Anesthesia & Analgesia Journal Publish Ahead of Print

DOI: 10.1213/ANE.0000000000005044

Reduced monocytic HLA-DR expression indicates immunosuppression in critically ill COVID-19 patients

Short title: mHLA-DR in COVID

Thibaud Spinetti*, Cedric Hirzel*, Michaela Fux, Laura N. Walti, Patrick Schober, Frank Stueber, Markus M. Luedi, Joerg C. Schefold

(* equally contributing first authors)

1. Thibaud Spinetti, PhD

Department of Intensive Care Medicine, Inselspital, Bern University Hospital, University of Bern, Switzerland, Email:thibaud.spinetti@insel.ch

Conflicts: This author reported no conflicts of interest

Attestation:This author has seen, reviewed and approved the final manuscript

2. Cedric Hirzel, MD

Department of Infectious Diseases, Inselspital, Bern University Hospital, University of Bern, Switzerland Email:cedric.hirzel@insel.ch

Conflicts: This author reported no conflicts of interest

Attestation: This author has seen, reviewed and approved the final manuscript

3. Michaela Fux, PhD

University Institute of Clinical Chemistry, Inselspital, University of Bern, Bern, Switzerland, Email: michaela.fux@insel.ch

Conflicts:This author reported no conflicts of interest

Attestation: This author has seen, reviewed and approved the final manuscript

4. Laura N. Walti, MD

Department of Infectious Diseases, Inselspital, Bern University Hospital, University of Bern, Switzerland Email: laura.walti@insel.ch

Conflicts:This author reported no conflicts of interest

Attestation: This author has seen, reviewed and approved the final manuscript

5. Patrick Schober, MD, PhD, MMedStat

Department of Anaesthesiology, Amsterdam University Medical Centres, Vrije Universiteit Amsterdam, Amsterdam, Netherlands Email: p.schober@amsterdamumc.nl

Conflicts: This author reported no conflicts of interest

Attestation: This author has seen, reviewed and approved the final manuscript

6. Frank Stueber, MD

Department of Anaesthesiology and Pain Medicine, Inselspital, Bern University Hospital, University of Bern, Switzerland Email: frank.stueber@insel.ch

Conflicts: This author reported no conflicts of interest

Attestation: This author has seen, reviewed and approved the final manuscript

7. Markus M. Luedi, MD, MBA

Department of Anaesthesiology and Pain Medicine, Inselspital, Bern University Hospital, University of Bern, Switzerland Email: markus.luedi2@insel.ch

Conflicts: This author reported no conflicts of interest

Attestation: This author has seen, reviewed and approved the final manuscript

8. Joerg C. Schefold, MD

Department of Intensive Care Medicine, Inselspital, Bern University Hospital, University of Bern, Switzerland Email: joerg.schefold@insel.ch

Conflicts: This author reported no conflicts of interest

Attestation: This author has seen, reviewed and approved the final manuscript

Counts: 326 words in abstract, 2119 words in main text, 3 figures, 1 table, 28 references

Declaration of interests: Cedric Hirzel declares no financial or non-financial conflict of interest

Michaela Fux declares no financial or non-financial conflict of interest

Laura N. Walti declares no financial or non-financial conflict of interest

Patrick Schober declares no financial or non-financial conflict of interest

Frank Stueber declares no financial or non-financial conflict of interest

Markus M. Luedi declares no financial or non-financial conflict of interest

Thibaud Spinetti and **Joerg C. Schefold** declare that the Department of Intensive Care Medicine, Inselspital, Bern, has received research or other grants from (full departmental disclosure): Orion Pharma, Abbott Nutrition International, B. Braun Medical, CSEM, Edwards Lifesciences Services, Kenta Biotech, Maquet Critical Care, Omnicare Clinical Research, Nestle, Pierre Fabre Pharma, Pfizer, Bard Medica, Abbott, Anandic Medical Systems, Pan Gas Healthcare, Bracco, Hamilton Medical, Fresenius Kabi, Getinge Group Maquet, Dräger, Teleflex Medical, Glaxo Smith Kline, Merck Sharp and Dohme, Eli Lilly and Company, Baxter, Astellas, Astra Zeneca, CSL Behring, Novartis, Covidien, Hemotune, Phagenesis, and Nycomed outside the submitted work. The money was paid into departmental funds. No personal financial gain applies.no financial or non-financial conflict of interest

Data transparency: All available data and materials are available upon reasonable non-commercial request to the corresponding author

Code availability: All software application or custom code are available upon request to the corresponding author

Authors' contributions:

