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RESEARCH ARTICLE

Bio-adrenomedullin as a marker of congestion in patients with new-onset and worsening heart failure

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Background Secretion of adrenomedullin (ADM) is stimulated by volume overload to maintain endothelial barrier function, and higher levels of biologically active (bio-) ADM in heart failure (HF) are a counteracting response to vascular leakage and tissue oedema. This study aimed to establish the value of plasma bio-ADM as a marker of congestion in patients with worsening HF.

Methods and results The association of plasma bio-ADM with clinical markers of congestion, as well as its prognostic value was studied in 2179 patients with new-onset or worsening HF enrolled in BIOSTAT-CHF. Data were validated in a separate cohort of 1703 patients. Patients with higher plasma bio-ADM levels were older, had more severe HF and more signs and symptoms of congestion (all $P < 0.001$). Amongst 20 biomarkers, bio-ADM was the strongest predictor of a clinical congestion score ($r^2 = 0.198$). In multivariable regression analysis, higher bio-ADM was associated with higher body mass index, more oedema, and higher fibroblast growth factor 23. In hierarchical cluster analysis, bio-ADM clustered with oedema, orthopnoea, rales, hepatomegaly and jugular venous pressure. Higher bio-ADM was independently associated with impaired up-titration of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers after 3 months, but not of beta-blockers. Higher bio-ADM levels were independently associated with an increased risk of all-cause mortality and HF hospitalization (hazard ratio 1.16, 95% confidence interval 1.06–1.27, $P = 0.002$, per log increase). Analyses in the validation cohort yielded comparable findings.

Conclusions Plasma bio-ADM in patients with new-onset and worsening HF is associated with more severe HF and more oedema, orthopnoea, hepatomegaly and jugular venous pressure. We therefore postulate bio-ADM as a congestion marker, which might become useful to guide decongestive therapy.

Keywords Bio-adrenomedullin • Pro-adrenomedullin • Adrenomedullin • Heart failure • Congestion

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Introduction

Pulmonary and systemic congestion play a major role in symptoms that are associated with worsening heart failure (HF).[1,2] Consequently, patients who are hospitalized for worsening HF with a poor diuretic response and incomplete decongestion at discharge have a higher risk of death and hospital re-admission.[3,4] Therefore, an easy-to-use surrogate biomarker for the assessment of a patient's congestion status might be of great clinical value.

A promising novel biomarker in this context is the biologically active form of adrenomedullin (bio-ADM), a 52-amino acid ringed, vasodilatory peptide hormone that is elevated in acute and chronic HF.[5–8] The adrenomedullin (ADM) gene encodes a pre-prohormone, which after cleavage generates a pro-ADM peptide, which by proteolytic fragmentation becomes a glycine-extended, inactive ADM. This is enzymatically converted to bio-ADM. Secretion of ADM has been demonstrated from endothelial cells, cardiac myocytes, vascular smooth muscle cells, and leucocytes. ADM is cleared by neutral endopeptidase and through binding with its receptors. The most dominant role of ADM is thought to be the regulation of endothelial function, and ADM has been shown to play an essential role in maintaining endothelial barrier function and disruption hereof results in vascular leakage, and systemic and pulmonary oedema.[9,10] ADM expression is stimulated by volume overload, and increased plasma ADM reflects excessive fluid overload.[11] In a recent study in patients with acute HF, bio-ADM at baseline was associated with more signs of congestion and higher bio-ADM levels after 7 days of decongestive treatment were associated with significant residual congestion at this time point.[7] The findings from this study suggest that bio-ADM might be a potential marker of congestion both at admission and during/after a hospitalization for acute HF. In the current study, we aimed to further evaluate clinical and biological factors associated with bio-ADM in patients with worsening and new-onset HF, and assess associations with congestion and outcome, in order to gain greater insight into the potential role of bio-ADM in HF.

Methods

Study population

We studied plasma bio-ADM in patients enrolled in 'A systems BIOlogy Study to TAilored Treatment in Chronic Heart Failure' (BIOSTAT-CHF), a multicentre, multinational, prospective observational study. In the BIOSTAT-CHF index cohort, 2516 patients were enrolled with worsening signs and/or symptoms of HF from 11 European countries, who were on suboptimal guideline-recommended medical therapy [i.e. ≤ 50% of target doses of angiotensin-converting enzyme inhibitors (ACEi)/angiotensin receptor blockers (ARB) and beta-blockers]. Patients could be enrolled as in- and outpatients, however the majority of patients were hospitalized for worsening HF. A number of patients were admitted or presented to the hospital for other reasons, yet developed worsening or new-onset HF during hospitalization. The most frequent other reasons were rhythm disturbances, acute coronary syndrome, implantation of a device, or up-titration of HF medication. Patients should have objective evidence of cardiac dysfunction documented by either left ventricular ejection fraction ≤ 40% or a B-type natriuretic peptide (BNP) level ≥ 400 pg/mL or N-terminal pro BNP (NT-proBNP) ≥ 2000 pg/mL during screening. During the first 3 months after enrolment, investigators were expected to optimize treatment of HF with ACEi/ARB and beta-blockers, according to the doses indicated in the 2012 European Society of Cardiology guidelines.[12]

The results were subsequently validated in the BIOSTAT-CHF validation cohort, in which 1738 patients were recruited from six centres in Scotland, United Kingdom. In summary, in- and outpatients diagnosed with HF with a previous admission for HF and maintenance dose of ≥ 20 mg of furosemide per day were eligible for enrolment. Details on both cohorts have been previously published.[13–16]

All patients provided written informed consent to participate in the study and BIOSTAT-CHF was conducted in concordance with the Declaration of Helsinki. The study was also approved by national and local ethics committees.

