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# Neutrophil Gelatinase-Associated Lipocalin and depression in patients with chronic heart failure

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ABSTRACT

Depression adversely affects prognosis in heart failure (HF) patients. Inflammation is indicated as potential biological pathway in this co-morbidity. Since increased levels of the cytokine Neutrophil Gelatinase-Associated Lipocalin (NGAL) are predictive for HF prognosis, and recently indicated in patients with major depression, this study examined the association of serum NGAL levels with symptoms of depression in patients with HF. Serum NGAL levels were measured in 104 patients with HF (left ventricular ejection fraction, LVEF $\leqslant 40$). Depression, evaluated using the Beck Depression Inventory (BDI; total score, somatic and cognitive component), and the Hamilton Depression Rating scale (HAMD), at baseline and 12 months follow-up, was associated with NGAL levels using mixed model analysis. Analyses were adjusted for demographics measures, disease severity indicators, inflammation, comorbidity and medication. Increased serum NGAL levels were significantly associated with depression measured by HAMD (baseline: $r = 0.25$, $p < .05$) and BDI (baseline: $r = 0.22$, $p < .05$; 12 months: $r = 0.37$, $p < .01$). This association remained significant after adjustment for covariates; age, sex, time, LVEF, and creatinine (HAMD, $t = 2.01$, $p = .047$; BDI, $t = 2.28$, $p = .024$). NGAL was significantly associated with somatic- ($p = 0.004$), but not cognitive depressive symptoms ($p = 0.32$). NGAL levels were associated with the experienced HF-related functional limitations (6 min walk test), rather than the severity of cardiac dysfunction (LVEF). This study indicates that depression in patients with chronic HF is associated with elevated NGAL levels, independent of clinical severity of the underlying disease.



## 1. Introduction

Depression is a common comorbid condition in patients with chronic heart failure (HF). Symptoms of depression are observed in 19–34% of the HF patients, depending on the diagnostic instruments used (Rutledge et al., 2006). Depression in HF is associated with adverse disease outcomes, including a further reduction in the already poor health status in conjunction with an increased morbidity and mortality (Nemeroff and Goldschmidt-Clermont, 2012). Optimal diagnostic procedures and anti-depressive interventions are therefore important goals in the clinical management of HF, but these efforts are hampered by the lack of knowledge on the pathophysiology of the association between depression and HF.

Abnormal levels of inflammatory proteins have been found in HF, in particular increased pro-inflammatory cytokines and products of inflammatory processes (Anker and von Haehling, 2004; Mommersteeg et al., 2010) and depression (Krishnadas and Cavanagh, 2012), with clear overlap (Pasic et al., 2003). The co-existence of depression and HF has been found to be related to higher levels of circulating pro-inflammatory proteins (Johansson et al., 2011; Kupper et al., 2012). However, due to the wide variation in expression of symptoms of depression and the variable aetiology of HF, detailed information on more specific inflammatory markers associated with symptoms of depression in HF is still lacking.

NGAL, also known as Lipocalin 2, siderocalin, 24p3, or uterocalin, is a 25 kDa acute phase inflammatory protein, upregulated via various pro-inflammatory stimuli and produced by numerous cell types (Liu and Nilsen-Hamilton, 1995). NGAL can induce a pro-apoptotic environment in myocardial- (Xu et al., 2012) and neuronal cells (Naudé et al., 2012) and can lead to reduced hippocampal neuronal growth during stress (Mucha et al., 2011). Increased

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circulating NGAL levels have been observed in HF (Damman et al., 2008). A 2 year follow-up study in patients with HF showed that NGAL levels were significantly increased in parallel to clinical severity, as determined by New York Heart Association (NYHA) classification, and were associated with increased mortality (Bolignano et al., 2009). Very recently, our research group found that increased circulating NGAL levels were significantly associated with symptoms of late-life depression, independent of clinical and life-style confounders (Naudé et al., 2013). Interestingly, increased NGAL levels found in depressed patients were not affected by the use of antidepressant medication (Naudé et al., 2013) as opposed to other inflammatory markers such at tumor necrosis factor alpha, interleukin-6, interferon gamma and C-reactive protein as previously reported (Hannestad et al., 2011; Maes et al., 1999; Vogelzangs et al., 2012). NGAL is therefore an interesting inflammatory component since circulating levels are increased and play important functions in the pathophysiology of both depression and HF. However, the association of NGAL with depressive symptoms in HF is still unknown. Data on the regulation of NGAL levels in depressed patients with HF will provide important insights to the existing knowledge of immunological abnormalities in HF and depression.

Therefore, the aim of the present study was to investigate whether depressive symptoms are associated with serum NGAL levels in patients with HF.

