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Inflammation Research

Adrenomedullin and endothelin-1 are related to inflammation in chronic heart failure

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Abstract. *Background:* Adrenomedullin (ADM) and endothelin-1 (ET-1) are novel promising peptide biomarkers in chronic heart failure (CHF). According to recent studies among their pleiotropic effect they play roles in the regulation of inflammation. The aim of the study was to measure the above mentioned two vasoactive peptides in parallel in a well characterized population of patients with CHF, and study their associations with inflammatory markers.
Materials and methods: A total of 186 patients (138 male, 48 female) with <45 % left ventricular ejection fraction (LV-EF), and without acute inflammatory disease, were enrolled. Plasma midregional-proADM (MR-proADM) and C-terminal-proET-1 (CT-proET-1) were determined by a novel sandwich immunoluminomertic assay.
Results: Increased MR-proADM and CT-proET-1 plasma levels were measured in patients with severe CHF (NYHA III-IV) as compared to the group of NYHA I-II ($p<0.0001$). MR-proADM and CT-proET-1 levels showed significant negative correlation with serum albumin and prealbumin levels ($p\leq0.0001$), while positive correlations were found with levels of CRP, TNF-alpha, soluble TNF receptors and IL-6 ($p\leq0.0001$). In multiple linear regression models after adjustments for several potential confounders (disease severity [LV-EF, NYHA classes, NT-proBNP], ion and water homeostasis [sodium and presence of peripheral oedema], renal function [serum creatine]) the relationship between ADM and albumin, CRP, soluble TNF receptors and between ET-1 and CRP, TNF receptors and IL-6 remained significant.
Conclusions: Vasoregulation and inflammation may be connected in heart failure patients independently of the disease severity. The observed link may contribute to the understanding of the complex pathomechanism in CHF.

Key words: Heart failure – Inflammation – Adrenomedullin – Endothelin-1

Introduction

Chronic heart failure (CHF) is recognized as a major and escalating public health problem in industrialized countries with ageing populations [1]. The understanding of CHF has developed from the model of mere pump failure to that of a multisystem disorder which affects not only the cardiovascular system but also the musculoskeletal, renal, neuroendocrine and immune systems. Several hypotheses have been suggested to describe the origin of immune activation in CHF. The myocardium itself can produce pro-inflammatory cytokines (for example tumor necrosis factor alpha) [2], and some evidences suggest that the production may be augmented by catecholamines [1]. Furthermore, translocation of bacterial endotoxins from the gut into the circulation has been described in patients with CHF, exerting strong pro-inflammatory stimuli [3–5]. Recent studies suggest that neurohormonal activation, such as of the renin-angiotensin-aldosteron system (RAAS), the adrenergic system and diminished cholinergic signaling could represent

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other mechanisms for immune activation and inflammation in CHF [6, 7].

Several biomarkers help clinical decision making on patients with heart failure, N-terminal brain natriuretic peptide (NT-proBNP) being the most widely used and investigated one. However, multimarker strategies are emerging to provide complementary and additional information for this challenge. Among the new peptide markers novel vascular regulatory factors are increasingly investigated allowed by innovative and highly reliable assay methods. Despite multiple recent studies focused on the biomarkers of CHF less is known about their biological correlates in the clinical setting.

Adrenomedullin (ADM) is a 52-amino-acid, multifunctional regulatory peptide that has homology with calcitonin gene-related peptide, and is produced and secreted by various types of cells [8]. Plasma ADM levels are increased in patients with various cardiovascular diseases, including hypertension, heart failure, acute myocardial infarction, peripheral arterial occlusive disease and renal failure [9]. ADM production can be stimulated by hypoxia, oxidative and shear stress, inflammatory cytokines and aldosteron [10]. The biological activity of adrenomedullin in the cardiovascular system is similar to that of brain natriuretic peptides (BNP), causing vasodilatation via production of nitric oxide, increasing cardiac output, and inducing diuresis and natriuresis [11]. *In vitro* and animal model studies provide some evidence that adrenomedullin has antihypertrophic, anti-apoptotic, antifibrotic, antioxidant effects and plays a role in modulation of angiogenesis and inflammation [11]. ADM is a clinically useful marker of prognosis after acute myocardial infarction [12] and has similar predictive properties compared with BNP determinations for one-year all-cause mortality in acute destabilized heart failure [13].