Study assessments

Plasma bio-ADM was measured at baseline in 2179 patients in the index cohort and in 1703 patients in the validation cohort, using an immunoassay (sphingotest® bio-ADM®) developed by sphingotec GmbH (Hennigsdorf, Germany).[8,17] Included patients were generally comparable to excluded patients for both cohorts (online supplementary *Tables S1* and *S2*). In brief, the bio-ADM immunoassay is a one-step sandwich chemiluminescence immunoassay based on acridinium NHS-ester labelling for the detection of human ADM in unprocessed, neat plasma. It uses two mouse monoclonal antibodies, one directed against the mid-region and the other directed against the amidated C-terminal moiety of ADM. The assay uses 50 μL of plasma samples/calibrators and 220 μL of labelled detection antibody. Upon storage at room temperature, bio-ADM in EDTA plasma is stable for up to 24 h, and samples are unaffected by at least up to four freeze–thaw cycles. The analytical assay sensitivity is 2 pg/mL. The lower detection limit is 3 pg/mL, and intra- and interassay coefficients were 5–10%, and 4–8%, respectively, in the above normal measuring range. This assay is highly specific to bio-ADM as it only reacts with the mature amidated C-terminus of ADM, and not to other variants of (pro-) ADM.[17] In healthy subjects, median bio-ADM concentration was 20.7 pg/mL (99th percentile: 43 pg/mL).[18]

A great number of other biomarkers from multiple pathophysiological domains, including markers of inflammation, apoptosis, remodelling, myocyte stress, angiogenesis, endothelial function and renal function were measured. Pro-enkephalin (PENK) was measured using a sandwich immunoassay (sphingotest® penKid®) targeting PENK A amino acids 119–159 developed by sphingotec GmbH (Hennigsdorf, Germany). Pro-ADM was measured using a sandwich ELISA on a Luminex® platform (Alere Inc., San Diego, CA, USA). Interleukin-6 and endothelin-1 were measured in frozen plasma by Singulex Inc. (Alameda, CA, USA) using high-sensitive single molecule counting (SMC™) technology (RUO, Erenna® Immunoassay System, Singulex Inc., Alameda, CA, USA). NT-proBNP was measured using electrochemiluminescence on a Cobas e411 analyser, using standard methods (Roche Diagnostics GmbH, Mannheim, Germany). Measurement of other biomarkers was performed as previously described.[15,19] From all available biomarkers, 20 were selected for these analyses based on their known involvement in volume overload, neurohormonal activation, renal function, and outcome (online supplementary *Table S3*).

A congestion score was calculated as the sum of peripheral oedema depending on the extent (0 to 1/3 to 2/3 to 1), jugular venous pressure

(JVP) (0 to 1), and orthopnoea (0 to 1) for the index cohort. For the validation cohort, the maximum score is 2, as orthopnoea was not routinely collected in this cohort. A modified congestion score, where oedema was scored 1 point if oedema extended above the knee, was used in a sensitivity analysis to balance the impact of oedema on the congestion score.

Outcomes

The endpoints selected for these analyses were all-cause mortality, cardiovascular mortality, and the combined endpoint of all-cause mortality or first occurrence of HF hospitalization. Cardiovascular mortality was based on the narrative provided for each death by a small committee of cardiologists. Additionally, the association between bio-ADM and therapy optimization for ACEi/ARB and beta-blockers at 3 months was evaluated.

Statistical analysis

Baseline clinical variables and biomarkers were evaluated over tertiles of bio-ADM levels. Frequency (percentage) was used to summarize categorical variables while normally distributed continuous variables were summarized with mean ± standard deviations and non-normally distributed continuous variables with median [interquartile range]. Trends over tertiles of bio-ADM were statistically tested with Cochran–Armitage trend test, Jonckheere–Terpstra, or a linear regression model for categorical variables, non-normally distributed continuous variables, and normally distributed continuous variables, respectively. Uni- and multivariable linear regression analysis was performed with log transformed bio-ADM as a dependent variable. Transformations were checked using multifractional polynomials. Multivariable linear regression analysis, including all variables with $P < 0.10$ in univariable analysis, were constructed via backward elimination and validated using bootstrap re-sampling with 1000 replicates. The model was tested for collinearity and checked by plotting residuals. The correlation heat maps and dendrograms were constructed using the ggplot2, reshape2, fastcluster, Hmisc, and sparcl packages in R. Cox proportional hazards regression analysis was performed to examine associations with clinical outcomes. Bio-ADM was investigated as a continuous variable and by quintiles. Multivariable models were adjusted for an outcome model specifically developed and validated in the BIOSTAT-CHF index and validation cohort.[14] The added value of bio-ADM for estimating the risk of poor outcome was assessed by examining gain in Harrell's C-index (a measure of model discrimination, higher values are better), using likelihood ratio tests for nested survival models, and assessment of continuous net reclassification improvement (a category-independent measure quantifying the degree of improvement in model-based risk estimates obtained by adding a marker to a model). Logistic regression was used to investigate the association between bio-ADM and ACEi/ARB use, and whether target dose was reached. A two-tailed *P*-value of <0.05 was considered statistically significant. All analyses were performed using R: A Language and Environment for Statistical Computing, version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics

Median bio-ADM at baseline was 33.8 [22.6–53.9] pg/mL. Baseline characteristics over tertiles of bio-ADM are presented in *Table 1*. Patients with higher bio-ADM levels were older, had a higher body mass index (BMI), and more severe HF (higher New York Heart Association class, higher NT-proBNP levels, and more frequently a previous HF hospitalization) (all $P < 0.006$). Additionally, higher bio-ADM levels were associated with the presence of signs and symptoms of congestion, lower systolic and diastolic blood pressure, poorer renal function, and higher levels of biomarkers such as fibroblast growth factor 23 (FGF23), PENK, and interleukin-6 (all $P < 0.009$). Interestingly, patients with higher bio-ADM levels used higher doses of diuretics ($P < 0.001$). After 9 months, patients in the highest tertile were more likely to use loop diuretics (98.2% vs. 92.2%, $P = 0.018$ for first vs. third tertile), and used slightly higher doses (40 [40–50] mg vs. 60 [40–125] mg of furosemide or equivalent, $P < 0.001$ for first vs. third tertile).

Association between bio-adrenomedullin and clinical variables

The result of multivariable linear regression analysis for log bio-ADM is shown in the online supplementary *Table S4*. The strongest associations with higher log bio-ADM were higher BMI, higher log FGF23, more frequent oedema, higher interleukin-6 and endothelin-1 ($r^2 = 0.580$). Log pro-ADM was significantly associated with log bio-ADM levels, yet explained only 30% of the variance of log bio-ADM. Pro-ADM was not significant in a multivariable model for bio-ADM; forcing this variable into the multivariable model increased the r^2 significantly to 0.594 ($P < 0.001$).