Thibaud Spinetti: This author helped with the conception and design of the study, analysis and interpretation of data, drafting the article and approved the final version to be submitted, and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Cedric Hirzel: This author helped with the conception and design of the study, analysis and interpretation of data, drafting the article and approved the final version to be submitted, and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Michaela Fux: This author helped with the conception and design of the study, analysis and interpretation of data, drafting the article and approved the final version to be submitted, and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Laura N. Walti: This author helped with the conception and design of the study, analysis and interpretation of data, drafting the article and approved the final version to be submitted, and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Patrick Schober: This author helped with the conception and design of the study, analysis and interpretation of data, drafting the article and approved the final version to be submitted, and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Frank Stueber: This author helped with the conception and design of the study, analysis and interpretation of data, drafting the article and approved the final version to be submitted, and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Markus M. Luedi: This author helped with the conception and design of the study, analysis and interpretation of data, drafting the article and approved the final version to be submitted, and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Joerg C. Schefold: This author helped with the conception and design of the study, analysis and interpretation of data, drafting the article and approved the final version to be submitted, and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Funding No funding involved

Correspondence: Joerg C. Schefold, MD, Department of Intensive Care Medicine, Inselspital, Bern University Hospital, University of Bern, Freiburgstrasse 18, 3010 Bern, Switzerland, Phone: +41 31 632 5397, Email: joerg.schefold@insel.ch.

Abstract

Background: The cellular immune system is of pivotal importance with regard to the response to severe infections. Monocytes / macrophages are considered key immune cells in infections and downregulation of the surface expression of monocytic human leukocyte antigen-DR (mHLA-DR) expression within the major histocompatibility complex class II reflects a state of immunosuppression, also referred to as injury-associated immunosuppression. As the role of immunosuppression in coronavirus disease 2019 (COVID-19) disease is currently unclear, we seek to explore the level of mHLA-DR expression in COVID-19 patients.

Methods: In a preliminary prospective monocentric observational study, 16 COVID-19 positive patients (75% male, median age: 68 [interquartile range 59-75], APACHE-II score in 9 ICU patients: 30 [interquartile range 25-32] with acute respiratory failure were included. Standardized quantitative assessment of mHLA-DR on CD14+ cells was performed using calibrated flow cytometry at baseline (ICU admission), and at days 3 and 5 after ICU admission. Baseline data was compared to hospitalized non-critically ill COVID-19 patients.

Results: While normal mHLA-DR expression was observed in all hospitalized non-critically ill patients (n=7), 89% (8/9) critically ill patients with COVID-19- induced acute respiratory failure showed signs of downregulation of mHLA-DR at ICU admission. Monocytic HLA-DR expression at admission was significantly lower in critically ill patients (median, [quartiles]: 9280 antibodies/cell [6114, 16567]) as compared to the non-critically ill patients (30900 antibodies/cell [26777, 52251]), with a median difference of 21508 antibodies/cell (95% CI: 14118 to 42971), P=0.002. Reduced monocytic HLA-DR expression was observed to persist until day 5 after ICU admission.

Conclusions: When compared to non-critically ill hospitalized COVID-19 patients, ICU patients with severe COVID-19 disease showed reduced mHLA-DR expression on circulating CD14+ monocytes at ICU admission, indicating a dysfunctional immune response. This immunosuppressive (monocytic) phenotype remained unchanged over the ensuing days after ICU

admission. Strategies aiming for immunomodulation in this population of critically ill patients should be guided by an immune-monitoring program in an effort to determine who might benefit best from a given immunological intervention.

Key words: SARS-CoV-2, COVID-19, intensive care unit, ICU, critical illness, immunodepression, immunosuppression, sepsis-associated immunosuppression.

Glossary of Terms:

CD (14+): Cluster of differentiation (14+)

COVID-19: Coronavirus Disease 2019

EDTA: Ethylenediaminetetraacetic acid

FACS: Fluorescence-activated cell sorting

FSC: Forward scatter

ICU: Intensive care unit

PCR: polymerase chain reaction

PE: Phycoerythrin

SARS -CoV-2: Severe acquired respiratory syndrome coronavirus-2

SSC: Side scatter

Key points:

Question: Is severe COVID-19 disease associated with an immunosuppressive phenotype of key innate immune cells ?

Findings: We observed considerable reduction of mHLA-DR, a key marker of monocytic immune function, in critically ill COVID-19 patients with acute respiratory failure and this effect remained unchanged over the first days on the ICU.

