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BRIEF REPORT

NGAL and other markers of inflammation as competitive or complementary markers for depressive symptom dimensions in heart failure

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Abstract

Objectives. Neutrophil gelatinase-associated lipocalin (NGAL) is an inflammatory marker associated with the pathophysiology of heart failure (HF), the psychopathology of depression and the co-existing symptoms of depression in HF patients. The aim of this study is to determine whether the association of serum NGAL levels with depressive symptoms dimensions in HF is independent of well-known inflammatory markers. *Methods.* Serum NGAL, high sensitive C-reactive protein (hsCRP), tumour necrosis factor-α (TNF-α), its two soluble receptors; sTNFR1, sTNFR2, Interleukin-6 (IL-6) and leukocytes were measured in 104 patients with HF at baseline and 12 months. Depressive symptoms were evaluated using the Beck Depression Inventory (BDI) at both timepoints. Correlations between NGAL and inflammatory markers and depressive symptoms dimensions were determined. The effect of hsCRP, IL-6, TNF-α, sTNFR1, sTNFR2 and leukocytes on the association of NGAL with depressive symptoms was determined and adjusted for time, demographics, cardiac disease severity, and kidney function. *Results.* NGAL levels were significantly correlated with hsCRP, TNF-α, sTNFR1, sTNFR2 and leukocytes. NGAL was significantly associated with somatic depressive symptoms, independent of above-mentioned markers. *Conclusions.* Serum NGAL is an independent inflammatory marker for somatic depressive symptoms in HF and may function as an immunopathogen linking somatic symptoms of depression to HF.

Key words: *Lipocalin 2, cognitive and somatic depressive symptoms, tumor necrosis factor alpha, interleukin-6, C reactive protein*

Introduction

Mounting evidence from fundamental and clinical research supports that pro-inflammatory processes are involved in the pathophysiology of heart failure (HF; Kop et al. 2011; Hartupee and Mann 2013), as well as depression (Eyre et al. 2014). Co-existing symptoms of depression is a common phenomenon in HF (Rolls et al. 2007), and strongly associated with adverse cardiovascular prognosis and quality of life in patients with cardiovascular disease (de Miranda Azevedo et al. 2014). In this respect, differences have been observed for symptoms relating to cognitive-affective and somatic-affective dimensions of depression (de Miranda Azevedo et al. 2014). Inflammation has been indicated as a physiological mechanism underlying depressive symptoms in cardiovascular

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disease (Poole et al. 2011) and may hence play a role in its differential associations with depressive symptom dimensions in HF (Kupper et al. 2012).

A relatively new inflammatory marker in this field is neutrophil gelatinase-associated lipocalin (NGAL). NGAL, also known as Lipocalin 2, siderocalin, 24p3, or uterocalin, is a 25-kDa acute phase inflammatory protein, produced by numerous cell types (Kjeldsen et al. 1993). Increased circulating levels of NGAL have a high prognostic value in patients with heart failure (van Deursen et al. 2014). Moreover, recently we showed that increased serum NGAL levels in heart failure patients are associated with depressive symptoms, independent of cardiac- and renal dysfunction (Naude et al. 2014).

NGAL is upregulated via various pro-inflammatory stimuli including tumour necrosis factor (TNF-α; Zhao et al. 2014), potentially by interleukin 6 (IL-6) (Ransohoff and Brown 2012) and produced by leukocytes in HF (Yndestad et al. 2009). TNF-α is an inflammatory marker that is commonly associated with depression (Dowlati et al. 2010). Mechanistically, TNF-α and NGAL are interconnected given that TNF-α upregulates NGAL specifically via activation of TNFR1, and increased NGAL in turn silences TNFR2-mediated pathways (Naudé et al. 2012). Furthermore, follow-up human studies showed that increased circulating levels of TNF-α, its two soluble receptors (Rauchhaus et al. 2000), as well as levels of NGAL (van Deursen et al. 2014) are predictors of mortality in HF. These findings collectively indicate that NGAL and markers of the TNF-α pathway may function as biological constituents underlying depression in HF.

