



Circulating dipeptidyl peptidase 3 and bio-adrenomedullin levels are associated with impaired outcomes in critically ill COVID-19 patients: a prospective international multicentre study

Dirk van Lier^{1,2}, Benjamin Deniau^{3,4}, Karine Santos⁵, Oliver Hartmann^{5,6}, Emmanuel Dudoignon^{3,4}, François Depret^{3,4}, Benoit Plaud^{3,4}, Pierre-François Laterre⁷, Alexandre Mebazaa³ and Peter Pickkers^{1,2}

¹Radboud University Medical Center, Department Intensive Care, Nijmegen, The Netherlands. ²Radboud University Medical Center, Radboud Center for Infectious Diseases, Nijmegen, The Netherlands. ³Department of Anesthesia, Burn and Critical Care, University Hospitals Saint-Louis – Lariboisière, AP-HP, Paris, France. ⁴Université de Paris Cité, Paris, France. ⁵4TEEN4 Pharmaceuticals GmbH, Hennigsdorf/Berlin, Germany. ⁶Sphingotec GmbH, Hennigsdorf/Berlin, Germany. ⁷Cliniques Universitaires Saint-Luc, UCL Bruxelles, Brussels, Belgium.

Peter Pickkers (Peter.Pickkers@radboudumc.nl)



Shareable abstract (@ERSpublications)

cDPP3 and bio-ADM responses are associated with short-term outcome in critically ill #COVID19 patients. These findings suggest that treatment with specific antibodies against these biomarkers may improve outcome of COVID-19 patients. <https://bit.ly/3SWx3EE>

Cite this article as: van Lier D, Deniau B, Santos K, *et al.* Circulating dipeptidyl peptidase 3 and bio-adrenomedullin levels are associated with impaired outcomes in critically ill COVID-19 patients: a prospective international multicentre study. *ERJ Open Res* 2023; 9: 00342-2022 [DOI: 10.1183/23120541.00342-2022].

Copyright ©The authors 2023

This version is distributed under the terms of the Creative Commons Attribution Non-Commercial Licence 4.0. For commercial reproduction rights and permissions contact permissions@ersnet.org

Received: 11 July 2022
Accepted: 4 Oct 2022

Abstract

Introduction Dipeptidyl peptidase-3 (DPP3) is a protease involved in the degradation of several cardiovascular mediators. Adrenomedullin (bio-ADM) is a peptide essential for regulation of endothelial barrier function. In different shock-pathologies, both biomarkers are associated with disease severity, organ dysfunction and mortality. Associations with outcome in critically ill COVID-19 patients are unknown. The objectives of the present study were to investigate associations of bio-ADM and “circulating DPP3” (cDPP3) with short-term outcome in critically ill COVID-19 patients (n=80).

Methods A multicentre prospective cohort study was performed. The primary end-point was 28-day mortality. Secondary end-points included different severities of acute kidney injury (AKI).

Results cDPP3 levels were mainly associated with 28-day mortality; Area under the receiver operating characteristics (AUROCs) of 0.69 (0.56–0.82, p=0.023), 0.77 (0.64–0.90, p<0.001) and 0.81 (0.65–0.96, p<0.001) at admission, day 3 and day 7, respectively. In contrast, bio-ADM levels were mainly associated with AKI, with AUROCs of 0.64 (0.51–0.77, p=0.048), 0.75 (0.64–0.86, p<0.001) and 0.83 (0.74–0.93, p<0.001) for day 1, 3 and 7, respectively. Interestingly, patients with high levels of both cDPP3 and bio-ADM at day 7 had an additionally increased risk of 28-day mortality (hazard ratio 11.8; 95% CI 2.5–55.3, p<0.001).

Conclusions cDPP3 and bio-ADM responses were associated with short-term mortality and AKI in critically ill COVID-19 patients, respectively. These findings suggest that treatment with specific antibodies modulating cDPP3 or bio-ADM-related pathways may improve outcome of COVID-19.

Introduction

Since late 2019, the world has been hit by an unprecedented pandemic crisis due to SARS-CoV-2. Ever since the pandemic started, there has been a global effort to search for predictors of COVID-19 disease severity and clinical outcome [1, 2]. While early studies provided an initial understanding on broad risk factors associated with detrimental outcome in SARS-CoV-2 infection, including age and cardiovascular disease, these were all non-modifiable risk factors, not amendable to therapeutic interventions [2]. The use of interleukin-6 (IL-6) antagonist therapies in combination with steroids has effectively improved survival of critically ill COVID-19 patients [3]. Interestingly, the beneficial effects of dexamethasone appear to be



most pronounced in patients with a hyperinflammatory phenotype [4], while IL-6 concentrations predict the therapeutic efficacy of tocilizumab [5]. These studies not only suggest that severe COVID-19 pathology is accompanied by elevated levels of several (inflammatory) parameters, but also that the levels of differently activated molecular pathways can predict the therapeutic efficacy of interventions.

Dipeptidyl peptidase-3 (DPP3) is a ubiquitous cytosolic metallopeptidase involved in blood pressure regulation, inflammation and pain regulation [5, 6]. When cell injury occurs, intracellular DPP3 is released into the circulation (coined cDPP3 for “circulating DPP3”). The subsequent rapid degradation of cardiovascular mediators such as angiotensin II by cDPP3 may contribute to haemodynamic instability [6]. Recent experimental and clinical studies demonstrated that high levels of cDPP3 are associated with organ dysfunction, such as renal and cardiac failure, and mortality in patients with sepsis [7, 8]. Biologically active adrenomedullin (bio-ADM) is a 52 amino-acid peptide essential for the regulation of vascular tone and endothelial barrier function [6, 9]. High concentrations of bio-ADM were observed in different shock-associated pathologies, with highest levels observed in septic shock. During sepsis, bio-ADM kinetics are strongly associated with sepsis severity and development of organ dysfunction, as well as mortality [6, 10].

Interestingly, combining measurements of both these biomarkers in *e.g.* cardiac surgery [11] or septic shock patients [6] improved accuracy to predict clinical outcomes, compared to separate assessment of individual biomarkers. Lastly, both biomarkers represent promising druggable targets, as bio-ADM- and cDPP3-modulating therapies have already shown beneficial effects in animal models of cardiogenic and septic shock [12–14], with clinical studies currently planned or ongoing [15].

Understanding the temporal and quantitative response patterns of bio-ADM and cDPP3 in critically ill patients with COVID-19 may improve our understanding of COVID-19-associated molecular pathophysiology. It may also provide important information on the likelihood of therapeutic efficacy of treatments related to these pathways. The main objective of this study was to clarify the associations between bio-ADM and cDPP3 and the occurrence of 28-day mortality in critically ill patients with COVID-19. Secondary end-points included the biomarkers’ association with acute kidney injury (AKI) (as a representative of organ failure), as well as to investigate the kinetics of both biomarkers in patients with COVID-19.