Correlation and network analysis

The results of hierarchical clustering of variables and a correlation heat map are presented in *Figures 1* and *2*. The heat map (*Figure 1*) illustrates that bio-ADM levels are most strongly correlated with FGF23 (Spearman's rho: 0.57, $P < 0.001$), pro-ADM (Spearman's rho: 0.54, $P < 0.001$), growth differentiation factor 15 (GDF15) (Spearman's rho: 0.49, $P < 0.001$), and the presence of oedema (Spearman's rho: 0.43, $P < 0.001$). In a hierarchical cluster analysis (*Figure 2*), bio-ADM clustered with oedema, as well as orthopnoea, rales, hepatomegaly and JVP – all signs of congestion. Interestingly, pro-ADM did not cluster with bio-ADM, yet clustered with FGF23, GDF15, and NT-proBNP.

Bio-adrenomedullin and congestion

Amongst 20 markers, bio-ADM was the strongest variable associated with a higher clinical congestion score ($r^2 = 0.198$, $P < 0.001$), compared with for instance pro-ADM ($r^2 = 0.120$, $P < 0.001$), and NT-proBNP ($r^2 = 0.114$, $P < 0.001$). The modified congestion score yielded similar findings. This was also observed for the presence of oedema where bio-ADM had an area under the curve of 0.768, compared to 0.658 for pro-ADM, and 0.641 for NT-proBNP. Other signs and symptoms of congestion such as JVP and orthopnoea yielded comparable findings. *Table 2* shows the

Table 1 Baseline characteristics over tertiles of bio-adrenomedullin in the index cohort

	Tertile 1	Tertile 2	Tertile 3	*P*-value for trend
Patients (*n*)	719	719	741	
Bio-ADM (pg/mL)	19.2 [15.1–22.5]	33.3 [29.1–38.1]	67.2 [53.2–100.6]	
Demographics				
Male sex (%, *n*)	76.1 (547)	72.6 (522)	70.9 (525)	0.024
Age (years)	66.9 ± 12.5	69.4 ± 11.7	70.4 ± 11.7	<0.001
Caucasian race (%, *n*)	98.9 (711)	98.7 (710)	99.1 (734)	0.756
BMI (kg/m^2)	25.8 ± 4.3	27.8 ± 5	29.9 ± 6.3	<0.001
Weight (kg)	76.1 ± 15.6	81.3 ± 16.7	87.7 ± 21	<0.001
Height (kg)	171.3 ± 9.4	170.8 ± 9.2	170.9 ± 9.1	0.402
NYHA class (%, *n*)				<0.001
I	3.2 (23)	2.9 (21)	0.1 (1)	
II	47.4 (341)	38.4 (276)	20.4 (151)	
III	40.3 (290)	45.1 (324)	57.1 (423)	
IV	6.5 (47)	11.1 (80)	18.9 (140)	
LVEF (%)	30.5 ± 9.4	31 ± 10.7	31.9 ± 12.2	0.015
HFpEF (%, *n*)	5.1 (35)	8.3 (53)	10.3 (65)	<0.001
Clinical profile				
Oedema (%, *n*)	10.7 (60)	22.7 (131)	52.4 (349)	<0.001
Orthopnoea (%, *n*)	23.8 (171)	31.8 (228)	48.2 (356)	<0.001
Rales > 1/3 up lung fields (%, *n*)	16.8 (48)	19.6 (76)	22 (103)	0.084
Jugular venous pressure (%, *n*)	21.1 (103)	31.5 (156)	49.4 (239)	<0.001
Hepatomegaly (%, *n*)	8.5 (61)	12.8 (92)	20.8 (154)	<0.001
Third heart tone (%, *n*)	10.6 (76)	8 (57)	11.1 (82)	0.731
Clinical congestion score	0 [0–1]	0.67 [0–1.33]	1.67 [0.67–2.33]	<0.001
Systolic blood pressure (mmHg)	125.6 ± 22.2	125.4 ± 21.9	122.5 ± 21.9	0.008
Diastolic blood pressure (mmHg)	76.1 ± 12.8	75 ± 13.8	72.9 ± 13.1	<0.001
Heart rate (b.p.m.)	77 ± 17.7	80.5 ± 20.6	82.6 ± 19.9	<0.001
Hospitalization (%, *n*)				
Type of visit				<0.001
Scheduled outpatient clinic	33.9 (244)	25.5 (183)	14.3 (106)	
Unscheduled outpatient clinic	6 (43)	3.9 (28)	3.6 (27)	
Inpatient hospitalization	60.1 (432)	70.7 (508)	82.1 (608)	
Reason for visit				<0.001
Worsening HF	45.1 (324)	51.9 (373)	63.8 (473)	
New-onset HF	30.6 (220)	30.5 (219)	26.5 (196)	
Other	24.3 (175)	17.7 (127)	9.7 (72)	
Diuretics i.v.	98.7 (310)	97.1 (372)	98.6 (545)	0.918
Inotropes i.v.	10.9 (34)	8.9 (34)	13.2 (73)	0.195
Nitrates i.v.	26.8 (83)	23.2 (89)	17.2 (95)	0.001
Heart failure history				
Years since first diagnosis	0.4 [0.1–1.3]	3 [0.3–8.8]	3.7 [0.7–7.2]	0.026
Ischaemic heart disease	56.2 (357)	62.6 (402)	61.6 (405)	0.048
Hypertension	54.2 (371)	59.7 (410)	54.9 (389)	0.785
Cardiomyopathy	52.8 (349)	38.7 (251)	38.7 (259)	<0.001
Valvular heart disease	37.1 (251)	40.5 (277)	44.4 (310)	0.006
NYHA class prior to decompensation/worsening HF				<0.001
I	11 (79)	8.5 (61)	6.1 (45)	
II	51.6 (371)	46.6 (335)	40.4 (299)	
III	24.3 (175)	27.7 (199)	33.7 (250)	
IV	1.9 (14)	4 (29)	4.5 (33)	
Previous HF hospitalization	28.4 (204)	29.9 (215)	35.1 (260)	0.005
Medical history (%, *n*)				
Hypertension	58.3 (419)	65.1 (468)	62.6 (464)	0.091
Atrial fibrillation	33.4 (240)	46.2 (332)	57.2 (424)	<0.001
Coronary artery disease	38.1 (274)	46.5 (334)	47.1 (349)	0.001