The aim of the present study was to determine whether the association of serum NGAL levels with depressive symptoms in HF as reported previously in this cohort (Naude et al. 2014) depends on the TNF-α pathway; TNF-α, its two soluble receptors; sTNFR1 and sTNFR2, as well as other inflammatory markers, IL-6, high sensitive C-reactive protein (hsCRP) and number of leukocytes. We hypothesize that elevated levels of TNF pathway markers could partly explain the association of NGAL with depressive symptoms.

Methods and materials

Patients and procedures

Patients with HF were enrolled between October 2003 and January 2005 at the Tweesteden Hospital Tilburg using the following inclusion criteria: left ventricular ejection fraction (LVEF) $< 40\%$, age < 80 years, and stable medication regimen over the past 3 months. Details of this cohort have been described previously (Denollet et al. 2009; Mommersteeg et al. 2010; Kupper et al. 2012; Naude et al. 2014). Briefly, the average age of this study population was 65.8 ($\pm$ 8.4) years and 72% were males. Complete data at baseline (blood and questionnaires) were available for 104 patients in the present study. Follow-up data at 12 months were available for 89/104 patients for questionnaires, and 81 (inflammatory markers), or 71 (NGAL) for blood analysis. The medical ethics committee Tilburg (Tweesteden Hospital) and Eindhoven (Catharina Hospital) approved the study protocol (protocol number M03/1376) and all patients provided written informed consent.

Blood assays

Blood samples were drawn with vacuum tubes by trained hospital personnel during visiting hours (08:00–17:00 h), as part of the patient routine HF check-up and collected at baseline and at 12-month follow-up. Blood samples were allowed to clot at room temperature, centrifuged, and serum samples were aliquoted and stored at $-80°$C until analysis and were thawed once upon analysis. All analyses were performed blinded in coded samples.

ELISA analyses of NGAL (R&D Systems, Minneapolis, MN) (Naude et al. 2014), TNF-α, sTNFR1, sTNFR2, IL-6, and hsCRP (Turnhout, Belgium) (Kupper et al. 2012) were measured in accordance with manufacturer recommendations. The intra- and inter-assay coefficients of variation for NGAL were 3 and 5% and intra-assay variation was less than 10%, and the inter-assay variation less than 11% for the other markers. Leukocyte count was measured using an automated procedure with a Beckman Coulter analyzer (Beckman Coulter, Inc., CA).

Depressive symptoms

Depressive symptoms were assessed with the Beck Depression Inventory (BDI; Beck and Steer 1993), 21-item version. BDI questionnaires were completed at home. The somatic subscale (eight items) and the cognitive-affective subscale (13 items) were examined separately to distinguish between somatic and cognitive components of depressive symptoms. Cronbach's alpha for the BDI total score was 0.86, for the somatic subscale 0.67 and cognitive-affective subscales 0.85, indicating a good internal consistency. A total BDI score ≥ 10 is considered indicative of clinically significant depressive symptoms.

Covariates

Information about clinical variables, including age, sex, disease severity, comorbid conditions,

medication use and standard laboratory assessment was obtained from medical records as previously described (Kupper et al. 2012; Naude et al. 2014). HF severity was based on Left Ventricular Ejection Fraction (LVEF), performance on the 6-min walk test (6MWT; meters walked), and NYHA functional classification (recoded into two categories I/II and III/IV). Information about weight and height (for body mass index BMI) was based on patients' self-report. Creatinine levels were recoded into the estimated glomerular filtration rate (eGFR in ml/min/1.73 m^2) (Levey et al. 2006), and a cutoff of ≤ 60 ml/min/1.73 m^2 was used to describe presence of impaired renal function.

