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**Risk and Adverse Factors in Heart Failure – from Immune and
Neurohormonal Activation to Medical Therapy**

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Abbreviations

ADHF	Acute decompensated heart failure
AHF	Acute heart failure
ANOVA	Analysis of variance
ANP	Atrial natriuretic peptide
BMI	Body mass index
BNP	Brain natriuretic peptide
CHF	Chronic heart failure
CI	Confidence interval
CrCl	Creatinine clearance
CRP	C-reactive protein
DIG	Digitalis Investigation Group
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
HDL	High-density lipoprotein
IL-6	Interleukin-6
LPS	Bacterial lipopolysaccharide, endotoxin
LVEDD	Left ventricular end-diastolic diameter
LVEF	Left ventricular ejection fraction
NYHA class	New York Heart Association class
PVO ₂	Peak oxygen consumption
sTNF-R1	Soluble tumour necrosis factor receptor 1
sTNF-R2	Soluble tumour necrosis factor receptor 2
TNF	Tumour necrosis factor- α
VE/VCO ₂ -slope	Minute ventilation / carbon dioxide production
WRF	Worsening renal function

Abstract

Chronic heart failure (CHF) is characterized by impaired cardiac pump function. However, it has also been recognised that in addition to the haemodynamic changes, the activation of neurohormonal, immune, and metabolic systems is associated with symptomatic severity and progression of this disease. During the studies performed on the one hand, immune and neurohormonal activation in CHF patients was studied and, on the other hand, predictors of outcome after hospitalization for acute decompensated heart failure (ADHF) were characterized.

We first investigated the relationship between tumour necrosis factor- α (TNF) and atrial and brain natriuretic peptides (ANP and BNP) in 25 CHF (7 cachectic) and 8 control patients. We found CHF, and in particular cachectic patients to present higher levels of BNP, ANP, epinephrine, and norepinephrine compared to controls. After adjustment for New York Heart Association (NYHA) class, creatinine clearance (CrCl), and age, TNF correlated with BNP and ANP.

With the aim to further investigate immune activation in CHF, we examined the relationship between bacterial lipopolysaccharide (LPS), lipoproteins, and cytokines. We analysed 25 CHF patients (10 with ADHF) and 10 healthy controls and found ADHF patients to have the highest LPS and cytokine levels and lowest high-density lipoprotein (HDL). A correlation between HDL with TNF and LPS was observed for all patients. The LPS/HDL ratio, indicating biologically active LPS, was highest in oedematous patients and related to TNF concentration, independently of NYHA class, CrCl, hepatic function, and age.

In order to identify predictors of mortality after admission for ADHF, we retrospectively analysed 128 patients. Creatinine, NYHA class, and left ventricular ejection fraction emerged as independent predictive factors of mortality after one year. After five years, admission creatinine and NYHA class independently predicted all-cause mortality. Digoxin and diuretics use was related to poor outcome.

We therefore conclude that, firstly, strong neurohormonal activation is observed in CHF and is stronger in cachectic subjects. Moreover, ANP and BNP strongly correlate with TNF, which could point to a causal relationship between neurohormonal and immune activation in CHF that might contribute to disease progression and cachexia. Secondly, an augmented cytokine level is positively related to LPS and inversely to HDL with increased disease severity, suggesting a beneficial effect of lipoproteins. Lastly, serum creatinine has been identified as an inexpensive and convenient marker of worse outcome in ADHF and moreover, a deleterious effect of the use of digoxin and diuretics is observed in these patients.

Abstract in German

Die chronische Herzinsuffizienz (CHI) ist durch Einschränkungen der Herzleistung definiert. Es setzt sich jedoch zunehmend die Erkenntnis durch, dass der Schweregrad der Symptome und die Progression der Erkrankung neben der Hämodynamik maßgeblich durch eine Aktivierung des Immunsystems, der Neurohormone und des Stoffwechsels beeinflusst werden. Ziel dieser Studie war erstens, den Stellenwert der immunologischen und neurohormonellen Aktivierung bei Patienten mit CHI herauszustellen. Zweitens sollte untersucht werden, welche Faktoren sich auf die Mortalität nach Hospitalisierung aufgrund einer akuten Dekompensation der Herzinsuffizienz (ADHI) auswirken.

