Letter to the Editor

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C-terminal alpha-1-antitrypsin peptides as novel predictor of hospital mortality in critically ill COVID-19 patients

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To the Editor,

The COVID-19 pandemic caused by the SARS-CoV-2 virus has resulted in over 771 million confirmed cases and more than 6 million deaths worldwide by the end of 2023 [1]. While the pandemic has since slowed down due to vaccination and natural immunity, understanding the molecular mechanisms behind infection and disease progression remains crucial not only for the remaining COVID-19 patients but also to further our understanding of other severe infectious diseases like sepsis.

One major shortcoming during the COVID-19 pandemic was the prediction of disease severity in SARS-CoV-2 positive patients. While well-known risk factors like age, BMI and pre-existing conditions were also associated with COVID-19 disease severity, also many young and seemingly healthy people developed severe conditions [2]. Furthermore, classic laboratory parameters of inflammation and disease severity like interleukin-6 (IL-6) and procalcitonin (PCT) were of limited value. Treatment with corticosteroids like dexamethasone, which is standard of care for ventilated COVID-19 patients, severely downregulates these two markers [3].

To overcome these shortcomings, we evaluated the prognostic potential of C-terminal alpha-1-antitrypsin peptides (CAAPs). These peptides are proteolytic cleavage products of alpha-1-antitrypsin (AAT), an important immunomodulatory protease inhibitor. As acute phase protein, AAT is highly upregulated during inflammation and has multifaceted roles in the innate immune response [4]. Several endogenous as well as pathogen-derived proteases have been described to interact with and cleave AAT, resulting in the release of CAAPs. Importantly, CAAPs have already been found in several infectious and inflammatory conditions. C42 has been previously proposed as sepsis marker, and we already showed in a pilot study that both C36 and C42 are found to be elevated in the plasma of COVID-19 patients [5, 6].

In this work, we measured CAAPs plasma concentrations in 84 patients with severe COVID-19 on day one to four after their admission to the ICU. In our cohort, we observed a mortality rate of 26.2 % (Table 1). Non-survivors were older than survivors (median 74.5 vs. 63 years), while BMI and sex ratio did not differ significantly between the groups. In accordance with disease severity, non-survivors had higher SOFA scores and needed ECMO, dialysis and mechanical ventilation interventions more often than survivors. We also determined the levels of IL-6 and PCT as gold standard and found them significantly elevated in non-survivors (42.1 vs. 18.5 ng/L and 0.29 vs. 0.14 µg/L, respectively). These concentrations are in accordance with previously published reports, except for the PCT levels in non-survivors, which were usually higher than in our cohort.

Regarding the C-terminal AAT peptides, we observed a trend of elevated plasma concentrations in non-survivors, with the exception of one peptide, C44, which showed an opposite trend. In addition, we calculated CAAP/IL-6 ratios analogous to Philippe et al. investigating the AAT/IL-6 ratio as a measure of immune system deregulation [7]. Ratios of

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Table 1: Characteristics at sampling timepoint (day 1–4 after ICU admission) and pre-existing conditions of patients who later died in hospital or survived and were discharged.

	Non-survivor, n=22[a]	Survivor, n=62[a]	p-Value[b]
Age, years	74.5 (68.0, 83.0)	63.0 (55.0, 75.8)	0.008
Sex			0.9
f	6 (27 %)	18 (29 %)	
m	16 (73 %)	44 (71 %)	
BMI	28.0 (25.0, 30.0)	29.0 (25.3, 35.8)	0.2
Arterial hypertension	13 (59 %)	51 (82 %)	0.028
COPD	1 (4.5 %)	4 (6.5 %)	>0.9
Diabetes mellitus	5 (23 %)	24 (39 %)	0.2
Liver cirrhosis	1 (4.5 %)	0 (0 %)	0.3
SOFA	5.5 (4.0, 7.0)	4.0 (3.0, 6.0)	0.010
ECMO	7 (32 %)	5 (8.1 %)	0.012
Dialysis	14 (64 %)	10 (16 %)	<0.001
Mechanical ventilation	18 (82 %)	21 (34 %)	<0.001
PCT, µg/L	0.29 (0.10, 0.64)	0.14 (0.06, 0.25)	0.040
IL-6, ng/L	42.10 (26.90, 89.73)	18.50 (9.20, 36.08)	0.001
C36, nM	200 (169, 240)	167 (135, 213)	0.071
C37, nM	39 (35, 62)	39 (28, 59)	0.4
C40, nM	18 (14, 24)	15 (11, 19)	0.028
C42, nM	177 (149, 226)	174 (143, 202)	0.4
C43, nM	15 (14, 16)	14 (12, 16)	0.3
C44, nM	19 (17, 25)	22 (18, 27)	0.2
C36/IL-6	4.5 (1.7, 7.0)	8.6 (4.4, 20.1)	0.005
C37/IL-6	0.9 (0.4, 1.6)	2.2 (1.1, 4.6)	0.003
C40/IL-6	0.4 (0.1, 0.7)	0.8 (0.3, 1.7)	0.022
C42/IL-6	3.7 (1.9, 6.0)	8.6 (4.4, 20.1)	0.002
C43/IL-6	0.3 (0.2, 0.5)	0.7 (0.4, 1.7)	0.002
C44/IL-6	0.4 (0.2, 0.8)	1.4 (0.5, 2.7)	<0.001