Endothelin-1 (ET-1) is a 21-amino-acid peptide, the most abundant member of a family of endothelins [14]. There is general agreement that ET-1 plays important physiological roles in the regulation of normal cardiovascular function, and excessive generation of ET isopeptides has been linked to major cardiovascular pathologies, including hypertension and heart failure [15]. ET-1, through its ET_A receptor, has vasoconstrictor, cell proliferative, angiogenetic, and remodeling activities but through ET_B receptor, vasodilative, antiproliferative effects dominate. Moreover, ET-1 may influence the cardiac inotropic state, may be arrhythmogenic and exacerbate myocardial failure, and stimulates the secretion of other neurohormones and potentates their effects [15]. There are accumulating data to support a role for ET-1 as a pro-inflammatory cytokine and fibrotic factor [16, 17]. Plasma ET-1 concentrations in patients with CHF correlate with both morbidity and mortality, prompting investigators to pursue the therapeutic potential of endothelin blockade [18]. Short term haemodynamic studies were promising, although according to recent clinical trials endothelin antagonists showed no significant effects in terms of mortality and symptoms in CHF patients [19, 20].

Since the potential interaction between vasoactive peptides and inflammatory mechanisms has never been investigated in clinical setting, we aimed to investigate whether ADM and ET-1 levels are related to inflammatory markers in CHF. Since both pathways (vasoregulation and inflammation) are known to be increasingly activated with the progression of CHF, we constructed adjusted multiple regression models to see whether the correlation between vasoregulation and inflammation is independent from disease severity.

Methods

Patient selection

The study was carried out in accordance with the Helsinki Declaration at the IIIrd Department of Internal Medicine, Semmelweis University, based on a study protocol approved by the highest Ethical Committee of Hungary. Patients with CHF were diagnosed according to the guideline of the European Society of Cardiology [21]: all of the enrolled patients had the symptoms of heart failure, typically breathlessness and fatigue; and as an objective evidence a left ventricular ejection fraction less than 45 %, determined by trans-thoracic echocardiography. Patients who provided written informed consent were consecutively included independently of the etiology of the disease, both from the out- or inpatient cardiology departments. A total of 195 patients (147 men, 48 women), all white Caucasians, were enrolled between February 2005, and April 2007, Nine patients with clinical signs of acute respiratory or urinary infections (as determined by a morning body temperature higher than 37.5 °C) were excluded. The full clinical record of the patients was registered at inclusion with the detailed physical status and results of routine laboratory tests.

Blood samples were taken after 6 hours of fasting between 8 and 10 AM by antecubital venipuncture into native, EDTA- or sodium citrate anticoagulated tubes. The samples were processed to obtain genomic DNA, and aliquots of serum and plasma, later stored at -70 °C until further analysis.

Determination of the laboratory parameters

Fragments of the ADM and ET-1 prohormones with the greatest stability – MR-proADM (midregional-proADM) and CT-proET-1 (C-terminal-proET-1) – were measured using sandwich immunoluminometric assays using 2 polyclonal antibodies to amino acids 45–92 of proADM and amino acids 168–212 of pre-proET-1 (BRAHMS AG, Hennigsdorf, Germany), as previously described [22, 23]. Levels of NT-proBNP (Biomedica ELISA kit (Cat. No. BI-20852)), serum TNF-alpha (R&D System high sensitivity ELISA kit (Cat. No. HSTA00C)), soluble TNF-receptor-I (sTNF-RI) (R&D Systems Human sTNF RI/TNFRSF1A Quantikine ELISA Kit (Cat. No. DRT100)), sTNF-RII (R&D Systems Human sTNF RII/TNFRSF1B Quantikine ELISA Kit (Cat. No. DRT200)) and serum IL-6 (R&D System high sensitivity ELISA kit (Cat. No. HS600B)) were measured according to the manufacturer's instructions. Standard laboratory parameters were measured by Roche Integra 800 (clinical chemistry, CRP), or by Cell-Dyn 3500 hematology analyzer (complete blood count).

