JIM Research Letter

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Circulating dipeptidyl peptidase (cDPP3)—A marker for end-stage heart failure?

Dear Editor,

Dipeptidyl dipeptidase (DPP3) is a protease involved in the degradation of cardiovascular mediators. In preclinical models, increased levels of circulating DPP3 (cDPP3) have been shown to be associated with impaired myocardial contraction, whereas inhibition by the specific antibody Procizumab may restore cardiac function [1]. Elevated cDPP3 is associated with therapy refractoriness in cardiogenic shock [2]. Recently, cDPP3 levels were determined in 2314 advanced heart failure (HF) patients of the BIOSTAT-CHF observational cohort, revealing an increase in the combined endpoint of all-cause mortality and HF hospitalizations in the highest quartile [3].

This study evaluated cDPP3 in patients with advanced heart failure with reduced ejection fraction (HFrEF) on up-titrated guideline-directed optimal medical therapy (GDMT) to correlate cDPP3 with a wide array of biomarkers of neurohumoral derangement, confirm previous results and, at the same time, extend the spectrum of the HFrEF population studied. The study protocol complies with the Declaration of Helsinki and was approved by the local ethics committee of the Medical University of Vienna.

Consecutive patients with advanced HFrEF and GDMT were enrolled prospectively from the HF outpatient clinic at the Medical University of Vienna between February 2016 and December 2017. Routine laboratory parameters including NT-proBNP and active plasma renin concentration (ARC), and additionally a set of other established HF neurohumoral biomarkers—norepinephrine (NE), growth differentiation factor 15 (GDF-15), copeptin, big endothelin 1 (big-ET-1) and plasma neprilysin (NEP) activity—were measured in plasma by specific immunoassays and a fluorokinetic assay as described earlier [3]. Bioactive adrenomedullin (bio-ADM) and pro-enkephalin-A 119–159 (PENK) were determined in serum samples by the sphingotest® assay. Blinded cDPP3 measurements in serum were organized by 4TEEN4 Pharmaceuticals GmbH (Hennigsdorf, Germany) using a luminometric immunoassay (DPP3-LIA) [3]. Because DPP3 is classified as a primary cytosolic enzyme and can be released from erythrocytes during hemolysis, hemolytic samples were excluded from the analysis. The combined endpoint of all-cause mortality or unplanned HF hospitalizations was assessed as the primary outcome. Mortality data were retrieved from the Austrian Statistics Agency. The correlation of cDPP3 levels with demographic parameters, routine laboratory tests and HF biomarkers was assessed by calculating the Spearman´s Rho correlation coefficient. The association between cDPP3 and outcome was analysed by cubic spline analysis. Additionally, Cox regression analysis was performed. The cohort was divided by a cut-off at cDPP3 concentrations of 15 ng/mL, identified by spline analysis, and baseline characteristics were compared for low and high cDPP3 patients.

A total of 365 patients were included in the study. Samples were hemolytic in 40 cases; therefore, cDPP3 measurements were analysed for a total of 325 patients. Baseline characteristics of the study cohort are depicted in Table 1. The median age was 65 years (IQR 54–73), 77% of patients were male and 54% of patients had an ischemic etiology of HF. NT-proBNP was elevated with a median of 1927pg/mL (IQR 845–4126). HF therapy was well established with 95%, 90%, 73% and 6% of patients receiving beta-blockers, RAS-inhibitors, MRA and ivabradine. A high percentage of patients received greater than 50% of target dosages.

The distribution of cDPP3 is displayed in Fig. 1A; median cDPP3 was 11.36 ng/mL (IQR: 8.87–14.48, range 5.04–71.10). cDPP3 levels were comparable for ischemic and non-ischemic etiology of HF and for different classes of RAS-inhibitors (Fig. 1B). cDPP3 showed a modest correlation with neurohumoral dysregulation as shown in Fig. 1C. Increasing cDPP3 was associated with worse outcome, whereas significance was driven by the mortality endpoint (Fig. 1D). Association with the endpoints was significant beyond cDPP3 values of the 3rd

Table 1. *Baseline characteristics of stable HFrEF patients on optimal medical therapy. Continuous variables are displayed as median and interquartile range, categorical data as counts and percentages*

