwan CJ, et al. Acute kidney injury in COVID-19: multicentre prospective analysis of registry data. Clin Kidney J. nov 2021;14(11):2356-64.

121. Hoogenboom WS, Pham A, Anand H, Fleysher R, Buczek A, Soby S, et al. Clinical characteristics of the first and second COVID-19 waves in the Bronx, New York: A retrospective cohort study. Lancet Reg Health Am. nov 2021;3:100041.
122. Cai X, Wu G, Zhang J, Yang L. Risk Factors for Acute Kidney Injury in Adult Patients With COVID-19: A Systematic Review and Meta-Analysis. Front Med. 2021;8:719472.
123. Martín-Del-Campo F, Ruvalcaba-Contreras N, Velázquez-Vidaurri AL, Cueto-Manzano AM, Rojas-Campos E, Cortés-Sanabria L, et al. Morbid obesity is associated with mortality and acute kidney injury in hospitalized patients with COVID-19. Clin Nutr ESPEN. oct 2021;45:200-5.
124. Page-Wilson G, Arakawa R, Nemeth S, Bell F, Girvin Z, Tuohy MC, et al. Obesity is independently associated with septic shock, renal complications, and mortality in a multiracial patient cohort hospitalized with COVID-19. PloS One. 2021;16(8):e0255811.
125. Gupta S, Coca SG, Chan L, Melamed ML, Brenner SK, Hayek SS, et al. AKI Treated with Renal Replacement Therapy in Critically Ill Patients with COVID-19. J Am Soc Nephrol JASN. janv 2021;32(1):161-76.
126. Zamoner W, Santos CA da S, Magalhães LE, de Oliveira PGS, Balbi AL, Ponce D. Acute Kidney Injury in COVID-19: 90 Days of the Pandemic in a Brazilian Public Hospital. Front Med. 2021;8:622577.
127. Bowe B, Cai M, Xie Y, Gibson AK, Maddukuri G, Al-Aly Z. Acute Kidney Injury in a National Cohort of Hospitalized US Veterans with COVID-19. Clin J Am Soc Nephrol CJASN. 31 déc 2020;16(1):14-25.
128. Nakeshbandi M, Maini R, Daniel P, Rosengarten S, Parmar P, Wilson C, et al. The impact of obesity on COVID-19 complications: a retrospective cohort study. Int J Obes 2005. sept 2020;44(9):1832-7.
129. Schiffl H. Obesity and the Survival of Critically Ill Patients with Acute Kidney Injury: A Paradox within the Paradox? Kidney Dis Basel Switz. janv 2020;6(1):13-21.
130. Schetz M, De Jong A, Deane AM, Druml W, Hemelaar P, Pelosi P, et al. Obesity in the critically ill: a narrative review. Intensive Care Med [Internet]. 1 juin 2019 [cité 3 nov 2021];45(6):757-69. Disponible sur: https://doi.org/10.1007/s00134-019-05594-1
131. Prospective Studies Collaboration, Whitlock G, Lewington S, Sherliker P, Clarke R, Emberson J, et al. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. Lancet Lond Engl. 28 mars 2009;373(9669):1083-96.
132. Di Angelantonio E, Bhupathiraju SN, Wormser D, Gao P, Kaptoge S, de Gonzalez AB, et al. Body-mass index and all-cause mortality: individual-participant-data meta-analysis of 239 prospective studies in four continents. The Lancet. août 2016;388(10046):776-86.
133. Naderi N, Kleine CE, Park C, Hsiung JT, Soohoo M, Tantisattamo E, et al. Obesity Paradox in Advanced Kidney Disease: From Bedside to the Bench. Prog Cardiovasc Dis. août 2018;61(2):168-81.
134. Sharma A, Lavie CJ, Borer JS, Vallakati A, Goel S, Lopez-Jimenez F, et al. Meta-analysis of the relation of body mass index to all-cause and cardiovascular mortality and hospitalization in patients with chronic heart failure. Am J Cardiol. 15 mai 2015;115(10):1428-34.
135. Nie W, Zhang Y, Jee SH, Jung KJ, Li B, Xiu Q. Obesity survival paradox in pneumonia: a meta-analysis. BMC Med. 10 avr 2014;12:61.

136. Tlayjeh H, Arabi YM, Ferguson ND, Zhou Q, Lamontagne F, Arroliga A, et al. Body Mass Index and Mortality in Subjects With ARDS: Post-hoc Analysis of the OSCILLATE Trial. Respir Care. sept 2019;64(9):1042-8.
137. Pepper DJ, Sun J, Welsh J, Cui X, Suffredini AF, Eichacker PQ. Increased body mass index and adjusted mortality in ICU patients with sepsis or septic shock: a systematic review and meta-analysis. Crit Care Lond Engl. 15 juin 2016;20(1):181.
138. Pepper DJ, Demirkale CY, Sun J, Rhee C, Fram D, Eichacker P, et al. Does Obesity Protect Against Death in Sepsis? A Retrospective Cohort Study of 55,038 Adult Patients*: Crit Care Med. mai 2019;47(5):643-50.
139. Zhi G, Xin W, Ying W, Guohong X, Shuying L. « Obesity Paradox » in Acute Respiratory Distress Syndrome: Asystematic Review and Meta-Analysis. PloS One. 2016;11(9):e0163677.
140. Manolis AS, Manolis AA, Manolis TA, Apostolaki NE, Melita H. COVID-19 Infection and Body Weight: A Deleterious Liaison in a J-Curve Relationship. Obes Res Clin Pract [Internet]. 3 nov 2021 [cité 11 nov 2021]; Disponible sur: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8563353/
141. Huang HK, Bukhari K, Peng CCH, Hung DP, Shih MC, Chang RHE, et al. The J-shaped relationship between body mass index and mortality in patients with COVID-19: A dose-response meta-analysis. Diabetes Obes Metab. juill 2021;23(7):1701-9.
142. Yang J, Ma Z, Lei Y. A meta-analysis of the association between obesity and COVID-19. Epidemiol Infect [Internet]. 22 déc 2020 [cité 10 juin 2022];149:e11. Disponible sur: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7844214/
143. Zhao X, Gang X, He G, Li Z, Lv Y, Han Q, et al. Obesity Increases the Severity and Mortality of Influenza and COVID-19: A Systematic Review and Meta-Analysis. Front Endocrinol [Internet]. 21 déc 2020 [cité 10 juin 2022];11:595109. Disponible sur: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7779975/
144. Gupta S, Hayek SS, Wang W, Chan L, Mathews KS, Melamed ML, et al. Factors Associated With Death in Critically Ill Patients With Coronavirus Disease 2019 in the US. JAMA Intern Med [Internet]. nov 2020 [cité 11 mai 2022];180(11):1-12. Disponible sur: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7364338/
145. Abumayyaleh M, Núñez Gil IJ, El-Battrawy I, Estrada V, Becerra-Muñoz VM, Aparisi A, et al. Does there exist an obesity paradox in COVID-19? Insights of the international HOPE-COVID-19-registry. Obes Res Clin Pract. juin 2021;15(3):275-80.
146. Zhang X, Lewis AM, Moley JR, Brestoff JR. A systematic review and meta-analysis of obesity and COVID-19 outcomes. Sci Rep. 30 mars 2021;11(1):7193.
147. Banack HR, Stokes A. The 'obesity paradox' may not be a paradox at all. Int J Obes. août 2017;41(8):1162-3.
148. Yong SJ. Long COVID or post-COVID-19 syndrome: putative pathophysiology, risk factors, and treatments. Infect Dis Lond Engl [Internet]. [cité 10 nov 2021];1-18. Disponible sur: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8146298/
149. Pavli A, Theodoridou M, Maltezou HC. Post-COVID Syndrome: Incidence, Clinical Spectrum, and Challenges for Primary Healthcare Professionals. Arch Med Res. août 2021;52(6):575-81.
150. Maltezou HC, Pavli A, Tsakris A. Post-COVID Syndrome: An Insight on Its Pathogenesis. Vaccines. 12 mai 2021;9(5):497.

151. Fernández-de-Las-Peñas C, Palacios-Ceña D, Gómez-Mayordomo V, Cuadrado ML, Florencio LL. Defining Post-COVID Symptoms (Post-Acute COVID, Long COVID, Persistent Post-COVID): An Integrative Classification. Int J Environ Res Public Health. 5 mars 2021;18(5):2621.
152. Greenhalgh T, Knight M, A'Court C, Buxton M, Husain L. Management of post-acute covid-19 in primary care. BMJ. 11 août 2020;370:m3026.
153. Amenta EM, Spallone A, Rodriguez-Barradas MC, El Sahly HM, Atmar RL, Kulkarni PA. Postacute COVID-19: An Overview and Approach to Classification. Open Forum Infect Dis [Internet]. 21 oct 2020 [cité 27 avr 2022];7(12):ofaa509. Disponible sur: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7665635/
154. Becker RC. COVID-19 and its sequelae: a platform for optimal patient care, discovery and training. J Thromb Thrombolysis. avr 2021;51(3):587-94.
155. Living with Covid19 – Second review [Internet]. NIHR Evidence. 2021 [cité 28 avr 2022]. Disponible sur: https://evidence.nihr.ac.uk/themedreview/living-with-covid19-second-review/
156. COVID-19 rapid guideline: managing the long-term effects of COVID-19 [Internet]. London: National Institute for Health and Care Excellence (NICE); 2020 [cité 28 avr 2022]. (National Institute for Health and Care Excellence: Clinical Guidelines). Disponible sur: http://www.ncbi.nlm.nih.gov/books/NBK567261/
157. Havervall S, Rosell A, Phillipson M, Mangsbo SM, Nilsson P, Hober S, et al. Symptoms and Functional Impairment Assessed 8 Months After Mild COVID-19 Among Health Care Workers. JAMA. 18 mai 2021;325(19):2015-6.
158. Carvalho-Schneider C, Laurent E, Lemaignen A, Beaufils E, Bourbao-Tournois C, Laribi S, et al. Follow-up of adults with noncritical COVID-19 two months after symptom onset. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. févr 2021;27(2):258-63.
159. Halpin SJ, McIvor C, Whyatt G, Adams A, Harvey O, McLean L, et al. Postdischarge symptoms and rehabilitation needs in survivors of COVID-19 infection: A cross-sectional evaluation. J Med Virol. févr 2021;93(2):1013-22.
160. Huang C, Huang L, Wang Y, Li X, Ren L, Gu X, et al. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. Lancet Lond Engl. 16 janv 2021;397(10270):220-32.
161. Carfì A, Bernabei R, Landi F, Gemelli Against COVID-19 Post-Acute Care Study Group. Persistent Symptoms in Patients After Acute COVID-19. JAMA. 11 août 2020;324(6):603-5.
162. Yelin D, Margalit I, Nehme M, Bordas-Martínez J, Pistelli F, Yahav D, et al. Patterns of Long COVID Symptoms: A Multi-Center Cross Sectional Study. J Clin Med. 9 févr 2022;11(4):898.
163. Moreno-Pérez O, Merino E, Leon-Ramirez JM, Andres M, Ramos JM, Arenas-Jiménez J, et al. Post-acute COVID-19 syndrome. Incidence and risk factors: A Mediterranean cohort study. J Infect. mars 2021;82(3):378-83.
164. Nittas V, Gao M, West EA, Ballouz T, Menges D, Wulf Hanson S, et al. Long COVID Through a Public Health Lens: An Umbrella Review. Public Health Rev [Internet]. 15 mars 2022 [cité 28 avr 2022];43:1604501. Disponible sur: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8963488/
165. Davis HE, Assaf GS, McCorkell L, Wei H, Low RJ, Re'em Y, et al. Characterizing long COVID in an international cohort: 7 months of symptoms and their impact. EClinicalMedicine. août 2021;38:101019.

166. Garrigues E, Janvier P, Kherabi Y, Le Bot A, Hamon A, Gouze H, et al. Post-discharge persistent symptoms and health-related quality of life after hospitalization for COVID-19. J Infect. déc 2020;81(6):e4-6.
167. Anaya JM, Rojas M, Salinas ML, Rodríguez Y, Roa G, Lozano M, et al. Post-COVID syndrome. A case series and comprehensive review. Autoimmun Rev [Internet]. nov 2021 [cité 20 mars 2022];20(11):102947. Disponible sur: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8428988/
168. Bellan M, Soddu D, Balbo PE, Baricich A, Zeppegno P, Avanzi GC, et al. Respiratory and Psychophysical Sequelae Among Patients With COVID-19 Four Months After Hospital Discharge. JAMA Netw Open. 4 janv 2021;4(1):e2036142.
169. Alemanno F, Houdayer E, Parma A, Spina A, Del Forno A, Scatolini A, et al. COVID-19 cognitive deficits after respiratory assistance in the subacute phase: A COVID-rehabilitation unit experience. PloS One. 2021;16(2):e0246590.
170. Tserel L, Jõgi P, Naaber P, Maslovskaja J, Häling A, Salumets A, et al. Long-Term Elevated Inflammatory Protein Levels in Asymptomatic SARS-CoV-2 Infected Individuals. Front Immunol. 2021;12:709759.
171. Ortelli P, Ferrazzoli D, Sebastianelli L, Engl M, Romanello R, Nardone R, et al. Neuropsychological and neurophysiological correlates of fatigue in post-acute patients with neurological manifestations of COVID-19: Insights into a challenging symptom. J Neurol Sci. 15 janv 2021;420:117271.
172. Davogustto GE, Clark DE, Hardison E, Yanis AH, Lowery BD, Halasa NB, et al. Characteristics Associated With Multisystem Inflammatory Syndrome Among Adults With SARS-CoV-2 Infection. JAMA Netw Open. 3 mai 2021;4(5):e2110323.
173. Patel P, DeCuir J, Abrams J, Campbell AP, Godfred-Cato S, Belay ED. Clinical Characteristics of Multisystem Inflammatory Syndrome in Adults: A Systematic Review. JAMA Netw Open. 1 sept 2021;4(9):e2126456.
174. Ong SWX, Fong SW, Young BE, Chan YH, Lee B, Amrun SN, et al. Persistent Symptoms and Association With Inflammatory Cytokine Signatures in Recovered Coronavirus Disease 2019 Patients. Open Forum Infect Dis. juin 2021;8(6):ofab156.
175. Acosta-Ampudia Y, Monsalve DM, Rojas M, Rodríguez Y, Zapata E, Ramírez-Santana C, et al. Persistent Autoimmune Activation and Proinflammatory State in Post-Coronavirus Disease 2019 Syndrome. J Infect Dis [Internet]. 25 janv 2022 [cité 20 mars 2022];jiac017. Disponible sur: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8903340/
176. Jacobs JJL. Persistent SARS-2 infections contribute to long COVID-19. Med Hypotheses. avr 2021;149:110538.
177. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. N Engl J Med. 9 juill 2020;383(2):120-8.
178. Fogarty H, Townsend L, Morrin H, Ahmad A, Comerford C, Karampini E, et al. Persistent endotheliopathy in the pathogenesis of long COVID syndrome. J Thromb Haemost JTH. oct 2021;19(10):2546-53.
179. Loosen SH, Jensen BEO, Tanislav C, Luedde T, Roderburg C, Kostev K. Obesity and lipid metabolism disorders determine the risk for development of long COVID syndrome: a cross-sectional study from 50,402 COVID-19 patients. Infection. 30 mars 2022;

180. Sudre CH, Murray B, Varsavsky T, Graham MS, Penfold RS, Bowyer RC, et al. Attributes and predictors of long COVID. Nat Med [Internet]. 1 avr 2021 [cité 7 nov 2021];27(4):626-31. Disponible sur: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7611399/
181. Vimercati L, De Maria L, Quarato M, Caputi A, Gesualdo L, Migliore G, et al. Association between Long COVID and Overweight/Obesity. J Clin Med. 14 sept 2021;10(18):4143.
181. Fernández-de-las-Peñas C, Torres-Macho J, Elvira-Martínez CM, Molina-Trigueros LJ, Sebastián-Viana T, Hernández-Barrera V. Obesity is associated with a greater number of long-term post-COVID symptoms and poor sleep quality: A multicentre case-control study. Int J Clin Pract [Internet]. [cité 11 nov 2021];n/a(n/a):e14917. Disponible sur: https://onlinelibrary.wiley.com/doi/abs/10.1111/ijcp.14917
183. Whitaker M, Elliott J, Chadeau-Hyam M, Riley S, Darzi A, Cooke G, et al. Persistent COVID-19 symptoms in a community study of 606,434 people in England. Nat Commun. 12 avr 2022;13(1):1957.

Part II

BIOMARKERS AND PHENOTYPING IN CRITICALLY ILL COVID-19 PATIENTS

CHAPTER 6

Cytokine levels in critically ill patients with COVID-19 and other conditions

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Introduction

An abnormally strong pro-inflammatory response known as "cytokine storm" may play an important role in the pathophysiology of coronavirus disease 2019 (COVID-19), although cytokine storm remains ill-defined[1]. Sinha and colleagues[2] reported that, although interleukin (IL)-6 levels are elevated in severe COVID-19, they are lower than levels usually observed in (non-COVID) acute respiratory distress syndrome (ARDS). However, this comparison is limited by the use of different assays, which are not well standardized[3]. We compared cytokine levels in critically ill COVID-19 patients with levels in patients with other critical illnesses.

Methods

All patients in this study were admitted to the intensive care unit (ICU) of Radboud university medical center. Plasma concentrations of pro-inflammatory cytokines tumor necrosis factor (TNF)-α, IL-6, and IL-8 were determined in consecutive mechanically ventilated COVID-19 patients with ARDS (PaO_2/FiO_2 ratio <300; sampled within 48 hours after ICU admission), bacterial septic shock with or without ARDS (sampled within 24 hours after septic shock diagnosis), out-of-hospital cardiac arrest (OHCA; sampled within 24 hours after ICU admission), and multiple trauma (sampled within 24 hours after trauma). The sepsis and trauma patients are part of larger published cohorts[4,5], whereas data of 14 OHCA patients were published previously[6]. Sampling occurred between 2010 and 2020 (Table 1). Patients with immunological insufficiencies were excluded, defined as chronic/concomitant use of immunosuppressive medication, chemo/radiotherapy in the last year, or in the past for (non)-Hodgkin lymphoma, or humoral/cellular deficiencies. Cytokines in all cohorts were determined using the same methodology (Milliplex assay, Millipore, on a MAGPIX instrument, Luminex Corporation) by the same technician using the same protocol.
Patient characteristics were analyzed using Fisher exact tests or Kruskal-Wallis tests followed by Dunn's post-hoc tests. Cytokine data are presented as geometric mean [95% confidence interval] and analyzed using one-way analysis of variance on log-transformed data followed by Dunnet's post-hoc tests. Data were analyzed using Graphpad Prism v8.3.0 (Graphpad Software). A 2-sided p-value <0.05 was considered statistically significant. The study was carried out in accordance with the applicable rules concerning the review of research ethics committees and informed consent in the Netherlands. All patients or legal representatives were informed about the study details and allowed to abstain from participation.

Results

There were 46 COVID-19 patients with ARDS, 51 with septic shock with ARDS, 15 with septic shock without ARDS, 30 with OHCA, and 62 with multiple trauma. There were no significant differences in sex or age between COVID-19 and other patient groups (Table 1). COVID-19 patients had a higher BMI and prevalence of diabetes than OHCA and trauma patients. In COVID-19, cardiovascular insufficiency was more common, overall disease severity and leukocyte counts lower, and lung injury more severe compared to the other groups. Levels of all 3 cytokines were significantly lower in patients with COVID-19 than in septic shock ARDS patients (geometric mean [95% confidence interval] in pg/mL: TNF-α: 22 [18-27] vs. 40 [30-55], p<0.01; IL-6: 48 [35-66] vs. 376 [190-744], p<0.001; IL-8: 27 [23-33] vs. 215 [133-347], p<0.001, respectively, depicted in Figure 1 on a log-scale). COVID-19 patients also displayed significantly lower IL-6 and IL-8 concentrations compared with septic shock patients without ARDS (Figure 1). TNF-α levels in COVID-19 patients were higher than those in trauma patients, whereas no differences between COVID-19 and OHCA or trauma patients were present for IL-6. For IL-8, lower concentrations were found in COVID-19 patients compared with OHCA patients, while no differences versus the trauma group were observed.

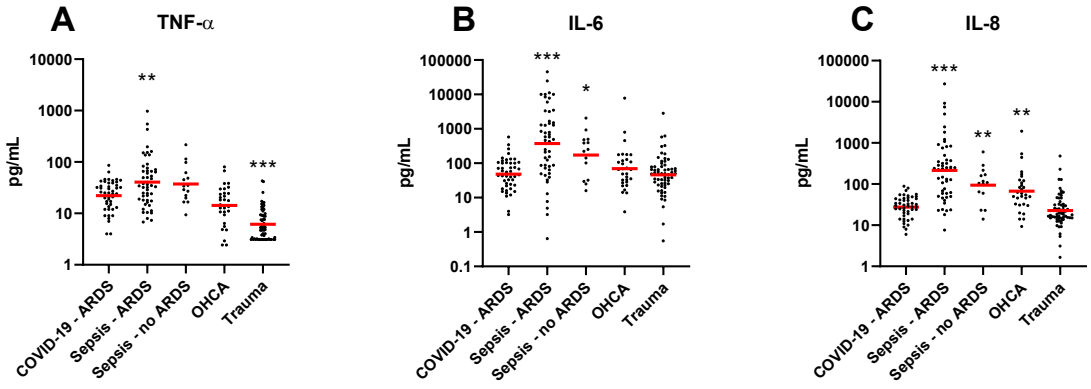

Figure 1. Cytokine levels in critically ill patients with COVID-19 and other conditions. *Panels A-C: Plasma concentrations of tumor necrosis factor (TNF)-α* ***(A)****, interleukin (IL)-6* ***(B)****, and IL-8* ***(C)*** *in patients with COVID-19 and acute respiratory distress syndrome (ARDS) (n=46), septic shock with ARDS (n=51), septic shock without ARDS (n=15), out-of-hospital cardiac arrest (OHCA, n=30), and multiple trauma (n=62). Data are presented as scatter plots with red horizontal bars indicating the geometric mean levels. *p<0.05, **p<0.01, ***p<0.001 vs. COVID-19-ARDS.*

Table 1. Patient characteristics.

	COVID-19 with ARDS (n=46)	Septic shock with ARDS (n=51)	Septic shock without ARDS (n=15)	Out-of-hospital cardiac arrest (n=30)	Trauma (n=62)
Sampling dates	March 11th 2020 - April 27th 2020	March 15th 2013 - March 28th 2017		February 5th 2010 - December 12th 2013	March 19th 2011 - May 30th 2013
Sex, n (%)					
Male	34 (74)	36 (71)	6 (40)	22 (73)	44 (71)
Female	12 (26)	15 (29)	9 (60)	8 (27)	18 (29)
Age, years	67 [57-71]	62 [53-72]	73 [64-78]	65 [52-75]	58 [37-72]
BMI, kg/m^2	27.5 [25.0-29.3]	26.4 [23.8-30.5]	25.0 [21.5-30.3]	25.1* [23.4-26.9]	24.7** [23.2-27.4]
Medical history, n (%)					
Cardiovascular insufficiency	12 (26)	2** (4)	2 (13)	1* (3)	1*** (2)
Respiratory insufficiency	3 (7)	1 (2)	0 (0)	0 (0)	0 (0)
COPD	3 (7)	5 (10)	0 (0)	0 (0)	0 (0)
Renal insufficiency	0 (0)	5 (10)	0 (0)	0 (0)	0 (0)
Metastatic neoplasm	4 (9)	1 (2)	2 (13)	1 (3)	0* (0)
Diabetes	13 (28)	8 (16)	1 (7)	1** (3)	4** (6)
Hematologic malignancy	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
APACHE II score	14 [12-18]	21*** [17-26]	24*** [18-31]	27*** [20-34]	20** [14-25]
PaO_2/FiO_2 ratio	139 [107-171]	206*** [162-260]	354*** [328-424]	246*** [159-370]	253*** [201-361]
Leukocytes, 10^9/L	8.2 [6.4-11.1]	14.0*** [9.8-20.8]	15.4** [7.2-24.4]	12.9*** [10.0-16.7]	11.8** [8.9-14.0]

*Data were obtained at the same day that blood was obtained for cytokine determination and presented as n (%) or median [interquartile range]. COVID-19: coronavirus disease 2019; ARDS: acute respiratory distress syndrome; BMI: Body mass index; COPD: Chronic obstructive pulmonary disease; APACHE II: Acute Physiology And Chronic Health Evaluation II (ICU score of overall disease severity ranging from 0-71; a higher score indicates more severe disease). PaO2: partial pressure of oxygen; FiO2: fraction of inspired oxygen. *p<0.05, **p<0.01, ***p<0.001, vs. COVID-19 with ARDS.*

Discussion

In this study, critically ill COVID-19 patients with ARDS had circulating cytokine levels that were lower compared with bacterial sepsis patients and similar to other critically ill patients. These findings are in line with lower leukocyte counts observed in COVID-19 patients, and are possibly due to lower overall disease severity, despite the presence of severe pulmonary injury. The findings of this preliminary analysis suggest the COVID-19 may not be characterized by cytokine storm. Whether anti-cytokine therapies will benefit COVID-19 patients remains to be determined. Limitations of the study include the small sample sizes, single center involved, and the use of different lots of the same assays without data on lot-to-lot variability.

References

1. Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR, Katze MG. Into the eye of the cytokine storm. Microbiol Mol Biol Rev. 2012;76(1):16-32.
2. Sinha P, Matthay MA, Calfee CS. Is a "Cytokine Storm" Relevant to COVID-19? JAMA Intern Med. 2020. doi: 10.1001/jamainternmed.2020.3313.
3. Khan SS, Smith MS, Reda D, Suffredini AF, McCoy JP, Jr. Multiplex bead array assays for detection of soluble cytokines: comparisons of sensitivity and quantitative values among kits from multiple manufacturers. Cytometry B Clin Cytom. 2004;61(1):35-39.
4. Habes QLM, van Ede L, Gerretsen J, Kox M, Pickkers P. Norepinephrine Contributes to Enterocyte Damage in Septic Shock Patients: A Prospective Cohort Study. Shock. 2018;49(2):137-143.
5. Timmermans K, Kox M, Vaneker M, et al. Plasma levels of danger-associated molecular patterns are associated with immune suppression in trauma patients. Intensive Care Med. 2016;42(4):551-561.
6. Timmermans K, Kox M, Gerretsen J, et al. The Involvement of Danger-Associated Molecular Patterns in the Development of Immunoparalysis in Cardiac Arrest Patients. Crit Care Med. 2015;43(11):2332-2338.

CHAPTER 7

Dexamethasone and tocilizumab treatment considerably reduces the value of C-reactive protein and procalcitonin to detect secondary bacterial infections in COVID-19 patients

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Abstract

Background

Procalcitonin (PCT) and C-reactive protein (CRP) were previously shown to have value for the detection of secondary infections in critically ill COVID-19 patients. However, since the introduction of immunomodulatory therapy, the value of these biomarkers is unclear. We investigated PCT and CRP kinetics in critically ill COVID-19 patients treated with dexamethasone with or without tocilizumab, and assessed the value of these biomarkers to detect secondary bacterial infections.

Methods

In this prospective study, 190 critically ill COVID-19 patients were divided into three treatment groups: *no dexamethasone, no tocilizumab (D-T-)*, *dexamethasone, no tocilizumab (D+T-)*, and *dexamethasone and tocilizumab (D+T+)*. Serial data of PCT and CRP were aligned on the last day of dexamethasone treatment, and kinetics of these biomarkers were analyzed between 6 days prior to cessation of dexamethasone and 10 days afterwards. Furthermore, the D+T- and D+T+ groups were subdivided into secondary infection and no-secondary infection groups to analyze differences in PCT and CRP kinetics and calculate detection accuracy of these biomarkers for the occurrence of a secondary infection.

Results

Following cessation of dexamethasone, there was a rebound in PCT and CRP levels, most pronounced in the D+T- group. Upon occurrence of a secondary infection, no significant increase in PCT and CRP levels was observed in the D+T- group ($p=0.052$ and $p=0.08$, respectively). Although PCT levels increased significantly in patients of the D+T+ group who developed a secondary infection ($p=0.0003$), this rise was only apparent from day 2 post-infection onwards. CRP levels remained suppressed in the D+T+ group. Receiver operating curve analysis of PCT and CRP levels yielded area under the curves of 0.52 and 0.55, respectively, which are both markedly lower than those found in the group of COVID-19 patients not treated with immunomodulatory drugs (0.80 and 0.76, respectively, with p-values for differences between groups of 0.001 and 0.02, respectively).

Conclusions

Cessation of dexamethasone in critically ill COVID-19 patients results in a rebound increase in PCT and CRP levels unrelated to the occurrence of secondary bacterial infections. Furthermore, immunomodulatory treatment with dexamethasone and tocilizumab considerably reduces the value of PCT and CRP for detection of secondary infections in COVID-19 patients.

Introduction

Coronavirus Disease 2019 (COVID-19) is characterized by inflammatory damage to various tissues, particularly the lung. Hence, a wide range of circulating inflammatory markers are elevated in COVID-19 patients, correlating with disease severity and outcomes (1). This observation also holds true for procalcitonin (PCT) and C-reactive protein (CRP) (2, 3). As has been studied repeatedly in non-COVID-19 patients, these biomarkers also have discriminatory potential for bacterial (super)infections in critically ill patients and are frequently used to assist antibiotic clinical decision making (4, 5). In addition, once antibacterial therapy is started, repeated measurements of PCT every 48-72 hours may help guide the duration of therapy (6).

We previously investigated the natural course of PCT and CRP and their value to identify secondary infections in critically ill COVID-19. We showed that COVID-19 patients have elevated concentrations at ICU admission, that gradually decline, while a later increase in these biomarkers indicate a secondary bacterial infection (5). However, since then, pharmacological treatment of COVID-19-patients admitted to the ICU has changed considerably. The immunomodulatory drugs dexamethasone (7, 8) and the human anti-interleukin (IL)-6 receptor antibody tocilizumab (9, 10) have been shown to exert beneficial clinical effects in patients with severe COVID-19 and consequently have become part of standard care. The effects of these therapies on PCT and CRP levels in critically ill COVID-19 patients are largely unclear, but were previously assessed in non-COVID-19 patients. For instance, in patients with severe community-acquired pneumonia, corticosteroids attenuate induction of CRP, while the suppressive effect on PCT levels appears to be less pronounced (11). In critically ill sepsis patients, treatment with corticosteroids lead to a significant decrease in CRP levels (12). This effect of corticosteroids is independent of SOFA scores, illustrating the direct immunomodulatory effect independent of the clinical condition of the patient (13). Interestingly, it was also reported that withdrawal of hydrocortisone treatment results in an inflammatory rebound phenomenon in septic shock patients (14). Like corticosteroids, tocilizumab treatment also reduced CRP levels in patients with rheumatoid arthritis and in those suffering from giant cell arteritis (15). Also in COVID-19 patients, reduction in PCT and CRP levels was observed in patients treated with tocilizumab (16). However, differentiation between the immunomodulatory and beneficial effect on the clinical condition of the patient is difficult. Currently, the effects of dexamethasone and tocilizumab on the kinetics of PCT and CRP in COVID-19 patients are unknown. Furthermore, whether the value to detect secondary bacterial infections of these biomarkers is jeopardized by these treatments is unclear as well. In the present study, we investigated serial PCT and CRP levels in critically ill COVID-19 patients treated with dexamethasone only or in combination with tocilizumab, and compared the natural course and accuracy to detect bacterial infections to the data obtained from patients that did not receive these immunomodulatory treatments (5).

Material & Methods

Study design and participants

All patients admitted to the ICU of the Radboud university medical center (Nijmegen, The Netherlands) between March 11th, 2020 and April 29th, 2020 ('first cohort') and between August 10th, 2021 and February 5th, 2021 ('second cohort') were screened. Adult patients with COVID-19 proven by a positive SARS-CoV-2 PT-PCR test in nasopharyngeal and throat swabs were eligible for inclusion. Patients that were immunocompromised based on pre-existent comorbidity or treatment were excluded. Also, because of multiple transfers between different hospitals during the second inclusion period, patients who stayed in another ICU for ≥7 days prior to admission to the ICU of our center were not included. See Figure 1 for an overview of the total patient selection. In the first cohort, treatment was largely supportive and patients did not receive any immunomodulating therapy. The complete second cohort received dexamethasone (DEXA) treatment as part of standard care, which was administered for a total of 10 days following hospital admission (6 mg daily, intravenously). A subgroup of the second cohort was also treated with tocilizumab (TOCI, single dose of 8mg/kg, intravenously). First, patients were treated with tocilizumab when they were randomized in the tocilizumab subgroup of the international REMAP-CAP trial (17). Later, when results of the REMAP-CAP trial showed beneficial effects of tocilizumab in severely ill COVID-19 patients (9), this treatment became part of standard care.

To investigate the natural course and effects of cessation of DEXA on PCT and CRP levels, patients were divided into three groups (see flowchart in Figure 1): (1) patients from the first cohort who did not receive DEXA nor TOCI ('D-T-'); data of this group was published previously (5), (2) patients from the second cohort who were treated with DEXA only ('D+T-'), and (3) patients from the second cohort who were treated with both DEXA and TOCI ('D+T+'). Patients in whom the DEXA treatment was already completed when admitted to the ICU, were excluded for this analysis. Serially measured PCT and CRP data of the latter two groups were aligned on the last day of DEXA treatment which was designated day 0. Data of the D-T- group were aligned on the median day of cessation of DEXA, relatively to ICU admission. To confirm that possibly observed effects were not based on a small group of patients with a more complicated clinical course and a longer stay in ICU, a sensitivity analysis was performed including only patients with no missing data throughout the complete study period.

In addition, to explore the predictive value of PCT and CRP to detect secondary infections in critically ill COVID-19 patients treated with DEXA with or without TOCI, patients of the

second cohort were further divided into 'secondary infection groups' and 'no-secondary infection groups' (see flowchart in Figure 1). Next to the general exclusion criteria, patients who were still admitted to the ICU on moment of data analysis (March 2021) were excluded for this analysis. A secondary bacterial infection was defined as an infectious episode confirmed by a positive culture, which was taken in case of a suspected secondary infection based on clinical signs of a new infectious episode (fever, respiratory failure, hemodynamic instability, elevated white blood cell counts). A positive culture in absence of clinical signs of a secondary infection was not scored as a secondary infection, but interpreted as colonization, as these patients were also not treated with antibiotics. The infectious episodes were determined from the electronical medical records by three ICU physicians (MvdB, TF and JS). Information about the type of infection and causative agents was retrieved from the medical records. Serially measured PCT and CRP levels of the secondary infection groups were aligned on the day the positive culture was taken, which was designated day 0. In case of multiple sequential secondary infections, only the first day of the first infectious episode was used as alignment day. Data of the no-secondary infections groups were aligned on the median day that secondary infections occurred in the affected groups following ICU admission. Following data alignment, patients in the no-secondary infection groups who were discharged from the hospital on alignment day were excluded because no data was available. To illustrate possible differences with our previously published results of the first cohort (5), data of the secondary infection group of this study (D-T-) were also included in the figures. Finally, all patients who developed a secondary infection were divided into an early infection group (secondary infection occurred ≤4 days following cessation of DEXA therapy) and a late infection group (secondary infection occurred > 4 days following cessation of DEXA therapy). Serially measured PCT and CRP, again aligned on day of secondary infection, were compared between the early and late infection groups to investigate whether the predictive accuracy of PCT and CRP differed between patients who developed a secondary infection during or early after DEXA treatment and patients who developed a secondary infection at a later stage in the ICU.

This study was carried out in accordance with the applicable rules concerning the review of research ethics committees and informed consent in the Netherlands. All patients or legal representatives were informed about the details of this cohort study and could decline to participate.

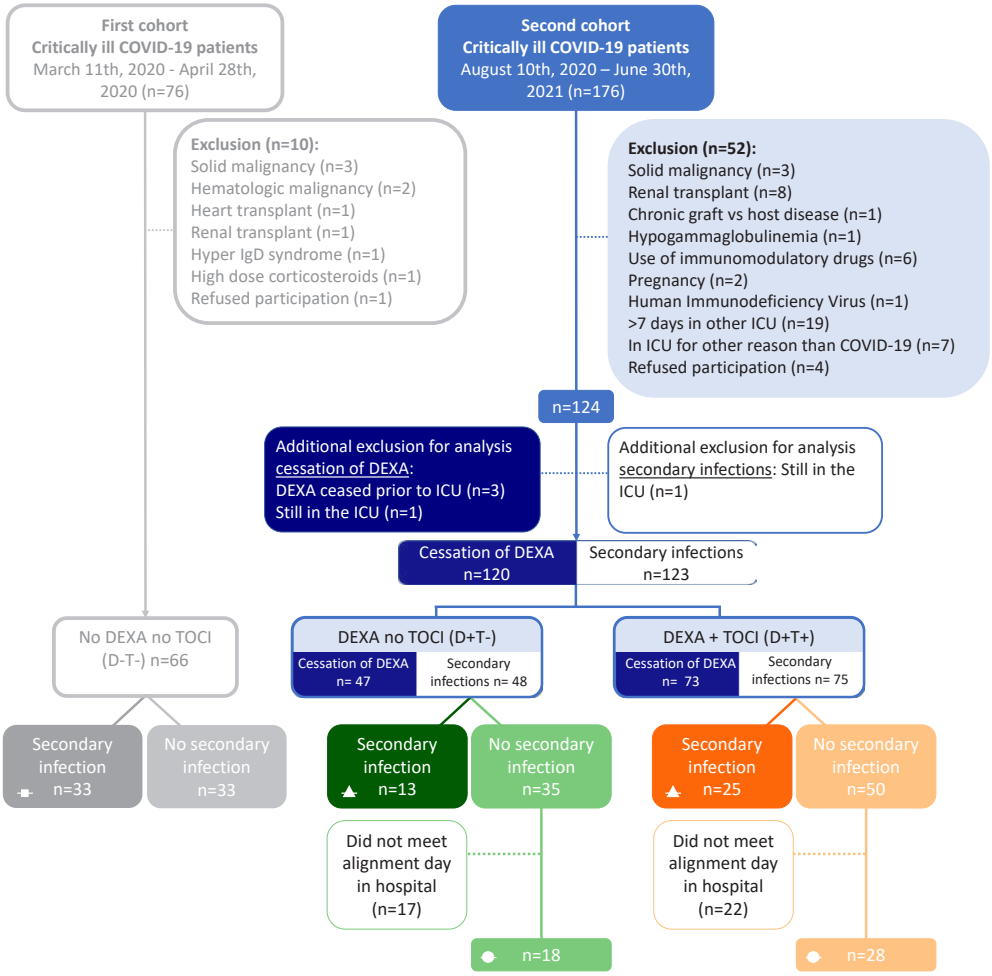

Figure 1. Patient flowchart. *Patients who were immunocompromised based on pre-existent comorbidity or treatment and patients of the second cohort who stayed in another ICU for ≥7 days prior to admission to the ICU were excluded. For the analysis of PCT and CRP kinetics following cessation of dexamethasone (DEXA) treatment, patients in whom the dexamethasone treatment was already completed when admitted to the ICU, were excluded. For the analysis of PCT and CRP kinetics in patients developing a secondary infection, patients who were still admitted to the ICU on moment of data analysis (July 2021) were excluded. The remaining patients were divided into a dexamethasone-only group (D+T-) and a dexamethasone and tocilizumab (TOCI) group (D+T+), which were again subdivided into a secondary infection group and a no-secondary infection group. Following data alignment, patients in the no secondary infection groups who were discharged from the hospital on alignment day were excluded because no data was available.*

Data collection

Data collection was carried out as part of a cohort study in critically ill COVID-19 patients in the ICU of the Radboud university medical center. Data of patient characteristics, medical history and clinical parameters were collected from the electronic patient files (Epic, Epic Systems Corporation, Verona, Wisconsin, USA) and recorded in the Good Clinical Practice-certified data management system Castor (Castor EDC, Amsterdam, the Netherlands). For clinical purposes, PCT and CRP were determined every 48 hours. PCT was determined using the Elecsys BRAHMS procalcitonin assay (Thermo Fisher Scientific, Waltham, MA, USA) and CRP was determined using an immunoturbidimetric assay, both on a Cobas 8000 immuno-analyzer (Roche Diagnostics, Rotkreuz, Switzerland).

Statistical analysis

Serial PCT and CRP data were aligned (see above) and binned into bins spanning two days using a script made in RStudio v3.6.2 (RStudio, PBC, Boston, USA). Differences in patient characteristics between the D-T-, D+T- and D+T+ groups were analyzed using Kruskal Wallis and chi-square tests followed by post-hoc Dunn's multiple comparisons tests or pairwise chi-square tests, respectively. Patient characteristics of the secondary infection groups and no-secondary infection groups (within the D-T-, D+T- and D+T+ groups) were analyzed using chi-square and Mann Whitney U tests. Between-group differences over time were analyzed using linear mixed effects model analysis on log-transformed data followed by post-hoc analyses using Sidak's multiple comparisons tests. For comparisons of PCT and CRP kinetics in patients with and without secondary infections, and to compare the early and late secondary infection groups, data were analyzed from day -10 until day 10 relative to the day of secondary infection. For these analyses, we performed the Last Observation Carried Forward (LOCF) method for data of patients who were discharged from the hospital between day +1 and day +10. To illustrate sensitivity and specificity of PCT and CRP levels to predict secondary infections, receiver operating curve (ROC) analyses were performed using binned data of day -1 and 0. Differences in area's under the receiver operating curves (AUROCs) between patients treated with and without immunomodulatory drugs were assessed using the following strategy:

First, AUROCs and corresponding standard errors (SE) of separate groups (D-T-, D+T-, D+T+ and D+T-/+) were determined. Subsequently, z-scores for differences between AUROCs were calculated using the following formula: $z = \frac{(ROCAUC1 - ROCAUC2)}{\sqrt{SE1^2 + SE2^2}}$

Finally, two-tailed p-values were determined using the following function in Excel 2016 (Microsoft Corporation): 2*(1-NORMSDIST(Z)).

Data are displayed as number (%), median with interquartile ranges [IQR], or geometric mean with 95% confidence intervals (CI). All statistical analyses were performed in SPSS Statistics 25 (IBM SPSS Statistics, Version 25.0. Armonk, NY: IBM Corp) and Graphpad Prism 8 Software (GraphPad Software, La Jolla California USA, www.graphpad.com).

Results

Natural course and rebound of PCT and CRP following cessation of dexamethasone treatment

We first assessed the natural course and effects of cessation of dexamethasone (DEXA) treatment on PCT and CRP levels in critically ill COVID-19 patients. This was also analyzed in DEXA-treated patients who also received tocilizumab (TOCI). Sixty-six, 47, and 73 patients were included in the no DEXA no TOCI (D-T-), DEXA no TOCI (D+T-), and DEXA and TOCI (D+T+) groups, respectively (Fig. 1). Patients in the D+T- and D+T+ groups had a significant higher body mass index (BMI) compared to those in the D-T- group (p=0.02, Table 1). As a result of treatment, levels of both PCT and CRP were lower in patients in the D+T- and D+T+ groups compared to the D-T- group at ICU admission (p=0.001 and p<0.0001, respectively). DEXA therapy ended 9 days [7-10] following ICU admission (median [IQR]); this is designated alignment day 0 in Figure 2. Following cessation of DEXA therapy, PCT significantly increased in the D+T- and D+T+ groups compared to the D-T- group (p<0.0001 and p=0.006, respectively, Fig. 2a). In the D+T- group, this rebound effect was even more pronounced for CRP levels (Fig. 2b). Compared to the continuously declining CRP levels in the D-T- group, CRP markedly increased in the D+T- group during the first four days after cessation of DEXA treatment (p<0.0001). In contrast, in the D+T+ group, CRP levels did not show any rebound following day 0 and remained significantly lower compared to the D-T- and D+T- groups at all subsequent timepoints (p<0.0001, Fig. 2b). To exclude the possibility that the observed rebound effects were due to the patients with a more complicated clinical course that remained in the ICU (while patients recovered were discharged), a sensitivity analysis was performed using patients with no missing data throughout the complete study period. This analysis yielded comparable rebound effects (data not shown), indicating that this effect was not due to case-mix changes.

Table 1. Patient characteristics and circulating levels of C-reactive protein and procalcitonin on ICU admission in the D-T-, D+T- and D+T+ groups.

	No DEXA, no TOCI (D-T-, n=66)	DEXA, no TOCI (D+T-, n=47)	DEXA+TOCI (D+T+, n=73)	P-value
Sex, male	49 (74)	35	46	0.26
Age, years	66 [59-72]	66 [56-72]	64 [57-71]	0.77
Body mass index, kg/m^2	27.6 [24.9-30.9] §	29.0 [26.3-32.4]	29.5 [26.2-34.4]	**0.02**
APACHE II	15 [12-19]	15 [13-18]	16 [14-21]	0.06
Time from first COVID-19 symptoms until ICU admission, days	11 [7-13]	9 [6-12]	10 [8-12]	0.19
Medical history				
Renal insufficiency	1 (2)	2	1	0.52
Metastatic neoplasm	5 (8)	1	1	0.13
Immunological insufficiency	1 (2)	2	3	0.62
COPD	6 (9)	6	7	0.80
Diabetes mellitus	15 (23)	14	17	0.65
Hypertension	33 (50)	24	38	0.97
Biomarkers at ICU admission				
Procalcitonin, µg/L	0.62 [0.26-1.05] * §	0.24 [0.17-0.46]	0.26 [0.11-0.42]	**0.001**
C-reactive protein, mg/L	222 [133-275] * §	94 [68-133]	90 [43-146]	**<0.0001**

. P-values were calculated using Kruskal Wallis or chi-square tests, followed by Dunn's multiple comparison tests and pairwise chi-square tests, respectively. Data are presented as n (%) or median [IQR].
** p<0.05 compared to D+T- group. § p<0.05 compared to D+T+ group.*

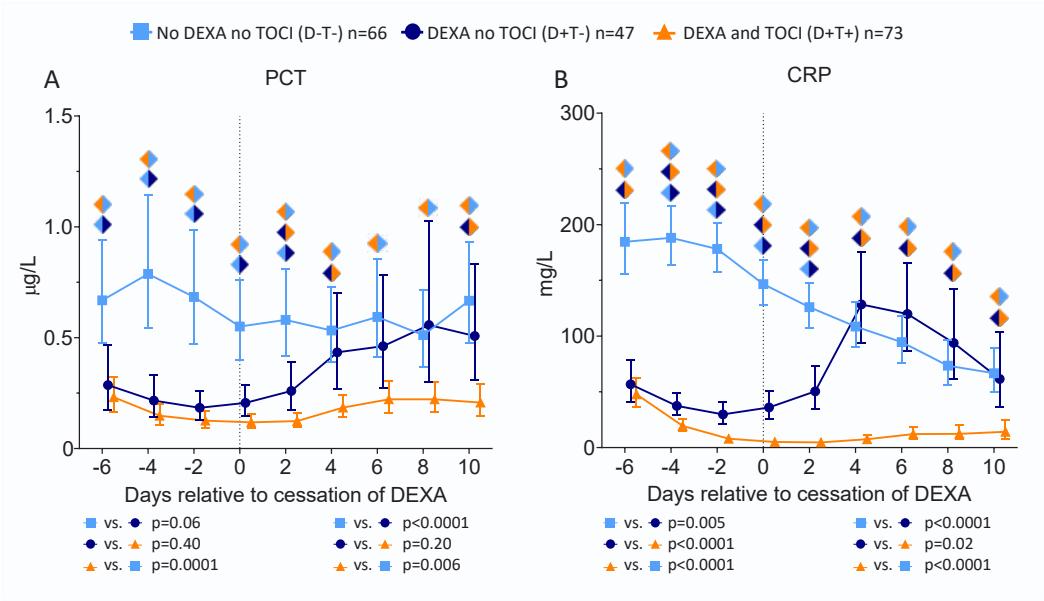

Figure 2. *Levels of* ***A)*** *procalcitonin (PCT) and* ***B)*** *C-reactive protein (CRP) over time within 6 days prior to and 10 days following cessation of dexamethasone (DEXA) in the group of patients treated with neither dexamethasone nor tocilizumab (D-T- group) as well as in the D+T- and D+T+ groups. Day of cessation of dexamethasone was designated day 0 (alignment day). Data of the D-T- group were aligned on the median alignment day, which was day 9 following ICU admission. Data are presented as geometric mean with 95% confidence intervals. P-values were calculated using mixed-models analyses (time*group interaction factor). P-values left and right below each panel reflect between-group differences in kinetics from day -6 until day 0 and from day 0 until day 10, respectively. Colored diamonds reflect p-values of <0.05 between the corresponding groups (D-T- light blue, D+T- dark blue, D+T+ orange) on the individual timepoint, calculated using Sidak's post-hoc multiple comparisons tests. D-T- : patients treated with neither dexamethasone nor tocilizumab, D+T- : patients treated with dexamethasone but no tocilizumab, D+T+ : patients treated with both drugs.*

PCT and CRP levels in patients who developed secondary infections

One hundred twenty three patients included in the second cohort, all of whom were treated with DEXA, were divided into D+T- (n=48) and D+T+ (n=75) groups (Fig. 1). In the D+T- group, 13 patients (27%) developed a secondary infection, whereas this was the case for 25 patients (33%) of the D+T+ group (Fig. 1). Secondary infections consisted of pulmonary tract infections and (catheter-associated) bloodstream infections with a wide variety of causative pathogens (depicted in additional figure 1). No differences in patient characteristics were observed between the secondary infection and no-secondary infection groups for the D-T-, D+T- and D+T+ groups (Table 2). In patients who developed a secondary infection, a positive culture was obtained on median [IQR] day 14 [11-19] following ICU

admission, which was designated day 0 for the following analyses. In contrast to the results of our previously published study in COVID-19 patients who did not receive immunomodulatory therapy (5) (depicted by the light grey line in Fig. 3a), no significant increase in PCT and CRP levels was observed in patients of the D+T- group who developed a secondary infection compared to patients who did not (p=0.052 and p=0.08, respectively, Fig. 3a-b). PCT levels in the D+T+ group significantly increased from day 2 onwards in patients who developed a secondary infection (Fig. 3c, p=0.0003). In contrast, CRP induction remained completely suppressed in the D+T+ group (Fig. 3d). When comparing the early and late secondary infection groups (development of secondary infection at 1 [-1-2] and 12 [9-16] days after cessation of DEXA, respectively), the late infection group displayed a more pronounced increase in levels of both PCT and CRP following alignment day, with significantly higher levels of PCT on days 0 and 2 (Additional figure 2).