Zu diesem Zweck wurde an 25 Patienten mit CHI (7 davon kachektisch) und 8 gesunden Kontrollen die Beziehung zwischen Tumornekrosefaktor- α (TNF) und den natriuretischen Peptiden ANP (atriales natriuretisches Peptid) und BNP (brain natriuretisches Peptid) untersucht. Alle Patienten, jedoch insbesondere die kachektischen, wiesen höhere Werte für BNP, ANP, Adrenalin und Noradrenalin im Vergleich zur Kontrollgruppe auf. Nach Adjustierung für New York Heart Association (NYHA)-Klasse, Kreatinin-Clearance (CrCl) und Alter korrelierte TNF mit BNP sowie mit ANP. Weiterhin wurde die Beziehung zwischen bakteriellem Lipopolysaccharid (LPS), Lipoproteinen und Zytokinen dargestellt. Es wurden 25 Patienten (10 mit ADHI) und 10 Gesunde analysiert. Patienten mit ADHI hatten die höchsten LPS- und Zytokinewerte sowie die niedrigsten Werte für High-Density Lipoprotein (HDL). Eine Korrelation zwischen HDL mit TNF und LPS wurde bei allen Patienten beobachtet. Das Verhältnis von LPS zu HDL, welches das biologisch aktive LPS anzeigt, war bei den Patienten mit ADHI am höchsten und stand in Zusammenhang mit der TNF-Konzentration, unabhängig von der NYHA-Klasse, CrCl, Leberfunktion und dem Alter.

Um Prädiktoren für die Mortalität nach einem Krankenhausaufenthalt aufgrund einer ADHI zu identifizieren, wurden 128 Patienten retrospektiv analysiert. Kreatinin, die NYHA-Klasse und die linksventrikuläre Ejektionsfraktion waren unabhängige prädiktive Faktoren der Mortalität nach einem Jahr. Nach fünf Jahren waren Kreatinin und die NYHA-Klasse unabhängige Prädiktoren der Mortalität. Zudem bestand eine positive Korrelation zwischen Mortalität und der Nutzung von Digitalispräparaten und Diuretika.

Aus den oben aufgeführten Untersuchungen lässt sich bei CHI-Patienten eine gewisse neurohormonelle Aktivierung konstatieren. Diese war intensiver bei den kachektischen Patienten. Ferner wurde eine positive Korrelation von ANP und BNP mit TNF festgestellt, was für einen Kausalzusammenhang zwischen neurohormoneller und immunologischer Aktivierung bei CHI spricht. Der chronische Krankheitsverlauf und die Ausprägung einer Kachexie werden bei diesen Patienten möglicherweise beeinflusst. Außerdem korrelierten

Zytokine direkt mit LPS und umgekehrt proportional mit einem erhöhten HDL. Dieser Zusammenhang deutet auf die protektive Wirkung der Lipoproteine hin, wie mit der „Endotoxin-Lipoprotein-Hypothese“ postuliert. Darüber hinaus wurde das Aufnahme-
kreatinin als preiswerter, überall verfügbarer und einfacher Prognosemarker bei ADHI beschrieben. Zusätzlich konnte ein weiterer Beweis dafür erbracht werden, dass die gemeinsame Einnahme von Digitalispräparaten und Diuretika bei diesen Patienten negative Auswirkungen haben kann.

Summary

1 Introduction

Chronic Heart Failure (CHF) is a complex syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the heart to support a physiological circulation. Patients with CHF are characterized by typical signs and symptoms of CHF and there is objective evidence of structural or functional abnormality of the heart at rest.¹ It is estimated that there are 15 million people in Europe with CHF, representing a prevalence of 2-3% of the population that increases to between 10 to 20% in persons over 70 years of age.¹ Furthermore, acute heart failure (AHF), defined as a rapid onset or change in signs and symptoms of CHF requiring urgent treatment, is the leading cause for hospitalization of people over 65 years.^{1;2} Among these patients, nearly 40% die within one year after first hospitalization and about 50% are dead or re-admitted to hospital within one year.³ It has been recognized that CHF is characterized not only by structural and haemodynamic changes, but also by activation of a heterogeneous group of maladaptive mechanisms such as neurohormonal, immune, and metabolic systems.⁴⁻⁸