## 2. Methods

### 2.1. Patients and procedures

In total, 227 patients who attended the outpatient heart failure clinic of the TweeSteden hospital, Tilburg, the Netherlands between October 2003 and January 2005 were approached for participation in the TweeSteden Heart Failure I cohort, as has been described previously (Kupper et al., 2012; Mommersteeg et al., 2010). Inclusion criteria were LVEF < 40%, being stable in medication 1 month before inclusion, and <80 years. A total of 21 patients were excluded due to either life threatening comorbidities, acute infections or insufficient proficiency of the Dutch language. Out of 206 eligible patients, 165 (80%) agreed to participate. Blood collection at baseline did not take place in 51 patients, since the blood collection sub-study started in January 2004. Five patients were excluded due to missing depression scores at baseline, and six patients were excluded due to missing blood samples at baseline. The patients who were excluded in the present study ($N = 61$) were more often categorized in NYHA-class III/IV, were more often overweight (BMI > 30), and reported increased cognitive depressive symptoms according to the BDI. Patients completed a psychological survey assessing depressive symptoms at the time of inclusion and 12 months later. Complete data at baseline (blood and questionnaires) were available in the present study for 104 patients. Nineteen patients were lost to follow-up; 4 died, 8 refusal, 2 excluded, the rest unknown causes. Complete questionnaire data were available for 89 patients at 12 months, and blood data were available for 71 patients at 12 months. The study protocol was approved by the local medical ethics committee in Tilburg, the Netherlands. The study was conducted conforming to the Helsinki Declaration, and all patients provided written informed consent.

### 2.2. Neutrophil Gelatinase-Associated Lipocalin

Blood samples were drawn with vacuum-tubes by trained hospital personnel at the hospital during visiting hours (8.00–17.00 h), as part of the patient's routine HF check-up and collected at both measurement occasions. Blood collection as well as questionnaire assessment were on average within 2 weeks of inclusion in the present study (mean date of inclusion – date of blood collection = −0.49 month, 95% CI of mean = −1.01–0.02 month). The blood was allowed to clot at room temperature and centrifuged. Aliquoted serum samples were stored at −80 °C. A blinded ELISA analysis was performed on coded samples. ELISA analysis was performed as described previously (Naudé et al., 2012). Briefly, quantification of NGAL from serum was performed via a sandwich ELISA using human Lipocalin-2/NGAL ELISA capture antibody (R&D Systems), recombinant human Lipocalin-2/NGAL (R&D Systems) for the internal standard and biotinylated human Lipocalin-2/NGAL detection antibody (R&D Systems) according to the manufacturers' protocol. Serum was diluted 1:100. The intra- and inter-assay coefficients of variation were 3% and 5%, respectively.

### 2.3. Depressive symptoms

Supervised and trained psychologists assessed depression according to a structured interview, using the Hamilton Depression Rating scale (HAMD) interview (Hamilton, 1960). The HAMD interview comprises 17 items, of which nine on a 5-point scale (0 = not present – 4 = severe), and eight items on a 0–2 scale (Hamilton, 1960). The summed score was used, and Cronbach's alpha in the present study was 0.75.

Self-reported depressive symptoms were assessed with the 21-item (0–3 range) version of the Beck Depression Inventory (BDI) (Beck and Steer, 1993). In addition to the total (sum) score, the cognitive subscale (13 items) and the somatic subscale (8 items) were examined to distinguish between somatic and cognitive aspects of depressive symptoms. Cronbach's alpha was 0.86 for the BDI total score, 0.85 for the cognitive subscale, and 0.67 for the somatic subscale. To examine consistent patterns, analyses were run separately for all operationalized depressive symptoms.

### 2.4. Co-variants

Information on sex, age, education level, marital status, smoking and weight and height were assessed by means of self-report. Medical records and information obtained from the patient's treating cardiologist were used to determine clinical variables, including disease severity, comorbid conditions, medication use and standard laboratory assessment. Disease severity status included left ventricular ejection fraction (LVEF) as a continuous measure, and an impaired LVEF < 25% was reported for descriptive purposes, ischemic aetiology of HF (yes/no), presence of cardiac devices (comprising implantable cardioverter–defibrillator (ICD), pacemaker (PM), biventricular pacemaker (BVP)), cardiac history (comprising myocardial infarct (MI), percutaneous coronary event (PCI), coronary artery bypass graft surgery (CABG)), time since HF diagnosis (years). Perception of the disease severity was measured by NYHA functional class (four categories, recoded in two categories I/II and III/IV), and performance on the 6-min walk test (6MWT; meters walked). Clinical comorbid conditions were presence of chronic obstructive pulmonary disease (COPD), diabetes mellitus, gastrointestinal disease, hypertension, hyperlipidaemia, renal disease (based on glomerular filtration rate (GFR) of creatinine $\leqslant$60 mL/min/1.73 $m^2$), liver disease, peripheral artery disease, rheumatoid arthritis, and overweight. Since NGAL is a clinical marker for renal dysfunction, creatinine levels (mg/dl) were included in the present study, and recalculated into an estimated glomerular filtration rate, eGFR (mL/min/1.73 $m^2$) according to Levey et al. (2006).

