Circulating dipeptidyl peptidase-3 at admission is associated with circulatory failure, acute kidney injury and death in severely ill burn patients.
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Abstract

Background: Dipeptidyl peptidase-3 (DPP3) is a metallopeptidase which cleaves bioactive peptides, notably angiotensin II, and is involved in inflammation regulation. DPP3 has been proposed to be a myocardial depressant factor and to be involved in circulatory failure in acute illnesses, possibly due to angiotensin II cleavage. In this study, we evaluated the association between plasmatic DPP3 level and outcome (mortality and hemodynamic failure) in severely ill burn patients.

Methods: In this biomarker analysis of a prospective cohort study, we included severely ill adult burn patients in two tertiary burn intensive care units. DPP3 was measured at admission (DPP3admin) and 3 days after. The primary endpoint was 90-day mortality. Secondary endpoints were hemodynamic failure and acute kidney injury (AKI).

Results: One hundred and eleven consecutive patients were enrolled. The median age was 48 (32.5–63) years, with a median total body surface area burned of 35% (25–53.5) and Abbreviated Burn Severity Index (ABSI) of 8 (7–11). Ninety-day mortality was 32%. The median DPP3admin was significantly higher in non-survivors versus survivors (53.3 ng/mL [IQR 28.8–103.5] versus 27.1 ng/mL [IQR 19.4–38.9]; \( p < 0.0001 \)). Patients with a sustained elevated DPP3 had an increased risk of death compared to patients with high DPP3admin but decreased levels on day 3. Patients with circulatory failure had higher DPP3admin (39.2 ng/mL [IQR 25.9–76.1] versus 28.4 ng/mL [IQR 19.8–39.6]; \( p = 0.001 \)) as well as patients with AKI (49.7 ng/mL [IQR 30.3–87.3] versus 27.6 ng/mL [IQR 19.4–41.4]; \( p = 0.001 \)). DPP3admin added prognostic value on top of ABSI (added chi2 12.2, \( p = 0.0005 \)), Sequential Organ Failure Assessment (SOFA) score at admission (added chi2 4.9, \( p = 0.0268 \)), and plasma lactate at admission (added chi2 6.9, \( p = 0.0086 \)) to predict circulatory failure within the first 48 h.

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Conclusions: Plasma DPP3 concentration at admission was associated with an increased risk of death, circulatory failure, and AKI in severely burned patients. Whether DPP3 plasma levels could identify patients who would respond to alternative hemodynamic support strategies, such as intravenous angiotensin II, should be explored.

Keywords: Dipeptidyl peptidase-3, Burn patients, Mortality, Acute kidney injury, Biomarkers

Background
Severe burn injury is associated with an early and profound hypovolemia followed by an intense systemic inflammatory response. Hemodynamic management, including fluid resuscitation, has long been recognized as the cornerstone of the early management and hemodynamic resuscitation of severely burned patients [1–3]. However, a systemic inflammatory response may be associated with distributive shock and/or acute myocardial dysfunction [4]. Dipeptidyl peptidase-3 (DPP3), also named enkephalinase B or red cell angiotensinase, is a predominantly intracellular, ubiquitously expressed, zinc-dependent metallopeptidase involved in the metabolism of peptides [5] implicated in many different pathways (e.g., blood pressure regulation, inflammation). DPP3 cleaves bioactive peptides, notably angiotensin II, enkephalins, and endomorphins [6–8]. We hypothesize that cleavage of angiotensin II by DPP3 may promote vasodilatation and circulatory failure. Severe burn patients are at high risk of developing vasodilatory shock with systemic inflammatory response after the early phase of hypovolemic shock. The main objective of this study was therefore to assess the association between DPP3 at admission (DPP3admin) and day 3 (DPP3Day3) with 90-day mortality in severely burned patients. The secondary objective was to evaluate the association between DPP3 and organ dysfunction (i.e., circulatory failure and acute kidney injury (AKI)).