Meaning: Severe COVID-19 disease is associated with reduced HLA-DR expression on circulating CD14+ cells, indicating a dysfunctional immune response. Future strategies aiming for immunomodulation in this population of ICU patients should be guided by an immune-monitoring program in an effort to determine who might potentially benefit from a targeted immunological intervention.

INTRODUCTION

In higher life forms, the immune system is organized in complex social network architecture-like structures [1] and its dysfunction is associated with adverse outcomes in various clinical scenarios. Although detection and monitoring of various organ dysfunctions is a key challenge for physicians involved in the care for the critically ill, the immune system may currently be regarded somewhat overlooked as it is typically not monitored within the clinical routines of most intensive care units (ICUs). This may be of particular importance in critically ill patients with severe infections (e.g. patients with bacterial septic shock) [2-7].

Data show that monocytes / macrophages play key roles in critically ill patients with severe infections and constitute a first line cellular response that initiates and promotes a targeted, i.e. adaptive, immune response [2,3,8]. In this regard, flow cytometry-based standardized assessment of the surface expression of monocytic human leukocyte antigen-DR (mHLA-DR) was proposed by us and others [2,9,10] to serve as a global marker of (monocytic) immune function as it reflects key cell-mediated immune functions including major histocompatibility complex (MHC) class II-mediated antigen-presentation, ex-vivo cytokine release, and phagocytosis) [2,3,6,8,10]. Monocytic HLA-DR expression can be assessed in a quantitative fashion using a standardized assay (coefficient of variation <4% intra-lab and 15% inter-lab) [11], allowing for multi-center data comparison [9]. Importantly, mounting data from critically ill patients with (bacterial) sepsis/ septic shock show that reduced mHLA-DR expression (indicating "injury-associated immunosuppression" [3]) is associated with adverse clinical outcomes in ICU patients, including increased rates of secondary infections, and increased mortality [2,12-15]. Further, mHLA-DR previously served to guide targeted immunological interventions e.g. using immunostimulatory approaches [16-18] or via reduction of inhibitory factors [19]. Such biomarker enrichment [20] may allow for identification of which patient might benefit best from a given immunomodulatory intervention [2,6,7,14].

From an epidemiological perspective, COVID-19 (SARS -CoV-2) patients appear (clinically) well characterized [21-24]. However, the role of virus- induced immunosuppression remains incompletely understood [25]. We therefore embarked to investigate the role and course of monocytic HLA-DR expression in ICU patients with severe COVID-19 disease, i.e. critically ill patients. This is performed as understanding of the immunologic phenotype will be important e.g. when immunomodulatory immunotherapies are evaluated.

METHODS

In a preliminary prospective monocentric observational study, patients with confirmed COVID-19 disease were included from March until April 2020 and followed up until ICU-/ hospital discharge and/or death. Patients were initially assessed for eligibility in the emergency rooms (non-critically ill patients), or at the ICU (in cases of direct ICU admission). The study was performed in an 900+ bed tertiary care academic medical center (Inselspital, Bern University Hospital, Switzerland). In this center, the Department of Intensive Care Medicine is the sole provider of intensive care medicine for adults.

Adult (aged ≥ 18 years) patients with confirmed SARS-CoV-2 infection (detected by PCR in nasopharyngeal swabs) were consecutively included in the study after provision of informed consent (in case of lack of capacity and/or inability to provide consent, consent followed the local procedures for research projects in emergency situations). Screening of patients was performed in daily clinical practice by the research team. The following exclusion criteria applied: no confirmed SARS-CoV2 infection or confirmed SARS-CoV2 infection > 3 days before inclusion, age <18 years, lack of consent. No financial compensation applied for participants. Hospitalized patients and ICU patients were followed up until ICU or hospital discharge, or death (while in hospital). Laboratory data was recorded with missing data indicated. Lab samples were drawn at the scheduled visits within ±36 hours. Data available until April 30, 2020 (censor date) was included. The trial was performed in accordance with the "Declaration of Helsinki" and approved by the Kantonale Ethikkommission KEK, Bern, Switzerland, Nr. 2020-00877.

Flowcytometric Assessment of mHLA-DR expression

Flow-cytometric assessment of mHLA-DR expression (primary outcome) was performed from EDTA samples within 4 hours, as previously reported [11] (discussed in [3,9]). In brief, a mixture that contains beads with pre-defined amounts of conjugated antibodies (Phycoerythrin molecules, PE) is measured using the same FACS instrument settings (as the cells of interest) using a mixture of anti-human HLA-DR-PE, anti-human CD14-PerCP-Cy5.5, and an inhibitor of HLA-DR turnover (Quantibrite™ HLA-DR/ Monocyte reagent, Becton Dickinson, Franklin Lakes, USA) [11]. The known ratio of PE to anti-HLA-DR antibody is applied to convert PE molecules/ cell into antibodies/ cell [3,9,11] with the anti-CD14 antibody detecting all monocytes (CD14 bright and weak positive) [11]. The gating strategy is given in Figure 1.