### 2.5. Statistical analysis

The distribution of NGAL data are depicted in the scatterplots in Fig. 1. Because of the skewed distribution of NGAL,

ln-transformed NGAL (ln-NGAL) levels were used for further statistical analyses. Preliminary analyses were done by Pearson correlations, paired samples *t*-tests and One-Way Anova. Pearson correlations were used to examine the association between depressive symptoms (either HAMD, BDI total score, cognitive and somatic subscales) with ln-NGAL values at baseline and 12 months. Paired samples *t*-tests were used to examine changes over time in ln-NGAL and depressive symptoms. One-Way ANOVA was used to examine differences in presence or absence of the dichotomized covariates with ln-NGAL levels at either baseline or 12 months.

To examine the multivariable association of depressive symptoms and ln-NGAL levels, a linear mixed model procedure was used: unstructured covariance matrix, maximum likelihood (ML) estimate in SPSS version 19.0. The dependent variable was ln-NGAL at both baseline and 12 months. Both time-points were used to preserve statistical power since more data points were used. Given that the variables did not change significantly over time, no further interactions with time were explored, and time was considered a covariate in the model. Separate analyses were run for the HAMD total score, BDI total score, BDI cognitive score and the BDI somatic score, using both baseline and 12-month data in each analysis. Each mixed multivariable analysis comprised three consecutive models. In the first model (Model 1) depressive symptoms (either HAMD, BDI total, BDI cognitive or BDI somatic) were entered as fixed variables together with age, sex and time. In the second model (Model 2), indicators of disease severity were added: LVEF and creatinine levels at baseline. In the third model (model 3), model 2 was expanded with the dichotomized NYHA class (I/II vs. III/IV). We choose NYHA category as indicator of functional capacity, and we choose creatinine levels over the GFR, since GFR is already adjusted for age and sex. No post hoc corrections were made for multiple comparisons due to the high probability of finding small effect sizes, the interdependency of the depressive subscales, and the explorative nature of the analyses. Therefore a Bonferroni correction for the number of tests ($\alpha/n = 0.05/4 = 0.0125$) for the multivariable analyses could be considered too conservative.

## 3. Results

### 3.1. Patient characteristics and NGAL levels

Patient characteristics at baseline are shown in Table 1. The study group consisted of mainly male CHF patients (72%) with an average LVEF of 30%, in total 17% of the patients ($n = 18$) had an LVEF below 25%, 43% of the patients were classified in NYHA class III/IV, over 50% had a cardiac history (MI, PCI, CABG), and a large variety of co-morbidities and prescribed medication. Although NYHA classification and 6-min walking distance were not significantly altered at 12 months vs. baseline, representing a stable HF population, the decreased ratio NYHA I,II/III,VI (0.41 ± 0.053 vs. 0.38 ± 0.052, 2-tailed, $p = 0.083$) suggests progression of heart failure. Accordingly, NGAL of 12 months were not significantly different from baseline, and NGAL levels at baseline and 12 months showed a significant and high correlation (Table 2: $r = 0.84$). Similarly, no significant differences were observed between baseline and 12 months for the HAMD score ($t(df = 84) = 1.19$, $p = 0.24$), BDI total score ($t(df = 87) = 0.18$, $p = .857$), BDI cognitive score ($t(df = 87) = 0.11$, $p = .914$), or BDI somatic score ($t(df = 87) = 0.19$, $p = .853$). Depression scores measured by both methods were significantly correlated ($r$ values ranging from 0.44 to 0.66). Furthermore, none of the parameters depicted in Table 1 differed between baseline and 12 months follow-up.