Tab. 1. Baseline clinical and demographic characteristics of the patients, divided according to disease severity

	NYHA I-II (n=95)	NYHA III-IV (n=91)	p-value*
Age (years)	67.1 (58.1–77.2)	71.0 (63.4–77.9)	0.126
Sex (male/female) (%)	68 (71.6)/27 (28.2)	70 (76.9)/21 (23.1)	0.405
Peripheral oedema (%)	30 (31.6)	49(53.8)	**0.003**
Pulmonary congestion (%)	20 (21.1)	57 (62.6)	**<0.0001**
Heart rate (1/min)	72 (66–85)	80 (70–93)	**0.039**
Systolic blood pressure (mmHg)	122 (120–140)	120 (105–140)	**0.022**
Diastolic blood pressure (mmHg)	80 (70–80)	70 (62–80)	**<0.001**
LV EF (%)	38 (30–41)	30.5 (25–36)	**<0.0001**
BMI (kg/m^2)	26.8 (24.4–30.9)	26.1 (23.8–31.2)	0.388
Etiology IHD (%)	56 (58.9)	60 (66.7)	0.276
MR-proADM (nmol/l)	0.85 (0.62–1.08)	1.3 (0.9–1.8)	**<0.0001**
CT-proET-1 (pmol/l)	94.9 (69.1–122)	139 (91.1–188)	**<0.0001**
NT-proBNP (pmol/ml)	0.49 (0.28–1)	1.2 (0.48–2.22)	**<0.0001**
Haemoglobin (g/l)	145 (135–155)	137.5 (121–149)	**0.001**
Se. sodium (mmol/l)	141 (139–143)	139 (135–142)	**<0.001**
Se. creatinine (umol/l)	91 (75–108)	106 (85–157)	**<0.001**
Se. bilirubin (umol/l)	13.3 (8.9–18.6)	14.5 (9.5–22.0)	0.255
Albumin (g/l)	42 (40–45)	40 (36–43)	**<0.0001**
Total protein (g/l)	74 (69–78)	70 (64–74)	**<0.001**
CRP (mg/l)	4.4 (2.3–9.4)	8.0 (3.8–15.8)	**0.003**
TNF-alpha (pg/ml)	1.9 (1.2–3.2)	2.8 (1.9–4.3)	**0.001**
TNF-RI (ng/ml)	4.9 (3.5–7.0)	7.5 (4.1–11.2)	**<0.0001**
TNF-RII (ng/ml)	3.5 (2.8–4.6)	5.0 (3.3–6.4)	**<0.0001**
IL-6 (pg/ml) (n=158=83+75)	8.94 (4.71–14.20)	11.01 (6.97–17.04)	**0.021**

*Mann-Whitney U-test at continuous and Pearson's χ^2 test at categorical variables
NYHA – New York Heart Association, LV EF – left Ventricular Ejection Fraction, BMI – Body Mass Index, IHD – Ischemic Heart Disease, MR-proADM – Mid-regional pro-Adrenomedullin, CT-proET-1 – C-Terminal pro-Endothelin-1, NT-proBNP – N-terminal pro-Brain-Natriuretic-Peptide, TNF-RI – Tumor Necrosis Factor Receptor I, IL-6 – Interleukin-6,