Methods
Study design and population
We conducted a double-center cohort study in the Burn Unit of Saint Louis Hospital (Assistance Publique Hôpitaux de Paris), Paris, and in the Burn Unit of Edouard Herriot Hospital, Lyon, France. The study was approved by our local ethic committee (PRONOBURN study, comité de protection des personnes IV, St-Louis Hospital; Institutional Review Board 00003835, protocol 2013/17NCIB). All patients admitted to our intensive care burn units (ICBU) between April 2014 and April 2016 and meeting the inclusion criteria were included. Inclusion criteria were the following:

- A total body surface area [TBSA] burned > 15%
- Admission in the ICBU within the 72 h following burn injury
- No decision to withdraw life support
- Available plasma sample at admission

Outcome
The endpoints were 90-day mortality, circulatory failure in the first 48 h, and AKI.

Measurements
The following data have been collected: sex, age, body mass index (BMI), TBSA, full-thickness body surface area (BSA) burned, mechanism of injury and patients’ characteristics, Abbreviated Burn Severity Index (ABSI) [9], Unit Burn Standard (UBS) [10], Sequential Organ Failure Assessment (SOFA) score [11], 28- and 90-day mortality, and AKI. Patients were resuscitated according to the Intensive Care Burn Unit resuscitation protocol [12]. A transthoracic or transesophageal echocardiography was performed at admission of patients according to the decision of the physician in charge. When performed, left ventricular ejection fraction (LVEF) was visually evaluated and systolic cardiac dysfunction was defined by a LVEF < 50% [13]. Circulatory failure was defined as a need for hemodynamic support with inotropic and/or a vasopressor (i.e., dobutamine, epinephrine, or norepinephrine) despite appropriate fluid resuscitation in the first 48 h. We choose this time frame to identify circulatory failure related to burn injury (as opposed to sepsis or surgical procedures which occur later during the course of burn injuries). AKI was defined and staged according to the Kidney Disease: Improving Global Outcomes (KDIGO) criteria [14] during the first 7 days post admission. Admission serum creatinine e (Screatadmin) was used as baseline Screat.

Venous blood samples were collected at admission and at day 3 in tubes containing ethylene-diamine-tetra-acetic acid. After centrifugation, plasma was kept frozen at –80 °C until assayed. DPP3 was measured using the recently developed DPP3 luminescence immunoassay [15].

Statistical analysis
Values are expressed as medians and interquartile ranges (IQR) or counts and percentages as appropriate. Group comparisons of continuous variables were performed using Kruskal-Wallis test. Categorical data were compared using Pearson’s chi-squared test for count data. DPP3 data was log-transformed. Cox proportional-
hazards regression was used to analyze the effect of risk factors on survival in uni- and multivariable analyses, and logistic regression was used for dichotomous endpoints. In both cases, to demonstrate independence from other variables, the added value of DPP3 on top of these was evaluated based on the likelihood ratio chi-squared test for nested models. The concordance index (C index or AUC) is given as an effect measure for uni- and multivariable models. For multivariable models, a bootstrap-corrected version of the C index is given. For continuous variables, hazard ratios (HR) or odds ratios (OR), as appropriate, were standardized to describe the HR/OR for a change of one IQR. HR (Cox regression) are used if time-to-event data is available; OR (logistic regression) are used if endpoints have event data (yes/no) only. Survival curves plotted by the Kaplan-Meier method were used for illustrative purposes. For dichotomous endpoints, receiver operating characteristic (ROC) curves were constructed for illustration. All statistical tests were 2-tailed and a two-sided p-value of 0.05 was considered for significance. The statistical analyses were performed using R version 3.4.3 (http://www.r-project.org, library rms, Hmisc, ROCR) and Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, Illinois, USA).

Results
Study population
From April 2014 to April 2016, 208 consecutive patients met the inclusion criteria; 55 patients had missing plasma at admission and were not included in the final analysis, resulting in 111 patients that were analyzed. The characteristics of the patients included in this study are summarized in Table 1. The median age was 48 (32.5–63) years, with a median TBSA of 35% (25–53.5) and median ABSI of 8 (7–11). All patients had a DPP3 admission measurement and 79 patients (71%) had DPP3 Day3 (10 patients died before day 3 and 22 patients had missing measurements at day 3).