Statistical analysis

Data are presented as *N* and %, mean $\pm$ standard deviation (SD), or median and inter-quartile range. All markers of inflammation were *ln*-transformed prior to analyses. Previous findings reported of this dataset have shown that neither the depressive symptoms, NGAL levels (Naude et al. 2014), nor the levels of markers of inflammation (Kupper et al. 2012) changed significantly over time. To make optimal use of the available data at baseline and 12 months, both time points were used in correlation analysis and multivariate mixed model analyses, adjusting for time, e.g., time-adjusted Pearson's correlations of NGAL with the markers of inflammation were reported. Linear mixed model multivariate analyses (covariance model, unstructured, Maximum Likelihood) were performed to examine the association between baseline and 12-month levels of depressive symptoms (outcome variable; either BDI total, BDI cognitive-affective symptoms, or BDI somatic-affective symptoms) with NGAL at baseline and 12 months and a marker of inflammation at baseline and 12 months, adjusted for time (baseline or 12 months) and covariates. Covariates measured at baseline included age, sex, an indicator of cardiac disease severity (LVEF), renal disease severity (creatinine level), since NGAL levels are known to be associated with kidney function, and BMI, which is known to affect markers of inflammation (O'Connor et al. 2009; Kupper et al. 2012; Naude et al. 2014). Separate models were run for markers of inflammation TNF-α, sTNFR1, sTNFR2, IL-6, hsCRP and number of leukocytes. Estimates B with 95% confidence intervals of B and *P* values were reported. In a post-hoc sensitivity analysis 6MWT, NYHA-category, and eGFR were used as covariates instead of LVEF, creatinine and BMI, as these disease severity and kidney function associated factors are potential confounders for the relation between depression and markers of inflammation as well. Data were analysed using SPSS 19.0 (IBM Corp, Armonk, NY) and a *P* value of < 0.05 was considered statistically significant.

Results

Patient characteristics and NGAL levels

The study group consisted of mainly male CHF patients (72%) with an average LVEF of 30%, 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 as was previously reported in more detail (Naude et al. 2014).

Correlation of NGAL with inflammatory markers

The median and interquartile range of NGAL and the markers of inflammation are described in Table I. NGAL was significant positively associated with the markers of inflammation ($n = 104$, adjusted for time); TNF-α ($r = 0.25$, $P = 0.001$), sTNFr1 ($r = 0.38$, $P < 0.001$), sTNFr2 ($r = 0.50$, $P < 0.001$),

Table I. Median and interquartile range of the markers of inflammation at baseline and 12 months.

	Baseline			12 months		
	Median	IQR25%	IQR75%	Median	IQR25%	IQR75%
NGAL [ng/ml]	142.8	110.7	184.3	140.1	106.0	183.9
TNF-α [pg/ml]	6.22	3.63	9.04	5.32	3.03	8.81
sTNFr1 [ng/ml]	3.57	2.77	5.15	3.53	2.64	5.03
sTNFr2 [ng/ml]	2.14	1.58	3.41	2.16	1.62	2.97
IL-6 [pg/ml]	1.30	0.82	2.70	1.25	0.82	3.04
hsCRP [mg/l]	5.40	2.87	10.09	5.17	1.69	10.70
Leukocytes [10^{E3}/µl]	7.95	6.53	9.18	7.80	6.50	8.70

NGAL, Neutrophil Gelatinase-Associated Lipocalin; TNF-α, Tumor Necrosis Factor alpha; sTNFr1, soluble Tumor Necrosis Factor alpha receptor 1; sTNFr2, soluble Tumor Necrosis Factor alpha receptor 2; IL-6, Interleukin-6; hsCRP, high sensitivity C Reactive Protein; IQR, Interquartile Range.

hsCRP ($r = 0.23$, $P = 0.002$) and leukocytes ($r = 0.31$, $P < 0.001$), but not IL-6 ($r = 0.15$, $P = 0.052$).