Statistical analysis

As most of the variables were not normally distributed, data are presented in the text and in the tables as median (25^{th}-75^{th} percentile), or as number (percent). Non-parametric tests were used for group comparisons; continuous variables between two groups were compared with the Mann-Whitney U test, whereas categorical variables were compared with the Pearson's χ^2 test. Multiple logistic regression analysis was performed to estimate interrelationship between variables as categorical predictors and disease severity (NYHA I-II *versus* NYHA III-IV) as dependent variable. Spearman rank correlation coefficients were calculated for estimation of interrelations between MR-proADM, CT-proET-1 and NT-proBNP and other variables. Linear regression models on log-transformed vasoactive peptide levels and laboratory parameters were analysed. Statistical analyses were carried out using the software STATISTICA 7.0 (StatSoft Inc., Tulsa, OK, USA) and GraphPad Prism 4.03 (GraphPad Software, San Diego, CA, USA). Two-tailed p values were calculated and the significance level was put at a value of $p<0.05$.

Results

Patient characteristics

The baseline clinical and demographic features of the patients, categorized according the NYHA functional classes, are described in Table 1. Table 2. contains data about medications and comorbidities. One hundred and eighty-six patients were recruited, with male predominance (74 %) and the mean age was 68.5 ± 11.4 years. The median MR-proADM, CT-proET-1 and NT-proBNP were 0.984 nmol/l, 109 pmol/l and 0.722 pmol/ml, respectively. The participants belonging to NYHA functional classes III and IV had peripheral oedema, pulmonary congestion more frequently, and a significantly lower systolic and diastolic blood pressure, and a decreased left ventricular ejection fraction as compared to patients in NYHA I and II classes. Plasma MR-proADM, CT-proET-1 and serum NT-proBNP, creatinine and urea levels were significantly higher in the patients with more severe disease. Serum level of the negative acute phase

reactant albumin was decreased in NYHA III-IV patients, while other inflammatory markers like CRP, TNF-alpha, sTNF-RI and RII and IL-6 were significantly increased in the NYHA III-IV patient group compared to the patients with NYHA I-II (Table 1). The mean time interval between inclusion and acute coronary event, percutaneous transluminal coronary angioplasty (PTCA) and coronary artery bypass graft surgery (CABG) was 11.08 (SD 10.56), 2.1 (SD 2.88) and 6.22 (SD 5.21) years, respectively.

Relationship of high MR-proADM, CT-proET-1, NT-proBNP levels with advanced heart failure

MR-proADM, CT-proET-1 and NT-proBNP progressively increased through NYHA classes I to IV (data not shown). If vasoactive peptide levels were stratified according to tertiles, patients with MR-proADM, CT-proET-1 and NT-proBNP levels in the highest tertile (MR-proADM>1.28nmol/l, CT-proET>141pmol/l, NT-proBNP>1.25pmol/ml) had a 8.09 (95 % CI 3.59–18.20) , 5.71 (2.61–12.51) and 5.43 (2.50–11.80) fold risk, respectively, to have advanced heart failure (NYHA III-IV), when compared to patients belonging to the lowest tertile group (MR-proADM<0.84mol/l, CT-proET<88.8mol/l, NT-proBNP<0.45mol/ml). These univariate associations remained significant for each peptide even after adjustment for sex, age, presence of oedema, ischaemic etiology, BMI, serum sodium, serum creatinine and serum albumin levels (Figure 1). According to these adjusted models, patients in the middle tertile had also a significantly increased risk of having advanced HF if compared to the low tertile group (Figure 1.) for all of the peptides.