Table 1 Continued

	Tertile 1	Tertile 2	Tertile 3	*P*-value for trend
Myocardial infarction	32.8 (236)	39.5 (284)	38.3 (284)	0.031
PCI	19.1 (137)	22 (158)	21.1 (156)	0.351
CABG	11.7 (84)	17.5 (126)	21.7 (161)	<0.001
Pacemaker	5.7 (41)	9.5 (68)	7.2 (53)	0.301
ICD	5.7 (41)	7.5 (54)	10.4 (77)	0.001
Biventricular pacer (CRT)	1.3 (9)	1.3 (9)	3 (22)	0.014
Biventricular pacer (CRT) and ICD	5.4 (39)	6.1 (44)	8.6 (64)	0.014
Diabetes mellitus	23.5 (169)	31.6 (227)	41.4 (307)	<0.001
COPD	13.1 (94)	18.2 (131)	20.2 (150)	<0.001
Peripheral artery disease	7.5 (54)	12.5 (90)	13.6 (101)	<0.001
Stroke	8.1 (58)	9.9 (71)	10.4 (77)	0.130
Medication				
ACEi or ARB (%, *n*)	78 (561)	73.2 (526)	64.2 (476)	<0.001
Target dose (%, *n*)	14.9 (107)	12.4 (89)	10.9 (81)	0.024
Beta-blockers (%, *n*)	85.1 (612)	83.9 (603)	80.4 (596)	0.017
Target dose (%, *n*)	4 (29)	6.3 (45)	6.3 (47)	0.055
Loop diuretics (%, *n*)	99.3 (714)	99.6 (716)	99.6 (738)	0.436
Loop diuretic dose (mg furosemide)	40 [40–62]	40 [40–80]	80 [40–150]	<0.001
Aldosterone antagonists (%, *n*)	53.5 (385)	53.1 (382)	50.6 (375)	0.259
Digoxin (%, *n*)	16 (115)	18.9 (136)	20 (148)	0.050
Laboratory				
Haemoglobin (g/dL)	13.7 [12.4–14.8]	13.4 [12.1–14.5]	12.7 [11.4–14]	<0.001
Creatinine (μmol/L)	90.2 [76.9–112]	103.8 [87–125.6]	116 [93–153]	0.708
Urea (mmol/L)	9.3 [6.7–14.7]	10.8 [7.5–17.4]	13.5 [8.8–23.8]	<0.001
eGFR (mL/min/1.73 m^2)	71.1 [53.6–86]	58.6 [44.8–75.5]	49.7 [34.7–67.1]	<0.001
Sodium (mmol/L)	140 [138–142]	140 [137–142]	139 [136–141]	<0.001
Potassium (mmol/L)	4.3 [4–4.6]	4.2 [3.9–4.6]	4.2 [3.8–4.6]	0.003
Calcium (mmol/L)	1.8 [1.5–2.1]	1.8 [1.5–2]	1.7 [1.4–2]	<0.001
Phosphate (mmol/L)	0.9 [0.7–1]	0.8 [0.7–1]	0.8 [0.7–1]	0.611
Albumin (g/L)	34 [29–39]	32 [28–37]	31 [25–35]	<0.001
Iron (µmol/L)	10 [6–14]	8.5 [6–12]	6 [4–10]	<0.001
Ferritin (µg/L)	116 [53.2–204.8]	106 [51.5–192]	81 [44–165]	0.057
Transferrin (g/L)	2 [1.6–2.4]	2 [1.6–2.4]	2 [1.5–2.5]	0.890
Aldosterone (pg/mL)	94 [43–187]	93 [44.5–180]	90 [42–211]	0.002
Renin (IU/mL)	68 [23.9–191.7]	89.1 [27.6–229]	125.1 [36.2–374.5]	<0.001
NGAL (ng/mL)	50.8 [33.0–80.2]	57.8 [35.7–88.3]	76.3 [45.6–125.4]	<0.001
Interleukin-6 (pg/mL)	3.4 [1.9–6.3]	4.9 [2.7–8.8]	8.6 [4.9–17]	<0.001
Troponin I (ng/L)	10.4 [5.9–22.8]	12.4 [6.6–27.7]	16.3 [9–33.2]	0.785
Endothelin-1 (pg/mL)	4.5 [3.5–5.8]	5 [4–6.7]	6.7 [5.1–9]	<0.001
FGF23 (RU/mL)	132.3 [90.7–217.5]	201.9 [121.9–401.1]	621.9 [274.6–1745.6]	<0.001
Galectin-3 (ng/mL)	17.6 [13.0–25.0]	20.3 [15.5–28.0]	26.0 [18.8–36.7]	<0.001
Pro-enkephalin (pmol/L)	73.7 [56.1–97.6]	85.1 [64.5–116.4]	105 [77.2–151.1]	<0.001
Pro-ADM (ng/mL)	0.4 [0.2–0.5]	0.5 [0.3–0.7]	0.8 [0.5–1.3]	<0.001
NT-proBNP (pg/mL)	1826 [825–3959]	2563.5 [1142–5101.8]	4313 [2067–9355.5]	<0.001
GDF15 (pg/mL)	1974 [1306–2912]	2573 [1723.2–3980.2]	4324 [2694–7366]	<0.001

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; Bio-ADM, biologically active adrenomedullin; BMI, body mass index; CABG, coronary artery bypass graft; COPD, chronic obstructive pulmonary disease; CRT, cardiac resynchronization therapy; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; GDF15, growth differentiation factor 15; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; ICD, implantable cardioverter defibrillator; LVEF, left ventricular ejection fraction; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal pro B-type natriuretic peptide; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; PENK, pro-enkephalin, Pro-ADM, pro-adrenomedullin.