	Stable HFrEF patients ($n = 325$)	Low cDPP3 (≤15 ng/mL) ($n = 254$)	High cDPP3 (>15 ng/mL) (n = 71)	*p*-value
cDPP3, ng/mL (IQR)	11.36 (8.87–14.48)	10.28 (8.31–12.33)	18.34 (16.28–20.33)	-
Basic demographics				
Age, years (IQR)	65 (54–73)	65 (53–72)	65 (57–75)	0.194
Male gender, *n* (%)	245 (77%)	193 (76%)	52 (73%)	0.633
BMI, kg/m^2 (IQR)	26.7 (23.9–31.0)	27.5 (24.2–31.2)	25.5 (22.0–30.9)	**0.039**
Systolic blood pressure, mmHg (IQR)	125 (115–145)	125 (110–145)	125 (115–135)	0.653
Heart rate, bpm (IQR)	70 (61–80)	70 (60–80)	71 (65–84)	0.102
NYHA class, I/II/III (%)	45 (14%)/149 (47%)/126 (39%)	10 (14%)/32 (46%)/28 (40%)	35 (14%)/117 (47%)/98 (39%)	0.976
Comorbidities				
Ischemic etiology of HF, *n* (%)	147 (54%)	135 (54%)	38 (54%)	1.000
Atrial fibrillation, *n* (%)	96 (30%)	56 (22%)	22 (31%)	0.156
Diabetes mellitus, *n* (%)	103 (32%)	80 (32%)	23 (33%)	0.886
Arterial Hypertension, *n* (%)	133 (42%)	106 (42%)	27 (39%)	0.586
Medication and device therapy				
Beta-blocker, *n* (%)	299 (95%)	235 (95%)	64 (93%)	0.544
Dose ≥ 50% of targetdose, *n* (%)	236 (79%)	188 (80%)	48 (75%)	
ACEi/ARB/ARNi, *n* (%)	193 (63%)/86 (30%)/23 (7%)	155 (65%)/62 (28%)/18 (7%)	38 (58%)/24 (37%)/5 (7%)	0.315/ 0.167/ 1.000
Dose ≥ 50% of target dose, *n* (%)	136 (70%)/62 (72%)/17 (74%)	107 (69%)/43 (69%)/14 (78%)	29 (76%)/19 (79%)/3 (60%)	
MRA, *n* (%)	224 (73%)	174 (48%)	50 (76%)	0.753
Dose ≥ 50% of target dose, *n* (%)	216 (96%)	168 (92%)	48 (96%)	
Ivabradin, *n* (%)	18 (6%)	16 (6%)	2 (3%)	0.381
Loop diuretics, *n* (%)	152 (52%)	117 (52%)	33 (51%)	0.888
PM/ICD/CRT, *n* (%)	86 (26%)	66 (26%)	20 (29%)	0.761
Laboratory parameters				
CREA, mg/dL (IQR)	1.20 (0.95–1.56)	1.17 (0.94–1.54)	1.27 (1.02–1.58)	0.248
BUN, mg/dL (IQR)	24 (17–33)	23 (17–32)	24 (18–36)	0.165
Sodium, mmol/L (IQR)	139 (138–141)	139 (138–141)	139 (137–141)	0.296
Albumin, g/L (IQR)	43.3 (40.5–45.9)	43.3 (40.7–45.9)	43.7 (39.5–45.5)	0.600
BChE, kU/L (IQR)	6.95 (5.54–8.27)	7.01 (5.61–8.30)	6.55 (4.94–8.08)	0.306
AST (GOT), U/L (IQR)	25 (21–32)	23 (20–29)	34 (25–42)	**<0.001**
ALT (GPT), U/L (IQR)	24 (17–34)	21 (16–31)	31 (22–42)	**<0.001**
GGT, U/L (IQR)	50 (27–106)	42 (24–91)	79 (42–189)	**<0.001**
Bilirubin, mg/dL (IQR)	0.61 (0.46–0.90)	0.58 (0.43–0.85)	0.73 (0.55–0.98)	**0.002**
Total cholesterol, mg/dL (IQR)	166 (136–195)	167 (136–195)	158 (132–197)	0.768
Hemoglobin, g/dL (IQR)	13.5 (12.1–14.7)	13.3 (12.1–14.4)	14.2 (12.2–15.0)	**0.013**
Thrombocytes, G/L (IQR)	220 (178–267)	221 (181–271)	208 (161–246)	0.078
Leukocyte count, G/L (IQR)	7.43 (6.20–8.91)	7.3 (6.2–8.8)	7.9 (6.5–9.6)	0.139

(*Continued*)