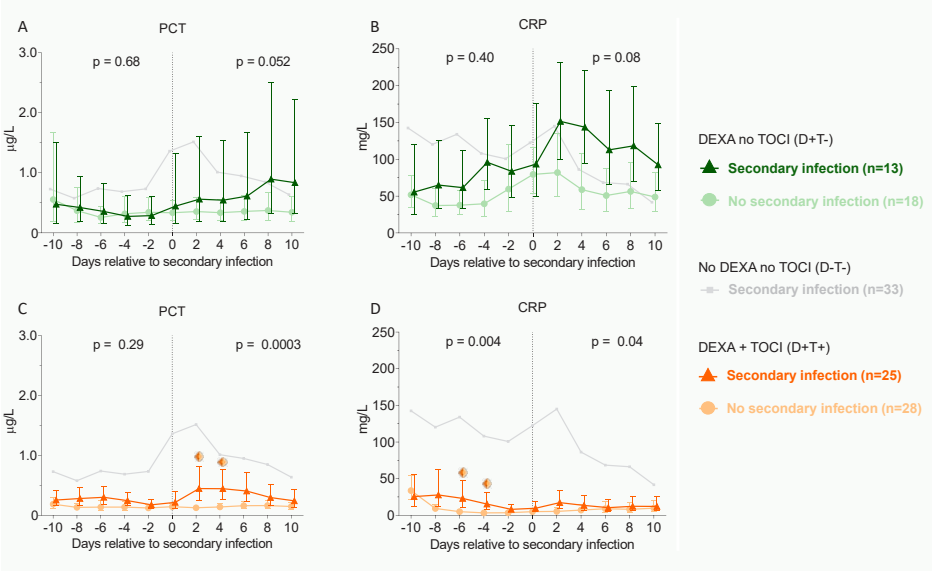

Figure 3. *Levels of procalcitonin (PCT) and C-reactive protein (CRP) over time within 10 days prior to and 10 days following the day of secondary infection in the group of patients treated with dexamethasone (TOCI) but not with tocilizumab (D+T- group, panels **A** and **B**), and in the D+T+ group (panels **C** and **D**). Day of secondary infection was designated day 0 (alignment day). Data of the no secondary infection groups were aligned on the median alignment day, which was day 14 following ICU admission. The light grey line indicates previously reported data of D-T- patients as a reference (5). Data are presented as geometric mean with 95% confidence intervals. P-values were calculated using mixed-models analyses (time*group interaction factor). P-values in left and right parts of each panel reflect between-group differences in kinetics from day -10 until day 0 and from day 0 until day 10, respectively. Colored diamonds reflect p-values of <0.05 on the individual timepoints, calculated using Sidak's post-hoc multiple comparisons tests.*
D-T- : patients treated with neither dexamethasone nor tocilizumab, D+T- : patients treated with dexamethasone but no tocilizumab, D+T+ : patients treated with both drugs.

Table 2. Patient characteristics and clinical parameters on ICU admission and on the day patients developed a secondary infection (alignment day) within the D-T-, D+T- and D+T+ groups.

	No DEXA, no TOCI (D-T-)			DEXA, no TOCI (D+T-)			DEXA and TOCI (D+T+)		
	Secondary infection (n=33)	No secondary infections (n=33)	p-value	Secondary infection (n=13)	No secondary infections (n=18)	p-value	Secondary infection (n=25)	No secondary infections (n=28)	p-value
Sex, male	26 (79)	23 (70)	0.57	9 (69)	12 (67)	1.00	19 (76)	18 (64)	0.39
Age, years	67 [60-73]	65 [56-70]	0.17	67 [59-73]	71 [60-73]	0.76	68 [59-71]	63 [56-72]	0.38
BMI, kg/m^2	27.6 [25.4-31.1]	27.7 [24.3-30.7]	0.40	29.4 [25.5-32.5]	29.8 [27.5-32.0]	0.48	27.5 [26.0-34.4]	29.4 [26.3-35.1]	0.54
APACHE II	15 [13-19]	15 [10-19]	0.77	15 [12-22]	17 [13-21]	0.32	19 [15-22]	16 [12-22]	0.20
Time from first COVID-19 symptoms until ICU admission, days	10 [7-13]	10 [6-14]	0.96	9 [6-12]	10 [8-12]	0.45	9 [5-12]	10 [9-11]	0.40
Medical history									
Renal insufficiency	0 (0)	1 (3)	1.00	1 (8)	1 (6)	1.00	0 (0)	1 (4)	1.00
Metastatic neoplasm	2 (6)	3 (9)	1.00	0 (0)	1 (6)	1.00	1 (4)	0 (0)	0.47
Immunological insufficiency	0 (0)	1 (3)	1.00	0 (0)	1 (6)	1.00	1 (4)	3 (11)	0.61
COPD	3 (9)	3 (9)	1.00	1 (8)	4 (22)	0.37	3 (12)	3 (11)	1.00
Diabetes mellitus	11 (33)	4 (12)	0.08	4 (31)	8 (44)	0.48	7 (28)	7 (25)	1.00
Hypertension	17 (52)	16 (48)	1.00	8 (62)	12 (67)	1.00	13 (52)	15 (54)	1.00
Clinical parameters on alignment day									
Temperature, Celsius	38.9 [37.8-40.0]	38.2 [37.3-38.9]	**0.03**	38.0 [37.2-38.6]	37.6 [36.7-38.2]	0.33	37.0 [36.7-38.6]]	37.3 [36.9-37.9]	0.90
Leukocytes, *10^9/L	16.1 [12.9-20.2]	11.7 [9.3-13.0]	**0.001**	10.7 [8.1-16.1]	10.9 [9.9-14.5]	1.00	10.0 [7.3-14.6]	9.1 [6.1-14.6]	0.48

Data of the no secondary infection groups were aligned on the median day following ICU admission that the secondary infections occurred in the affected groups. P-values were calculated using Mann-Whitney or Chi-square tests. Data are presented as n (%) or median [IQR].

Value of PCT and CRP for early detection of secondary infections

The value of PCT and CRP to detect secondary infections in critically ill COVID-19 patients treated with DEXA was investigated for both D+T- and D+T+ groups as well as for the total second cohort (D+T-/+). In the D+T- group, receiver operating curve (ROC) analysis of PCT and CRP levels on the day of secondary infection yielded area under the receiver operating curves (AUROC) of 0.50 and 0.57, respectively (Fig. 4a-b). These AUROCs are markedly lower than those reported in our previous study in COVID-19 patients not treated with DEXA (0.80 and 0.76, p=0.02 and p=0.15, respectively) (5). Data of the D+T+ group showed comparable results, with AUROCs of 0.55 and 0.61 for PCT and CRP, respectively (p=0.01 and p=0.15, respectively, Fig.4c-d). In the D+T-/+, the AUROC of PCT was 0.52 (p=0.001 compared to AUROC of D-T-, Fig.4e), whereas the AUROC of CRP was 0.55 (p=0.02 compared to AUROC of D-T-, Fig.4f). In accordance, positive predictive value (PPV) and negative predictive value (NPV) were lower compared to patients from the first cohort who did not receive immunomodulating therapy (5). In the D+T+ group, PPV could not be calculated because no patients displayed CRP levels >150 mg/L.

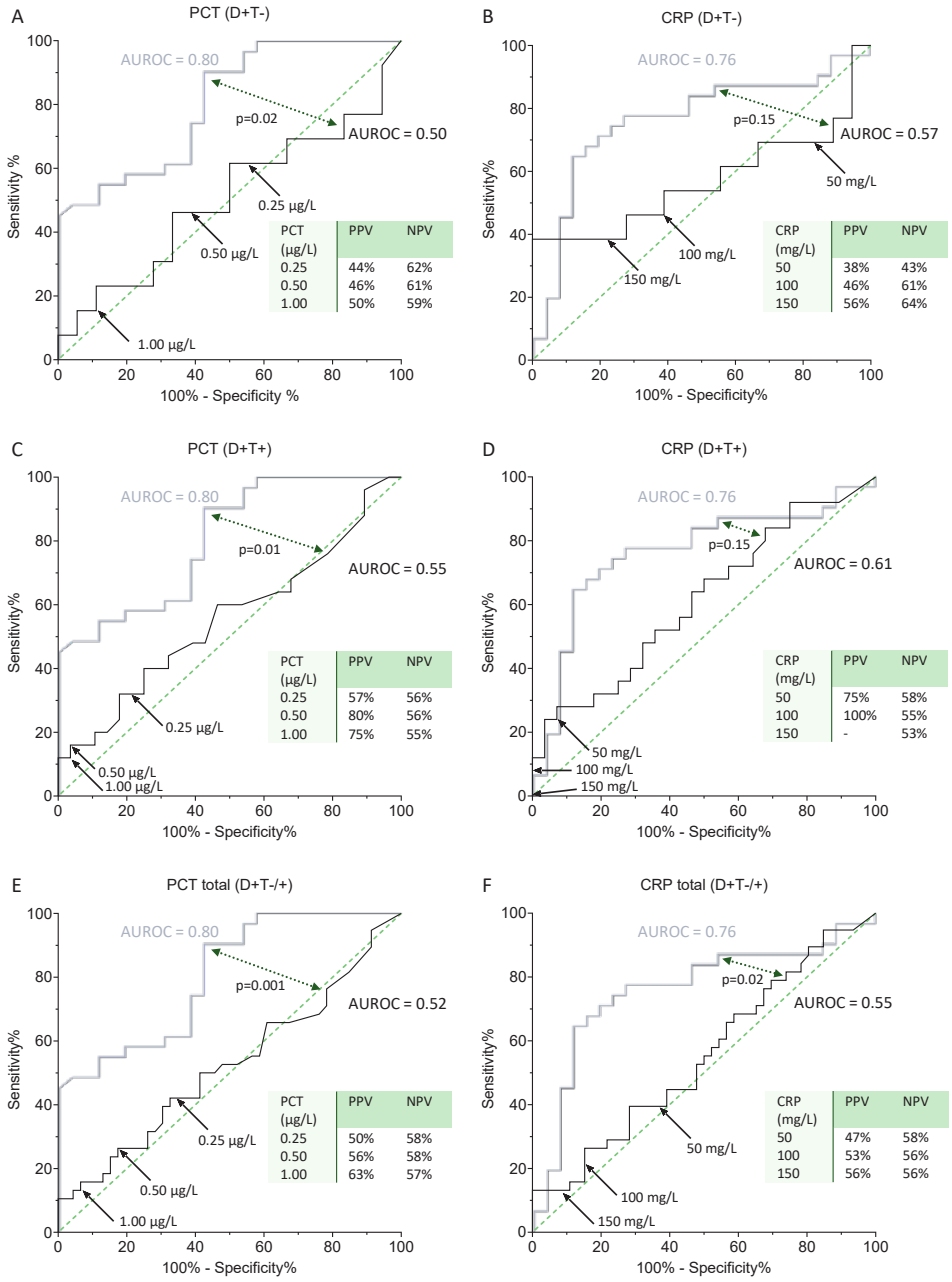

Figure 4. *Receiver operating curves (ROC) of procalcitonin (PCT) and C-reactive protein (CRP) in the group of patients treated with dexamethasone but not with tocilizumab (D+T- group, panels* ***A*** *and* ***B****), the D+T+ group (panels* ***C*** *and* ***D****), and in all patients of the second cohort (D+T-/+ group, panels* ***E*** *and* ***F****) to illustrate sensitivity and specificity to predict the occurrence of a secondary infection in critically ill COVID-19 patients. Binned data of PCT and CRP of days -1 and 0 were used for these analyses. The grey lines illustrate the previously published ROCs of D-T- patients (5). P-values reflect differences between the two areas under the receiver operating curves (AUROCs). Positive predictive value (PPV) and negative predictive value (NPV) are provided for the concentrations of PCT and CRP indicated by the arrows.*

Discussion

In the present study, we investigated the effects of dexamethasone and tocilizumab treatment on PCT and CRP kinetics, as well as the values of these inflammatory biomarkers for early detection of secondary infections in critically ill COVID-19 patients. We demonstrate that PCT and CRP levels are suppressed by dexamethasone treatment and that, after completion of the dexamethasone course, a clear inflammatory rebound effect was observed for both biomarkers, but particularly for CRP. In patients treated with both dexamethasone and tocilizumab, PCT also increased following cessation of dexamethasone, albeit less pronounced than in patients treated with dexamethasone only. Combined treatment with dexamethasone and tocilizumab resulted in suppressed CRP levels, an effect which persisted for the total observation period. Finally, the value of both biomarkers for the early detection of secondary infections was considerably reduced following immunotherapy.

In accordance with our results, a meta-analysis of biomarker levels before and after administration of tocilizumab in COVID-19 patients showed a reduction of CRP and a -non statistically significant- reduction of PCT (16). Also, dexamethasone therapy resulted in markedly reduced CRP levels in COVID-19 patients (18). However, except for one explorative study describing reduced inflammatory markers in COVID-19 patients treated with immunomodulatory drugs as a secondary endpoint (19), no studies have related overall suppression of PCT and CRP to their ability to detect secondary bacterial infections in COVID-19 patients. Furthermore, while so-called 'rebound effects' on inflammatory biomarkers after cessation of corticosteroid therapy have been observed in other conditions (14), they have not been studied in COVID-19 patients yet. The relatively swift CRP rebound after cessation of dexamethasone therapy is in line with its biological half-life of 36–54 h (20). A sensitivity analysis indicating that the rebound effect is not caused by differences in case mix strengthens this finding. The observed rebound-effect in COVID-19 patients is of clinical relevance, as it reflects a potential false positive signal for the development of a secondary infection. In addition, despite the apparent return of PCT and CRP levels to elevated levels after cessation of dexamethasone therapy, a further increase caused by secondary infections remained limited over the entire observation period and consequently we observed that these biomarkers lost their diagnostic ability to detect secondary infections, representing a false negative signal. This may be explained by other, more prolonged anti-inflammatory effects of dexamethasone (21). The long half-life of tocilizumab (approximately 10 days) explains the much more prolonged suppression of both PCT and CRP levels observed in our study and the complete absence of a CRP rebound-effect.

Our study clearly demonstrates that the values of PCT and CRP to detect secondary infections in COVID-19, which we previously showed to be helpful in patients who did not receive

immunomodulatory treatment (5), is considerably reduced by use of dexamethasone, whether or not in combination with tocilizumab. The potent and long-lasting anti-inflammatory effects of dexamethasone and tocilizumab appear to directly attenuate PCT and CRP to such extent that they are no longer sufficiently induced in response to bacterial infection. These findings take us back to the question often asked in daily ICU practice: how can we reliably recognize ICU-acquired infections and hence decide on appropriate antimicrobial treatment? The increased use of immunomodulatory agents in the ICU, during COVID-19 times but also in our non-COVID-19 population prevents the use of an important tool for antimicrobial stewardship in the ICU. In an era of increasing antimicrobial resistance due to consistent overuse of antibiotics in ICU, we are now thrown back to 'basic clinical reasoning' when deciding to start antimicrobial treatment or not. Our findings indicate that decisions based on the levels of these biomarkers may be false positive (rebound effect) as well as false negative (especially within 4 days following cessation of immunomodulatory treatment). Interestingly, when comparing patients who developed a secondary infection early after cessation of dexamethasone (≤4 days) to patients who developed such an infection later on (>4 days), a more pronounced increase in PCT and CRP was apparent in the late infection group. These findings may indicate that both biomarkers regain some value to detect secondary infections at a later stage after cessation of dexamethasone therapy in critically ill COVID-19 patients. Further research is needed to confirm this hypothesis. Also in decision making on stopping antibiotic therapy (6), immunomodulatory drugs likely affect the predictive characteristics of PCT and CRP, but this still needs to be confirmed. Nevertheless, since a delayed peak in CRP was observed within 2-4 days following the day of secondary infection in patients treated with only dexamethasone (not tocilizumab), the absence of an increase in CRP during the first 2-4 days could possibly be used in the decision to cease antibiotic therapy.

This study has several limitations. First, the observational design with two different periods of inclusion may lead to possible time-related bias when comparing these groups. It cannot be excluded that the differences between both groups can be partly explained by the fact that knowledge about COVID-19 improved over time and medical staff have become more experienced in caring for COVID-19 patients. Second, steroid treatment in the ward likely prevented most patients from deteriorating, implying that selection bias for those patients that did not respond and were transmitted to the ICU might be present. This might have resulted in a different population in the ICU and different outcomes and complications, although no major differences in disease severity, clinical parameters and patient characteristics between groups on ICU admission were observed. Third, the relatively small number of patients in our cohort resulted in rather limited sample sizes after further division into different subgroups (e.g., secondary infection vs. no-secondary infection). Nevertheless, this is, to

our knowledge, the first study to assess the predictive value of PCT and CRP for detection of secondary infection after the introduction of dexamethasone and tocilizumab treatment in critically ill COVID-19 patients. Finally, in the present study we focused on well-established biomarkers of bacterial infection. Therefore, it remains to be determined to what extent the immunomodulatory treatment would influence the predictive value of other markers or for other types of infections, such as beta-d-glucan and galactomannan for invasive candidiasis and CAPA.

Conclusions

Our study shows that in critically ill COVID-19 patients, the inflammatory biomarkers CRP and PCT show a rebound increase upon cessation of dexamethasone treatment, potentially leading to false-positive findings. Furthermore, the use of immunomodulatory treatments in critically ill COVID-19 patients considerably reduces the value of PCT and CRP for detection of secondary infections, reflecting a false-negative finding. As a result, clinicians should not rely on these biomarkers, but assess basic clinical infection signs and cultures to detect secondary infections in COVID-19 patients that received these immunomodulating treatments.

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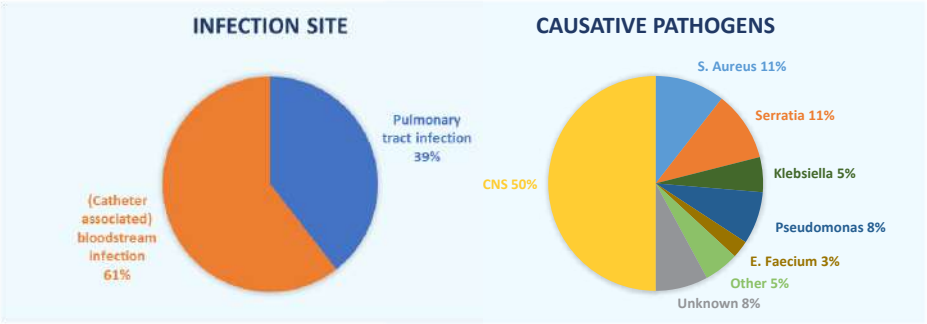
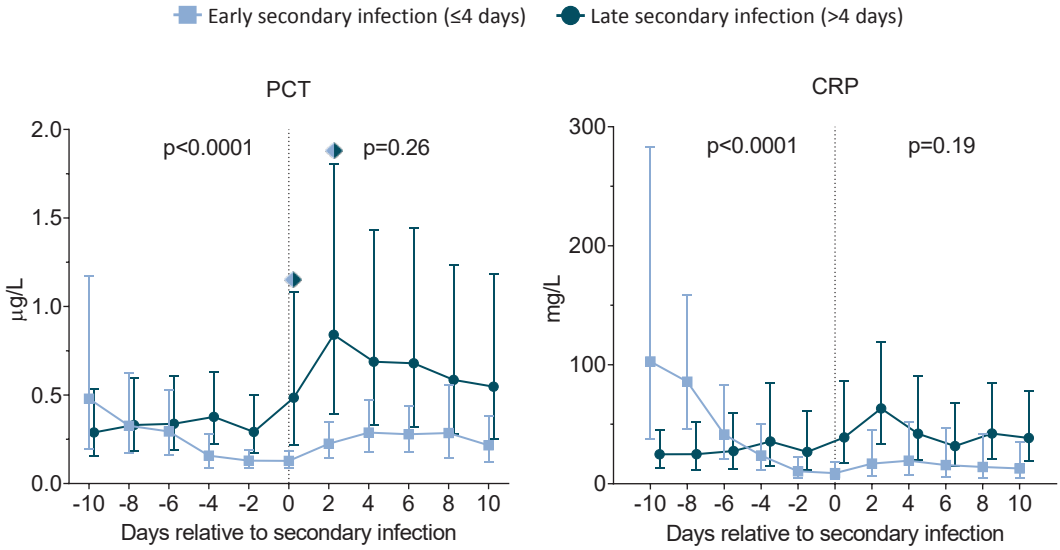
References

1. Hodges G, Pallisgaard J, Schjerning Olsen AM, McGettigan P, Andersen M, Krogager M, et al. Association between biomarkers and COVID-19 severity and mortality: a nationwide Danish cohort study. BMJ Open. 2020;10(12):e041295.
2. Hu R, Han C, Pei S, Yin M, Chen X. Procalcitonin levels in COVID-19 patients. Int J Antimicrob Agents. 2020;56(2):106051.
3. Lippi G, Plebani M. Procalcitonin in patients with severe coronavirus disease 2019 (COVID-19): A meta-analysis. Clin Chim Acta. 2020;505:190-1.
4. Schuetz P, Beishuizen A, Broyles M, Ferrer R, Gavazzi G, Gluck EH, et al. Procalcitonin (PCT)-guided antibiotic stewardship: an international experts consensus on optimized clinical use. Clin Chem Lab Med. 2019;57(9):1308-18.
5. van Berkel M, Kox M, Frenzel T, Pickkers P, Schouten J. Biomarkers for antimicrobial stewardship: a reappraisal in COVID-19 times? Crit Care. 2020;24(1):600.
6. de Jong E, van Oers JA, Beishuizen A, Vos P, Vermeijden WJ, Haas LE, et al. Efficacy and safety of procalcitonin guidance in reducing the duration of antibiotic treatment in critically ill patients: a randomised, controlled, open-label trial. Lancet Infect Dis. 2016;16(7):819-27.
7. Angus DC, Derde L, Al-Beidh F, Annane D, Arabi Y, Beane A, et al. Effect of Hydrocortisone on Mortality and Organ Support in Patients With Severe COVID-19: The REMAP-CAP COVID-19 Corticosteroid Domain Randomized Clinical Trial. Jama. 2020;324(13):1317-29.
8. Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, et al. Dexamethasone in Hospitalized Patients with Covid-19. N Engl J Med. 2021;384(8):693-704.
9. Gordon AC, Mouncey PR, Al-Beidh F, Rowan KM, Nichol AD, Arabi YM, et al. Interleukin-6 Receptor Antagonists in Critically Ill Patients with Covid-19. N Engl J Med. 2021;384(16):1491-502.
10. Horby PW, Pessoa-Amorim G, Peto L, Brightling CE, Sarkar R, Thomas K, et al. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): preliminary results of a randomised, controlled, open-label, platform trial. medRxiv. 2021:2021.02.11.21249258.
11. Confalonieri M, Urbino R, Potena A, Piattella M, Parigi P, Puccio G, et al. Hydrocortisone infusion for severe community-acquired pneumonia: a preliminary randomized study. Am J Respir Crit Care Med. 2005;171(3):242-8.
12. Müller B, Peri G, Doni A, Perruchoud AP, Landmann R, Pasqualini F, et al. High circulating levels of the IL-1 type II decoy receptor in critically ill patients with sepsis: association of high decoy receptor levels with glucocorticoid administration. J Leukoc Biol. 2002;72(4):643-9.
13. Rinaldi S, Adembri C, Grechi S, De Gaudio AR. Low-dose hydrocortisone during severe sepsis: effects on microalbuminuria. Crit Care Med. 2006;34(9):2334-9.
14. Keh D, Boehnke T, Weber-Cartens S, Schulz C, Ahlers O, Bercker S, et al. Immunologic and hemodynamic effects of "low-dose" hydrocortisone in septic shock: a double-blind, randomized, placebo-controlled, crossover study. Am J Respir Crit Care Med. 2003;167(4):512-20.
15. Berman M, Ben-Ami R, Berliner S, Anouk M, Kaufman I, Broyde A, et al. The Effect of Tocilizumab on Inflammatory Markers in Patients Hospitalized with Serious Infections. Case Series and Review of Literature. Life (Basel). 2021;11(3):258.

16. Ivan Hariyanto T, Kurniawan A. Tocilizumab administration is associated with the reduction in biomarkers of coronavirus disease 2019 infection. J Med Virol. 2021;93(3):1832-6.
17. Angus DC, Berry S, Lewis RJ, Al-Beidh F, Arabi Y, van Bentum-Puijk W, et al. The REMAP-CAP (Randomized Embedded Multifactorial Adaptive Platform for Community-acquired Pneumonia) Study. Rationale and Design. Ann Am Thorac Soc. 2020;17(7):879-91.
18. Fatima SA, Asif M, Khan KA, Siddique N, Khan AZ. Comparison of efficacy of dexamethasone and methylprednisolone in moderate to severe covid 19 disease. Ann Med Surg (Lond). 2020;60:413-6.
19. Giacobbe DR, Battaglini D, Ball L, Brunetti I, Bruzzone B, Codda G, et al. Bloodstream infections in critically ill patients with COVID-19. European Journal of Clinical Investigation. 2020;50(10):e13319.
20. Melby JC. Clinical pharmacology of systemic corticosteroids. Annu Rev Pharmacol Toxicol. 1977;17:511-27.
21. Meijvis SC, Hardeman H, Remmelts HH, Heijligenberg R, Rijkers GT, van Velzen-Blad H, et al. Dexamethasone and length of hospital stay in patients with community-acquired pneumonia: a randomised, double-blind, placebo-controlled trial. Lancet. 2011;377(9782):2023-30.



Additional figure 1. *Pie charts illustrating the sites of secondary bacterial infections and the causative pathogens in the second cohort (patients treated with dexamethasone with or without tocilizumab, D+T-/+ group).*



Additional figure 2. *Levels of* ***A)*** *procalcitonin (PCT) and* ***B)*** *C-reactive protein (CRP) over time within 10 days prior to and 10 days following the day of secondary infection in all patients of the second cohort treated with dexamethasone with or without tocilizumab (D+T-/+ group) who developed a secondary infection early (≤4 days) and late (>4 days) following cessation of dexamethasone. Day of secondary infection was designated day 0 (alignment day). Data are presented as geometric mean with 95% confidence intervals. P-values were calculated using mixed-models analyses (time*group interaction factor). P-values in left and right parts of each panel reflect between-group differences in kinetics from day -10 until day 0 and from day 0 until day 10, respectively. Colored diamonds reflect p-values of <0.05 on the individual timepoints, calculated using Sidak's post-hoc multiple comparisons tests.*

CHAPTER 8

Molecular mechanisms and treatment responses of pulmonary fibrosis in severe COVID-19

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Abstract

Background

Coronavirus disease 2019 (COVID-19) patients can develop pulmonary fibrosis (PF), which is associated with impaired outcome. We assessed specific leukocytic transcriptome profiles associated with PF and the influence of early dexamethasone (DEXA) treatment on the clinical course of PF in critically ill COVID-19 patients.

Methods

We performed a pre-post design study in 191 COVID-19 patients admitted to the Intensive Care Unit (ICU) spanning two treatment cohorts: the *pre-DEXA*- (n=67) and the *DEXA-cohort* (n=124). PF was identified based on radiological findings, worsening of ventilatory parameters and elevated circulating PIIINP levels. Longitudinal transcriptome profiles of 52 *pre-DEXA* patients were determined using RNA sequencing. Effects of prednisone treatment on clinical fibrosis parameters and outcomes were analyzed between PF- and no-PF-patients within both cohorts.

Results

Transcriptome analyses revealed upregulation of inflammatory, coagulation and neutrophil extracellular trap-related pathways in PF-patients compared to no-PF patients. Key genes involved included *PADI4, PDE4D, MMP8, CRISP3*, and *BCL2L15*. Enrichment of several identified pathways was associated with impaired survival in a external cohort of patients with idiopathic pulmonary fibrosis. Following prednisone treatment, PF-related profiles reverted towards those observed in the no-PF-group. Likewise, PIIINP levels decreased significantly following prednisone treatment. PF incidence was 28% and 25% in the pre-DEXA- and DEXA-cohort, respectively (p=0.61). ICU length-of-stay (*pre-DEXA*: 42 (29-49) vs. 18 (13-27) days, p<0.001; *DEXA*: 42 (28-57) vs. 13 (7-24) days, p<0.001) and mortality (pre-DEXA: 47% vs. 15%, p=0.009; DEXA: 61% vs. 19%, p<0.001) were higher in the PF-groups compared to the no-PF-groups within both cohorts. Early dexamethasone therapy did not influence these outcomes.

Conclusions

ICU patients with COVID-19 who develop PF exhibit upregulated coagulation, inflammation, and neutrophil extracellular trap-related pathways as well as prolonged ICU length-of-stay and mortality. This study indicates that early dexamethasone treatment neither influences the incidence or clinical course of PF, nor clinical outcomes.

Background

Patients with Coronavirus Disease 2019 (COVID-19)-induced Acute Respiratory Distress Syndrome (ARDS) are at risk of subsequent complications such as a pathological fibroproliferative response (1, 2). Pulmonary fibrosis (PF) is associated with challenges in mechanical ventilation, prolonged length of stay (LOS) in ICU, higher mortality rates, and chronic symptoms in survivors (3-7).

It is challenging to detect PF at an early stage of ARDS. High N-terminal pro-peptide of type III procollagen (PIIINP) levels in bronchoalveolar lavage (BAL) fluid (8), as well as PIIINP in blood may be used. Also, other circulating fibrosis biomarkers such as hepatocyte growth factor (HGF) (9) and Macrophage Inflammatory Protein-3 alpha (MIP-3α) (10) could be of value. The mechanisms underlying the development of PF are largely unexplored, while knowledge of these pathways may aid early diagnosis and novel treatment targets. Currently, PF in patients with non-COVID-19 ARDS is treated with corticosteroids, which is effective in reducing time on mechanical ventilation and ICU-LOS, especially in those with elevated biomarker concentrations (11, 12).

Initially, care for critically ill COVID-19 patients was limited to supportive treatment. However, since early treatment with the corticosteroid dexamethasone (DEXA) was shown to be beneficial (13), hospitalized COVID-19 patients requiring oxygen suppletion were all treated with DEXA. It is however unknown whether DEXA treatment influences the incidence or severity of PF and whether or not it affects the therapeutic efficacy of later corticosteroid treatment in patients who develop PF. The primary aim of this study in critically ill COVID-19 patients was therefore twofold: 1) to explore transcriptome profiles associated with PF and the response to treatment using longitudinal RNA sequencing of circulating leukocytes. 2) to determine the influence of early dexamethasone treatment on the incidence and time to development of PF, and to assess the therapeutic efficacy of steroids to treat PF both before and after the introduction of early dexamethasone as standard care for critically ill COVID-19 patients.

Methods

Study design and participants

In this prospectively designed pre-post design cohort study, all adult COVID-19 patients admitted to the ICU of Radboud University Medical Center (Radboudumc, Nijmegen, The Netherlands) between March 2020 and April 2021 were screened for inclusion. Pa-

tients with comorbidities that might significantly influence the disease course and clinical outcomes (e.g. immunocompromised patients) were excluded. This study was carried out in accordance with the applicable rules concerning the review of research ethics committees and informed consent in the Netherlands. All patients or legal representatives were informed about the details of this cohort study and could decline to participate.

Included patients were divided into two cohorts: patients who were not treated with DEXA (pre-DEXA-cohort, March 2020-August 2020) and patients who received DEXA (6 mg/day, intravenously for 10 days) as part of standard COVID-19 care in accordance to the RECOVERY criteria (13) (DEXA-cohort, August 2020-April, 2021). A subgroup of the DEXA-cohort was also treated with the interleukin (IL)-6 receptor antagonist tocilizumab as part of standard COVID-19 care (single dose of 8 mg/kg, intravenously) (14). Details on the sensitivity analyses performed in this subgroup are provided in Additional file 1 (Additional methods). Both cohorts were subdivided into groups of patients who were assessed to have developed PF while still in ICU and were treated with prednisone (start dose of 1 mg/kg twice daily, intravenously, PF-groups) and groups of patients who were not (no-PF-groups). In the absence of validated diagnostic criteria of PF, the diagnosis, and therefore the indication for prednisone treatment was at the discretion of the treatment team. All patients were discussed daily in a multidisciplinary meeting including over 15 medical experts, suspicion of PF and initiation of prednisone treatment was based on a combination of radiological findings, worsening ventilatory parameters (e.g. lower PaO2/FiO2 ratio, lower lung compliance and increased ventilatory ratio as a measure of impaired ventilation and increase in dead space ventilation), and an increase in PIIINP plasma levels that were measured three times per week. To analyze the kinetics of fibrosis biomarkers in the days prior to and following the day on which prednisone treatment for PF was initiated, serial data were aligned on the first day of prednisone treatment for PF (PF-day 0). For patients of the no-PF-groups, data were aligned on the median start day of late prednisone treatment in both cohorts separately to correct for time-dependent effects in this group (15, 16).

RNA sequencing

To explore underlying molecular mechanisms of PF development and treatments responses, we performed RNA sequencing on leukocytes isolated from a total of 52 PF- and no-PF-patients of the pre-DEXA-cohort. We used co-expression network analysis on these longitudinal RNA sequencing data using our established hCocena pipeline (17) to identify similarly regulated genes across samples and group these genes into modules. We applied this approach to samples obtained up to day 0 (when prednisone treatment was initiated in PF patients), to identify genes associated with the development of PF (pre-alignment day

analysis). To assess the transcriptome response to treatment of PF with prednisone, we applied the same analysis pipeline to samples obtained from day 0 onwards (post-alignment day analysis). See Additional file 1 (Additional methods) for a detailed description of RNA sequencing and analysis procedures.

Clinical data and biomarker measurements
See Additional file 1 (Additional methods).

Statistical analysis
Differences in baseline characteristics and clinical outcomes between the PF- and no-PF-groups were analyzed using Mann-Whitney U and Fisher's exact tests for continuous and categorical data, respectively. Differences in kinetics of serially measured data were analyzed using linear mixed effect model analysis on log-transformed data followed by post-hoc Sidak's multiple comparisons tests. ICU-LOS and mortality were analyzed using log-rank tests during 60 days following ICU admission. A more detailed description of the statistical analysis is presented in Additional file 1 (additional methods).

Results

Patient characteristics
The pre-DEXA-cohort and the DEXA-cohort consisted of 67 and 124 patients, respectively (Figure 1). Baseline characteristics of both cohorts are listed in Table 1. Prednisone treatment for PF was initiated on day 16 (12-21) and day 19 (14-23) following ICU admission in the pre-DEXA- and DEXA-cohorts, respectively (p=0.11, Table 1). No relevant baseline demographic differences were present between the PF- and no-PF-groups within both cohorts. Furthermore, within the DEXA-cohort, no difference in the proportion of patients who were also treated with tocilizumab as standard COVID-19 care was present between the PF- and no-PF-groups (65% vs. 60%, p=0.83, Table 1). Also, when comparing the PF-groups between both cohorts, no significant differences were present in patient characteristics and PF-free days from hospital admission onwards (Table 1).

Data presented as n (%) or median with interquartile ranges ([IQR]). P-values were calculated using Mann-Whitney U and two-sided Fisher's exact tests for continuous and categorical data, respectively.
DEXA: dexamethasone, PF: pulmonary fibrosis, BMI: body mass index, COVID-19: corona virus disease 2019, ICU: intensive care unit, COPD: chronic obstructive pulmonary disease

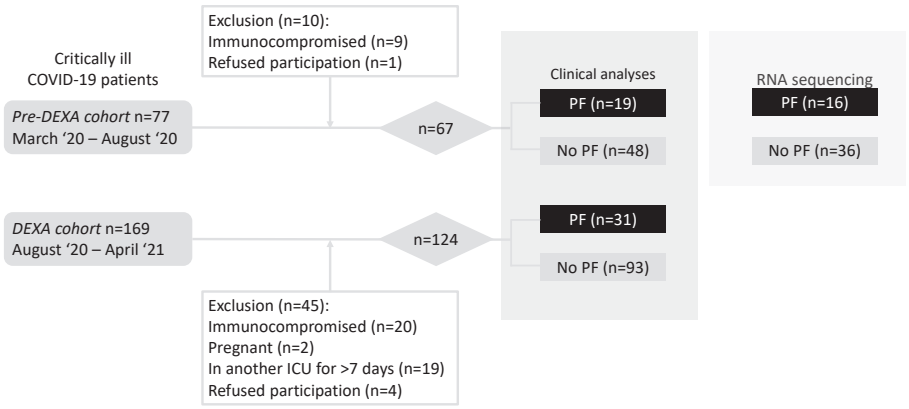


Figure 1. Patient flowchart.
COVID-19: Coronavirus Disease 2019, DEXA: dexamethasone, ICU: intensive care unit, PF: pulmonary fibroproliferation

Table 1. Patient characteristics of the pre-DEXA- and DEXA-cohorts.

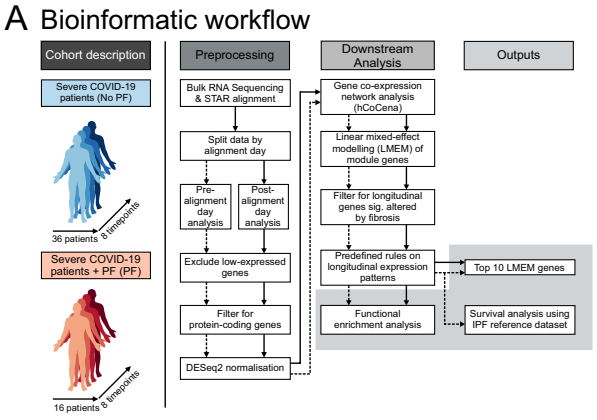
	Pre-DEXA-cohort (n=67)	DEXA-cohort (n=124)	p-value
Age, years	65 [58-72]	65 [56-72]	0.89
Sex, male	50 (75)	84 (68)	0.41
BMI, kg/m^2	27.7 [24.9-30.8]	29.4 [26.2-33.3]	0.005
APACHE II	15 [12-19]	16 [13-20]	0.08
Days first COVID-19 signs until ICU admission	11 [7-13]	10 [7-12]	0.46
Medical history			
Renal insufficiency	1 (1)	3 (2)	1.00
Metastatic neoplasm	5 (7)	2 (2)	0.053
Immunological insufficiency	1 (1)	6 (5)	0.43
COPD	6 (9)	13 (10)	0.81
Diabetes Mellitus	15 (22)	32 (26)	0.73
Hypertension	33 (49)	63 (51)	0.88

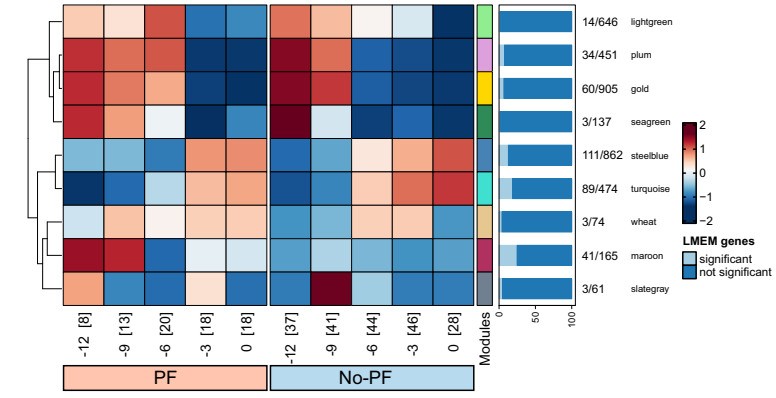
Data presented as n (%) or median with interquartile ranges ([IQR]). P-values were calculated using Mann-Whitney U and two-sided Fisher's exact tests for continuous and categorical data, respectively.
DEXA: dexamethasone, PF: pulmonary fibrosis, BMI: body mass index, COVID-19: corona virus disease 2019, ICU: intensive care unit, COPD: chronic obstructive pulmonary disease

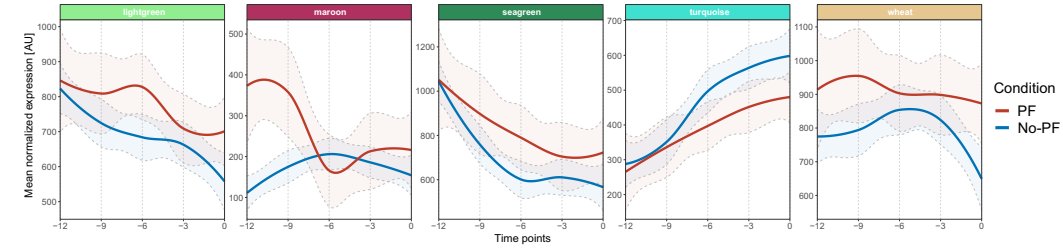
Transcriptome analysis

In blood samples obtained up to day 0 (when prednisone treatment was initiated in PF patients), we identified nine co-expressed modules associated with the development of PF across a total of 3775 genes included in the analysis (pre-alignment day analysis, Figure 2ab). These modules are designated by colors gold to wheat (Figure 2b). Based on linear regression analysis and a predefined set of rules (see Figure 2a and supplemental material), we focused on five modules associated with PF: seagreen, lightgreen, maroon, and wheat

(upregulated in PF-patients) and turquoise (downregulated in PF-patients). Differential expressed genes over time were visualized by wave plots (Figure 2c, wave plots of all modules provided in Additional file 1; Figure S1) and heatmaps of the top 10 significant genes ranked by effect size (Figure 2d, genes of all modules provided in Additional file 1; Figure S1). Functional Enrichment Analysis (FEA) on these modules identified associated gene signatures with distinct functional characteristics related to fibrosis (Figure 2e, all associated gene signatures provided in Additional file 1; Figure S1). For instance, 'inflammatory response', 'interferon (IFN)-αresponse', 'IFN-αresponses', 'response to virus', 'COVID-19' and 'influenza' are enriched in the seagreen module, in keeping with the fact that inflammation is an important driver of fibrotic processes (18). Hence, these data suggest a more pronounced response to (viral) infections, leading to more severe inflammation in COVID-19 patients who developed PF compared to COVID-19 patients who did not. In accordance, 'regulation of interleukin-6 production' and 'myeloid cell differentiation' were enriched in the lightgreen module and play key roles in both inflammation and development of PF (19-21). The wheat module showed enrichment for 'coagulation' and 'platelet activation', and previous work has shown that the coagulation pathway is involved in fibroproliferative responses (22). Accordingly, prevalence rates of pulmonary embolisms (PE) during stay in ICU and use of therapeutic low molecular weight heparins (LMWH) were compared between PF and no-PF groups. No differences in prevalence rates of PE were present between PF and no-PF groups (71% vs. 65%, respectively, p=1.00). Therapeutic dosage of LMWH were administered in 75% of PF patients compared to 42% of no-PF patients (p=0.04). 'Neutrophil extracellular trap (NET) formation' and 'chromatin assembly', were enriched in the maroon module. Interestingly, the release of NETs has been shown to play a role in the development of organ fibrosis (23) and their release is dependent on histone modification by peptidylarginine deiminase 4 (PADI4) (23, 24), which was one of the top 10 genes in the lightgreen module (Figure 2d). The turquoise module which was downregulated in PF-patients, showed enrichment of 'proteasomal protein catabolic process' and 'ubiquitin-mediated proteolysis'. Dysregulation of the ubiquitin-proteasome pathway is linked to multiple conditions, including fibrotic diseases (25), implicating that the ubiquitin-proteasome pathway is less functional in COVID-19 patients with PF. Finally, several specific genes which were distinctly upregulated in PF patients have previously been linked to fibrotic processes, including PDE4D (26), MMP8 (27), CRISP3 (28), and BCL2L15 (29) (all in maroon module, Figure 2d). Additionally, to explore relationships between the gene modules and clinical outcomes of fibrosis, we performed gene set variation analysis on leukocyte gene expression data of a published cohort of patients with idiopathic pulmonary fibrosis (IPF, Figure 2a) (30). Four-year survival of IPF patients who showed enrichment of the genes in each module was compared to outcome of patients who exhibited no enrichment. Strikingly, survival of IPF patients who showed enrichment for genes in the maroon module was significantly worse (p=0.019, Figure-2f, survival plots for all modules provided in Additional file 1; Figure S2).


A Bioinformatic workflow
Cohort description
Preprocessing
Downstream Analysis
Outputs
Severe COVID-19 patients (No PF)
36 patients 8 timepoints
Severe COVID-19 patients + PF (PF)
16 patients 8 timepoints
Bulk RNA Sequencing & STAR alignment
Split data by alignment day
Pre-alignment day analysis
Post-alignment day analysis
Exclude low-expressed genes
Filter for protein-coding genes
DESeq2 normalisation
Gene co-expression network analysis (hCoCena)
Linear mixed-effect modelling (LMEM) of module genes
Filter for longitudinal genes sig. altered by fibrosis
Predefined rules on longitudinal expression patterns
Functional enrichment analysis
Top 10 LMEM genes
Survival analysis using IPF reference dataset


B Gene co-expression network analysis
14/646 lightgreen
34/451 plum
60/905 gold
3/137 seagreen
111/862 steelblue
89/474 turquoise
3/74 wheat
41/165 maroon
3/61 slategray
2
1
0
−1
−2
LMEM genes
significant
not significant
-12 [8]
-9 [13]
-6 [20]
-3 [18]
0 [18]
-12 [37]
-9 [41]
-6 [44]
-3 [46]
0 [28]
Modules
0
50
100
PF
No-PF


C Longitudinal mean expression
lightgreen
maroon
seagreen
turquoise
wheat
Mean normalized expression [AU]
Time points
Condition
PF
No-PF

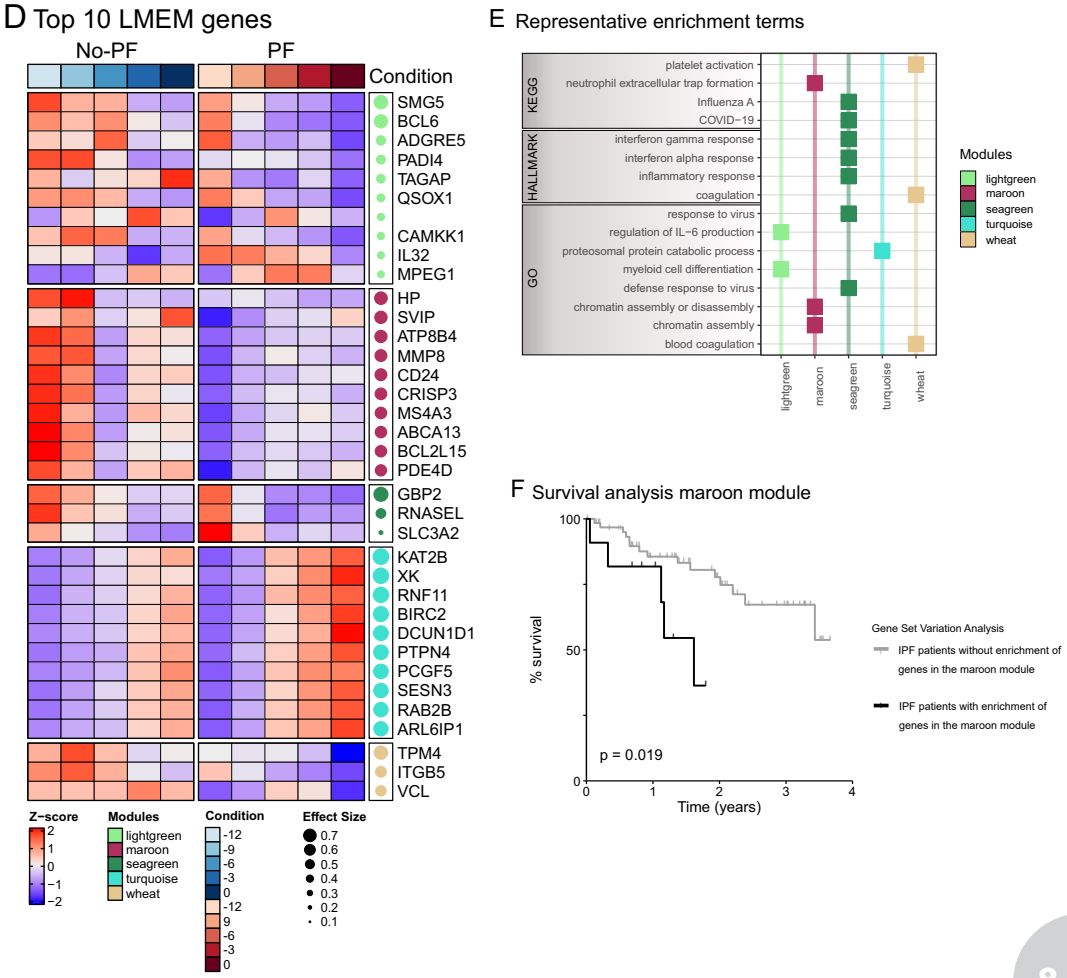


Figure 2. Summary of bulk RNA-seq data pre-alignment day (day 0, the day prednisone treatment was initiated in patients with PF).

(a) *depicts an overview of the complete cohort and general workflow per data set (dashed: pre-alignment day data set; solid: post-alignment day data set).* ***(b)*** *shows the expression profile across the time points prior to the alignment day per module in a heatmap split by condition. The amount of samples per timepoint is displayed in brackets. Tiles are colored based on the group fold change (GFC) and modules are represented by their respective colors. Percentage of LMEM genes per module are depicted in a barplot (colored based on significance in the LMEM) and total numbers are shown as a ratio of LMEM genes to module genes.* ***(c)*** *displays the mean expression per fibrosis-related module filtered by the LMEM genes over time prior to the alignment day. Lines and confidence intervals are colored according to the condition.* ***(d)*** *depicts the mean expression of the top 10 LMEM genes per fibrosis-related module ordered by effect size for all conditions and time points prior to the alignment day. Modules are colored accordingly and effect size is indicated by the dot size.* ***(e)*** *shows significant representative functional enrichment terms from GO and KEGG database as well as the hallmark gene set of the Molecular Signature Database per fibrosis-related module. Modules names are displayed on the x-axis and the respectively colored squares indicate the enrichment of a functional term in the module.* ***(f)*** *displays the Kaplan-Meier plot of patients with idiopathic pulmonary fibrosis with and without enrichment of genes in the maroon module. Lines are colored based on the enrichment of the LMEM genes in the maroon module in the reference dataset using GSVA.*

The transcriptome response to treatment of PF with prednisone was assessed in blood samples obtained from day 0 onwards (post-alignment day analysis, see Figure 2a). This analysis revealed nine co-expression modules (Figure 3a). Applying the linear regression analysis and the predefined set of rules, led us to focus on two modules: slategray and wheat (Figure 3bc, wave plots and top 10 genes of all modules are provided in Additional file 1; Figure S3). Genes in both modules were upregulated in the PF-group on day 0 and converged towards the no-PF-group afterwards, suggesting a treatment effect. Similar to the pre-alignment day analysis, both modules are enriched for multiple inflammatory and coagulation pathways (Figure 3d, all associated gene signatures provided in Additional file 1; Figure S3). Furthermore, the slategray module showed enrichment for 'epithelial mesenchymal transition', which was previously implicated in the development of organ fibrosis (31-33).

Fibrosis biomarkers and ventilatory parameters

Peak PIIINP levels in the PF-groups were observed on day 3 following start of prednisone treatment and on the day prednisone treatment was initiated for PF (day 0) for the pre-DEXA- and DEXA-cohorts, respectively. PIIINP levels decreased significantly following prednisone treatment (Figure 4ab). These kinetics were not observed in the no-PF-groups (Figure 4ab). Unlike PIIINP, no significant between-group differences in circulating levels of HGF and MIP-3αwithin both cohorts were present (Additional file 1; Figure S4). Following initiation of prednisone treatment, the dynamic lung compliance remained lower in the PF-groups of both cohorts compared with the no-PF-groups during the entire follow-up period (Figure 4cd). In the pre-DEXA-cohort, the ventilatory ratio decreased following initiation of prednisone treatment in the PF-group, whereas no relevant changes in ventilatory ratio were

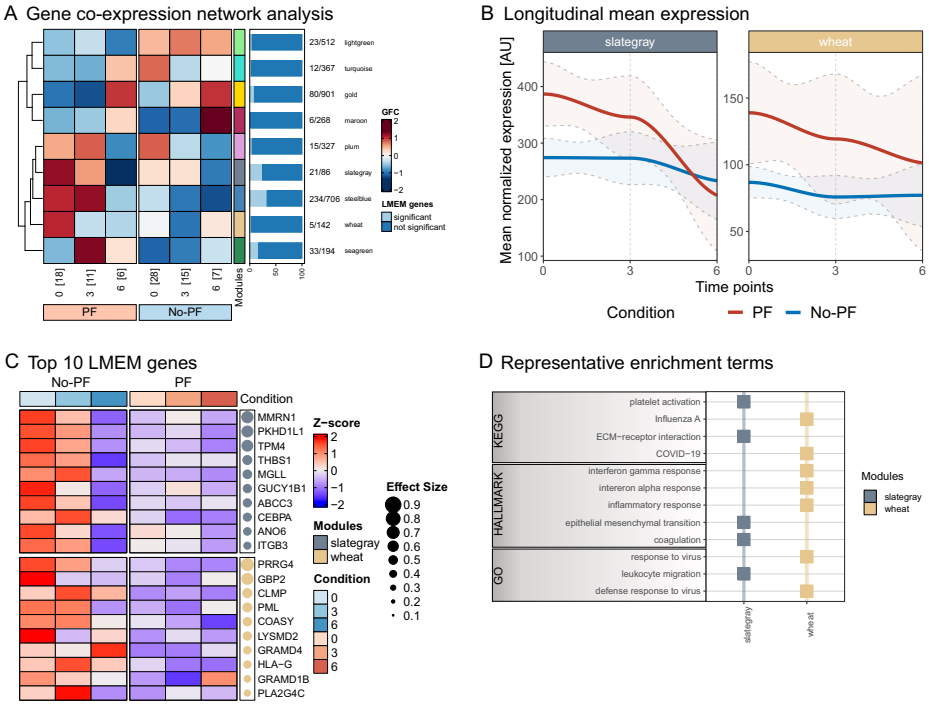


Figure 3. Summary of bulk RNA-seq data post-alignment day (day 0, the day prednisone treatment was initiated in patients with PF).