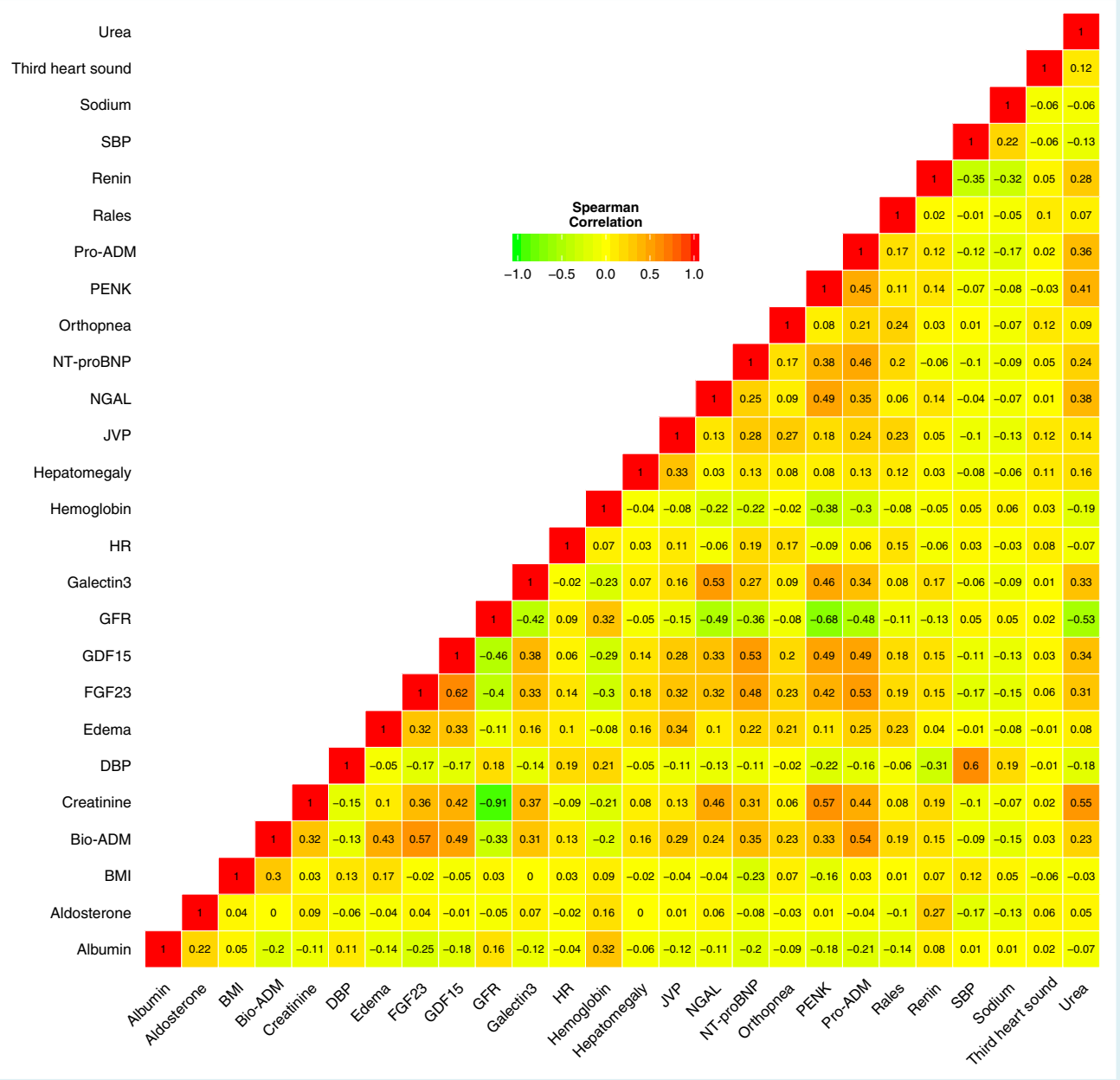

Figure 1 Biomarker position of bio-adrenomedullin depicted in a correlation heat map. Bio-ADM, biologically active adrenomedullin; BMI, body mass index; DBP, diastolic blood pressure; FGF23, fibroblast growth factor 23; GDF15, growth differentiation factor 15; GFR, glomerular filtration rate; HR, heart rate; JVP, jugular venous pressure; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal pro B-type natriuretic peptide; PENK, pro-enkephalin; Pro-ADM, pro-adrenomedullin; SBP, systolic blood pressure.

comparison between associations of bio-ADM and NT-proBNP with clinical markers of congestion. In particular, the association between bio-ADM and oedema was stronger than the association between NT-proBNP and oedema. The distribution of bio-ADM over quartiles of NT-proBNP (for both cohorts) is plotted in the online supplementary *Figure S1*. Using receiver operating characteristic (ROC) analysis, the best cut-off value of bio-ADM to assess congestion (defined as a congestion score > 1) was 34 pg/mL.

Bio-adrenomedullin and therapy optimization

Higher levels of log bio-ADM were associated with lower rates of ACEi/ARB and beta-blocker use at baseline (*Table 1*). Also, patients with higher bio-ADM levels less frequently used guideline-recommended doses of ACEi/ARB and beta-blockers (*Table 1*). Furthermore, higher bio-ADM levels were inversely associated with ACEi/ARB use and dosage after 3 months of

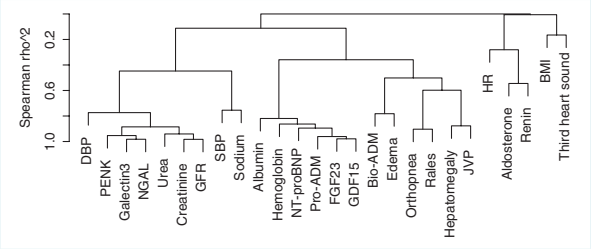

Figure 2 Biomarker position of bio-adrenomedullin depicted in hierarchical cluster analysis. Bio-ADM, biologically active adrenomedullin; BMI, body mass index; DBP, diastolic blood pressure; FGF23, fibroblast growth factor 23; GDF15, growth differentiation factor 15; GFR, glomerular filtration rate; HR, heart rate; JVP, jugular venous pressure; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal pro B-type natriuretic peptide; PENK, pro-enkephalin; Pro-ADM, pro-adrenomedullin; SBP, systolic blood pressure.

up-titration (*Table 3*). This association remained significant after adjustment for BMI, FGF23, PENK, NT-proBNP, estimated glomerular filtration rate, age, sex, oedema and ACEi/ARB or beta-blocker use at baseline. In contrast, there was no significant independent association between bio-ADM levels and beta-blocker dosage after 3 months of up-titration.