[a]Median (IQR); n (%), [b]Wilcoxon rank sum test; Pearson's Chi-squared test; BMI, body mass index; COPD, chronic obstructive pulmonary disease; SOFA, sequential organ failure assessment score; ECMO, extracorporeal membrane oxygenation support; PCT, procalcitonin; IL-6, interleukin-6, f, female; m, male.

C42, C43 and C44 with IL-6 were significantly decreased in non-survivors (Table 1). We further confirmed that those ratios offer additive predictive value regarding hospital mortality compared to IL-6 or PCT alone. Logistic regression models using IL-6 or PCT as predictors performed better regarding their akaike information criterion (AIC) and McFadden's R^2 when C36, C40, C42, C44 or any CAAP/IL-6 ratio was added (Supplement Table 1).

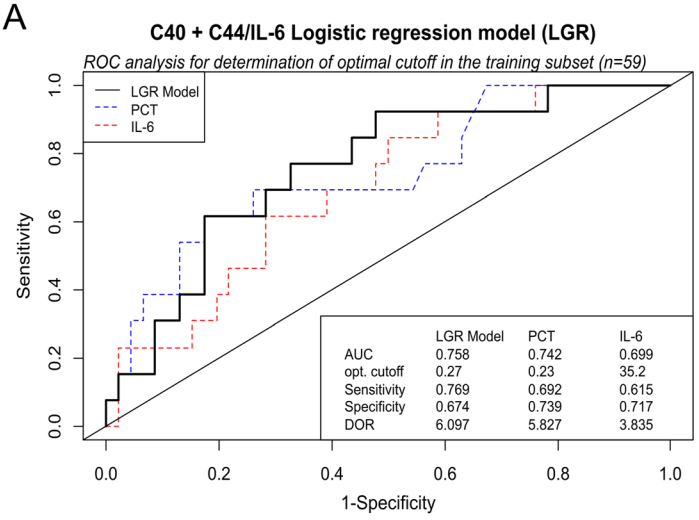
Lastly, we identified the most informative predictors of hospital mortality among CAAPs and CAAP/IL-6 ratios by logistic regression. A forward and backward selection based on the AIC was performed using the *stepAIC* function of the *MASS* package in R. The final model consisted of C40 and C44/IL-6 as predictors and was compared to models using only IL-6 or PCT as predictors (Figure 1, Supplement Table 2). We trained all models in a random 70 % split of the cohort (training set) and evaluated them in the remaining 30 % (test set). In the training set, best performance characterized by highest area under the receiver operating characteristic curve (AUC) was achieved by our model (AUC=0.758), followed by PCT (AUC=0.742) and IL-6 (AUC=0.699). Next, we calculated optimal cutoffs for prediction of hospital mortality as determined by Youden's index for all three models and applied them in the test set. Here, our model containing CAAPs (sensitivity 78 %, specificity 63 %) and the IL-6 model (sensitivity 67 %, specificity 75 %) both performed comparably well (Figure 1B). In contrast, PCT only displayed a sensitivity and specificity of 44 % and 50 %, respectively.

These findings add to a growing list of diagnostic and prognostic value of AAT and CAAPs. Our observation that most CAAP concentrations, especially of C36 and C42, are elevated in non-survivors is in accordance with previous findings linking CAAPs to various inflammatory conditions. Like in bacterial sepsis, the main clinical driver of disease severity in COVID-19 seems to be a deregulated immune response [2]. An overwhelmingly inflammatory state is reportedly correlated with poor outcome and higher CAAPs concentrations are therefore to be expected in those patients.