Table 1. *Continued*

	Stable HFrEF patients ($n = 325$)	Low cDPP3 (≤15 ng/mL) ($n = 254$)	High cDPP3 (>15 ng/mL) (n = 71)	p-value
CRP, mg/dL (IQR)	0.39 (0.14–0.86)	0.32 (0.14–0.74)	0.56 (0.15–1.12)	0.096
Cardiac and HF biomarkers				
NT-proBNP pg/mL (IQR)	1927 (845–4126)	1923 (781–3975)	2038 (1134–6070)	0.157
Renin, uIE/mL (IQR)	153 (29–527)	146 (28–430)	189 (29–829)	0.208
Aldosterone, pmol/L (IQR)	99 (52–176)	91 (52–163)	113 (52–233)	0.166
GDF-15, pg/mL (IQR)	430 (251–749)	410 (230–734)	516 (324–759)	0.057
Big-ET-1, pmol/mL (IQR)	0.63 (0.42–1.10)	0.61 (0.41–1.09)	0.56 (0.51–1.23)	0.061
PENK, pmol/L (IQR)	78.3 (59.5–119.6)	77.6 (59.4–118.9)	81.4 (60.4–121.1)	0.658
bio-ADM, pg/mL (IQR)	26.0 (16.2–46.7)	24.2 (14.5–43.7)	28.6 (19.7–59.4)	**0.045**
NEP activity, nmol/mL/min (IQR)	2.39 (1.16–4.61)	2.21 (1.03–4.21)	3.92 (1.97–6.72)	**<0.001**
Copeptin, pmol/L (IQR)	11.5 (5.4–23.4)	10.1 (4.8–22.3)	14.3 (7.5–31.2)	**0.009**
Norepinephrine, pg/mL (IQR)	1055 (794–1375)	1004 (781–1355)	1296 (952–1438)	**0.001**

quartile at approximately 15 ng/mL. Patients with cDDP3 values greater than 15 ng/mL had lower BMI, and notably higher transaminase and bilirubin levels. Univariate Cox regression analysis was significant for the primary endpoint (crude HR 1.15 [95% CI: 1.03–1.28], $p = 0.012$ for an increase of 5 ng/mL cDPP3). The association remained a trend after adjustment for NT-proBNP (adj. HR 1.12 [95% CI: 1.00–1.25], $p = 0.060$).

cDPP3 levels in stable, advanced HFrEF on GDMT are similar to the BIOSTAT-CHF cohort and lower compared to critically ill patients with hemodynamic instability (stable advanced HFrEF on GDMT: 11.4[IQR 8.9–14.5] advanced HFrEF from BIOSTAT-CHF: 11.5[range 2.8–84.9] < cardiogenic shock, refractory 76.1[IQR 37.9–238.7] and non-refractory 32.8[IQR 23.9–47.6] or sepsis, 26.5[IQR 16.2–40.4]; in ng/mL). As such, cDPP3 level distribution in stable HFrEF basically resembles that of healthy volunteers. cDDP3 correlated generally positively with neurohumoral derangement, whereas no particularly strong relationship with RAS activation or RAS-inhibitor therapy was observed. A more marked relationship might have been anticipated given the role of DPP3 in AngII metabolism, yet RAS activation, chronic regulation of AngII response and AngII degradation are still not fully understood in HFrEF. Mechanisms for elevated cDPP3 levels in shock should be different, as this condition is characterized by excessive release of intracellular DPP3 into the circulation and rapid alterations in cDPP3 levels upon stabilization. In contrast, liver enzymes, bilirubin and plasma NEP activity correlated with cDPP3. The association of plasma NEP activity with liver failure has been established in preclinical models. Plasma NEP is similarly elevated in critically ill conditions, as out-of-hospital cardiac arrest is associated with increased mortality in the highest quartile, yet is not a good biomarker for stable HFrEF [4], suggesting that both NEP and DPP3 may enter circulation upon cell sequestration or indicating reduced clearance for these peptidases due to functional liver impairment. cDPP3 is a risk factor for worse outcome in stable HFrEF as demonstrated in other more critical conditions. The source of cDPP3, its cardiodepressive effects and therapeutic potential of cDPP3 as a biotarget within this population need to be investigated in further studies. The increase in cDPP3 levels is not as marked as in sepsis or cardiogenic shock, which puts into question a direct role of cDPP3 in bad outcomes in HFrEF. Still, a causative role of cDPP3 should not be excluded given the complex and fine neurohumoral balance in HFrEF. The prognostic value of cDPP3 lies predominantly in predicting mortality, which increases beyond a cut-off of 15 ng/mL, similar to the cut-off found in BIOSTAT-CHF on population-dependent quartiles. Because cDPP3 is only elevated in late stages, it could not only serve as a biomarker for cardiogenic shock, but could specify end-stage HFrEF, helping to identify a vulnerable and difficult-to-define patient population. Recently, Butler and Braunwald urgently asked for a better definition of the stages of HF using objective parameters, and biomarkers such as cDPP3 could be one of them [5].

