Letter to the Editor

Arite Bigalke, Charles Neu, Ricardo Esper Treml, Sina M. Coldewey* and Michael Kiehntopf*

Fragments of alpha-1-antitrypsin in patients with severe COVID-19 and bacterial pulmonary sepsis

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To the Editor,

We read with great interest the recent article by Zerimech et al. proposing a protease-antiprotease imbalance as a key pathophysiological mechanism in the progression of COVID-19 to severe ARDS (acute respiratory distress syndrome) [1]. In this correspondence, we would like to offer new evidence relating to this finding by providing the concentrations of two C-terminal protease cleavage products of alpha-1-antitrypsin (AAT) in plasma.

A deficiency in alpha-1-antitrypsin, a protease inhibitor and acute-phase protein with anti-inflammatory properties, has been proposed to play a role in the pathogenesis of COVID-19. A first association was observed by Vianello et al. who found regions in Italy with higher incidence of hereditary AAT deficiency to be affected more severely by the pandemic [2]. Aside from its anti-inflammatory effects, AAT also has antiviral properties. It has been shown that AAT inhibits the transmembrane protease serine subtype 2 (TMPRSS2), which is necessary for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to enter the cell [3]. In patients with COVID-19, the ratio of pro-inflammatory IL-6 and AAT was able to predict disease severity and mortality [4]. Similarly to chronic lung disease [5], an antiprotease–protease imbalance has been proposed to contribute to disease severity in patients with COVID-19 by Zerimech et al. [1]. The study found higher peak values of neutrophil elastase (NE) and matrix metalloprotease-12 (MMP-12) in patients who died from severe COVID-19.

A variety of enzymes and pathophysiological conditions has been identified that are associated with the formation of C-terminal peptides of AAT (CAAPs) including NE and MMP-12. Some CAAPs have also been proposed to serve as putative biomarkers for a variety of diseases. Analysis of sepsis patients demonstrate that certain CAAPs are significantly increased in blood during systemic inflammation compared to healthy individuals [6]. To the best of our knowledge, there is hitherto no evidence for the formation of CAAPs during viral infections. However, a recent proteomic study comparing mild and severe patients with COVID-19 found different concentrations of AAT peptides in the urine, indicating a distinct AAT cleavage pattern during host response to viral infection [7].

To clarify the role of CAAPs during severe COVID-19, we have analyzed plasma concentrations of two AAT fragments, C-36 (cleavage product of i.a. NE) and C-42 (cleaved i.a. by MMP-12), using our newly developed LC/MS-MS method [6]. Patients samples were obtained from a subgroup of an ongoing single-center prospective cohort study on days three (T1) and seven (T2) after onset of severe disease in patients with severe COVID-19 (n=10) and compared with patients with bacterial sepsis of pulmonary origin (n=10) and healthy controls (n=10) [8]. All three cohorts showed no significant differences in sex (all cohorts 30% female) and age (median healthy: 65 y, sepsis: 63 y, COVID-19: 61.5 y). Patients had a similar disease

Arite Bigalke and Charles Neu (first authors) as well as Michael Kiehntopf and Sina Coldewey (senior authors) contributed equally to this work.

***Corresponding authors: Sina M. Coldewey**, Department of Anaesthesiology and Intensive Care Medicine, Jena University Hospital, Jena, Germany; and Septomics Research Centre, Jena University Hospital, Jena, Germany, Phone: +49 3641 9323200,
E-mail: sina.coldewey@med.uni-jena.de; and **Michael Kiehntopf**, Institute of Clinical Chemistry and Laboratory Diagnostics and Integrated Biobank Jena (IBBJ), Jena University Hospital, Jena, Germany, Phone: +49 36419325000,
E-mail: michael.kiehntopf@med.uni-jena.de
Arite Bigalke, Institute of Clinical Chemistry and Laboratory Diagnostics and Integrated Biobank Jena (IBBJ), Jena University Hospital, Jena, Germany. https://orcid.org/0000-0003-1283-3117
Charles Neu and Ricardo Esper Treml, Department of Anaesthesiology and Intensive Care Medicine, Jena University Hospital, Jena, Germany; and Septomics Research Centre, Jena University Hospital, Jena, Germany