In contrast, one novel and interesting finding of this study is the reduced plasma concentration of C44 in patients who later died in hospital, in contrast to other CAAPs increasing in those patients. Only one protease, the matrix metalloprotease 11 (MMP-11, also known as stromelysin 3), is known to produce C44 upon cleavage of AAT *in vitro* [8]. One possible explanation for reduced plasma levels of C44 in patients with poor outcome could be that the immune response in those patients is more severely deregulated than in surviving patients. Overwhelming inflammation is known to correlate with increased serum levels of proteases that cleave AAT to produce e.g. C42 and C37 (neutrophil and macrophage associated MMPs), C36 (neutrophil elastase) or C40 (cathepsin L) [9]. Increased abundance and activity of those inflammation-associated proteases could lead to a preferred cleavage of AAT at the corresponding cleavage sites, leaving less AAT to be cleaved at the C44 site. Furthermore, it has been shown that expression of MMP-11 in pulmonary microvascular endothelial cells (PMVEC) is significantly reduced in an *in vitro* sepsis model [10]. This might hint towards lower circulatory MMP-11 levels and therefore reduced production of C44 under inflammatory conditions.

To summarize, C-terminal AAT peptide levels at ICU submission tend to be different in patients who later died in hospital compared to those who survived. Some of these peptides, as well as CAAP/IL-6 ratios provided additional

A



B

Application of cut-offs in the test subset (n=25)

	Cut-off	Sensitivity	Specificity	DOR
LGR Model	0.27	0.78	0.63	4.85
PCT	0.23	0.44	0.5	0.82
IL-6	35.2	0.67	0.75	5.16

Figure 1: Comparison of logistic regression model with PCT and IL-6 levels for hospital mortality prediction. (A) Depicts the ROC analysis of our final logistic regression (LGR) model combining C40 and C44/IL-6 in comparison with PCT and IL-6 levels in the training subset (n=59) and lists AUC as well as sensitivity, specificity and diagnostic odds ratio (sorry, my fault, I overlooked the 's) (DOR) at the optimal cut-off as determined by Youden's statistic. These cut-offs were applied in the test subset to determine sensitivity, specificity and DOR of the LGR model, PCT or IL-6 levels, respectively (B). AUC: area under the curve; ROC: receiver operating characteristic, PCT: procalcitonin, IL-6: interleukin 6.

predictive value compared to classic parameters IL-6 or PCT in our cohort. Especially C40 and C44/IL-6 were promising markers in our analysis underscoring them as worthwhile targets for future analyses. Although this study is a retrospective analysis of a small, monocentric cohort, our findings provide novel insights into the prognostic value of C-terminal alpha-1-antitrypsin peptides in critically-ill COVID-19 patients and might also spark new investigations in other systemic inflammation disorders like sepsis. Replicating our findings in larger cohorts and exploring the mechanistic links between CAAPs, AAT, and the pathogenesis of various infectious diseases may shed light on the underlying biological processes driving severe disease courses.

Research ethics: This study was approved by the Ethics Board of the Jena University Hospital (ref. No. 2020_1797) and was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Informed consent: Informed consent was obtained from all individuals included in this study, or their legal guardians or wards.

Author contributions: FS: study design; method development; acquisition, analyses and interpretation of the data; writing the manuscript. DS: methods, data curation, interpretation of results, writing – review & editing. DT-R: data collection, data curation, interpretation of results, writing – review & editing, MB: study design; discussion and critical reviewing of data, writing – review & editing, MK: study design; discussion, interpretation and critical reviewing of data, writing – review & editing. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Michael Kiehntopf is an inventor of a patent application covering the utilized LC-MS/MS method as a tool for characterizing systemic inflammation (applicant: Jena University Hospital; inventors: Arite Bigalke and Michael Kiehntopf; published as EP4224163A1). Jena University Hospital is owner of a patent related to methods determining the origin of an infection (EP3239712: granted; inventors: Michael Kiehntopf, Diana Schmerler). A patent covering the initial identification of C42 was granted as well (published as CN104204808B, JP6308946B2, US10712350B2, EP2592421B1, EP2780719B1; owner: Jena University Hospital; Inventors: Michael Kiehntopf, Diana Schmerler, Thomas Deufel, Frank Brunkhorst). All other authors state no conflict of interest.

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Data availability: The raw data can be obtained on request from the corresponding author.

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Supplementary Material: This article contains supplementary material (https://doi.org/10.1515/cclm-2024-0920).