Neurohormonal activation as reflected by augmented atrial and brain natriuretic peptides (ANP and BNP) increases with higher functional class and predicts poor outcome.^{5;9;10} An immune response evidenced by elevated tumour necrosis- α (TNF) levels has been observed in these patients^{11;12} but an association between ANP and BNP with TNF in patients with advanced disease and cachexia has not been analysed yet. One of the hypotheses explaining increased immune activation observed in symptomatic CHF patients presumes the entrance of bacterial lipopolysaccharide (endotoxins, LPS) into the bloodstream triggering systemic inflammation.^{13;14} Moreover, elevated concentrations of LPS and cytokines have been found in CHF patients during acute oedematous exacerbation.¹⁵ Besides, it is known that lipoproteins can bind LPS thereby diminishing its bioactivity.¹⁶ Additionally, diverse factors have been identified to predict worse outcome after hospitalization for AHF in the short and medium term.¹⁷⁻²¹ However, little is known about factors predicting long-term mortality in these patients.

Thus, the main objectives of this work were as follows:

1. To examine the relationship between ANP and BNP with TNF particularly in cachectic CHF patients.
2. To investigate the association between endotoxin, immune activation, and lipoprotein levels, especially in acute decompensated heart failure (ADHF) patients.
3. To determine whether impaired renal function as well as other admission factors predict long-term mortality after hospitalization due to ADHF.

2 Material and Methods

2.1 Study Population

The patients were recruited as follows:

- In the neurohormonal and immune activation group, 25 CHF patients and 8 healthy volunteers were evaluated. In 7 patients from the CHF group, cardiac cachexia defined as documented, non-intentional, and non-oedematous weight loss of >6% of their pre-morbid body weight in at least six months²² was diagnosed.
- For the endotoxaemia subgroup, 25 consecutive patients with CHF and 10 healthy controls were recruited. Of the CHF patients, 10 patients were admitted with ADHF with peripheral oedema but without clinical evidence of central congestion and the other 15 were clinically stable.
- For the analysis of predictors of mortality in ADHF, 128 patients admitted to the hospital due to worsening of CHF were evaluated.

All patients had a history of CHF of at least six months with symptomatic exercise intolerance, cardiomegaly, and objective evidence of left ventricular dysfunction. The patients had no clinical signs of acute infection or other chronic inflammatory conditions, rheumatoid arthritis, or cancer and all patients were receiving standard medical therapy in variable combinations. The study protocols were approved by the local Ethics Committees and all participants gave written informed consent.

2.2 Laboratory Measurements

Laboratory measurements were performed using routine hospital analysis. Renal function was calculated by using the Cockcroft-Gault formula for creatinine clearance (CrCl).²³

For endotoxin measurement, venous blood was drawn into endotoxin-free tubes (Endo Tube ET®, Chromogenix AB, Mölndal, Sweden) and stored at -80°C until analysis. Endotoxin concentrations were measured using a commercially available test kit (Limulus Amebocyte Lysate QCL-1000 test kit, BioWhittaker, Inc., Walkersville, USA). In healthy subjects the normal level of LPS in this assay is <0.50 EU/ml.

Systemic concentrations of TNF were determined by ELISA (Quantikine® HS human TNF, sensitivity 0.18 pg/ml, R&D Systems, Minneapolis, MN, USA) from EDTA plasma samples. Soluble tumour necrosis factor receptors 1 (sTNF-R1, sensitivity 25 pg/ml) and 2 (sTNF-R2, sensitivity 2 pg/ml) were measured by ELISA (R&D Systems, Minneapolis, MN, USA).²⁴

For the quantitative determination of plasma ANP and BNP levels, blood samples were collected into EDTA-Na plastic tubes at 1.5 mg/mL and aprotinin at 500 KIU/mL. A solid-

phase “sandwich” immunoradiometric assay with two monoclonal antibodies (Shionoria ANP/BNP, Shionogi & Co, LTD, Osaka, Japan) was used. The detection limit for ANP and BNP was 2.5 and 2.0 pg/mL, respectively.