Methods

Study design and population

We performed a multicentre prospective cohort study including all COVID-19 patients admitted to an intensive care unit (ICU) from March to April 2020. Participating centres included the Radboudumc University Medical Center in Nijmegen (Netherlands) and the Lariboisiere and Saint-Louis hospitals in Paris (Université de Paris Cité, assistance Publique des Hôpitaux de Paris, France). All patients admitted to the ICU for severe COVID-19 were eligible. Patients aged under 18 years or those who were pregnant were excluded from the analysis. The diagnosis of COVID-19 was based on the detection of SARS-CoV-2 from nasopharyngeal samples by PCR and the clinical picture of COVID-19.

This study was carried out 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 details of this cohort study and could decline to participate. Clinical and biological data were recorded at admission, as well as days 3, 7, 14, 21 and 28 of ICU admission. Patient discharge status and mortality were recorded up to day 28 after ICU admission, regardless of patient admission status. Primary end-points were 28-day mortality and AKI. AKI was defined and staged according to the Kidney Disease: Improving Global Outcomes (KDIGO) criteria [16]. The kinetics of both biomarkers during the course of the first week of admission represented a secondary end-point.

Measurements

Ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood for determination of cDPP3 and bio-ADM concentrations was sampled within 24 h after ICU admission; follow-up samples were acquired on day 3 and 7 after ICU admission. After centrifugation, plasma was stored at -80°C until blinded bio-ADM and cDPP3 analysis using luminescence immunoassays for bio-ADM (Sphingotec GmbH, Hennigsdorf, Germany) and cDPP3 (4TEEN4 Pharmaceuticals GmbH, Hennigsdorf, Germany) [17, 18]. After patient enrolment, the following data were collected at baseline and on days 3, 7, 14 and 28: need for renal replacement therapy, need for vasopressor therapy and need for invasive mechanical ventilation. The following routine laboratory parameters were also collected: leukocytes, serum creatinine, troponin-T, creatinine-kinase, aspartyl-aminotransferase (ASAT), alanine-aminotransferase (ALAT), interleukin-6 (IL-6) and lactate.

Statistical analyses

Data are presented as median (interquartile range (IQR)) or mean (95% confidence interval (CI)), according to distribution, and as counts and percentages for categorical variables. Group comparisons of continuous variables were performed using Mann–Whitney U-tests or Kruskal–Wallis tests, depending on the number of groups. If Kruskal–Wallis tests yielded overall significance, Tukey’s *post hoc* tests were performed. Data were log-transformed if not normally distributed. Categorical data were compared using Fisher’s exact tests. Correlations between continuous variables were calculated using Pearson’s correlation. If correction for repeated observations was necessary, the Bland–Altman method was used [19]. Area under the receiver operating characteristics (AUROCs) with 95% CIs are reported as an effect measure of the discriminative capacity of different predictor variables. Differences between AUROCs were assessed with the Hanley–McNeil method. To assess whether combining biomarker measurements with other known predictor variables improved predictive value, the added value of these biomarkers was evaluated using logistic regression modelling. Different variables with significant predictive value were entered as independent variables, with binary outcomes as dependent variables. To test for added predictive value, we used the likelihood ratio chi-square test for nested models to assess whether a biomarker added predictive value to a clinical model or a risk score. When dichotomising the population based on a continuous biomarker measurement was necessary, cut-offs defined by earlier studies were used for both cDPP3 (40 ng·mL⁻¹; [6, 7]) and bio-ADM (70 pg·mL⁻¹; [20, 21]). Sensitivity, specificity and accuracy implementing these cut-offs are reported as additional biomarker effect measures. Kaplan–Meier curves implementing these cut-offs were generated for illustrative purposes.

A two-sided p-value of <0.05 was considered statistically significant. All analyses were performed using Statistical Package for the Social Sciences (SPSS) version 25.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 8.0 (GraphPad Software, La Jolla, CA, USA).

Results

Study population

cDPP3 and bio-ADM were measured in a cohort of 80 patients hospitalised in the ICU for COVID-19, from March 2020 to April 2020. Baseline characteristics of studied patients are depicted in table 1. Over

TABLE 1 Admission baseline characteristics of the total cohort, as well as admission characteristics of 28-day survivors *versus* non-survivors

	Total	Non-survivors	Survivors	p-value
Subjects n	80	18	62	
Demographics				
Sex, male	60 (75.0)	14 (77.8)	46 (74.2)	1.000
Age years	65 (57–70)	64 (58–71)	65 (57–69)	0.809
BMI kg·m ⁻²	27.5 (25.4–31.0)	29.6 (27.0–32.1)	27.0 (24.6–30.6)	0.037
Medical history				
COPD	4 (5.0)	0 (0.0)	4 (6.5)	0.570
Diabetes	31 (38.8)	7 (38.9)	24 (38.7)	1.000
Hypertension	41 (51.2)	9 (50.0)	32 (51.6)	1.000
Immune-deficiency	7 (8.8)	3 (16.7)	4 (6.5)	0.185
Characteristics during hospitalisation				
Leukocytes	8.5 (6.2–12.4)	9.5 (7.3–11.5)	8.4 (6.0–12.5)	0.541
Creatinine _{day1} μmol·L ⁻¹	80 (61–119)	125 (72–269)	77 (58–100)	0.004
Lactate _{day1} mmol·L ⁻¹	1.2 (1.0–1.6)	1.5 (1.0–1.9)	1.2 (1.0–1.6)	0.102
Troponin-T _{day1} ng·L ⁻¹	23 (14–56)	68 (19–130)	22 (11–42)	0.013
P/F ratio	146 (96–200)	88 (66–183)	165 (118–203)	0.036
Vasopressors	38 (47.5)	7 (38.9)	31 (50.0)	0.289
Inotropics	3 (3.8)	1 (5.6)	2 (3.2)	0.540
Mechanical ventilation	66 (82.5)	15 (83.3)	51 (82.3)	1.000
AKI (any KDIGO stage)	34 (42.5)	9 (50.0)	25 (40.3)	0.590
AKI (KDIGO stage 3)	15 (18.8)	7 (38.9)	8 (12.9)	0.034
Severity scores				
Admission SOFA	6 (4–7)	6 (3–9)	6 (4–7)	0.731

Data presented as n (%) or median (IQR) unless stated otherwise. BMI: body mass index; COPD: chronic obstructive pulmonary disease; P/F ratio: P_{aO_2}/F_{iO_2} ratio; AKI: acute kidney injury; KDIGO: Kidney Disease: Improving Global Outcomes; SOFA: sequential organ failure assessment.

the 28-day follow-up period, a total of 18 (22.5%) patients died. As patients were included during the first COVID-19 surge, none of the patients received treatment with dexamethasone or IL-6-targeted antibodies.