Statistics

Data were analyzed with Stata 16.1 (StataCorp, College Station, TX, USA). The distribution of continuous data was assessed with histograms, Q-Q plots and Shapiro-Wilk tests. All continuous data departed from the normal distribution and are presented as median [quartiles]. Baseline demographic data, laboratory data and follow-up data were compared between the groups with Mann-Whitney U tests (continuous data) or with Fisher's exact test (categorical data; all had expected counts <5 in $\geq 25\%$ of cells).

The primary outcome, mHLA-DR expression at admission, was compared between the groups (patients admitted to the general ward versus patients admitted to the ICU) using a Mann-Whitney U test [26]. The median difference between the groups and its 95% confidence interval were estimated with the Hodges-Lehmann estimator. Being aware that our small dataset does not lend itself to multiple regression modeling, we yet performed explorative analyses to gauge potential confounding due to differences in baseline characteristics between groups [27,28]. We used quantile (median) regression, as well as linear regression with bootstrapped standard errors (10,000 replications), to adjust for age, sex and BMI.

One patient was first admitted to the normal ward and subsequently to the ICU after clinical deterioration. According to the primary admission, this patient is considered a non-critically ill patient for the comparison of mHLA-DR expression at admission. We also performed a sensitivity analysis in which the patient was analyzed as being an ICU patient.

In ICU patients, follow up data for mHLA-DR expression were obtained at days 3 and 5 of their ICU admission. Differences over time were tested with the Skillings-Mack test. This test is an extension of the Friedman test (nonparametric equivalent to one-way repeated measures analysis of variance) that allows for missing data. Two-sided $p<0.05$ was considered statistically significant. For this preliminary investigation, a formal a priori power analysis/sample size calculation was not performed, and the sample size is based on the available number of patients.

RESULTS

Sixteen patients (12 male, median age 68 years) were included in this preliminary observational study, of which 9 were primarily admitted to the ICU for mechanical ventilation, and 7 with primary hospitalization at the general ward. Table 1 shows demographic data, comorbidities, laboratory data, as well as follow-up data for both patient groups. While the table suggests clinically relevant differences in terms of infection parameters and length of hospital stay in our *sample* of patients, there was insufficient evidence to claim a significant difference in the *population* of patients from which the data were sampled (all P-values >0.05).

Monocyte HLA-DR expression at admission was significantly lower in the ICU-group (9280 antibodies / cell [6114, 16567]) as compared to the non-ICU group (30900 antibodies /cell [26777, 52251]), with a median difference of 21508 antibodies /cell (95% CI: 14118 to 42971), P=0.002 (Figure 2). Similarly, the adjusted quantile regression and linear regression provided evidence for a significant between-group difference (P=0.001 and P<0.001, respectively).

The sensitivity analysis, in which one patient of the non-ICU group was counted towards the ICU group as described above, provides consistent results in the unadjusted analysis (median difference 19419 antibodies/cell, 95% CI: 8487 to 43578, P=0.016) as well as in the adjusted analyses (P=0.001 and P=0.007, respectively).

In ICU patients, median mHLA-DR expression was 9280 antibodies / cell [6114, 16567] at admission (N=9), 9672 antibodies / cell [8253, 10511] at 3 days (N=9), and 7334 antibodies / cell [5241, 11022] at 5 days (N=6), without evidence for a change over time (P=0.33, Figure 3). Including the admission-measurement of the patient who was initially admitted to the normal ward and who was later admitted to the ICU, median HLA-DR expression was 9944 antibodies / cell [6114, 16782] at admission (N=10), still without evidence for a change over time (P=0.19).

DISCUSSION

We demonstrate immunosuppression of key innate immune cells in critically ill patients with (severe) COVID-19. Monocytic HLA-DR expression was reduced on circulating CD14+ cells, and this was not observed in hospitalized COVID-19 patients without critical illness. Importantly, this initial effect persisted over the ensuing days of ICU treatment (until day 5). Of note, all study individuals were treatment-naïve regarding immunomodulatory agents and/ or high-dose corticosteroids. In the light of the current multiple immunomodulatory interventional approaches tested in COVID-19 [25], it seems essential that patients are adequately immunologically characterized using immunological read-outs in an effort to determine which patient might benefit best from a given immunomodulatory intervention. Thus, injury-associated immunosuppression should be taken into account when novel immunomodulatory interventions are designed and tested in critically ill patients with COVID-19.