Correlations of ln-NGAL concentration with other parameters are summarized in Table 2. ln-NGAL levels were positively correlated with age, plasma creatinine concentrations, and inversely with glomerular filtration rate (GFR) and distance on the 6MWT. NGAL levels at baseline and 12 months were significantly related to NYHA-class at baseline (NYHA I–II = 146.1 ± 54.3 vs. NYHA III–IV = 181.7 ± 90.4, $F(1,99) = 6.0$, $p = .016$) and at 12 months (NYHA I–II = 133.2 ± 60.3 vs. NYHA III–IV = 188.3 ± 80.2, $F(1,70) = 10.9$, $p = .002$). NGAL levels were higher in patients with renal disease (based on GFR cut-off <60 (mL/min/1.73 $m^2$) (baseline: 182.0 ± 84.0; 12 months: 172.6 ± 79.3), compared to those without renal disease (baseline: 137.5 ± 47.6, $F(1,96) = 10.6$, $p = .002$; 12 months: 132.0 ± 47.0, $F(1,67) = 6.76$, $p = .012$). However,

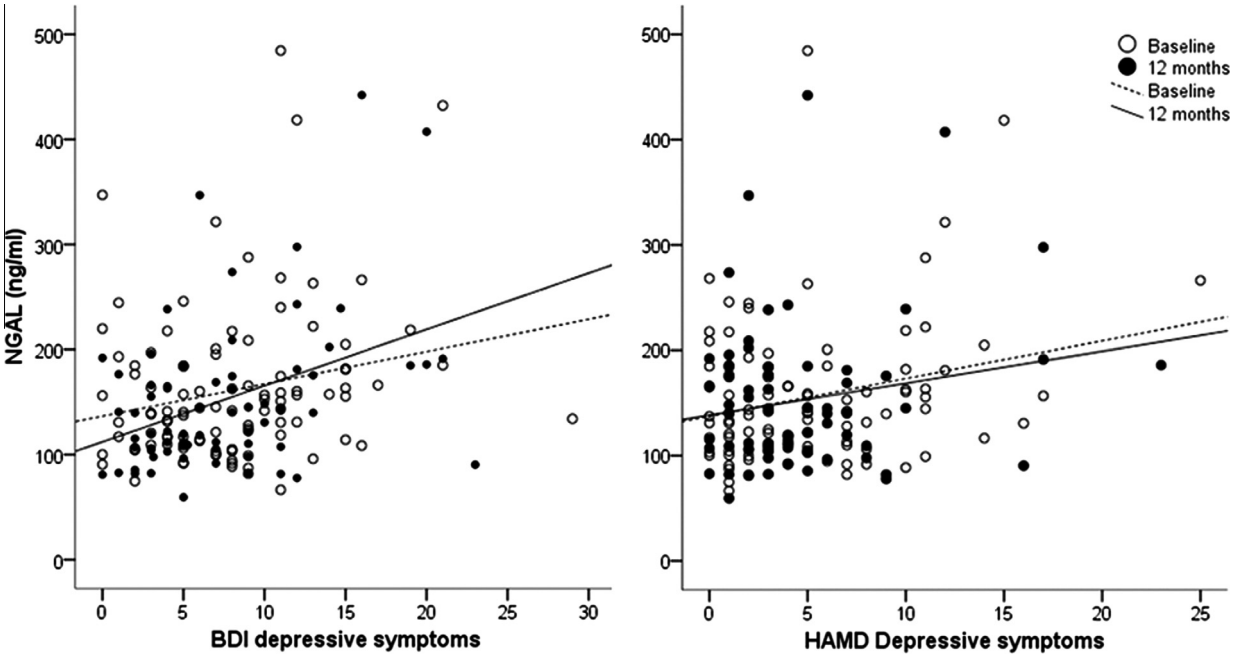


**Fig. 1.** Scatterplot of depressive symptoms and NGAL levels at baseline and 12 months. BDI baseline $r = 0.22$, $p = 0.03$; 12 months $r = 0.391$, $p = 0.001$; HAMD baseline $r = 0.25$, $p = 0.02$; 12 months $r = 0.18$, $p = 0.12$.