Both cDPP3 and bio-ADM levels were associated with 28-day mortality in critically ill patients with COVID-19

At ICU admission, patients that would eventually not survive already presented with higher plasma creatinine (median: 125 (IQR: 72–269) versus 77 (58–100), $p=0.004$), higher troponin-T (68 (19–130) versus 22 (11–42), $p=0.013$), and a worse arterial oxygen tension (P_{aO_2})/inspiratory oxygen fraction (F_{IO_2}) ratio (88 (66–183) versus 165 (118–203), $p=0.036$) (table 1). At ICU admission, median (IQR) cDPP3 and bio-ADM concentrations were $35.7 \text{ ng}\cdot\text{mL}^{-1}$ (25.0–54.3) and $47.7 \text{ pg}\cdot\text{mL}^{-1}$ (33.5–78.8), respectively. 28-day non-survivors presented with higher admission cDPP3 levels compared to survivors, and these differences became more pronounced over time (figure 1a) with AUROCs of 0.69 (0.56–0.82, $p=0.023$), 0.77 (0.64–0.90, $p<0.001$) and 0.81 (0.65–0.96, $p<0.001$) for admission, day 3 and day 7, respectively (figure 2a). In multivariate analyses (implementing creatinine and P_{aO_2}/F_{IO_2} ratio, as these were found to be significantly associated with 28-day mortality as well), the more pronounced predictive value of DPP3 over time remained present: DPP3 day 1 standardised hazard ratio (HR) 2.02 (95% CI 0.77–5.29), $p=0.152$; day 3 standardised HR 2.49 (95% CI 0.98–6.31), $p=0.054$; and day 7 standardised HR 3.51

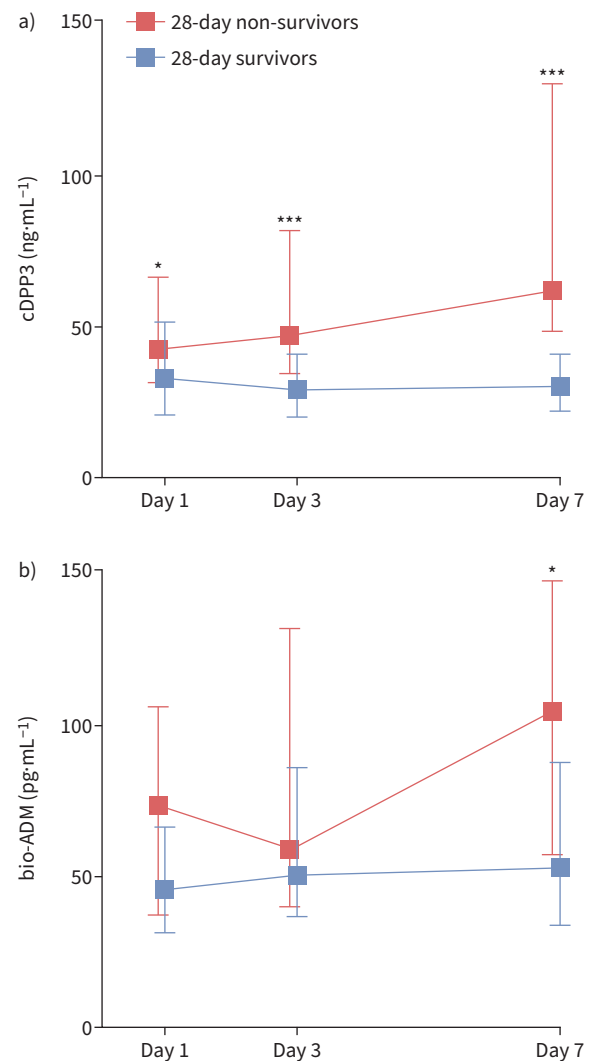


FIGURE 1 Biomarker measurements of a) cDPP3 and b) bio-ADM on day 1, 3 and 7 of ICU admission. Results of 28-day survivors and non-survivors are displayed, respectively. The median, interquartile ranges and Tukey's hinges are displayed. cDPP3: circulating dipeptidyl peptidase 3; bio-ADM: biologically active adrenomedullin. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

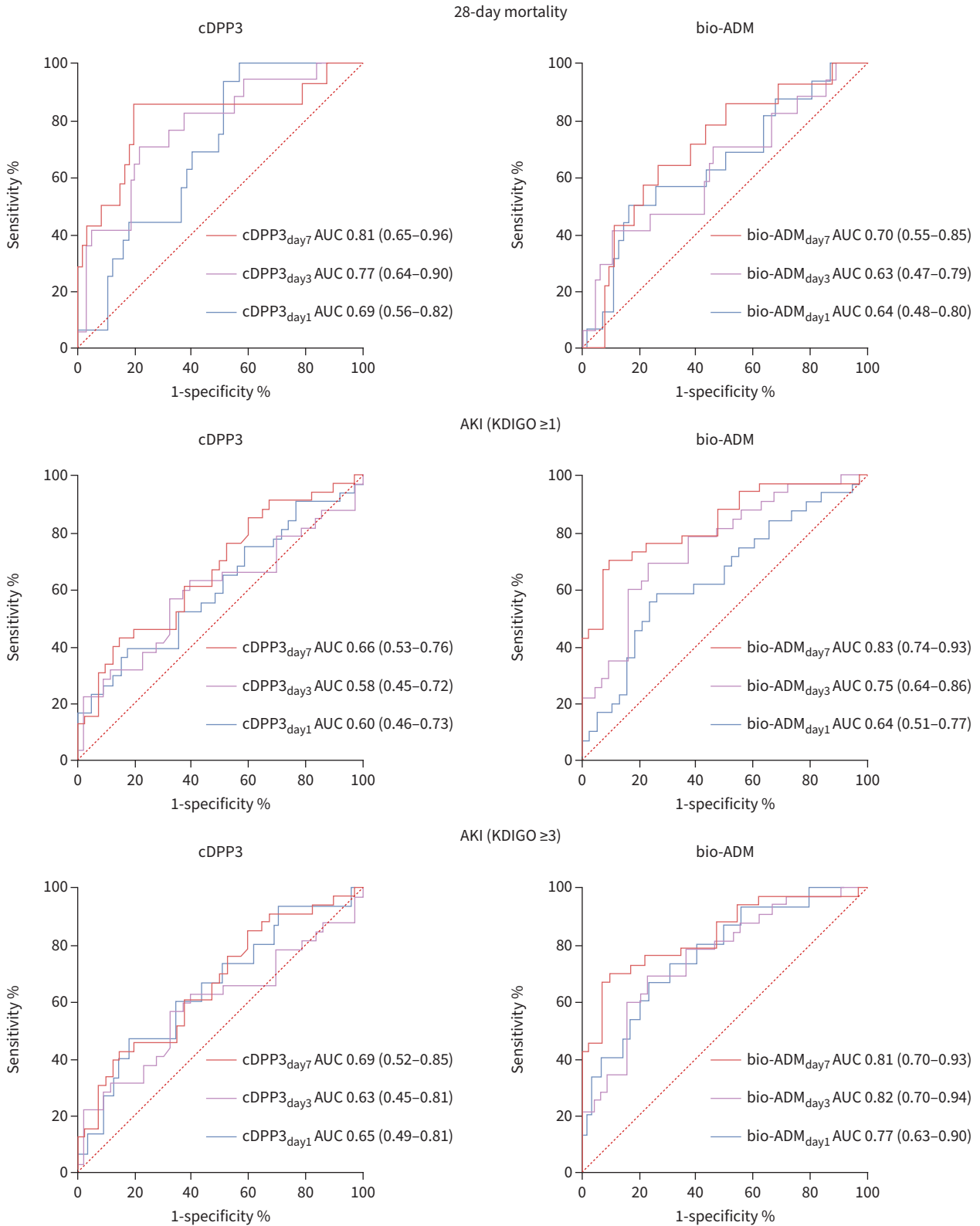


FIGURE 2 Areas under the receiver operating characteristic curve (AUC) of cDPP3 and bio-ADM on days 1, 3 and 7 of ICU admission. Separate graphs display different study outcomes. Acute kidney injury (AKI) was defined according to the Kidney Disease: Improving Global Outcomes (KDIGO) criteria. Bio-ADM: bioactive adrenomedullin; cDPP3: circulating dipeptidyl peptidase 3.