(a) *shows the expression profile across the time points post-alignment day per module in a heatmap split by condition. The amount of samples per timepoint is displayed in brackets. Tiles are colored based on the group fold change (GFC) and modules are represented by their respective colors. Percentage of LMEM genes per module are depicted in a barplot (colored based on significance in the LMEM) and total numbers are shown as a ratio of LMEM genes to module genes.* ***(b)*** *displays the mean expression per fibrosis-related module filtered by the LMEM genes over time post-alignment day. Lines and confidence intervals are colored according to the condition.* ***(c)*** *depicts the mean expression of the top 10 LMEM genes per fibrosis-related module ordered by effect size for all conditions and time points post-alignment day. Modules are colored accordingly and effect size is indicated by the dot size.* ***(d)*** *shows significant representative functional enrichment terms from GO and KEGG database as well as the hallmark gene set of the Molecular Signature Database per fibrosis-related module. Modules names are displayed on the x-axis and the respectively colored squares indicate the enrichment of a functional term in the module.*

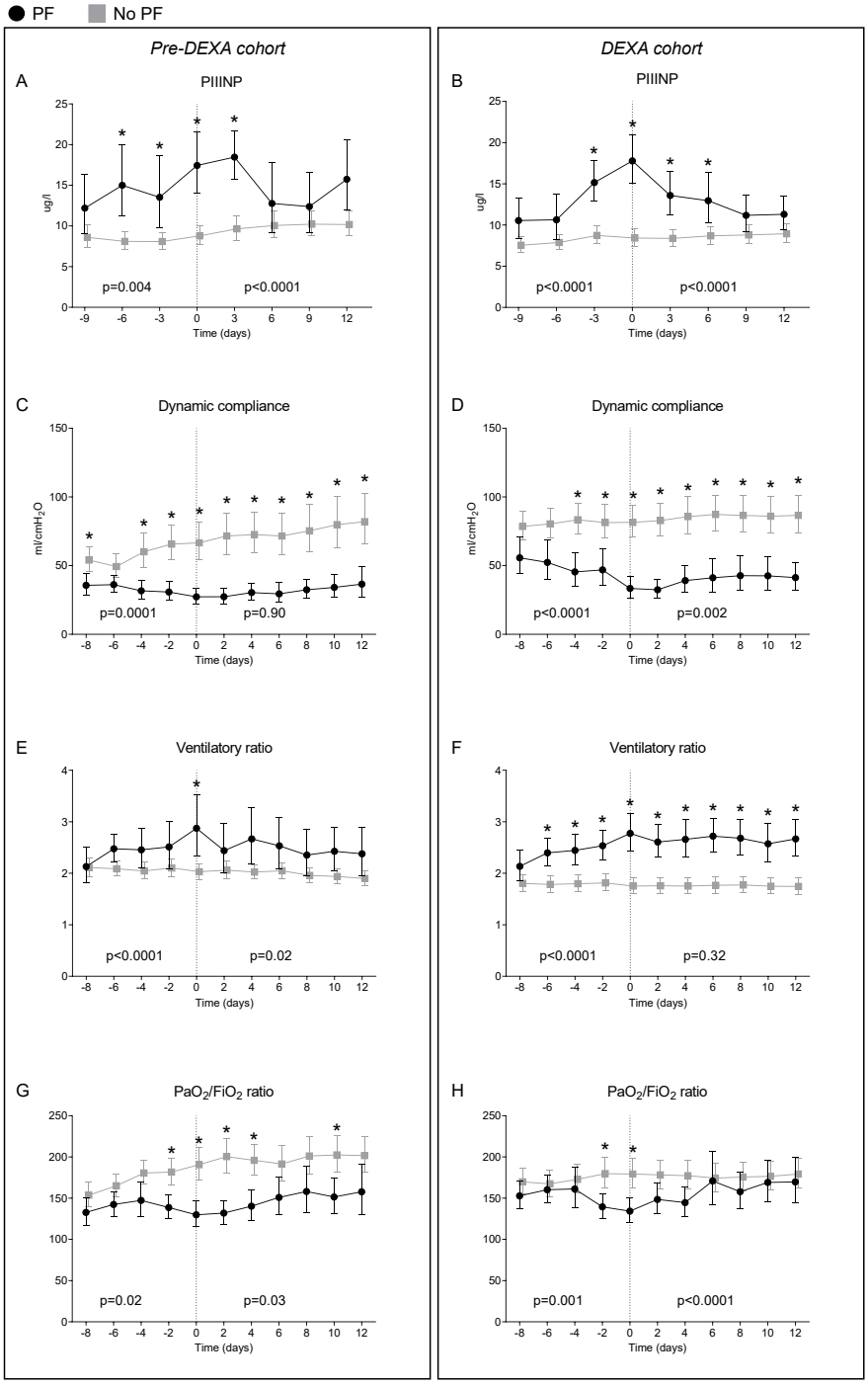

PF
No PF
Pre-DEXA cohort
DEXA cohort
A
PIIINP
ug/l
p=0.004
p<0.0001
B
PIIINP
ug/l
p<0.0001
p<0.0001
C
Dynamic compliance
ml/cmH2O
p=0.0001
p=0.90
D
Dynamic compliance
ml/cmH2O
p<0.0001
p=0.002
E
Ventilatory ratio
p<0.0001
p=0.02
F
Ventilatory ratio
p<0.0001
p=0.32
G
PaO2/FiO2 ratio
p=0.02
p=0.03
H
PaO2/FiO2 ratio
p=0.001
p<0.0001
Time (days)

Figure 4. Circulating procollagen type III concentrations and clinical parameters.
Differences between the pulmonary fibrosis (PF)- and no-PF-groups in kinetics of circulating procollagen type III (PIIINP) in ***(a)*** *pre-DEXA-cohort and* ***(b)*** *DEXA-cohort, dynamic lung compliance in* ***(c)*** *pre-DEXA-cohort and* ***(d)*** *DEXA-cohort, ventilatory ratio in* ***(e)*** *pre-DEXA-cohort and* ***(f)*** *DEXA-cohort, and* PaO_2/FiO_2 *ratio in* ***(e)*** *pre-DEXA-cohort and* ***(f)*** *DEXA-cohort within 9 days (PIIINP) or 8 days (ventilatory parameters) prior to and 12 days following the alignment day (PF-day 0, start of prednisone treatment in the PF-groups). P-values on the left and the right of each panel reflect between-group differences over time for the days prior to and following PF-day 0, respectively, and were calculated using linear mixed models analysis (time*group interaction factor). Data presented as geometric mean with 95% confidence intervals. * p-value <0.05 on the corresponding timepoint, calculated using Sidak's post-hoc multiple comparisons tests.*

observed in the no-PF-group (Figure 4e). In the DEXA-cohort, the ventilatory ratio of the PF-group remained higher compared to the no-PF-group on all ensuing timepoints following initiation of prednisone treatment (Figure 4f). In the pre-DEXA-cohort, PaO2/FiO2 ratio of the PF-group remained lower compared to the no-PF-group for several days following start of prednisone treatment, while this was not the case in the DEXA-cohort (Figure 4gh). Kinetics and values on individual timepoints of all ventilatory parameters were similar between PF patients of both cohorts (Additional file 1; Figure S5). So, overall, early DEXA treatment did not influence the subsequent response to steroid therapy in PF patients.

Clinical outcomes

PF incidence was 28% and 25% in the pre-DEXA- and DEXA-cohorts, respectively (p=0.61). Time on ventilator, LOS in ICU and mortality were higher in the PF-groups compared to the no-PF-groups within both cohorts (Table 1, Figure 5). Furthermore, within both cohorts, PF-patients who survived their ICU stay had a prolonged time on mechanical ventilation and ICU stay compared to no-PF-patients who survived. None of the clinical outcomes differed between the PF-groups of both cohorts (Table 1), again indicating no influence of early DEXA treatment on these clinical response to steroid treatment for PF. When dividing the DEXA-cohort into subgroups of patients who were also treated with tocilizumab and those who were not, similar differences in clinical outcomes between the PF- and no-PF-groups were observed as in the main analysis (Additional file 1; Table S1). Furthermore, no differences in clinical outcomes were present between PF-patients who were co-treated with tocilizumab and PF-patients who were not (Additional file 1; Table S1).

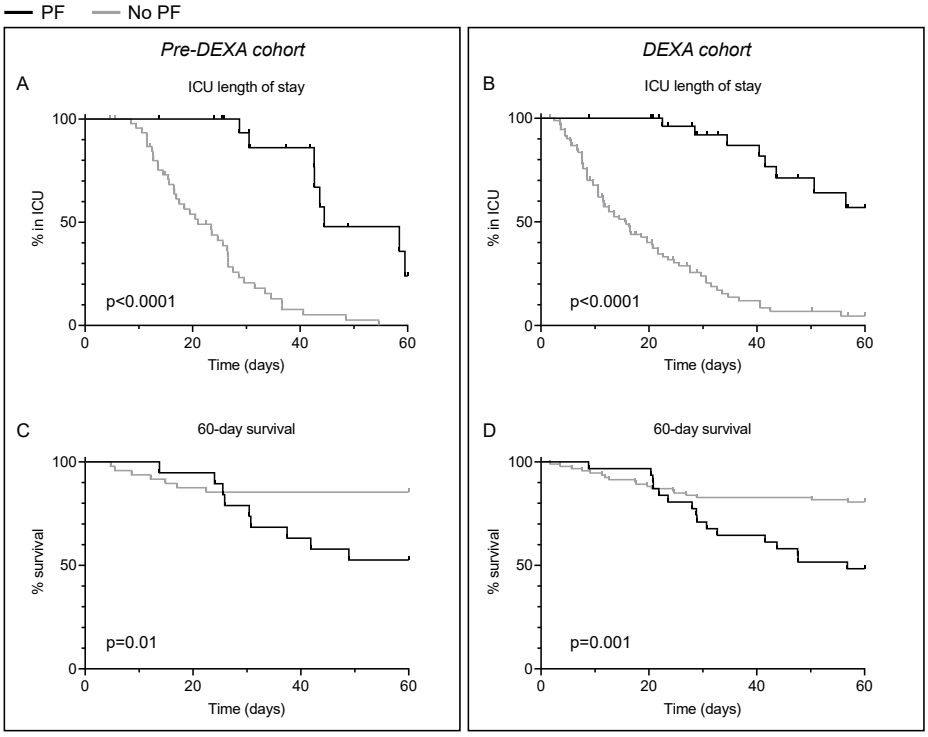


Figure 5. Clinical outcomes.
Differences between the pulmonary fibrosis (PF)- and no PF-groups in length of stay (LOS) in the intensive care unit (ICU) in ***(a)*** *the pre-DEXA-cohort and* ***(b)*** *the DEXA-cohort, and 60-day hospital mortality in* ***(c)*** *the pre-DEXA-cohort and* ***(d)*** *the DEXA-cohort. Kaplan-Meier curves are depicted and p-values were calculated using log-rank tests. For analysis of 60-day hospital mortality, patients who were discharged alive from the hospital or were still in the ICU or hospital on day 60 were censored at day 60. Numbers at risk are shown below graphs.*

Discussion

Our study reveals that several genes and signaling pathways that were previously linked to organ fibrosis are upregulated in critically ill COVID-19 patients who develop PF, including inflammatory processes, coagulation, and NET-related pathways. Furthermore, we demonstrate that some of the identified pathways are associated with worse long-term outcomes of fibrotic diseases. Following initiation of steroid treatment for PF, multiple upregulated pathways in the PF-group converged towards expression levels observed in the no-PF-group. Likewise, circulating PIIINP levels reverted to concentrations similar to those observed in the no-PF-group following treatment of PF with steroids. Whereas several clinical ventilatory parameters also stabilized or improved after treatment, these largely remained worse in PF-patients compared to no-PF-patients. Importantly, this treatment response was not influenced by early dexamethasone treatment. Finally, PF was associated with a prolonged length of stay in the ICU and higher mortality rates, which was also not influenced by early dexamethasone treatment or co-treatment with tocilizumab.

Up to now, multiple studies have described long-term symptoms of COVID-19 in both ICU and non-ICU COVID-19 patients, including pulmonary sequalae (34-37). The exact underlying mechanisms for these long-lasting symptoms are still unclear, but the development of PF likely plays a role. Therefore, and because of its high mortality, it is of paramount importance to detect and treat PF at an early stage. In non-COVID-19 ARDS patients, it was shown that corticosteroid treatment is effective in shortening ICU-LOS and reducing mortality rates (11). Accordingly, we hypothesized that early dexamethasone treatment would result in a lower incidence or less severe course of excessive PF and subsequent more pronounced improvement of clinical pulmonary outcomes in patients of our DEXA-cohort compared to the pre-DEXA-cohort. In contrast, we did not observe differences in incidence rates or clinical outcomes between both cohorts.

Although it did not reach statistical significance, time from ICU admission until the initiation of prednisone to treat PF was three days later in the DEXA-cohort. One may argue that prolongation of the early dexamethasone treatment as standard care in this subgroup of critically ill COVID-19 patients could further delay or even prevent the development of PF. Additionally, the dose of dexamethasone used as standard treatment for COVID-19 is considerably lower than the equivalent corticosteroid dose of prednisone used for the treatment of PF. For example, a patient with PF of 80 kg would be treated with 160 mg prednisone daily, approximately equivalent to 24 mg dexamethasone (38), and thus several times higher than the 6 mg dexamethasone dose used as standard treatment for COVID-19. Therefore,

we cannot exclude that prolongation of treatment, or increasing the dosage of early dexamethasone may mitigate the development of PF and improve clinical outcomes in critically ill COVID-19 patients. Of interest, a recently published study compared the effects of daily administration of 6 mg and 12 mg dexamethasone for 10 days in 982 severely ill COVID-19 patients and showed better clinical outcomes in the 12 mg group, while the incidence of serious adverse effects was similar (39). To investigate the effects of early dexamethasone treatment on incidence rates and mortality of all hospitalized COVID-19 patients and the effects of prolongation/intensification of dexamethasone treatment on the development of PF in critically ill COVID-19 patients, randomized controlled trials should be performed.

Our longitudinal transcriptome analysis provided clues for novel therapeutic targets for prevention or treatment of PF in critically ill COVID-19 patients. One of the most strongly upregulated genes in PF-patients, MMP8, is related to bleomycin-induced fibrosis in mice (27, 40), that treatment with MMP8 inhibitors may be beneficial. Similar, inhibition of PDE4, also markedly upregulated in PF patients, prevented PF in bleomycin-treated mice (26). Interestingly, the PDE4 inhibitor roflumilast is already licensed for the treatment of severe COPD and asthma (41, 42).

This study has several limitations. First, early dexamethasone treatment was not randomized. As a consequence, bias related to the initial response to dexamethasone is likely present, especially because this treatment is often started on the ward. Therefore, it is possible that several patients of the pre-DEXA-cohort would not have required ICU admission if they would have received dexamethasone on the ward. On the other hand, our data are as observed in current clinical practice. Second, later on, patients were treated with prednisone when PF was identified based on radiological findings, worsening of ventilatory parameters and elevated circulating PIIINP levels which were available to the treating physicians. Ideally, PF should be diagnosed based on high PIIINP levels in BAL fluids and typical high-resolution computed tomography (HRCT) images (4, 8). However, during the COVID-19 pandemic, it was not feasible to perform repeated BALs and HRCTs, and circulating PIIINP levels have shown promise for evaluation of disease progression and treatment efficacy in non-COVID-19 ARDS patients (12, 43). Also, the differences in transcriptome profiles between PF- and no-PF-patients support the PF diagnosis, and the changes in PIIINP kinetics, pulmonary parameters, and gene expression patterns following prednisone treatment illustrate therapeutic efficacy. Third, the transcriptome analyses were performed on total leukocytes. With this approach, changes in blood count differential (i.e. the percentages of e.g. monocytes, lymphocytes, and neutrophils) influence gene expression levels. Unfortunately, we do not have data available on blood count differentials to assess the magnitude of this

effect in our analyses. Fourth, since the relatively small sample size, our study was possibly underpowered to draw conclusions on the effects of early dexamethasone treatment on incidence and clinical outcomes of COVID-19 patients with PF. However, we did not find any (non-significant) indications to a major influence of early dexamethasone treatment on these outcomes. Last, the observational design of our study could have introduced confounding because of the rapidly increasing knowledge of the disease and the so-called learning curve during the pandemic. Since we compared data of two cohorts of COVID-19 patients who were admitted to the ICU during different periods in time, the possibility that the introduction of dexamethasone and tocilizumab as standard treatment in COVID-19 was not the only difference in treatment between both cohorts has to be acknowledged. For example, differences in virulence of the dominant SARS-CoV-2 strain during each period of time might also be of influence on our study outcomes. Preferably, a RCT in hospitalized COVID-19 patients should be performed to more accurately determine the effects of early dexamethasone treatment on the incidence, clinical course and outcomes of PF, although this would now raise ethical dilemmas.

Conclusions

In critically ill COVID-19 patients who develop PF, coagulation, inflammation and NET-related pathways are upregulated, ICU-LOS is prolonged and mortality is higher. This study indicates that early dexamethasone treatment neither influences the incidence or clinical course of PF, nor the outcomes of this subgroup of critically ill COVID-19 patients. Steroid treatment normalized PF-related RNA profiles and PIIINP levels, while clinical parameters stabilized, but remained aberrant compared to critically ill COVID-19 patients without signs of PF.

References

1. Burnham EL, Janssen WJ, Riches DWH, Moss M, Downey GP: The fibroproliferative response in acute respiratory distress syndrome: mechanisms and clinical significance. The European respiratory journal 2014, 43:276-285.
2. Cabrera-Benitez NE, Laffey JG, Parotto M, Spieth PM, Villar J, Zhang H, Slutsky AS: Mechanical ventilation-associated lung fibrosis in acute respiratory distress syndrome: a significant contributor to poor outcome. Anesthesiology 2014, 121:189-198.
3. Martin C, Papazian L, Payan M-J, Saux P, Gouin F: Pulmonary Fibrosis Correlates With Outcome in Adult Respiratory Distress Syndrome: A Study in Mechanically Ventilated Patients. Chest 1995, 107:196-200.
4. Ichikado K, Muranaka H, Gushima Y, Kotani T, Nader HM, Fujimoto K, Johkoh T, Iwamoto N, Kawamura K, Nagano J, et al: Fibroproliferative changes on high-resolution CT in the acute respiratory distress syndrome predict mortality and ventilator dependency: a prospective observational cohort study. BMJ Open 2012, 2:e000545.
5. Gao J, Chu W, Duan J, Li J, Ma W, Hu C, Yao M, Xing L, Yang Y: Six-Month Outcomes of Post-ARDS Pulmonary Fibrosis in Patients With H1N1 Pneumonia. Front Mol Biosci 2021, 8:640763.
6. Herridge MS, Cheung AM, Tansey CM, Matte-Martyn A, Diaz-Granados N, Al-Saidi F, Cooper AB, Guest CB, Mazer CD, Mehta S, et al: One-year outcomes in survivors of the acute respiratory distress syndrome. N Engl J Med 2003, 348:683-693.
7. Masclans JR, Roca O, Muñoz X, Pallisa E, Torres F, Rello J, Morell F: Quality of life, pulmonary function, and tomographic scan abnormalities after ARDS. Chest 2011, 139:1340-1346.
8. Forel JM, Guervilly C, Hraiech S, Voillet F, Thomas G, Somma C, Secq V, Farnarier C, Payan MJ, Donati SY, et al: Type III procollagen is a reliable marker of ARDS-associated lung fibroproliferation. Intensive Care Med 2015, 41:1-11.
9. Yamanouchi H, Fujita J, Yoshinouchi T, Hojo S, Kamei T, Yamadori I, Ohtsuki Y, Ueda N, Takahara J: Measurement of hepatocyte growth factor in serum and bronchoalveolar lavage fluid in patients with pulmonary fibrosis. Respiratory Medicine 1998, 92:273-278.
10. Chu X, Jin Q, Chen H, Wood GC, Petrick A, Strodel W, Gabrielsen J, Benotti P, Mirshahi T, Carey DJ, et al: CCL20 is up-regulated in non-alcoholic fatty liver disease fibrosis and is produced by hepatic stellate cells in response to fatty acid loading. J Transl Med 2018, 16:108.
11. Meduri GU, Golden E, Freire AX, Taylor E, Zaman M, Carson SJ, Gibson M, Umberger R: Methylprednisolone infusion in early severe ARDS: results of a randomized controlled trial. Chest 2007, 131:954-963.
12. Meduri GU, Tolley EA, Chinn A, Stentz F, Postlethwaite A: Procollagen types I and III aminoterminal propeptide levels during acute respiratory distress syndrome and in response to methylprednisolone treatment. Am J Respir Crit Care Med 1998, 158:1432-1441.
13. Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, Staplin N, Brightling C, Ustianowski A, Elmahi E, et al: Dexamethasone in Hospitalized Patients with Covid-19. N Engl J Med 2021, 384:693-704.
14. Gordon AC, Mouncey PR, Al-Beidh F, Rowan KM, Nichol AD, Arabi YM, Annane D, Beane A, van Bentum-Puijk W, Berry LR, et al: Interleukin-6 Receptor Antagonists in Critically Ill Patients with Covid-19. N Engl J Med 2021, 384:1491-1502.

15. Kooistra EJ, Waalders NJB, Grondman I, Janssen NAF, de Nooijer AH, Netea MG, van de Veerdonk FL, Ewalds E, van der Hoeven JG, Kox M, Pickkers P: Anakinra treatment in critically ill COVID-19 patients: a prospective cohort study. Crit Care 2020, 24:688.
16. Kooistra EJ, van Berkel M, van Kempen NF, van Latum CRM, Bruse N, Frenzel T, van den Berg MJW, Schouten JA, Kox M, Pickkers P: Dexamethasone and tocilizumab treatment considerably reduces the value of C-reactive protein and procalcitonin to detect secondary bacterial infections in COVID-19 patients. Crit Care 2021, 25:281.
17. Aschenbrenner AC, Mouktaroudi M, Krämer B, Oestreich M, Antonakos N, Nuesch-Germano M, Gkizeli K, Bonaguro L, Reusch N, Baßler K, et al: Disease severity-specific neutrophil signatures in blood transcriptomes stratify COVID-19 patients. Genome Medicine 2021, 13:7.
18. Ueha S, Shand F, Matsushima K: Cellular and Molecular Mechanisms of Chronic Inflammation-Associated Organ Fibrosis. Frontiers in Immunology 2012, 3.
19. Saito F, Tasaka S, Inoue K-i, Miyamoto K, Nakano Y, Ogawa Y, Yamada W, Shiraishi Y, Hasegawa N, Fujishima S, et al: Role of Interleukin-6 in Bleomycin-Induced Lung Inflammatory Changes in Mice. American Journal of Respiratory Cell and Molecular Biology 2008, 38:566-571.
20. Le TT, Karmouty-Quintana H, Melicoff E, Le TT, Weng T, Chen NY, Pedroza M, Zhou Y, Davies J, Philip K, et al: Blockade of IL-6 Trans signaling attenuates pulmonary fibrosis. J Immunol 2014, 193:3755-3768.
21. Florez-Sampedro L, Song S, Melgert BN: The diversity of myeloid immune cells shaping wound repair and fibrosis in the lung. Regeneration (Oxford, England) 2018, 5:3-25.
22. Chambers RC, Scotton CJ: Coagulation Cascade Proteinases in Lung Injury and Fibrosis. Proceedings of the American Thoracic Society 2012, 9:96-101.
23. Chrysanthopoulou A, Mitroulis I, Apostolidou E, Arelaki S, Mikroulis D, Konstantinidis T, Sivridis E, Koffa M, Giatromanolaki A, Boumpas DT, et al: Neutrophil extracellular traps promote differentiation and function of fibroblasts. The Journal of Pathology 2014, 233:294-307.
24. Suzuki M, Ikari J, Anazawa R, Tanaka N, Katsumata Y, Shimada A, Suzuki E, Tatsumi K: PAD4 Deficiency Improves Bleomycin-induced Neutrophil Extracellular Traps and Fibrosis in Mouse Lung. Am J Respir Cell Mol Biol 2020, 63:806-818.
25. Inui N, Sakai S, Kitagawa M: Molecular Pathogenesis of Pulmonary Fibrosis, with Focus on Pathways Related to TGF-αand the Ubiquitin-Proteasome Pathway. International Journal of Molecular Sciences 2021, 22.
26. Udalov S, Dumitrascu R, Pullamsetti SS, Al-tamari HM, Weissmann N, Ghofrani HA, Guenther A, Voswinckel R, Seeger W, Grimminger F, Schermuly RT: Effects of phosphodiesterase 4 inhibition on bleomycin-induced pulmonary fibrosis in mice. BMC Pulmonary Medicine 2010, 10:26.
27. Craig VJ, Quintero PA, Fyfe SE, Patel AS, Knolle MD, Kobzik L, Owen CA: Profibrotic activities for matrix metalloproteinase-8 during bleomycin-mediated lung injury. J Immunol 2013, 190:4283-4296.
28. Mathai SK, Cardwell J, Metzger F, Powers J, Walts AD, Kropski JA, Eickelberg O, Hauck SM, Yang IV, Schwartz DA: Preclinical Pulmonary Fibrosis Circulating Protein Biomarkers. American Journal of Respiratory and Critical Care Medicine 2020, 202:1720-1724.
29. Safaeian L, Abed A, Vaseghi G: The role of Bcl-2 family proteins in pulmonary fibrosis. European Journal of Pharmacology 2014, 741:281-289.

30. Herazo-Maya Jose D, Noth I, Duncan Steven R, Kim S, Ma S-F, Tseng George C, Feingold E, Juan-Guardela Brenda M, Richards Thomas J, Lussier Y, et al: Peripheral Blood Mononuclear Cell Gene Expression Profiles Predict Poor Outcome in Idiopathic Pulmonary Fibrosis. Science Translational Medicine 2013, 5:205ra136-205ra136.
31. Kalluri R, Neilson EG: Epithelial-mesenchymal transition and its implications for fibrosis. J Clin Invest 2003, 112:1776-1784.
32. Stone RC, Pastar I, Ojeh N, Chen V, Liu S, Garzon KI, Tomic-Canic M: Epithelial-mesenchymal transition in tissue repair and fibrosis. Cell and tissue research 2016, 365:495-506.
33. Guarino M, Tosoni A, Nebuloni M: Direct contribution of epithelium to organ fibrosis: epithelial-mesenchymal transition. Hum Pathol 2009, 40:1365-1376.
34. Gamberini L, Mazzoli CA, Prediletto I, Sintonen H, Scaramuzzo G, Allegri D, Colombo D, Tonetti T, Zani G, Capozzi C, et al: Health-related quality of life profiles, trajectories, persistent symptoms and pulmonary function one year after ICU discharge in invasively ventilated COVID-19 patients, a prospective follow-up study. Respiratory Medicine 2021, 189:106665.
35. Morin L, Savale L, Pham T, Colle R, Figueiredo S, Harrois A, Gasnier M, Lecoq AL, Meyrignac O, Noel N, et al: Four-Month Clinical Status of a Cohort of Patients After Hospitalization for COVID-19. Jama 2021, 325:1525-1534.
36. Latronico N, Peli E, Calza S, Rodella F, Novelli MP, Cella A, Marshall J, Needham DM, Rasulo FA, Piva S: Physical, cognitive and mental health outcomes in 1-year survivors of COVID-19-associated ARDS. Thorax 2021.
37. Heesakkers H, van der Hoeven JG, Corsten S, Janssen I, Ewalds E, Simons KS, Westerhof B, Rettig TCD, Jacobs C, van Santen S, et al: Clinical Outcomes Among Patients With 1-Year Survival Following Intensive Care Unit Treatment for COVID-19. Jama 2022.
38. Schimmer BP, Funder JW: ACTH, Adrenal Steroids, and Pharmacology of the Adrenal Cortex. In Goodman & Gilman's: The Pharmacological Basis of Therapeutics, 12e. Edited by Brunton LL, Chabner BA, Knollmann BC. New York, NY: McGraw-Hill Education; 2015
39. Granholm A, Munch MW, Myatra SN, Vijayaraghavan BKT, Cronhjort M, Wahlin RR, Jakob SM, Cioccari L, Kjær MN, Vesterlund GK, et al: Dexamethasone 12 mg versus 6 mg for patients with COVID-19 and severe hypoxaemia: a pre-planned, secondary Bayesian analysis of the COVID STEROID 2 trial. Intensive Care Med 2021:1-11.
40. García-Prieto E, González-López A, Cabrera S, Astudillo A, Gutiérrez-Fernández A, Fanjul-Fernandez M, Batalla-Solís E, Puente XS, Fueyo A, López-Otín C, Albaiceta GM: Resistance to Bleomycin-Induced Lung Fibrosis in MMP-8 Deficient Mice Is Mediated by Interleukin-10. PLOS ONE 2010, 5:e13242.
41. Pagès L, Gavaldà A, Lehner MD: PDE4 inhibitors: a review of current developments (2005 – 2009). Expert Opinion on Therapeutic Patents 2009, 19:1501-1519.
42. Li H, Zuo J, Tang W: Phosphodiesterase-4 Inhibitors for the Treatment of Inflammatory Diseases. Frontiers in Pharmacology 2018, 9.
43. Steinberg KP, Hudson LD, Goodman RB, Hough CL, Lanken PN, Hyzy R, Thompson BT, Ancukiewicz M, National Heart L, Blood Institute Acute Respiratory Distress Syndrome Clinical Trials N: Efficacy and safety of corticosteroids for persistent acute respiratory distress syndrome. N Engl J Med 2006, 354:1671-1684.

Additional file 1

ADDITIONAL METHODS

Sensitivity analysis in tocilizumab-treated patients

To exclude that treatment with tocilizumab in the subgroup of the dexamethasone (DEXA)-cohort could possibly affect our results, we performed a sensitivity analysis by comparing the number of patients treated with tocilizumab between the pulmonary fibrosis (PF) and no-PF groups of the DEXA-cohort. Also, the DEXA-cohort was subdivided into patients who were treated with tocilizumab and patients who were not and differences in clinical outcomes between the PF and no-PF groups were analyzed within both subgroups (treated with tocilizumab/not treated with tocilizumab).

Data collection

Patient characteristics, laboratory data and daily clinical measurements including dynamic lung compliance, PaO2/FiO2 ratio, minute ventilation, and PaCO2 were collected from the electronic patient files (EPF, Epic, Epic Systems Corporation, Verona, Wisconsin, USA) during stay in ICU with a maximum follow up of 40 days. Clinical outcome data (time on ventilator, LOS in ICU and hospital mortality) were also collected from the EPFs. All clinical data were recorded in the Good Clinical Practice (GCP)-certified data management system Castor (Castor EDC, Amsterdam, The Netherlands). Data of minute ventilation and PaCO2 were used to calculate daily ventilatory ratio: [minute ventilation (ml/min) × PaCO2 (mm Hg)]/(predicted body weight × 100 × 37.5).1

Biomarker measurements

Circulating PIIINP levels were measured in serum and stored at -20ºC until analysis, three times per week as part of routine care as instructed by the manufacturer using the PIIINP radio immune assay (UniQ®, cat no. 68570, Aidian Oy, Espoo, Finland) and collected from the electronic patient files (EPF). Circulating HGF and MIP-3αlevels were measured afterwards by enzyme-linked immunosorbent assays (ELISA) in residual serum samples used for clinical purposes which were stored at -30 oC according to the manufacturer's instructions (R&D systems Duosets, Minneapolis, Minnesota, USA).

RNA sequencing

Of 52 patients of the pre-DEXA-cohort, whole blood samples for RNA-seq analysis were collected in PAXgene tubes three times a week following ICU admission. Total RNA was converted into double-stranded cDNA libraries using the TruSeq Stranded Total RNA with Ribo-Zero Globin kit (Illumina). In brief, ribosomal and globin mRNA were depleted from 750 ng purified total RNA using biotinylated, target-specific oligos combined with Ribo-Zero rRNA removal beads; remaining RNA was fragmented using divalent cations under elevated temperature. First-strand was generated using SuperScript2 RT (Invitrogen) supplemented with actinomycin D, followed by second-strand synthesis with dUTP replacing dTTP. 3α ends were adenylated and index adapters were ligated before subsequent PCR amplification to yield the final library. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities, and enzymes were removed. Selective enrichment of DNA fragments with ligated adaptor molecules was performed using Illumina PCR primers in a 15-cycle PCR reaction, followed by purification cDNA using SPRIBeads (Beckman Coulter). Libraries were quantified by Qubit dsDNA HS Assay (Thermo Fisher Scientific), and fragment size distribution was determined using the HS D1000 assay on a Tapestation 4200 system (Agilent). High-throughput sequencing was carried out with a NovaSeq™ 6000 Sequencing System S2 (50bp paired-end reads), and data was converted into fastq files using bcl2fastq2 v2.20. The data are made available at the European Genome-Phenome Archive (EGA) under accession numbers EGAS00001005735 and EGAS00001006407, which is hosted by the EBI and the CRG.

Sequencing alignment and pre-processing

Sequenced reads were aligned and quantified using STAR: ultrafast universal RNA-seq aligner (v2.7.3a) 2 and the human reference genome, GRCh38p13, from the Genome Reference Consortium. For PF-patients, the day prednisone was started was used as alignment day (day 0). For no-PF-patients, the median alignment day of the PF-patients was designated day 0. Raw counts were imported using DESeqDataSetFromHTSeqCount function from DESeq2 (v1.34.0) 3. The data was split into two data sets (pre-alignment day: day -12 to day 0; post-alignment day: day 0 to day 6). Both data sets were preprocessed identically. Genes with a lower count than the number of samples were excluded from the analysis. DESeq2 was used for the calculation of normalized counts for protein-coding genes and further relevant gene types using default parameters.

Gene co-expression network analysis and longitudinal fibrosis-related gene identification

The gene co-expression network analysis was performed using the hcocena package. Estimation of the number of top variable genes was performed by identifying the inflection point of a curve of the logged variance of the ranked genes. Pearson's correlation coefficient cut-offs of 0.656 and 0.714 were chosen for the prior to and post-alignment day network, respectively. Gene modules were calculated based on the group fold change (GFC) with the Leiden algorithm iterating it ten times. Database enrichment utilizing the Bioconductor R package clusterProfiler (v4.2.2) 4 was performed using the default parameters for the KEGG 5 enrichment and HALLMARK 6,7 enrichment from the Molecular Signature Database (MSigDB) and using the biological process ontology for the Gene Ontology (GO) 8,9 enrichment. Longitudinal genes strongly altered due to fibrosis onset were identified by comparing two linear mixed-effect models (LMEM) for each gene computed using the R package lme4 (v1.1-28) 10, of which one includes the fibrosis categorization, over the respective time span using an asymptotic likelihood ratio test from the R package lmtest (v0.9-40) 11. Genes with a likelihood ratio χ^2-statistics < 0.1 were defined as statistically significant. Effect size was calculated per gene by squaring the correlation coefficient of the fitted and observed values.

Decision rules for which CoCena modules to focus on

For the analysis up to day 0 (pre-alignment day analysis), we focused on modules for which the wave plots showed no intersection of the PF- and no-PF-groups at any timepoint or which showed significant differences (non-overlapping confidence intervals) at day -12 and converged afterwards. For the analysis from day 0 onwards (post-alignment day analysis), we focused on modules for which the PF- and no-PF-groups were significantly different on day 0 and converged afterwards (indicating normalization of the PF-group towards the no-PF-group).

Survival analysis

A survival analysis was conducted with the prior to alignment day data set using a microarray data set of idiopathic pulmonary fibrosis (GSE28042, replication cohort) 12 as reference data. Gene set variation analysis (GSVA, v1.42.0) 13 with the z-score method was used to check the enrichment of the modules filtered for the significant LMEM genes per patient of the reference dataset. Modules with a z-score > 1.645 (p-value ~ 0.1) were spec-

ified to be significantly enriched in a patient. Utilizing the survival package (v3.3-1) 14 the probability of survival of patients with or without significant enrichment of the gene set were computed per module. P-values were calculated with a log-rank-test and the Kaplan-Meier curves visualized using the survminer package (v0.4.9) 15.

Statistical analysis

To analyze kinetics of serially measured data, variables were binned into bins spanning two or three days because not all variables were measured daily in all patients. Because of the relatively small size of the cohorts, a normal distribution of data was not assumed. Data are displayed as median with interquartile ranges [IQR], numbers with percentages, or geometric means with 95% confidence intervals (CI). P-values <0.05 were considered to indicate statistical significance. Differences in baseline characteristics and clinical outcomes between the PF- and no-PF-groups were analyzed using Mann-Whitney U and Fisher's exact tests for continuous and categorical data, respectively. Differences in kinetics of serially measured data were analyzed using linear mixed effect model analysis on log-transformed data followed by post-hoc Sidak's multiple comparisons tests. Because data collection was only performed during stay in ICU, we performed Last Observation Carried Forward (LOCF) for data of patients who were discharged from ICU or who deceased. LOS in ICU and mortality were analyzed using log-rank tests during 60 days following ICU admission. For the log-rank test of 60-day mortality, patients who were discharged alive from the hospital or were still in the ICU or hospital on day 60 were censored at day 60. Statistical analysis was performed using SPSS 25 (IBM SPSS statistics version 25.0. Armonk, NY: IBM Corp) and GraphPad Prism 8 (GraphPad Software, La Jolla California, USA).

References Additional methods

1. Sinha P, Calfee CS, Beitler JR, et al. Physiologic Analysis and Clinical Performance of the Ventilatory Ratio in Acute Respiratory Distress Syndrome. American Journal of Respiratory and Critical Care Medicine. 2018;199(3):333-341.
2. Dobin A, Davis CA, Schlesinger F, et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics. 2013;29(1):15-21.
3. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology. 2014;15(12):550.
4. Wu T, Hu E, Xu S, et al. clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. Innovation (Camb). 2021;2(3):100141.
5. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Research. 2017;45(D1):D353-D361.
6. Subramanian A, Tamayo P, Mootha Vamsi K, et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. Proceedings of the National Academy of Sciences. 2005;102(43):15545-15550.
7. Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov Jill P, Tamayo P. The Molecular Signatures Database Hallmark Gene Set Collection. Cell Systems. 2015;1(6):417-425.
8. Ashburner M, Ball CA, Blake JA, et al. Gene Ontology: tool for the unification of biology. Nature Genetics. 2000;25(1):25-29.
9. The Gene Ontology C. The Gene Ontology resource: enriching a GOld mine. Nucleic Acids Research. 2021;49(D1):D325-D334.
10. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software. 2015;67(1):1 - 48.
11. Zeileis A, Hothorn T. Diagnostic Checking in Regression Relationships. 2001;2.
12. Herazo-Maya Jose D, Noth I, Duncan Steven R, et al. Peripheral Blood Mononuclear Cell Gene Expression Profiles Predict Poor Outcome in Idiopathic Pulmonary Fibrosis. Science Translational Medicine. 2013;5(205):205ra136-205ra136.
13. Hänzelmann S, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-Seq data. BMC Bioinformatics. 2013;14(1):7.
14. Therneau TM, Grambsch PM. Modeling Survival Data: Extending the Cox Model Springer. 2000.
15. Kassambara A, Kosinski M, Biecek P, Scheipl F. Drawing Survival Curves using 'ggplot2'. 2021.

ADDITIONAL TABLES

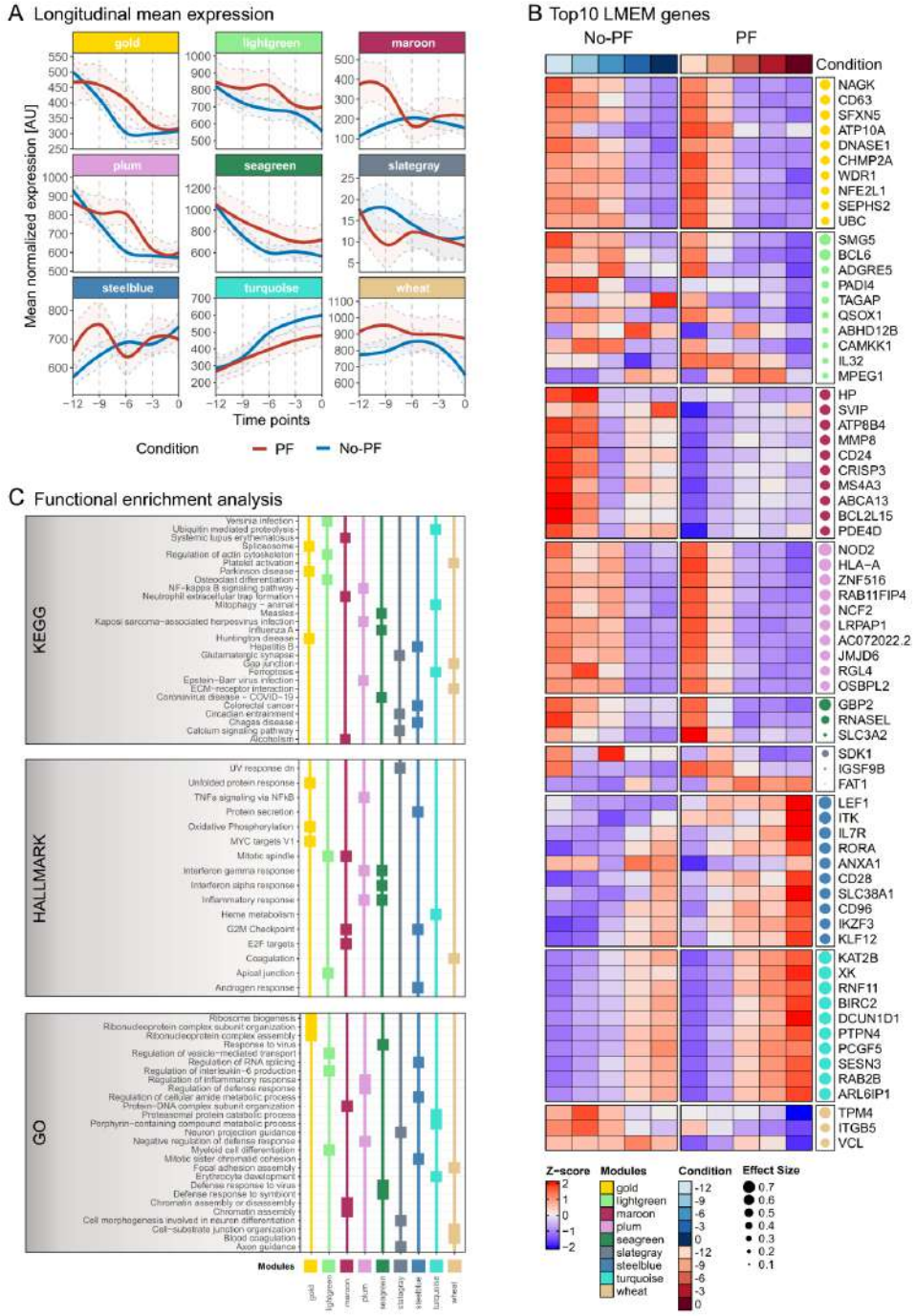
Table S1. Clinical outcomes within tocilizumab subgroups of the DEXA-cohort.
Data presented as n (%) median with interquartile ranges ([IQR]). P-values were calculated using Mann-Whitney U and two-sided Fisher's exact tests for continuous and categorical data, respectively.
DEXA: dexamethasone, PF: pulmonary fibrosis, ICU: intensive care unit

	Not treated with tocilizumab			**Treated with tocilizumab**			**PF + no tocilizumab vs. PF + tocilizumab**
	PF (n=11)	No-PF (n=37)	p-value	PF (n=20)	No-PF (n=56)	p-value	p-value
Hospital mortality	7 (64)	9 (24)	0.02	12 (60)	9 (16)	<0.001	1.00
Length of stay in ICU (days)	42 [22-70]	12 [8-25]	<0.001	41 [28-50]	15 [8-24]	<0.001	0.68
Survivors	56 [27-73]	11 [8-22]	0.005	47 [36-66]	16 [8-23]	<0.001	1.00
Non-survivors	29 [21-64]	18 [12-39]	0.02	32 [22-47]	12 [7-25]	0.007	0.61
Time on ventilator (days)	34 [20-61]	8 [5-20]	<0.001	30 [21-49]	12 [3-20]	<0.001	0.80
Survivors	49 [24-62]	7 [4-18]	0.005	40 [23-61]	11 [4-18]	0.001	0.87
Non-survivors	28 [20-59]	13 [8-36]	0.04	30 [21-44]	14 [0-22]	0.004	1.00

Figure S1. Complete analysis of bulk RNA-seq data pre-alignment day (day 0, the day prednisone treatment was initiated in patients with PF). (a) *displays the mean expression per module filtered by the LMEM genes over time prior to the alignment day. Lines and confidence intervals are colored according to the condition.* ***(b)*** *depicts the mean expression of the top 10 LMEM genes per module ordered by effect size for all conditions and time points prior to the alignment day. Modules are colored accordingly and effect size is indicated by the dot size.* ***(c)*** *shows the top 3 significant functional enrichment terms from GO and KEGG database as well as the hallmark gene set of the Molecular Signature Database per module. Modules names are displayed on the x-axis and the respectively colored squares indicate the enrichment of a functional term in the module.*

→

ADDITIONAL FIGURES



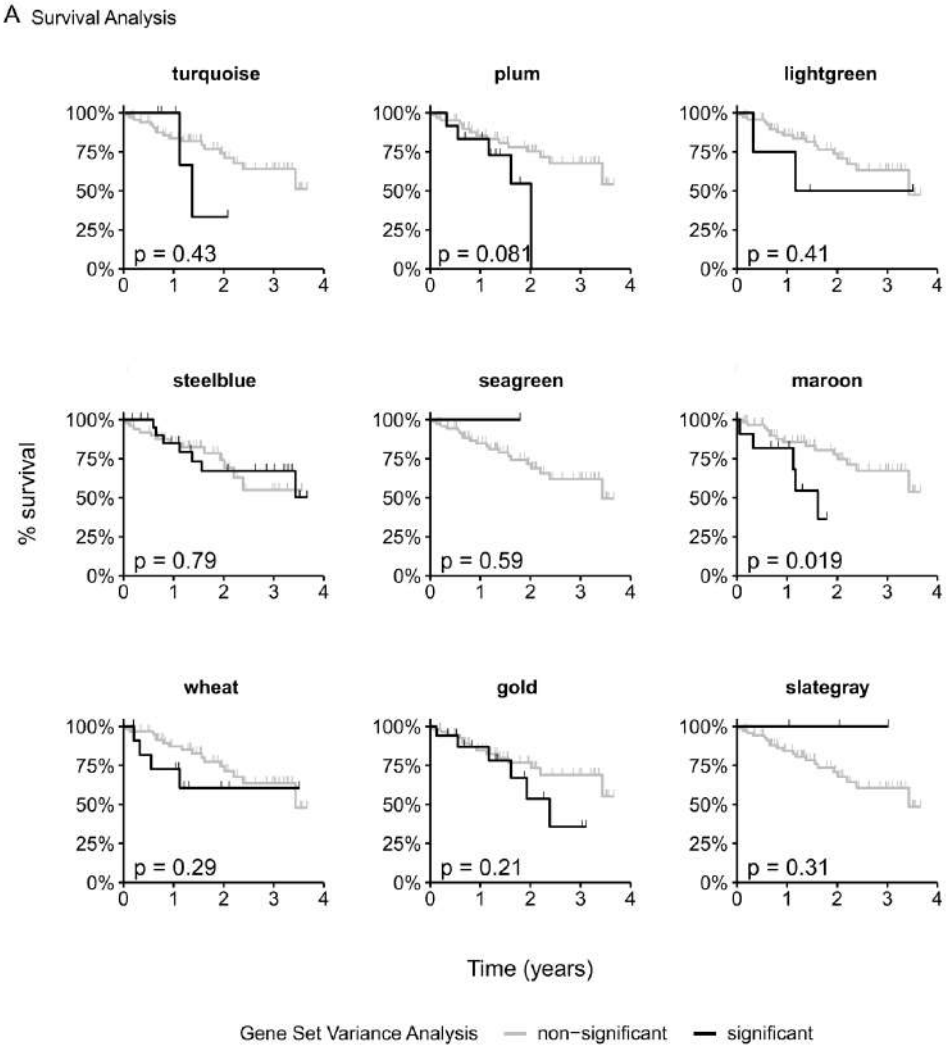
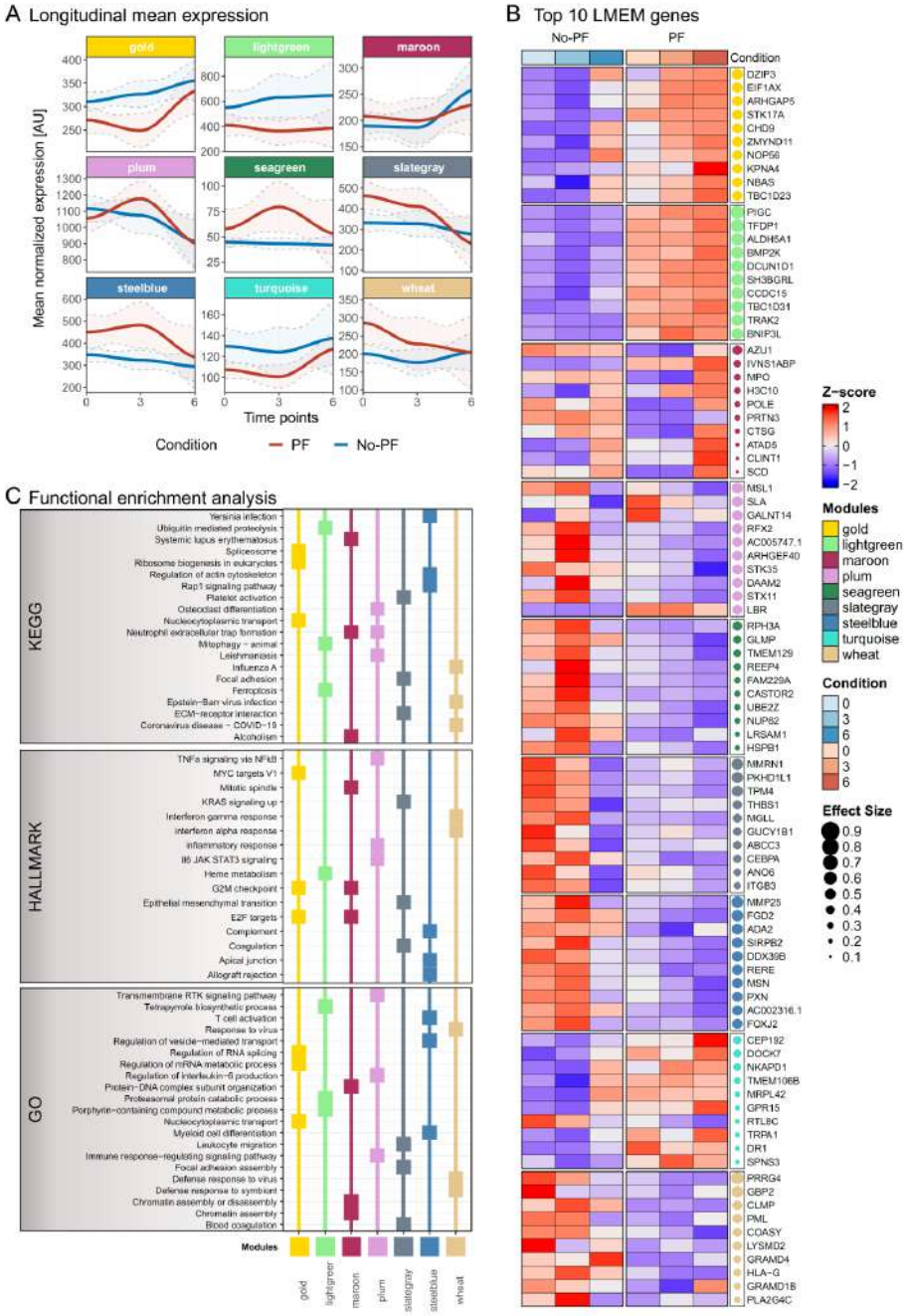