Bio-adrenomedullin and outcomes

During a median follow-up of 21 [15–27] months, 583 (26.7%) patients died and 914 (41.9%) patients experienced the combined endpoint of all-cause mortality or HF hospitalization. In univariable Cox regression analysis, log bio-ADM was significantly associated with an increased risk of all-cause mortality [hazard ratio (HR) 1.79, 95% confidence interval (CI) 1.63–1.97; $P < 0.001$ per log increase], and the combined endpoint (HR 1.71, 95% CI 1.58–1.85; $P < 0.001$ per log increase). This remained significant for both endpoints after adjustment for the BIOSTAT-CHF risk model (*Table 4*), as well as after adjustment for the BIOSTAT-CHF risk model with addition of NT-proBNP and the clinical congestion score (HR 1.19, 95% CI 1.03–1.36; $P = 0.015$ per log increase for the combined endpoint). The association of bio-ADM with outcome was also analysed over quintiles of bio-ADM, where after multivariable adjustment quintile 4 and 5, i.e. the highest bio-ADM levels, remained significantly associated with an increased risk of adverse outcome (*Table 3*). Kaplan–Meier curves for all-cause mortality per quintile of bio-ADM are shown in *Figure 3*, illustrating a higher risk over increasing quintiles of bio-ADM (log-rank $P < 0.001$). Higher levels of bio-ADM were independently associated with an increased risk of cardiovascular mortality (HR 1.27, 95% CI 1.04–1.56; $P = 0.020$ per log increase).

When comparing the predictive value of NT-proBNP to bio-ADM for outcome, NT-proBNP was a stronger predictor of all-cause mortality (univariable c-index 0.673 for NT-proBNP vs. 0.636 for bio-ADM), as well as the combined endpoint (univariable c-index 0.652 for NT-proBNP vs. 0.624 for bio-ADM). Bio-ADM did not improve the net reclassification index on top of the BIOSTAT-CHF risk model (including NT-proBNP) for both outcomes (online supplementary *Table S5*).

Validation of bio-adrenomedullin

The value of bio-ADM in worsening or new-onset HF was subsequently validated in a separate cohort of 1703 HF patients. Median bio-ADM was 27.3 [18.0–42.1] pg/mL. Baseline characteristics over tertiles of bio-ADM in the validation cohort are shown in the online supplementary *Table S6*. The strongest associations with higher bio-ADM levels in multivariable linear regression analysis were observed for oedema, higher levels of urea, PENK and higher heart rate (adjusted $r^2 = 0.357$) (online supplementary *Table S7*).

Correlation and network analysis

The heat map (online supplementary *Figure S2*) illustrates that bio-ADM levels are most strongly correlated with the clinical congestion score (Spearman's rho: 0.37, $P < 0.001$), the presence of oedema (Spearman's rho: 0.34, $P < 0.001$), and creatinine

Table 2 Comparison of associations with congestion variables between bio-adrenomedullin and N-terminal pro B-type natriuretic peptide

	Bio-ADM		NT-proBNP		*P*-value (comparison bio-ADM vs. NT-proBNP)
	Correlation coefficient (*r*)	*P*-value	Correlation coefficient (*r*)	*P*-value	
Oedema (above knee)	0.43	<0.001	0.22	<0.001	<0.001
Orthopnoea	0.24	<0.001	0.17	<0.001	0.036
JVP	0.29	<0.001	0.28	<0.001	0.767
Third heart sound	0.03	0.168	0.05	0.039	0.630
Hepatomegaly	0.16	<0.001	0.12	<0.001	0.157
Rales	0.19	<0.001	0.20	<0.001	0.587

Presented correlations are Spearman's.
Bio-ADM, biologically active adrenomedullin; JVP, jugular venous pressure; NT-proBNP, N-terminal pro B-type natriuretic peptide.

Table 3 Bio-adrenomedullin and angiotensin-converting enzyme inhibitor/angiotensin receptor blocker or beta-blocker use after 3 months of up-titration in the index cohort

Log bio-ADM	ACEi/ARB use OR (95% CI)	*P*-value	Target dose OR (95% CI)	*P*-value	Beta-blocker use OR (95% CI)	*P*-value	Target dose OR (95% CI)	*P*-value
Univariable	0.49 (0.42–0.57)	<0.001	0.67 (0.57–0.78)	<0.001	0.56 (0.47–0.66)	<0.001	0.94 (0.79–1.12)	0.500
Multivariable[a]	0.60 (0.47–0.79)	<0.001	0.64 (0.47–0.87)	0.005	0.57 (0.42–0.77)	<0.001	0.97 (0.70–1.33)	0.850

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; bio-ADM, biologically active adrenomedullin; CI, confidence interval; OR, odds ratio.
[a]Adjusted for body mass index, fibroblast growth factor 23, pro-enkephalin, N-terminal pro B-type natriuretic peptide, estimated glomerular filtration rate, age, sex, oedema, and ACEi/ARB or beta-blocker use at baseline.

Table 4 Cox regression analysis for bio-adrenomedullin and all-cause mortality, and the combined endpoint in the index cohort

	All-cause mortality	*P*-value	Multivariable[a]	*P*-value	Combined endpoint		Multivariable[b]	*P*-value
	Univariable HR (95% CI)		HR (95% CI)		Univariable HR (95% CI)	*P*-value	HR (95% CI)	
Log bio-ADM	1.78 (1.62–1.95)	<0.001	1.14 (1.02–1.28)	0.015	1.71 (1.58–1.85)	<0.001	1.16 (1.06–1.27)	0.002
Quintile 1	1.0 (Ref)	Ref	1.0 (Ref)	Ref	1.0 (Ref)	Ref	1.0 (Ref)	Ref
Quintile 2	1.42 (1.02–1.96)	0.037	1.20 (0.87–1.66)	0.275	1.27 (0.99–1.61)	0.062	1.06 (0.83–1.35)	0.670
Quintile 3	1.69 (1.23–2.31)	0.001	1.11 (0.80–1.53)	0.532	1.56 (1.23–1.97)	<0.001	1.11 (0.88–1.41)	0.376
Quintile 4	2.41 (1.79–3.26)	<0.001	1.35 (1.00–1.83)	0.056	2.06 (1.64–2.58)	<0.001	1.23 (0.98–1.56)	0.076
Quintile 5	3.82 (2.87–5.09)	<0.001	1.39 (1.02–1.90)	0.036	3.23 (2.60–4.01)	<0.001	1.33 (1.05–1.68)	0.019

bio-ADM, biologically active adrenomedullin; CI, confidence interval; HR, hazard ratio.
[a]Adjusted for the BIOSTAT-CHF risk model for all-cause mortality (age, log blood urea nitrogen, log N-terminal pro B-type natriuretic peptide, haemoglobin, and beta-blocker use at baseline).
[b]Adjusted for the BIOSTAT-CHF risk model for the combined endpoint (age, heart failure hospitalization in previous year, systolic blood pressure, log N-terminal pro B-type natriuretic peptide, haemoglobin, high-density lipoprotein, sodium, and beta-blocker use at baseline).