(95% CI 1.21–10.15), $p=0.021$. Bio-ADM concentrations did not differ significantly between non-survivors and survivors on day 1 and 3, but concentrations were higher at day 7 in non-survivors (figure 1b). Consequently, day 1 and 3 bio-ADM levels did not significantly predict 28-day mortality, with AUROCs of 0.64 (0.48–0.80, $p=0.089$) and 0.63 (0.47–0.79, $p=0.110$), respectively (figure 2a). At day 7, significant predictive capacity was present, with an AUROC of 0.70 (0.55–0.85, $p=0.020$) (figure 2a). Combining cDPP3 (cut-off at $40 \text{ ng}\cdot\text{mL}^{-1}$) and bio-ADM (cut-off at $70 \text{ pg}\cdot\text{mL}^{-1}$) measured at day 7 showed additional prognostic value to predict 28-day mortality compared to separate assessment of both biomarkers alone; HR 11.8 (2.5–55.3, $p<0.001$) compared to patients with one of the two, or both biomarkers below the cut-off value (figure 3). An overview of the sensitivity, specificity and accuracy of associations with 28-day mortality of both biomarkers implementing predefined cut-offs is provided in figure 4a and b.

Bio-ADM levels were associated with the development of AKI

An important proportion of COVID-19 patients ($n=34$, 42.5%) developed AKI during the 28-day follow-up (median diagnosis day 3 (3–7) days). A total of 16 (20.0%), 3 (3.8%) and 15 (18.8%) patients developed KDIGO stage 1, 2 and 3, respectively. Of note, patients that developed KDIGO stage 3 AKI were more likely to die during admission; 7 out of 15 (46.7%) versus 11 out of 65 (16.9%) ($p=0.034$), while no differences in survival status were present in patients that developed KDIGO stage 1 or 2 AKI (table 1). No associations between cDPP3 and creatinine were found (table 2). As depicted by figure 2b and c, cDPP3 concentrations at day 1 and day 3 were not associated with the development of AKI. Associations with day 7 cDPP3 levels and AKI were present, albeit only weakly; AUROC of 0.66 (0.53–0.76, $p=0.019$) for all stage AKI, and AUROC of 0.69 (0.52–0.85, $p=0.032$) for stage 3 AKI, respectively (figure 2b and c).

By contrast, bio-ADM levels were associated with the occurrence of all-stage AKI (figure 2b). Bio-ADM levels correlated most strongly with creatinine ($r=0.62$, $p<0.001$). Association between bio-ADM levels and all stage AKI became more pronounced from day 1 to day 7 (Hanley–McNeil $p=0.022$), with AUROCs of 0.64 (0.51–0.77, $p=0.048$), 0.75 (0.64–0.86, $p<0.001$) and 0.83 (0.74–0.93, $p<0.001$) for day 1, 3 and 7, respectively (figure 2b). An overview of the sensitivity, specificity and accuracy of associations with all-stage AKI of both biomarkers implementing predefined cut-offs is provided in figure 4c and d. Associations between bio-ADM and stage 3 AKI were also present for all time points, with good discriminative capacity already being present for day 1 and 3 time points (figure 2c). Lastly, to further investigate factors influencing biomarker kinetics, a combined approach (with subgroups based on the

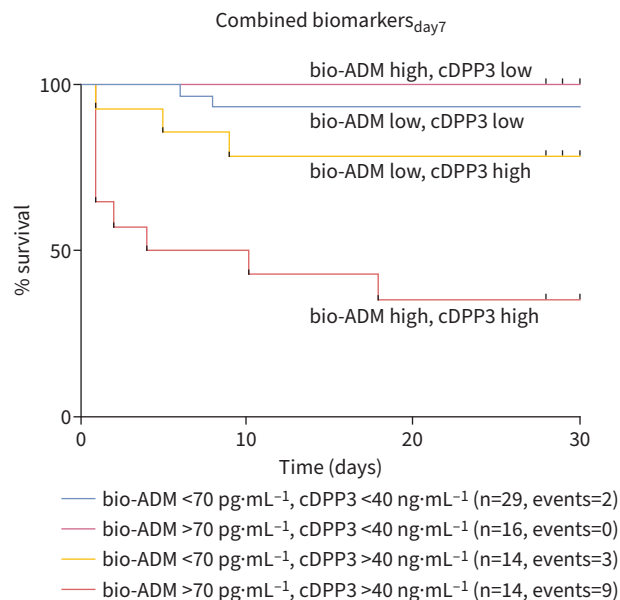


FIGURE 3 Kaplan–Meier analysis of 28-day mortality in critically ill COVID-19 patients on day 7 after ICU admission. Groups are based on high cDPP3 ($>40 \text{ ng}\cdot\text{mL}^{-1}$) versus low cDPP3 ($<40 \text{ ng}\cdot\text{mL}^{-1}$), as well as high bio-ADM ($>70 \text{ pg}\cdot\text{mL}^{-1}$) versus low bio-ADM ($>70 \text{ pg}\cdot\text{mL}^{-1}$). The reported hazard ratio compares the group with high cDPP3 and high bio-ADM levels to the other three groups (curves displayed separately in figure for illustrative purposes). Bio-ADM: bioactive adrenomedullin; cDPP3: circulating dipeptidyl peptidase 3.