Our current data show that immunosuppression presented as early as at ICU admission and the observed downregulation of mHLA-DR did not change significantly over the ensuing days of ICU treatment (i.e. at least until day 5 after ICU admission). This seems of particular interest, as when compared to ICU patients with (bacterial) septic shock, decreased mHLA-DR expression is

mostly observed after a few days following ICU admission and may be most prominent after about 72 hours (reviewed in [3]). Although the exact onset of disease can mostly not be elucidated in critically ill patients, it is known that the median incubation period of SARS-CoV-2 is about 4-5 days [21-24] and about 97.5% of patients diagnosed with COVID-19 develop symptoms within the first 11.5 days [22]. In the light of the specific disease investigated, it thus seems tempting to speculate that downregulation of mHLA-DR would typically occur prior to development of acute respiratory failure, i.e. before ICU admission in severe COVID-19 disease and mHLA-DR assessment could theoretically provide early prognostic information. Moreover, and importantly, the immunosuppression reflected in decreased mHLA-DR might theoretically be a relevant contributor to progression of increased viral replication and/or the severity of the viral disease. Previous data from ICU patients with bacterial septic shock show that secondary infection rates are increased in patients with persistent downregulation of mHLA-DR, i.e. injury-associated immunosuppression (reviewed in [3,6]). Thus, as secondary infections contribute to increased morbidity and mortality in ICU patients with COVID-19 disease, the potential association of mHLA-DR downregulation with secondary infection rates should be investigated in subsequent investigations. Importantly, however, in the current analysis, we are unable to conclude back on causality and/or effects on secondary infection rates due to the limited sample size and the preliminary, observational nature of the investigation.

Further, our observations might challenge the concept of a general macrophage activation syndrome as being a primary driver of severe COVID-19 disease. In the current observational study, we observed macrophage "deactivation" as indicated by reduced expression of MHC class II (HLA-DR) on CD14+ cells, rather than macrophage activation. However, due to the limited sample size and monocentric character of the investigation, this awaits confirmation in subsequent larger analyses.

A number of additional limitations of this analysis deserve discussion. First, consecutive patients were included in a single-center observational study and all respective limitations (driven by study design) apply. Second, the sample size was limited and results await confirmation in subsequent larger cohorts. However, the limited sample size was partly due to the fact that informed consent cannot easily be achieved during a pandemic in the cohort investigated. Nevertheless, observed effects on mHLA-DR expression were consistent and strong, likely pointing to an important disease-immanent process.

Third, final outcome data was not available for 3 patients with long-term ICU stay. However, although a final data set might have been preferable, it may underline the relevance of immunosuppression in this context. Fourth, follow-up data at days 3 and 5 of non-critically ill hospitalized patients would have been interesting, but were unavailable (also for the fact that patients improved rapidly) to obtain such samples as patients were discharged from our institution. Fifth, longer follow-up, i.e. until all ICU patients have recovered, would have been preferable, however, this was initially deemed not possible and out of the scope of the current investigation. Nevertheless, we are therefore unable to conclude on the potential recovery slope of mHLA-DR in survivors of critical illness and suppose that this should be investigated in subsequent larger studies. Sixth, one might speculate whether monocyte deactivation would be a phenomenon of the blood compartment, rather than to reflect monocytic immune function in "solid immune organs". However, it was previously shown in patients with bacterial sepsis, that HLA-DR downregulation would not only be observed in the blood compartment, but would rather be paralleled in respective "solid immune organs" [15].

In conclusion, in a prospective monocentric study with a limited sample size, we observed that severe COVID-19 disease is associated with considerable and sustained immunosuppression of key innate immune cells (monocytes / macrophages), indicating a dysfunctional immune response in a majority of critically ill patients. This phenotype persisted over the initial days of ICU treatment and may underline the urgent need for an adequate immunological characterization (using appropriate immunological read-outs) when targeted immunomodulatory interventions are tested in respective critically ill patients.