Epinephrine and norepinephrine were measured with high-performance liquid chromatography (sensitivity 0.1 ng/mL for both).⁴

2.3 Determination of Left Ventricular Function

Left ventricular ejection fraction (LVEF) was assessed by coronary catheterization, or otherwise determined by echocardiography or radionuclide ventriculography. The left ventricular end-diastolic diameter (LVEDD) was estimated by echocardiography.

2.4 Exercise Testing

All patients in the neurohormonal and immune activation group underwent maximal cardiopulmonary exercise testing (modified Bruce protocol, Amis 2000, Odense, Denmark) as described before.²⁵

2.5 Follow-Up

In the group of patients hospitalized with ADHF, one- and five-year follow-ups were carried out via telephone interviews with the patient or his or her local physician. Seven patients were lost to five-year follow-up and for 20 patients the exact date of death could not be determined. These patients were censored at the date of last available information.

2.6 Statistical Analysis

ANOVA with Fisher’s post hoc test and Student’s t-test were used to compare quantitative group results. The relationship between variables was analysed by simple and multivariate regression analysis. Cox proportional hazards analyses and χ^2 -test were used as appropriate. A probability value of $P < 0.05$ was considered statistically significant. Normal distribution was tested by Kolmogorov-Smirnov test. Due to skewed distribution, ANP, BNP, C-reactive protein (CRP), and creatinine levels were log-transformed before analysis. Hazard ratios with 95% confidence interval (CI) and probability values by the likelihood ratio test were used. Kaplan-Meier survival analyses and log-rank tests were used to assess differences in survival. Data were analysed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) and StatView 4.5 (Abacus Concepts Inc., Berkeley, CA, USA).

3 Results

3.1 Relationship Between Natriuretic Peptides and Immune Activation

We analysed 25 CHF patients, 7 of whom with diagnosed cachexia as defined in the methods section, and compared them with 8 healthy control subjects. The main objective of this analysis was to find out whether there is an association between cardiac functional and structural damage reflected by high levels of natriuretic peptides and immune activation with increased TNF.²⁶

CHF patients' characteristics did not differ significantly from those of the control group in age, body mass index (BMI), and CRP. In contrast to this, CHF patients had significantly lower peak oxygen consumption (PVO_2 , $P=0.003$), sodium ($P=0.026$), potassium ($P=0.001$), and higher LVEDD ($P<0.03$), and serum creatinine ($P=0.04$) compared with healthy controls.

Furthermore, in CHF patients, BNP ($P=0.013$), ANP ($P=0.02$), and norepinephrine ($P=0.006$) levels were significantly higher than in the control group. In contrast, we found no significant differences between the two groups regarding TNF and epinephrine. We found cachectic CHF patients to have overall higher levels of these variables compared with controls, although TNF did not reach statistical significance. Moreover, when comparing with non-cachectic subjects, cachectic ones had the highest levels of ANP ($P=0.2$) and BNP ($P=0.02$). Post hoc analysis revealed differences between all groups regarding BNP ($P=0.02$) and epinephrine ($P=0.01$) levels.

We found strong positive correlations of TNF with ANP ($r=0.60$, $P=0.0016$) and BNP ($r=0.64$, $P=0.0006$), also independently of LVEDD. Even after adjustment for age, New York Heart Association (NYHA) class and CrCl, TNF was independently related to ANP and BNP. Furthermore, ANP and BNP also correlated with NYHA class ($P=0.0009$ and $P<0.0001$), PVO_2 ($P=0.001$ and $P=0.0002$), LVEED ($P=0.0006$ and $P=0.0025$), and LVEF ($P=0.0004$ and $P<0.0001$). Uric acid, epinephrine, and norepinephrine correlated also with increased natriuretic peptides.

3.2 Association Between High-Density Lipoprotein, Endotoxin, and Cytokines

In order to validate the hypothesis relating lipoproteins with endotoxin activity and immune activation, we analysed these parameters in 25 CHF patients and compared them to 10 healthy controls.²⁷ The CHF patients were divided into those presenting ADHF with peripheral oedema ($N=10$) and clinically stable CHF patients ($N=15$).