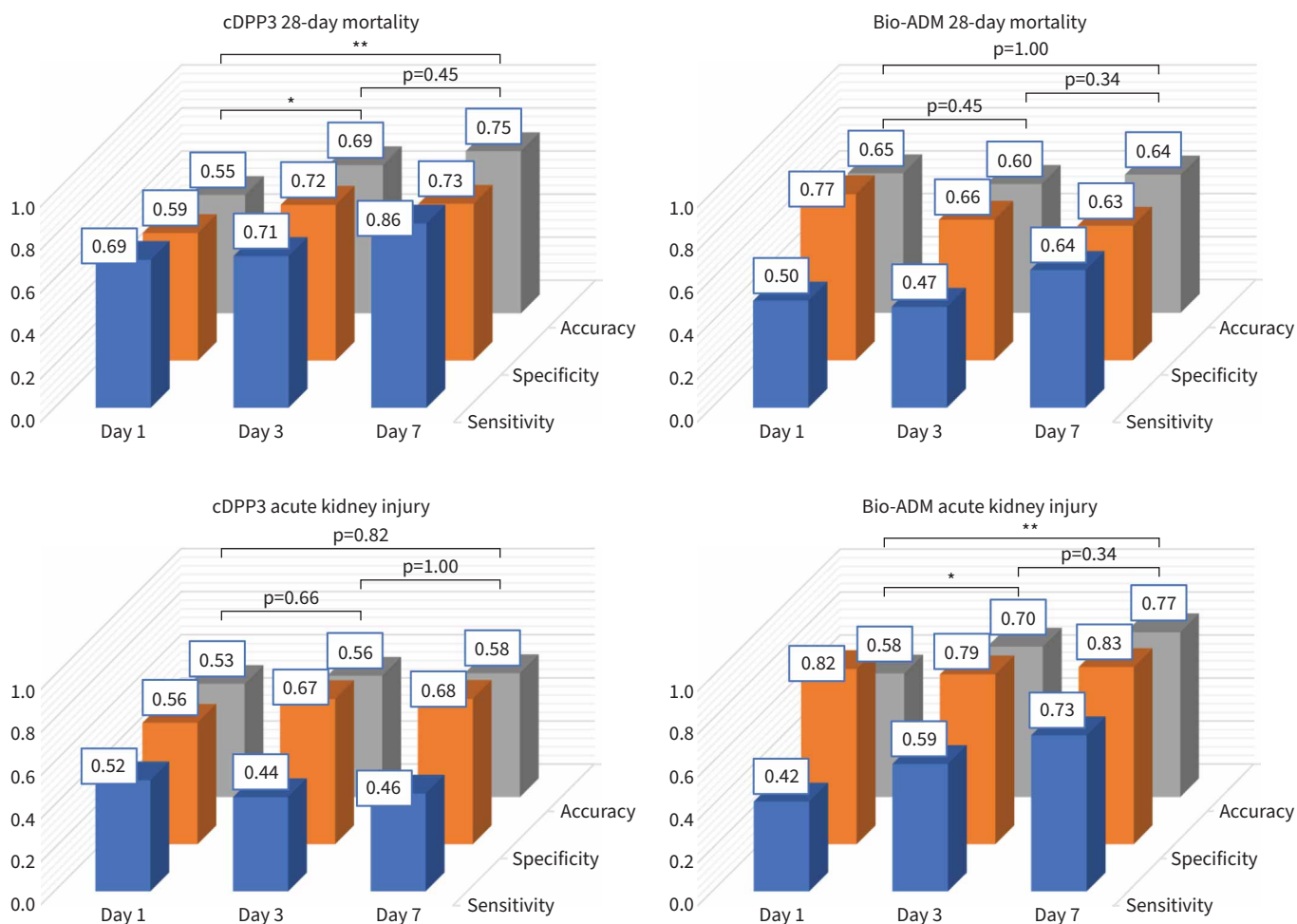


FIGURE 4 Graphical overview of sensitivity, specificity and accuracy for associations of cDPP3 and bio-ADM with 28-day mortality and all-stage AKI. Cut-offs for cDPP3 and bio-ADM were $>40 \text{ ng}\cdot\text{mL}^{-1}$ and $>70 \text{ pg}\cdot\text{mL}^{-1}$, respectively. Bio-ADM: bioactive adrenomedullin; cDPP3: circulating dipeptidyl peptidase 3. *: $p<0.05$; **: $p<0.01$.

occurrence of both 28-day mortality and AKI) was also performed. An overview of these analyses is presented in figure 5. Results should be interpreted with caution, as further subgroup stratification severely limits statistical power given the study cohort size.

Additional association analyses with other measurements of disease severity

At corresponding time points, cDPP3 levels correlated most strongly with ALAT ($r=0.53$) and ASAT ($r=0.71$), as well as serum lactate ($r=0.37$) (all $p<0.001$) (table 2). cDPP3 levels did not correlate with time-corresponding measurements of bio-ADM (table 2). Correlations of bio-ADM with troponin ($r=0.22$, $p=0.046$), lactate ($r=0.33$, $p<0.001$), IL-6 ($r=0.35$, $p=0.023$) and bilirubin ($r=0.196$, $p<0.001$) were also present (table 2).

Discussion

The main finding of this multicentre prospective cohort study is that cDPP3 and bio-ADM show distinctly different kinetics and associations with outcome in critically ill COVID-19 patients. cDPP3 levels were mainly associated with short-term mortality, with measurements performed later during ICU admission showing the strongest associations. In contrast, the association of bio-ADM concentrations was most pronounced with the development of AKI, with measurements performed early during ICU admission already strongly associating with development of severe AKI. Interestingly, patients with high levels of both biomarkers 1 week into ICU admission demonstrated an incrementally increased risk of 28-day mortality compared to patients with either high levels of only one biomarker or low levels of both of them.