**Table 1**
General descriptive statistics of patients with chronic heart failure.

| | *N* (present) | % or mean | *n* or SD |
|---|---|---|---|
| *Study substance* | | | |
| NGAL baseline [ng/ml] | 104 | 160.6 | 72.9 |
| NGAL 12 months [ng/ml] | 71 | 154.1 | 73.1 |
| *Demographics* | | | |
| Age [years] | 104 | 65.8 | 8.4 |
| Men | 104 | 72% | 75 |
| Education [lower education] | 104 | 35% | 36 |
| Smoking | 104 | 26% | 27 |
| BMI [kg/m$^2$] | 96 | 28.6 | 9.5 |
| *HF disease status* | | | |
| LVEF [mean %] | 104 | 30.4 | 6.0 |
| NYHA III/IV | 104 | 43% | 45 |
| Six minutes walk test [m] | 104 | 270.6 | 168.4 |
| Time between diagnosis and baseline [years] | 102 | 4.2 | 4.5 |
| Cardiac history (MI/PCI/CABG) | 104 | 57% | 59 |
| *Depressive symptoms* | | | |
| HAMD | 95 | 5.4 | 4.8 |
| BDI | 104 | 7.98 | 5.20 |
| BDI (cognitive) | 104 | 2.85 | 2.72 |
| BDI (somatic) | 104 | 5.13 | 3.11 |
| *Renal disease* | | | |
| Creatinine [mg/dl] | 93 | 1.18 | 0.43 |
| GFR [mL/min/1.73 m$^2$] | 100 | 63.58 | 20.60 |
| *Comorbidity* | | | |
| COPD | 104 | 11% | 11 |
| Diabetes mellitus | 104 | 24% | 25 |
| Dyslipidaemia | 104 | 51% | 53 |
| Hypertension | 104 | 32% | 33 |
| Kidney failure based on $GFR_{creat} \leqslant 60$ | 100 | 46% | 46 |
| *Medication use* | | | |
| ACE-inhibitor | 104 | 79% | 82 |
| AII-antagonists | 104 | 16% | 17 |
| Aspirin use | 104 | 43% | 45 |
| Beta-blocker | 104 | 64% | 66 |
| Diuretics use | 104 | 20% | 21 |
| Digoxine use | 104 | 35% | 36 |
| Oral anticoagulants | 104 | 50% | 52 |
| Psychotropic medication | 104 | 11% | 11 |
| Statin use | 104 | 44% | 46 |

NGAL = Neutrophil Gelatinase-Associated Lipocalin; GFR = glomerular filtration rate; HAMD = Hamilton Depression Rating scale; BDI = Beck Depression Inventory.

patients with and without renal disease did not differ in depression scores (BDI total: 7.7 ± 0.6 vs. 8.0 ± 0.9; HAMD: 5.2 ± 0.6 vs. 5.2 ± 0.8 for patients with no renal disease vs. renal disease patients, resp.). No significant associations were found for NGAL with sex, marital status, education level, smoking, BMI, LVEF, time since diagnosis, cardiac history, devices, ischemic aetiology, comorbid conditions or any of the medication use (data not shown).

### 3.2. Primary association of depressive symptoms and NGAL

Fig. 1 depicts the association between depressive symptoms and non-transformed NGAL levels at baseline and 12 months. NGAL levels were significantly correlated with total depressive symptoms, according to the HAMD as well as the total BDI scores at baseline, and to total BDI at 12 months. Similarly ln-transformed NGAL levels were significant positively correlated with total depressive symptoms at baseline (HAMD and BDI scores). Whereas the BDI somatic subscale at baseline was also associated with increased NGAL levels, the BDI cognitive–affective subscale showed a trend towards significance. Similar results were obtained at 12 months, except for the correlation between the HAMD scores and NGAL (Table 2).

### 3.3. Multivariate analysis

Mixed model multivariable analyses (Table 3) shows that depressive symptoms measured by the BDI total score and the BDI somatic subscale score were significantly associated with ln-NGAL levels, when adjusting for age, sex and time. After further adjustment for LVEF and plasma creatinine concentrations, interview assessed HAMD depression scores, BDI total score, and BDI somatic component, but not BDI cognitive component, were significantly associated with NGAL levels. Subsequent adjustment for NYHA functional class slightly attenuated these findings for the HAMD score, which became non-significant ($p = .08$), but did not affect the significant association between ln-NGAL and BDI total and somatic subscale scores. Model fit scores showed that Model 3 (complete adjusted) was to be preferred over Models 1 and 2, based on the AIC and BIC criteria, and a log-likelihood ratio test showed a significant improvement in the model after further adjustment for the covariates. In the fully adjusted model, covariates plasma creatinine concentration was significantly associated with ln-NGAL levels ($t(df = 85–90) = 5.97–6.10$, $p < .001$), as well as NYHA class I/II ($t(df = 90–97) = -2.62$ to $-2.65$, $p = .010–.012$). No significant effect of sex, age, duration of disease or LVEF on ln-NGAL were observed in the adjusted models.

## 4. Discussion

In the present study the association of serum NGAL levels with symptoms of depression was examined in patients with chronic HF. Results showed that serum NGAL levels are significantly associated with symptoms of depression, obtained from a structured interview (HAMD) as well as self-report (BDI). This association remained significant after adjustment for age, sex, LVEF, and plasma creatinine concentrations. Data further indicates that the experienced burden of HF, rather than the heart disease itself was associated with the final NGAL levels. The steadiness of intrapatient NGAL levels observed in this study over 12 months and the physical properties of NGAL strengthen the abovementioned discussed properties of NGAL as a biological stable marker over time.