We found ADHF patients to have the highest levels of endotoxin ($P=0.016$ and $P=0.013$), TNF ($P<0.001$ and $P<0.001$), sTNF-R1 ($P<0.0001$ and $P<0.0001$), and sTNF-R2 ($P<0.001$

and $P<0.0001$), and lowest high-density lipoprotein (HDL) levels ($P=0.08$ and $P=0.017$) compared to non-oedematous patients and healthy controls, respectively.

For all patients, HDL was inversely related to endotoxin ($r=-0.31$, $P=0.08$) and TNF ($r=-0.53$, $P=0.001$). Other parameters related to TNF concentrations were age ($r=0.35$, $P=0.04$), NYHA class ($r=0.50$, $P=0.02$), and CrCl ($r=-0.43$, $P=0.07$). In CHF patients there was a relationship of HDL levels with endotoxin ($r=-0.50$, $P=0.01$) and TNF ($r=-0.60$, $P=0.0016$), but these were not observed in the control group.

We found the endotoxin/HDL ratio, indicating biologically active unbound endotoxin, to be significantly increased in CHF patients compared to controls ($P=0.03$), and particularly in ADHF patients compared with non-oedematous patients ($P=0.006$) and healthy controls ($P=0.003$). Furthermore, TNF concentrations were strongly related to the endotoxin/HDL ratio ($r=0.87$, $P<0.0001$) and NYHA class ($r=0.50$, $P=0.002$) in the entire group. This correlation between TNF and endotoxin/HDL ratio was stronger when analysing only the CHF group ($r=0.88$, $P<0.0001$) and especially when considering ADHF patients alone ($r=0.91$, $P<0.001$). The endotoxin/HDL ratio significantly predicted TNF concentration ($P=0.04$) in multivariate analysis, independently of NYHA class ($P=0.45$) and CrCl ($P=0.13$).

3.3 Predictors of Mortality in Acute Decompensated Heart Failure

For the analysis of predictors of medium- and long-term mortality in ADHF, a total of 128 consecutive patients admitted to the hospital due to worsening of CHF were studied.²⁸

The mean NYHA functional class was 2.6 ± 0.7 and patients were distributed as follows: NYHA class II (N=65), III (N=49), and IV (N=14) and 24% were women. Of 128 patients, 70 (55%) died after 31 to 3123 days (median 1474 days). Cumulative mortality rate for all patients was 10% (13 deaths) at 6 months, 20% (25 deaths) at 12 months, 31% (38 deaths) at 24 months, and 39% (50 deaths) at 60 months.

Patients dying within five years were more likely to be older (66 ± 11 vs. 60 ± 12 , $P=0.02$) and had significantly lower BMI than survivors (26 ± 4 vs. 28 ± 5 , $P=0.02$). Non-survivors also presented higher levels of urea (11 ± 7 vs. 8 ± 5 , $P=0.02$) and creatinine (124 ± 63 vs. 97 ± 29 , $P=0.005$), and lower CrCl (64 ± 32 vs. 91 ± 43 , $P=0.001$) values than survivors. The two groups also differed in NYHA class distribution at baseline.

Creatinine at admission best correlated with urea ($r=0.63$, $P<0.001$) followed by age ($r=0.31$, $P<0.001$), sodium ($r=-0.30$, $P=0.001$), CRP ($r=0.27$, $P=0.002$), BMI ($r=-0.23$, $P=0.008$), potassium ($r=-0.20$, $P=0.02$), and NYHA class ($r=0.20$, $P=0.02$).

In univariate analysis we found high admission serum creatinine to best predict mortality after one ($P<0.001$) and five years ($P=0.001$). Other variables predicting medium- and long-

term mortality were lower CrCl ($P=0.002$ and $P=0.001$), higher NYHA class ($P=0.007$ and $P=0.004$), lower BMI ($P=0.017$ and $P=0.01$), increased urea ($P=0.006$ and $P=0.018$), as well as the use of loop diuretics ($P=0.037$ and $P=0.001$) and digoxin ($P=0.001$ and $P=0.002$). The intake of aspirin was associated with better prognosis both after one ($P=0.005$) and five years ($P=0.001$). While NYHA class only predicted overall mortality after one year ($P=0.02$), age did so only after five years ($P=0.017$).

