No indications for overt innate immune suppression in critically ill COVID-19 patients

Authors
Matthijs Kox[1,2], Tim Frenzel[1,2], Jeroen Schouten[1,2], Frank L. van de Veerdonk[2,3], Hans J.P.M. Koenen[2,4], and Peter Pickkers[1,2], on behalf of the RCI-COVID-19 study group.

Affiliations
[1]Dept. of Intensive Care Medicine, Radboud university medical center, Nijmegen, The Netherlands.
[2]Radboud Center for Infectious Diseases (RCI), Radboud university medical center, Nijmegen, The Netherlands.
[3]Dept. of Internal Medicine, Radboud university medical center, The Netherlands.
[4]Dept. of Medical Immunology, Radboud university medical center, Nijmegen, The Netherlands.

Correspondence
Matthijs Kox PhD
E-mail: Matthijs.Kox@radboudumc.nl

Abstract
At the end of March 2020, there were in excess of 800.000 confirmed cases of coronavirus disease 2019 (COVID-19) worldwide. Several reports suggest that, in severe cases, COVID-19 may cause a hyperinflammatory "cytokine storm". However, unlike SARS-CoV infection, high levels of anti-inflammatory mediators have also been reported in COVID-19 patients. One study reported that 16% of COVID-19 patients who died developed secondary infection, which might indicate an immune-suppressed state. We explored kinetics of mHLA-DR expression, the most widely used marker of innate immune suppression in critically ill patients, in COVID-19 patients admitted to the ICU.
Twenty-four confirmed COVID-19 patients were included, of which 75% was male and 79% had comorbidities. All patients were mechanically ventilated and exhibited large high levels of inflammatory parameters such as CRP and PCT. mHLA-DR expression levels were mostly within the normal range of 15000-45000 mAb/cell and showed no change over time. COVID-19 patients displayed notably higher mHLA-DR expression levels compared with bacterial septic shock patients. None of the COVID-19 patients developed a secondary infection.
In conclusion, despite a pronounced inflammatory response, mHLA-DR expression kinetics indicate no overt innate immune suppression in COVID-19 patients. These data signify that innate immune suppression as a negative feedback mechanism following PAMP-induced inflammation appears not to be present in COVID-19.

Key words: HLA-DR, mHLA-DR, COVID, COVID-19, SARS-CoV-2, monocytes, immune suppression.

Low monocytic (m)HLA-DR expression is the most widely used marker of innate immune suppression in critically ill patients. We recently showed that, in bacterial septic shock patients, low mHLA-DR expression is prevalent and associated with the development of secondary infections [1]. At the end of March 2020, there were in excess of 800.000 confirmed cases of coronavirus disease 2019 (COVID-19) worldwide, of whom more than 12.000 from the Netherlands. Several reports suggest that patients with severe COVID-19 may suffer from a hyperinflammatory "cytokine storm" [2, 3]. However, unlike SARS-CoV infection, high levels of anti-inflammatory mediators (e.g. IL-10 and IL-4) have also been reported in COVID-19 [3]. Although there are few indications that secondary infections are common in COVID-19 patients, one study reported that 16% of COVID-19 patients who died developed secondary infections [4], which might indicate an immune-suppressed state. In the present work, we explored mHLA-DR expression kinetics in a cohort of 24 COVID-19 patients admitted to the Intensive Care Unit (ICU).

Between March 18th-27th, all COVID-19 patients admitted to our ICU were included in this prospective observational study. COVID-19 was confirmed by two positive RT-PCR tests for SARS-CoV-2 in throat swabs and by CT-scan findings. Fourteen patients were transferred from other ICUs. The median [IQR] ICU length of stay at the time of study inclusion was 3 [3-4] days. The study was carried out in accordance with the applicable rules concerning the review of research ethics committees and informed consent in the Netherlands. The ethics committee (CMO Arnhem-Nijmegen) board waived the need for informed consent because of the observational nature of the study and the non-invasiveness of blood withdrawal (all patients had an arterial canula in place, so no venapunctures were performed). All patients or legal representatives were informed about the study details and could abstain from participation. Ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood was stored at 4-8 ^{o}C until mHLA-DR expression analysis (performed within 2 hours after withdrawal). Expression levels were determined using the Anti-HLA-DR/Anti-Monocyte Quantibrite assay (BD Biosciences, San Jose, USA) on a Navios flow cytometer and software (Beckman Coulter, Brea, USA). Total number of antibodies bound per cell (AB/cell) were quantified using a standard curve constructed with BD Quantibrite phycoerythrin (PE) beads (BD Biosciences). All other data were extracted from the electronic patient record. For patients who were transferred from other ICUs, patient characteristics (listed in the Table) were obtained at admission to our ICU. Data were analysed using SPSS Statistics version 22 (IBM, Armonk, USA), and Graphpad Prism version 8.3.0 (Graphpad Software, La Jolla, USA).

Patient characteristics are listed in Table 1. In line with previous observations [3], the majority of patients was male and many had comorbidities. The median [IQR] time from onset of COVID-19 symptoms to ICU admission was 11 [8-13] days. All patients were mechanically ventilated and exhibited increases in inflammatory parameters (Table 1). As of March 27th 2020, two patients died (at 3 and 4 days post-ICU admission, data of only one timepoint of these patients was recorded), and 22 patients were still in the ICU.