Figure S2. Survival analysis of bulk RNA-seq data pre-alignment day (day 0, the day prednisone treatment was initiated in patients with PF).
(a) *Survival curves of all modules. Lines are colored based on the enrichment of the LMEM genes in the respective module in the reference dataset using GSVA.*

Figure S3. Complete analysis of bulk RNA-seq data post-alignment day (day 0, the day prednisone treatment was initiated in patients with PF). (a) *displays the mean expression per module filtered by the LMEM genes over time post-alignment day. Lines and confidence intervals are colored according to the condition.* ***(b)*** *depicts the mean expression of the top 10 LMEM genes per module ordered by effect size for all conditions and time points post-alignment day. Modules are colored accordingly and effect size is indicated by the dot size.* ***(c)*** *shows the top 3 significant functional enrichment terms from GO and KEGG database as well as the hallmark gene set of the Molecular Signature Database per module. Modules names are displayed on the x-axis and the respectively colored squares indicate the enrichment of a functional term in the module.*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wheat
Mean normalized expression [AU]
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B Top 10 LMEM genes
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Condition
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EIF1AX
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STK17A
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Rap1 signaling pathway
Platelet activation
Osteoclast differentiation
Nucleocytoplasmic transport
Neutrophil extracellular trap formation
Mitophagy - animal
Leishmaniasis
Influenza A
Focal adhesion
Ferroptosis
Epstein-Barr virus infection
ECM-receptor interaction
Coronavirus disease - COVID-19
Alcoholism
HALLMARK
TNFa signaling via NFkB
MYC targets V1
Mitotic spindle
KRAS signaling up
Interferon gamma response
Interferon alpha response
Inflammatory response
Il6 JAK STAT3 signaling
Heme metabolism
G2M checkpoint
Epithelial mesenchymal transition
E2F targets
Complement
Coagulation
Apical junction
Allograft rejection
GO
Transmembrane RTK signaling pathway
Tetrapyrrole biosynthetic process
T cell activation
Response to virus
Regulation of vesicle-mediated transport
Regulation of RNA splicing
Regulation of mRNA metabolic process
Regulation of interleukin-6 production
Protein-DNA complex subunit organization
Proteasomal protein catabolic process
Porphyrin-containing compound metabolic process
Nucleocytoplasmic transport
Myeloid cell differentiation
Leukocyte migration
Immune response-regulating signaling pathway
Focal adhesion assembly
Defense response to virus
Defense response to symbiont
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Blood coagulation
Modules
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maroon
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slategray
steelblue
wheat

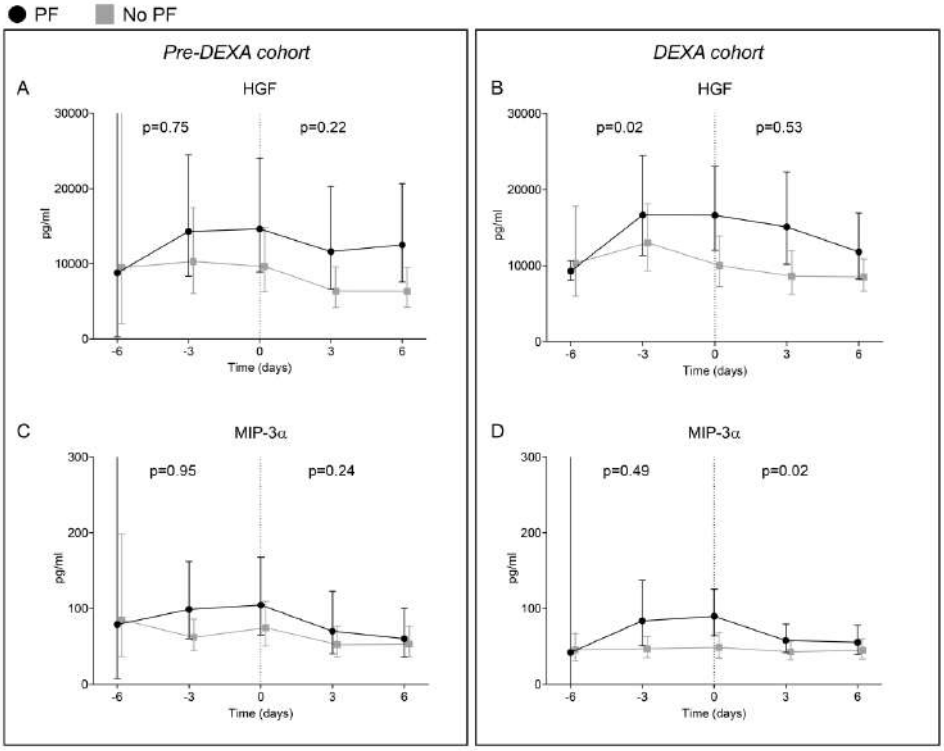

Figure S4. Circulating fibrosis markers.
Differences between the pulmonary fibrosis (PF)- and no-PF groups in kinetics of circulating hepatocyte growth factor (HGF) in ***(a)*** *pre-DEXA-cohort and* ***(b)*** *DEXA-cohort and*
Macrophage Inflammatory Protein 3 Alpha (MIP-3α) in ***(c)*** *pre-DEXA-cohort and* ***(d)*** *DEXA-cohort within 6 days prior to and following the alignment day (PF-day 0, start of prednisone treatment in the PF-groups). P-values on the left and the right of each panel reflect between-group differences over time for the days prior to and following PF-day 0, respectively, and were calculated using linear mixed models analysis (time*group interaction factor). Data presented as geometric mean with 95% confidence intervals. * p-value <0.05 on the corresponding timepoint, calculated using Sidak's post-hoc multiple comparisons test.*

Figure S5. Ventilatory parameters of the pulmonary fibrosis groups.
Differences between the pulmonary fibrosis (PF)-groups of both the pre-DEXA- and DEXA-cohorts in kinetics of ***(a)*** *dynamic lung compliance,* ***(b)*** *ventilatory ratio, and* ***(c)*** *PaO_2/FiO_2 ratio within 8 days prior to and 12 days following the alignment day (PF-day 0, start of prednisone treatment in the PF-groups). P-values on the left and the right of each panel reflect between-group differences over time for the days prior to and following PF-day 0, respectively, and were calculated using linear mixed models analysis (time*group interaction factor). Data presented as geometric mean with 95% confidence intervals.*

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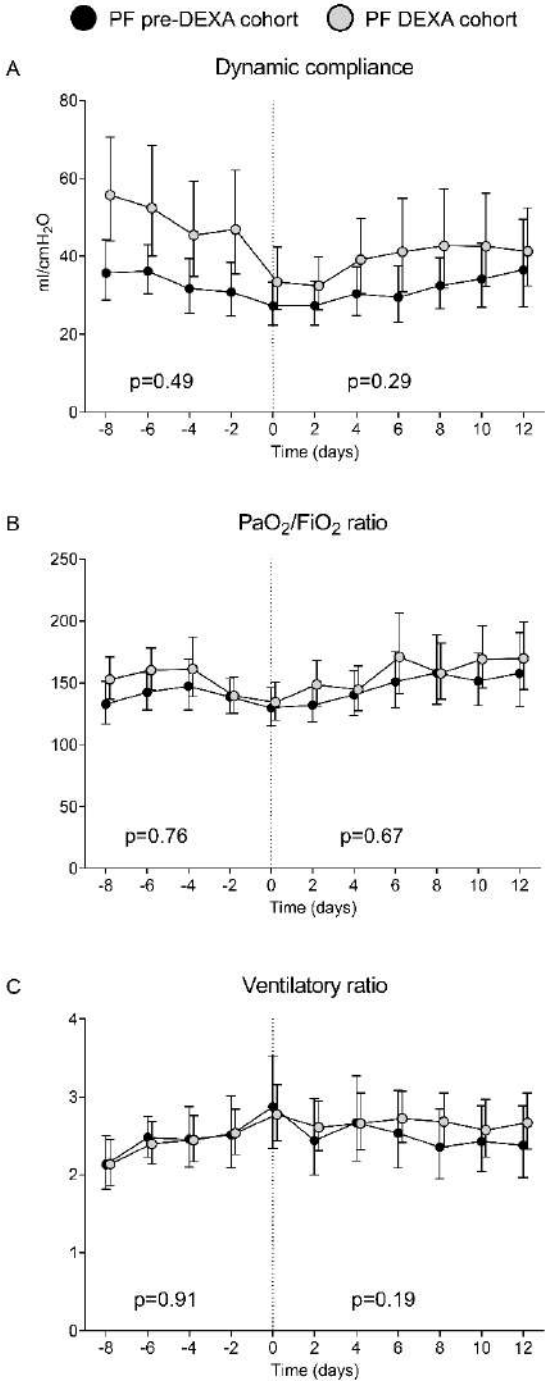
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PF DEXA cohort
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p=0.76
p=0.67
Time (days)
C
Ventilatory ratio
p=0.91
p=0.19
Time (days)

CHAPTER 9

Clinical sepsis phenotypes in critically ill COVID-19 patients

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Introduction

The current undifferentiated treatment approach for coronavirus disease 2019 (COVID-19) may be inadequate, as mainly patients with high levels of inflammatory markers appear to benefit from immunosuppressive medication (1,2). Therefore, personalized medicine may be warranted, for which a better understanding of disease heterogeneity is pivotal (3). In non-COVID-19 sepsis patients, four clinical phenotypes with differential outcomes and treatment responses were previously identified (4). We applied these phenotypes to critically ill COVID-19 sepsis patients and three non-COVID-19 sepsis cohorts to determine possible differences in proportions of phenotypes and their relation with clinical outcome.

Methods

Data of 52,274 patients admitted to 82 Dutch Intensive Care Units (ICUs) from January 2016 to November 2021 recorded in the national ICU registry (NICE) were used (5). Cohorts were: COVID-19 sepsis divided into a pre-dexamethasone (n=2288) and post-dexamethasone (6) cohort (n=8596), non-COVID-19 viral pneumonia sepsis (n=3460), bacterial pneumonia sepsis (n=19,947), and bacterial sepsis of non-pulmonary origin (n=17,983). We mapped patient data to previously validated clinical sepsis phenotypes by Euclidean distance (4). The NICE dataset contained 17 of the 29 features used in the original phenotyping model (4), measured within the first 24 hours after ICU admission (see eMethods).

Results

Characteristics of all five cohorts are listed in the Table.
The proportion of patients with the β phenotype was small in all cohorts (Figure). The introduction of dexamethasone as standard treatment did not importantly affect phenotype distribution in critically ill COVID-19 patients, apart from a larger proportion of patients with the δ phenotype in the post-dexamethasone cohort (11%) compared to the pre-dexamethasone cohort (6%, p<0.00001) at the expense of the γ phenotype (72% versus 81%, p<0.00001). Outcome differences between phenotypes were most pronounced for the pre-dexamethasone COVID-19 cohort and least pronounced in the non-COVID-19 viral pneumonia sepsis cohort. Of interest, 90-day survival of the δ phenotype was markedly better following the introduction of dexamethasone therapy (62% vs 41%, p<0.00001, Figure), whereas no relevant differences in survival were observed for the other phenotypes.
Phenotype distribution was very similar between the COVID-19 and non-COVID-19 viral pneumonia sepsis cohorts. In both bacterial sepsis cohorts (pulmonary and non-pulmonary), the proportion of patients with the δ phenotype was greater than in the viral sepsis

cohorts at the expense of the α- and especially the γ phenotype. This was particularly apparent for patients with bacterial sepsis of non-pulmonary origin. In all cohorts, the α phenotype displayed the lowest mortality, while patients with the δ phenotype generally displayed the highest mortality.

Table 1: Patient characteristics and outcomes.

	COVID-19 pre-dexamethasone (n=2288)	COVID-19 post-dexamethasone (n=8596)	non-COVID-19 viral pneumonia (n=3460)	Bacterial pneumonia (n=19947)	Sepsis of non-pulmonary origin (n=17983)
Sex, male	1672 (73%)	5914 (69%)***	1823 (53%)***	12147 (61%)***	10373 (58%)***
BMI, kg/m²	27.8 (25.4 - 31.2)	29.2 (26.1 - 33.0)***	25.7 (22.6 - 29.7)***	25.2 (22.4 - 29.1)***	26.2 (23.2 - 30.3)***
Age, years	65 (56 - 72)	64 (55 - 71)*	66 (57 - 74)***	69 (60 - 77)***	69 (59 - 77)***
APACHE IV score	58 (47 - 71)	59 (49 - 71)	64 (51 - 80)***	73 (58 - 91)***	78 (62 - 98)***
APACHE IV APS[a] score	46 (38 - 57)	48 (40 - 58)*	50 (39 - 65)***	57 (44 - 74)***	62 (47 - 81)***
Aids	1 (0%)	7 (0%)	6 (0%)	87 (0%)*	42 (0%)
Cardiovascular insuffiency	23 (1%)	117 (1%)	115 (3%)***	877 (4%)***	754 (4%)***
Chronic dialysis	3 (0%)	53 (1%)*	33 (1%)***	244 (1%)***	471 (3%)***
Chronic renal insuffiency	60 (3%)	377 (4%)***	237 (7%)***	1769 (9%)***	2389 (13%)***
Cirrhosis	3 (0%)	40 (0%)	22 (1%)*	286 (1%)***	542 (3%)***
COPD	183 (8%)	809 (9%)	1551 (45%)***	6605 (33%)***	2248 (13%)***
Diabetes mellitus	435 (19%)	2050 (24%)***	693 (20%)	4186 (21%)	4696 (26%)***
Hematological malignancy	35 (2%)	138 (2%)	180 (5%)***	1030 (5%)***	995 (6%)***
Immunological insufficiency	167 (7%)	776 (9%)*	640 (18%)***	3683 (18%)***	3467 (19%)***
Metastatic neoplasm	16 (1%)	55 (1%)	68 (2%)***	1054 (5%)***	1280 (7%)***
Respiratory insufficiency	92 (4%)	361 (4%)	594 (17%)***	2336 (12%)***	581 (3%)
Comorbidity index[b]	0.25 (0.53)	0.31 (0.59)**	0.88 (0.79)***	0.84 (0.86)***	0.70 (0.88)***
Mechanical ventilation	1848 (81%)	5030 (59%)***	2449 (71%)***	11321 (57%)***	5323 (30%)***
PaO_2/FiO_2 ratio	125 (90 - 173)	90 (69 - 124)***	167 (112 - 233)***	149 (98 - 221)***	257 (164 - 344)***

Table 1: Continued.

No ARDS (>300 mmHg)	100 (4%)	176 (2%)***	316 (9%)***	1657 (8%)***	5352 (30%)***
Mild ARDS (>200 - ≤300 mmHg)	238 (10%)	372 (4%)***	792 (23%)***	3617 (18%)***	4053 (23%)***
Moderate ARDS (>100 - ≤200 mmHg)	1058 (46%)	2575 (30%)***	1315 (38%)***	7216 (36%)***	3647 (20%)***
Severe ARDS (≤100 mmHg)	684 (30%)	4645 (54%)***	623 (18%)***	4512 (23%)***	1366 (8%)***
$PaCO_2$, mmHg	41 (35 - 48)	36 (32 - 43)***	47 (37 - 60)***	41 (33 - 52)	34 (29 - 40)***
Respiratory rate (max), breaths/min	31 (26 - 38)	33 (28 - 39)***	32 (27 - 39)***	32 (27 - 38)***	30 (25 - 35)***
Vasoactive medication	1569 (69%)	3945 (46%)***	1541 (45%)***	9872 (49%)***	12140 (68%)
Hematocrit (min)	0.37 (0.34 - 0.39)	0.38 (0.35 - 0.41)***	0.37 (0.32 - 0.41)	0.34 (0.29 - 0.38)***	0.31 (0.27 - 0.35)***
Heart rate (max), beats/min	103 (91 - 116)	98 (87 - 112)***	116 (101 - 132)***	118 (102 - 135)***	116 (100 - 135)***
Mean arterial pressure (max), mmHg	62 (57 - 68)	66 (59 - 73)***	61 (54 - 69)**	59 (52 - 67)***	55 (48 - 62)***
Mean arterial pressure (min), mmHg	107 (97 - 121)	108 (98 - 121)	106 (94 - 121)**	100 (89 - 114)***	94 (84 - 106)***
Acute renal failure	204 (9%)	493 (6%)***	402 (12%)**	3650 (18%)***	6283 (35%)***
Creatinine, max, µmol/L	78 (63 - 102)	73 (60 - 98)**	84 (60 - 127)***	97 (67 - 156)***	146 (93 - 237)***
Blood urea nitrogen, mg/dL	18 (13 - 25)	23 (17 - 31)***	23 (15 - 35)***	28 (18 - 43)***	35 (22 - 54)***
Urinary output, L	1.20 (0.82 - 1.72)	1.60 (1.19 - 2.20)***	1.53 (1.00 - 2.23)***	1.48 (0.92 - 2.23)***	1.35 (0.66 - 2.20)***
Bilirubin, µmol/L	9 (6 - 12)	8 (6 - 12)***	8 (5 - 12)***	10 (6 - 16)***	14 (8 - 27)***
Sodium, max, mmol/L	138 (136 - 141)	139 (137 - 141)***	139 (136 - 142)***	139 (136 - 142)***	139 (136 - 142)***
Potassium (max), mmol/L	4.1 (3.8 - 4.4)	4.3 (4.0 - 4.6)***	4.4 (4.0 - 4.8)***	4.3 (4.0 - 4.8)***	4.3 (4.0 - 4.9)***

Table 1: Continued.

Glucose (max), mmol/L	8.4 (7.1 - 10.9)	11.5 (9.2 - 15.2)***	9.9 (7.9 - 12.5)***	9.5 (7.5 - 12.5)***	9.0 (7.1 - 12.1)***
pH (min)	7.39 (7.32 - 7.45)	7.44 (7.38 - 7.48)***	7.36 (7.29 - 7.43)***	7.38 (7.29 - 7.44)***	7.38 (7.30 - 7.44)***
Bicarbonate (max), mmol/L	26 (24 - 28)	26 (24 - 28)	28 (24 - 32)***	25 (22 - 29)***	22 (19 - 24)***
Albumin (min), g/L	26 (23 - 30)	28 (25 - 32)***	29 (25 - 34)***	26 (22 - 31)	24 (20 - 28)***
White blood cell count (max), x10^9/L	9.1 (6.9 - 12.0)	9.9 (7.2 - 13.2)***	11.0 (7.5 - 15.3)***	13.8 (9.4 - 19.6)***	15.9 (10.2 - 23.4)***
Thrombocytes (min), x10^9/L	228 (172 - 296)	240 (185 - 305)***	198 (143 - 261)***	208 (148 - 285)***	169 (104 - 254)***
Temperature, °C	38.7 (38.0 - 39.4)	37.5 (37.0 - 38.2)***	38.1 (37.4 - 38.8)***	38.0 (37.4 - 38.8)***	38.0 (37.3 - 38.9)***
ICU length-of-stay survivors, days	15 (9 - 29)	8 (4 - 17)***	4 (2 - 8)***	3 (2 - 7)***	2 (1 - 5)***
ICU length-of-stay nonsurvivors, days	11 (5 - 21)	16 (8 - 25)***	6 (2 - 12)***	3 (1 - 8)***	2 (1 - 5)***
ICU mortality	609 (27%)	1965 (23%)***	583 (17%)***	3831 (19%)***	3250 (18%)***
Hospital length-of-stay survivors, days	29 (18 - 45)	19 (12 - 33)***	11 (7 - 20)***	13 (8 - 22)***	13 (7 - 24)***
Hospital length-of-stay nonsurvivors, days	15 (8 - 24)	20 (12 - 30)***	9 (4 - 17)***	8 (3 - 16)***	7 (2 - 17)***
In-hospital mortality	666 (29%)	2181 (25%)**	755 (22%)***	5257 (26%)*	4456 (25%)***
28-day in-hospital mortality	554 (24%)	1588 (18%)***	676 (20%)***	4705 (24%)	3932 (22%)
90-day in-hospital mortality	664 (29%)	2168 (25%)**	753 (22%)***	5228 (26%)*	4407 (25%)***

*Data are presented as median (interquartile range) or number (%). * indicates p=0.01 -0.05, ** indicates p=0.001 - 0.01, *** indicates p=0 - 0.001 compared with the COVID-19 pre-dexamethasone cohort, calculated using Dunn's post-hoc tests (following Kruskall Wallis tests across all cohorts which all yielded p<0.05) or Bonferroni-corrected pairwise Chi-square tests (following Chi-square tests across all cohorts which all yielded p<0.05). [a]Acute physiology score. [b]Calculated by adding one point for each of the following comorbidities present: Aids, metastatic neoplasm, cardiovascular insufficiency, chronic dialysis, chronic renal insufficiency, cirrhosis, hematologic malignancy, immune insufficiency, and COPD or respiratory insufficiency. COVID-19: coronavirus disease 2019, BMI: body mass index, APACHE IV: Acute Physiology and Chronic Health Evaluation IV, COPD: chronic obstructive pulmonary disease, ARDS: acute respiratory distress syndrome, ICU: intensive care unit*

Discussion

We applied previously established clinical sepsis phenotypes (4) to 52,274 critically ill patients with COVID-19 or sepsis of other etiologies. Overall, the characteristics and gradation in mortality associated with the phenotypes were comparable across the cohorts and also similar to those reported in the original work (4), with the α phenotype showing the most favorable outcome and δ associated with the highest mortality. Of interest, following the introduction of dexamethasone treatment, a significantly higher proportion of COVID-19 patients exhibited the δ phenotype, while at the same time, survival of this phenotype was better compared to the pre-dexamethasone cohort. It is likely that the use of dexamethasone (and later on also tocilizumab) importantly contributed to improved outcome of this particular phenotype because it was associated with the highest white blood cell counts in our COVID-19 cohort and with the most elevated IL-6 and TNF levels in sepsis patients (4). Intriguingly, compared to COVID-19 sepsis patients, the differentiation in outcome between phenotypes was less pronounced in patients with non-COVID-19 viral pneumonia sepsis. This study underlines that classification of critically ill COVID-19 patients into phenotypes may aid prognostication, predict treatment efficacy, and thereby facilitates personalized medicine.

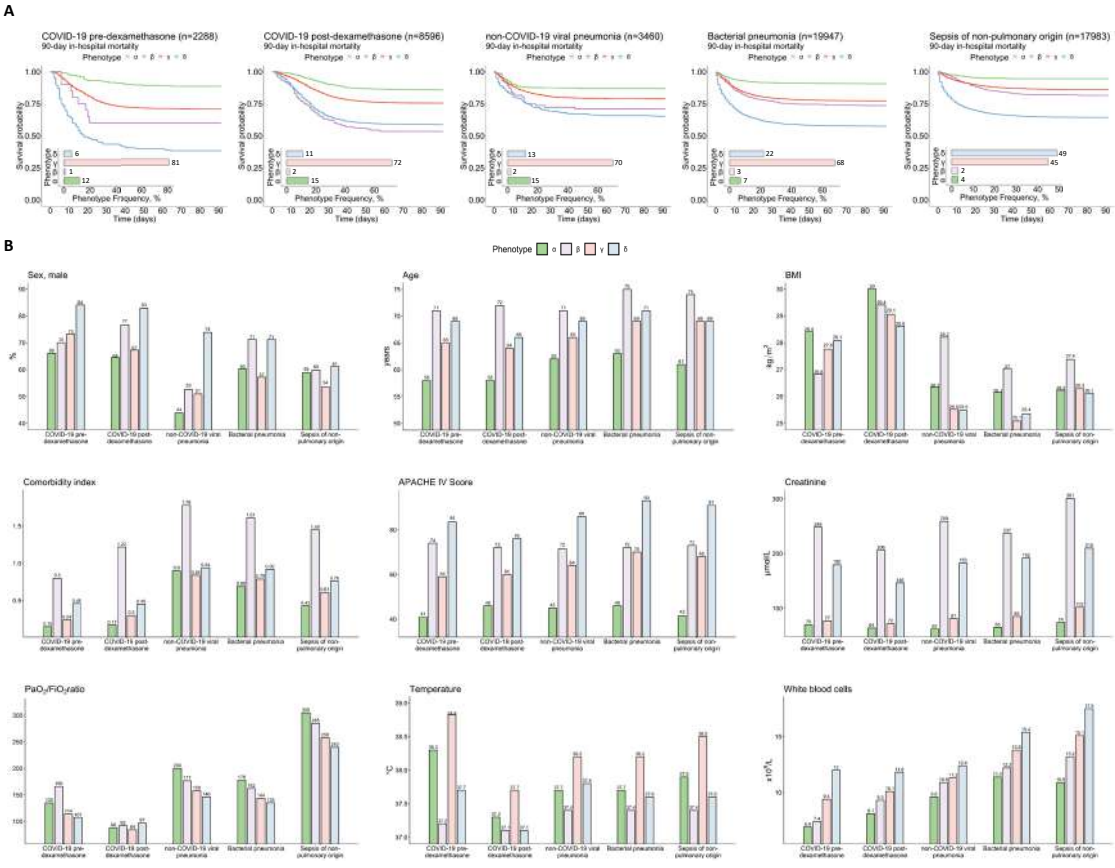

***Figure 1. Distribution, characteristics, and outcome of phenotypes in patients with COVID-19 sepsis and sepsis of other origins. A**: Phenotype distribution and 90-day in-hospital mortality Kaplan Meier curves for patients with COVID-19 in the pre-dexamethasone period, COVID-19 in the post-dexamethasone period, non-COVID-19 viral pneumonia sepsis, bacterial pneumonia sepsis, and bacterial sepsis of non-pulmonary origin. **B**: Sex, body mass index (BMI), comorbidity index, Acute Physiology and Chronic Health Evaluation (APACHE) IV score, creatinine, PaO_2/FiO_2 ratio, temperature, and white blood cell count for each phenotype across all abovementioned cohorts.*

References

1. Galvan-Roman, J. M. et al. IL-6 serum levels predict severity and response to tocilizumab in COVID-19: An observational study. J Allergy Clin Immunol 147, 72-80 e78, doi:10.1016/j.jaci.2020.09.018 (2021).
2. Sinha, P. et al. Latent Class Analysis Reveals COVID-19-related Acute Respiratory Distress Syndrome Subgroups with Differential Responses to Corticosteroids. Am J Respir Crit Care Med 204, 1274-1285, doi:10.1164/rccm.202105-1302OC (2021).
3. Demerle, K., Angus, D. C. & Seymour, C. W. Precision Medicine for COVID-19. JAMA 325, 2041, doi:10.1001/jama.2021.5248 (2021).
4. Seymour, C. W. et al. Derivation, Validation, and Potential Treatment Implications of Novel Clinical Phenotypes for Sepsis. JAMA 321, 2003-2017, doi:10.1001/jama.2019.5791 (2019).
5. Arts, D., de Keizer, N., Scheffer, G. J. & de Jonge, E. Quality of data collected for severity of illness scores in the Dutch National Intensive Care Evaluation (NICE) registry. Intensive Care Med 28, 656-659, doi:10.1007/s00134-002-1272-z (2002).
6. Group, R. C. et al. Dexamethasone in Hospitalized Patients with Covid-19. N Engl J Med 384, 693-704, doi:10.1056/NEJMoa2021436 (2021).

ADDITIONAL METHODS

Data collected in the Nationale Intensive Care Evaluatie (NICE) registry includes demographic characteristics, medical history, disease severity scores, cardiorespiratory parameters, and clinical laboratory values obtained during the first 24 hours after ICU admission as well as clinical outcomes. Data collection is completely standardized using strict definitions and subject to data quality checks1. All collected data were anonymized by NICE and, in accordance with Dutch legislation and compliant with the European General Data Protection Regulation, there is no need to obtain consent when anonymous data are used. Patients were included in the following periods: pre-dexamethasone cohort: January - April 2020; post-dexamethasone cohort: September 2020 - mid-November 2021; non-COVID-19 viral pneumonia sepsis: January 2016 – September 2019; bacterial pneumonia sepsis: January 2016 – September 2019; bacterial sepsis of non-pulmonary origin: January 2016 – September 2019. The period spanning May to August 2020 was used as a 'washout period' between the pre-dexamethasone and post-dexamethasone periods. During this period, very few COVID-19 patients were admitted to the ICU due to low numbers of SARS-CoV-2 infections in this summer period in the Netherlands (see https://www.stichting-nice.nl/). For the COVID-19 cohorts, SARS-CoV-2 infection had to be the primary reason for ICU admission, otherwise the patient was excluded. The 17 variables obtained during the first 24 hours after ICU admission that were used for clustering were:

Variable	Min/Max
Age	
Albumin	Maximum during first 24 hours of ICU admission
Bicarbonate	Minimum during first 24 hours of ICU admission
Bilirubin	
Blood urea nitrogen	
Comorbidity index[a]	
Creatinine	Maximum during first 24 hours of ICU admission
Gender	
Glucose	Maximum during first 24 hours of ICU admission
Heartrate	Maximum during first 24 hours of ICU admission
PaO2	
Respiratory rate	Maximum during first 24 hours of ICU admission
Sodium	Maximum during first 24 hours of ICU admission
Systolic blood pressure	Minimum during first 24 hours of ICU admission
Temperature	Maximum during first 24 hours of ICU admission
Thrombocytes	Minimum during first 24 hours of ICU admission
White blood cell count	

[a]Calculated by adding one point for each of the following comorbidities present: Aids, metastatic neoplasm, cardiovascular insufficiency, chronic dialysis, chronic renal insufficiency, cirrhosis, hematologic malignancy, immune insufficiency, and COPD or respiratory insufficiency.

Variables were filtered for missing values and values that were out of range for each parameter according to the NICE data dictionary. Patients with more than five missing clustering variables were excluded. For the remaining patients, missing data was imputed using multiple imputation with chained equations. After log-transformation, scaling, and centering, clustering was performed using the same approach as previously described for sepsis phenotype validation in external cohorts2. All analyses were performed using R 3.6.1.

Additional references

1. Arts, D., de Keizer, N., Scheffer, G. J. & de Jonge, E. Quality of data collected for severity of illness scores in the Dutch National Intensive Care Evaluation (NICE) registry. Intensive Care Med 28, 656-659, doi:10.1007/s00134-002-1272-z (2002).
2. Seymour, C. W. et al. Derivation, Validation, and Potential Treatment Implications of Novel Clinical Phenotypes for Sepsis. JAMA 321, 2003, doi:10.1001/jama.2019.5791 (2019).

Part III

IMMUNOMODULATORY TREATMENT IN CRITICALLY ILL COVID-19 PATIENTS

CHAPTER 10

Effect of anakinra in COVID-19

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We read with interest the article by Giulio Cavalli and colleagues that described the effects of the interleukin-1 receptor antagonist anakinra in patients who were critically ill with Coronavirus Disease 2019 (COVID-19) (1). The authors reported promising effects of high-dose anakinra, including improvement of respiratory function and better survival compared with a historical control group that received standard treatment. Although these results are encouraging, there are notable differences in patient characteristics between both groups which may have confounded the observed effects.

First, the authors indicate that they have studied a group of patients with COVID-19 and hyperinflammation. This makes sense, as anakinra was shown to be effective in sepsis patients with features of macrophage activation syndrome (MAS) in a post-hoc analysis of a randomized clinical trial performed more than two decades ago (2). MAS is characterized by an excessive inflammatory response, most specifically elevated ferritin concentrations (3). According to a validated diagnostic score of MAS, a ferritin level higher than 2000 ng/mL increases the probability of MAS (4). Notably, in the study by Cavalli and colleagues, median ferritin levels were only 1237 ng/mL in the anakinra group compared with 2218 ng/mL in the control group, indicating a much less pronounced inflammatory state (and thus better prognosis) in the anakinra group.

Second, it is well-established that advanced age is an important risk factor for severe disease course in patients with COVID-19, with a hazard ratio for mortality of 1.7 (95% CI 1.1-2.7) for patients aged 65 years or older (5). In the study by Cavalli and colleagues, the median age of patients treated with anakinra was 62 years, 8 years lower than that of the control group, representing another substantial disadvantage for the control group. The less pronounced inflammatory state together with this age discrepancy represent relevant confounders that might explain the improvement in respiratory function and clinical outcome of this group, irrespective of anakinra treatment.

A commonly used method to prevent disbalance in patient characteristics (and thereby bias) is propensity score matching, which is used to make groups more comparable in non-randomised studies. Therefore, it would be interesting to learn if the effects of anakinra observed in the study by Cavalli and colleagues hold up to scrutiny in a propensity score matched design.

In conclusion, there is clear rationale for using anakinra in the treatment of patients who are critically ill with COVID-19 and hyperinflammation. However, additional cohorts using propensity score matching or, ideally, a randomised controlled trial are needed to confirm its putative benefits.

References

1. Cavalli G, De Luca G, Campochiaro C, Della-Torre E, Ripa M, Canetti D, et al. Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study. Lancet Rheumatol. 2020;2(6):e325-e31.
2. Shakoory B, Carcillo JA, Chatham WW, Amdur RL, Zhao H, Dinarello CA, et al. Interleukin-1 Receptor Blockade Is Associated With Reduced Mortality in Sepsis Patients With Features of Macrophage Activation Syndrome: Reanalysis of a Prior Phase III Trial. Crit Care Med. 2016;44(2):275-81.
3. Kyriazopoulou E, Leventogiannis K, Norrby-Teglund A, Dimopoulos G, Pantazi A, Orfanos SE, et al. Macrophage activation-like syndrome: an immunological entity associated with rapid progression to death in sepsis. BMC Med. 2017;15(1):172.
4. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395(10229):1033-4.
5. Li X, Xu S, Yu M, Wang K, Tao Y, Zhou Y, et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. J Allergy Clin Immunol. 2020.

CHAPTER 11

Anakinra treatment in critically ill COVID-19 patients: a prospective cohort study

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Abstract

Background

A subset of critically ill COVID-19 patients develop a hyperinflammatory state. Anakinra, a recombinant interleukin-1 receptor antagonist, is known to be effective in several hyperinflammatory diseases. We investigated the effects of anakinra on inflammatory parameters and clinical outcomes in critically ill, mechanically ventilated COVID-19 patients with clinical features of hyperinflammation.

Methods

In this prospective cohort study, twenty-one critically ill COVID-19 patients treated with anakinra were compared to a group of standard care. Serial data of clinical inflammatory parameters and concentrations of multiple circulating cytokines were determined and aligned on start day of anakinra in the treatment group, and median start day of anakinra in the control group. Analysis were performed for day -10 to +10 relative to alignment day. Clinical outcomes were analyzed during 28 days. Additionally, three sensitivity analyses were performed: (1) using propensity score-matched groups, (2) selecting patients who did not receive corticosteroids and (3) using a subset of the control group aimed to match the criteria (fever, elevated ferritin) for starting anakinra treatment.

Results

Baseline patient characteristics and clinical parameters on ICU admission were similar between groups. As a consequence of bias by indication, plasma levels of aspartate aminotransferase (ASAT) (p=0.0002), ferritin (p=0.009), and temperature (p=0.001) were significantly higher in the anakinra group on alignment day. Following treatment, no relevant differences in kinetics of circulating cytokines were observed between both groups. Decreases of clinical parameters, including temperature (p=0.03), white blood cell counts (p=0.02), and plasma levels of ferritin (p=0.003), procalcitonin (p=0.001), creatinine (p=0.01), and bilirubin (p=0.007) were more pronounced in the anakinra group. No differences in duration of mechanical ventilation or ICU length of stay were observed between groups. Sensitivity analyses confirmed these results.

Conclusions

Anakinra is effective in reducing clinical signs of hyperinflammation in critically ill COVID-19 patients. A randomized controlled trial is warranted to draw conclusion about the effects of anakinra on clinical outcomes.

Background

In December 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified in Wuhan, China, which led to a pandemic in 2020. The clinical spectrum of SARS-CoV-2 infection ranges from asymptomatic cases to severe viral sepsis leading to organ dysfunction and high mortality. In the absence of a vaccine, effective treatments for corona virus disease 2019 (COVID-19) are highly warranted.

Inflammation plays a key role in the pathophysiology of COVID-19. Initial reports have shown that patients exhibit strongly elevated concentrations of several circulating pro-inflammatory cytokines and chemokines (1, 2). Additionally, critically ill COVID-19 patients display higher plasma concentrations of pro-inflammatory cytokines than mild cases (3), suggestive of a relationship between inflammation and disease severity. A subset of severe COVID-19 patients may even develop severe hyperinflammation, showing similarities with macrophage activation syndrome (MAS), which is characterized by features such as hyperferritinemia, fever, pancytopenia, hepatobiliary dysfunction (HBD), and diffuse intravascular coagulation (DIC) (4-7). The pro-inflammatory cytokine interleukin (IL)-1α plays a critical role in MAS through enhancing production of cytokines, including IL-1α itself, activation of endothelium with fluid extravasation, hypotension, and even death (4). This positive feedback loop creates a vicious circle of inflammation and tissue pathology (4).

Anakinra, a recombinant IL-1 receptor antagonist (IL-1RA) which is currently used in the treatment of patients with rheumatoid arthritis, cryopyrin associated periodic syndrome (CAPS), and Still's disease, has been shown to be effective for the treatment of a subgroup of severe bacterial sepsis patients that demonstrate features of MAS (8). Recently, small studies suggest that anakinra may also be effective in treating COVID-19 patients with features of a MAS-like syndrome, including persistent high fever and elevated plasma levels of ferritin. COVID-19 patients that received treatment with anakinra showed improvement of respiratory function and a decreased mortality risk compared to patients that did not receive anakinra (9-13). Although these results are promising, limitations related to the retrospective comparison with historical control data are clear. Also, the number of treated patients remains small and mainly focussed on non-critically ill patients. Finally, data of the putative immunomodulatory effects of anakinra are limited.

In the present study, our primary objective was to investigate the effects of anakinra on the inflammatory response in critically ill COVID-19 patients with features of hyperinflammation, compared to a contemporary control group receiving standard care. The secondary objective was to explore differences in clinical outcomes between these groups. In addition, we performed three sensitivity analyses: (1) using propensity score-matched groups, (2) selecting patients who did not receive corticosteroids and (3) using a subset of the control group aimed to match the criteria (fever, elevated ferritin) for starting anakinra treatment.

Methods

Study design and participants

In this prospective cohort study, consecutive mechanically ventilated COVID-19 patients admitted to the Intensive Care Unit (ICU) in the Radboud university medical center (Nijmegen, The Netherlands) and Bernhoven Hospital (Uden, The Netherlands) between March 11th and April 27th were screened for inclusion. COVID-19 was diagnosed by a positive SARS-CoV-2 RT-PCR test in nasopharyngeal and throat swabs and by typical chest CT-scan findings. Patients with a pre-existing immunosuppressed status or other comorbidities that could strongly influence prognosis were excluded. Indication for starting treatment with anakinra was based on clinical judgement of features of hyperinflammation (including persistent high fever and/or a high plasma level of ferritin and/or progressive organ dysfunction with no apparent reason apart from hyperinflammation). Patients who were not treated with anakinra were designated to the control group. Treatment was started with 300 mg anakinra intravenously (i.v.), followed by 100 mg i.v. every six hours. Blood sampling was carried out as part of a cohort study in critically ill COVID-19 patients, which was carried out in accordance with the applicable rules concerning the review of research ethics committees and informed consent in the Netherlands. All patients or legal representatives were informed about the details of this cohort study and could decline to participate.

All included patients who received anakinra (n=21) were compared to included patients who received standard care (control group, n=39). We also performed three sensitivity analyses: one in which we compared all anakinra-treated patients to a propensity score-matched control group receiving standard care (matched on baseline demographic characteristics), another in which only patients who did not receive corticosteroids were included, and a third in which the anakinra group was compared to a subgroup of the control group aimed to match criteria used to start treatment with anakinra (either fever >38.5°C for at least two days or high ferritin plasma levels [>1800 µg/L]). Details are provided in Additional File 1.

Data collection

Details on collection of clinical data are provided in Additional File 1. In the anakinra group, serial data were aligned on the day anakinra treatment was started, which was designated day 0. Data of the control group were aligned on the median day anakinra was started in the anakinra group (day 12 after ICU admission).

Inflammatory parameters

The analysis methods for circulating cytokines and inflammatory proteomics are provided in Additional File 1. Body temperature, as well as circulating white blood cell counts and blood levels of ferritin, C-reactive protein (CRP) and procalcitonin were recorded between

ten days before and after alignment day (day -10 until day 10). In case patients were actively cooled when they had a fever of >40.0°C, body temperature was imputed as 40.0°C. In addition, serial values of creatinine, bilirubin, platelets, norepinephrine infusion rates, and PaO_2/FiO_2 (P/F) ratio were collected to calculate the sequential organ failure assessment (SOFA) score. As all patients were sedated, the Glasgow Coma Score was omitted from the SOFA score. Because a relevant proportion of patients were transferred to the regular ward at day 7 after alignment day, PaO_2/FiO_2 ratio and SOFA-score data were not recorded. To prevent case-mix-related effects, data of these parameters are shown until day 6 after alignment day. Use of corticosteroids, remdesivir, and chloroquine was recorded between ten days before and after alignment day.

Clinical outcome data

Clinical outcomes (development of secondary infections, time on mechanical ventilation, ICU length of stay (LOS), and mortality) were recorded until 28 days after alignment day. Secondary infection was defined as 'any infectious episode' evidenced by the presence of positive culture(s) and time-stamped at the day the culture was performed. Infectious episodes were independently determined by two ICU physicians. In case of incongruency, a third ICU physician was consulted.

Statistical analysis

Details are provided in Additional File 1. Briefly, data are displayed as median with interquartile range (IQR) or geometric mean with 95% confidence interval (CI). Between-group differences were assessed using Fisher's exact tests, Mann-Whitney-U tests, linear mixed effects model analysis on log-transformed data, log-rank tests, and t-tests (indicated in legends).

Results

Patient characteristics

Seventy-eight consecutive critically ill patients with proven COVID-19 were screened. Nine patients were excluded because of a pre-existing immunocompromising condition. One patient refused participation. On alignment day, eight control patients were no longer in the ICU and were therefore excluded. Of the remaining 60 patients, 21 received anakinra (Supplementary Fig. 1, Additional File 2). Median age was 63 [55-71] and 67 [59-72] years in the anakinra and control groups, respectively (p=0.42). No significant differences in other patient characteristics, medical history, and laboratory and clinical parameters were present on ICU admission (Table 1). All patients required mechanical ventilation and sedation.

On the alignment day, as a result of indication bias, patients in the anakinra group exhibited higher levels of ASAT (96 [67-142] vs. 64 [46-97] U/L, p=0.009), and ferritin (2365 [1272-3713] vs. 1410 [713-1680] µg/L, p=0.001, respectively) and a higher temperature (39.1 [38.3-40.0] °C) compared to the control group (37.8 [37.0-38.3] °C, p=0.0002). No other significant differences between groups were present on alignment day (Table 1). APACHE II scores for the anakinra and control group on the day of alignment were 12 [9-13] and 10 [7-14], respectively (p=0.41).

Table 1. Patient characteristics and clinical parameters at Intensive Care Unit admission and on alignment day.

	Anakinra (n=21)	Control (n=39)	p-value
Sex, male	14 (67)	33 (85)	0.19
Age, years	63 [55-71]	67 [59-72]	0.42
Body Mass Index, kg/m^2	27.7 [25.9-29.9]	26.8 [24.3-31.1]	0.50
APACHE II	15 [13-18]	16 [12-20]	0.46
Time from first COVID symptoms until ICU admission, days	13 [9-14]	10 [7-15]	0.33
Medical history			
Cardiovascular insufficiency	4 (19)	10 (26)	0.75
Hypertension	8 (38)	22 (56)	0.28
Respiratory insufficiency	1 (5)	2 (5)	1.00
Renal insufficiency	0 (0)	1 (3)	1.00
Metastatic neoplasm	2 (10)	2 (5)	0.61
Immunological insufficiency	0 (0)	1 (3)	1.00
Chronic obstructive pulmonary disease	0 (0)	5 (13)	0.15
Diabetes mellitus	7 (33)	7 (18)	0.21
Hematologic malignancy	1 (5)	0 (0)	0.35
Clinical parameters on admission day			
D-dimer, ng/mL	3380 [2028-18343]	2950 [1605-4610]	0.28
Creatinine, µmol/L	84 [68-96]	82 [65-110]	0.70
Alanine transaminase, U/L	47 [21-62]	39 [30-56]	0.95
Aspartate transaminase, U/L	55 [35-76]	51 [39-63]	0.96
Bilirubin, µmol/L	8 [6-13]	7 [5-14]	0.53
Lactate dehydrogenase, U/L	380 [322-493]	406 [324-507]	0.92
White blood cells, x10^9/L	8.2 [7.0-12.0]	9.8 [6.0-11.4]	0.95
Thrombocytes, x10^9/L	247 [189-324]	244 [181-325]	0.61
C-reactive protein, mg/L	254 [188-297]	198 [133-292]	0.22
Procalcitonin, µg/L	0.66 [0.18-1.39]	0.86 [0.32-2.88]	0.44
Ferritin, µg/L	1842 [1313-2767]	1439 [815-2396]	0.19
Temperature, °Celsius	38.4 [37.8-38.9]	38.6 [37.5-39.3]	0.56
PaO_2/FiO_2 ratio, mmHg	138 [105-199]	139 [101-178]	0.94
SOFA score	7 [4-7]	6 [5-8]	0.27

Clinical parameters on alignment day			
D-dimer, ng/mL	4063 [2585-6285]	3918 [2349-5943]	0.70
Creatinine, µmol/L	92 [76-106]	79 [58-148]	0.55
Alanine transaminase, U/L	89 [55-119]	67 [49-114]	0.24
Aspartate transaminase, U/L	96 [67-142]	64 [46-97]	0.009
Bilirubin, µmol/L	6 [5-12]	5 [4-9]	0.18
Lactate dehydrogenase, U/L	371 [317-450]	348 [256-406]	0.11
White blood cells, x10⁹/L	12.4 [10.4-15.9]	13.5 [11.4-16.6]	0.49
Thrombocytes, x10⁹/L	351 [314-494]	366 [263-453]	0.34
C-reactive protein, mg/L	130 [90-237]	92 [59-170]	0.13
Procalcitonin, µg/L	0.66 [0.39-1.88]	0.48 [0.23-0.78]	0.07
Ferritin, µg/L	2365 [1272-3713]	1410 [713-1680]	0.001
Temperature, °Celsius	39.1 [38.3-40.0]	37.8 [37.0-38.3]	0.0002
PaO_2/FiO_2, mmHg	188 [133-268]	157 [128-212]	0.18
SOFA score	6 [4-8]	5 [4-7]	0.75
APACHE II	12 [9-13]	10 [7-14]	0.41
Time from first COVID symptoms until alignment day, days	22 [19-27]	21 [18-26]	0.62

Data are presented as n (%) or median [IQR]. P-values were calculated using Fisher's exact tests and Mann-Whiney-U tests.

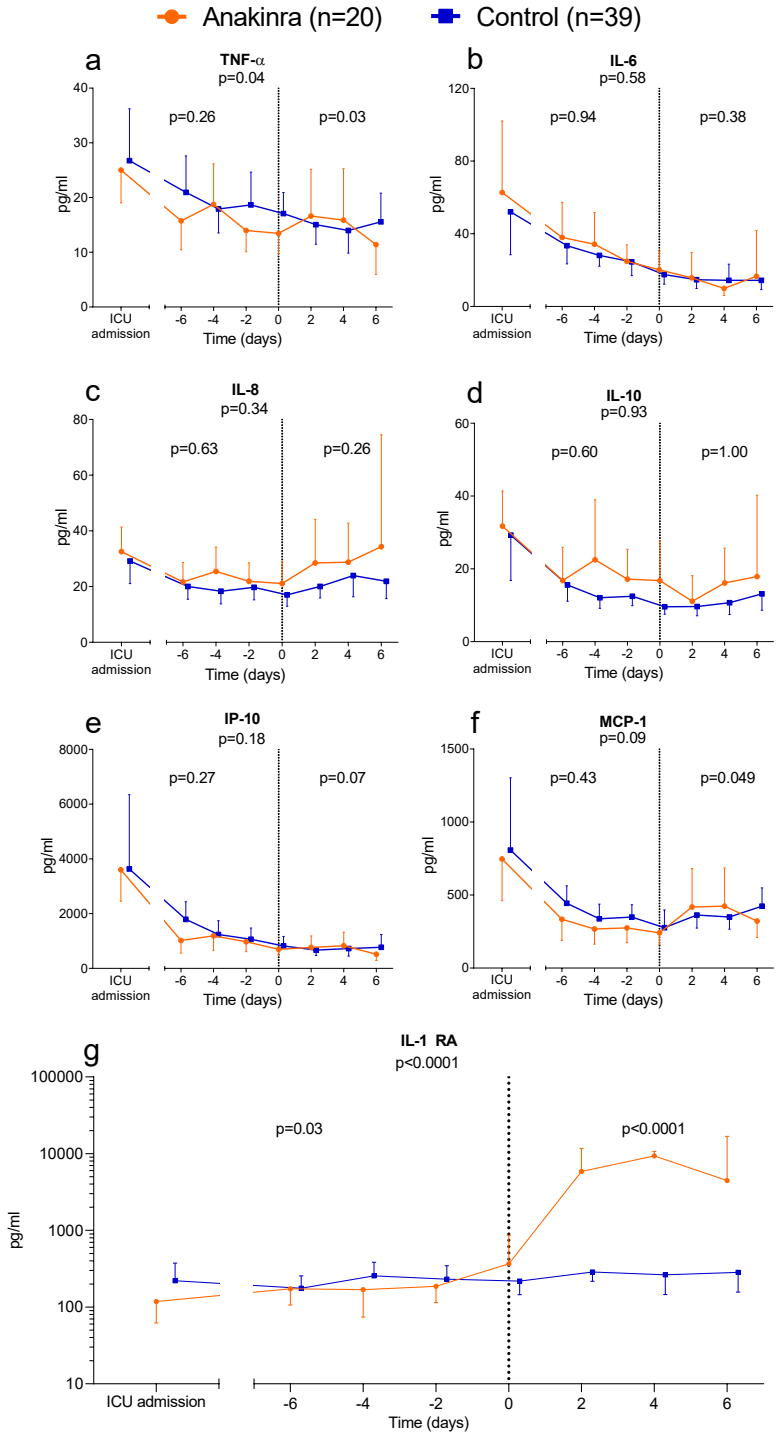
Inflammatory response

In both groups, concentrations of all circulating cytokines decreased from ICU admission until alignment day (Fig. 1). Expectedly, administration of anakinra significantly increased circulating IL-1RA concentrations following treatment ($p<0.0001$, Fig. 1g). After alignment day, tumor necrosis factor (TNF)-α and monocyte chemoattractant protein (MCP)-1 showed a significant difference between the anakinra and control groups ($p=0.03$ and $p=0.049$, respectively, Fig. 1a, f). However, a clear direction of these significant differences was absent. The kinetics of the other measured cytokines did not reveal significant differences between the anakinra group and control group (Fig. 1). Subgroups used for inflammatory proteomic analysis revealed no relevant differences in patient characteristics and clinical parameters on admission day (Supplementary Table 1, Additional File 3). Of the 75 inflammatory mediators measured, 17 pro-inflammatory proteins decreased in the anakinra group post-treatment, whereas no changes were observed in the control group (Fig. 2a). Because of the large number of measured proteins and correction for multiple testing, statistical significance was not reached.