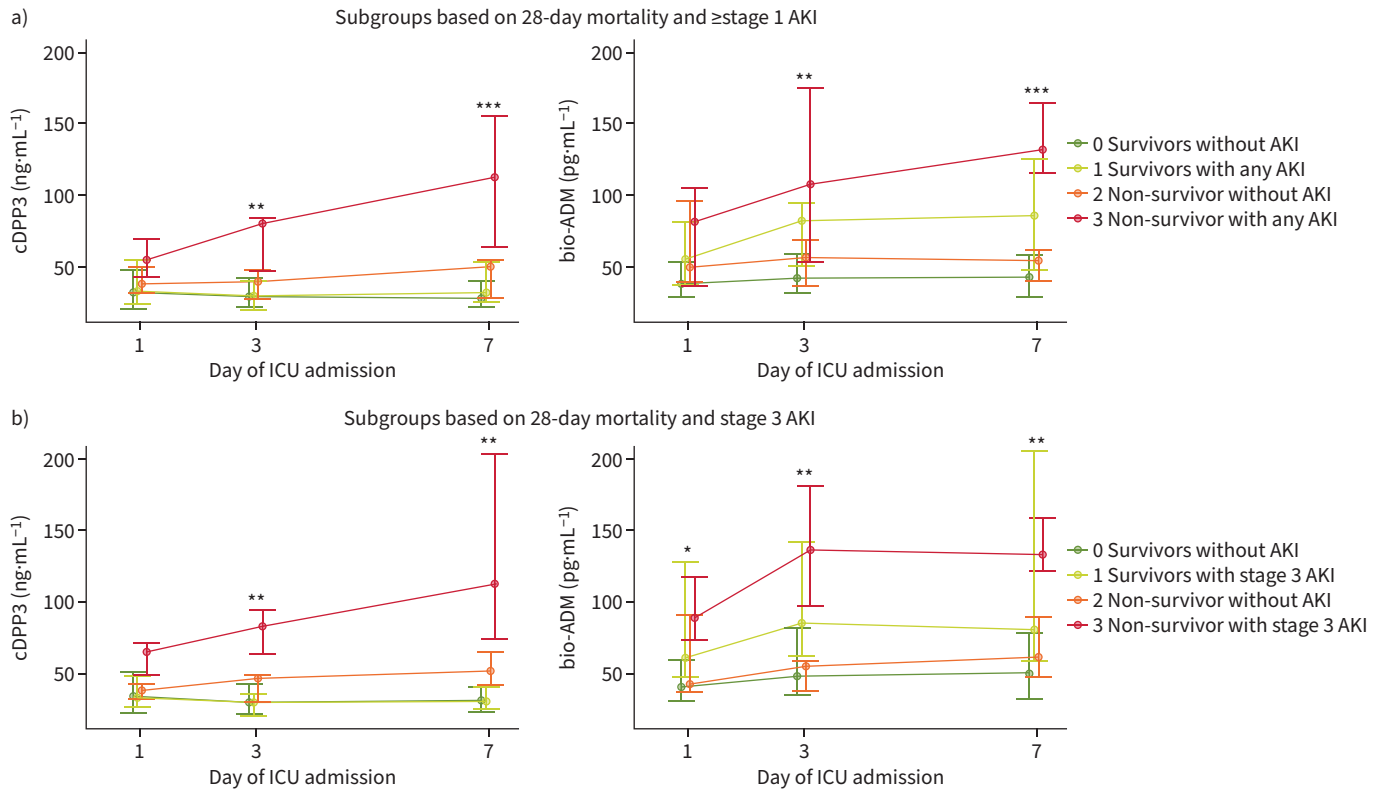


FIGURE 5 Biomarker kinetics of cDPP3 and bio-ADM based on subgroups combining study outcomes of 28-day mortality and AKI. **a)** Subgroups based on survival status and occurrence of KDIGO stage 1 AKI. **b)** Subgroups based on survival status and occurrence of KDIGO stage 3 AKI. Median and interquartile ranges are displayed. AKI: acute kidney injury; bio-ADM: bioactive adrenomedullin; cDPP3: circulating dipeptidyl peptidase 3; ICU: intensive care unit. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

These observed differences in outcome prediction in critically ill COVID-19 patients might be explained by the different pathophysiological pathways involved in the release of these biomarkers.

DPP3 only reaches high levels in the circulation if significant cell injury occurs [6]. Interestingly, until now, high cDPP3 levels were mainly described in different etiologies of circulatory shock, including sepsis, cardiogenic shock and severe burn injury [7, 8, 22, 23]. Apart from circulatory shock, high cDPP3 levels also appear to be induced by major surgical trauma, albeit less sustained than during circulatory shock [11, 24]. However, both these mechanisms cannot readily explain the sustained high cDPP3 levels observed in our critically ill COVID-19 cohort. While close to 50% of patients required a low dose of vasopressor therapy, the majority of the cohort did not meet circulatory shock definitions. Moreover, observational studies describe circulatory shock in $<20\%$ of critically ill COVID-19 patients [25]. Thus, the absence of shock makes it unlikely that cellular lysis induced by tissue hypoperfusion is the main cause of cDPP3 release in our cohort. DPP3 is however also expressed in different pulmonary tissues [26]. Putatively, the increasing cDPP3 levels observed in our study are associated with pulmonary cell damage, incurred because of the ongoing infection, as well as ventilator-associated pulmonary injury. Interestingly, a recent study that investigated endothelial pulmonary injury biomarkers described markers already peaking at ICU admission [27]. All these endothelial injury markers subsequently only decreased up until the second week of ICU admission [27]. These results make it unlikely that COVID-19-induced pulmonary endothelial injury alone can explain the further increase in cDPP3 levels observed after 1 week of ICU admission. Another possible source of high cDPP3 levels could be liver cell damage. Hepatocytes are known to contain large amounts of DPP3 [26]. Moreover, cDPP3 levels were already found to correlate with the severity of liver failure in a cohort of liver cirrhosis patients [28], as well as liver sequential organ failure assessment (SOFA) scores in sepsis patients [7, 29]. In accordance, we describe notable associations between cDPP3 levels and the transaminases ALAT and ASAT. However, transaminases have been described as poor measures of liver function disorder during critical illness, rather reflecting overall disease severity [30]. Also, significant correlations between cDPP3 and bilirubin, a measure of hepatic

TABLE 2 Correlation coefficients between different predictor variables compared to cDPP3 and bio-ADM measurements

Variable	r-coefficient	p-value
cDPP3 correlation		
Bio-ADM	0.144	0.089
Creatinine	0.162	0.058
Troponin-T	0.176	0.103
ALAT	0.530	<0.001
ASAT	0.714	<0.001
Bilirubin	0.181	0.065
Lactate	0.374	<0.001
P/F ratio	-0.138	0.138
Urine output	-0.310	0.027
IL-6	0.277	0.075
Bio-ADM correlation		
cDPP3	0.144	0.089
Creatinine	0.617	<0.001
Troponin-T	0.215	0.046
ALAT	0.005	0.910
ASAT	0.008	0.363
Bilirubin	0.196	<0.001
Lactate	0.334	<0.001
P/F ratio	0.104	0.268
Urine output	-0.009	0.937
IL-6	0.353	0.023

cDPP3: circulating dipeptidyl peptidase 3; Bio-ADM: biologically active adrenomedullin; ALAT: alanine-aminotransferase; ASAT: aspartyl-aminotransferase; P/F ratio: P_{aO_2}/F_{IO_2} ratio; IL-6: interleukin-6.

dysfunction with higher specificity, were not present. Therefore, correlations between cDPP3 and transaminases might well be explained because they are markers of general cellular damage. This would mean they are associated with disease severity, rather than being associated with a specific hepatic origin.

Regardless of aetiology, there are multiple ways by which high cDPP3 levels would be able to cause further clinical deterioration in COVID-19 patients. Circulating DPP3 is able to rapidly metabolise essential cardiovascular mediators, most notably angiotensin II [6]. Recent research has already demonstrated markedly reduced angiotensin-II levels in critically ill COVID-19 patients, also compared to other acute respiratory distress syndrome etiologies [31, 32]. Based on our results, it might well be that high cDPP3 levels represent an additional pathway leading to failing angiotensin-II responses in critically ill COVID-19 patients.

Several studies have already assessed associations of mid-regional-pro-adrenomedullin (MR-pro-ADM), another biomarker of the adrenomedullin pathway, with outcome in COVID-19 patients [33–35]. Interestingly, while most of these studies performed only one measurement within a short timeframe after admission, they report moderate associations with short-term mortality [33–35]. Interestingly, the strength of these reported associations appears quite similar to those we report. At the same time, they contrast with the weaker associations we report for the two measurements performed early during admission.

Interestingly, none of the aforementioned studies investigated associations between adrenomedullin and development of AKI, despite its frequent occurrence in COVID-19 patients [36]. Despite numerous recent studies on the potential mechanisms of COVID-19-associated AKI, its pathophysiology remains elusive [36]. It is thought to involve both local and systemic inflammatory responses, endothelial injury and a dysregulation of the renin–angiotensin system [36]. Adrenomedullin is well known as a hormone essential for endothelial homeostasis, aimed at maintaining endothelial integrity during inflammation [9]. Increased levels of adrenomedullin are also found during acute episodes of capillary leak syndrome, a disease aetiology which is mainly characterised by endotheliitis [37]. Considering the strong relation between bio-ADM and the development or presence of AKI found in our study, it might well be that these levels are mainly related to COVID-associated endothelial injury as well. As bio-ADM mitigates the loss of endothelial barrier function present during septic shock, it is thought that increased levels represent a

compensatory response [9]. However, because circulatory shock was not present in the majority of patients with AKI, haemodynamic compromise alone cannot serve as an explanation for the high bio-ADM levels found in our cohort.