References

1. Rieckmann JC, Geiger R, Hornburg D, et al. Social network architecture of human immune cells unveiled by quantitative proteomics. Nature immunology 2017;18:583-93.
2. Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. Nature reviews Immunology 2013;13:862-74.
3. Pfortmueller CA, Meisel C, Fux M, Schefold JC. Assessment of immune organ dysfunction in critical illness: utility of innate immune response markers. Intensive care medicine experimental 2017;5:49.
4. Rubio I, Osuchowski MF, Shankar-Hari M, et al. Current gaps in sepsis immunology: new opportunities for translational research. The Lancet Infectious diseases 2019;19:e422-e36.
5. Schefold JC, Hasper D, Reinke P, Monneret G, Volk HD. Consider delayed immunosuppression into the concept of sepsis. Critical care medicine 2008;36:3118.
6. Venet F, Monneret G. Advances in the understanding and treatment of sepsis-induced immunosuppression. Nature reviews Nephrology 2018;14:121-37.
7. Schefold JC, Hasper D, Volk HD, Reinke P. Sepsis: time has come to focus on the later stages. Medical hypotheses 2008;71:203-8.
8. Roche PA, Furuta K. The ins and outs of MHC class II-mediated antigen processing and presentation. Nature reviews Immunology 2015;15:203-16.
9. Monneret G, Venet F, Meisel C, Schefold JC. Assessment of monocytic HLA-DR expression in ICU patients: analytical issues for multicentric flow cytometry studies. Critical care 2010;14:432.
10. Schefold JC. Measurement of monocytic HLA-DR (mHLA-DR) expression in patients with severe sepsis and septic shock: assessment of immune organ failure. Intensive care medicine 2010;36:1810-2.

11. Docke WD, Hoflich C, Davis KA, et al. Monitoring temporary immunodepression by flow cytometric measurement of monocytic HLA-DR expression: a multicenter standardized study. Clinical chemistry 2005;51:2341-7.
12. Cheron A, Monneret G, Landelle C, Floccard B, Allaouchiche B. [Low monocytic HLA-DR expression and risk of secondary infection]. Annales francaises d'anesthesie et de reanimation 2010;29:368-76.
13. Monneret G, Lepape A, Voirin N, et al. Persisting low monocyte human leukocyte antigen-DR expression predicts mortality in septic shock. Intensive care medicine 2006;32:1175-83.
14. Venet F, Lukaszewicz AC, Payen D, Hotchkiss R, Monneret G. Monitoring the immune response in sepsis: a rational approach to administration of immunoadjuvant therapies. Current opinion in immunology 2013;25:477-83.
15. Boomer JS, To K, Chang KC, et al. Immunosuppression in patients who die of sepsis and multiple organ failure. Jama 2011;306:2594-605.
16. Nierhaus A, Montag B, Timmler N, et al. Reversal of immunoparalysis by recombinant human granulocyte-macrophage colony-stimulating factor in patients with severe sepsis. Intensive care medicine 2003;29:646-51.
17. Meisel C, Schefold JC, Pschowski R, et al. Granulocyte-macrophage colony-stimulating factor to reverse sepsis-associated immunosuppression: a double-blind, randomized, placebo-controlled multicenter trial. American journal of respiratory and critical care medicine 2009;180:640-8.
18. Payen D, Faivre V, Miatello J, et al. Multicentric experience with interferon gamma therapy in sepsis induced immunosuppression. A case series. BMC infectious diseases 2019;19:931.

19. Schefold JC, von Haehling S, Corsepius M, et al. A novel selective extracorporeal intervention in sepsis: immunoadsorption of endotoxin, interleukin 6, and complement-activating product 5a. Shock 2007;28:418-25.

20. Spinetti T, Meisel C, von Gunten S, Schefold JC. Immune biomarker-based enrichment in sepsis trials. Critical care 2020;24:58.

21. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. The New England journal of medicine 2020;382:1708-20.

22. Lauer SA, Grantz KH, Bi Q, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. Annals of internal medicine 2020.

23. Li Q, Guan X, Wu P, et al. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. The New England journal of medicine 2020;382:1199-207.

24. Pung R, Chiew CJ, Young BE, et al. Investigation of three clusters of COVID-19 in Singapore: implications for surveillance and response measures. Lancet 2020;395:1039-46.

25. Tay MZ, Poh CM, Renia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. Nature reviews Immunology 2020.

26. Schober P, Vetter TR. Two-Sample Unpaired t Tests in Medical Research. Anesthesia and analgesia 2019;129:911.

27. Vetter TR, Schober P. Regression: The Apple Does Not Fall Far From the Tree. Anesthesia and analgesia 2018;127:277-83.