During the ten-day period before start of anakinra treatment, body temperature significantly diverted between the two groups, with higher temperatures in the anakinra group ($p=0.02$, Fig. 2b). After anakinra treatment, temperature curves converged ($p=0.03$, Fig. 2b). Similar

effects of anakinra were observed for ferritin and procalcitonin plasma levels (Fig. 2c-d). White blood cell count decreased in the anakinra group after treatment, whereas a further increase was observed in the control group (p=0.02, Fig. 2e). No significant differences in the kinetics of CRP plasma levels were present between both groups, although the decrease in CRP post-treatment appeared to be more pronounced in the anakinra group (Fig. 2f).
The decrease in plasma levels of creatinine and bilirubin was significantly more pronounced in the anakinra group compared to the control group after start of treatment (p=0.01 and p=0.007, respectively, Fig. 3a-b). Norepinephrine infusion rate was significant higher in the anakinra group before treatment (p=0.005, Fig. 3e) whereas no significant differences were present afterwards (p=0.61, Fig. 3e). No significant differences in thrombocyte counts, PaO_2/FiO_2 ratio, or total SOFA score were observed (Fig. 3).
The use of corticosteroids tended to be lower in the anakinra group (5%) compared to the control group (26%) before alignment day (p=0.08, Supplementary Fig. 2a, Additional File 4). This difference became smaller after start of treatment (anakinra group 14% and control group 28%, p=0.14, Supplementary Fig. 2a, Additional File 4). No differences between both groups were present pertaining to the use of remdesivir or chloroquine (Supplementary Fig. 2b-c, Additional File 4).

Figure 1. Circulating cytokine concentrations.
*Concentrations of circulating **(a)** tumor necrosis factor (TNF)-α, **(b)** interleukin (IL)-6, **(c)** IL-8, **(d)** IL-10, **(e)** interferon gamma-induced protein (IP)-10, **(f)** monocyte chemoattractant protein (MCP)-1, and **(g)** IL-1 receptor antagonist (IL-1RA) on day of intensive care unit (ICU) admission and serial data within six days pre- and post-alignment day (day 0). Data are presented as geometric mean with 95% confidence intervals and were analyzed using mixed-models analysis (time*group interaction factor) to evaluate differences between groups over time. P-values under graph titles reflect overall between-group differences (day -6 until day 6). Between-group p-values for day -6 until day 0 and day 0 until day 6 are shown on the left and right of each panel, respectively.*


Anakinra (n=20)
Control (n=39)
a
TNF-α
p=0.04
p=0.26
p=0.03
pg/ml
ICU admission
Time (days)
b
IL-6
p=0.58
p=0.94
p=0.38
c
IL-8
p=0.34
p=0.63
p=0.26
d
IL-10
p=0.93
p=0.60
p=1.00
e
IP-10
p=0.18
p=0.27
p=0.07
f
MCP-1
p=0.09
p=0.43
p=0.049
g
IL-1 RA
p<0.0001
p=0.03
p<0.0001

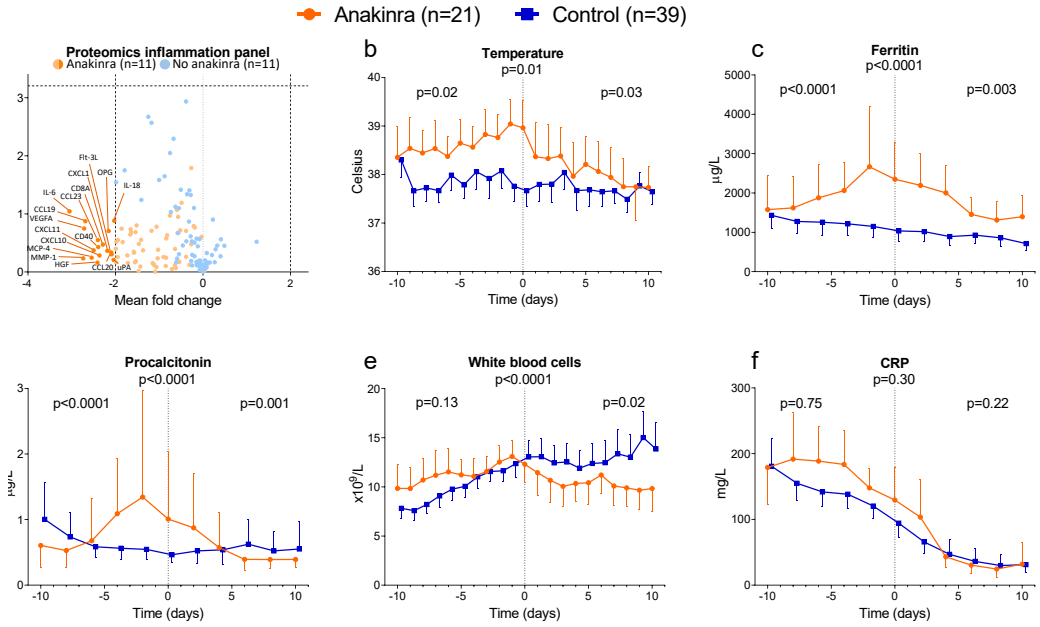


Figure 2. Proteomics inflammation panel and kinetics of inflammatory parameters. (a) *Volcano plot of proteomics inflammation panel for 75 proteins in both groups. Mean fold change and -10log(p-value) were shown on the x-axis and y-axis, respectively. P-values were calculated using t-tests. A mean fold change of ≤ -2 or ≥ 2 was considered relevant. A -10log(p-value) > 3.204 was considered significant (p<0.000625).* ***(b)*** *Body temperature and plasma levels of* ***(c)*** *ferritin,* ***(d)*** *procalcitonin,* ***(e)*** *white blood cell counts, and* ***(f)*** *C-reactive protein (CRP) over time within 10 days pre- and post- start anakinra alignment day (day 0). Data are presented as geometric mean with 95% confidence intervals and were analyzed using mixed-models analysis (time*group interaction factor) to evaluate differences between groups over time. P-values under graph titles reflect overall between-group differences (day -10 until day 10). Between-group p-values for day -10 until day 0 and day 0 until day 10 are shown on the left and right of each panel, respectively.*

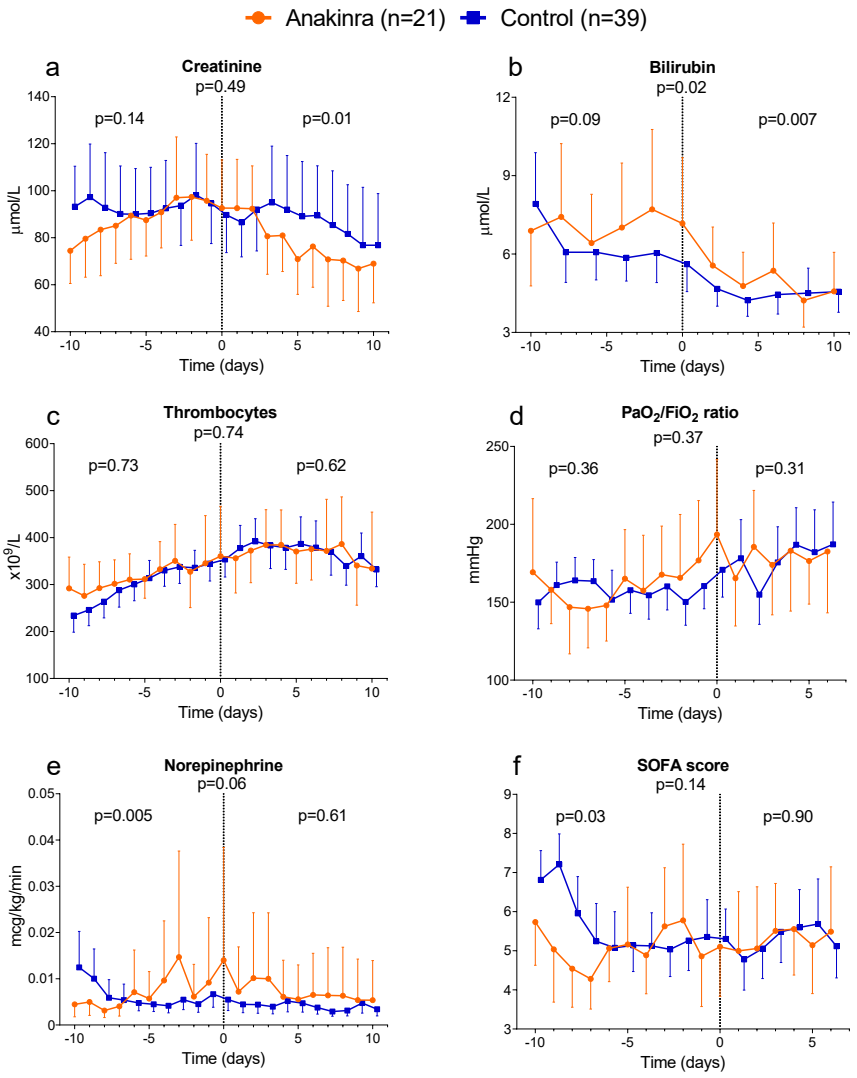


Figure 3. Individual parameters of sequential organ failure assessment (SOFA) score and total SOFA score.

*Plasma levels of **(a)** creatinine, **(b)** bilirubin, and **(c)** thrombocytes and **(d)** PaO2/FiO2 (P/F) ratio, **(e)** infusion rate of norepinephrine, and **(f)** SOFA score over time within 10 days pre- and post-alignment day (day 0). PaO2/FiO2 ratio and SOFA score were presented until day 6. Data are presented as geometric mean with 95% confidence intervals and were analyzed using mixed-models analysis (time*group interaction factor) to evaluate differences between groups over time. P-values under graph titles reflect overall between-group differences (day -10 until day 6 or 10). Between-group p-values for day -10 until day 0 and day 0 until day 6 or 10 are shown on the left and right of each panel, respectively.*

Clinical outcomes

A total of seven patients (33%) of the anakinra group developed a secondary infection during the first 28 days after alignment day versus nine patients (23%) of the control group (p=0.54). Time on mechanical ventilation was 23 [10-29] days in the anakinra group and 17 [7-29] days in the control group (p=0.79). ICU length of stay was 24 [10-29] days in the anakinra group and 17 [6-29] days in the control group (p=0.59). In the anakinra group, 28-day mortality was 19% (n=4) vs. 18% (n=7) in the control group (p=0.87). Kaplan-Meier curves are shown in Fig. 4.

Sensitivity analyses

The propensity score-matched analysis, the analysis restricted to patients who did not receive corticosteroids, and the analysis using a subgroup of control patients aimed to match criteria to start anakinra all yielded comparable results as the primary analysis, as detailed in the additional results, Supplementary Tables 2-4, and Supplementary Figs. 3-15 (Additional Files 5-21).

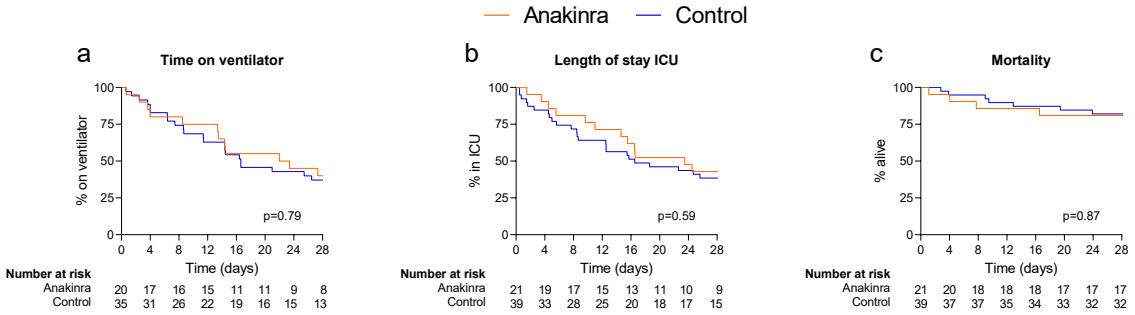


Figure 4. Clinical outcomes. *Kaplan-Meier graphs of* ***(a)*** *time on mechanical ventilator,* ***(b)*** *length of stay in the Intensive Care Unit (ICU), and* ***(c)*** *mortality. Data are presented for the first 28 days after anakinra alignment day. Patients who were no longer mechanically ventilated on alignment day were not included in time on ventilator graph. P-values were calculated using log-rank tests. Numbers at risk on each timepoint per group are shown below graphs.*

Discussion

In the present study, the effects of anakinra in critically ill COVID-19 patients with clinical features of MAS/hyperinflammation were compared to critically ill COVID-19 patients that did not display these features. Upon start of treatment, body temperature and plasma levels of ferritin and ASAT were significantly higher in the anakinra group, which supports the indications to initiate this therapy. Anakinra treatment resulted in attenuation of the clinical inflammatory response compared with the control group, including a decrease in body temperature, ferritin, white blood cell counts, and procalcitonin plasma levels. Furthermore, a more pronounced improvement in kidney and liver function was observed in the anakinra group. No significant difference was found for the overall SOFA-score, likely due to limited statistical power. These findings on clinical parameters of inflammation support anti-inflammatory effects of anakinra in critically ill COVID-19 patients with features of hyperinflammation. No differences in clinical outcome parameters were observed.

Apart from IL1-RA, no relevant between-group differences in circulating IL-6 or other cytokines over time were observed. It is likely that the significant increase in plasma IL-1RA levels after start of anakinra treatment is due to detection of the drug compound itself, as anakinra is recombinant IL-1RA and was administered intravenously. Although anakinra blocks IL-1β signalling, this cytokine is of little theragnostic use, because of its short half-life which renders it undetectable in the circulation in most cases (14). Based on our current findings, circulating concentrations of other cytokines appear to be a poor reflection of the immunomodulating effects of anakinra as well, and are therefore not likely to be useful as biomarkers to start treatment and/or monitor the effects of anakinra in critically ill COVID-19 patients.

Our study did not show an obvious clinical benefit of the anti-inflammatory effects of anakinra treatment. However, it is of paramount importance to realize that this was not a randomized controlled trial, and that patients in the anakinra and control group may not have had the same prognosis at the moment of alignment. Patients that were treated with anakinra clearly exhibited signs of hyperinflammation, while this was not the case for patients in the control group. It is well known that hyperinflammation/MAS in sepsis patients is associated with impaired clinical prognosis (15). In accordance, in the post-hoc analysis of the anakinra trial in bacterial sepsis patients, presence of MAS was associated with a mortality of 65% (8). Treatment with anakinra in this subgroup of MAS patients was associated with a significantly reduced mortality of 35%, which was similar to the 30% mortality observed in sepsis patients without MAS in the same trial. In line with these results, clinical outcomes of

COVID-19 patients treated with anakinra in the present study were comparable with those of the control group, who did not display hyperinflammation and may have had a better a priori prognosis. Nevertheless, our study design does not allow for conclusions of possible clinical benefits of anakinra treatment.

While the use of corticosteroids between the two arms of the study did not reach statistical significance, it was 5-fold more prevalent in the group without anakinra before the start of the intervention, and remained twice as high after start of anakinra therapy. Because corticosteroids can affect inflammatory parameters and were recently reported to decrease mortality in COVID-19 (16, 17), we performed a sensitivity analysis in patients who did not receive corticosteroids during the study period. This analysis yielded similar results to our primary analysis, ruling out corticosteroids as an important confounder.

The absence of significant respiratory improvement and/or reduced mortality in the anakinra group contrasts previous findings (9-12). This might be due to the fact that we included critically ill mechanically ventilated COVID-19 patients, whereas previous studies were performed in less severe cases with either no ventilatory support or non-invasive ventilation (9-12). Possibly, earlier or preventive treatment with anakinra may be more effective and/or disease severity is of relevance for the therapeutic efficacy of anakinra. Also, circulating concentrations of all measured cytokines in our study were markedly lower on alignment day than on ICU admission in both groups. It might be speculated that anakinra is more effective in an earlier, more pro-inflammatory stage of COVID-19 pre-ICU admission, during which higher concentrations of cytokines are present. Furthermore, the previously reported clinical improvements in COVID-19 patients treated with anakinra may be due to notable differences in patient characteristics between treatment groups in earlier studies. For example, anakinra was started in case of mild hyperinflammation (e.g. ferritin ≥900 ng/ml or CRP ≥100mg/L, or both), whereas the control group had substantially higher levels of both ferritin and CRP compared to the anakinra group (9) or were compared to historical data of bacterial sepsis patients with MAS in absence of a COVID-19 control group (10). These issues might have confounded the clinical outcome results demonstrated in these studies. So, in the absence of a prospective controlled trial randomizing patients that fulfil a hyperinflammatory profile, we feel it is too early to judge any possible clinical effects and to decide what would be the optimal timing to start treatment with anakinra in critically ill COVID-19 patients.

This study has several limitations. First, as inclusion criteria were applied to start treatment with anakinra and these criteria were not present in the control group, bias by indication was

clearly present. This difference is likely of importance for the prognosis of the patients, so as a consequence, no direct link can be deducted between the use of anakinra and the clinical results. Nevertheless, we did use a contemporary control group, which was not the case in previous studies (9-12). Also, we performed propensity score matching based on patient characteristics which yielded similar results. Additionally, aimed to address this possible bias by indication, an additional sensitivity analysis using a subgroup of control patients who partially met the criteria to start anakinra treatment was performed. Although this matching was not perfect (as presence of fever was shorter, ferritin was somewhat less elevated and patients had no signs of progressing organ failure), this group was better matched to the anakinra group than the control group used for the main analyses. This additional sensitivity analysis also showed a more pronounced decrease of clinical inflammatory markers in the anakinra group compared to the control group. Of relevance, and confirming the main analyses, no trends towards differences in clinical outcome parameters were found. Second, limited statistical power because of the relatively small number of patients preclude conclusions related to the presence or absence of efficacy of anakinra on clinical outcome parameters. Nevertheless, the inflammation-related clinical endpoints show a clear signal in accordance with the mechanism of action of anakinra and kidney and liver function appear to improve with anakinra therapy.

Conclusions

Anakinra reduces clinical inflammatory parameters in severe COVID-19 patients with features of hyperinflammation. Results of this study, including three sensitivity analyses, do not indicate efficacy of anakinra on clinical outcome parameters. A larger multi-centre randomized controlled trial is warranted to demonstrate the presence/absence of clinical efficacy of anakinra in severely ill COVID-19 patients.

References

1. 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.
2. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med. 2020.
3. Henry BM, de Oliveira MHS, Benoit S, Plebani M, Lippi G. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. Clin Chem Lab Med. 2020;58(7):1021-8.
4. Schulert GS, Grom AA. Pathogenesis of macrophage activation syndrome and potential for cytokine- directed therapies. Annu Rev Med. 2015;66:145-59.
5. Zhang C, Wu Z, Li JW, Zhao H, Wang GQ. Cytokine release syndrome in severe COVID-19: interleukin-6 receptor antagonist tocilizumab may be the key to reduce mortality. Int J Antimicrob Agents. 2020;55(5):105954.
6. Giamarellos-Bourboulis EJ, Netea MG, Rovina N, Akinosoglou K, Antoniadou A, Antonakos N, et al. Complex Immune Dysregulation in COVID-19 Patients with Severe Respiratory Failure. Cell Host Microbe. 2020;27(6):992-1000 e3.
7. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395(10229):1033-4.
8. Shakoory B, Carcillo JA, Chatham WW, Amdur RL, Zhao H, Dinarello CA, et al. Interleukin-1 Receptor Blockade Is Associated With Reduced Mortality in Sepsis Patients With Features of Macrophage Activation Syndrome: Reanalysis of a Prior Phase III Trial. Crit Care Med. 2016;44(2):275-81.
9. Cavalli G, De Luca G, Campochiaro C, Della-Torre E, Ripa M, Canetti D, et al. Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study. Lancet Rheumatol. 2020;2(6):e325-e31.
10. Dimopoulos G, de Mast Q, Markou N, Theodorakopoulou M, Komnos A, Mouktaroudi M, et al. Favorable anakinra responses in severe COVID-19 patients with secondary hemophagocytic lymphohistocytosis. Cell Host Microbe. 2020.
11. Huet T, Beaussier H, Voisin O, Jouveshomme S, Dauriat G, Lazareth I, et al. Anakinra for severe forms of COVID-19: a cohort study. The Lancet Rheumatology. 2020.
12. Navarro-Millán I, Sattui SE, Lakhanpal A, Zisa D, Siegel CH, Crow MK. Use of Anakinra to Prevent Mechanical Ventilation in Severe COVID-19: A Case Series. Arthritis Rheumatol. 2020.
13. Aouba A, Baldolli A, Geffray L, Verdon R, Bergot E, Martin-Silva N, et al. Targeting the inflammatory cascade with anakinra in moderate to severe COVID-19 pneumonia: case series. Ann Rheum Dis. 2020.
14. Lopez-Castejon G, Brough D. Understanding the mechanism of IL-1β secretion. Cytokine Growth Factor Rev. 2011;22(4):189-95.

15. Kyriazopoulou E, Leventogiannis K, Norrby-Teglund A, Dimopoulos G, Pantazi A, Orfanos SE, et al. Macrophage activation-like syndrome: an immunological entity associated with rapid progression to death in sepsis. BMC Med. 2017;15(1):172.
16. Ramiro S, Mostard RLM, Magro-Checa C, van Dongen CMP, Dormans T, Buijs J, et al. Historically controlled comparison of glucocorticoids with or without tocilizumab versus supportive care only in patients with COVID-19-associated cytokine storm syndrome: results of the CHIC study. Annals of the Rheumatic Diseases. 2020:annrheumdis-2020-218479.
17. Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, et al. Dexamethasone in Hospitalized Patients with Covid-19 - Preliminary Report. N Engl J Med. 2020.

Additional files

Additional file 1

ADDITIONAL METHODS

Sensitivity analyses
To correct for possible baseline differences in demographic characteristics, we also compared all anakinra-treated patients to a propensity score-matched control group receiving standard care (n=21). Propensity score matching was performed using the MatchIt package in R-studio v3.6.2 (http://www.r-project.org). The nearest neighbor method was employed using the following variables: age, sex, APACHE II score, body mass index (BMI) and medical history. Data of a patient of the control group was aligned on the day anakinra was started in his/her respective matched patient from the anakinra group.
Several patients included in this study were treated with corticosteroids for (suspected) pulmonary fibrosis, with a start dose of 25-100 mg i.v. twice daily. Because corticosteroids can modulate inflammatory parameters and possibly clinical outcome, a subgroup analysis was performed in which only patients who did not receive corticosteroids were included.
Aimed to address possible bias by indication, a third sensitivity analysis was performed using a subgroup of the control group including patients who partially met the criteria to start treatment with anakinra (either fever >38.5°C for at least two days or high ferritin plasma levels [>1800 μg/L]). Data of the control group was aligned on the first day of the fever episode or high ferritin plasma levels (median: day 6 of ICU stay). Because alignment day of the control group was earlier during stay in ICU, data of this sensitivity analysis are shown from day -4 onwards.

Data collection
Clinical data were collected from the electronic patient files (EPIC, EPIC Systems Corporation, Verona, Wisconsin, USA or NEXUS/PDMS, Nieuwegein, the Netherlands) and recorded in the good clinical practice (GCP)-compliant data management system Castor (Castor EDC, Amsterdam, the Netherlands).

Plasma cytokines
A baseline blood sample was obtained within the first 48 hours following ICU admission and serial samples were collected every other day within six days pre- and post-alignment day. Ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood was centrifuged (2000g, 10 min, 4 °C), after which plasma was stored at -80 °C until analysis. Concentrations of tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-8, IL-10, interferon gamma-induced protein

(IP)-10, monocyte chemoattractant protein (MCP)-1, and IL-1 receptor antagonist (IL-1RA) were determined in one batch using a Luminex assay (Milliplex, Millipore, Billerica, USA). The lower detection limit was 3.2 pg/mL for all cytokines.

Inflammatory proteomics

A total of 92 circulating inflammatory proteins were determined in 11 randomly sampled anakinra patients and 11 random patients of the control groups within three days before and after the alignment day using a multiplex proximity extension assay (PEA, inflammation panel, Olink Proteomics AB, Uppsala, Sweden). Proteins are expressed on a log2-scale as normalized protein expression (NPX) values, which were normalized using bridging samples to correct for batch variation. A quality control was performed per sample by Olink Proteomics. A sample that deviated < 0.3 NPX from the median passed the quality control. Proteins which did not pass the quality control or were detected in less than 80% of the samples were excluded from analysis. This led to exclusion of 17 proteins, resulting in analysis of 75 proteins in both groups.

Statistical analysis

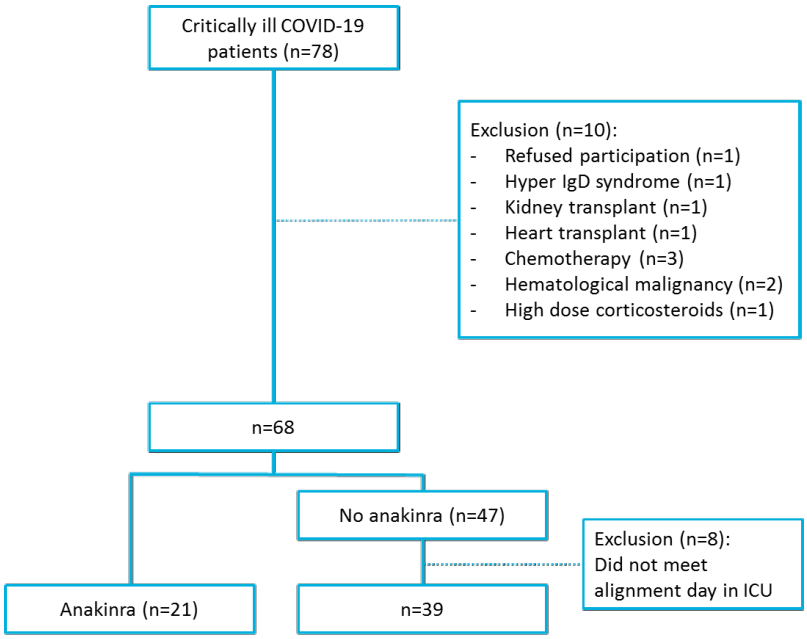
Variables that were not measured daily were binned into bins spanning two or three days, depending on the frequency of measurements, using a custom script made in R-studio v3.6.2 (https://www.r-project.org).https://www.r-project.org). If more than one value was present in a two- or three-day period, the mean value was used. Because of the relatively small group size, normality was not assumed. Baseline characteristics and differences in clinical parameters on ICU admission and alignment day were analyzed using Fisher's exact tests and Mann-Whitney-U tests. Between-group differences over time of clinical measurements and concentrations of circulating cytokines were analyzed using linear mixed effects model analysis on log-transformed data. Three mixed model analysis were performed for each variable: the overall between-group differences (day -10 until day 10), differences pre-alignment day (day -10 until day 0), and differences post-alignment (day 0 until day 10, representing the treatment effect). Differences in use of corticosteroids, remdesivir, and chloroquine, and differences in the proportion of patients who developed a secondary infection were tested using Fisher's exact test. Time on mechanical ventilation, ICU LOS, and mortality were analyzed using log-rank tests from alignment day onwards. Patients who died in the hospital or those who were still in the ICU and/or receiving mechanical ventilation on day 28 were censored at day 29 for the analysis of time on mechanical ventilation and ICU LOS. For the mortality analysis, patients who were discharged alive from the hospital or were still in the ICU or hospital on day 28 were censored at day 29.

Proteomics data were binned into one value per protein before alignment day and one

value after alignment day for each patient using the aforementioned R-script. Differences between these two values were analyzed within the anakinra and control group using t-tests. To correct for multiple testing, a p-value of <0.000625 (0.05/80 proteins) was considered as statistically significant. Fold changes (after vs. before alignment day) of ≤ -2 or ≥ 2 were considered relevant. Statistical analysis was performed using SPSS 25 (IBM) and GraphPad Prism 8 software (GraphPad Software).

Additional file 2

Supplementary figure 1. Patient flowchart.



Additional file 3

Supplementary table 1. Characteristics and clinical parameters at ICU admission and on alignment day for patients included in the inflammatory proteomics analysis. *Data are presented as n (%) or median [IQR]. P-values were calculated using Fisher's exact tests and Mann-Whitney-U tests.*

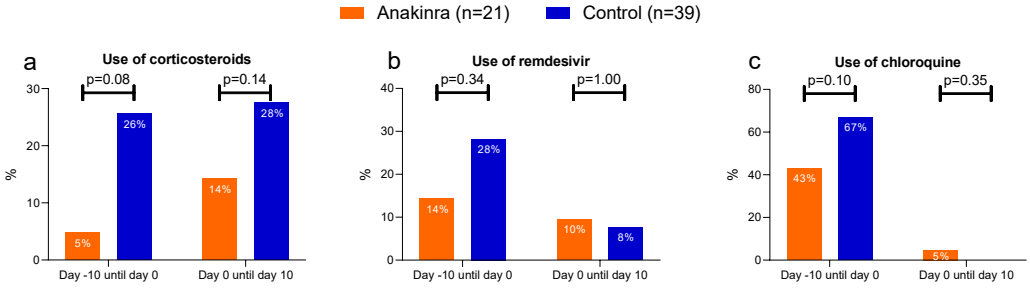
	Anakinra (n=11)	Control (n=11)	p-value
Sex, male	9 (82)	11 (100)	0.48
Age, years	60 [53-73]	72 [64-73]	0.19
BMI, kg/m^2	27.2 [25.6-27.8]	26.7 [24.7-34.0]	0.90
Apache II	14 [13-15]	19 [15-20]	0.03
Time from first COVID symptoms until ICU admission, days	13 [10-15]	10 [7-13]	0.10
Medical history			
Cardiovascular insufficiency	2 (18)	7 (64)	0.08
Hypertension	3 (27)	8 (73)	0.09
Respiratory insufficiency	1 (9)	0 (0)	1.00
Renal insufficiency	0 (0)	1 (9)	1.00
Metastatic neoplasm	2 (18)	1 (9)	1.00
Immunological insufficiency	0 (0)	1 (9)	1.00
Chronic obstructive pulmonary disease	0 (0)	1 (9)	1.00
Diabetes mellitus	4 (36)	3 (27)	1.00
Hematologic malignancy	1 (9)	0 (0)	1.00
Clinical parameters on admission day			
D-dimer, ng/mL	3350 [2320-48340]	2860 [1840-4600]	0.44
Creatinine, µmol/L	90 [70-94]	104 [79-119]	0.10
Alanine transaminase, U/L	47 [21-55]	35 [21-44]	0.30
Aspartate transaminase, U/L	44 [37-67]	53 [33-63]	0.90
Bilirubin, µmol/L	8 [6-13]	7 [6-10]	0.56
Lactate dehydrogenase, U/L	380 [310-516]	388 [359-436]	0.90
White blood cells, x109/L	8.2 [6.5-13.4]	9.1 [5.2 -9.9]	0.30
Thrombocytes, x109/L	289 [217-377]	227 [161-264]	0.08
C-reactive protein, mg/L	236 [192-311]	170 [118-245]	0.11
Procalcitonin, µg/L	0.69 [0.30-1.03]	0.99 [0.26-2.18]	0.47
Ferritin, µg/L	1780 [1265-2626]	1663 [1129-2055]	0.65
Temperature, °Celsius	38.6 [38.4-40.0]	38.6 [37.6-39.5]	0.80
PaO2/FiO2 ratio, mmHg	164 [108-206]	125 [108-158]	0.37
SOFA score	7 [4-7]	6 [4-7]	0.90
Clinical parameters on alignment day			
D-dimer, ng/mL	4800 [3909-6840]	3905 [2450-7190]	0.56
Creatinine, µmol/L	92 [79-106]	128 [91-212]	0.07
Alanine transaminase, U/L	91 [48-120]	51 [38-114]	0.22

Supplementary table 1. Continued.

Aspartate transaminase, U/L	97 [67-132]	45 [42-89]	0.008
Bilirubin, µmol/L	6 [4-7]	6 [3-11]	0.70
Lactate dehydrogenase, U/L	383 [348-450]	302 [243-375]	0.03
White blood cells, x109/L	14.6 [11.5-16.4]	13.4 [8.5-16.1]	0.80
Thrombocytes, x109/L	442 [329-515]	363 [263-470]	0.24
C-reactive protein, mg/L	177 [84-246]	126 [87-240]	0.75
Procalcitonin, µg/L	0.56 [0.34-1.41]	0.51 [0.41-0.76]	0.90
Ferritin, µg/L	2030 [1081-3516]	1371 [902-2217]	0.17
Temperature, °Celsius	39.7 [38.5-40.0]	37.6 [37.0-38.3]	0.001
PaO_2/FiO_2 ratio, mmHg	164 [126-268]	143 [125-212]	0.75
SOFA score	5 [5-6]	5 [4-7]	0.65
Time from first COVID symptoms until alignment day, days	22 [19-27]	21 [18-24]	0.45

Additional file 4

Supplementary figure 2. Use of medication. *Differences of use of* ***(a)*** *corticosteroids,* ***(b)*** *remdesivir, and* ***(c)*** *chloroquine between anakinra group and control group during ten days before and ten days after alignment day (day 0). P-values were calculated using Fisher's exact tests.*



Additional file 5

Additional results

Propensity score matched analysis

Propensity score matching increased the similarity in patient characteristics between the anakinra and control groups (Supplementary Table 2, Additional File 6). Analysis of circulating cytokine concentrations showed results comparable to the primary analysis (Supplementary Fig. 3, Additional File 9). Unlike the results of the primary analysis, significance was reached for the decrease in CRP plasma levels in the anakinra group after alignment day (p=0.003) (Supplementary Fig. 4e, Additional File 10). Kinetics of all other clinical parameters were on a par with results of the primary analysis (Supplementary Fig. 4 and 5, Additional Files 10 and 11). No significant differences in use of corticosteroids, remdesivir and chloroquine were present (Supplementary Fig. 6, Additional File 12). Also, analysis of clinical outcomes in propensity score matched groups yielded comparable results (Supplementary Fig. 7, Additional File 13).

Sensitivity analysis in patients who did not receive corticosteroids

During the study period, three patients of the anakinra group and 14 patients of the control group were treated with corticosteroids and therefore excluded for this analysis. No significant differences in patient characteristics and clinical parameters on ICU admission day were present (Supplementary Table 3, Additional File 7). Circulating cytokine concentrations showed no significant between-group differences in TNF-αand MCP-1 anymore (Supplementary Fig. 8a, f, Additional File 14). Kinetics of other circulating cytokine concentrations were comparable to those in the primary analysis (Supplementary Fig. 8, Additional File 14). The clinical inflammatory parameters showed largely comparable results to the primary analysis (Supplementary Fig. 9, Additional File 15), with the decrease in CRP after start of anakinra treatment becoming significant (p=0.001), whereas significance was lost for temperature. The decrease in creatinine after start of anakinra treatment remained present, albeit slightly less pronounced than in the primary analysis (p=0.06, Supplementary Fig. 10a, Additional File 16). Kinetics of bilirubin plasma levels, thrombocyte counts, PaO2/FiO2 ratio, norepinephrine infusion rate and total SOFA score were comparable to the results in our primary analysis (Supplementary Fig. 10, Additional File 16). As in the primary analysis, no differences in time on mechanical ventilation, ICU length of stay and mortality were present (Supplementary Fig. 11, Additional File 17).

Sensitivity analysis using control patients with persisting fever/high ferritin plasma levels

A total of 33 patients of the control group of the main analysis developed a period of fever

(n=17) or high ferritin plasma levels (n=16) during their ICU stay. The median alignment day of this subgroup was day 6 post-ICU admission. No significant differences in patient characteristics and clinical parameters on ICU admission were present between this control group and the anakinra group (Supplementary Table 4, Additional File 8). On alignment day, PaO_2/FiO_2 ratio was 188 [133-268] mmHg in the anakinra group versus 155 [124-170] mmHg in the control group (p=0.03). The time between the start of COVID-19 symptoms and alignment day was 22 [19-27] days in the anakinra group and 18 [13-23] days in the control group (p=0.008). No other significant differences between both groups were present on alignment day (Supplementary Table 4, Additional File 8). In general, effects of anakinra treatment on inflammatory parameters in this sensitivity analysis were comparable to those of the main analysis, although statistical significance was lost for some parameters (temperature) and emerged for others (CRP, several cytokines, Supplementary Fig. 12-13, Additional Files 18-19). The significant between-group differences in several cytokines in this sensitivity analysis are likely due to the earlier day of alignment of the control group (day 6 vs. day 12 in the main analyses). As cytokine levels are highest on ICU admission and gradually decrease thereafter (see Fig. 1), the 6-day difference in day of alignment is a plausible explanation for the higher concentrations of circulating cytokines on alignment day in the control group (Supplementary Fig. 12, Additional File 18). Results of other clinical inflammatory parameters, SOFA-score (and its individual components) and clinical outcomes were comparable to the results of the main analysis (Supplementary Fig. 13-15, Additional Files 19-21).

Additional file 6

Supplementary table 2. Patient characteristics and clinical parameters at ICU admission and on alignment day in propensity score matched groups. *Data are presented as n (%) or median [IQR]. P-values were calculated using Fisher's exact tests and Mann-Whitney-U tests.*

	Anakinra (n=21)	**Control (n=21)**	**p-value**
Sex, male	14 (67)	15 (71)	1.00
Age, years	63 [55-71]	65 [61-72]	0.50
BMI, kg/m^2	27.7 [25.9-29.9]	25.7 [24.2-28.2]	0.13
Apache II	15 [13-18]	15 [12-19]	0.74
Days first COVID symptoms until ICU admission, days	13 [9-14]	11 [8-15]	0.67
Medical history			
Cardiovascular insufficiency	4 (19)	8 (38)	0.31
Hypertension	8 (38)	11 (52)	0.54
Respiratory insufficiency	1 (5)	2 (10)	1.00
Renal insufficiency	0 (0)	0 (0)	1.00
Metastatic neoplasm	2 (10)	2 (10)	1.00
Immunological insufficiency	0 (0)	1 (5)	1.00
Chronic obstructive pulmonary disease	0 (0)	1 (5)	1.00
Diabetes mellitus	7 (33)	4 (19)	0.48
Hematologic malignancy	1 (5)	0 (0)	1.00
Clinical parameters on admission day			
D-dimer, ng/mL	3380 [2028-18343]	2950 [1835-5765]	0.38
Creatinine, µmol/L	84 [68-96]	94 [57-111]	0.80
Alanine transaminase, U/L	47 [21-62]	40 [28-57]	0.90
Aspartate transaminase, U/L	55 [37-76]	52 [37-63]	0.89
Bilirubin, µmol/L	8 [6-13]	7 [5-10]	0.28
Lactate dehydrogenase, U/L	380 [322-493]	388 [324-469]	0.82
White blood cells, x10^9/L	8.2 [7.0-12.0]	9.1 [6.3-11.7]	0.86
Thrombocytes, x10^9/L	247 [189-324]	263 [168-344]	0.07
C-reactive protein, mg/L	254 [188-297]	196 [158-296]	0.40
Procalcitonin, µg/L	0.66 [0.18-1.39]	1.11 [0.41-4.05]	0.19
Ferritin, µg/L	1842 [1313-2767]	1452 [1026-1879]	0.11
Temperature, °Celsius	38.4 [37.8-38.9]	38.3 [37.7-39.7]	0.71
PaO_2/FiO_2 ratio, mmHg	138 [105-199]	125 [103-182]	0.77
SOFA score	7 [4-7]	6 [5-7]	0.56
Clinical parameters on alignment day			
D-dimer, ng/mL	4063 [2585-6285]	4730 [2970-6855]	0.51
Creatinine, µmol/L	92 [76-106]	100 [55-154]	0.85
Alanine transaminase, U/L	89 [55-119]	83 [54-208]	0.95
Aspartate transaminase, U/L	96 [67-142]	77 [46-122]	0.23

Bilirubin, µmol/L	6 [5-12]	4 [3-6]	0.04
Lactate dehydrogenase, U/L	371 [317-450]	358 [276-403]	0.26
White blood cells, x10^9/L	12.4 [10.4-15.9]	12.9 [10.2-16.1]	0.97
Thrombocytes, x10^9/L	351 [314-494]	391 [280-468]	0.40
C-reactive protein, mg/L	130 [90-237]	97 [46-173]	0.16
Procalcitonin, µg/L	0.66 [0.39-1.88]	0.62 [0.24-1.38]	0.42
Ferritin, µg/L	2365 [1272-3713]	1123 [805-2011]	0.005
Temperature, °Celsius	39.1 [38.3-40.0]	37.8 [37.2-38.8]	0.009
PaO_2/FiO_2 ratio, mmHg	188 [133-268]	163 [120-255	0.41
SOFA score	6 [4-8]	6 [4-8]	0.78
Time from first COVID symptoms until alignment day, days	22 [19-27]	23 [18-27]	0.84

Additional file 7

Supplementary table 3. Patient characteristics and clinical parameters at ICU admission and on alignment day for the subgroup analysis in patients who received no corticosteroids. *Data are presented as n (%) or median [IQR]. P-values were calculated using Fisher's exact tests and Mann-Whitney-U tests.*

	Anakinra (n=18)	**Control (n=25)**	**p-value**
Sex, male	12 (67)	21 (84)	0.28
Age, years	60 [52-68]	65 [57-71]	0.44
BMI, kg/m2	27.7 [25.4-31.0]	27.4 [24.1-31.1]	0.49
Apache II	15 [13-18]	15 [11-20]	0.68
Time from first COVID symptoms until ICU admission, days	13 [8-14]	10 [7-15]	0.42
Medical history			
Cardiovascular insufficiency	3 (0.17)	6 (24)	0.71
Hypertension	6 (33)	15 (60)	0.12
Respiratory insufficiency	1 (6)	2 (8)	1.00
Renal insufficiency	0 (0)	0 (0)	1.00
Metastatic neoplasm	2 (11)	1 (4)	0.56
Immunological insufficiency	0 (0)	1 (4)	1.00
COPD	0 (0)	3 (12)	0.25
Diabetes mellitus	6 (33)	5 (20)	0.48
Hematologic malignancy	1 (6)	0 (0)	0.42
Clinical parameters on admission day			
D-dimer, ng/mL	3410 [1905-33450]	2905 [1592-4388]	0.18
Creatinine, µmol/L	91 [69-100]	82 [63-111]	0.89
Alanine transaminase, U/L	48 [21-58]	40 [31-61]	0.76
Aspartate transaminase, U/L	50 [36-79]	51 [38-63]	0.94
Bilirubin, µmol/L	8 [6-14]	7 [5-13]	0.69
Lactate dehydrogenase, U/L	367 [318-506]	421 [333-523]	0.71
White blood cells, $x10^9$/L	8.2 [6.5-10.5]	9.3 [5.6-11.0]	0.99
Thrombocytes, $x10^9$/L	240 [197-313]	227 [176-314]	0.61
C-reactive protein, mg/L	254 [190-295]	186 [120-292]	0.12
Procalcitonin, µg/L	0.69 [0.19-1.37]	0.74 [0.33-1.98]	0.81
Ferritin, µg/L	1695 [1210-2985]	1311 [556-2535]	0.13
Temperature, °Celsius	38.5 [37.7-39.0]	38.5 [37.5-39.2]	0.82
PaO_2/FiO_2 ratio, mmHg	143 [103-209]	143 [103-179]	0.89
SOFA score	7 [5-7]	7 [5-8]	0.13
Clinical parameters on alignment day			
D-dimer, ng/mL	4063 [2775-6055]	3935 [3160-5900]	0.91
Creatinine, µmol/L	93 [73-118]	72 [46-148]	0.51
Alanine transaminase, U/L	91 [65-118]	67 [51-107]	0.23
Aspartate transaminase, U/L	97 [67-139]	64 [48-97]	0.02
Bilirubin, µmol/L	6 [5-12]	5 [4-9]	0.21

Lactate dehydrogenase, U/L	383 [318-450]	349 [258-425]	0.22
White blood cells, x10⁹/L	11.9 [10.3-16.7]	13.3 [9.2-16.1]	0.61
Thrombocytes, x10⁹/L	379 [319-497]	376 [263-431]	0.25
C-reactive protein, mg/L	149 [76-248]	87 [59-145]	0.07
Procalcitonin, µg/L	0.46 [0.30-0.68]	0.58 [0.29-0.83]	0.42
Ferritin, µg/L	2344 [1353-4397]	1247 [447-1680]	0.002
Temperature, °Celsius	39.2 [38.3-40.0]	37.8 [37.0-38.5]	0.001
PaO_2/FiO_2 ratio, mmHg	206 [136-275]	157 [143-231]	0.24
SOFA score	6 [4-8]	5 [4-7]	0.28
Time from first COVID symptoms until alignment day, days	22 [19-26]	21 [18-26]	0.92

Additional file 8

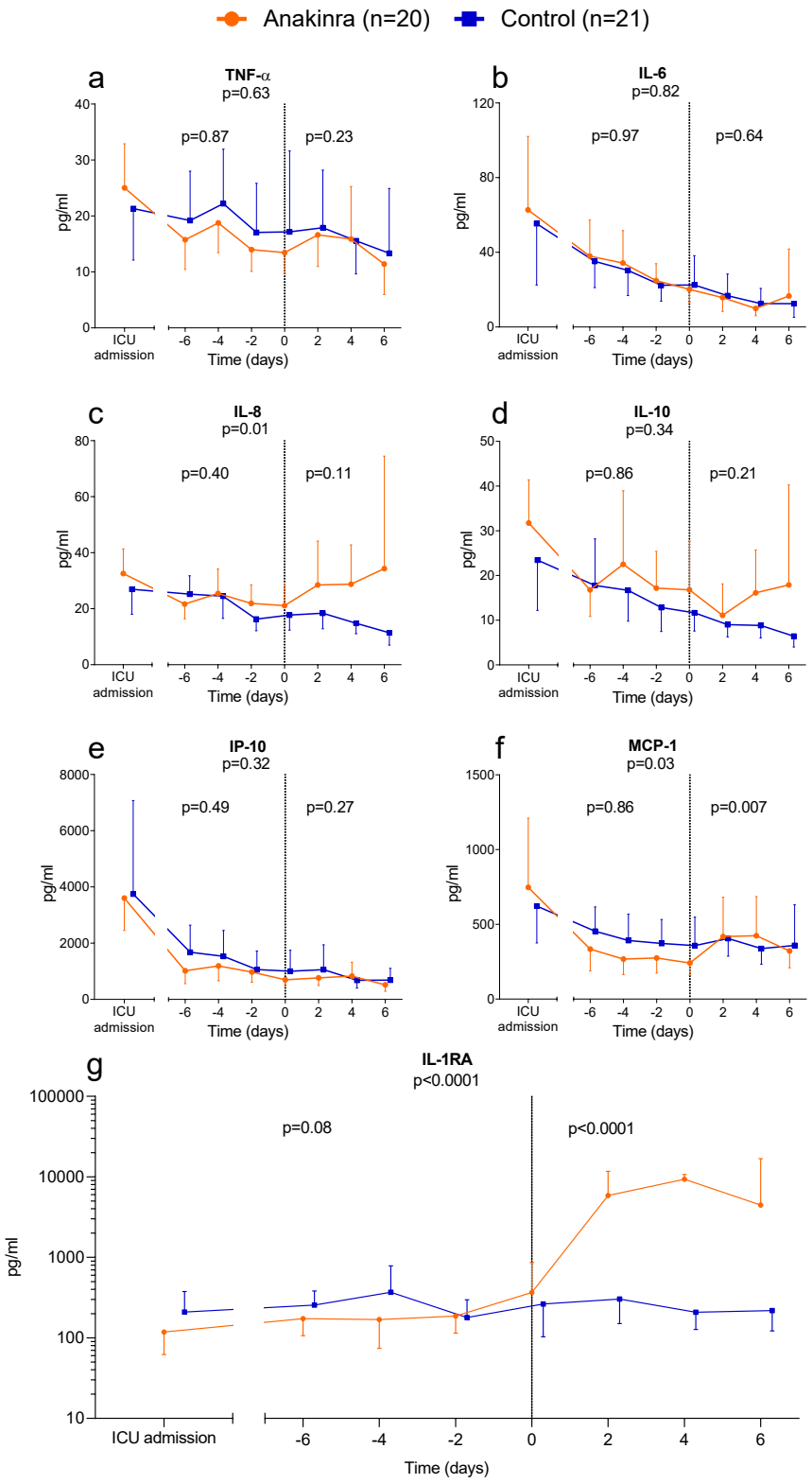
Supplementary table 4. Patient characteristics and clinical parameters at ICU admission and on alignment day for the subgroup analysis with control patients who partially met the criteria to start anakinra treatment. *Data are presented as n (%) or median [IQR]. P-values were calculated using Fisher's exact tests and Mann-Whitney-U tests.*