(Spearman's rho: 0.34, $P < 0.001$). Again, the modified congestion score yielded similar findings. In a hierarchical cluster analysis (online supplementary *Figure S3*), bio-ADM clustered with NT-proBNP and diastolic blood pressure. When for the index cohort exactly the same variables as in the validation cohort are entered into a heat map and hierarchical cluster analysis, bio-ADM clustered with oedema, JVP, hepatomegaly, and the clinical congestion score (online supplementary *Figures S4* and *S5*).

Bio-adrenomedullin and outcomes

In the validation cohort during a median follow-up of 21 [12–33] months, 519 (30.5%) patients died and 715 (42.0%) experienced the combined endpoint. Cox regression analyses for (quintiles of) bio-ADM yielded comparable findings to the index cohort (online supplementary *Table S8* and *Figure S6*). Similar to the index cohort, when comparing the prognostic value of bio-ADM to NT-proBNP, NT-proBNP was a stronger predictor of both endpoints.

Discussion

In patients with new-onset and worsening HF, higher levels of plasma bio-ADM were strongly associated with more severe HF, and signs and symptoms of congestion, and independently associated with an increased risk of all-cause mortality and HF hospitalization. Using network analysis tools, such as hierarchical cluster analysis, bio-ADM clustered with markers of congestion. Interestingly, patients with higher bio-ADM levels were less likely to receive target doses of ACEi/ARBs after 3 months of up-titration. These findings were externally validated in a separate cohort of patients with HF.

Bio-adrenomedullin as a marker of congestion

Bio-ADM is a vasodilatory peptide hormone that has been shown to play an essential role in maintaining endothelial barrier function, and disruption of this regulatory system results in vascular leakage, and systemic and pulmonary oedema.[9] ADM acts both intravascularly as well as in the interstitium. Intravascular ADM acts on the endothelial cells, improves vascular integrity and decreases vascular permeability, whereas interstitial ADM causes vasodilatation through its effect on vascular smooth muscle cells.[20] Furthermore, the expression of ADM is stimulated by volume overload, and vice versa increased plasma ADM reflects excessive fluid overload.[11] These elevated levels of bio-ADM in HF are reflective of a counteracting response to volume overload, as an attempt to limit

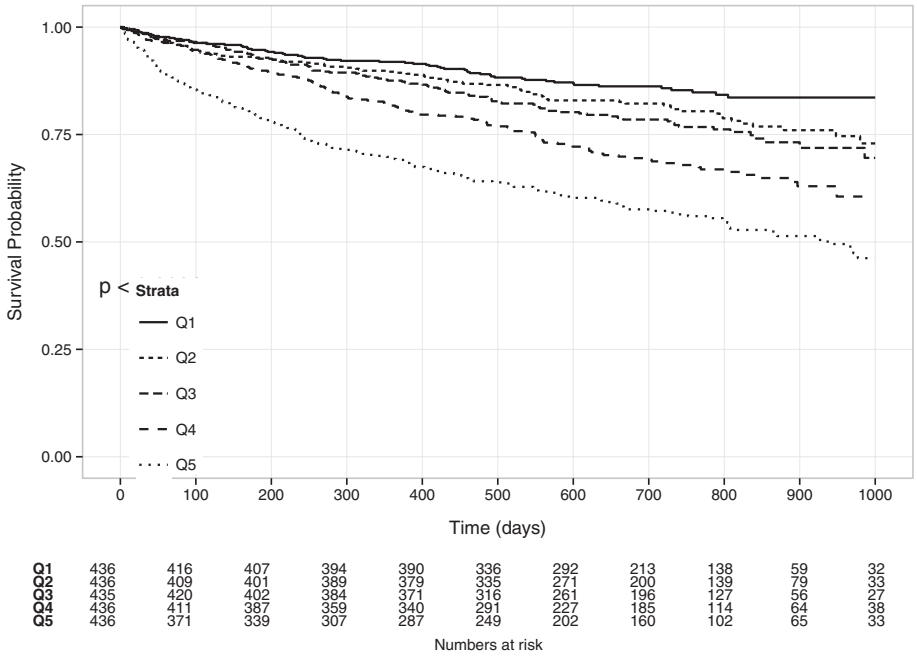

Figure 3 Kaplan–Meier curve for all-cause mortality for quintiles of bio-adrenomedullin in the index cohort.

tissue fluid overload by stabilizing the endothelial barrier function. We recently showed that in patients with acute HF, bio-ADM at baseline was strongly associated with signs and symptoms of congestion.[7] Interestingly, higher bio-ADM levels after 7 days of decongestive treatment were associated with significant residual congestion, this was not observed for NT-proBNP. These findings suggest that bio-ADM might be a marker of congestion that provides additional information over NT-proBNP and could possibly be used to guide decongestive therapy. In this study in patients with new-onset or worsening HF, slightly lower levels of bio-ADM were observed compared to the previous study in patients with acute HF (median bio-ADM 33.8 vs. 44.1 pg/mL). This might confirm that in patients with more fluid overload, i.e. acute HF, bio-ADM levels are more elevated. In the present study, however, bio-ADM levels were increased compared to healthy controls where a median value of 20.7 pg/mL has been described.[18]

Despite the fact that this study investigated patients with new-onset or worsening HF with less fluid overload, the strong association of bio-ADM with congestion is evident. In hierarchical cluster analysis bio-ADM, as the only biomarker, clustered with symptoms of congestion. The slight difference in results between the index and validation cohort might be due to the fact that patients in the validation cohort were generally less congested and for instance had significantly lower NT-proBNP levels. Yet, compared to NT-proBNP, bio-ADM was a stronger predictor of a clinical congestion score in both cohorts, and for the presence of oedema in particular. The association of bio-ADM with congestion is further supported by the finding that patients with higher bio-ADM levels used higher doses of loop diuretics and were more likely to use loop diuretics after 9 months of follow-up. In addition to an association with congestion markers, bio-ADM also showed a strong association with FGF23. FGF23 is a phosphaturic hormone that is associated with renin–angiotensin–aldosterone activation and has been postulated as a regulator of sodium hemostasis through upregulation of the sodium–chloride co-transporter in the distal tubule.[21] As such the association of bio-ADM with FGF23 might be indicative of congestion and could also suggest less effective decongestive therapy. Using ROC analyses, the best cut-off for bio-ADM to assess congestion was 34 pg/mL, which is well above the median described in healthy volunteers, and if confirmed in future studies could be used to identify patients with (residual) congestion. As congestion is notoriously difficult to assess reliably and interobserver variability is considerable, there is a great need for (bio)markers that aid in reliable assessment of congestion. Our study suggests that bio-ADM is a biomarker that might have unique characteristics in this aspect, and could have clinical utility in a broad range of HF patients, i.e. from new-onset to acute HF. Future studies, preferentially using more objective measures to assess congestion, such as echography, in addition to physical examination will have to show whether bio-ADM does indeed provide additional information on top of this. Interestingly, patients with higher bio-ADM levels were less well up-titrated with ACEi or ARB, which may be due to possible residual congestion and diuretic use, or more severe HF, hypotension and haemodynamic intolerance of these drugs. The association of bio-ADM with ACEi or ARB up-titration after 3 months was however independent of several markers of HF severity, such as NT-proBNP, and baseline use of ACEi or ARB.