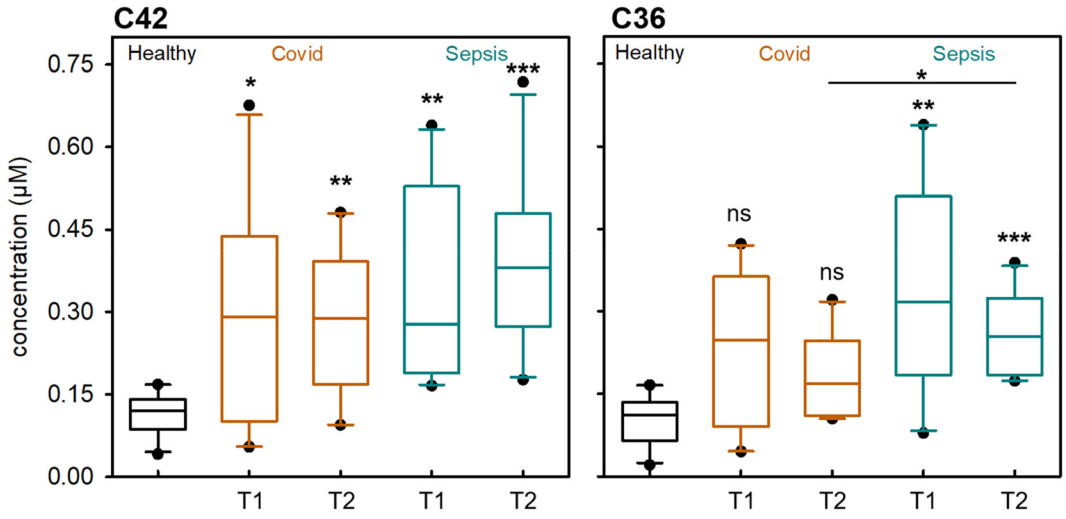

Figure 1: Concentrations of C36 and C42 detected in EDTA plasma (n=10 in each group).
T1: three days and T2: seven days after onset of severe disease. Significance was determined using one-way-ANOVA on ranks with Dunn's multiple comparison against control for each time point. t-Test was used for comparison between sepsis and COVID-19. ***p<0.001, **p<0.01, *p<0.05.

severity at T1 (median SOFA sepsis: 5.5, COVID-19: 3.5). Patients with severe COVID-19 had a higher body weight than healthy controls (median healthy: 74.35, sepsis: 84, COVID-19: 93.5).

Both patient groups (COVID-19 and bacterial sepsis) showed significantly higher concentrations of C-42 compared with healthy controls (Figure 1). Conversely, C-36 was significantly increased only in patients with bacterial sepsis compared with healthy controls, though there was a non-significant trend towards elevated C-36 levels also in patients with COVID-19. Patients with bacterial sepsis also showed significantly higher levels of C-36 at T2 compared to patients with COVID-19 at this time point.

To the best of our knowledge, this is the first report of elevated C-36 and C-42 concentrations in plasma of patients with severe COVID-19 and viral infections in general. Our results are in line with the findings by Zerimech et al., who demonstrated higher concentrations of plasma protease levels, including MMP-12 and NE, in more severe cases of COVID-19, supporting the proposed imbalance between proteases and antiprotease [1]. Similarly, we found no differences in C-36 or C-42 concentrations between three and seven days after onset of severe disease, indicating a steady state during the acute phase. The main cellular sources of MMP-12 and NE, macrophages and neutrophils, have been shown to be drivers of ARDS in both COVID-19 and bacterial ARDS [9]. However, the differences in C-36 peptide concentrations may indicate a notable difference in the pathophysiology of these two syndromes, e.g. a less pronounced role of neutrophils in severe COVID-19 compared with bacterial pulmonary sepsis. Hitherto, no study has compared the plasma activities of NE of both patient groups at defined time points, making an interpretation of our results in a broader context more difficult.

The results should be interpreted in light of the limitations of this study. A limitation of this study is its small sample size, possibly limiting the generalizability of the results. Also, concentrations of proteases in the blood may only partially reflect concentrations in the pulmonary tissue.

Therefore, larger studies are needed comparing the activities of these proteases and their cleavage products in these two syndromes. A distinct molecular pattern distinguishing COVID-19 from bacterial sepsis would be vital to the development of specific therapies for this syndrome.

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Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission. SMC and MK designed and supervised the study. AB, CN and RET acquired and analyzed the data. All authors drafted and revised the manuscript before submission.

Competing interests: MK and AB are inventors of a patent covering a method for quantification of C-terminal peptides of AAT (applicant: University Hospital Jena (JUH); number: 22154836.5; status of application: submitted). JUH is also applicant of other published patents related to methods determining the origin of an infection (EP17719610.2; EP16167699.4) or covering the initial identification of C42 (CN104204808, EP2592421, EP2780719, US20170242035; Applicant: University Hospital Jena; Inventors: M. Kiehntopf, D. Schmerler, T. Deufel, F. Brunkhorst). CN, RET and SMC declare no competing interests.

Informed consent: Written informed consent was obtained from all individuals or their legal representative before inclusion in this study.

Ethical approval: The study was approved by the Ethics Committee of the Friedrich Schiller University Jena (5276-09/17) and conducted in accordance with the Declaration of Helsinki.

Data availability: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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