	Anakinra (n=21)	Control (n=33)	p-value
Sex, male	14	28	0.18
Age, years	63 [55-71]	67 [59-72]	0.46
BMI, kg/m^2	27.7 [25.9-29.9]	25.8 [24.2-29.5]	0.30
Apache II	15 [13-18]	16 [12-20]	0.51
Days first COVID symptoms until ICU admission, days	13 [9-14]	10 [7-16]	0.38
Medical history			
Cardiovascular insufficiency	4 (19)	7 (21)	1.00
Hypertension	8 (38)	20 (61)	0.16
Respiratory insufficiency	1 (5)	1 (3)	1.00
Renal insufficiency	0 (0)	0 (0)	1.00
Metastatic neoplasm	2 (10)	2 (6)	0.64
Immunological insufficiency	0 (0)	1 (3)	1.00
Chronic obstructive pulmonary disease	0 (0)	3 (9)	0.27
Diabetes mellitus	7 (33)	7 (21)	0.36
Hematologic malignancy	1 (5)1	0 (0)	0.39
Clinical parameters on admission day			
D-dimer, ng/mL	3380 [2028-18343]	2905 [1668-4568]	0.24
Creatinine, µmol/L	84 [68-96]	82 [70-110]	0.67
Alanine transaminase, U/L	47 [21-62]	39 [30-62]	0.88
Aspartate transaminase, U/L	55 [35-76]	51 [41-63]	0.91
Bilirubin, µmol/L	8 [6-13]	7 [6-13]	0.53
Lactate dehydrogenase, U/L	380 [322-493]	414 [324-488]	0.98
White blood cells, x10^9/L	8.2 [7.0-12.0]	9.8 [6.5-11.2]	0.89
Thrombocytes, x10^9/L	247 [189-324]	244 [175-325]	0.77
C-reactive protein, mg/L	254 [188-297]	210 [167-292]	0.33
Procalcitonin, µg/L	0.66 [0.18-1.39]	0.87 [0.39-2.95]	0.37
Ferritin, µg/L	1842 [1313-2767]	1469 [879-2457]	0.31
Temperature, °Celsius	38.4 [37.8-38.9]	38.9 [37.7-39.8]	0.35
PaO_2/FiO_2 ratio, mmHg	138 [105-199]	139 [93-178]	0.84
SOFA score	7 [4-7]	6 [5-8]	0.38
Clinical parameters on alignment day			
D-dimer, ng/mL	4063 [2585-6285]	3580 [2545-6380]	0.96
Creatinine, µmol/L	92 [76-106]	80 [62-153]	0.65
Alanine transaminase, U/L	89 [55-119]	78 [38-187]	0.74
Aspartate transaminase, U/L	96 [67-142]	75 [57-119]	0.40
Bilirubin, µmol/L	6 [5-12]	6 [4-10]	0.68

Lactate dehydrogenase, U/L	371 [317-450]	383 [301-479]	0.82
White blood cells, x10⁹/L	12.4 [10.4-15.9]	11.5 [9.1-13.4]	0.09
Thrombocytes, x10⁹/L	351 [314-494]	320 [224-426]	0.11
C-reactive protein, mg/L	130 [90-237]	185 [85-283]	0.52
Procalcitonin, µg/L	0.48 [0.33-0.69]	0.66 [0.24-1.32]	0.62
Ferritin, µg/L	2365 [1272-3713]	1929 [654-3287]	0.13
Temperature, °Celsius	39.1 [38.3-40.0]	38.7 [38.3-39.4]	0.32
PaO_2/FiO_2 ratio, mmHg	188 [133-268]	155 [124-170]	**0.03**
SOFA score	6 [4-8]	6 [4-8]	0.59
Time from first COVID symptoms until alignment day, days	22 [19-27]	18 [13-23]	**0.008**

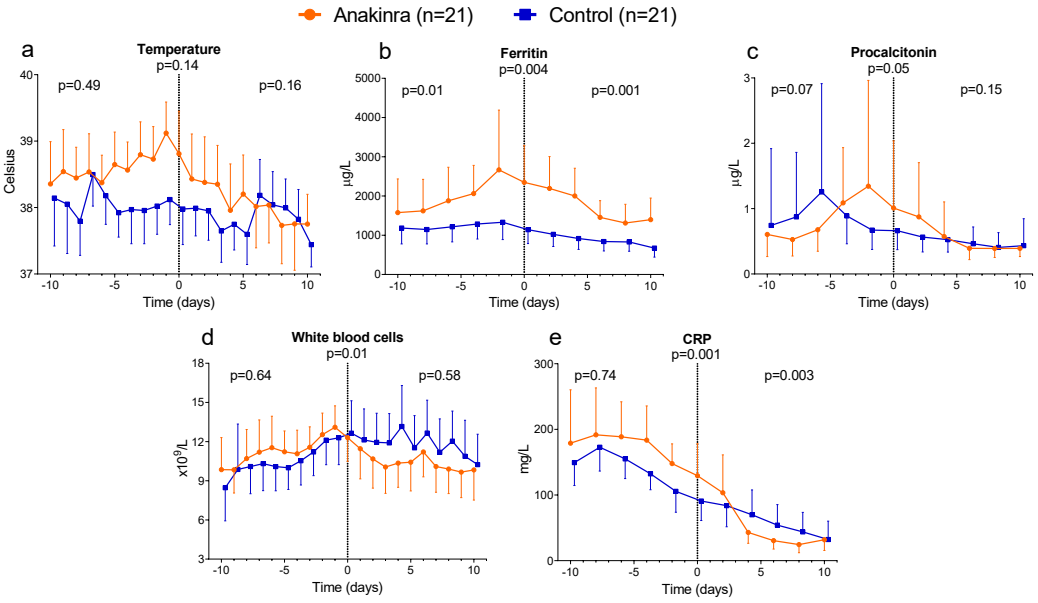
Additional file 9

Supplementary figure 3. Circulating cytokine concentrations in propensity score matched groups. *Concentrations of circulating* ***(a)*** *tumor necrosis factor (TNF)-α* ***(b)*** *interleukin (IL)-6,* ***(c)*** *IL-8,* ***(d)*** *IL-10,* ***(e)*** *interferon gamma-induced protein (IP)-10,* ***(f)*** *monocyte chemoattractant protein (MCP)-1, and* ***(g)*** *IL-1 receptor antagonist (IL-1RA) on day of intensive care unit (ICU) admission and serial data within six days pre- and post-alignment day (day 0). Data are presented as geometric mean with 95% confidence intervals and were analyzed using mixed-models analysis (time*group interaction factor) to evaluate differences between groups over time. P-values under graph titles reflect overall between-group differences (day -6 until day 6). Between-group p-values for day -6 until day 0 and day 0 until day 6 are shown on the left and right of each panel, respectively.*


Anakinra (n=20)
Control (n=21)
a
TNF-α
p=0.63
p=0.87
p=0.23
pg/ml
ICU admission
Time (days)
b
IL-6
p=0.82
p=0.97
p=0.64
c
IL-8
p=0.01
p=0.40
p=0.11
d
IL-10
p=0.34
p=0.86
p=0.21
e
IP-10
p=0.32
p=0.49
p=0.27
f
MCP-1
p=0.03
p=0.86
p=0.007
g
IL-1RA
p<0.0001
p=0.08
p<0.0001

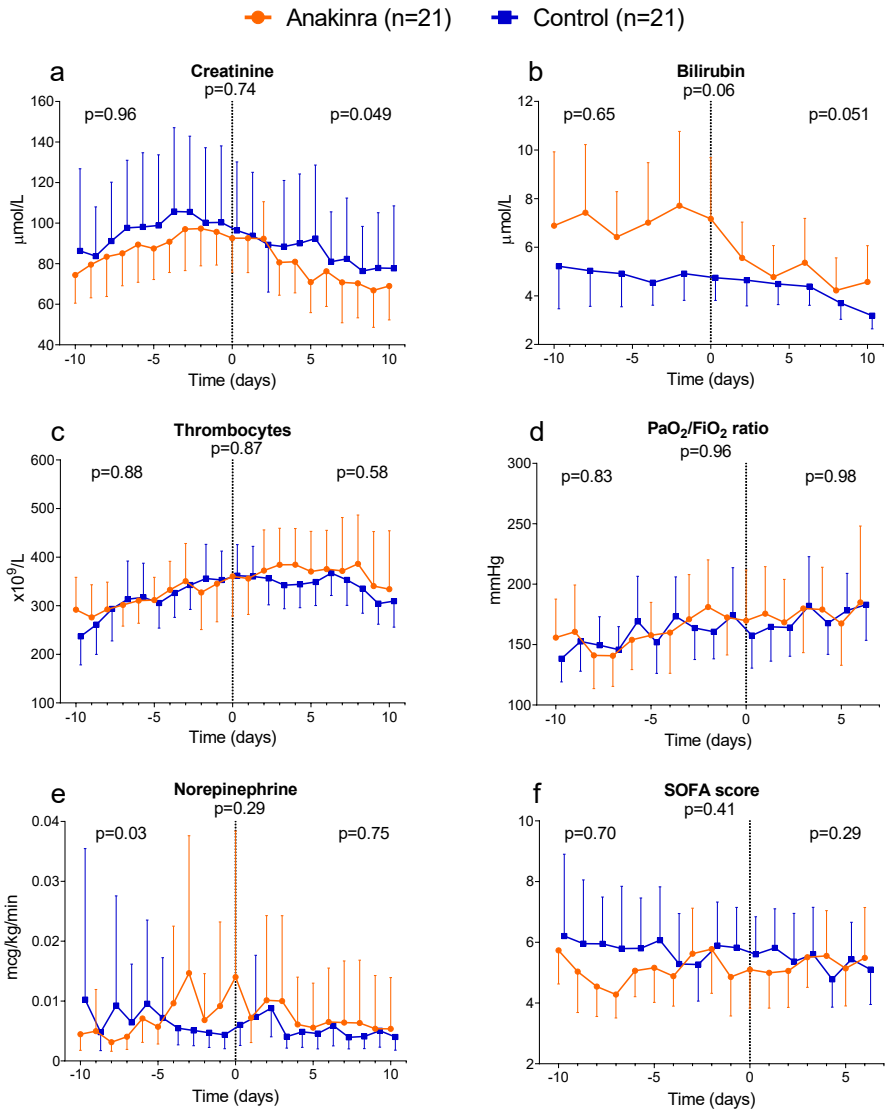
Additional file 10

Supplementary figure 4. Inflammation parameters over time in propensity score matched groups. ***(a)*** *Body temperature and plasma levels of* ***(b)*** *ferritin,* ***(c)*** *procalcitonin,* ***(d)*** *white blood cell counts, and* ***(e)*** *C-reactive protein (CRP) over time within 10 days pre- and post- alignment day (day 0) in propensity scored matched groups (n=21 both). Data are presented as geometric mean with 95% confidence intervals and were analyzed using mixed-models analysis (time*group interaction factor) to evaluate differences between groups over time. P-values under graph titles reflect overall between-group differences (day -10 until day 10). Between-group p-values for day -10 until day 0 and day 0 until day 10 are shown on the left and right of each panel, respectively.*



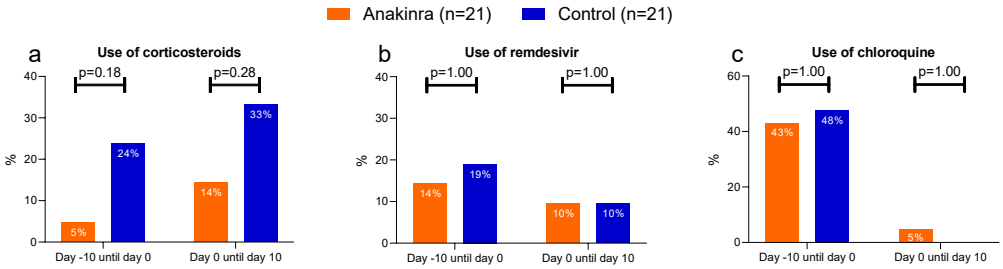
Additional file 11

Supplementary figure 5. Individual parameters of sequential organ failure assessment (SOFA) score and total SOFA score in propensity score matched groups. *Plasma concentrations of* ***(a)*** *creatinine,* ***(b)*** *bilirubin, and* ***(c)*** *thrombocytes and* ***(d)*** *PaO_2/FiO_2 (P/F)-ratio,* ***(e)*** *infusion rate of norepinephrine, and* ***(f)*** *SOFA score over time within 10 days pre- and post-alignment day (day 0) in propensity score matched groups (n=21 both). PaO_2/FiO_2 ratio and SOFA score were presented until day 6. Data are presented as geometric mean with 95% confidence intervals and were analyzed using mixed-models analysis (time*group interaction factor) to evaluate differences between groups over time. P-values under graph titles reflect overall between-group differences (day -10 until day 6 or 10). Between-group p-values for day -10 until day 0 and day 0 until day 6 or 10 are shown on the left and right of each panel, respectively.*



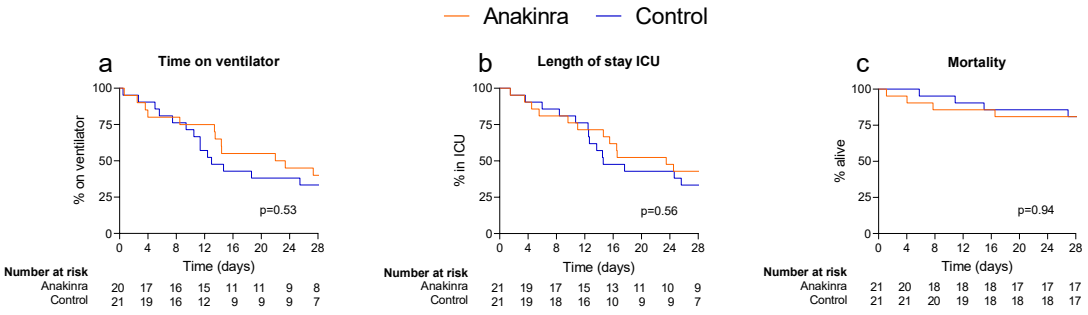
Additional file 12

Supplementary figure 6. Use of medication in propensity score matched groups*. Differences of use of* ***(a)*** *corticosteroids,* ***(b)*** *remdesivir, and* ***(c)*** *chloroquine between anakinra group and control group during ten days before and ten days after alignment day (day 0). P-values were calculated using Fisher's exact tests.*



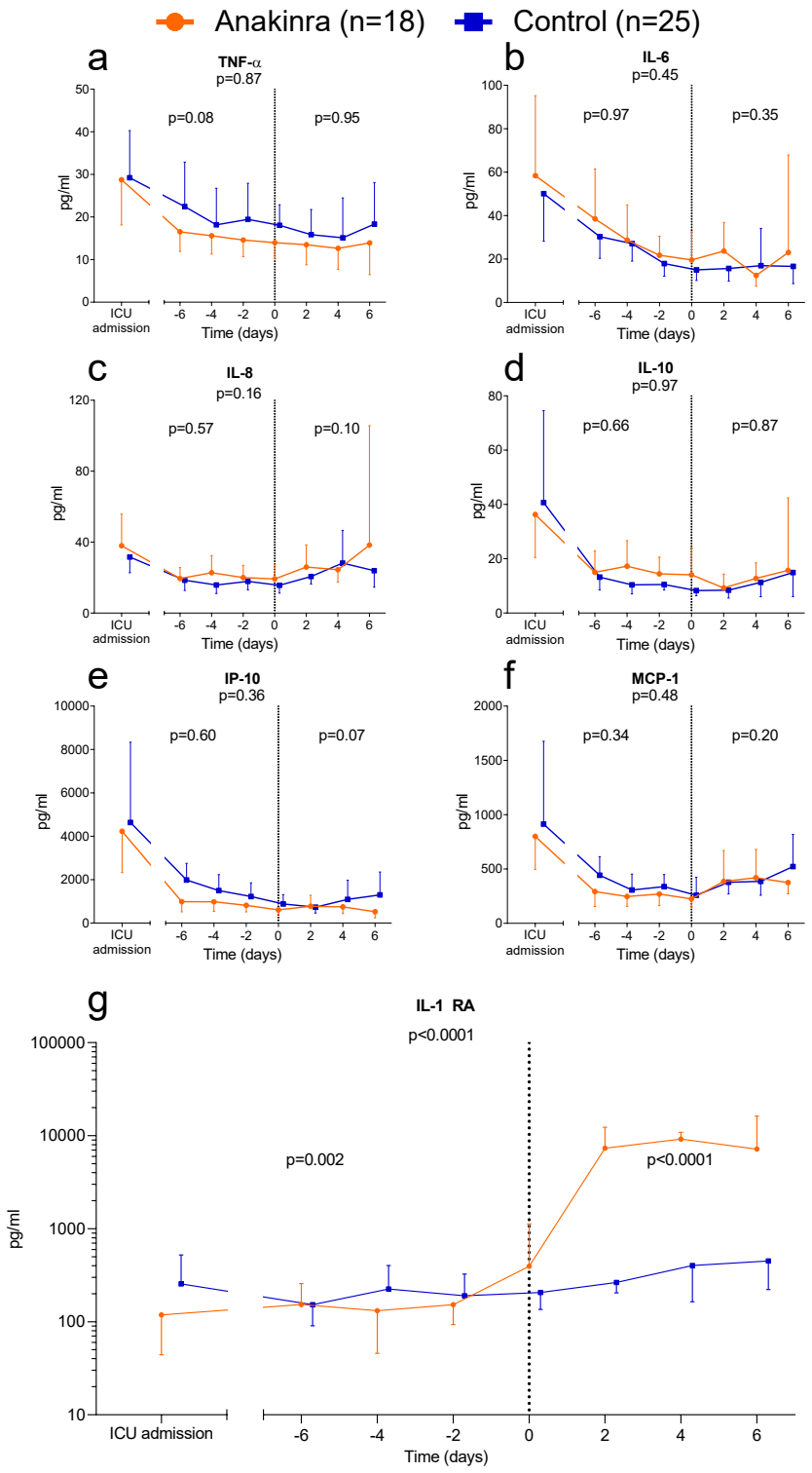
Additional file 13

Supplementary figure 7. Clinical outcomes in propensity score matched groups. *Kaplan-Meier graphs of* ***(a)*** *time on mechanical ventilator,* ***(b)*** *length of stay in the Intensive Care Unit (ICU), and* ***(c)*** *mortality for propensity score matched groups. Data are presented for the first 28 days after anakinra alignment day. Patients who were no longer mechanically ventilated on alignment day were not included in time on ventilator graph. P-values were calculated using log-rank tests. Numbers at risk on each timepoint per group are shown below graphs.*



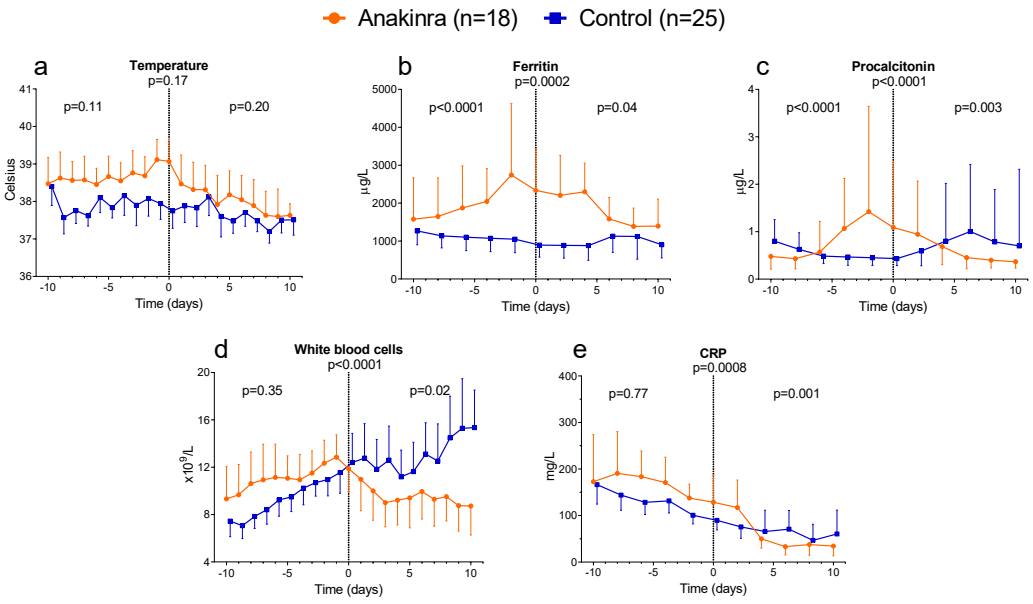
Additional file 14

Supplementary figure 8. Circulating cytokine concentrations for the subgroup analysis in patients who did not receive corticosteroids*. Concentrations of circulating* ***(a)*** *tumor necrosis factor (TNF)-α* ***(b)*** *interleukin (IL)-6,* ***(c)*** *IL-8,* ***(d)*** *IL-10,* ***(e)*** *interferon gamma-induced protein (IP)-10,* ***(f)*** *monocyte chemoattractant protein (MCP)-1, and* ***(g)*** *IL-1 receptor antagonist (IL-1RA) on day of intensive care unit (ICU) admission and serial data within six days pre- and post-alignment day (day 0). Data are presented as geometric mean with 95% confidence intervals and were analyzed using mixed-models analysis (time*group interaction factor) to evaluate differences between groups over time. P-values under graph titles reflect overall between-group differences (day -6 until day 6). Between-group p-values for day -6 until day 0 and day 0 until day 6 are shown on the left and right of each panel, respectively.*


Anakinra (n=18)
Control (n=25)
a
TNF-α
p=0.87
p=0.08
p=0.95
b
IL-6
p=0.45
p=0.97
p=0.35
c
IL-8
p=0.16
p=0.57
p=0.10
d
IL-10
p=0.97
p=0.66
p=0.87
e
IP-10
p=0.36
p=0.60
p=0.07
f
MCP-1
p=0.48
p=0.34
p=0.20
g
IL-1 RA
p<0.0001
p=0.002
p<0.0001
pg/ml
ICU admission
Time (days)

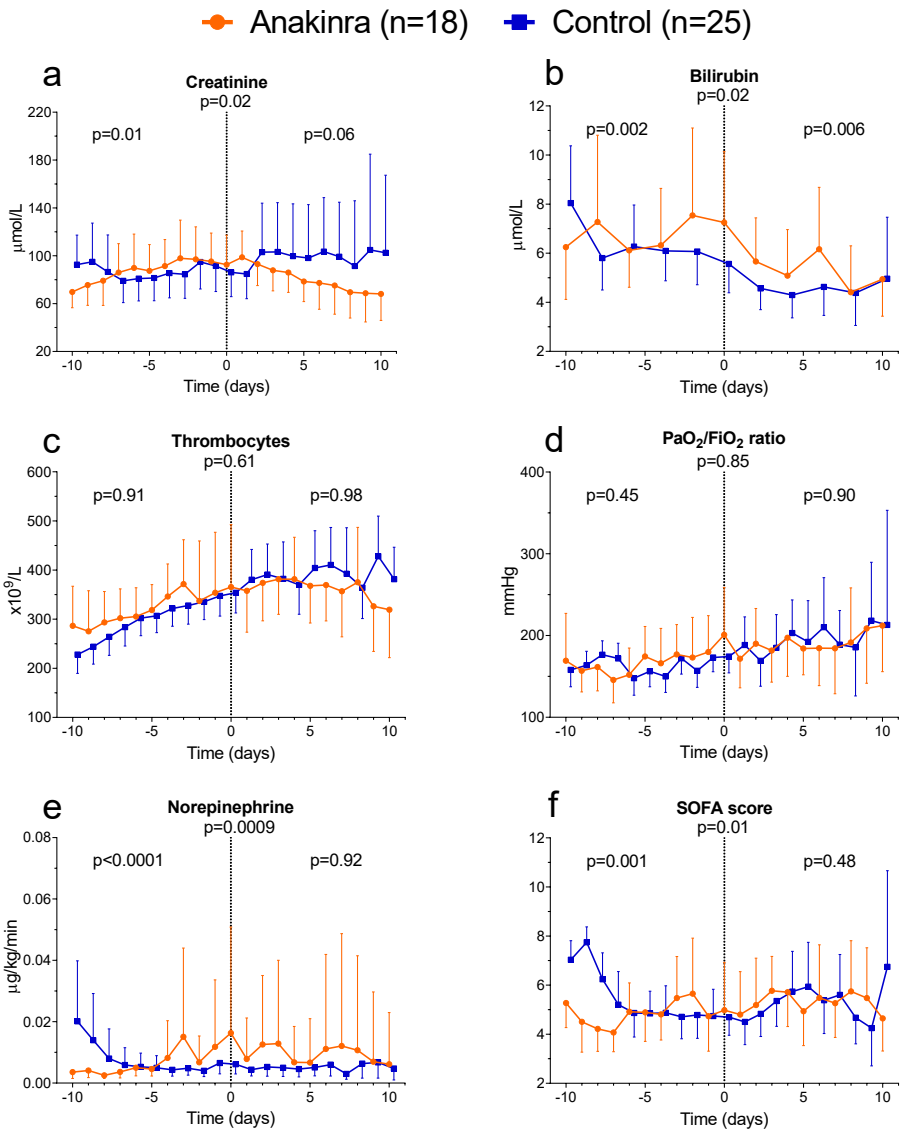
Additional file 15

Supplementary figure 9. Inflammation parameters over time for the subgroup analysis in patients who did not receive corticosteroids. ***(a)*** *Body temperature and plasma levels of* ***(b)*** *ferritin,* ***(c)*** *procalcitonin,* ***(d)*** *white blood cell counts, and* ***(e)*** *C-reactive protein (CRP) over time within 10 days pre- and post- alignment day (day 0). Data are presented as geometric mean with 95% confidence intervals and were analyzed using mixed-models analysis (time*group interaction factor) to evaluate differences between groups over time. P-values under graph titles reflect overall between-group differences (day -10 until day 10). Between-group p-values for day -10 until day 0 and day 0 until day 10 are shown on the left and right of each panel, respectively.*



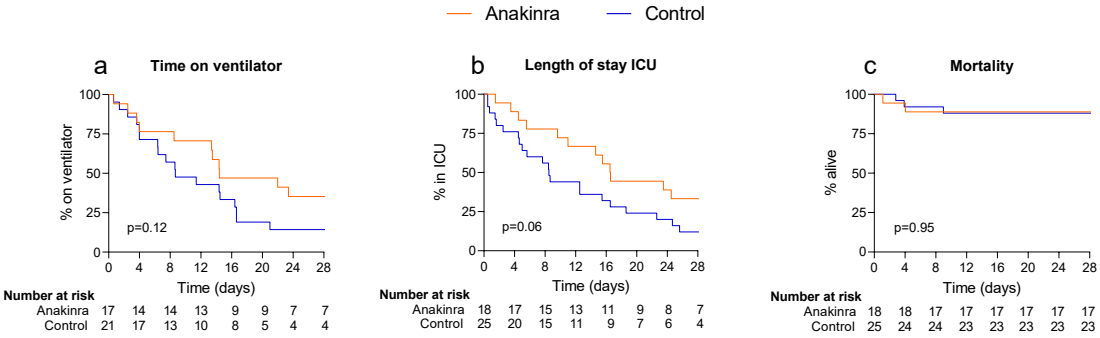
Additional file 16

Supplementary figure 10. Individual parameters of sequential organ failure assessment (SOFA) score and total SOFA score for the subgroup analysis in patients who did not receive corticosteroids. *Plasma concentrations of* ***(a)*** *creatinine,* ***(b)*** *bilirubin, and* ***(c)*** *thrombocytes and* ***(d)*** *PaO_2/FiO_2 (P/F)-ratio,* ***(e)*** *infusion rate of norepinephrine, and* ***(f)*** *SOFA score over time within 10 days pre- and post-alignment day (day 0). PaO_2/FiO_2 ratio and SOFA score were presented until day 6. Data are presented as geometric mean with 95% confidence intervals and were analyzed using mixed-models analysis (time*group interaction factor) to evaluate differences between groups over time. P-values under graph titles reflect overall between-group differences (day -10 until day 6 or 10). Between-group p-values for day -10 until day 0 and day 0 until day 6 or 10 are shown on the left and right of each panel, respectively.*



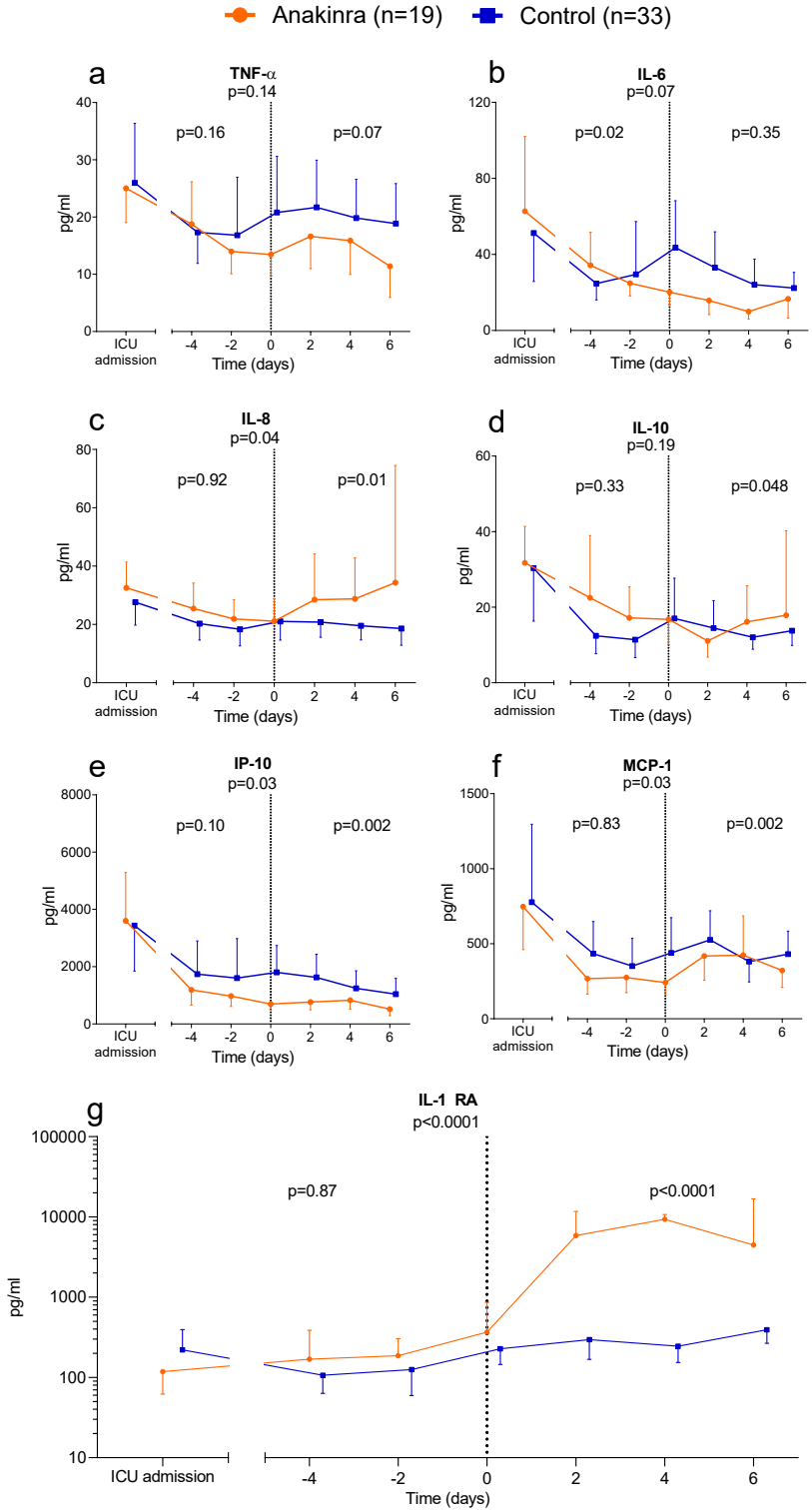
Additional file 17

Supplementary figure 11. Clinical outcomes for the subgroup analysis in patients who did not receive corticosteroids. *Kaplan-Meier graphs of* ***(a)*** *time on mechanical ventilator,* ***(b)*** *length of stay in the Intensive Care Unit (ICU), and* ***(c)*** *mortality. Data are presented for the first 28 days after anakinra alignment day. Patients who were no longer mechanically ventilated on alignment day were not included in time on ventilator graph. P-values were calculated using log-rank tests. Numbers at risk on each timepoint per group are shown below graphs.*



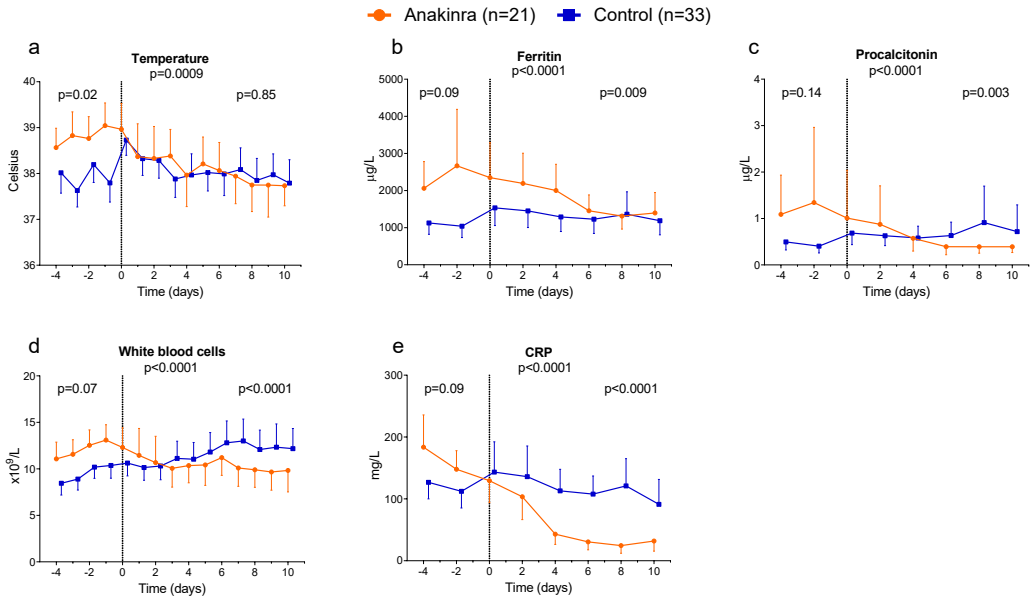
Additional file 18

Supplementary figure 12. Circulating cytokine concentrations for the subgroup analysis with control patients who partially met the criteria to start anakinra treatment. *Concentrations of circulating* ***(a)*** *tumor necrosis factor (TNF)-α* ***(b)*** *interleukin (IL)-6,* ***(c)*** *IL-8,* ***(d)*** *IL-10,* ***(e)*** *interferon gamma-induced protein (IP)-10,* ***(f)*** *monocyte chemoattractant protein (MCP)-1, and* ***(g)*** *IL-1 receptor antagonist (IL-1RA) on day of intensive care unit (ICU) admission and serial data within four days pre- and six days post-alignment day (day 0). Data are presented as geometric mean with 95% confidence intervals and were analyzed using mixed-models analysis (time*group interaction factor) to evaluate differences between groups over time. P-values under graph titles reflect overall between-group differences (day -6 until day 6). Between-group p-values for day -6 until day 0 and day 0 until day 6 are shown on the left and right of each panel, respectively.*


Anakinra (n=19)
Control (n=33)
a
TNF-α
p=0.14
p=0.16
p=0.07
pg/ml
ICU admission
Time (days)
b
IL-6
p=0.07
p=0.02
p=0.35
c
IL-8
p=0.04
p=0.92
p=0.01
d
IL-10
p=0.19
p=0.33
p=0.048
e
IP-10
p=0.03
p=0.10
p=0.002
f
MCP-1
p=0.03
p=0.83
p=0.002
g
IL-1 RA
p<0.0001
p=0.87
p<0.0001

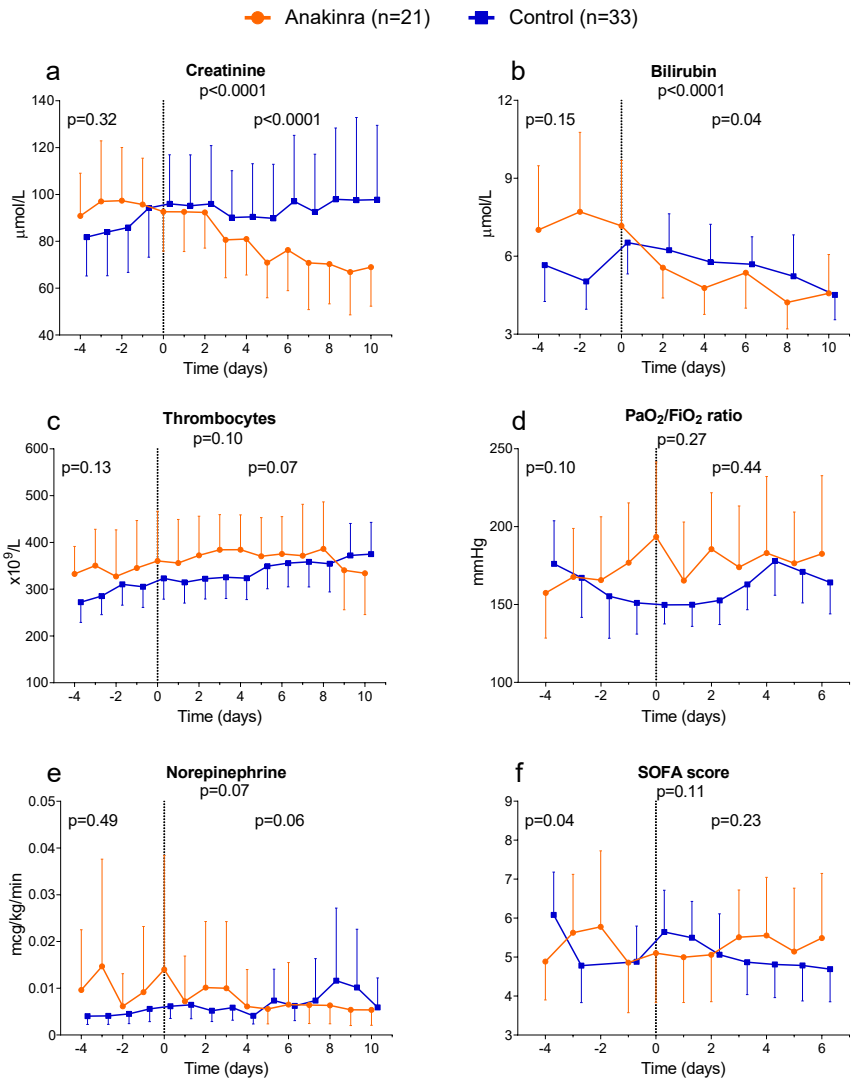
Additional file 19

Supplementary figure 13. Inflammation parameters over time for the subgroup analysis with control patients who partially met the criteria to start anakinra treatment. ***(a)*** *Body temperature and plasma levels of* ***(b)*** *ferritin,* ***(c)*** *procalcitonin,* ***(d)*** *white blood cell counts, and* ***(e)*** *C-reactive protein (CRP) over time within 10 days pre- and post- alignment day (day 0). Data are presented as geometric mean with 95% confidence intervals and were analyzed using mixed-models analysis (time*group interaction factor) to evaluate differences between groups over time. P-values under graph titles reflect overall between-group differences (day -10 until day 10). Between-group p-values for day -10 until day 0 and day 0 until day 10 are shown on the left and right of each panel, respectively.*



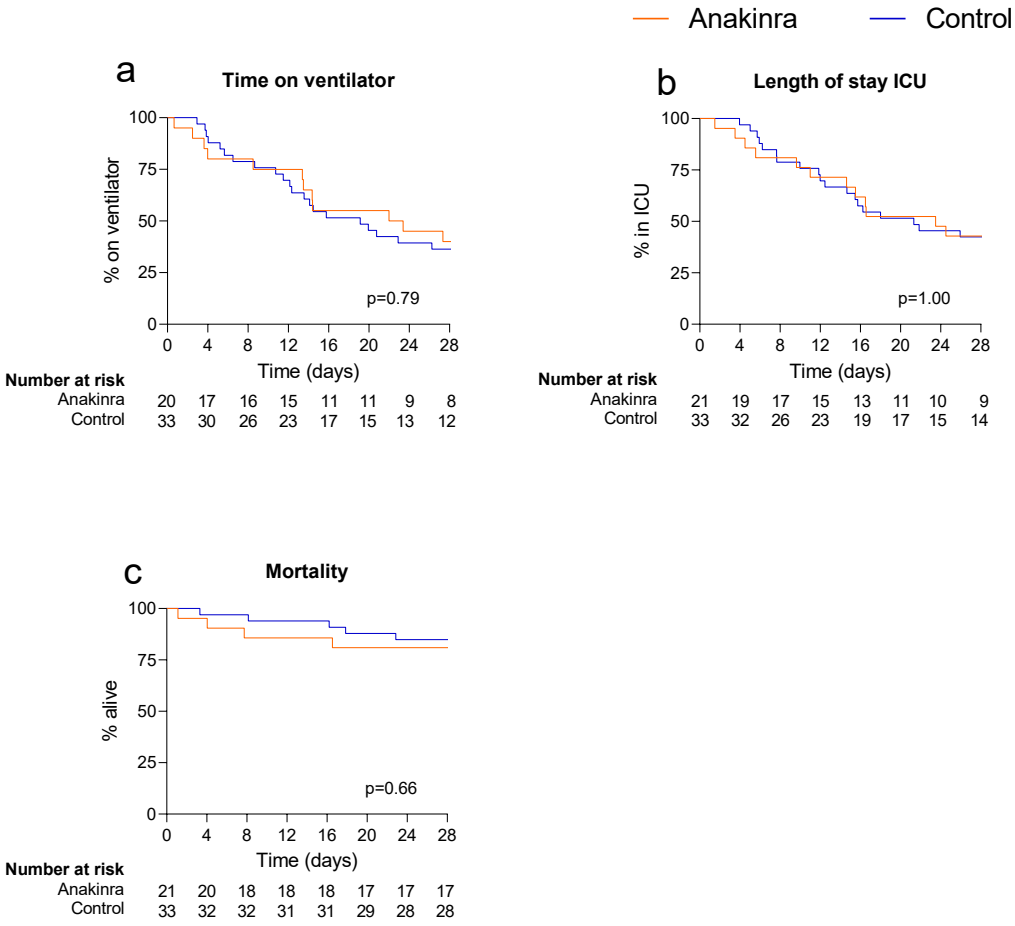
Additional file 20

Supplementary figure 14. Individual parameters of sequential organ failure assessment (SOFA) score and total SOFA score for the subgroup with control patients who partially met the criteria to start anakinra treatment. *Plasma concentrations of* ***(a)*** *creatinine,* ***(b)*** *bilirubin, and* ***(c)*** *thrombocytes and* ***(d)*** *PaO_2/FiO_2 (P/F)-ratio,* ***(e)*** *infusion rate of norepinephrine, and* ***(f)*** *SOFA score over time within 10 days pre- and post-alignment day (day 0). PaO_2/FiO_2 ratio and SOFA score were presented until day 6. Data are presented as geometric mean with 95% confidence intervals and were analyzed using mixed-models analysis (time*group interaction factor) to evaluate differences between groups over time. P-values under graph titles reflect overall between-group differences (day -10 until day 6 or 10). Between-group p-values for day -10 until day 0 and day 0 until day 6 or 10 are shown on the left and right of each panel, respectively.*



Additional file 21

Supplementary figure 15. Clinical outcomes for the subgroup with control patients who partially met the criteria to start anakinra treatment. *Kaplan-Meier graphs of* ***(a)*** *time on mechanical ventilator,* ***(b)*** *length of stay in the Intensive Care Unit (ICU), and* ***(c)*** *mortality. Data are presented for the first 28 days after anakinra alignment day. Patients who were no longer mechanically ventilated on alignment day were not included in time on ventilator graph. P-values were calculated using log-rank tests. Numbers at risk on each timepoint per group are shown below graphs.*



CHAPTER 12

Interferon gamma immunotherapy in five critically ill COVID-19 patients with impaired cellular immunity: a case series

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* These authors share first authorship
# These authors share last authorship

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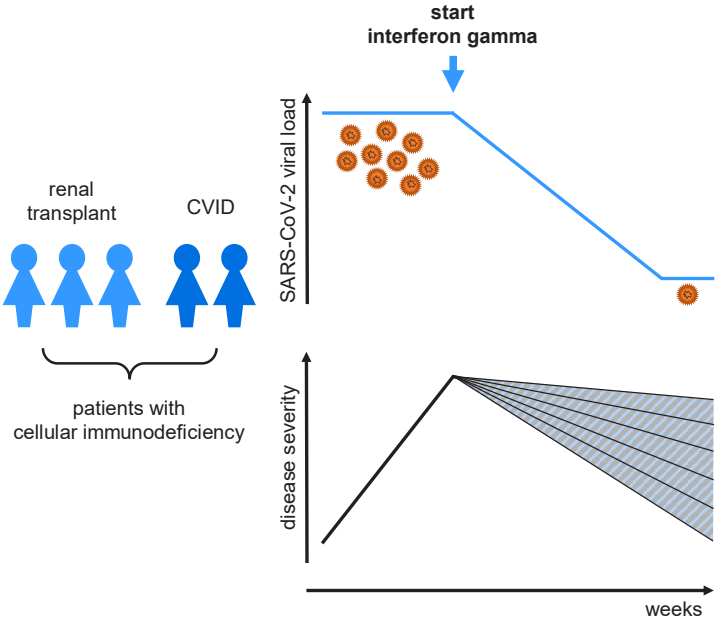


Abstract

Background: Prolonged SARS-CoV-2 shedding has been described in immunocompromised COVID-19 patients, resulting in protracted disease and poor outcome. Specific therapy to improve viral clearance and outcome for this group of patients is currently unavailable.
Methods: Five critically ill COVID-19 patients with severe defects in cellular immune responses, high SARS-CoV-2 viral RNA loads, and no respiratory improvement were treated with interferon gamma, 100 µg subcutaneously, thrice weekly. Bronchial secretion was collected every 48 hours for routine diagnostic SARS-CoV-2 RT-PCR and viral culture.
Findings: Interferon gamma administration was followed by a rapid decline in SARS-CoV-2 load and a positive to negative viral culture conversion. Four patients recovered and no signs of hyperinflammation were observed.
Conclusions: Interferon gamma may be considered as adjuvant immunotherapy in a subset of immunocompromised COVID-19 patients.

Introduction

Exuberant immunopathology plays an important role in Coronavirus Disease 2019 (COVID-19) mortality (1), and indeed, immunosuppressive therapy has demonstrated to improve outcome (2). However, in patients with pre-existent immunodeficiencies, replication-competent Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) may be shed well after 21 days after disease onset (3,4). Convalescent plasma or remdesivir is used in immunocompromised patients, but has not been shown to promote viral clearance. Our patients had conditions reducing their interferon response, which is likely important for viral clearance (4). In addition, interferon-mediated immunity is known to be impaired by COVID-19 itself (5,6). Clinical data however failed to demonstrate positive effects of systemic interferon beta-1a in COVID-19 patients (7),while interferon gamma has been thus far avoided due to its potential proinflammatory effects. However, in patients with severe cellular immune defects, therapeutic stimulation of the antiviral host defence may benefit SARS-CoV-2 clearance.

Results

Five immunocompromised COVID-19 patients were treated with adjuvant interferon gamma, 100 µg subcutaneously, thrice weekly. The patients were monitored for SARS-CoV-2 viral load, culture conversion (patient 1-3), and signs of hyperinflammation.

Patient 1. A 50-year old woman with a history of splenectomy and common variable immunodeficiency (Table 1), was admitted to the intensive care unit (ICU). Mechanical ventilation was initiated on day 22 after COVID-19 symptom onset. She had developed an invasive pulmonary *Aspergillus nidulans* infection and *Pseudomonas aeruginosa* pneumonia. Because of an absent SARS-CoV-2 antibody response, convalescent plasma was administered (Figure 1) without clinical response. Five days after admission, she was transferred to our centre because of acute kidney injury and severe acute respiratory distress syndrome. She developed a fulminant herpes simplex stomatitis, with temporary response to acyclovir. Even though lymphocyte counts were only moderately decreased, the *Aspergillus* and herpes virus complications pointed to an impaired Th1/interferon gamma response, in addition to her common variable immunodeficiency-related humoral defects. SARS-CoV-2 viral load in bronchial secretion remained high after administration of a third dose of convalescent plasma at day 44. Interferon gamma was initiated on day 47, after which SARS-CoV-2 viral loads decreased. Virus culture was negative at all time points tested, including a sample taken before the initiation of interferon gamma treatment. Although her respiratory status im-

proved, she remained in need of mechanical ventilation as a result of severe ICU-acquired weakness. She died on day 87 after she persisted in her wish to stop supportive treatment.

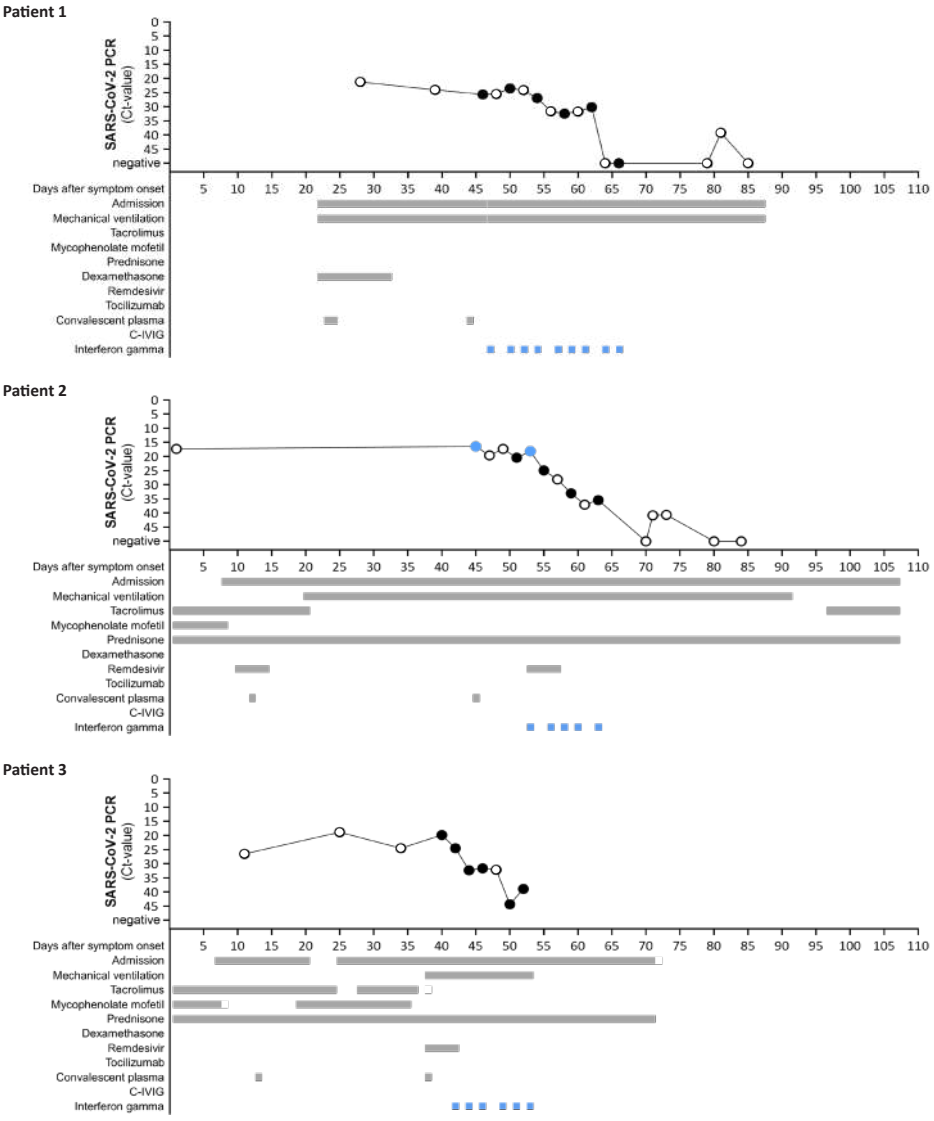
Patient 2. A 32-year old woman using tacrolimus, mycophenolate mofetil and prednisone because of a renal transplantation 9 months earlier, developed COVID-19 symptoms 9 days after alemtuzumab treatment of graft rejection. She was admitted with respiratory distress, transient renal insufficiency and diarrhoea another 8 days later. Mycophenolate mofetil was stopped that day and tacrolimus at day 21 after symptom onset. She was treated with remdesivir and convalescent plasma because of an absent SARS-CoV-2 antibody response. Mechanical ventilation was initiated when high-flow nasal oxygen therapy failed at day 20. She was treated for a suspected bacterial pulmonary superinfection at day 42, and remained severely lymphopenic (Supplementary table 1), with high SARS-CoV-2 loads and no signs of clinical improvement. Interferon gamma and a second course of remdesivir were started at day 53. Viral loads subsequently declined and viral culture became negative. Although treated for bacteraemia with extended spectrum beta-lactamase *Klebsiella pneumoniae* at day 57 and 90, she recovered with preserved renal function (eGFR CKD-EPI > 90ml/min/1.73m^2) and started weaning from mechanical ventilation through a tracheostomy tube and was discharged at day 107. After discharge, the creatinine steadily increased, for which a kidney biopsy was performed on day 211, showing signs of an antibody-mediated rejection. It cannot be excluded that this is related to the discontinuation of the mycophenolate mofetil and tacrolimus and the treatment with interferon gamma during the hospital admission for COVID-19.