In multivariate analysis after one year, creatinine ($P=0.004$) and LVEF ($P=0.019$) independently predicted mortality whereas NYHA class ($P=0.05$) and BMI ($P=0.138$) did not reach significance. When adjusted for the use of diuretics, NYHA class gained significance ($P=0.04$), creatinine and LVEF remained significant ($P=0.032$ and $P=0.04$, respectively), and BMI continued to be non-significant ($P=0.085$).

Creatinine ($P=0.03$) and NYHA class ($P=0.035$) independently predicted mortality in the five-year follow-up analysis but BMI and age did not achieve significance. However, following adjustment for the intake of diuretics, creatinine lost its significance ($P=0.308$), age remained non-significant ($P=0.528$), NYHA class continued to show statistical significance ($P=0.011$) and BMI and the use of diuretics were independently associated with poor prognosis ($P=0.006$ and $P<0.001$, respectively).

After stratification by sex, creatinine ($P=0.049$), the use of diuretics ($P=0.007$), and digoxin ($P=0.037$) independently predicted mortality after five years.

Following Kaplan-Meier analysis and after stratifying patients according to local laboratory normal creatinine values (male ≤ 102 $\mu\text{mol/L}$ and female ≤ 88 $\mu\text{mol/L}$), patients with high creatinine were found to have worse prognosis after five years ($P=0.045$). Similarly, CrCl below 60 mL/min/1.73m^2 was associated with impaired survival ($P=0.006$). According to the medication at admission, the use of diuretics and digoxin was related to long-term mortality by Kaplan Meier analyses ($P<0.001$ and $P=0.002$, respectively).

4 Discussion

4.1 Neurohormonal and Immune Activation in Chronic Heart Failure

We have found a strong correlation of ANP and BNP with TNF for all CHF patients, independently of LVEDD, age, NYHA class, and CrCl. Neurohormonal and immune derangements are known to be activated in CHF.^{10;29} On the one hand, natriuretic peptides are important biomarkers for the diagnosis of CHF^{9;30} and have been further related to CHF severity and poor outcome.^{5;31} On the other hand, immune activation reflected by increased levels of TNF, interleukin-6 (IL-6), sTNF-R1, and sTNF-R2 predicts increased mortality in

patients with advanced CHF.^{32;33} Torre-Amione et al.²⁹ compared proinflammatory cytokine levels with functional class and neurohormonal activation in patients with symptomatic and asymptomatic left ventricular dysfunction (NYHA I to III) and found a relationship between plasma TNF levels and increasing NYHA class. However, they found a weak correlation between TNF and ANP ($r=0.35$, $P=0.005$) and concluded that neurohormonal activation is unlikely to explain immune activation observed in CHF. Nonetheless, patients in functional class IV were not evaluated and thus, considering the association between increased levels of ANP with worsening CHF and survival, the authors hypothesized that TNF, IL-6, and ANP could serve as biomarkers for the development of symptomatic CHF. In our analysis²⁶ the correlation between ANP and BNP with TNF was strong and highly significant so that we have demonstrated for the first time a relationship between neurohormonal and immune activation in CHF. However, the mechanisms involved in this association remain unclear.

4.2 Cachexia and Body Mass Index in Heart Failure

Cachexia is a heterogeneous syndrome associated with underlying illness and characterized by loss of muscle with or without loss of fat mass.³⁴ Cardiac cachexia is known to be associated with worse prognosis in CHF independently of age, functional class, LVEF, and exercise capacity.^{24;35;36} Moreover, immune and neurohormonal activation reflected in sympathetic activation and catabolic/anabolic imbalance is observed in these patients.^{4;12} Although increased concentrations of ANP and BNP are known to be directly related to worsening CHF,^{10;29} the presence of cardiac cachexia was not taken into consideration in these studies. We have found cachectic CHF patients to have the highest levels of ANP and BNP, epinephrine, norepinephrine, and TNF.²⁶ Thus, cachexia in these patients may contribute to their high ANP and BNP levels. The underlying mechanism that might explain the relationship between neurohormonal activation and increased circulating levels of TNF and cardiac cachexia remains unclear but different assumptions have been made.²⁶ For example, recently it has been shown that both ANP and BNP increase the production of adiponectin in vitro and in CHF subjects.³⁷ Furthermore, high levels of adiponectin have been related to cardiac cachexia^{38;39} and moreover, adiponectin has correlated positively not only with BNP but also with TNF.³⁹