Serum NGAL levels were significantly associated with symptoms of depression, obtained from a structured interview (HAMD) as well as self-report (BDI). The significant association of increased circulating NGAL levels with symptoms of depression found in this study, is further supported by recent findings by our group from the *Netherlands Study of Depression in Older people* (NESDO) cohort with 350 depressed and 129 non-depressed older persons (Comijs et al., 2011). A significant association of increased plasma NGAL levels with major depressive disorder diagnosis according to DSM-IV-R criteria was observed. This association remained significant after adjustments for confounding factors; age, sex, smoking and waist circumference were made (Naudé et al., 2013). Furthermore, NGAL levels were attenuated in recently remitted depressed patients and increased in those suffering from recurrent depression irrespective of the age of onset (Naudé et al., 2013). In this regard, the present study shows that the association between NGAL levels and symptoms of depression remained similar over a 12 months period. These findings indicate that NGAL levels may be closely related to current symptoms of depression.

Interestingly, data from this study show that increased NGAL levels are significantly associated with somatic-, rather than with cognitive symptoms of depression measured by the BDI, after adjusting for covariates. These findings remain significant after a conservative Bonferroni correction for multiple testing as well.

**Table 2**
Correlations between baseline and 12 months ln-NGAL, HAMD, BDI depression and continuous covariates.

| | NGAL [ln ng/ml] | | HAMD | HAMD | Baseline BDI depression | | | 12 month BDI depression | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Baseline | 12 months | Baseline | 12 months | Total | Cognitive | Somatic | Total | Cognitive | Somatic |
| NGAL 12 months | **0.84**$^{***}$ | | | | | | | | | |
| HAMD baseline | **0.25**$^{*}$ | **0.23**$^{\dagger}$ | | | | | | | | |
| HAMD 12 months | 0.12 | 0.17 | **0.44**$^{***}$ | | | | | | | |
| BDI total baseline | **0.22**$^{*}$ | **0.27**$^{*}$ | **0.37**$^{***}$ | **0.36**$^{***}$ | | | | | | |
| BDI cognitive baseline | **0.18**$^{\dagger}$ | **0.22**$^{\dagger}$ | **0.37**$^{***}$ | **0.29**$^{**}$ | **0.87**$^{***}$ | | | | | |
| BDI somatic baseline | **0.21**$^{*}$ | **0.25**$^{*}$ | **0.30**$^{**}$ | **0.34**$^{**}$ | **0.90**$^{***}$ | **0.58**$^{***}$ | | | | |
| BDI total 12 months | **0.28**$^{**}$ | **0.37**$^{**}$ | **0.45**$^{***}$ | **0.65**$^{***}$ | **0.62**$^{***}$ | **0.60**$^{***}$ | **0.49**$^{***}$ | | | |
| BDI cognitive 12 months | **0.19**$^{\dagger}$ | **0.21**$^{\dagger}$ | **0.35**$^{**}$ | **0.58**$^{***}$ | **0.45**$^{***}$ | **0.59**$^{***}$ | **0.24**$^{*}$ | **0.85**$^{***}$ | | |
| BDI somatic 12 months | **0.30**$^{**}$ | **0.42**$^{***}$ | **0.42**$^{***}$ | **0.52**$^{***}$ | **0.60**$^{***}$ | **0.43**$^{***}$ | **0.61**$^{***}$ | **0.84**$^{***}$ | **0.42**$^{***}$ | |
| *Covariates* | | | | | | | | | | |
| Age | 0.15 | **0.30**$^{**}$ | −0.02 | 0.21 | −0.01 | −0.01 | 0.00 | **0.23**$^{*}$ | 0.17 | **0.22**$^{*}$ |
| BMI | −0.13 | −0.02 | 0.09 | −0.08 | −0.11 | 0.09 | 0.10 | 0.01 | −0.01 | 0.02 |
| LVEF | −0.09 | −0.11 | 0.04 | 0.05 | 0.03 | 0.02 | 0.03 | −0.01 | 0.03 | −0.05 |
| 6MWT [m] | **−0.30**$^{**}$ | **−0.29**$^{*}$ | **−0.29**$^{**}$ | −0.12 | **−0.24**$^{*}$ | **−0.18**$^{\dagger}$ | **−0.23**$^{*}$ | **−0.28**$^{**}$ | −0.13 | **−0.35**$^{**}$ |
| Time since diagnosis [yrs] | 0.02 | 0.01 | 0.09 | 0.15 | −0.06 | −0.02 | −0.07 | **0.21**$^{\dagger}$ | **0.19**$^{\dagger}$ | 0.16 |
| GFR [mL/min/1.73 m$^2$] | **−0.46**$^{***}$ | **−0.40**$^{**}$ | 0.06 | −0.03 | −0.10 | −0.12 | −0.06 | −0.08 | −0.12 | −0.08 |
| Creatinin [mg/dl] | **0.55**$^{***}$ | **0.56**$^{**}$ | −0.03 | −0.01 | 0.10 | 0.14 | 0.03 | 0.07 | 0.13 | 0.02 |