Patient 3. A 45-year old woman was admitted 7 days after COVID-19 symptom onset. One month earlier a blood group-incompatible renal transplantation had been performed, for which she had received rituximab, immunoadsorption, intravenous immune globulin and basiliximab. She used tacrolimus, mycophenolate mofetil and prednisone as maintenance therapy, and immune suppression was adjusted during admission. She developed end-stage renal failure with thrombotic microangiopathy and tubular damage, but no signs of rejection on biopsy. Because of negative SARS-CoV-2 serology, she received convalescent plasma at day 12. High-flow nasal oxygen therapy could be stopped at day 20 and she was discharged at her own request despite persistent requirement for oxygen therapy. At day 25 she was re-admitted and at day 30, a suspected bacterial pneumonia was treated. On day 38, renal replacement therapy and mechanical ventilation were initiated, a second dose of convalescent plasma was administered, and remdesivir was started without clinical improvement. At day 42 the persistent lymphopenia, high SARS-CoV-2 load, and lack of clinical improvement prompted us to start interferon gamma, after which the viral load swift-

ly declined and respiratory function improved. Virus culture was negative before initiation of interferon gamma treatment and remained negative at other time points tested. She was extubated at day 53 and discharged at day 71. Circulating cytokines did not show signs of hyperinflammation in patient 1-3 (Supplementary Figure 1).

Patient 4. A 55-year old woman with common variable immunodeficiency, was admitted 6 days after COVID-19 symptom onset and 17 days after receiving her first mRNA COVID-19 vaccination. Mechanical ventilation was started 8 days after COVID-19 symptom onset. She received tocilizumab and hyperimmune anti-COVID-19 intravenous immunoglobulin (C-IVIG) at day 8 and 9 respectively. She developed portal hypertension and was treated with high-dose steroids for pulmonary fibrosis, and with acyclovir for herpes simplex virus pneumonia. Because of persistent high SARS-CoV-2 loads, interferon gamma was initiated at day 23 after which the viral load declined. She received a second and third dose of C-IVIG at respectively day 30 and 54 after symptom onset. Her respiratory function improved and she started weaning through tracheostomy at day 41. Still weaning, she was transferred to another hospital on day 57.

Patient 5. A 21-year old woman using mycophenolate mofetil and prednisone because of a renal transplantation 15 years earlier, and who received alemtuzumab treatment for graft rejection 17 months prior, developed COVID-19 symptoms 4 months after asymptomatic COVID-19 and 7 days after receiving her second mRNA COVID-19 vaccination. She was admitted with renal insufficiency, metabolic acidosis, and diarrhoea 9 days after symptom onset, and was discharged two days later. At 18 days after symptom onset, she was readmitted because of renal insufficiency, metabolic dysregulation, and diarrhoea. CT-thorax showed signs of a pneumomediastinum and she received low-flow oxygen therapy. Prednisone was switched for dexamethasone. Mycophenolate mofetil was stopped at 20 days after symptom onset and tocilizumab was administered once. Mechanical ventilation was initiated when high-flow nasal oxygen therapy failed at day 23. Because of clinical deterioration and persistent high SARS-CoV-2 load, interferon gamma was started at day 23 and C-IVIG was administered at day 25. Viral loads declined and her respiratory function improved. She was extubated at day 34 and discharged at day 42. Her renal transplant status remained poor.


Patient 1
SARS-CoV-2 PCR (Ct-value)
0 5 10 15 20 25 30 35 40 45 negative
Days after symptom onset
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110
Admission
Mechanical ventilation
Tacrolimus
Mycophenolate mofetil
Prednisone
Dexamethasone
Remdesivir
Tocilizumab
Convalescent plasma
C-IVIG
Interferon gamma
Patient 2
SARS-CoV-2 PCR (Ct-value)
0 5 10 15 20 25 30 35 40 45 negative
Days after symptom onset
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110
Admission
Mechanical ventilation
Tacrolimus
Mycophenolate mofetil
Prednisone
Dexamethasone
Remdesivir
Tocilizumab
Convalescent plasma
C-IVIG
Interferon gamma
Patient 3
SARS-CoV-2 PCR (Ct-value)
0 5 10 15 20 25 30 35 40 45 negative
Days after symptom onset
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110
Admission
Mechanical ventilation
Tacrolimus
Mycophenolate mofetil
Prednisone
Dexamethasone
Remdesivir
Tocilizumab
Convalescent plasma
C-IVIG
Interferon gamma

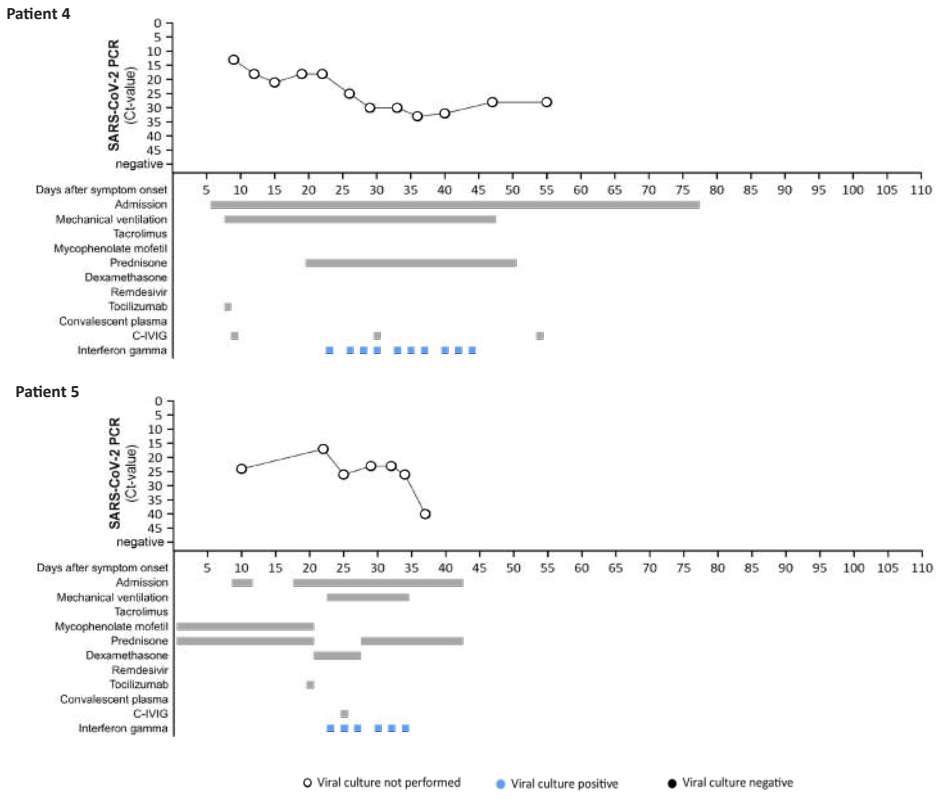


Figure 1. Clinical course and SARS-CoV-2 viral loads.
SARS-CoV-2 RNA was measured in bronchial secretions by routine PCR against the E-gene, expressed as Ct-value. Interferon gamma was administered as 100 µg subcutaneously, thrice weekly. Convalescent plasma and C-IVIG were provided by the Dutch national blood supply Sanquin, and was administered intravenously in doses of approximately 200 mL (plasma) and 50 to 100 mL (C-IVIG). Remdesivir was adminstered intravenously with a 200 µg loading dose, followed by daily doses of 100 µg for four days. Tocilizumab was administered intravenously in a dose of 400 mg. Filled circles indicate time points at which virus culture was performed. Blue circles represent samples that were virus culture positive, black circles are samples that were virus culture negative.

Table 1. Patient characteristics.

	Patient 1	**Patient 2**	**Patient 3**
Sex	Female	Female	Female
Age at admission (years)	50	32	45
BMI (kg/m2)	19	27	18
Comorbidities			
Cardiovascular disease	Aortic prosthesis (18 years prior) after aortitis	No	No
Diabetes mellitus	No	No	No
Systemic hypertension	No	No	No
Pulmonary disease	Bronchiectasis	No	No
Other	Common variable immunodeficiency based on a *NFKB1*-mutation Splenectomy (25 years prior) Primary sclerosing cholangitis Recurrent sinopulmonary infections	Renal transplant (9 months before admission)	Renal transplant (1 month before admission)
Immunosuppressive medication	None	Alemtuzumab at the time of transplantation and 9 days prior to COVID-19 onset. Maintenance immunosuppression with tacrolimus mycophenolate mofetil and Prednisone	Rituximab, immunoadsorption, intravenous immune globulin and basiliximab at the time of transplantation. Maintenance immunosuppression with tacrolimus, mycophenolate mofetil, Prednisone
Time from COVID-19 symptom onset (days)			
to first hospital admission	22	8	7
to mechanical ventilation	22	20	38
COVID-19 immunomodulatory treatment			
Dexamethasone	Yes	No	No
Prednisone	No	Yes	Yes
Tocilizumab	No	No	No
Outcome	Deceased following patient requested policy limitations	Tracheostomy; extubated; discharged	Extubated; discharged

Patient 4	**Patient 5**
Female	Female
55	21
23	19
Yes	No
No	No
No	No
Bronchiectasis	No
Common variable immunodeficiency Splenomegaly Immune thrombocytopenic purpura Autoimmune haemolytic anaemia Recurrent pulmonary infections	Renal transplant (15 years before admission). Mayer Rokitansky MDA type 1 GJA5 mutation
None	Alemtuzumab 17 months before admission. Maintenance immunosuppression with mycophenolate mofetil and prednisone
6	9
8	23
Yes	Yes
Yes	Yes
Yes	Yes
Weaning through tracheostomy; transferred to other hospital; extubated	Extubated; discharged

Discussion

We report five critically ill immunocompromised COVID-19 patients, with persistent failure of respiratory improvement accompanied by sustained high SARS-CoV-2 viral loads for a prolonged period of time, despite repeated convalescent plasma or C-IVIG administration and remdesivir as adjuvant therapy. The persistent high SARS-CoV-2 viral loads and infectious complications suggested severe immune dysfunction in these patients. Last-resort adjuvant treatment with interferon gamma was well tolerated, followed by a rapid decline in SARS-CoV-2 viral loads, and four patients have subsequently recovered.

Immunotherapeutic trials in COVID-19 have shown benefit of inhibition of exaggerated inflammation (2). However, no therapy is established to augment viral clearance, including convalescent plasma therapy which was safely used in small series of immunocompromised patients. Monoclonal neutralising antibodies may have a role in seronegative hospitalised patients (8), but an effect on mortality in severe COVID-19 patients later in the course of disease has not yet been shown. Remdesivir has not demonstrated a clinical antiviral effect and it is therefore doubtful whether its administration in two of our patients contributed to viral clearance. In critically ill COVID-19 cases, type I interferon pathways are impaired (6), and although these mediators are likely important for viral clearance (4), subcutaneous interferon beta-1a was not associated with survival benefit (7). Because of the lack of effectivity of adjuvant type I interferons, and because type I interferons can have suppressive effects on T-helper function (9), we deemed administration of type I interferons undesirable in our patients, as Th1 immunity was already impaired in our patients as illustrated by their *Aspergillus* and herpes simplex virus infections.

Type II interferons are less well studied in COVID-19. Although circulating concentrations are not altered in COVID-19 patients, the interferon-gamma production upon *ex-vivo* stimulation is reduced (5,10), as is the interferon gamma responsive gene signature in critically ill COVID-19 patients (6). Interferon gamma immunotherapy has been applied previously in patients with pulmonary tuberculosis (11) and pulmonary aspergillosis (12), but no published data were available for use in COVID-19. We considered application of adjuvant interferon gamma therapy a logical choice of last-resort therapy because of its potent immunostimulatory effects including on tissue macrophages that are likely important for COVID-19 immunity (1). In our patients, interferon gamma administration was indeed followed by viral clearance and clinical improvement in four out of five patients. A theoretical adverse effect of interferon gamma administration could be the development of hyperinflammation in the spectrum of a macrophage activation syndrome or hemophagocytic lymphohistiocytosis in which pathophysiology interferon gamma is causally involved (13). Favourably though, follow-up samples showed stable concentrations of plasma C-reactive protein, se-

rum ferritin (Supplementary Table 1), as well as monocyte-derived pro-inflammatory cytokines involved in hyperinflammation such as IL-1α IL-6 and IL-18. Only the lymphocyte stimulating IL-12 showed an increase during interferon gamma treatment in patient 1-3, which might have contributed to viral clearance (Supplementary Figure 1). No early or cell-mediated anti-allograft immunity was observed in the patients with a functioning renal allograft, but it cannot be excluded that the interferon gamma administration played a role in the antibody-mediated rejection that was later identified in patient 2. A series of seven renal transplant patients with invasive fungal infections treated with adjuvant interferon gamma showed that among the four patients with stable graft function before interferon gamma initiation, the graft function remained stable (12). Future studies on interferon gamma immunotherapy in COVID-19 could include patients with impaired cellular immunity caused by either immunosuppressive medication, primary immunodeficiencies or malignancies. For patients with auto-immune or auto-inflammatory disorders, extra caution is warranted to monitor for signs of hyperinflammation.
We thus propose interferon gamma immunotherapy as a study candidate for adjuvant therapy in severely immunocompromised COVID-19 patients with persistent high SARS-CoV-2 loads and lack of clinical improvement.

Inherent to a case series, limitations of the study include the small sample size of five patients with the specific phenotype of high SARS-CoV-2 viral loads and impaired cellular immunity. Moreover, because interferon gamma was used off-label as last-resort therapy instead of in a randomised setting, we cannot attribute the viral clearance with certainty to interferon gamma immunotherapy. Lastly, functional immunological data was not available for these patients.

References

1. Wiersinga, W.J., Rhodes, A., Cheng, A.C., Peacock, S.J., and Prescott, H.C. (2020). Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19). JAMA 324.
2. RECOVERY Collaborative Group, Horby, P., Lim W S, and et al. (2021). Dexamethasone in Hospitalized Patients with Covid-19. New England Journal of Medicine 384.
3. Avanzato, V.A., Matson, M.J., Seifert, S.N., Pryce, R., Williamson, B.N., Anzick, S.L., Barbian, K., Judson, S.D., Fischer, E.R., Martens, C., et al. (2020). Case Study: Prolonged Infectious SARS-CoV-2 Shedding from an Asymptomatic Immunocompromised Individual with Cancer. Cell 183.
4. Bastard, P., Rosen, L.B., Zhang, Q., Michailidis, E., Hoffmann, H.-H., Zhang, Y., Dorgham, K., Philippot, Q., Rosain, J., Béziat, V., et al. (2020). Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science 370.
5. Janssen, N.A.F., Grondman, I., de Nooijer, A.H., Boahen, C.K., Koeken, V.A.C.M., Matzaraki, V., Kumar, V., He, X., Kox, M., Koenen, H.J.P.M., et al. (2021). Dysregulated Innate and Adaptive Immune Responses Discriminate Disease Severity in COVID-19. The Journal of Infectious Diseases 223.
6. Hadjadj, J., Yatim, N., Barnabei, L., Corneau, A., Boussier, J., Smith, N., Péré, H., Charbit, B., Bondet, V., Chenevier-Gobeaux, C., et al. (2020). Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. Science 369.
7. WHO Solidarity Trial Consortium, Pan, H., Peto, R., Henao-Restrepo, A.M., Preziosi, M.P., Sathiyamoorthy, V., Abdool Karim, Q., Alejandria, M.M., Hernández García, C., Kieny, M.P., et al. (2021). Repurposed Antiviral Drugs for Covid-19 — Interim WHO Solidarity Trial Results. New England Journal of Medicine 384.
8. RECOVERY Collaborative Group, Horby, P.W., Mafham, M., Peto, L., Campbell, M., Pessoa-Amorim, G., Spata, E., Staplin, N., Emberson, J.R., Prudon, B., et al. (2021). Casirivimab and imdevimab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. MedRxiv.
9. McNab, F., Mayer-Barber, K., Sher, A., Wack, A., and O'Garra, A. (2015). Type I interferons in infectious disease. Nature Reviews Immunology 15.
10. Rother, N., Yanginlar, C., Lindeboom, R.G.H., Bekkering, S., van Leent, M.M.T., Buijsers, B., Jonkman, I., de Graaf, M., Baltissen, M., Lamers, L.A., et al. (2020). Hydroxychloroquine Inhibits the Trained Innate Immune Response to Interferons. Cell Reports Medicine 1.
11. Dawson, R., Condos, R., Tse, D., Huie, M.L., Ress, S., Tseng, C.-H., Brauns, C., Weiden, M., Hoshino, Y., Bateman, E., et al. (2009). Immunomodulation with Recombinant Interferon-α1 b in Pulmonary Tuberculosis. PLoS ONE 4.
12. Armstrong-James, D., Teo, I.A., Shrivastava, S., Petrou, M.A., Taube, D., Dorling, A., and Shaunak, S. (2010). Exogenous Interferon-αImmunotherapy for Invasive Fungal Infections in Kidney Transplant Patients. American Journal of Transplantation 10.
13. Brisse, E., Wouters, C.H., and Matthys, P. (2015). Hemophagocytic lymphohistiocytosis (HLH): A heterogeneous spectrum of cytokine-driven immune disorders. Cytokine & Growth Factor Reviews 26.

14. Wölfel, R., Corman, V.M., Guggemos, W., Seilmaier, M., Zange, S., Müller, M.A., Niemeyer, D., Jones, T.C., Vollmar, P., Rothe, C., et al. (2020). Virological assessment of hospitalized patients with COVID-2019. Nature 581.
15. Varghese, F., van Woudenbergh, E., Overheul, G., Eleveld, M., Kurver, L., van Heerbeek, N., van Laarhoven, A., Miesen, P., den Hartog, G., de Jonge, M., et al. (2021). Berberine and Obatoclax Inhibit SARS-Cov-2 Replication in Primary Human Nasal Epithelial Cells In Vitro. Viruses 13.

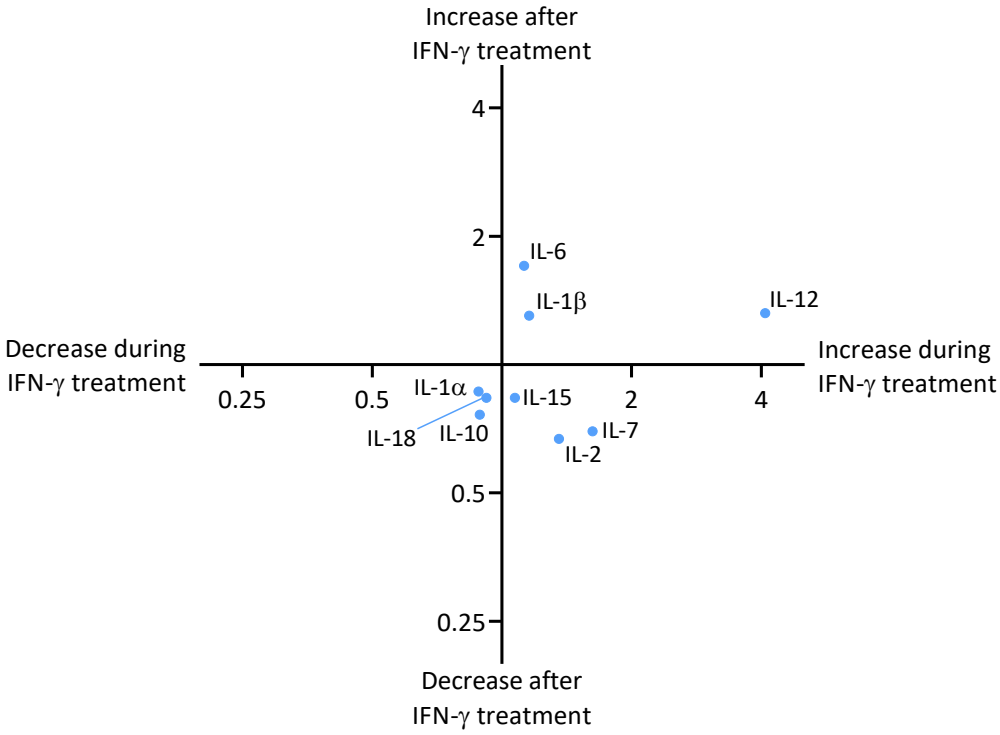
Additional method details

Patients were admitted to the ICU department and received additional treatment before and during interferon gamma therapy as discussed. Interferon gamma (Immukine, Clinigen Healthcare Ltd) was administered as 100 µg subcutaneously, thrice weekly. During interferon gamma immunotherapy, bronchial secretion was collected every 48 hours for routine diagnostic SARS-CoV-2 RT-PCR using commercially available systems targeting the E gene, and in patient 1-3 for inoculation of Vero E6 cells using methods adapted from Wölfel et al14. Bronchial secretion was diluted and mixed 1:1 in viral transport medium, of which 100 µl was used to infect Vero E6 cells, which were seeded in 24 well plates at a density of 2.5x105 cells/well. After 1 hour, the inoculum was replaced with 1 ml of Dulbecco's Modified Eagle Medium (DMEM, Gibco) containing 2% fetal bovine serum (Sigma), 100 µg/ml gentamycin (Gibco), 2.5 µg/ml amphotericin-B (Gibco), 100 µg/ml streptomycin and 100 U/ml penicillin (Gibco) and culture medium was collected directly and at 48 hours and at 96 hours post infection for RNA isolation and RT-qPCR using primers targeting the E gene,14 as described previously.15 A positive viral culture was defined as increased levels of SARS-CoV-2 over time. Patients were monitored clinically for signs of hyperinflammation and inflammatory parameters were measured every 48 h. Circulating cytokines were measured in serum samples of patient 1-3 using the Olink Explore 1536 platform a multiplex proximity extension assay technique (Olink Proteomics, Uppsala, Sweden) and cytokines involved in hyperinflammation and T-cell function were analyzed.
No statistical analyses were undertaken. GraphPad Prism version 8.0.2. was used for graphical analysis of data.

Supplementary table 1. Laboratory measurements, related to results.

	Patient 1			**Patient 2**			**Patient 3**			**Patient 4**			**Patient 5**		
Timing relative to IFN-γ treatment	Before	During	After	Before	During	After	Before	During	After	Before	During	After	Before	During	After
Time from symptom onset (days)	47	52	66	45	59	65	42	49	55	22	34	46	22	28	36
Haemoglobin (mmol/L)	4.6	6.3	4.6	5.6	5.4	5.4	4.5	5.4	4.4	6.6	6.7	5.5	6.0	4.5	6.2
Leucocyte count (10^9/L)	9.4	8.4	16.1	3.9	5.8	16.5	13.2	11.7	12.5	14.4	6.5	4.1	4.6	6.4	4.5
neutrophils (10^9/L)	7.1	4.0	8.9	3.5	5.3	15.5	12.7	9.7	10.4	12.9	5.3	3.4	4.4	5.2	3.0
lymphocytes (10^9/L)	0.9	2.2	2.7	0.2	0.1	0.1	0.1	0.8	0.8	0.6	0.5	0.4	0.0	0.3	0.4
monocytes (10^9/L)	1.2	2.0	4.2	0.1	0.3	0.7	0.1	0.8	0.5	0.9	0.7	0.3	0.2	0.8	0.8
eosinophils (10^9/L)	0.2	0.1	0.3	0.1	0.1	0.1	0.3	0.3	0.8	0.0	0.0	0.2	0.0	0.2	0.2
basophils (10^9/L)	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Thrombocyte count (10^9/L)	126	190	215	177	128	242	261	226	293	193	156	105	183	189	197
C-reactive protein (mg/L)	110	79	56	48	33	100	217	102	287	1	12	25	42	9	4
Ferritin (µg/L)	560	796	831	429	703	977	3563	1857	3917	391	278	328	1910	1337	1141
D-dimer (µg/L)	2320	2820	3000	3450	2430	2020	1010	3610	7750	810	<500	1790	3950	6850	2420

Supplementary figure 1. Circulating cytokines before, during and after adjuvant interferon gamma therapy, related to results. *Circulating cytokines were measured in serum samples of patient 1-3 at three time-points: 2 (±1) days before interferon gamma therapy (IFN-γ) was started, 6 (±1 day) after initiation of interferon gamma therapy, and 2 (±1) days after cessation of interferon gamma therapy. Mean ratios of samples taken during and before interferon gamma therapy are indicated at the x-axis and mean ratios of samples after cessation and during interferon gamma therapy are indicated at the y-axis.*



CHAPTER 13

Summary, general discussion and future perspectives

This thesis focuses on different aspects of coronavirus disease 2019 (COVID-19) in critically ill patients with specific emphasis on **(I)** obesity as a risk factor for poor clinical outcomes and long-term symptoms, **(II)** the use of biomarkers and phenotyping, and **(III)** immunomodulatory therapy. The studies described in this thesis were conducted from the very beginning of the pandemic onwards. During the course of the pandemic, multiple treatments were proven effective. This allowed us to evaluate the effects of these treatments on specific aspects of COVID-19 using data collected before and after the introduction of these treatments in several chapters. **Chapter 1** provides a general introduction on the topics of this thesis, first outlining the global importance of scientific research into this new pandemic disease caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).

Part I: Obesity in critically ill COVID-19 patients

Summary

In **chapter 2** we investigated putative differences in the inflammatory response, respiratory function, and clinical outcomes between obese and non-obese critically ill COVID-19 patients. This study was conducted prior to the introduction of immunomodulatory therapy as standard care for patients with severe COVID-19. During the first ten days in the ICU, a significant decline in circulating cytokine levels over time was observed in all patients, but no differences between obese and non-obese patients were present. Also, no correlation between BMI and circulating cytokine levels on ICU admission were observed. Kinetics of clinical inflammatory parameters, including white blood cell counts, body temperature and C-reactive protein (CRP) and ventilatory parameters did not differ between obese and non-obese COVID-19 ICU patients as well. Also, both time on ventilator, ICU length of stay (LOS) and ICU mortality were comparable between obese and non-obese patients. Although the sample size of this study was relatively small, no relevant differences in the immune response and clinical outcomes appear to be present between obese and non-obese patients once admitted to the ICU.

Early on during the pandemic, it became clear that obese patients were more likely to be admitted to the ICU compared to non-obese patients. It was thought that their chances to survive would also be related to the presence of obesity. In **chapter 3** we describe the results of a study conducted in 35.506 critically ill patients admitted to 82 Dutch ICU's of which the primary aim was to investigate whether BMI is related to clinical outcomes in critically ill COVID-19 patients. For comparison, the relationship between BMI and outcome was also explored in large cohorts of critically ill patients with viral pneumonia of other origin (mainly influenza virus), bacterial pneumonia, and multiple trauma, as a non-infectious comparison

group. Compared to the other three cohorts, COVID-19 patients were more likely male, had a higher BMI, a lower PaO_2/FiO_2 ratio and were more likely to require mechanical ventilation during the first 24 hours in the ICU. Nevertheless, the APACHE III severity score of COVID-19 patients was lower compared to that of the non-SARS-CoV-2 viral pneumonia and bacterial pneumonia cohorts. Clinical outcomes of COVID-19 patients were less favourable compared to the other groups, including a more prolonged length of stay in the ICU and hospital, as well as a higher in-hospital mortality rate. Odds ratios for mortality were calculated for each BMI category within each cohort using multivariable logistic regression analyses including multiple confounding factors which were selected based on expert opinion and available literature. This analysis revealed significantly lower in-hospital mortality rates in higher BMI categories in both the non-SARS-CoV-2 viral pneumonia and bacterial pneumonia cohorts, confirming the presence of the so-called *obesity paradox* in these patients. In COVID-19 on the other hand, BMI was not related to in-hospital mortality, indicating absence of the obesity paradox for this type of ICU patients. In conclusion, BMI is no longer associated with impaired outcomes once COVID-19 patients become critically ill. In other words, while patients with obesity may be more likely to become infected and have a more serious disease course, BMI does not appear to influence their outcome once they are admitted to the ICU. Nevertheless, a higher BMI is also not associated with improved outcome as is observed in other critically ill patients with respiratory infections.
Later on during the pandemic, it became clear that many patients who survive severe COVID-19 may suffer from persistent health issues.

Chapter 4 entails a study on differences in long-term sequalae between COVID-19 patients of different BMI categories who survived their critical illness. Symptoms of physical, mental, and cognitive impairments were collected using validated questionnaires on three timepoints (pre-ICU [in retrospect], and 3 months and 12 months following ICU admission). No significant differences in symptoms on any of the three domains were present between BMI categories before patients developed COVID-19. However, compared to the other BMI categories, patients with severe obesity (BMI>35 kg/m^2) were more likely to experience prolonged physical and mental impairments following their ICU admission. Therefore, we advocate that specifically in COVID-19 ICU survivors with obesity, monitoring long-term impairments is warranted.

In **chapter 5**, several aspects regarding the role of obesity in the development and clinical course of severe COVID-19 in the intensive care unit (ICU) were reviewed. Obesity emerged as a risk factor for severe COVID-19 and long-term complications, but the underlying mechanisms remain unclear. In contrast to the higher risk of ICU admission of COVID-19 patients

with obesity compared to non-obese COVID-19 patients, no clear differences in clinical outcomes appear apparent between COVID-19 patients with different body mass indices (BMI) once they are admitted to the ICU. To possibly explain the observation that obese patients are more likely to need ICU admission, a difference in immunity between obese and non-obese patients was advocated. Furthermore, it is advocated that recognition of obesity as a risk factor and close clinical monitoring of obese patients with COVID-19 is important for prognostication and treatment.

General discussion

Early on in the COVID-19 pandemic, obesity was recognized as an important risk factor for both susceptibility towards infection with SARS-CoV-2 and a more severe disease course of COVID-19. The latter includes need for invasive mechanical ventilation (IMV) in the ICU and overall increased mortality rates (1-3). Also, compared to non-obese patients, hospitalised COVID-19 patients with obesity were more likely to develop severe complications such as acute kidney injury (4, 5), septic shock (4, 6), and venous thromboembolisms (7, 8). SARS-CoV-2 enters cells by binding to the angiotensin-converting enzyme 2 (ACE2) receptor, of which the expression is higher in adipose tissue than in lung tissue (9, 10). Theoretically, the higher expression of ACE2 in adipose tissue could lead to increased viral shedding and a more pronounced immune response in COVID-19 patients with obesity, putatively explaining the higher susceptibility towards infection with SARS-CoV-2 and the more severe disease course compared to non-obese patients, respectively. Furthermore, it was suggested that the endocrine function of adipose tissue, including production of pro- and anti-inflammatory mediators (adipocytokines), may contribute to dysregulation of the immune response in COVID-19 patients with obesity (11-13). These hypotheses appear not to be correct. We showed that there are no relevant differences in circulating cytokines between obese and non-obese COVID-19 patients in the ICU. Later on during the pandemic, this result was confirmed in other studies with larger sample sizes that reported similar levels of clinical inflammatory markers at ICU admission between different BMI groups (14, 15). Furthermore, in collaboration with another research group from our centre, we showed that circulating concentrations of the adipocytokines leptin and adiponectin were associated with BMI in COVID-19 patients, but not with disease severity of mortality (16). Vice versa, circulating concentrations of the adipocytokine resistin were associated with disease severity and mortality, but not with BMI (16). These findings indicate that BMI-related differences in adipocytokine concentrations are not associated with COVID-19 severity or outcome. Collectively, the hypothesis that BMI-related differences in immunity explain why obesity is a risk factor for severe COVID-19 appears sufficiently rejected. Therefore, based on existing data, it is more likely that the increased risk of ICU admission in COVID-19 in patients with

obesity is explained by other factors than modulation of immunity. Since obesity is associated with an increased risk of acute respiratory distress syndrome (ARDS), difficult intubation, and mechanical ventilation in general (17-19), as well as in COVID-19 specifically (3, 20, 21), it might be that this higher risk is mainly driven by the mechanical effects of a higher BMI on the respiratory system.

In multiple conditions of patients in the ICU other than COVID-19, the obesity paradox is a well-known phenomenon. It is characterized by a paradoxical J-shaped association between BMI and mortality (22-25). Increased BMI is not only related to better ICU survival, but also with higher 4-year survival after discharge from the ICU (26). Although the presence of the obesity paradox has frequently been described, the exact underlying mechanisms remain unclear. In COVID-19, there are conflicting reports on whether the obesity paradox is present (27) or not (28, 29). Our investigation in a very large cohort of critically ill COVID-19 patients showed that BMI was not related to mortality, while in non-SARS-CoV-2 viral pneumonia and bacterial pneumonia ICU patients, a higher BMI was associated with lower mortality. This latter finding confirms the obesity paradox in ICU patients other than those suffering from COVID-19, and therefore provides confidence that the methodology used is sound. Based on our findings, one may argue that critically ill COVID-19 patients with obesity have a relative disadvantage compared to obese patients in the ICU with other respiratory tract infections. As discussed above, patients with obesity have a higher risk of developing respiratory insufficiency with need for IMV compared to patients with a normal weight (3, 20, 21), which explains the large proportion of obese COVID-19 patients in the ICU. Possibly, this could also explain the fact that the obesity paradox is present in other infectious diseases and not in COVID-19, since the reason for ICU admission for COVID-19 is predominantly severe respiratory insufficiency, not other organ failure. In other severe infectious diseases, it might be that obese patients are more likely admitted to the ICU because of isolated respiratory insufficiency (e.g. severe single organ failure), while non-obese patients are more likely admitted to the ICU with multi-organ failure, resulting in a worse prognosis for non-obese patients compared to obese patients. In COVID-19, the main reason for ICU admission is virtually always respiratory insufficiency, irrespective of BMI. Therefore, the baseline characteristics and thus prognosis of different BMI groups once admitted to the ICU are more comparable. Interestingly however, when dividing severe COVID-19 patients in different age groups, the obesity paradox was observed in patients below 45 years of age (30). This indicates that the protective role of a higher BMI may be present in young adult COVID-19 patients but not in older patients. Still, the exact reasons for obesity as a risk factor for severe COVID-19 and the fact that the obesity paradox is not ubiquitously present in COVID-19 require further elucidation.

ICU survivors often experience new or worsening of mental, physical and/or cognitive long-lasting symptoms, a condition known as the post-intensive care syndrome (PICS) (31). When the first patients recovered from COVID-19, it appeared that many of them also suffered from long-term symptoms and that a notable number of ICU survivors developed PICS (32-34). We demonstrate that patients with severe obesity were more likely to suffer from mental and physical impairments one year following ICU admission for COVID-19 compared to other BMI categories. Again, the underlying mechanisms are not clear, we speculate that patients with severe obesity experienced more traumatic situations during their stay in the ICU compared to other patients because of challenges in intubation, mechanical ventilation or switching between prone and supine position. Also, although no differences in comorbidities and pre-ICU symptoms were present between BMI categories in our study, overall condition and muscle strength were not included in these scores, while one may assume that these factors may be less favourable in patients with obesity. If so, this represents a disadvantage for obese patients during recovery from critical illness, leading to BMI-dependent difficulties in rehabilitation and a higher prevalence of long-term impairments.

Future perspectives

Apart from the COVID-19 pandemic, obesity has been an pandemic by itself for many decades, leading to ever-increasing health-related problems across the globe (35). The emergence of the COVID-19 pandemic clearly highlighted these problems. The increased risk of a severe disease course and complications in (non-ICU) patients with obesity is also apparent in many other diseases, and this problem will grow with the increasing prevalence of obesity. Health programs to prevent overweight and obesity are of most importance, because these have the potential to mitigate medical and psychological problems. It is also important that healthcare workers are aware of the increased health risks of obese patients. Additional studies into BMI-related differences in pathophysiological aspects and the influence of BMI on the disease course and clinical outcome may help to personalize treatment for COVID-19 and many other diseases. Also, additional advice related to the prevention of COVID-19 for people with obesity could be advocated, such as recommendations for strict quarantine, early vaccination, and wearing of face masks for this specific group of patients. In the undesirable event of a new infectious pandemic, it is crucial to focus on possible differences in disease course and complications between BMI categories, so that healthcare workers can act on this information. However, this may potentially also lead to ethical issues with regard to discrimination and exclusion of specific populations.

COVID-19 placed an enormous burden on worldwide healthcare systems in general and ICUs in particular. Gaining knowledge about this new disease was necessary to provide

optimal care and to develop effective treatments. In overcrowded ICUs, triage decision rules were made based on patient characteristics, medical history, and acute problems. BMI was one of the suggested characteristics to focus on during triage, based on the assumption that a high BMI was associated with impaired outcome. However, we demonstrate that BMI is no longer a risk factor for impaired outcomes once COVID-19 patients are admitted to the ICU. Thus, the spectrum of risk factors may differ between the general population, patients on the normal ward, and patients in the ICU. As a result, triage decisions solely based on data obtained in non-ICU patients are not justified. Developing a prognostic tool for critically ill COVID-19 patients based on available data at ICU admission would be helpful for prediction of clinical outcomes and in triage decision making. The rapid developments in the field of artificial intelligence could facilitate such an approach.

Part II: Biomarkers and phenotyping in critically ill COVID-19 patients

Summary

From the beginning of the pandemic, COVID-19 patients were suspected to be hyperinflamed and therefore COVID-19 was thought to induce a cytokine storm. A direct comparison with cytokine levels in other inflammatory diseases was however missing. In **chapter 6**, circulating cytokine levels during the first 48 hours in the ICU were compared between COVID-19 patients and patients admitted because of other conditions: (bacterial) septic shock with and without ARDS, out-of-hospital cardiac arrest and multiple trauma. Using the exact same methodology (i.e. cytokine assays and laboratory protocols), this study showed that cytokine levels of COVID-19 patients were significantly lower compared to bacterial septic shock patients, and were more comparable to patients who were admitted to the ICU following out-of-hospital cardiac arrest or multiple trauma, conditions not associated with a cytokine storm.

Procalcitonin (PCT) and CRP and are often used as biomarkers of systemic inflammation and to detect a secondary bacterial infection in ICU patients, however the accuracy of these biomarkers to detect an infection is likely influenced by the use of immunomodulatory drugs. As dexamethasone and tocilizumab became standard of care for critically ill COVID-19 patients, we investigated the kinetics of PCT and CRP in critically ill COVID-19 patients who were not treated with immunomodulatory drugs, as well as in patients who received dexamethasone with or without tocilizumab as standard COVID-19 therapy in **chapter 7**. The predictive value of PCT and CRP to detect secondary infections was nullified by dexamethasone treatment, whether or not in combination with tocilizumab. Also, cessation of dexamethasone treatment after 10 days resulted in an increase in PCT and CRP levels, which may be a false positive signal for the presence of a secondary bacterial infection.

Early work from China indicated that circulating concentrations of inflammatory biomarkers have prognostic value for mortality prediction in patients with COVID-19.

Pulmonary fibrosis is a dreaded complication of COVID-19. In **chapter 8** we describe which leukocytic transcriptome profiles are associated with the development of pulmonary fibroproliferation (PF) in critically ill COVID-19 patients. Also, as PF is treated with steroids, the influence of early dexamethasone treatment (as part of standard COVID-19 care) on the clinical course and outcomes of PF in critically ill COVID-19 patients was investigated. PF was diagnosed based on radiologic findings, worsening of ventilatory parameters and elevated levels of PF-biomarker N-terminal pro-peptide of type III procollagen (PIIINP). Compared to patients who did not develop PF, inflammatory, coagulation and neutrophil extracellular trap (NET)-related pathways were upregulated in patients who developed PF. Following initiation of corticosteroids to treat PF, the PF-related RNA profiles and PIIINP levels normalized, but ventilatory parameters and clinical outcomes remained impaired compared to patients without PF. Furthermore, early dexamethasone treatment for COVID-19 did not influence the incidence, clinical course or clinical outcomes of PF in these critically ill patients.

One sepsis patient may appear similar to another one, yet one may survive while the other dies. Also, therapeutic efficacy of various treatments are highly variable in sepsis due to patient heterogeneity. Based on these observations, phenotyping of sepsis patients to identify more homogenous subgroups has become a hot topic in the field. In **chapter 9**, four previously identified clinical phenotypes in sepsis patients (36) were applied to two cohorts of critically ill COVID-19 patients (before and after the introduction of dexamethasone as standard-of-care) and three non-COVID-19 severe sepsis cohorts (non-COVID-19 viral pneumonia, bacterial pneumonia, and bacterial sepsis of non-pulmonary origin). The distribution of the four phenotypes was comparable between the COVID-19 and non-COVID-19 viral pneumonia sepsis cohorts. The phenotype including patients with a lower incidence of comorbidities, younger age and a higher BMI showed the most favourable outcomes across all cohorts, while the phenotype with higher creatinine levels and white blood cell counts, higher incidence of comorbidities, and a higher proportion of males showed the highest mortality. Based on previous work in sepsis patients, this latter phenotype was also associated with the highest cytokine levels, and had the most pronounced improvement of survival following the initiation of dexamethasone treatment.

General discussion

Elevated inflammatory markers, such as PCT, CRP, ferritin and interleukin (IL)-6 were as-

sociated with a more severe disease course and impaired clinical outcomes in COVID-19 patients who were hospitalised early in the COVID-19 pandemic (37-41) and many studies described that severe COVID-19 was characterized by a cytokine storm (42-44). However, in Chapter 6 we showed that the increase in circulating cytokine levels of COVID-19 patients were less pronounced compared to patients with bacterial septic shock and that cytokine levels were comparable to patients with out-of-hospital cardiac arrest and multiple trauma. These findings indicate that severe COVID-19 is not typically characterized by a cytokine storm such as present in critically ill bacterial sepsis patients. Data of other studies confirmed our findings that COVID-19 in the ICU is not characterized by higher levels of circulating cytokines compared to ARDS and sepsis patients (45). Of interest, multiple studies showed that cytokine concentrations in endotracheal aspiration fluids were 10-10000 times higher than in the circulation (46-48). Therefore, pulmonary hyperinflammation clearly plays a role in the pathophysiology of COVID-19. Randomized controlled trials (RCTs) were initiated to investigate the effects of immunomodulatory therapy in COVID-19 and demonstrated that anti-inflammatory treatment with dexamethasone and tocilizumab has beneficial effects in COVID-19 patients requiring additional nasal oxygen therapy (49-51). The therapeutic efficacy of these immunomodulatory treatments also illustrate that hyperinflammation does play a relevant role in COVID-19 patients. Following publication of these large RCT's, these drugs were introduced as standard-of-care in severe COVID-19 patients. Although high levels of PCT and CRP were previously reported to have value in detecting secondary bacterial infections in critically ill COVID-19 patients (52), the current prognostic and diagnostic value of inflammatory markers was unclear since the introduction of immunomodulatory drugs as treatment for severe COVID-19. Although high levels of PCT and CRP were previously reported to have value in detecting secondary bacterial infections in critically ill COVID-19 patients (52), we showed that this value is nullified in critically ill COVID-19 patients treated with dexamethasone with or without tocilizumab co-therapy. Furthermore, we showed that baseline PCT and CRP levels were considerably lower in critically ill COVID-19 patients treated with these immunomodulatory drugs, which has also been observed by others (53). Noteworthy, the rebound in increase of PCT and CRP after cessation of dexamethasone therapy observed in our study could be misinterpreted as a sign of a secondary infection. Others have found that, although CRP levels were significantly lower, clinical signs of secondary infections were not influenced by tocilizumab treatment in critically ill COVID-19 patients (54). Based on these available data, clinicians should focus on clinical signs and cultures to diagnose secondary bacterial infections in COVID-19 patients who are treated with immunomodulatory drugs. Additionally, we also showed that although PCT, CRP and ferritin levels were significantly lower in patients treated with immunomodulatory drugs, mortality rates did not decrease proportionally. This suggests that the relationship between

these systemic inflammatory markers (and possibly inflammation in general) and clinical outcomes is largely lost by treating COVID-19 patients with immunomodulatory therapy. Clinicians are therefore not advised to use these markers for prognostication.

Many patients develop long-term respiratory sequelae following the acute phase of COVID-19, of which PF is considered as one of the important causative factors (55). Multiple risk factors for the development of PF have been described in COVID-19 patients, including admission to the ICU and need for mechanical ventilation (56). Since almost half of COVID-19 survivors developed PF (56), it is important to investigate the pathophysiology of PF and therapeutic options to prevent or treat PF in COVID-19 patients. Up till now, administration of corticosteroids is the only treatment available for PF in critically ill patients. Therefore, early treatment with immunomodulatory drugs as part of standard care in COVID-19 could be of influence on the incidence and clinical course of PF in these patients. We describe multiple molecular pathways which were upregulated in COVID-19 patients with PF who did not receive dexamethasone as standard-of-care, including inflammatory, coagulation and NET-formation pathways. Therefore, our work may provide leads for novel therapies targeting these pathways. For instance PDE4 could be an attractive target, as this gene was markedly upregulated in PF-patients and PDE4 inhibitors were shown to confer benefit in mice with PF (57). The effects of early dexamethasone therapy on these upregulated pathways has not been investigated yet, but would be useful in elucidating the pathophysiological processes of PF in COVID-19, as well as therapeutic options to prevent or reverse it. We describe that early dexamethasone as standard-of-care in COVID-19 did not influence the therapeutic efficacy of subsequent PF-treatment with prednisone on PIIINP kinetics and ventilatory parameters. Clinical outcomes were impaired in PF patients compared to patients who did not develop PF and were also not affected by early dexamethasone treatment. One could argue that more prolonged and/or a higher dosage of the early dexamethasone therapy in critically ill COVID-19 patients is required to prevent the development of PF. Along these lines, it has been described that doubling the dosage of 6 mg dexamethasone to 12 mg might indeed improve clinical outcomes in severe COVID-19 patients, while it does not appear to increase the incidence of serious adverse events (58). Others reported that a higher dose of dexamethasone (20 mg/day for 5 days followed by 10 mg/day for 5 days) improved the clinical disease course compared to the standard dose (6 mg/day for 10 days) (59). However, a larger randomized-controlled trial conducted later on revealed that this higher dosing regimen led to clinical worsening and an increased mortality risk compared to the standard-dose group in hospitalised COVID-19 with clinical hypoxia (60). Based on the latter study, it is therefore not recommended to increase the dose of dexamethasone in these patients. Furthermore, results of a retrospective study suggest that prolonga-

tion of corticosteroid treatment may also lead to increased mortality (61). Additional studies into specific COVID-19 patient groups that may benefit most from corticosteroid treatment is warranted, taking different phenotypes/subgroups into account.

Along these lines, it needs to be acknowledged that COVID-19 is a heterogenous disease with a wide variety of risk factors, symptoms and clinical features, although perhaps to a lesser extent than bacterial sepsis. Since dexamethasone and IL-6 inhibitors are the only proven effective drug therapies up till now, all critically ill COVID-19 patients are treated with these drugs. However, it appears plausible that the therapeutic efficacy might be much lower, or treatment may even be harmful, in patients who do not display hyperinflammation. Later studies indeed suggest that dexamethasone and tocilizumab are mainly effective in patients with signs of hyperinflammation (62-65). A better understanding of the heterogeneity of COVID-19 is therefore warranted to provide a more personalized treatment (66). Identifying different phenotypes could help in such an approach. When we applied previously identified sepsis phenotypes (36) to critically ill COVID-19 patients, we observed that several phenotype characteristics were shared between sepsis and COVID-19. However, there were also major differences, likely due to varying disease aetiology and pathophysiology. Although the finding that one phenotype benefited the most from dexamethasone therapy is promising, these findings indicate that the sepsis phenotypes are an imperfect fit to COVID-19 patients. During the last years, multiple studies were published in which COVID-19-specific phenotypes were developed (67-69). However, these studies used different techniques to cluster and were performed using data of relatively small sample sizes from single countries. Also, these data were collected during the very beginning of the pandemic (prior to the introduction of immunomodulatory treatment for COVID-19), which might lead to outdated conclusions. Finally, it has been shown that COVID-19 patients do not usually retain their initial phenotype during ICU stay (70). This illustrates that phenotypes are highly dynamic in nature. They are probably also influenced by other factors, such as the strain of the virus and a patient's vaccination status. Therefore, it will likely be difficult to develop a generalizable phenotype model that can be used in clinical practice.

Future perspectives

Although the finding that immunomodulatory therapy had beneficial effects in severe COVID-19 patients was a major breakthrough during the COVID-19 pandemic, it has also resulted in new diagnostic, therapeutic and scientific challenges. Because most diseases and syndromes in the ICU are heterogeneous and multi-factorial, it is recognized that some treatments may be effective in a subgroup of patients, while it may be harmful in another subgroup of patients suffering from the same disease. From the moment that dexamethasone

and IL-6 inhibitors were proven to be effective in treating COVID-19 and immediately became the standard of care, it was considered unethical to withhold these treatments in severe COVID-19 patients. Therefore, in many publications describing the effects of immunomodulatory therapy in COVID-19, the control group consisted of patients who were suffering from severe COVID-19 in the period before the introduction of dexamethasone and tocilizumab. This led to study limitations because different study groups were included during different periods of time. As a result, time was an important factor of bias in these studies, because of the occurrence of viral mutations and increasing knowledge of and experience with (treatment of) this disease and thus overall improvement of care. Nevertheless, although the use of a historical control group is not ideal, these studies provided indications of which patients would or would not benefit from different therapies. In COVID-19, it is of value to identify new COVID-19-specific phenotypes in critically ill patients and investigate whether there is a subgroup of patients who do not benefit, or even experience harm, from immunomodulatory therapy. Also, it would be of clinical benefit to investigate the moment (if present at all) at which PCT and CRP are again of value in detecting secondary bacterial infections in COVID-19 patients after cessation of treatment with dexamethasone and tocilizumab. Especially for the latter therapy this is challenging, as it has a very long half-life.

Part III: Immunomodulatory treatment in critically ill COVID-19 patients

Summary

Another immunomodulating compound that sparked interest for the treatment of COVID-19 patients is the IL-1 receptor antagonist anakinra. **Chapter 10** provides a letter written in response to a publication describing the effects of anakinra in critically ill COVID-19 patients (71). In that study, patient characteristics of the intervention group and the (historical) control group differed significantly, with the control group displaying a much less favourable profile. We therefore propose to at least use propensity score matching when historical controls are used to confirm the described beneficial effects of anakinra in COVID-19.

In accordance, in our Radboudumc cohort, the effects of anakinra in critically ill COVID-19 patients were described and further supported by performing propensity score matching in **chapter 11**. Patients that showed persistent hyperinflammation during their ICU stay were treated with anakinra. Clinical markers of hyperinflammation, such as body temperature, white blood cell counts and plasma levels of ferritin and PCT reduced following initiation of anakinra treatment. However, differences in clinical outcomes between patients who were or were not treated with anakinra could not be detected, although the study was underpowered for these endpoints.