Mixed model of NGAL with depressive symptoms adjusted for inflammatory variables

NGAL levels were significantly associated with BDI total score (B = 3.34, 95%CI 0.55–6.13, $P = 0.019$) and BDI somatic symptoms of depression (B = 2.22, 95%CI 0.65–3.80, $P = 0.006$), but not cognitive-affective symptoms (B = 1.25, 95%CI –0.30–2.80, $P = 0.113$) after adjustment for time, age, sex, LVEF, creatinine and BMI (data not shown).

Table II shows that TNF-α, sTNFR1, sTNFR2, IL-6, and hsCRP did not affect the association of NGAL with depressive symptoms, with one exception: NGAL was no longer associated with total depressive symptoms in the model which included number of leukocytes. None of the markers of inflammation showed a significant association with depressive symptoms. The association between NGAL and somatic symptoms of depression persisted in the models with TNF-α, sTNFR1, sTNFR2, IL-6, hsCRP and leukocytes.

In the complete adjusted model, sex was significantly associated with total (B = 2.35, 95%CI 0.13–4.57, $P = 0.04$) and somatic depressive symptoms (B = 2.15, 95%CI 0.89–3.43, $P = 0.001$), and BMI was significantly associated with cognitive-affective symptoms (B = 0.17, 95%CI 0.04–0.29, $P = 0.02$).

A sensitivity analysis for 6MWT, NYHA-category, and eGFR as covariates in the model instead of LVEF, creatinine and BMI did not affect the main findings; NGAL remained associated with somatic depressive symptoms when adjusted for the markers of inflammation (Averaged B = 1.69, 95%CI 0.24–3.15, $P = 0.025$).

Discussion

The present study showed that increased serum NGAL levels are significantly associated with symptoms of depression, independent of markers of the TNF-α pathway; TNF-α, its two soluble receptors; sTNFR1 and sTNFR2, and IL-6, hsCRP and number of leukocytes after correcting for time, age, sex, LVEF, creatinine and BMI as confounding factors. These findings persisted after correcting for 6MWT, NYHA-category, and eGFR as covariates in the model instead of LVEF, creatinine and BMI. These associations were specific for somatic but not cognitive-affective symptoms of depression.

Unexpectedly, markers of the TNF-α pathway did not have an effect on the association of NGAL with symptoms of depression. Our findings are of interest considering the role of NGAL in inflammatory processes. TNF-α induces NGAL production in various tissues (Zhao et al. 2014). Elevated TNF-α levels mediate increased sTNFR1 and sTNFR2 release (Jansen et al. 1995) which bind to soluble TNF-α and subsequently stabilize and preserve the bio-active trimeric forms of TNF-α (Diez-Ruiz et al. 1995). Therefore, sTNFRs levels reflect the activity of TNF-α and may play a role in NGAL production.

Table II. Mixed model analysis of depressive symptoms with NGAL and inflammatory variables.

	BDI total depressive symptoms				BDI cognitive-affective				BDI somatic			
	B	95%CI		*P*	B	95%CI		*P*	B	95%CI		*P*
NGAL	**3.26**	**0.45**	**6.08**	**0.023**	1.23	−0.34	2.80	0.123	**2.16**	**0.58**	**3.74**	**0.008**
TNF-α	0.21	−0.81	1.24	0.679	0.05	−0.53	0.62	0.871	0.23	−0.35	0.81	0.441
NGAL	**3.39**	**0.58**	**6.20**	**0.018**	1.45	−0.11	3.00	0.068	**2.19**	**0.60**	**3.77**	**0.007**
sTNFr1	−0.15	−1.14	0.84	0.762	−0.44	−1.03	0.16	0.146	0.12	−0.42	0.66	0.668
NGAL	**2.91**	**0.06**	**5.75**	**0.045**	1.12	−0.47	2.71	0.165	**1.97**	**0.37**	**3.57**	**0.016**
sTNFr2	0.88	−0.38	2.14	0.170	0.27	−0.49	1.03	0.484	0.51	−0.18	1.19	0.143
NGAL	**3.29**	**0.51**	**6.08**	**0.021**	1.25	−0.30	2.80	0.113	**2.14**	**0.58**	**3.70**	**0.008**
IL-6	0.45	−0.60	1.51	0.394	−0.01	−0.61	0.59	0.964	0.52	−0.06	1.10	0.079
NGAL	**3.24**	**0.47**	**6.01**	**0.022**	1.19	−0.36	2.75	0.130	**2.18**	**0.62**	**3.74**	**0.006**
hsCRP	0.41	−0.28	1.09	0.241	0.16	−0.24	0.56	0.431	0.30	−0.08	0.68	0.116
NGAL	2.31	−0.60	5.23	0.119	0.63	−1.01	2.27	0.448	**1.83**	**0.18**	**3.49**	**0.030**
Leukocytes	0.32	−0.12	0.76	0.150	0.14	−0.10	0.39	0.244	0.16	−0.09	0.41	0.206