Biological associations of MR-proADM, CT-proET-1 and NT-proBNP in CHF

The Spearman rank correlations of MR-proADM, CT-proET-1, NT-proBNP and NYHA classes with laboratory and clinical parameters are shown in Table 3. Strong correlations were found between the vasoactive peptides ($p<0.0001$ for every pair) and they also strongly correlated with NYHA classes ($p<0.0001$). NT-proBNP, but not MR-proADM or CT-proET-1 correlated inversely with BMI ($p<0.0001$). Weak to moderate correlations between the peptides and age, peripheral and pulmonary oedema were also observed. The diameter of the vena cava inferior (IVC), measured by echocardiography, correlated strongly with the levels of vasoactive peptides, among them NT-proBNP showed the strongest association with left ventricular ejection fraction ($r=0.40$, $p<0.0001$). Albumin and prealbumin showed significant negative correlation with MR-proADM, CT-proET-1 and NT-proBNP level, total protein correlated significantly with MR-proADM and NT-proBNP. Levels of TNF-alpha, soluble TNF receptors and IL-6 showed significant correlations with vasoactive peptide concentrations, however, the relationships between NT-proBNP and CRP or TNF-alpha were less pronounced.

Tab. 2. Basic data of the study population regard to medication and comorbidities.

	(n=186)	
Medication		
Loop diuretics	138	(74.2 %)
ACE-I. ARB	129	(69.4 %)
Calcium antagonist	28	(15.1 %)
Digoxin	36	(19.4 %)
Amiodarone	32	(17.2 %)
β–blocker	121	(65 %)
Statin	72	(38.7 %)
Aspirin	73	(39.2 %)
Nitrate	61	(32.8 %)
Comorbidities		
Atrial fibrillation	89	(52.7 %)
Hypertension	122	(65.6 %)
Diabetes mellitus	63	(33.9 %)
AMI	77	(41.4 %)
CAGB	27	(14.5 %)
PTCA	26	(14.0 %)
GFR<60	76	(40.9 %)
Stroke	20	(19.7 %)

ACE-I angiotenzin converting enzyme inhibitor, ARB – angiotenzin receptor blocker, B-blocker – beta-adrenergic blocker, AMI – acute myocardial ischemia, PTCA – percutaneous transluminal coronary angioplastycoronary artery, CABG – bypass graft surgery, GFR – glomerular filtration rate (ml/min/1.73m^2)

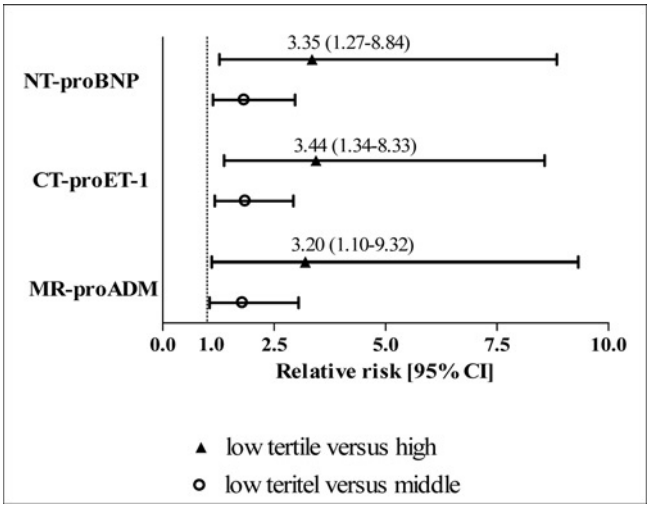

Fig. 1. Multivariable adjusted* relative risk (RR and 95 % CI) for advanced heart failure (NYHA III-IV) according to elevated vasoactive peptide levels.

* Adjusted for sex, age, presence of peripheral oedema, ischaemic etiology, BMI, serum sodium, serum creatinine and serum albumin levels.

MR-proADM – Mid-regional pro-Adrenomedullin, CT-proET-1 – C-Terminal pro-Endothelin-1, NT-proBNP – N-terminal pro-Brain-Natriuretic-Peptide

Tab. 3. Spearman rank correlation coefficients of disease severity, vasoactive peptide levels and clinical-, laboratory parameters. R and p-values are shown. Ns means not significant.