The focus of COVID-19 treatment has largely been on suppression of the immune response. However, in a specific subgroup of patients, immunosuppression may already be present. In these patients, anti-inflammatory treatment might not be of benefit and even lead to secondary infectious complications. In these cases, immunostimulatory treatment can be considered. **Chapter 12** reports a case series of five renal transplant patients who developed severe COVID-19 and were admitted to the ICU. Because of an impaired cellular immune response and the inability to clear the virus these patients were treated with the immunostimulatory drug interferon-gamma (IFN-γ). Following IFN-γ treatment these patients cleared the SARS-CoV-2 virus while none of them showed signs of hyperinflammation or transplant rejection following initiation of this treatment. Four of these patients recovered.

General discussion

The detrimental role of inflammatory processes in the pathophysiology of severe COVID-19 has extensively been studied since the early beginning of the COVID-19 pandemic. Therefore, the use of immunomodulatory drugs was suggested to attenuate the hyperinflammatory response and prevent a severe disease course. In severe bacterial sepsis, the effects of anakinra were previously investigated in a randomized placebo-controlled trial, and overall no effects on clinical outcomes were found (72). However, nearly two decades later, a post-hoc analysis was performed to investigate the effects of anakinra in a subgroup of sepsis patients with signs of the macrophage activation syndrome (MAS) (73). MAS comprises a hyperinflammatory state characterized by a severe cytokine storm and high circulating levels of ferritin. In this study, MAS was diagnosed based on the presence of both hepatobiliary dysfunction (high liver enzymes/prothrombin time) and disseminated intravascular coagulation (high prothrombin time/partial thromboplastin time and low platelet counts), but no data on ferritin levels were available. Intriguingly, in this post-hoc analysis anakinra conferred a large survival benefit in the subgroup of MAS patients. Accordingly, it was suggested to treat severe COVID-19 patients with signs of severe hyperinflammation or MAS with anakinra. Subsequently, multiple cohort studies were performed, which reported promising effects of anakinra in critically ill COVID-19 patients, but these studies had important limitations. In chapter 11, we proposed to perform propensity score matching to confirm the promising results of anakinra treatment in COVID-19. In chapter 12, we described that anakinra was effective in reducing clinical signs of hyperinflammation, a result which was confirmed also following propensity score matching. However, based on small cohort studies, no conclusions can be drawn on the effects of anakinra on clinical outcomes. Therefore, meta-analyses were performed using data of multiple of these small cohort studies using historical controls (74, 75). Perhaps not surprising in light of the positive effects of anakinra in the majority of the aforementioned small studies, both of these meta-analyses reported

reduced mortality risk in hospitalised COVID-19 patients who were treated with anakinra. Analogous to the aforementioned post-hoc analysis in sepsis patients, the most favourable effects were observed in patients with high levels of CRP, suggesting that anakinra treatment particularly benefits COVID-19 patients with (signs of) hyperinflammation. Of interest, one prospective randomized-controlled trial showed positive results of treatment with anakinra in hyperinflamed COVID-19 patients who were admitted to the ward (pre-ICU) (76). In this trial, hyperinflammation/disease severity was indicated using plasma levels of soluble urokinase plasminogen activator receptor (suPAR), which is currently not a commonly used marker in the clinic. Nevertheless, since SOFA scores decreased significantly and mortality was lower in anakinra-treated patients, this study indicates that anakinra might be useful in pre-ICU COVID-19 patients with signs of hyperinflammation. In contrast, in the Randomized Embedded Multifactorial Adaptive Platform (REMAP-CAP) trial, which did not use any form of enrichment to select hyperinflamed patients, anakinra was not effective in reducing mortality or disease severity in critically ill COVID-19 patients (77).

Treatment of immunocompromised patients with COVID-19 is a difficult puzzle to solve, as the standard-of-care immunosuppressive therapies (i.e. dexamethasone and tocilizumab) may (further) impair clearance of the virus and predispose these patients to secondary infections. In chapter 13, we described five patients with severely impaired immunity, no clinical improvement and a poor clearance of SARS-CoV-2. IFN-γ therapy rapidly resulted in a decline of the SARS-CoV-2 load and clinical improvement. This case series illustrates that, although immunosuppressive treatment is effective in the majority of critically ill COVID-19 patients, it might be beneficial to do the opposite and boost immunity in specific subgroups of patients. Up till now, no other studies were published describing the use of IFN-γ treatment in COVID-19 patients. Addressing other studied treatments for COVID-19, such as convalescent plasma, remdesivir, and hydrochloroquine are beyond the scope of this thesis.

Future perspectives

Although immunomodulatory treatments have proven to be beneficial in COVID-19, prevention remains key. In this respect, the introduction of SARS-CoV-2 vaccines represents the greatest medical advance achieved since the start of the pandemic. Upon widespread vaccination, the number of COVID-19 patients in hospitals across the globe has decreased tremendously (78). Vaccination may also reduce transmission of SARS-CoV-2, especially in people who also received booster vaccinations and/or were previously infected (79). At the moment of writing, COVID-19 is still present both in and outside of hospitals and the SARS-CoV-2 virus keeps mutating. Therefore, comparable to influenza, vaccinating against SARS-CoV-2 may be important for a very long period, especially in people who are at high risk of developing severe COVID-19.

Regarding the future of immunomodulatory therapy for patients with severe COVID-19 in the ICU, it is important to recognize that the beneficial effects of dexamethasone and tocilizumab in severe COVID-19 represent the first successful application of immunomodulatory therapy in a form of sepsis that has become standard-of-care. This success story is probably due to the fact that severe COVID-19 is less heterogenous than 'classic' sepsis, due to the involvement of a single pathogen and, in most cases, failure of a single organ, the lungs. Nevertheless, to maximize the chances of beneficial treatment responses, it will still be of additional value to identify subgroups with specific immunological phenotypes. In the future, artificial intelligence approaches will likely prove to be instrumental in identifying who benefits from immunomodulatory therapies and who does not. Based on the promising results presented in our case series, IFN-γ or other immunostimulatory drugs should be considered for the treatment of immunocompromised patients with COVID-19, although more elaborate studies are required to determine their place in the therapeutic arsenal available for COVID-19 and especially longer-term effect on e.g. renal function in kidney transplantation patients is warranted. Furthermore, similar to patients with bacterial sepsis, a subgroup of patients with no history of a compromised immune system may develop a severely immunosuppressed phenotype during the COVID-19 disease course. Future studies should evaluate whether these patients may also benefit from immunostimulatory treatments.

References

1. Ho JSY, Fernando DI, Chan MY, Sia CH. Obesity in COVID-19: A Systematic Review and Meta-analysis. Ann Acad Med Singap. 2020;49(12):996-1008.
2. Freuer D, Linseisen J, Meisinger C. Impact of body composition on COVID-19 susceptibility and severity: A two-sample multivariable Mendelian randomization study. Metabolism. 2021;118:154732.
3. Yang Y, Wang L, Liu J, Fu S, Zhou L, Wang Y. Obesity or increased body mass index and the risk of severe outcomes in patients with COVID-19: A protocol for systematic review and meta-analysis. Medicine (Baltimore). 2022;101(1):e28499.
4. Onder G, Palmieri L, Vanacore N, Giuliano M, Brusaferro S, the Italian National Institute of Health C-MG. Nonrespiratory Complications and Obesity in Patients Dying with COVID-19 in Italy. Obesity. 2021;29(1):20-3.
5. Jayanama K, Srichatrapimuk S, Thammavaranucupt K, Kirdlarp S, Suppadungsuk S, Wongsinin T, et al. The association between body mass index and severity of Coronavirus Disease 2019 (COVID-19): A cohort study. PLOS ONE. 2021;16(2):e0247023.
6. Page-Wilson G, Arakawa R, Nemeth S, Bell F, Girvin Z, Tuohy M-C, et al. Obesity is independently associated with septic shock, renal complications, and mortality in a multiracial patient cohort hospitalized with COVID-19. PLOS ONE. 2021;16(8):e0255811.
7. Hendren NS, de Lemos JA, Ayers C, Das SR, Rao A, Carter S, et al. Association of Body Mass Index and Age With Morbidity and Mortality in Patients Hospitalized With COVID-19. Circulation. 2021;143(2):135-44.
8. Wang SY, Singh A, Eder MD, Vadlamani L, Lee AI, Chun HJ, et al. Association of obesity with venous thromboembolism and myocardial injury in COVID-19. Obesity Research & Clinical Practice. 2021;15(5):512-4.
9. Al-Benna S. Association of high level gene expression of ACE2 in adipose tissue with mortality of COVID-19 infection in obese patients. Obes Med. 2020;19:100283.
10. Li MY, Li L, Zhang Y, Wang XS. Expression of the SARS-CoV-2 cell receptor gene ACE2 in a wide variety of human tissues. Infect Dis Poverty. 2020;9(1):45.
11. Muscogiuri G, Pugliese G, Laudisio D, Castellucci B, Barrea L, Savastano S, et al. The impact of obesity on immune response to infection: Plausible mechanisms and outcomes. Obes Rev. 2021;22(6):e13216.
12. Fantuzzi G. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol. 2005;115(5):911-9; quiz 20.
13. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol. 2011;11(2):85-97.
14. Friedman AN, Guirguis J, Kapoor R, Gupta S, Leaf DE, Timsina LR, et al. Obesity, inflammatory and thrombotic markers, and major clinical outcomes in critically ill patients with COVID-19 in the US. Obesity. 2021;29(10):1719-30.
15. Wolf M, Alladina J, Navarrete-Welton A, Shoults B, Brait K, Ziehr D, et al. Obesity and Critical Illness in COVID-19: Respiratory Pathophysiology. Obesity (Silver Spring). 2021;29(5):870-8.
16. 16. de Nooijer AH, Kooistra EJ, Grondman I, Janssen NAF, Joosten LAB, van de Veerdonk FL, et al. Adipocytokine plasma concentrations reflect influence of inflammation but not body mass index (BMI) on clinical outcomes of COVID-19 patients: A prospective observational study from the Netherlands. Clinical Obesity. 2023;13(2):e12568.

17. Gong MN, Bajwa EK, Thompson BT, Christiani DC. Body mass index is associated with the development of acute respiratory distress syndrome. Thorax. 2010;65(1):44-50.
18. Tafelski S, Yi H, Ismaeel F, Krannich A, Spies C, Nachtigall I. Obesity in critically ill patients is associated with increased need of mechanical ventilation but not with mortality. Journal of Infection and Public Health. 2016;9(5):577-85.
19. De Jong A, Molinari N, Pouzeratte Y, Verzilli D, Chanques G, Jung B, et al. Difficult intubation in obese patients: incidence, risk factors, and complications in the operating theatre and in intensive care units. Br J Anaesth. 2015;114(2):297-306.
20. Simonnet A, Chetboun M, Poissy J, Raverdy V, Noulette J, Duhamel A, et al. High Prevalence of Obesity in Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) Requiring Invasive Mechanical Ventilation. Obesity (Silver Spring). 2020;28(7):1195-9.
21. Lemyze M, Courageux N, Maladobry T, Arumadura C, Pauquet P, Orfi A, et al. Implications of Obesity for the Management of Severe Coronavirus Disease 2019 Pneumonia. Crit Care Med. 2020;48(9):e761-e7.
22. Pickkers P, de Keizer N, Dusseljee J, Weerheijm D, van der Hoeven JG, Peek N. Body mass index is associated with hospital mortality in critically ill patients: an observational cohort study. Crit Care Med. 2013;41(8):1878-83.
23. Zhi G, Xin W, Ying W, Guohong X, Shuying L. "Obesity Paradox" in Acute Respiratory Distress Syndrome: Asystematic Review and Meta-Analysis. PLoS One. 2016;11(9):e0163677.
24. Naderi N, Kleine CE, Park C, Hsiung JT, Soohoo M, Tantisattamo E, et al. Obesity Paradox in Advanced Kidney Disease: From Bedside to the Bench. Prog Cardiovasc Dis. 2018;61(2):168-81.
25. Sharma A, Lavie CJ, Borer JS, Vallakati A, Goel S, Lopez-Jimenez F, et al. Meta-analysis of the relation of body mass index to all-cause and cardiovascular mortality and hospitalization in patients with chronic heart failure. Am J Cardiol. 2015;115(10):1428-34.
26. Zhou D, Wang C, Lin Q, Li T. The obesity paradox for survivors of critically ill patients. Critical Care. 2022;26(1):198.
27. Dana R, Bannay A, Bourst P, Ziegler C, Losser MR, Gibot S, et al. Obesity and mortality in critically ill COVID-19 patients with respiratory failure. Int J Obes (Lond). 2021;45(9):2028-37.
28. Biscarini S, Colaneri M, Ludovisi S, Seminari E, Pieri TC, Valsecchi P, et al. The obesity paradox: Analysis from the SMAtteo COvid-19 REgistry (SMACORE) cohort. Nutr Metab Cardiovasc Dis. 2020;30(11):1920-5.
29. Abumayyaleh M, Núñez Gil IJ, El-Battrawy I, Estrada V, Becerra-Muñoz VM, Aparisi A, et al. Does there exist an obesity paradox in COVID-19? Insights of the international HOPE-COVID-19-registry. Obesity Research & Clinical Practice. 2021;15(3):275-80.
30. den Uil CA, Termorshuizen F, Rietdijk WJR, Sablerolles RSG, van der Kuy HPM, Haas LEM, et al. Age Moderates the Effect of Obesity on Mortality Risk in Critically Ill Patients With COVID-19: A Nationwide Observational Cohort Study. Crit Care Med. 2023;51(4):484-91.
31. Geense WW, Zegers M, Peters MAA, Ewalds E, Simons KS, Vermeulen H, et al. New Physical, Mental, and Cognitive Problems 1 Year after ICU Admission: A Prospective Multicenter Study. Am J Respir Crit Care Med. 2021;203(12):1512-21.
32. Heesakkers H, van der Hoeven JG, Corsten S, Janssen I, Ewalds E, Simons KS, et al. Clinical Outcomes Among Patients With 1-Year Survival Following Intensive Care Unit Treatment for COVID-19. Jama. 2022;327(6):559-65.

33. Nanwani-Nanwani K, López-Pérez L, Giménez-Esparza C, Ruiz-Barranco I, Carrillo E, Arellano MS, et al. Prevalence of post-intensive care syndrome in mechanically ventilated patients with COVID-19. Sci Rep. 2022;12(1):7977.
34. Ceban F, Ling S, Lui LMW, Lee Y, Gill H, Teopiz KM, et al. Fatigue and cognitive impairment in Post-COVID-19 Syndrome: A systematic review and meta-analysis. Brain Behav Immun. 2022;101:93-135.
35. W.H.O. Obesity: preventing and managing the global epidemic: World Health Organization; 2000.
36. Seymour CW, Kennedy JN, Wang S, Chang C-CH, Elliott CF, Xu Z, et al. Derivation, Validation, and Potential Treatment Implications of Novel Clinical Phenotypes for Sepsis. JAMA. 2019;321(20):2003-17.
37. Huang I, Pranata R, Lim MA, Oehadian A, Alisjahbana B. C-reactive protein, procalcitonin, D-dimer, and ferritin in severe coronavirus disease-2019: a meta-analysis. Therapeutic Advances in Respiratory Disease. 2020;14:1753466620937175.
38. Coomes EA, Haghbayan H. Interleukin-6 in Covid-19: A systematic review and meta-analysis. Rev Med Virol. 2020;30(6):1-9.
39. Meng Y, Wang J, Wen K, Da W, Yang K, Zhou S, et al. Clinical Features and Laboratory Examination to Identify Severe Patients with COVID-19: A Systematic Review and Meta-Analysis. Biomed Res Int. 2021;2021:6671291.
40. Katzenschlager S, Zimmer AJ, Gottschalk C, Grafeneder J, Schmitz S, Kraker S, et al. Can we predict the severe course of COVID-19 - a systematic review and meta-analysis of indicators of clinical outcome? PLoS One. 2021;16(7):e0255154.
41. 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.
42. Zhang C, Wu Z, Li JW, Zhao H, Wang GQ. Cytokine release syndrome in severe COVID-19: interleukin-6 receptor antagonist tocilizumab may be the key to reduce mortality. Int J Antimicrob Agents. 2020;55(5):105954.
43. Zhou Y, Fu B, Zheng X, Wang D, Zhao C, Qi Y, et al. Pathogenic T-cells and inflammatory monocytes incite inflammatory storms in severe COVID-19 patients. Natl Sci Rev. 2020;7(6):998-1002.
44. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395(10229):1033-4.
45. Remy KE, Mazer M, Striker DA, Ellebedy AH, Walton AH, Unsinger J, et al. Severe immunosuppression and not a cytokine storm characterizes COVID-19 infections. JCI Insight. 2020;5(17).
46. Zhang W, Chua B, Selva K, Kedzierski L, Ashhurst T, Haycroft E, et al. Immune responses in COVID-19 respiratory tract and blood reveal mechanisms of disease severity. Res Sq. 2021.
47. Jouan Y, Baranek T, Si-Tahar M, Paget C, Guillon A. Lung compartmentalization of inflammatory biomarkers in COVID-19-related ARDS. Crit Care. 2021;25(1):120.
48. Bendib I, Beldi-Ferchiou A, Schlemmer F, Surenaud M, Maitre B, Plonquet A, et al. Alveolar compartmentalization of inflammatory and immune cell biomarkers in pneumonia-related ARDS. Critical care (London, England). 2021;25(1):23-.
49. Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, et al. Dexamethasone in Hospitalized Patients with Covid-19. N Engl J Med. 2021;384(8):693-704.

50. Gordon AC, Mouncey PR, Al-Beidh F, Rowan KM, Nichol AD, Arabi YM, et al. Interleukin-6 Receptor Antagonists in Critically Ill Patients with Covid-19. N Engl J Med. 2021;384(16):1491-502.
51. RecoveryCollaborativeGroup. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. Lancet (London, England). 2021;397(10285):1637-45.
52. van Berkel M, Kox M, Frenzel T, Pickkers P, Schouten J. Biomarkers for antimicrobial stewardship: a reappraisal in COVID-19 times? Crit Care. 2020;24(1):600.
53. Szakmany T, Tuckwell W, Harte E, Wetherall N, Ramachandran S, Price S, et al. Differences in Inflammatory Marker Kinetics between the First and Second Wave of COVID-19 Patients Admitted to the ICU: A Retrospective, Single-Center Study. J Clin Med. 2021;10(15).
54. Taramasso L, Magnasco L, Portunato F, Briano F, Vena A, Giacobbe DR, et al. Clinical presentation of secondary infectious complications in COVID-19 patients in intensive care unit treated with tocilizumab or standard of care. European Journal of Internal Medicine. 2021;94:39-44.
55. Ahmed OF, Kakamad FH, Hama Amin BJ, Abdullah BA, Hassan MN, Salih RQ, et al. Post COVID-19 pulmonary complications; a single center experience. Ann Med Surg (Lond). 2021;72:103052.
56. Hama Amin BJ, Kakamad FH, Ahmed GS, Ahmed SF, Abdulla BA, Mohammed SH, et al. Post COVID-19 pulmonary fibrosis; a meta-analysis study. Ann Med Surg (Lond). 2022;77:103590.
57. Udalov S, Dumitrascu R, Pullamsetti SS, Al-tamari HM, Weissmann N, Ghofrani HA, et al. Effects of phosphodiesterase 4 inhibition on bleomycin-induced pulmonary fibrosis in mice. BMC Pulm Med. 2010;10:26.
58. Granholm A, Munch MW, Myatra SN, Vijayaraghavan BKT, Cronhjort M, Wahlin RR, et al. Dexamethasone 12 mg versus 6 mg for patients with COVID-19 and severe hypoxaemia: a pre-planned, secondary Bayesian analysis of the COVID STEROID 2 trial. Intensive Care Med. 2021:1-11.
59. Taboada M, Rodríguez N, Varela PM, Rodríguez MT, Abelleira R, González A, et al. Effect of high versus low dose of dexamethasone on clinical worsening in patients hospitalised with moderate or severe COVID-19 pneumonia: an open-label, randomised clinical trial. Eur Respir J. 2022;60(2).
60. Abani O, Abbas A, Abbas F, Abbas J, Abbas K, Abbas M, et al. Higher dose corticosteroids in patients admitted to hospital with COVID-19 who are hypoxic but not requiring ventilatory support (RECOVERY): a randomised, controlled, open-label, platform trial. The Lancet. 2023;401(10387):1499-507.
61. Viana MV, Pellegrini JAS, Perez AV, Schwarz P, da Silva D, Teixeira C, et al. Association between prolonged corticosteroids use in COVID-19 and increased mortality in hospitalized patients: a retrospective study with inverse probability of treatment weighting analysis. Critical Care. 2023;27(1):143.
62. Galván-Román JM, Rodríguez-García SC, Roy-Vallejo E, Marcos-Jiménez A, Sánchez-Alonso S, Fernández-Díaz C, et al. IL-6 serum levels predict severity and response to tocilizumab in COVID-19: An observational study. J Allergy Clin Immunol. 2021;147(1):72-80.e8.
63. Martínez-Sanz J, Muriel A, Ron R, Herrera S, Pérez-Molina JA, Moreno S, et al. Effects of tocilizumab on mortality in hospitalized patients with COVID-19: a multicentre cohort study. Clinical Microbiology and Infection. 2021;27(2):238-43.

64. Pagnesi M, Inciardi RM, Lombardi CM, Agostoni P, Ameri P, Barbieri L, et al. Determinants of the protective effect of glucocorticoids on mortality in hospitalized patients with COVID-19: Insights from the Cardio-COVID-Italy multicenter study. International Journal of Infectious Diseases. 2021;108:270-3.
65. Keller MJ, Kitsis EA, Arora S, Chen J-T, Agarwal S, Ross MJ, et al. Effect of Systemic Glucocorticoids on Mortality or Mechanical Ventilation in Patients With COVID-19. Journal of Hospital Medicine. 2020;15(8):489-93.
66. Bakkerus L, Pickkers P. Personalized medicine in COVID-19. Intensive Care Med. 2022;48(11):1607-10.
67. Gutiérrez-Gutiérrez B, Del Toro MD, Borobia AM, Carcas A, Jarrín I, Yllescas M, et al. Identification and validation of clinical phenotypes with prognostic implications in patients admitted to hospital with COVID-19: a multicentre cohort study. Lancet Infect Dis. 2021;21(6):783-92.
68. Dupont T, Caillat-Zucman S, Fremeaux-Bacchi V, Morin F, Lengliné E, Darmon M, et al. Identification of Distinct Immunophenotypes in Critically Ill Coronavirus Disease 2019 Patients. Chest. 2021;159(5):1884-93.
69. Lusczek ER, Ingraham NE, Karam BS, Proper J, Siegel L, Helgeson ES, et al. Characterizing COVID-19 clinical phenotypes and associated comorbidities and complication profiles. PLoS One. 2021;16(3):e0248956.
70. Siepel S, Dam TA, Fleuren LM, Girbes ARJ, Hoogendoorn M, Thoral PJ, et al. Evolution of Clinical Phenotypes of COVID-19 Patients During Intensive Care Treatment: An Unsupervised Machine Learning Analysis. J Intensive Care Med. 2023:8850666231153393.
71. Cavalli G, De Luca G, Campochiaro C, Della-Torre E, Ripa M, Canetti D, et al. Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study. Lancet Rheumatol. 2020;2(6):e325-e31.
72. Opal SM, Fisher CJ, Dhainaut J-FA, Vincent J-L, Brase R, Lowry SF, et al. Confirmatory interleukin-1 receptor antagonist trial in severe sepsis: A phase III, randomized, doubleblind, placebo-controlled, multicenter trial. Critical Care Medicine. 1997;25(7).
73. Shakoory B, Carcillo JA, Chatham WW, Amdur RL, Zhao H, Dinarello CA, et al. Interleukin-1 Receptor Blockade Is Associated With Reduced Mortality in Sepsis Patients With Features of Macrophage Activation Syndrome: Reanalysis of a Prior Phase III Trial. Crit Care Med. 2016;44(2):275-81.
74. Naveed Z, Sarwar M, Ali Z, Saeed D, Choudhry K, Sarfraz A, et al. Anakinra treatment efficacy in reduction of inflammatory biomarkers in COVID-19 patients: A meta-analysis. J Clin Lab Anal. 2022;36(6):e24434.
75. Kyriazopoulou E, Huet T, Cavalli G, Gori A, Kyprianou M, Pickkers P, et al. Effect of anakinra on mortality in patients with COVID-19: a systematic review and patient-level meta-analysis. The Lancet Rheumatology. 2021;3(10):e690-e7.
76. Kyriazopoulou E, Poulakou G, Milionis H, Metallidis S, Adamis G, Tsiakos K, et al. Early treatment of COVID-19 with anakinra guided by soluble urokinase plasminogen receptor plasma levels: a double-blind, randomized controlled phase 3 trial. Nat Med. 2021;27(10):1752-60.

77. Derde LPG. Effectiveness of Tocilizumab, Sarilumab, and Anakinra for critically ill patients with COVID-19 The REMAP-CAP COVID-19 Immune Modulation Therapy Domain Randomized Clinical Trial. medRxiv. 2021:2021.06.18.21259133.
78. Zheng C, Shao W, Chen X, Zhang B, Wang G, Zhang W. Real-world effectiveness of COVID-19 vaccines: a literature review and meta-analysis. International Journal of Infectious Diseases. 2022;114:252-60.
79. Tan ST, Kwan AT, Rodríguez-Barraquer I, Singer BJ, Park HJ, Lewnard JA, et al. Infectiousness of SARS-CoV-2 breakthrough infections and reinfections during the Omicron wave. Nature Medicine. 2023.

NEDERLANDSE SAMENVATTING

Dit proefschrift beschrijft verschillende aspecten van COVID-19 bij patiënten op de intensive care (IC), met specifieke aandacht voor (I) obesitas als risicofactor voor klinische uitkomsten in het ziekenhuis en langetermijngevolgen, (II) het gebruik van biomarkers en fenotypering, en (III) immuunmodulerende behandelingen. In **hoofdstuk 1** wordt een algemene introductie gegeven over de onderwerpen van dit proefschrift, waarbij het wereldwijde belang van wetenschappelijk onderzoek naar COVID-19, veroorzaakt door het Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), wordt beschreven.

Deel I: Obesitas bij ernstig zieke COVID-19 patiënten

In **hoofdstuk 2** werd onderzocht of er verschillen zijn in de ontstekingsreactie, ademhalingsfunctie en klinische uitkomsten in het ziekenhuis tussen ernstig zieke COVID-19-patiënten met en zonder obesitas. Dit onderzoek werd uitgevoerd voordat medicijnen die het immuunsysteem beïnvloeden standaard werden toegepast bij patiënten met ernstige COVID-19. Bij alle 67 onderzochte COVID-19 patiënten werd gedurende de eerste tien dagen op de IC een afname van de hoeveelheid ontstekingseiwitten in het bloed waargenomen ten opzichte van de hoeveelheid bij opname, maar er waren daarbij geen verschillen tussen patiënten met en zonder obesitas. Er werd ook geen verband gevonden tussen de body mass index (BMI) en de hoeveelheid ontstekingseiwitten in het bloed bij opname op de IC. Daarnaast waren het verloop van klinische en ademhalingsparameters, de duur van beademing en opname op de IC, en de overleving vergelijkbaar tussen patiënten met en zonder obesitas. Hieruit concluderen we dat er, ondanks de beperkte omvang van de studie, geen aanwijzingen zijn voor relevante verschillen in de ontstekingsreactie, ademhalingsparameters en klinische uitkomsten in het ziekenhuis tussen COVID-19-patiënten met en zonder obesitas op de IC.

In **hoofdstuk 3** beschrijven we de resultaten van een onderzoek bij 35.506 ernstig zieke patiënten opgenomen op 82 IC's in Nederland. Deze patiënten hadden COVID-19, andere virale of bacteriële longontstekingen, of meervoudig trauma. We onderzochten het BMI verband houdt met klinische uitkomsten in het ziekenhuis bij deze patiëntgroepen. Vergeleken met patiënten met andere virale of bacteriële longontstekingen, of meervoudig trauma, hadden COVID-19-patiënten vaker obesitas en vertoonden ze meer ademhalingsproblemen. Verder waren de klinische ziekenhuisuitkomsten van COVID-19-patiënten minder gunstig, met een langere opnameduur op de IC en hogere sterftecijfers. Binnen de groepen patiënten met andere virale en bacteriële longontstekingen vonden we een lager sterftecijfer bij patiënten met obesitas dan bij patiënten met een normaal gewicht, een fenomeen wat bekend staat als de obesitas-paradox. Bij COVID-19-patiënten werd echter

geen verband gevonden tussen BMI en sterfte, wat aangeeft dat de obesitas-paradox hier niet van toepassing is. Kortom, hoewel obesitas het risico op het ontwikkelen van ernstige COVID-19 vergroot, lijkt het geen invloed te hebben op de uitkomst als de patiënt eenmaal is opgenomen op de IC.

In **hoofdstuk 4** hebben we onderzocht of COVID-19-patiënten met obesitas na hun IC-opname meer kans lopen op langetermijngevolgen dan patiënten met een normaal BMI. Symptomen zoals fysieke, mentale en cognitieve beperkingen werden op drie momenten onderzocht bij patiënten van verschillende BMI-categorieën: vóór de IC-opname, en 3 en 12 maanden na de IC-opname. Vóórdat patiënten COVID-19 kregen, waren er geen significante verschillen in deze symptomen tussen de verschillende BMI-categorieën. Echter, patiënten met ernstige obesitas (BMI> 35 kg/m^2) hadden 12 maanden na hun IC-opname meer fysieke en mentale beperkingen. Dit benadrukt het belang om bij COVID-19-patienten met obesitas langetermijngevolgen goed in de gaten te houden na de IC-opname.

Hoofdstuk 5 is een reviewartikel waarin we verschillende aspecten met betrekking tot de rol van obesitas in de ontwikkeling en het klinische beloop van COVID-19 op de IC uiteenzetten. Obesitas is een bekende risicofactor voor het ontwikkelen van ernstige COVID-19 en langetermijngevolgen, maar de onderliggende mechanismen blijven onduidelijk. In tegenstelling tot het hogere risico op IC-opname van COVID-19-patiënten met obesitas, lijken er na opname op de IC geen duidelijke verschillen in klinische uitkomsten te zijn tussen COVID-19-patiënten met verschillende BMI. Een afwijkende ontstekingsreactie wordt vaak als verklaring aangedragen voor het feit dat patiënten met obesitas vaker op de IC terecht komen dan patiënten zonder obesitas, maar tot nu toe is er geen bewijs voor deze hypothese. Verder dringen we erop aan dat goede monitoring van vitale parameters en algehele conditie extra belangrijk is voor de behandeling en prognose van patiënten met COVID-19 en obesitas.

Deel II: Biomarkers en fenotypering bij ernstig zieke COVID-19 patiënten

Vanaf het begin van de pandemie bestaat het vermoeden dat er bij ernstig zieke COVID-19-patiënten een sterke ontstekingsreactie speelt, een zogenaamde “cytokinestorm”. Hier was echter geen bewijs voor. In **hoofdstuk 6** hebben we de hoeveelheid ontstekingseiwitten (biomarkers die een cytokinestorm aantonen) in het bloed gemeten gedurende de eerste 48 uur na IC-opname bij zowel COVID-19-patiënten als patiënten met andere ernstige aandoeningen, zoals septische shock, post-reanimatie en meervoudig trauma. We vonden dat de hoeveelheid ontstekingseiwitten juist aanmerkelijk lager was bij CO-

VID-19-patiënten dan bij patiënten met septische shock. De hoeveelheid die we vonden bij COVID-19 patiënten was meer vergelijkbaar met die bij post-reanimatiepatiënten en patienten met meervoudig trauma, aandoeningen waar geen sprake is van een cytokinestorm. Procalcitonine (PCT) en C-reactive protein (CRP) worden vaak gebruikt als biomarkers om bacteriële infecties bij IC-patiënten aan te tonen. In **hoofdstuk 7** onderzochten we het beloop van PCT en CRP in het bloed van ernstig zieke COVID-19-patiënten die al dan niet behandeld werden met dexamethason en tocilizumab, twee medicijnen die het immuunsysteem onderdrukken. Het bleek dat deze biomarkers sterk onderdrukt werden door deze middelen, waardoor ze hun waarde voor het opsporen van bacteriële infecties verliezen.

In **hoofdstuk 8** beschrijven we een ander soort biomarkers, namelijk genexpressieprofielen, die geassocieerd zijn met de ontwikkeling van pulmonale fibrose (PF) bij ernstig zieke COVID-19-patiënten. Verder onderzochten we hoe behandeling met corticosteroiden (medicijnen die het immuunsysteem onderdrukken) het beloop van PF beïnvloeden. COVID-19-patiënten met PF vertoonden veranderingen in de expressie van ontstekings- en stollingsgerelateerde genen. Hoewel behandeling met corticosteroïden leidde tot normalisatie van de PF-gerelateerde genexpressieprofielen en andere biomarkers, verbeterde het de klinische uitkomsten in het ziekenhuis niet.

In **hoofdstuk 9** werden vier klinische fenotypes die eerder bij sepsispatiënten geïdentificeerd zijn, toegepast op twee groepen ernstig zieke COVID-19-patiënten (vóór en na de start van standaard behandeling met dexamethason) en op drie groepen patiënten met ernstige sepsis met een andere oorzaak (niet-COVID-19 virale longontsteking, bacteriële longontsteking en bacteriële sepsis vanuit een ander orgaan dan de longen). De verdeling van de vier fenotypen was vergelijkbaar tussen de COVID-19 en niet-COVID-19 virale longontsteking groepen. Patiënten met het fenotype dat zich kenmerkte door minder comorbiditeiten, een lagere leeftijd en een hoger BMI hadden de meest gunstige klinische uitkomsten in alle groepen. De sterfte was het hoogst bij patiënten met het fenotype dat zich kenmerkte door hogere creatininewaarden (duidend op nierfunctiestoornissen) en witte bloedcellen (duidend op meer uitgesproken ontsteking), meer comorbiditeiten en een hoger percentage mannen. In eerder onderzoek bij sepsispatiënten werden bij patiënten met dit laatste fenotype ook de hoogste hoeveelheden ontstekingseiwitten in het bloed gemeten. Behandeling met dexamethason gaf voor dit fenotype dan ook de meeste reductie in sterfte.

Deel III: Immuunmodulerende behandelingen voor ernstig zieke COVID-19-patiënten

In **hoofdstuk 10** reageren we op een artikel waarin de effecten van anakinra, een medicijn dat het immuunsysteem onderdrukt, bij ernstig zieke COVID-19-patiënten beschreven worden. In dit artikel wordt gebruik gemaakt van een zogenaamde historische controlegroep. Er waren echter aanzienlijke verschillen tussen de met anakinra behandelde groep en de historische controlegroep, wat de interpretatie ernstig bemoeilijkt. Daarom benadrukken we het belang van het gebruik van geschikte controlegroepen in dit soort studies. Wij stellen voor om hier propensity score matching voor te gebruiken, een methode om een groep met anakinra-behandelde patiënten zo vergelijkbaar mogelijk te krijgen met een controlegroep die niet met dit medicijn behandeld werd.

In **hoofdstuk 11** worden de effecten van anakinra bij COVID-19-patiënten opgenomen op de IC onderzocht met behulp van propensity score matching. Hoewel de ontstekingswaarden verbeterden na anakinra-behandeling, werden er geen significante verschillen gevonden in klinische uitkomsten in het ziekenhuis. Dit komt mogelijk omdat de bestudeerde groepen patiënten te klein waren om dit aan te tonen.

Hoofdstuk 12 beschrijft een reeks van vijf niertransplantatiepatiënten met ernstige COVID-19 die werden behandeld met interferon-gamma (IFN-ɣ), een medicijn dat het immuunsysteem stimuleert. Deze behandeling leidde tot snelle virusklaring zonder dat het de ontstekingswaarden verergerde. Bovendien herstelden vier van de vijf patiënten.

Dit proefschrift belicht verschillende aspecten van COVID-19 bij IC-patiënten, van obesitas tot biomarkers en immunomodulerende behandeling, en draagt bij aan ons begrip van deze complexe ziekte.

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Lieve **papa en mama**, ik heb meer dan een paar zinnen nodig om jullie te bedanken. Jullie zijn eigenlijk degenen die een onderzoeker van me gemaakt hebben. Ik kreeg als kind zo veel ruimte om de wereld te ontdekken. Hutten bouwen in het bos, bij scouting op avontuur, met mijn eigen microscoop bekeek ik papa's bloedcellen, tegen mijn zin in moest ik de Alpen beklimmen, we zetten ons in voor goede doelen en duurzaamheid, en nog zo veel meer. Met jullie onvoorwaardelijke steun en liefde komt alles altijd goed. Jullie staan dag en nacht voor ons klaar. Bedankt voor alles. Ik hoop dat ik word wat jullie zijn.

Liefste **Steven**, door jou leer ik wat echt belangrijk is. Bij jou ben ik thuis. Ik heb zin in alles wat we nog samen gaan meemaken. Ik hou van jou!

Liefs, Emma

CURRICULUM VITAE

Emma Kooistra werd geboren op 5 maart 1995 in Utrecht. Zij groeide op met haar ouders, een broertje, een zusje, en een kat in Wijk bij Duurstede. In 2013 behaalde ze haar Atheneumdiploma aan het Revius Lyceum in Doorn. Die zomer verhuisde ze naar Nijmegen om aan de studie Geneeskunde aan de Radboud Universiteit te beginnen.

Na het behalen van het bachelorsdiploma werkte ze een jaar fulltime als student-assistent op de onderzoeksafdeling van de Intensive Care (IC) in het Radboudumc, waar zij ondersteunde bij het uitvoeren van een klinisch, dubbelblind, gerandomiseerd medicijnonderzoek onder leiding van Dr. M. Kox en Prof. dr. P. Pickkers.

Tijdens de masterfase van de studie Geneeskunde bleef ze parttime betrokken bij verschillende onderzoeksprojecten op de IC. Gedurende de coschappen groeide haar interesse voor interne geneeskunde, acute zorg en wetenschappelijk onderzoek. In 2020 behaalde ze haar artsendiploma. Door de recente start van de COVID-19-pandemie kreeg zij toen de kans om te starten met een promotietraject gericht op COVID-19 op de IC, onder leiding van Dr. M. Kox en Prof. dr. P. Pickkers. De uitvoer van dit promotieonderzoek duurde twee jaar en resulteerde in dit proefschrift.

Vanaf september 2022 werkte zij een jaar als arts (ANIOS) bij de afdeling interne geneeskunde in het Canisius Wilhelmina Ziekenhuis te Nijmegen. Sinds september 2023 is zij terug bij de IC in het Radboudumc waar ze werkzaam is als arts-onderzoeker gericht op duurzaamheid in de acute zorg, in samenwerking met Dr. H. Touw en Dr. T. Stobernack.

In haar vrije tijd houdt ze zich graag bezig met volleybal, natuur, duurzaamheid en familie en vrienden.

PHD Portfolio

Name PhD candidate: Emma Kooistra
Department: Intensive Care
Graduate School: Radboud Institute for Health Sciences

PhD period: 01-07-2020 - 05-04-2024
PhD Supervisor: Prof. P. Pickkers
PhD Co-supervisor: Dr. M. Kox

TRAINING ACTIVITIES

a) Courses	Year(s)	ECTS
- RIMLS - Introduction course "In the lead of my PhD"	2020	15.00
- RU - Statistiek voor promovendi met SPSS	2020	60.00
- RU - Projectmanagement voor Promovendi	2021	45.00
- Radboudumc - Scientific integrity	2021	20.00
- Introduction day Radboudumc	2021	7.00
- Radboudumc - eBROK course (for Radboudumc researchers working with human subjects)	2021	26.00
- R introduction course	2021	28.00
- Statistiek en SPSS	2021	28.00
b) Seminars		
- ESICM DIGITAL 2020 - two poster presentations	2020	28.00
- NVIC conference 2021 - two poster presentations	2021	14.00
- ISICEM 2021 - two poster presentations	2021	28.00
- ISICEM 2021 - oral presentation	2021	14.00
- Weimar Sepsis Update 2021 - two poster presentations	2021	28.00
- ESICM LIVES 2021 - oral presentation	2021	14.00
- Oral presentation for local group of healthcareworkers in the ICU (four times)	2022	28.00
- ESICM LIVES 2022 - poster presentation	2022	14.00
- ISICEM 2022 - poster presentation	2022	14.00
c) Conferences		
- NVIC conference 2020	2020	7.00
- ESICM DIGITAL conference 2020	2020	28.00
- NVIC Intensivistendagen 2021	2021	14.00
- ISICEM 2021	2021	28.00
- Weimar Sepsis Update 2021	2021	21.00
- ESICM LIVES 2021	2021	28.00
- ISICEM 2022	2022	28.00
- ESICM LIVES 2022	2022	35.00
d) Other		
- Study monitoring training	2020	7.00

TEACHING ACTIVITIES

e) Lecturing

- Oral presentation COVID-19 in the ICU for local Internal Medicine group	2020	7.00
- Supervising Bachelor project Grant Proposal	2021	28.00
- Supervising Bachelor project Grant Proposal	2022	28.00
- Daily supervision of 6 master students	2022	168.00

TOTAL	**838.00**

List of publications

2023 **Kooistra EJ**, Dahm K, van Herwaarden AE, Gerretsen J, Nuesch Germano M, Mauer K, Smeets RL, van der Velde S, van den Berg MJW, van der Hoeven JG, Aschenbrenner AC, Schultze JL, Ulas T, Kox M, Pickkers P. Molecular mechanisms and treatment responses of pulmonary fibrosis in severe COVID-19. Respir Res. 2023 Aug 9;24(1):196. doi: 10.1186/s12931-023-02496-1. PMID: 37559053; PMCID: PMC10413531.

2023 de Nooijer AH, **Kooistra EJ**, Grondman I, Janssen NAF, Joosten LAB, van de Veerdonk FL, Kox M, Pickkers P, Netea MG; RCI-COVID-19 study group. Adipocytokine plasma concentrations reflect influence of inflammation but not body mass index (BMI) on clinical outcomes of COVID-19 patients: A prospective observational study from the Netherlands. Clin Obes. 2023 Apr;13(2):e12568. doi: 10.1111/cob.12568. Epub 2022 Nov 25. PMID: 36426776.

2022 Zoodsma M, de Nooijer AH, Grondman I, Gupta MK, Bonifacius A, Koeken VACM, **Kooistra E**, Kilic G, Bulut O, Gödecke N, Janssen N, Kox M, Domínguez-Andrés J, van Gammeren AJ, Ermens AAM, van der Ven AJAM, Pickkers P, Blasczyk R, Behrens GMN, van de Veerdonk FL, Joosten LAB, Xu CJ, Eiz-Vesper B, Netea MG, Li Y. Targeted proteomics identifies circulating biomarkers associated with active COVID-19 and post-COVID-19. Front Immunol. 2022 Nov 3;13:1027122. doi: 10.3389/fimmu.2022.1027122. PMID: 36405747; PMCID: PMC9670186.

2022 **Kooistra E**, Heesakkers H, Pickkers P, Zegers M, van den Boogaard M. Long-Term Impairments Are Most Pronounced in Critically Ill Patients with COVID-19 with Severe Obesity. Am J Respir Crit Care Med. 2022 Oct 15;206(8):1037-1039. doi: 10.1164/rccm.202202-0376LE. PMID: 35696647; PMCID: PMC9801993.

2022 Bruse N, **Kooistra EJ**, Jansen A, van Amstel RBE, de Keizer NF, Kennedy JN, Seymour C, van Vught LA, Pickkers P, Kox M. Clinical sepsis phenotypes in critically ill COVID-19 patients. Crit Care. 2022 Aug 9;26(1):244. doi: 10.1186/s13054-022-04118-6. PMID: 35945618; PMCID: PMC9361232.

2022 Frishberg A, **Kooistra E**, Nuesch-Germano M, Pecht T, Milman N, Reusch N, Warnat-Herresthal S, Bruse N, Händler K, Theis H, Kraut M, van Rijssen E, van Cranenbroek B, Koenen HJ, Heesakkers H, van den Boogaard M, Zegers M, Pickkers P, Becker M, Aschenbrenner AC, Ulas T, Theis FJ, Shen-Orr SS, Schultze JL, Kox M. Mature neutrophils and a NF-κB-to-IFN transition determine the unifying disease recovery dynamics in COVID-19. Cell Rep Med. 2022 Jun 21;3(6):100652. doi: 10.1016/j.xcrm.2022.100652. Epub 2022 May 17. PMID: 35675822; PMCID: PMC9110324.

2021 van der Heijden CDCC, Ter Heine R, **Kooistra EJ**, Brüggemann RJ, Walburgh Schmidt JWJ, de Grouw EPLM, Frenzel T, Pickkers P, Leentjens J. Effects of dalteparin on anti-Xa activities cannot be predicted in critically ill COVID-19 patients. Br J Clin Pharmacol. 2021 Dec 29. doi: 10.1111/bcp.15208.

2021 van Laarhoven A, Kurver L, Overheul GJ, **Kooistra EJ**, Abdo WF, van Crevel R,

Duivenvoorden R, Kox M, Ten Oever J, Schouten J, van de Veerdonk FL, van der Hoeven H, Rahamat-Langendoen J, van Rij RP, Pickkers P, Netea MG. Interferon gamma immunotherapy in five critically ill COVID-19 patients with impaired cellular immunity: A case series. Med (N Y). 2021 Oct 8;2(10):1163-1170.e2. doi: 10.1016/j.medj.2021.09.003.

2021 Kyriazopoulou E, Huet T, Cavalli G, Gori A, Kyprianou M, Pickkers P, Eugen-Olsen J, Clerici M, Veas F, Chatellier G, Kaplanski G, Netea MG, Pontali E, Gattorno M, Cauchois R, **Kooistra E**, Kox M, Bandera A, Beaussier H, Mangioni D, Dagna L, van der Meer JWM, Giamarellos-Bourboulis EJ, Hayem G; International Collaborative Group for Anakinra in COVID-19. Effect of anakinra on mortality in patients with COVID-19: a systematic review and patient-level meta-analysis. Lancet Rheumatol. 2021 Oct;3(10):e690-e697. doi: 10.1016/S2665-9913(21)00216-2.

2021 van den Berg MJW, Waanders D, **Kooistra EJ**, Kox M, Pickkers P. The value of D-dimer to predict pulmonary embolism in critically ill COVID-19 patients. J Crit Care. 2021 Aug;64:18-21. doi: 10.1016/j.jcrc.2021.03.002.

2021 **Kooistra EJ**, van Berkel M, van Kempen NF, van Latum CRM, Bruse N, Frenzel T, van den Berg MJW, Schouten JA, Kox M, Pickkers P. Dexamethasone and tocilizumab treatment considerably reduces the value of C-reactive protein and procalcitonin to detect secondary bacterial infections in COVID-19 patients. Crit Care. 2021 Aug 5;25(1):281. doi: 10.1186/s13054-021-03717-z. PMID: 34353339; PMCID: PMC8340482.

2021 de Nooijer AH, Grondman I, Lambden S, **Kooistra EJ**, Janssen NAF, Kox M, Pickkers P, Joosten LAB, van de Veerdonk FL, Derive M, Gibot S, Netea MG; RCI-COVID-19 study group. Increased sTREM-1 plasma concentrations are associated with poor clinical outcomes in patients with COVID-19. Biosci Rep. 2021 Jul 30;41(7):BSR20210940. doi: 10.1042/BSR20210940.

2021 **Kooistra EJ**, Brinkman S, van der Voort PHJ, de Keizer NF, Dongelmans DA, Kox M, Pickkers P. Body Mass Index and Mortality in Coronavirus Disease 2019 and Other Diseases: A Cohort Study in 35,506 ICU Patients. Crit Care Med. 2021 Jul 16. doi: 10.1097/CCM.0000000000005216. Epub ahead of print. PMID: 34374504.

2021 van Groenendael R, Beunders R, Hemelaar P, Hofland J, Morshuis WJ, van der Hoeven JG, Gerretsen J, Wensvoort G, **Kooistra EJ**, Claassen WJ, Waanders D, Lamberts MGA, Buijsse LSE, Kox M, van Eijk LT, Pickkers P. Safety and Efficacy of Human Chorionic Gonadotropin Hormone-Derivative EA-230 in Cardiac Surgery Patients: A Randomized Double-Blind Placebo-Controlled Study. Crit Care Med. 2021 May 1;49(5):790-803. doi: 10.1097/CCM.0000000000004847.

2021 **Kooistra EJ**, de Nooijer AH, Claassen WJ, Grondman I, Janssen NAF, Netea MG, van de Veerdonk FL, van der Hoeven JG, Kox M, Pickkers P; RCI-COVID-19 study group. A higher BMI is not associated with a different immune response and disease course in critically ill COVID-19 patients. Int J Obes (Lond). 2021 Mar;45(3):687-694. doi: 10.1038/s41366-021-00747-z. Epub 2021 Jan 25. PMID: 33495522; PMCID: PMC7829495.

2020 **Kooistra EJ**, Waalders NJB, Grondman I, Janssen NAF, de Nooijer AH, Netea MG, van de Veerdonk FL, Ewalds E, van der Hoeven JG, Kox M, Pickkers P; RCI-COVID-19 Study Group. Anakinra treatment in critically ill COVID-19 patients: a prospective cohort study. Crit Care. 2020 Dec 10;24(1):688. doi: 10.1186/s13054-020-03364-w. PMID: 33302991; PMCID: PMC7726611.

2020 Kox M, Waalders NJB, **Kooistra EJ**, Gerretsen J, Pickkers P. Cytokine Levels in Critically Ill Patients With COVID-19 and Other Conditions. JAMA. 2020 Sep 3;324(15):1565–7. doi: 10.1001/jama.2020.17052.

2020 **Kooistra EJ**, Waalders NJB, Kox M, Pickkers P. Effect of anakinra in COVID-19. Lancet Rheumatol. 2020 Sep;2(9):e523-e524. doi: 10.1016/S2665-9913(20)30235-6. Epub 2020 Jul 24. PMID: 32838316; PMCID: PMC7380916.

Research data management

Ethics and privacy

This thesis is based on the results of medical-scientific research with human participants. All studies described in this thesis were carried out in accordance with the applicable rules concerning the review of research ethics committees and informed consent in the Netherlands. All patients or legal representatives were informed about the details of this cohort study and could decline to participate. Technical and organizational measures were followed to safeguard the availability, integrity and confidentiality of the data (these measures include the use of independent monitoring, pseudonymization, access authorization and secure data storage).

Data/sample collection and storage

Clinical data used in chapters 2, 4, 6, 7, 8, 11, and 12 were collected from electronic patient files (EPD) and were anonymized and stored in CASTOR EDC. From Castor EDC data were exported to Microsoft Excel, SPSS, R, and GraphPad for tabulation, statistical analysis, and visualisation. Data will be archived at Radboudumc servers for 15 years after completion of the studies. Data used in chapters 3 and 9 were collected and stored by the Dutch national ICU registry (NICE, http://www.stichting-nice.nl). These pseudonymized data were owned by NICE, and data analysis was conducted in collaboration with NICE on their servers. Blood samples were collected by researchers or healthcare workers. These samples were processed by trained researchers and were pseudonymized and stored in designated freezers at the department of Intensive Care Medicine. Only the study team has access to these freezers. Likewise, sample logs are only accessible to the study team. All analyses on these samples, except for those described in chapter 8 were performed in-house at Radboudumc. RNA sequencing described in Chapter 8 was performed on pseudonymized samples at the University of Bonn, Germany.

Availability of data

Except for the data used in chapters 3 and 9, the pseudonymized datasets that were used for analysis are available from the corresponding author upon reasonable request. Furthermore, the RNA sequencing data described in Chapter 8 are available at the European Genome-Phenome Archive (EGA) under accession numbers EGAS00001005735 and EGAS00001006407, which is hosted by the European Bioinformatics Institute (EBI) and the Centre for Genomic Regulation (CRG).