NGAL = Neutrophil Gelatinase-Associated Lipocalin; HAMD = Hamilton Depression Rating scale; BDI = Beck Depression Inventory; BMI = body mass index; LVEF = left ventricular ejection fraction; 6MWT = 6-min walk test; GFR = glomerular filtration rate Pearsons' *r* is given.
$N_{range}$ = 66 (BMI baseline-NGAL 12 months) – 104 (BDI baseline-LVEF).
$^{\dagger}$ $p < .10$.
$^{*}$ $p < .05$.
$^{**}$ $p < .01$.
$^{***}$ $p < .001$.

**Table 3**
Mixed multivariate modelling of ln-NGAL levels by depressive symptoms.

| | HAMD | | | BDI total | | | BDI cognitive | | | BDI somatic | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *t* | *df* | *p* | *t* | *df* | *p* | *t* | *df* | *p* | *t* | *df* | *p* |
| Model 1: depressive symptoms, age, sex, and time | 1.61 | 92 | 0.111 | **2.33** | **133** | **0.021** | 1.24 | 118 | 0.216 | **2.75** | **123** | **0.007** |
| Model 2: model 1 + LVEF and creatinine | **2.01** | **104** | **0.047** | **2.28** | **151** | **0.024** | 1.00 | 132 | 0.320 | **2.97** | **142** | **0.004** |
| Model 3: model 2 + NYHA functional class | 1.77 | 114 | 0.080 | **2.27** | **153** | **0.025** | 1.13 | 130 | 0.259 | **2.81** | **149** | **0.006** |
| Model fit | AIC | BIC | | AIC | BIC | | AIC | BIC | | AIC | BIC | |
| Model 1: age, sex, time and depression | 58.44 | 82.78 | | 65.92 | 90.72 | | 69.74 | 94.54 | | 63.87 | 88.67 | |
| Model 2: model 1 + LVEF and creatinine | 31.12 | 61.56 | | 36.00 | 67.00 | | 40.10 | 71.10 | | 32.61 | 63.61 | |
| Model 3: model 2 + NYHA functional class | 27.35 | 60.83 | | 31.38 | 65.48 | | 35.14 | 69.24 | | 28.74 | 62.84 | |
| Likelihood ratio test | $\chi^2$ | *df* | *p* | $\chi^2$ | *df* | *p* | $\chi^2$ | *df* | *p* | $\chi^2$ | *df* | *p* |
| Model 1–2: effect of LVEF and creatinine | 31.31 | 2 | <0.001 | 33.93 | 2 | <0.001 | 33.64 | 2 | <0.001 | 35.26 | 2 | <0.001 |
| Model 2–3: effect of NYHA functional class | 5.77 | 1 | 0.016 | 6.62 | 1 | 0.010 | 6.96 | 1 | 0.008 | 5.87 | 1 | 0.015 |

Likelihood ratio test = Chi-square test of change in −2LogLikelihood between the models, *df* = change in degrees of freedom.
AIC = Aikake's information criteria; BIC = Schwarz's Bayesian information criterion, a lower scores indicates a better model fit.

This finding is of interest, since somatic symptoms of depression have been shown to be a significant risk factor for mortality in HF (Jiang et al., 2007), while somatic rather than cognitive depressive symptoms are associated with inflammation (Duivis et al., 2013). In this regard, somatic symptoms of depression measured by BDI have been proposed to be a useful psychiatric measurement for the identification of mortality in chronic heart disease (Schiffer et al., 2009). Moreover, our data illustrate that increased NGAL levels were strongly correlated with the perception of the burden of the disease as expressed in NYHA class and 6MWT, rather than with the severity of left ventricular dysfunction, measured by LVEF, per se. Accordingly, in one study (Yndestad et al., 2009) increased NGAL levels have been correlated with NYHA class, but not with LVEF, cardiac index, left ventricular end-systolic and end-diastolic dimension. In another study, symptoms of depression in patients with HF are also associated with the perception of the severity of the disease (NYHA class, 6MWT), rather than the severity of the disease measured by LVEF (Gottlieb et al., 2009). This finding, together with strong correlation of increased NGAL with depressive symptoms at baseline and 12-month follow-up further strengthens the link between increased NGAL and symptoms of depression in HF.