	NYHA		NT-proBNP		MR-proADM		CT-proET-1	
	r	p	r	p	r	p	r	p
age	0.14	ns	0.17	0.020	0.32	<0.0001	0.23	0.001
sex	0.03	ns	-0.16	0.027	-0.09	ns	-0.06	ns
BMI	-0.05	ns	-0.37	<0.0001	-0.07	ns	-0.08	ns
Peripherial oedema	0.29	<0.0001	0.24	0.001	0.44	<0.0001	0.41	<0.0001
NT-proBNP	0.44	<0.0001			0.58	<0.0001	0.43	<0.0001
MR-proADM	0.55	<0.0001	0.58	<0.0001			0.77	<0.0001
CT-proET-1	0.41	<0.0001	0.43	<0.0001	0.77	<0.0001		
EF	-0.31	<0.0001	-0.28	0.0002	-0.19	0.013	-0.11	ns
Albumin	-0.31	<0.0001	-0.47	<0.0001	-0.43	<0.0001	-0.31	<0.0001
CRP	0.28	0.0001	0.17	0.023	0.33	<0.0001	0.29	<0.0001
TNF-alpha	0.25	<0.001	0.16	0.032	0.30	<0.0001	0.21	0.004
TNF-RI	0.35	<0.0001	0.36	<0.0001	0.58	<0.0001	0.52	<0.0001
TNF-RII	0.37	<0.0001	0.28	0.0001	0.53	<0.0001	0.32	<0.0001
IL-6	0.27	0.001	0.32	<0.0001	0.50	<0.0001	0.42	<0.0001

MR-proADM – Mid-regional pro-Adrenomedullin, CT-proET-1 – C-Terminal pro-Endothelin-1, NT-proBNP – N-terminal pro-Brain-Natriuretic-Peptide, CRP – C-reactive Protein, TNF-RI – Tumor Necrosis Factor Receptor I, IL-6 – Interleukin-6

Relationship of MR-proADM and CT-proET-1 with inflammatory markers in heart failure

As shown in Table 3 multiple correlations between vasoactive peptide levels and inflammatory markers were observed in HF patients. To rule out the possible influence of the univariate correlations by disease severity and clinical parameters, adjusted linear regression models were built. Several potential confounders were considered to incorporate into these multiple linear regression models. Characteristic variables reflecting disease severity (LV-EF, NT-proBNP), ion- and water hoemeostasis (sodium and presence of peripheral oedema), renal function (creatinine), were included, besides basic clinical parameters (age, sex, BMI and diastolic blood pressure). As shown in Table 4, the relationships between ADM and albumin, CRP, sTNF-RI and –RII, and IL-6 remained highly significant in these adjusted models. Similar adjusted, significant relationships were found between CT-proET-1 and CRP, sTNF-RI, and IL-6. Nearly the same results were obtained when regarding disease severity the adjustment was preformed for NYHA functional classes and NT-proBNP (data not shown).

Discussion

The major novel finding of our study is the presentation of strong correlation between CT-proET-1 and MR-proADM levels with inflammation in CHF. Furthermore, we confirmed the observation that vasoactive peptide levels are increased and inflammatory mechanisms activated in advanced heart failure patients. Inflammatory mechanisms were assessed in a complex manner by measuring levels of positive and negative acute-phase reactants, cytokines and soluble cytokine receptors; among vasoregulatory peptides ADM, ET-1 and the 'gold-standard' NT-proBNP were measured. According to our knowledge this is the first study showing direct, multiple associations in heart failure patients between key vasoregulatory peptides and inflammatory molecules in the literature. Recognizing the requirement of multimarker strategies in clinical decision making for heart failure patients, our study may provide important observational information about the complex relationships and interactions between inflammatory and vasoregulatory mechanisms.