The results of our study may have several clinically relevant implications. First, both cDPP3 and bio-ADM could act as potential stratifying biomarkers during the first week of ICU admission, identifying COVID-19 patients with an increased risk of unfavourable outcome. Assessment of cDPP3 in conjunction with bio-ADM identifies patients with an even higher risk of impaired short-term outcomes. These results emphasise that the effects of multiple pathophysiological processes are associated with markedly heterogeneous disease phenotypes in COVID-19. This heterogeneity, already well known from different etiologies of critical illness like sepsis, means that assessment of one biomarker, as well as conventional disease severity indexes, are unlikely to accurately predict short-term outcome [38].

Both cDPP3- (preclinical stage) and bio-ADM (clinical stage [15])-associated therapeutics are being developed. The availability of specific therapeutics able to correct the dysregulated pathways associated with these biomarkers greatly increases their clinical relevance. Based on our results, it appears plausible that cDPP3 inhibition might serve to mitigate clinical outcomes in COVID-19 patients. Also, bio-ADM modulators might serve as an additional therapeutic intervention, aimed at preventing COVID-associated AKI. Of interest, the derangements in both pathways were not already present at ICU admission, but rather became apparent during the first week of admission [7, 10]. This would mean that compared to bacterial sepsis, there may be a wider therapeutic window in which bio-ADM- and cDPP3-associated therapies could exert therapeutic efficacy in COVID-19 patients. Clearly, the observational nature of the current study does not imply that modulation of these pathways would translate into a clinical benefit for the patient. It does however warrant confirmation in interventional studies. Correspondingly, a phase 2 study investigating the adrenomedullin-modulating antibody Adrecizumab in COVID-19 patients is currently being planned (<https://clinicaltrials.gov/ct2/show/NCT05156671>).

Our study has several limitations. One major limitation is the small sample size, as only 80 patients were recruited during a short time interval. This means our results should be interpreted as hypothesis-generating. However, because we included only critically ill COVID-19 patients, unfavourable outcomes occurred frequently, explaining why we were already able to find significant associations with cDPP3 and bio-ADM in this relatively small cohort. Second, between patient's recruitment and final results of our analysis, the clinical management of COVID-19 has markedly changed, most notably related to the immunomodulatory treatment (corticosteroids and tocilizumab). Future studies are needed to demonstrate to what extent biomarker kinetics are influenced by these immunomodulatory treatments. Lastly, we did not directly compare the prognostic performance of both cDPP3 and bio-ADM to other candidate biomarkers. Since other candidate biomarkers may be more readily available at the bedside, follow-up studies with larger sample sizes should assess whether cDPP3 and/or bio-ADM are able to further improve the prediction already provided by these biomarkers.

In conclusion, our study suggests that cDPP3 and bio-ADM, two biomarkers previously used to predict outcomes in bacterial septic shock, are associated with short-term mortality and development of AKI in critically ill COVID-19 patients, respectively. The two biomarkers show different kinetics and different predictive values for organ dysfunction or mortality, while an approach combining both biomarkers identified patients with the highest risk of unfavourable outcome. Based on our results, the therapeutics associated with both these biomarkers may hold promise at improving outcome of critically ill COVID-19 patients.

Provenance: Submitted article, peer reviewed.

Acknowledgements: We would like to thank Marie Céline Fournier (Hôpital Lariboisière, Paris, France), Badr Louadah (Hôpital Lariboisière, Paris, France), Fariza Abeud (Hôpital Saint-Louis, Paris, France), Adrien Picod (Hôpital Lariboisière, Paris, France) and Feriel Azibani (Hôpital Lariboisière, Paris, France) for their contributions to the study.

Data availability: The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Author contributions: P. Pickkers, P-F. Laterre and A. Mebazaa conceptualised the study. D. van Lier drafted the manuscript. D. van Lier and B. Deniau acquired and processed the samples. K. Santos performed sample analyses. D. van Lier, B. Deniau, K. Santos and O. Hartmann performed data quality control, assurance and

transformation. D. van Lier performed data analysis. All authors critically revised the manuscript. The authors read and approved the final manuscript.

Conflict of interest: Analyses of samples was performed free of charge by the respective biomarker companies (Sphingotec for bio-ADM and 4TEEN4 for cDPP3). No additional funding was provided for the execution of this study. Both biomarker-companies had no role in the design of the study, its execution or analyses, interpretation of the data, or the decision to submit results. D. van Lier was invited to a meeting in Berlin by 4TEEN4 Pharmaceuticals GmbH. B. Deniau was invited to a meeting in Henningsdorf by 4TEEN4 Pharmaceuticals GmbH. K. Santos is employed by 4TEEN4 Pharmaceuticals, the company holding patent rights for the DPP3 assay. O. Hartmann is employed by Sphingotec GmbH, the company holding patent rights for the bio-ADM assay and a licence to commercialise the cDPP3 assay. E. Dudoignon, F. Depret and B. Plaud have nothing to declare. P-F. Laterre, A. Mebazaa and P. Pickkers received travel and consultancy reimbursements from 4TEEN4 and Sphingotec, the companies holding patent rights for the DPP3 and bio-ADM assays used in the study, respectively.