In addition to its role in HF, NGAL receives extensive attention as biomarker for renal injury (Mishra et al., 2005). Accordingly, our data showed a strong correlation between increased NGAL levels and impaired renal function in HF, which further supports studies that recommend NGAL as a promising early marker of acute kidney injury in patients with or without HF (Clerico et al., 2012). However, we did not find an association of impaired renal function and symptoms of depression, neither did depression scores differ between patients with and without renal disease. Since we found that increased NGAL levels were significantly associated with symptoms of depression, even after adjustment for parameters of kidney function, it seems reasonable to presume that the association of increased NGAL levels with depression in HF cannot be attributed to impaired kidney function. In this regard, our data together with a study by Yndestad et al. (2009) show that, while a significant correlation of increased serum NGAL levels with higher serum creatinine was found in patients with HF, increased NGAL levels were also found in patients with normal creatinine

levels. These findings indicate that NGAL levels in HF do not only reflect impaired renal function. According to Triposkiadis and colleagues, inflammation could be regarded as common denominator of HF and associated non-cardiac co-morbidities (Triposkiadis and Skoularigis, 2012). Therefore, increased NGAL levels in depressed patients with HF may reflect inflammation associated with symptoms of depression independent of the presence of renal impairment.

Fundamental research on the mechanisms of NGAL in the heart and brain signifies that NGAL may play a role in the pathophysiology of HF that is often accompanied by depressive symptoms. Research in animals have shown that NGAL is upregulated in the brain after induction of a peripheral inflammatory response (injection of lipopolysaccharide, LPS) (Ip et al., 2011), as well as after psychological stressors (Mucha et al., 2011), indicating a brain-located NGAL response to peripheral inflammation. Increased brain NGAL levels may result in a reduction of hippocampal synaptic spine density (Mucha et al., 2011). In this regard, NGAL can induce a pro-apoptotic signaling cascade in neurons by attenuating Akt phosphorylation of the protein kinase B (PKB)/Akt pathway (Naudé et al., 2012). Cellular signaling via Akt has been proposed as an important signaling pathway for neuroplasticity in the hippocampus (Balu et al., 2012). Furthermore, decreased PKB/Akt activity were found in post mortem human brain tissue of suicide victims compared to non-depressed controls (Karege et al., 2011). Increased central nervous system NGAL levels can therefore attenuate Akt signaling that consequently can lead to reduced neuroplasticity. These data may provoke the thought that NGAL can locally impair neuronal growth and play a role in both the "impaired adult neurogenesis and plasticity hypothesis of depression" (Sahay and Hen, 2007) and "inflammation hypothesis of depression" (Krishnadas and Cavanagh, 2012). A study in cell cultures showed that NGAL could induce a pro-apoptotic effect in primary rat cardiomyocytes (Xu et al., 2012). In addition, mutant mice lacking NGAL showed improved recovery of cardiac function after cardiac ischemia–reperfusion (Yang et al., 2012). In essence, the abovementioned data together with the strong association of increased NGAL levels with somatic symptoms of depression in HF found in this study, collectively suggest that NGAL not only functions as a biomarker but could also play an important role in the pathophysiology of heart disease via its mechanistic functions in brain- and heart-cells, especially when co-existing somatic symptoms of depression are present.

In order to properly interpret the results presented in this study, study limitations ought to be acknowledged. Although this study is based on a relatively low number of patients, the very stable levels of NGAL as well as depression scores and its strong association, over a 12 months period merits its potential as marker for depression in HF. This study focuses on outpatients with chronic HF. Our findings may therefore not apply to hospitalized patients with HF. Given the increased NGAL levels in the NYHA-class III/IV, and its association with cognitive depressive symptoms according to the BDI, the excluded group because of missing blood collection, could potentially have affected the present findings. The present findings may underrepresent the actual association between NGAL and depressive symptoms in patients with heart failure. Immunological markers and associated hormones follow a circadian rhythm that affects their daily profile, which may also apply to NGAL. In this respect, time of blood collection may cause variations in circulating NGAL levels. However the strong correlation between baseline and 12 months NGAL levels in conjunction with similar mean values suggest stable assessment with low variability.

In conclusion, the present study shows that depressive symptoms were significantly related to NGAL levels in HF. These results appeared independent from other determinants of NGAL levels such as age, sex, severity of heart disease, and renal disease. As somatic rather than cognitive aspects of depression were associated with increased NGAL levels, and NGAL reflected experienced burden of HF rather than clinical measures of cardiac function, NGAL may provide additional information on psychological functioning in HF. NGAL additionally possesses ideal characteristics to be used as biological indicator. Data from this study strongly suggest that researchers should take co-existing depressive symptoms into account when investigating the association of NGAL with HF. The postulated involvement of NGAL as pathophysiological element in heart disease further merits NGAL as promising target for future research.

## Conflict of interest

The authors herein declare no conflict of interest.

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