Figure 1A illustrates the mHLA-DR expression kinetics; most values were within the normal range of 15000-45000 mAb/cell [5] and showed no change over time. COVID-19 patients exhibited notably higher mHLA-DR expression levels compared with bacterial septic shock patients (pink line in Figure 1A, data from [1], $p<0.0001$). Circulating C-reactive protein concentrations declined over time (Figure 1B), whereas no significant changes in circulating procalcitonin, leukocytes, or ferritin levels were observed (Figure 1C-E). None of the patients developed a secondary infection.

In conclusion, despite a pronounced inflammatory response in COVID-19 patients, our preliminary results of mHLA-DR expression kinetics indicate no overt innate immune suppression. These findings are in accordance with a low incidence of secondary infections. Therefore, innate immune suppression as a negative feedback mechanism following pathogen-associated molecular pattern-induced inflammation appears not to be present in COVID-19.

Table 1

Characteristics	**All patients (n=24)**
Age, years	69 [61-73]
Sex	
Female	6 (25%)
Male	18 (75%)
Body mass index, kg/m^2	27.5 [24.3-31.1]
Any comorbidities	19 (79%)
Diabetes	7 (29%)
Hypertension	6 (25%)
Cardiovascular disease	7 (29%)
Chronic obstructive pulmonary disease	3 (13%)
Malignancy	10 (42%)
Chronic liver disease	0 (0%)
Chronic kidney disease	1 (4%)
Immunocompromised*	5 (17%)
APACHE II	17 [11-21]
Time from illness onset to ICU admission, days	11 [8-13]
Medication use	
Norepinephrine use	20 (83%)
Maximum infusion rate in first 24h on ICU, µg/kg/min	0.11 [0.07-0.21]
Corticosteroids	1 (4%)
Remdesivir	3 (13%)
Chloroquine	19 (79%)
Anakinra	1 (4%)
Symptoms & (laboratory) parameters	
Heart rate, bpm	83 [71-112]
Mean arterial pressure, mmHg	77 [72-81]
Fluid balance in first 24h on ICU, mL	1348 [680-1881]
Urine output in first 24h on ICU, mL	1105 [888-1486]
Creatinine, µmol/L	86 [70-133]
Dialysis	0 (0%)
Mechanical ventilation (invasive)	24 (100%)
Tidal volume, mL/kg	5.3 [4.4-6.0]
Respiratory rate, bpm	21 [20-24]
PEEP, cm H_2O	12 [10-14]
FiO_2, %	50 [41-60]
P/F ratio	164 [136-189]
100-200	20 (83%)
200-300	4 (17%)
Thrombocytes, 10^9/L	239 [151-274]
Leukocytes, 10^9/L	8.2 [5.3-11.6]
C-reactive protein, mg/L	301 [157-316]
Procalcitonin, µg/L	0.72 [0.29-3.66]
Ferritin, µg/L	1216 [488-1834]
Lactate (highest over last 24h), mmol/L	1.2 [1.1-1.7]

D-dimer, ng/mL	3075 [1780-4598]
Troponin I, ng/L	23 [13-44]
Albumin, g/L	20 [17-22]
Alanine aminotransferase, U/L	34 [21-41]
Aspartate aminotransferase, U/L	48 [31-73]
Creatinine kinase, U/L	136 [56-357]
Lactate dehydrogenase, U/L	398 [303-499]
Outcome parameters	
Secondary infections	0 (0%)
Death	2 (8%)

Data were obtained at study inclusion and are presented as n (%) or median [IQR]. *Chronic use of immunosuppressive medication.

Declarations

Ethics approval and consent to participate

The study was carried out in accordance with the applicable rules concerning the review of research ethics committees and informed consent in the Netherlands. The ethics committee (CMO Arnhem-Nijmegen) board waived the need for informed consent because of the observational nature of the study and the non-invasiveness of blood withdrawal (all patients had an arterial canula in place, so no venapunctures were performed). All patients or legal representatives were informed about the study details and could abstain from participation.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

MK and PP designed the study. TF, JS, and FvdV were responsible for data collection. HK performed the flow cytometric analysis. MK performed the statistical analysis and drafted the manuscript. TF, JS, FvdV, HK, and PP critically revised the manuscript. All authors read and approved the final manuscript.

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Figure 1 legend

Kinetics of **(A)** mHLA-DR expression as well as **(B)** circulating C-reactive protein, **(C)** procalcitonin, **(D)** leukocyte numbers, and **(E)** ferritin in COVID-19 patients. Individual data are shown. The transparent grey line represents mean (panels A, B, and D) or geometric mean (panels C and E) values of the entire cohort. The transparent pink line in panel A represents data obtained from bacterial septic shock patients using the same methodology, as recently published [1] (geometric mean ± 95% CI, please note that values obtained from septic shock patients at days 1-2 (n=203), 3-4 (n=205), and 6-8 (n=133) are plotted at day 1-3, 4-5, and 6-7, respectively). The dotted lines in panel A indicate the reference range in healthy subjects. p-values next to the transparent grey line represent changes over time in COVID-19 patients calculated using mixed model analysis (on log transformed data for panels C and E). Differences between COVID-19 and sepsis patients were analysed using unpaired t-tests on log-transformed data ($p<0.0001$ on all three timepoints).