Bio-adrenomedullin as a marker for clinical outcome

In the present study, we found that higher bio-ADM levels were independently associated with poorer clinical outcome in patients with new-onset and worsening HF. This finding confirms previous studies that reported added prognostic value of bio-ADM for shorter-term outcomes.[7,8,22] We also found that higher levels of bio-ADM were independently associated with an increased risk of the combined endpoint, which includes both all-cause mortality and HF hospitalization. The strong association between higher levels of bio-ADM and a higher risk of hospital (re)admission is of particular interest, since the main cause of HF admissions for worsening HF is related to congestion. The addition of bio-ADM to a model that included NT-proBNP did however not improve the net reclassification index. Therefore, the main value of bio-ADM might not be in predicting outcome, yet in its role as a congestion marker. As such it would have novel and additive value, compared to more established markers such as troponin or NT-proBNP. Taken together, our findings suggest that bio-ADM might be used to guide (decongestive) therapy, and possibly assess low risk of (re)hospitalization at discharge. Further studies in which bio-ADM is assessed at multiple time points during HF hospitalization might be able to shed more light on this.

Adrenomedullin as a therapeutic target

Finally, bio-ADM might be a modifiable risk factor, for instance by administering adrecizumab, a humanized, monoclonal non-neutralizing antibody against the N-terminus of ADM, that increases plasma concentrations of bio-ADM in a dose-dependent manner.[23] In animal models of systemic inflammation and septic shock, adrecizumab has shown promising results by improving haemodynamics and renal function.[23,24] In HF, administration of adrecizumab might result in improved vascular integrity with consequent improvement of tissue congestion and possibly outcomes.[20] Of note, pharmacologic inhibition of neprilysin increased the plasma levels and potentiated the natriuretic and diuretic responses of ADM, when exogenously applied in a dog model.[25] On that background it could be hypothesized that the beneficial effects of sacubitril/valsartan in HF might be partly explained by this mechanism.

Bio- and pro-adrenomedullin

In this study, a relatively novel ADM assay was used that assesses bio-ADM. Until recently ADM activity was measured using a biomarker that assessed an ADM precursor peptide (pro-ADM), which is in contrast to bio-ADM.[17] In this study, pro-ADM explained merely 30% of the variance in bio-ADM levels. Hierarchical clustering showed that pro-ADM did not cluster with bio-ADM or with congestion markers, yet with FGF23 and GDF15. This suggests that bio-ADM and pro-ADM could provide insight into different underlying pathophysiological processes.

Strengths and limitations

This is the first study to assess the value of bio-ADM in patients with new-onset and worsening HF. Strengths of this study are the number of patients enrolled in this cohort, the validation in a separate cohort, as well as the extensive (bio)marker data available. Limitations of this study are the retrospective, observational design, the assessment of bio-ADM at one time point, and the lack of information regarding decongestion or decongestive treatment. Also, the congestion score used in this study is a modified score from the previously published congestion scores due to differences in recording of congestion variables.[26,27] Furthermore, bio-ADM was particularly strongly associated with easier to assess variables of congestion, i.e. oedema and orthopnoea; therefore, more objective markers to assess congestion, such as ultrasound, invasive haemodynamic measurements, or plasma volume should be studied in subsequent studies. These were unfortunately not available in this dataset. However, at the moment there is no gold standard for the assessment of congestion in HF.[28] Based on this study, only associations are described and causality cannot be proven. Although bio-ADM was associated with clinical outcome in univariable models, there was no strong additive value on top of existing markers of clinical outcome.

Future perspectives

Future studies should assess the value of bio-ADM to guide (decongestive) treatment and might improve assessment of congestion in order to reliably establish euvolaemia. Also, therapies aimed at increasing ADM levels, for instance by administration of adrecizumab or neprilysin inhibitors, could be considered in order to improve outcomes.

Conclusions

Elevated plasma bio-ADM levels in patients with new-onset and worsening HF are associated with typical signs and symptoms of congestion and with an increased risk of mortality and HF hospitalization. Bio-ADM might therefore be useful to guide decongestive therapy or might become a target for therapy.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Included vs. excluded patients from the index cohort based on available bio-ADM measurements.

Table S2. Included vs. excluded patients from the index cohort based on available bio-ADM measurements.

Table S3. Biomarkers used in analyses involving the clinical congestion score.

Table S4. Multivariable model for log bio-ADM in the index cohort.

Table S5. Prognostic performance of bio-ADM in both cohorts.

Table S6. Baseline characteristics per tertiles of bio-ADM in the validation cohort.

Table S7. Multivariable model bio-ADM in the validation cohort.
Table S8. Cox regression analysis for bio-ADM and all-cause mortality, and the combined endpoint in the validation cohort.
Figure S1. Distribution of bio-ADM vs. NT-proBNP in the index and validation cohort.
Figure S2. Biomarker position of bio-ADM depicted in a correlation heatmap in the validation cohort.
Figure S3. Biomarker position of bio-ADM depicted in a dendrogram in the validation cohort.
Figure S4. Biomarker position of bio-ADM depicted in a correlation heatmap in the index cohort with the same variables as the validation cohort.
Figure S5. Biomarker position of bio-ADM depicted in a dendrogram in the index cohort with the same variable as the validation cohort.
Figure S6. Kaplan–Meier curve for all-cause mortality for quintiles of bio-ADM in the validation cohort.

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