Model: NGAL, and inflammatory marker, adjusted for time, age, sex, LVEF, creatinine and BMI. For abbreviations see Table I.

Studies in mice showed that IL-6 may play a non-essential role in systemic NGAL production during inflammatory processes (Lee et al. 2012; Hamzic et al. 2013). To our best knowledge the effect of CRP on NGAL production is still unknown. However, similar to the present study, increased NGAL levels are positively correlated with CRP in patients with HF (Daniels et al. 2012). NGAL is produced by several leukocytes, especially by activated neutrophils that release NGAL into the circulation (Chakraborty et al. 2012). In this respect, neutrophils are an important source of NGAL in HF (Yndestad et al. 2009). Thus, the above-mentioned studies show that TNF-α and leukocytes play important roles in NGAL production during inflammatory conditions. Accordingly, we observed that NGAL significantly correlated with all of the inflammatory markers investigated in this study, except for IL-6.

Interestingly, our findings suggest that increased serum NGAL is a specific marker for somatic symptoms of depression in people with HF, independent of above-mentioned inflammatory markers, as well as the severity of the disease measured by LVEF, and serum creatinine. This supports the specificity of NGAL as marker for somatic symptoms of depression. Moreover, the findings persisted after performing a sensitivity analysis for eGFR and perception of the burden of HF as expressed by NYHA class and 6MWT (Naude et al. 2014), as confounding factors.

The specific association of NGAL with somatic symptoms of depression is of importance, considering that somatic symptoms of depression are strongly associated with adverse cardiovascular prognosis and mortality, compared to cognitive-affective symptoms of depression in patients with cardiovascular disease (de Miranda Azevedo et al. 2014). Moreover, somatic, rather than cognitive depressive symptoms are associated with inflammation (Dantzer et al. 2008). Since NGAL can play an important role in the pathophysiology of HF (Cruz et al. 2012), i.e., by inducing pro-apoptotic effects in cardiomyocytes (Xu et al. 2012), and is significantly increased in HF patients with co-existing somatic symptoms of depression, as shown in this study, it may function as an important biological constituent responsible for the adverse prognostic effects of co-existing somatic symptoms of depression in HF. In addition, neutralization of NGAL attenuated cardiac ischemic reperfusion injury in mice (Cheng et al. 2015), and may therefore function as a drug target in heart disease.

A limitation of the present study is its relatively small sample size, which warrants replication in a larger sample size. The high prevalence of men in this study limits the generalizability of these findings for women, although sex was not associated with depressive symptoms.

In conclusion, NGAL was associated with inflammatory markers in HF, but the association of NGAL with somatic depressive symptoms remained significant after adjustment for markers of the TNF-α pathway and other commonly reported markers of inflammation, and hence may represent an independent inflammatory marker for somatic depressive symptoms in HF. Regarding its roles in the pathophysiology of HF, NGAL may function as an immunopathogen linking somatic symptoms of depression to HF.

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Statement of Interest

None to declare.

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