DPP3 admission and 90-day mortality
Thirty-six (32%) patients died before day 90. Median DPP3 admission was significantly higher in non-survivors versus survivors (53.3 ng/mL [IQR 28.8–103.5] versus 27.1 ng/mL [IQR 19.4–38.9]; p < 0.0001). We observed a stepwise increase in mortality among quartile groups of DPP3 admission, the patients in the highest quartile having the highest mortality (Fig. 1). There was no interaction between DPP3 value and TBSA (p = 0.7132) (Supplementary Figure 1). The C index of DPP3 admission for 90-day mortality was 0.734 (0.653–0.815, p < 0.0001, standardized HR 2.6 (1.9–3.6)). DPP3 admission added prognostic value on top of ABSI (added chi² 24.5, p < 0.0001), SOFA score at admission (SOFA admission added chi² 15.4, p < 0.0001), and lactate at admission (added chi² 11.7, p = 0.0006) to predict 90-day mortality (Fig. 2). Furthermore, adding DPP3 Day3 to DPP3 admission provided added value to predict 90-day mortality (added chi² 5.6, p = 0.018; missing data at day 3 replaced with admission data). Patients with a high DPP3 admission that decreased at day 3 had a better prognosis than patients with high DPP3 admission and sustained DPP3 Day3 (Fig. 3).

DPP3 and circulatory failure
Fifty-three (48%) patients had circulatory failure during the first 48 h (44 patients received norepinephrine, five patients received dobutamine + norepinephrine, 4 patients received epinephrine). DPP3 admission was significantly higher in patients with circulatory failure compared to patients without (39.2 ng/mL [IQR 25.9–76.1] versus 28.4 ng/mL [IQR 19.8–39.6]; p = 0.001) (Fig. 4 left panel). DPP3 admission was associated with circulatory failure with an AUC of 0.680 (0.581–0.778, p < 0.0001, standardized OR 2.8 (1.6–4.9)). DPP3 admission provided value on top of ABSI (added chi² 12.2, p = 0.0005), SOFA score at admission (SOFA admission added chi² 4.9, p = 0.0268), and lactate at admission (added chi² 6.9, p = 0.0086) to predict hemodynamic support in the first 48 h. There was no correlation between DPP3 and the volume administered on day 1 (r = 0.17, p = 0.07).

Fifty-nine patients (53%) had an echocardiography performed at admission. Among them, 10 (17%) patients had a systolic cardiac dysfunction. DPP3 admission was significantly higher in patients with systolic cardiac dysfunction compared to patients without (62.4 ng/mL [IQR 40.4–112.3] versus 29.3 ng/mL [IQR 22.4–45.1]; p < 0.0122) (Fig. 4 middle panel). The area under the ROC curve for DPP3 admission to predict systolic cardiac dysfunction was 0.753 (95%CI 0.582–0.925, p = 0.0054).

DPP3 and acute kidney injury
Thirty-five (32%) patients developed AKI during the first 7 days. DPP3 admission was significantly higher in patients with AKI compared to patients without (49.7 ng/mL [IQR 30.3–87.3] versus 27.6 ng/mL [IQR 19.4–41.4]; p = 0.001) (Fig. 4 right panel). DPP3 admission was associated with AKI with an AUC of 0.735 (0.641–0.829, p = 0.0005, standardized OR 2.3 (1.4–4.0)). DPP3 admission added value on top of ABSI (added chi² 9.4, p = 0.0022), SOFA score at admission (SOFA admission added chi² 14.3, p = 0.0002), but not on top of creatinine at admission (added chi² 0.3, p = 0.5954) to predict AKI.

Discussion
In this biomarker analysis of a prospective cohort, we observed that DPP3 admission was strongly associated with 90-day mortality, circulatory failure, and AKI in severely burned patients. Furthermore, adding DPP3 admission to
SOFA$_{\text{admin}}$, lactate$_{\text{admin}}$, or ABSI outperformed these prognostic factors to predict 90-day mortality. Serial measurements of DPP3 have improved the prediction of outcome compared to DPP3$_{\text{admin}}$ alone.