In addition to cachexia, also BMI is known to predict worse long-term survival in CHF.⁴⁰ Nonetheless, the role of cachexia or weight loss in regard to long-term outcome after admission for ADHF has not yet been analysed. Even though we did not look for weight loss in our ADHF substudy, we did find an association between low BMI and medium- and long-term mortality by univariate Cox-regression analysis. Moreover, after adjusting for creatinine, NYHA class, age, and the use of diuretics, BMI independently predicted

outcome after five years. Thus, we think that weight loss might play an important role in the progression of CHF, particularly in ADHF, but this hypothesis needs to be verified. Furthermore, we believe that patients with body wasting should be clearly identified not only in the outpatient setting but also at admission due to acute worsening of CHF.

4.3 Evidence Supporting the Endotoxin-Lipoprotein Hypothesis

Bacterial endotoxins or lipopolysaccharide are known to stimulate the release of cytokines and particularly TNF.^{13;14} It has been shown that lipoproteins exert a protective action by binding and neutralizing endotoxins and thus reducing their bioactivity.^{16;41} The predictive importance of different cytokines and soluble cytokine receptors for mortality in CHF has been analysed, among others, by Rauchhaus et al.²⁴ In this study, sTNF-R1 best predicted 24-month mortality independent of NYHA class, PVO₂, VE/VCO₂ slope, LVEF, and cachexia.²⁴ In our study population we could confirm a strong immune activation as reflected by elevated TNF, sTNF-R1, and sTNF-R2, which was more pronounced in ADHF patients. Furthermore, higher cytokines have been associated with low cholesterol levels and poor outcome in CHF.⁴²

Like Niebauer et al.,¹⁵ we found oedematous CHF patients to have augmented endotoxin and cytokine levels. In addition, we observed ADHF patients to have lower HDL concentrations compared to healthy controls. Importantly, HDL inversely correlated with endotoxin and TNF and the endotoxin/HDL ratio emerged as a powerful predictor of TNF concentrations. Considering our results we can add some evidence to the hypothesis by which lipoproteins may diminish immune activation by binding endotoxins. Following this hypothesis it has been postulated,⁴³ that particularly in patients with advanced CHF, lipid lowering treatment with statins should be used with caution. Therefore, it seems obvious that randomised, controlled trials in CHF using statin treatment^{44;45} must have failed.

4.4 Renal Impairment and Mortality in Acute Decompensated Heart Failure

We have identified creatinine at admission as the most powerful factor to predict medium- and long-term mortality in ADHF. Moreover, lower CrCl, high urea, functional class, and lower BMI were also associated with worse prognosis over one and five years.

The influence of renal dysfunction in CHF and particularly, worsening renal function (WRF) defined by many authors as an increase in serum creatinine of at least 0.3 mg/dL during hospitalization, have been recurrently documented and associated with longer hospitalizations, rehospitalization rates, in-hospital and medium-term mortality, and higher costs.^{17;18;46-48}

Although we did not look for WRF in our evaluation, we did find a strong correlation between impaired renal function characterized by both high serum creatinine levels and low CrCl at admission to predict one- and five-year overall mortality. Thus, we add more information regarding the impact of impaired renal function on overall mortality and present a cheap and easily detectable prognosticator of five-year mortality in ADHF syndromes.

4.5 The Importance of Admission Medication for Long-Term Outcome

The use of diuretics and digoxin at admission for worsening CHF was associated with worse outcome. This association was observed for both medium- and long-term mortality in univariate analysis. Furthermore, in multivariate analysis, the use of digoxin and diuretics together with creatinine independently predicted mortality after five years.