28. Schober P, Vetter TR. Confounding in Observational Research. Anesthesia and analgesia 2020;130:635.

Figure Legends

Figure 1 Flow cytometric analysis of HLA-DR expression in COVID-19 patients Gating strategy: key steps in measurement of monocytic HLA-DR expression using standardized assessment. Beads are gated on their SSC and FSC characteristics (not shown), and PE fluorescence is plotted (A). Patient's monocytes are gated by CD14-binding and SSC properties (B), mHLA-DR expression is plotted against CD14 to calculate the median HLA-DR expression (C).

Figure 2 Monocytic HLA-DR expression in hospitalized patients with COVID-19 MHLA-DR expression (given in antibodies / cell, Ab / cell) at normal ward (n=7) versus primary ICU admission (n=9), p=0.002 in Mann-Whitney U test.

Figure 3 Expression of monocytic HLA-DR over time in patients hospitalized on the ICU Available data (presented in antibodies / cell, Ab/ cell) are given at ICU admission, and days 3 and 5. Discharge from ICU (until day 5) and transfer from the normal ward to ICU is included.

Table Legends

Table 1 Patient demographics, disease severity, and clinical outcomes Demographical data, baseline comorbidities, laboratory data, and clinical follow-up is given for patients with primary admission to ICU vs. normal ward (until censor date). G= Giga, L= Liters, APACHE-II= Acute Physiology and Chronic Health Evaluation- II score, SAPS-2= Simplified Acute Physiology Score-2, SOFA= Sepsis-related organ failure assessment score, COPD= chronic obstructive pulmonary disease, HIV= human immunodeficiency virus, AIDS= acquired immunodeficiency syndrome. Numbers (No.) with percentages are given, as indicated. Continuous data are reported as median [quartiles]. Between group p-values from Mann-Whitney U tests and Fisher's exact tests are given for ICU vs. non-ICU (normal ward) populations. * Mortality data available from 7 ICU patients. Between group p-values are given for ICU vs. non-ICU (normal ward) populations.

Table 1: Baseline demographics, disease severity, and clinical outcome.

		ICU patients with COVID-19 (n=9)	**Hospitalized COVID-19 patients** (n=7)	**Total cohort** (n=16)	between group p-value
Demographics	- Age (years)	66 [62, 77]	71 [55, 73]	68 [59, 75]	0.98
	- Gender (male, %)	6 (67%)	6 (86%)	12 (75%)	0.59
	- Body Mass Index	26.9 [26.0, 27.8]	26.3 [24.4, 27.9]	26.6 [25.1, 27.9]	0.59
	- APACHE-II score (first 24 hours)	30 [25, 32]	-	-	-
	- SAPS II score (first 24 hours)	69 [66, 78]	-	-	-
	- SOFA score (baseline)	13 [13, 15]	-	-	-
Comorbidity data	- Charleson Comorbidity Index (total score)	3 [2, 6]	-	-	-
	- Myocardial infarction (No./%)	1 (11%)	-	-	-
	- Chronic heart failure (No./%)	0 (0%)	-	-	-
	- Peripheral vascular disease (No./%)	1 (11%)	-	-	-
	- Cerebrovascular accident (No./%)	1 (11%)	-	-	-
	- Dementia (No./%)	0 (0%)	-	-	-
	- COPD (No./%)	3 (33%)	-	-	-
	- Connective tissue disease (No./%)	0 (0%)	-	-	-
	- Peptic ulcer disease (No./%)	0 (0%)	-	-	-
	- Liver disease (0-3) (No./%)	0 (0%)	-	-	-
	- Diabetes (0-2) (No./%)	2 (22%)	-	-	-
	- Hemiplegia (No./%)	0 (0%)	-	-	-
	- Moderate to severe CKD (No./%)	2 (22%)	-	-	-
	- Solid tumor (0-6) (No./%)	0 (0%)	-	-	-
	- Leukemia (No./%)	0 (0%)	-	-	-
	- Lymphoma (No./%)	0 (0%)	-	-	-
	- HIV/ AIDS (No./%)	0 (0%)	-	-	-
Laboratory data	- C-reactive protein (mg/L)	149 [96, 243]	43 [7, 126]	120 [31, 197]	0.07
	- Procalcitonin levels (ng/ml)	0.4 [0.2, 1.2]	0.2 [0.1, 0.3]	0.3 [0.2, 0.5]	0.09
	- Total leukocyte count (G/L)	7.0 [4.7, 7.7]	6.9 [5.2, 8.5]	6.9 [5.0, 8.1]	0.92
	- Total lymphocyte count (G/L)	0.8 [0.6, 0.9]	1.2 [1.0, 1.9]	1.0 [0.8, 1.7]	0.06
	- Platelet count (G/L)	192 [143, 225]	185 [174, 267]	189 [166; 234]	0.47
	- Serum potassium (mmol/L)	3.9 [3.6, 4.2]	3.7 [3.6, 4.2]	3.8 [3.6, 4.2]	0.42
	- Serum creatinine (µmol/L)	102 [76, 134]	79 [62, 97]	95 [67, 105]	0.14
	- D-Dimers (µg/L)	1340 [982, 1973]	490 [428, 2062]	1233[574, 2062]	0.28
Follow-up	- Days on ICU	11.8 [8.3, 23.0]	-	-	-
	- Days in hospital	17 [9, 24]	6 [4, 14]	10.5 [4.5, 20.0]	0.07
	- Total days on antibiotics	6 [4, 8]	4 [1, 12]	4.5 [1.5, 9.0]	0.49
	- Total days on mechanical ventilation	9 [5, 23]	-	-	-
	- Renal replacement at any time (No./%)	3 (33%)	-	-	-
	- On vasopressors at any time (No./%)	8 (89%)	-	-	-
	- Total norepinephrine dose (cumulative dose/ ICU days; mg)	3.4 [1.3, 6.8]	-	-	-
	- ICU Mortality (No./%)	2/7 (29%)*	-	-	-
	- Hospital mortality (No./%)	2/7 (29%)*	0 (0%)	2/14 (14%)	0.46