While the prognosis of burn patients has improved, the mortality of most severe patients remains high with many patients dying from circulatory failure and multiple organ failure [16, 17]. Initial hemodynamic management has long been considered critical in the treatment and prognosis of burn patients [18]. Burn injury is characterized by an initial hypodynamic state with low cardiac output due to hypovolemia followed by a hyperdynamic state with high cardiac output and low vascular resistance developing 12 to 24 h after the injury [1]. The severity of the distributive shock and occurrence of cardiac dysfunction may, however, vary greatly between

### Table 1 Patients characteristics

| Patient’s characteristics | Total, $N = 111$ | 90-day survivors, $N = 75$ | 90-day non-survivors, $N = 36$ | $p$ |
|--------------------------|-----------------|--------------------------|--------------------------|-----|
| Sex, male—n (%)          | 71 (64)         | 51 (68)                  | 20 (56)                  | 0.2858 |
| Age—year                 | 48 [32.5–63]    | 42 [29–58]               | 56.5 [42–79]             | 0.0013 |
| BMI—kg/m$^2$             | 25.2 [22.9–28.7]| 25.1 [23–28.3]           | 25.7 [22.4–29.1]         | 0.9673 |
| **Medical history**      |                 |                          |                          |     |
| CIC—n (%)                | 3 (2.7)         | 2 (3)                    | 1 (3)                    | 1.0000 |
| COPD—n (%)               | 3 (2.7)         | 2 (3)                    | 1 (3)                    | 1.0000 |
| CKD—n (%)                | 5 (4.5)         | 1 (1)                    | 4 (11)                   | 0.0374 |
| Chronic HBP—n (%)        | 25 (22.5)       | 12 (16)                  | 13 (36)                  | 0.0277 |
| Psychiatric—n (%)        | 34 (30.6)       | 22 (29)                  | 12 (33)                  | 0.6668 |
| **Burn characteristics** |                 |                          |                          |     |
| TBSA—%                   | 35 [25–53.5]    | 32 [22–45]               | 57 [31–70]               | < 0.0001 |
| Deep burn BSA—%          | 21 [10–40]      | 17 [7–30]                | 42 [15–61]               | 0.0001 |
| Inhalation injury—n (%)  | 54 (48.6)       | 28 (37)                  | 26 (72)                  | 0.0012 |
| **Characteristics during hospitalization** |                     |                          |                          |     |
| Mechanic ventilation—n (%) | 82 (73.9)       | 52 (69)                  | 30 (83)                  | 0.1799 |
| DPP3$_{\text{admin}}$ (ng/mL) | 30.6 [22.4–53.6]| 27.1 [19.4–40.2]         | 53.3 [29.5–104]          | < 0.0001 |
| DPP3$_{\text{day3}}$ (ng/mL) | 17.3 [11.8–25.2]| 14.1 [11.5–20.6]         | 22.1 [16.6–30.8]         | 0.0102 |
| Screat—μmol/L            | 72.5 [56.5–92]  | 70 [54.8–81.3]           | 90.5 [67.3–138.3]        | 0.0003 |
| Lactate—mmol/L           | 14.0 [9.3–21.3] | 12.9 [9–19.3]            | 18 [10.9–25.9]           | 0.0945 |
| Bilirubin—mmol/L         | 140 [9.3–13.3]  | 129 [9–19.3]             | 18 [10.9–25.9]           | 0.0945 |
| Platelet—G/L             | 250 [185–304]   | 236 [183–277]            | 279 [180–372]            | 0.3840 |
| Length of hospitalization—days | 90 [35.5–90]    | 41 [26–61]               | 18 [2–32.5]              | < 0.0001 |
| RRT—n (%)                | 24 (21.6)       | 5 (7)                    | 28 (78)                  | 0.0011 |
| **Severity scores**      |                 |                          |                          |     |
| SOFA                     | 4 [1–7]         | 2 [0–4]                  | 6.5 [3.3–9.8]            | < 0.0001 |
| ABSI                     | 8 [7–11]        | 8 [6–9]                  | 11 [9–13]                | < 0.0001 |
| SAPS2                    | 33 [23–47]      | 28 [20–42]               | 47 [33–62]               | < 0.0001 |
| UBS                      | 100 [52.5–166]  | 84 [45–132]              | 184 [86–249]             | < 0.0001 |
| **Hemodynamic on admission** |               |                          |                          |     |
| Echocardiography, n (%)  | 59 (53)         | 33 (44)                  | 26 (72)                  | 0.0078 |
| Systolic cardiac dysfunction, n (%) | 10 (9)  | 2 (3)                    | 8 (22)                   | 0.0163 |
| Circulatory failure, n (%) | 53 (48)         | 24 (32)                  | 29 (81)                  | < 0.0001 |
| MAP in mmHg              | 79 [70–95]      | 84 [73–97]               | 73 [64–85]               | 0.0104 |
| Volume of crystalloids at day 1 | 8250 [3700–15,000] | 6700 [3300–12,800] | 13,400 [6430–18,380] | 0.0018 |
| Volume of crystalloids at day 2 | 3000 [1000–5650] | 2500 [1000–5150] | 4000 [2000–7500] | 0.1078 |