Although medical treatment of CHF has considerably improved over the last decades, there is still a paucity of information regarding the efficacy of different treatment strategies. For instance, although diuretics can meliorate symptoms and control fluid overload in AHF, the favourable effects of its use on a long-term basis in stable CHF patients have not been demonstrated in a prospective randomized study.^{49;50} Indeed, non-potassium-sparing diuretics have been associated with increased risk of death, cardiovascular death, progressive CHF death, sudden cardiac death, and CHF hospitalization in a post-hoc analysis of 6797 patients in the Digitalis Investigation Group (DIG) trial.⁵⁰ Interestingly, the DIG study investigated the long-term effects of the intake of digoxin in CHF. In the main analysis of this study, the use of digoxin was not associated with a better prognosis of these patients but with reduced hospitalization rates.⁵¹ High serum digoxin concentrations are associated with a subsequent risk of toxicity and mortality.⁵² Consequently it is recommended to individualize the dose of digoxin, considering several factors such as age, body size, renal function, and concomitant medications.⁵³ Furthermore, the presence of hypokalaemia and hypomagnesaemia often observed in patients taking diuretics is known to diminish the toxic threshold of digoxin, further increasing the risk of adverse reactions.⁵⁴ This could explain our results, since 36% of the patients were taking both diuretics and digoxin at admission. Although we neither found low concentrations of potassium at admission nor an association of potassium with mortality, changes over time might have occurred.

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Statement of Contributions

The doctoral candidate has contributed to the following peer-reviewed original publications resumed in this work as follows:

- **Vaz Pérez A**, Doehner W, von Haehling S, Schmidt H, Zimmermann AV, Volk HD, Anker SD, Rauchhaus M. The relationship between tumor necrosis factor-alpha, brain natriuretic peptide and atrial natriuretic peptide in patients with chronic heart failure. *Int J Cardiol* 2010;141:39-43. Impact Factor (2009): 3.469

50% contribution

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- Zimmermann AV, Doehner W, **Vaz Pérez A**, Schmidt H, Volk HD, Anker SD, Rauchhaus M. The relationship between high-density lipoprotein, bacterial lipopolysaccharide, and tumor necrosis factor- α in patients with acute decompensated heart failure. *Arch Med Sci* 2008;4:380-85. Impact Factor (2009): 1.012

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Supervisor

María Amalia Vaz Pérez
Doctoral candidate

Selected Publications

The following publications are set out in pages 15 to 32:

1. Vaz Pérez A, Doehner W, von Haehling S, Schmidt H, Zimmermann AV, Volk HD, Anker SD, Rauchhaus M. The relationship between tumor necrosis factor- α , brain natriuretic peptide and atrial natriuretic peptide in patients with chronic heart failure. *Int J Cardiol* 2010;141:39-43
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Curriculum Vitae and Publication List

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Publications

Original Peer-Reviewed

Vaz Pérez A, Otawa K, Zimmermann AV, Stockburger M, Müller-Werdan U, Werdan K, Schmidt H, Ince H, Rauchhaus M. The impact of impaired renal function on mortality in patients with acutely decompensated chronic heart failure. *Eur J Heart Fail* 2010;12:122-28.

Vaz Pérez A, Doehner W, von Haehling S, Schmidt H, Zimmermann AV, Volk HD, Anker SD, Rauchhaus M. The relationship between tumor necrosis factor- α , brain natriuretic peptide and atrial natriuretic peptide in patients with chronic heart failure. *Int J Cardiol* 2010;141:39-43.

Zimmermann AV, Doehner W, **Vaz Pérez A**, Schmidt H, Volk HD, Anker SD, Rauchhaus M. The relationship between high-density lipoprotein, bacterial lipopolysaccharide, and tumor necrosis factor- α in patients with acute decompensated heart failure. *Arch Med Sci* 2008;4:380-85.

Hypothesis Peer-Reviewed

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Review

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Abstracts

Vaz Pérez A, Otawa K, Zimmermann AV, Herrmann R, Stockburger M, Doehner W, Werdan K, Schmidt H, Rauchhaus M. The importance of aggravated renal dysfunction on mortality in patients with acute heart failure. *Eur Heart J* 2009;30 (Suppl 1):1020.

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Declaration

Ich, María Amalia Vaz Pérez, erkläre, dass ich die vorgelegte Dissertation mit dem Thema: „Risk and Adverse Factors in Heart Failure – from Immune and Neurohormonal Activation to Medical Therapy“ selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.

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María Amalia Vaz Pérez

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