Demographical data, baseline comorbidities, laboratory data, and clinical follow-up is given for patients with primary admission to ICU vs. normal ward (until censor date). G= Giga, L= Liters, APACHE-II= Acute Physiology and Chronic Health Evaluation- II score, SAPS-2= Simplified Acute Physiology Score-2, SOFA= Sepsis-related organ failure assessment score, COPD= chronic obstructive pulmonary disease, HIV= human immunodeficiency virus, AIDS= acquired immunodeficiency syndrome. Numbers (No.) with percentages are given, as indicated. Continuous data are reported as median [quartiles]. Between group p-values from Mann-Whitney U tests and Fisher's exact tests are given for ICU vs. non-ICU (normal ward) populations. * Mortality data available from 7 ICU patients. Between group p-values are given for ICU vs. non-ICU (normal ward) populations.

Figure 1

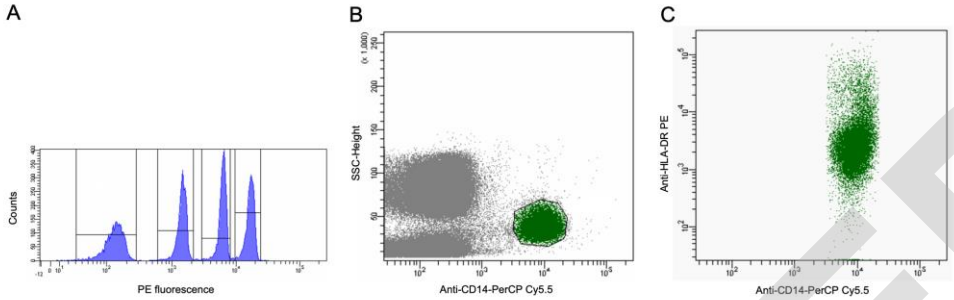

Figure 2

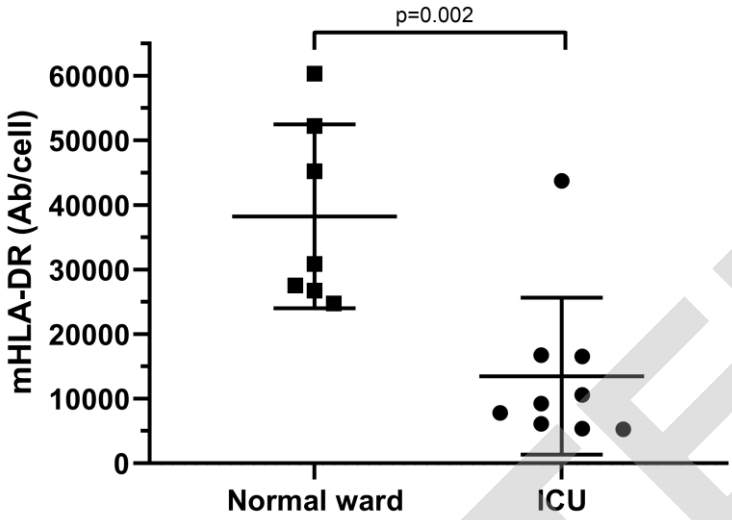

Figure 3