BMI body mass index, CIC chronic ischemic cardiopathy, COPD chronic obstructive pulmonary disease, CKD chronic kidney disease, HBP high blood pressure, TBSA total burn surface area, ECMO extracorporeal membrane oxygenation, Screat serum creatinine at admission, RRT renal replacement therapy, SOFA score simplified organ failure assessment, ABSI Abbreviated Burn Severity Index, UBS Unit Burn Standard, SAPS 2 The Simplified Acute Physiology Score
patients. The association between DPP3 levels, circulatory failure, and AKI is consistent with the current understanding of AKI in the critically ill, associating hemodynamic factors and inflammation/immune response [19, 20]. These results might also be expected in patients developing systemic inflammatory response from different causes (e.g., sepsis, post-cardiopulmonary bypass, post-cardiac arrest, pancreatitis), and it should be further explored.

In the current study, DPP3 was strongly associated with mortality and hemodynamic failure, even after adjustment for classic markers of severity and prognosis.
Recently, Deniau et al. observed an association between high plasmatic levels of DPP3 and high mortality and organ dysfunction in severe heart failure patients. Furthermore, I.V. administration of DPP3 rapidly deteriorated cardiac contraction in mice [21]. In an ancillary study of the OptimaCC study, Takagi et al. showed that high circulating DPP3 was associated with low cardiac index, refractory shock, and high mortality in patients with cardiogenic shock [22].

The results of the present study have several potential implications for future research. First, the identification of patients with high plasma DPP3 may trigger cardiac function assessment. Second, high DPP3 levels at admission may help to select candidate patients for

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**Fig. 3** Represents an illustration of the added value of DPP3 day 3 using a cut point of 53.65 ng/mL at admission and day 3. Patients without DPP3 data at day 3 were left in their subgroup assigned to on day 1. High at admission and high at day 3 (HH): patients above 53.65 ng/mL at admission and at day 3; high at admission and low at day 3 (HL): patients above 53.65 ng/mL at admission and below 53.65 ng/mL at day 3; low at admission and high at day 3 low high (LH): patients below 53.65 ng/mL at admission and above 53.65 ng/mL at day 3; low at admission and low at day 3 (LL): Patients below 53.65 ng/mL at admission and on day 3. Cut point identified is the third quartile (53.65 ng/mL)

**Fig. 4** Represents median DPP3 admin between patients with and without circulatory failure in the first 48 h (left panel), between patients with or without systolic cardiac dysfunction at admission (middle panel), and between patients with or without acute kidney injury (right panel)
alternative vasopressor therapies, especially for infusion of angiotensin II [23, 24]. Angiotensin II has been found to be downregulated in some forms of septic shock associated with poor prognosis [25]. DPP3 cleaves angiotensin II and may, therefore, play a role in vasoplegic shock by reducing angiotensin II levels. Since angiotensin II is not easy to measure in clinical practice, DPP3 may represent a potential candidate biomarker for selecting patients most likely to respond to angiotensin II infusion. Third, pharmacological inhibition of DPP3 by a specific antibody has been shown to promptly restore and sustain cardiac contraction in mice [21] and might be a therapeutic option in burn patients with high DPP3. All these strategies are hypothesis and require exploration and validation in well-designed prospective human studies.