ADM and ET-1 are potent vasoactive peptides, and new methodological developments provide reliable measurement of their plasma levels [22, 23]. Patients with CHF have been reported to show elevated ADM and ET-1 levels positively correlated with disease severity, suggesting that they can be new, promising biomarkers [24, 25]. In complete agreement with the previous results, our present data also show significant correlation not only between plasma MR-proADM and CT-proET-1 levels and NYHA classes, but also with NT-proBNP, the gold standard of CHF biomarkers. The association of higher ADM, ET-1 and BNP concentrations with advanced HF (NYHA classes III and IV) was shown even after an adjustment for sex, age, BMI and markers of ion homeostasis, kidney and liver function (Figure 1).

ADM has pleitropic effects extending to important roles in the immune system. Complement factor H is an ADM-binding protein. ADM and its prohormones have antimicrobial effects, and it exerts a regulatory effect on

Tab. 4. Adjusted* associations [beta and (p) values] of inflammatory markers with vasoactive peptide levels obtained in linear regression models

	Log MR-proADM	Log CT-proET-1
Log albumin	**-0.22 (<0.0001)**	-0.05 (0.451)
Log CRP	**0.20 (<0.0001)**	**0.18 (0.004)**
Log TNF-alpha	0.07 (0.078)	0.06 (0.353)
Log TNF-RI	**0.21 (<0.0001)**	**0.24 (0.0007)**
Log TNF-RII	**0.13 (0.008)**	-0.01 (0.901)
Log IL-6	**0.19 (<0.0001)**	**0.14 (0.047)**

*All models are adjusted for age, sex, left ventricular ejection fraction, NT-proBNP, BMI, presence of peripheral oedema, diastolic blood pressure, serum sodium and creatinine.
MR-proADM – Mid-regional pro-Adrenomedullin, CT-proET-1 – C-Terminal pro-Endothelin-1, NT-proBNP – N-terminal pro-Brain-Natriuretic-Peptide, CRP – C-reactive Protein, TNF-RI – Tumor Necrosis Factor Receptor I, IL-6 – Interleukin-6

the production of inflammatory cytokines [26]. According to our results, ADM showed strong positive correlation with the pro-inflammatory cytokine IL-6 in univariate and adjusted regression models (Tables 3 and 4). Similar association was observed by others in patients with peripheral arterial occlusive disease [27], in patients with atherosclerotic risks [28], liver cirrhosis [29] and after major surgery [30]. The positive correlation between TNF-alpha and ADM was no longer present after adjustment for serum creatinine, indicating indirect relationship between the two variables. To the best of our knowledge the significant negative association with albumin and the positive correlation with CRP levels, and the strong association of ADM with soluble TNF-receptor concentrations are new observations in cardiovascular diseases, and they may suggest that ADM is directly connected to the systematic acute-phase reaction of the body. Several *in vitro* and animal model experiments support the observed clinical relationship between ADM and inflammatory molecules, recently reviewed by Zudaire at al. [26]. ADM induced IL-6 production in a mouse fibroblast cell line, and up-regulated the LPS-induced TNF-alpha and IL-6 production in macrophages, but reduced TNF-alpha secretion in Swiss 3T3 cells. It appears that ADM can both promote and inhibit inflammation, depending on the cell type and disease of interest [11]. Inflammatory cytokines may also have effects on ADM expression. TNF-alpha seems to induce ADM production in rat vascular smooth muscle cells or nonmyocyte cardiac cells, while IL-6 increased ADM expression of gastric epithelial cells[10]. Less is known about the regulation of soluble TNF-Rs, and about their *in vivo* functions and their relationship to vasoregulatory mechanisms.