References

- 1 Williamson EJ, Walker AJ, Bhaskaran K, *et al.* Factors associated with COVID-19-related death using OpenSAFELY. *Nature* 2020; 584: 430–436.
- 2 Wiersinga WJ, Rhodes A, Cheng AC, *et al.* Pathophysiology, transmission, diagnosis, and treatment of Coronavirus Disease 2019 (COVID-19): a review. *JAMA* 2020; 324: 782–793.
- 3 Group RC. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. *Lancet* 2021; 397: 1637–1645.
- 4 Chen H, Xie J, Su N, *et al.* Corticosteroid therapy is associated with improved outcome in critically ill patients with COVID-19 with hyperinflammatory phenotype. *Chest* 2021; 159: 1793–1802.
- 5 Galvan-Roman JM, Rodriguez-Garcia SC, Roy-Vallejo E, *et al.* IL-6 serum levels predict severity and response to tocilizumab in COVID-19: an observational study. *J Allergy Clin Immunol* 2021; 147: 72–80 e8.
- 6 van Lier D, Kox M, Pickkers P. Promotion of vascular integrity in sepsis through modulation of bioactive adrenomedullin and dipeptidyl peptidase 3. *J Intern Med* 2021; 289: 792–806.
- 7 Blet A, Deniau B, Santos K, *et al.* Monitoring circulating dipeptidyl peptidase 3 (DPP3) predicts improvement of organ failure and survival in sepsis: a prospective observational multinational study. *Crit Care* 2021; 25: 61.
- 8 Takagi K, Blet A, Levy B, *et al.* Circulating dipeptidyl peptidase 3 and alteration in haemodynamics in cardiogenic shock: results from the OptimaCC trial. *Eur J Heart Fail* 2020; 22: 279–286.
- 9 Geven C, Kox M, Pickkers P. Adrenomedullin and adrenomedullin-targeted therapy as treatment strategies relevant for sepsis. *Front Immunol* 2018; 9: 292.
- 10 Mebazaa A, Geven C, Hollinger A, *et al.* Circulating adrenomedullin estimates survival and reversibility of organ failure in sepsis: the prospective observational multinational Adrenomedullin and Outcome in Sepsis and Septic Shock-1 (AdrenOSS-1) study. *Crit Care* 2018; 22: 354.
- 11 van Lier D, Beunders R, Kox M, *et al.* The value of bioactive adrenomedullin and dipeptidyl peptidase 3 to predict short-term unfavourable outcomes after cardiac surgery: a prospective cohort study. *Eur J Anaesthesiol* 2022; 39: 342–351.
- 12 Geven C, Peters E, Schroedter M, *et al.* Effects of the humanized anti-adrenomedullin antibody adrecizumab (HAM8101) on vascular barrier function and survival in rodent models of systemic inflammation and sepsis. *Shock* 2018; 50: 648–654.
- 13 Deniau B, Blet A, Santos K, *et al.* Inhibition of circulating dipeptidyl-peptidase 3 restores cardiac function in a sepsis-induced model in rats: a proof of concept study. *PLoS One* 2020; 15: e0238039.
- 14 Deniau B, Rehfeld L, Santos K, *et al.* Circulating dipeptidyl peptidase 3 is a myocardial depressant factor: dipeptidyl peptidase 3 inhibition rapidly and sustainably improves haemodynamics. *Eur J Heart Fail* 2020; 22: 290–299.
- 15 Laterre PF, Pickkers P, Marx G, *et al.* Safety and tolerability of non-neutralizing adrenomedullin antibody adrecizumab (HAM8101) in septic shock patients: the AdrenOSS-2 phase 2a biomarker-guided trial. *Intensive Care Med* 2021; 47: 1284–1294.
- 16 Kellum JA, Lameire N, Group KAGW. Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1). *Crit Care* 2013; 17: 204.
- 17 Rehfeld L, Funk E, Jha S, *et al.* Novel methods for the quantification of dipeptidyl peptidase 3 (DPP3) concentration and activity in human blood samples. *J Appl Lab Med* 2019; 3: 943–953.
- 18 Weber J, Sachse J, Bergmann S, *et al.* Sandwich immunoassay for bioactive plasma adrenomedullin. *J Appl Lab Med* 2017; 2: 222–233.
- 19 Bland JM, Altman DG. Calculating correlation coefficients with repeated observations: part 1 – correlation within subjects. *BMJ* 1995; 310: 446.
- 20 Marino R, Struck J, Maisel AS, *et al.* Plasma adrenomedullin is associated with short-term mortality and vasopressor requirement in patients admitted with sepsis. *Crit Care* 2014; 18: R34.

- 21 Caironi P, Latini R, Struck J, *et al.* Circulating biologically active adrenomedullin (bio-ADM) predicts hemodynamic support requirement and mortality during sepsis. *Chest* 2017; 152: 312–320.
- 22 Depret F, Amzallag J, Pollina A, *et al.* Circulating dipeptidyl peptidase-3 at admission is associated with circulatory failure, acute kidney injury and death in severely ill burn patients. *Crit Care* 2020; 24: 168.
- 23 Deniau B, Picod A, Van Lier D, *et al.* High plasma dipeptidyl peptidase 3 levels are associated with mortality and organ failure in shock: results from the international, prospective and observational FROG-ICU cohort. *Br J Anaesth* 2022; 128: e54–e57.
- 24 Gombert A, Barbati M, Kotelis D, *et al.* In-hospital mortality and organ failure after open and endovascular thoraco-abdominal aortic surgery can be predicted by increased levels of circulating dipeptidyl peptidase 3. *Eur J Cardiothorac Surg* 2021; 59: 750–757.
- 25 Varshney AS, Omar WA, Goodrich EL, *et al.* Epidemiology of cardiogenic shock in hospitalized adults with COVID-19: a report from the American Heart Association COVID-19 Cardiovascular Disease Registry. *Circ Heart Fail* 2021; 14: e008477.
- 26 Uhlen M, Fagerberg L, Hallstrom BM, *et al.* Proteomics. Tissue-based map of the human proteome. *Science* 2015; 347: 1260419.
- 27 Spadaro S, Fogagnolo A, Campo G, *et al.* Markers of endothelial and epithelial pulmonary injury in mechanically ventilated COVID-19 ICU patients. *Crit Care* 2021; 25: 74.
- 28 Voiosu AM, Wiese S, Goetze JP, *et al.* Dipeptidyl peptidase-3 is associated with severity of liver disease and circulatory complications in patients with cirrhosis. *Biomarkers* 2022; 27: 196–204.
- 29 Frigyesi A, Lengquist M, Spangfors M, *et al.* Circulating dipeptidyl peptidase 3 on intensive care unit admission is a predictor of organ dysfunction and mortality. *J Intensive Care* 2021; 9: 52.
- 30 Jenniskens M, Langouche L, Van den Berghe G. Cholestatic alterations in the critically ill: some new light on an old problem. *Chest* 2018; 153: 733–743.
- 31 Reindl-Schwaighofer R, Hodlmoser S, Eskandary F, *et al.* ACE2 elevation in severe COVID-19. *Am J Respir Crit Care Med* 2021; 203: 1191–1196.
- 32 van Lier D, Kox M, Santos K, *et al.* Increased blood angiotensin converting enzyme 2 activity in critically ill COVID-19 patients. *ERJ Open Res* 2021; 7: 00848-2020.
- 33 Sozio E, Tascini C, Fabris M, *et al.* MR-proADM as prognostic factor of outcome in COVID-19 patients. *Sci Rep* 2021; 11: 5121.
- 34 van Oers JAH, Kluiters Y, Bons JAP, *et al.* Endothelium-associated biomarkers mid-regional proadrenomedullin and C-terminal proendothelin-1 have good ability to predict 28-day mortality in critically ill patients with SARS-CoV-2 pneumonia: a prospective cohort study. *J Crit Care* 2021; 66: 173–180.
- 35 Oblitas CM, Galeano-Valle F, Ramirez-Navarro J, *et al.* Mid-regional pro-adrenomedullin, methemoglobin and carboxyhemoglobin as prognosis biomarkers in critically ill patients with COVID-19: an observational prospective study. *Viruses* 2021; 13: 2445.
- 36 Legrand M, Bell S, Forni L, *et al.* Pathophysiology of COVID-19-associated acute kidney injury. *Nat Rev Nephrol* 2021; 17: 751–764.
- 37 Xie Z, Chen WS, Yin Y, *et al.* Adrenomedullin surges are linked to acute episodes of the systemic capillary leak syndrome (Clarkson disease). *J Leukoc Biol* 2018; 103: 749–759.
- 38 van Lier D, Pickkers P. Circulating biomarkers to assess cardiovascular function in critically ill. *Curr Opin Crit Care* 2021; 27: 261–268.