Our study has several limitations. First, the observational design of the present study does not allow us to conclude on the causality between DPP3 and mortality or organ dysfunction. Second, the study contains a relatively low number of patients, even though this is one of the largest cohort studies among critically ill burn patients with sufficient power to identify an association between the biomarker levels and outcomes. Thirdly, factors influencing DPP3 metabolism are unknown and will need further exploration in critically ill burn patients. Finally, only half of our patients had an echocardiography at admission, limiting the interpretation of the association between DPP3 levels and cardiac dysfunction.

Conclusion
Plasma DPP3 concentration at admission was associated with an increased risk of death, circulatory failure, and AKI in severely burned patients. Whether DPP3 plasma levels could identify patients who would respond to alternative hemodynamic support strategies, such as intravenous angiotensin II, should be explored.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s13054-020-02888-5.

Additional file 1: Figure S1. represents interaction between Total Body Surface Area (TBSA) and DPP3levels (IF 43.49).

Additional file 2. Supplementary data Table 1. Patients characteristics according to TBSA (Total burn surface area).

Abbreviations
DPP3: Dipeptidyl peptidase-3; AKI: Acute kidney injury; ICBU: Intensive care burn unit; TBSA: Total body surface area burn; BMI: Body mass index; BSA: Body surface area; ABSI: Abbreviated Burn Severity Index; UBS: Unit Burn Standard; SOFA: Sequential Organ Failure Assessment; LVEF: Left ventricular ejection fraction; KIDGO: Kidney Disease: Improving Global Outcomes; IQR: Interquartile range; HR: Hazard ratio; OR: Odds ratio; ROC: Receiver operator characteristics

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Authors’ contributions
FD collected the data, contributed to the interpretation of the data, and drafted the manuscript. AP collected the data, contributed to the interpretation of the data, and drafted the manuscript. JA collected the data, contributed to the interpretation of the data, and drafted the manuscript. LFP collected the data, contributed to the interpretation of the data, and drafted the manuscript. MCo collected the data, contributed to the interpretation of the data, and drafted the manuscript. MCh collected the data, contributed to the interpretation of the data, and drafted the manuscript. AF collected the data, contributed to the interpretation of the data, and drafted the manuscript. HO collected the data, contributed to the interpretation of the data, and drafted the manuscript. AC collected the data, contributed to the interpretation of the data, and drafted the manuscript. MB collected the data, contributed to the interpretation of the data, and drafted the manuscript. KG collected the data, contributed to the interpretation of the data, and drafted the manuscript. MG performed DPP3 measurements, contributed to the interpretation of the data, and drafted the manuscript. OH performed analysis and contributed to the interpretation of the data, and drafted the manuscript. JT collected the data, contributed to the interpretation of the data, and drafted the manuscript. MJ collected the data, contributed to the interpretation of the data, and drafted the manuscript. BD collected the data, contributed to the interpretation of the data, and drafted the manuscript. LG collected the data, contributed to the interpretation of the data, and drafted the manuscript. HO collected the data, contributed to the interpretation of the data, and drafted the manuscript. AM conceived the study, contributed to the interpretation of the data, and drafted the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The study protocol was approved by our local research ethical committee (Comité de Protection des Personnes 2013/17/NICB).

Consent for publication
Not applicable.

Competing interests
FD has received research grants from the French ministry of health, Société française d’anesthésie réanimation, European Society of Intensive Care Medicine, and lecture fees Sedana medical.

JA has no conflict of interest.

MCo has no conflict of interest.

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KS and OH are employed by 4TEEN4 Pharmaceuticals GmbH, a company which holds patent rights in and commercializes the DPP3 assay.

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