There are accumulating data to support the role for ET-1 as a pro-inflammatory molecule and ET-1 has been implicated in different disease states accompanied by inflammation, such as atherosclerosis, inflammatory airway diseases, ischemia-reperfusion caused cystitis and viral myocarditis [16]. Our present results support the hypothesis on the direct relationship between ET-1 and inflammation. In the adjusted model (Table 4), the ET-1 level was associated with the serum concentration of IL-6, TNF-Rs and CRP independently from disease severity (NYHA classes, BNP) and clinical parameters. Similar observations on the correlation between ET-1 and CRP were made in septic patients [31], and modest correlation was found between TNF-alpha and bigET-1 in CHF [32]. In the heart, ET-1 may induce myocardial inflammation by recruiting leukocytes, stimulating production of adhesion molecules, and inducing cytokine expression, like IL-6, TNF-alpha, IL-1. It may also induce cardiac fibrosis by activating fibrotic factor TGF-β, increasing collagen synthesis, and stimulating fibroblast proliferation. Moreover, ET-1 exerts trophic effects in cardiomyocytes and leads to left ventricular remodelling [16]. According to a study on ET-1 producing transgenic mice, robust cardiac-specific expression of human ET-1 was sufficient to induce cardiac inflammation, hypertrophy, dilatation, dysfunction, CHF and death [33]. The explanation of the particularly strong association with soluble TNF-RI is unknown; the interaction of ET-1 and shedding of TNF receptors has not been studied. Appraising our results, it should be considered that ET-1 may have local autocrine and paracrine actions within the heart, though an increase in myocardial ET-1 expression can exist without an increase in circulating levels of ET-1 [33].

In the present cross-sectional study correlations were found between the levels of NT-proBNP and soluble TNF-Rs, IL-6 and albumin concentration. Our observations were in agreement with the results of Nozaki et al. [34] and Niethammer et al. [35] with respect to the positive correlation of TNF-Rs with BNP levels in CHF, and with results of Emdin at al. [36], on the positive correlation of BNP with IL-6 and TNF-alpha.

Several mutually non-exclusive mechanisms might explain the connection between inflammatory markers and vasoactive peptides. An indirect parallel activation of the two pathways, driven by common factors such as diseases severity, is supported by earlier observations [3, 7, 24, 25]. Importantly, since the correlation between ADM /ET-1 and inflammatory markers was independent from disease severity-related clinical parameters (Table 4), a direct molecular interaction-based relationship between the two systems seems also to be possible. Besides the clinical observations of the present study, this hypothesis is supported by earlier studies showing that cytokines might induce the expression and secretion of these peptides [16, 26], and high levels of the vasoactive peptides may also regulate cytokine production [26, 33]. Since a mutual regulation of inflammatory cytokine production and ADM or ET-1 expression may exist, the direct association between the inflammatory and vasoregulatory pathways is a potentially important arm in the pathophysiology of CHF.

A potential limitation of our study is its cross-sectional design not allowing to conclude for causality. Rather, we aimed to describe detailed clinical correlates for these novel peptide biomarkers and this is why NYHA functional class was chosen as clinical surrogate parameter. The clinical follow-up of this population is in progress and subsequent research will determine whether these peptide regulators are related to clinical events in inflammation dependent- or independent manner.

The clinical importance of inflammatory mechanisms in HF is increasingly recognized [6], although less is known about the mechanisms regulating these processes. Our observational clinical data provide a possible link between vasoregulation and inflammation in HF patients and may therefore contribute to the understanding of the complex pathomechanism in this disease. If follow-up *in vitro* or animal model research could convincingly support our hypothesis on the direct relationship between vasoregulation and inflammation new intervention points could be defined as therapeutic agents in CHF. Furthermore, these observations will possibly help to interpret the results of large prospective clinical studies on new biomarkers, especially in the emerging multimarker risk-stratification field.

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Conflict of interest. Dr Morgenthaler, Dr Kunde and Dr Papassotiriou are employees of BRAHMS AG, Hennigsdorf, Germany that commercializes immunoassays and has developed the MRproADM and CT-proET-1 assays, for which it owns patent rights. The present study was not financed by BRAHMS AG. The remaining authors report no conflicts.

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