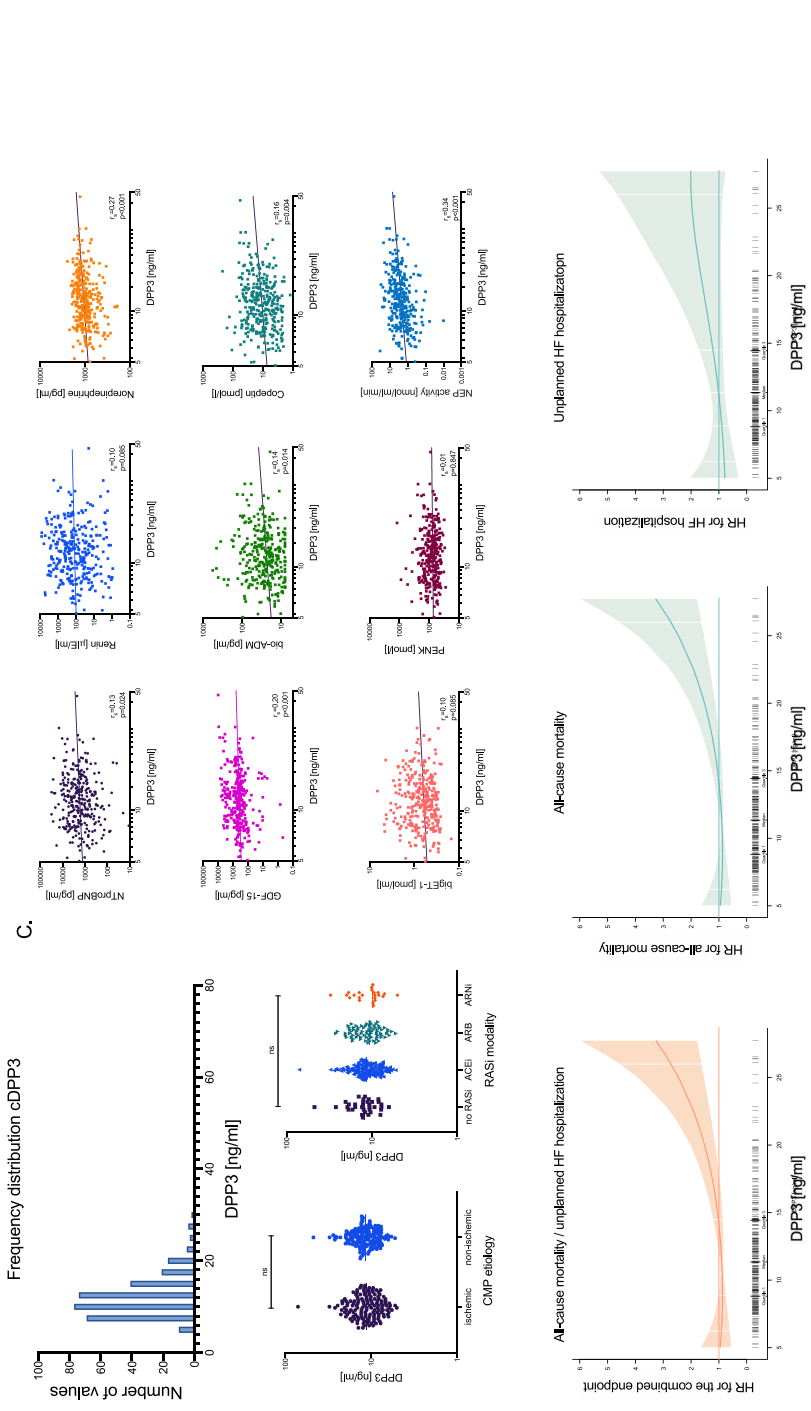

Fig 1 *cDPP3 in advanced HFrEF. (A) Distribution of cDDP3 in advanced stable HFrEF, (B) association of cDDP3 with HF etiology and (B) RAS-inhibitor therapy, (C) relationship between cDPP3 and neurohumoral derangement and (D) association of cDPP3 and outcome. For (C) scatter plots, linear regression lines and Spearman's Rho correlation coefficients with the level of significance are shown for the correlation between cDPP3 and NT-proBNP, renin, norepinephrine, GDF-15, bio-ADM, Copeptin, big-ET-1, PENK and plasma NEP activity. For (D) Cubic spline curves are shown for the combined endpoint of all-cause mortality and unplanned HF hospitalizations as well as for the endpoints separately. Distribution of cDPP3 levels with quartiles are displayed in the graphs. Hazard ratios (HR) and the 95% confidence intervals are shown for each curve.*

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Author contribution

NP, GG, PB and MH designed the experiment. AZ, NP, SP, GSp, RW and HA managed participant recruitment and were responsible for sample acquisition. KS was responsible for the blinded cDPP3 measurements. NP, MH, SP, GG, PB, JM, GSp and GSt analysed and interpreted the data. NP and MH wrote the manuscript. All authors revised the manuscript carefully for important intellectual content. MH was the supervisor of the study.

Conflicts of interest

KS is an employee of 4TEEN4 Pharmaceuticals GmbH. 4TEEN4 Pharmaceuticals GmbH holds the patent rights on the DPP3 biomarker and humanized antibody Procizumab. cDPP3 measurements were sponsored by 4TEEN4 Pharmaceuticals and were carried out in a blinded fashion. Matching of patient data to cDPP3 was performed by NP.

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