Original Paper

Neutrophil Gelatinase-Associated Lipocalin (NGAL) in Chronic Cardiorenal Failure is Correlated with Endogenous Erythropoietin Levels and Decreases in Response to Low-Dose Erythropoietin Treatment

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Abstract

Background: Neutrophil-gelatinase associated lipocalin (NGAL), a tubular injury marker, is associated with iron metabolism in hemodialysis patients. We investigated whether serum NGAL levels reflect iron metabolism in combined chronic heart failure and chronic kidney disease (CHF/CKD) and whether treatment with low-dose erythropoietin stimulating agent (ESA) modulates NGAL levels. *Methods:* In the EPOCARES trial (ClinTrialsNCT00356733) serum NGAL, hepcidin-25, transferrin saturation (TSAT), reticulocyte hemoglobin content (Ret-He) and endogenous erythropoietin (EPO) levels were measured. *Results:* Baseline serum NGAL levels correlated with cystatin C (r=0.767, p<0.001) and baseline EPO levels (r=-0.395, p=0.003). There was no correlation with baseline TSAT, Ret-He, and hepcidin-25 levels. After two weeks, NGAL levels decreased in the ESA-group (p=0.02), while there was no change in the no-ESA group (p=0.62). The magnitude in NGAL decrease in the ESA-group correlated with baseline

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EPO levels (r=0.431, p=0.01). **Conclusions:** In contrast to in HD patients, in combined CKD/CHF, serum NGAL levels did not correlate with iron metabolism, hence NGAL might reflect tubular damage in these patients. NGAL levels inversely correlated with baseline EPO levels and decreased in response to short-term ESA treatment, which might reflect an effect of ESA on tubular damage. These findings need to be confirmed and alternative explanations should be evaluated.

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Introduction

Neutrophil-gelatinase associated lipocalin (NGAL or lipocalin-2), a 25kDa protein of the lipocalin family, acts as a natural bacteriostatic agent by interfering with bacterial iron uptake and also increases in response to inflammation. In the setting of acute renal tubular injury, human serum NGAL levels increase 7 to 16-fold and urinary NGAL levels increase 25-100 fold [1]. Consequently, NGAL has been proposed as a biomarker for tubular damage to detect acute kidney injury at an early stage in various conditions [2-4]. However, NGAL has also been shown to be elevated in chronic conditions, such as chronic kidney disease (CKD) [5, 6] and chronic heart failure (CHF) [7], presumably reflecting chronic damage to tubular cells, irrespective of the glomerular filtration rate [8, 9].

In addition, NGAL levels might also reflect iron metabolism since the NGAL pathway acts as an alternative to the transferrin-mediated iron delivery pathway by cytoplasmic iron delivery into target cells [10]. Recently, Bolignano et al. and Malyszko et al. suggested that low NGAL levels in hemodialysis patients reflect reduced iron availability and transport; lower NGAL levels were associated with lower transferrin saturation (TSAT), lower ferritin levels and higher hepcidin levels [11-13].

Thus, NGAL levels might reflect inflammation, tubular damage or reduced iron availability. Iron deficiency plays an important role in the pathophysiology of CKD and CHF [14] and is related to a reduced quality of life and increased mortality [15]. In combined CHF/CKD, it is unknown whether NGAL reflects iron metabolism or tubular damage. Since ESA treatment induces increased iron utilization and reduces hepcidin one could hypothesize that during ESA treatment NGAL levels increase [16, 17]. Therefore, we investigated the hypothesis that (1) serum NGAL levels reflect iron availability in anemic patients with combined chronic heart and kidney failure and (2) that ESA treatment increases serum NGAL levels in accordance with its effect on hepcidin. To this end we measured NGAL and assessed markers of inflammation and iron metabolism including transferrin saturation (TSAT), reticulocyte hemoglobin content (Ret-He) and hepcidin-25 levels, using a mass spectrometry based assay, in the EPOCARES study.

Materials and Methods

Study design and patients

The study design of the EPOCARES study (ClinicalTrials.gov number NCT 00356733) has been published elsewhere [18]. In short, the EPOCARES study is an open-label, prospective, randomized trial, in which patients with CHF, CKD (estimated creatinine clearance (eCrCl) by Cockroft-Gault equation of 20-70 ml/min) and mild anemia (hemoglobin 10.3-12.6 g/dL for men and 10.3-11.9 g/dL for women) are included to test the erythropoietic and non-erythropoietic responses to low-dose ESA treatment. Exclusion criteria, amongst others, were ESA therapy within 6 months, bleeding, chronic inflammatory disease or malignancy. Hemoglobin (Hb) level for inclusion was measured after at least four weeks of oral iron supplementation, if tolerated. The diagnostic criteria for CHF were those recommended by the European Society of Cardiology guidelines [19]. Patients with heart failure with reduced left ventricular ejection fraction as well as patients with preserved left ventricular ejection fraction were included [20].

The subjects were randomized into 3 groups. The first two groups received a fixed dose of 50 IU/kg per week of ESA (Neorecormon; Roche Pharmaceuticals), After two weeks, the Hb level was let to increase to a maximum of 13.7 g/dL for men and 13.4 g/dL for women in one group (ESA-Hb-rise group) whereas in the other group the Hb levels were maintained at baseline level for 26 weeks by sequential blood withdrawal to a maximum of 250 mL per 2 weeks (ESA-Hb-stable group). The third group, the control group, did not receive ESA (the no-ESA group). In addition, a group of 25 healthy, age- and sex-matched controls were recruited for comparison of NGAL levels. The Medical-Ethical Committee approved the protocol of the study and informed consent was obtained from all subjects. Procedures were in accordance with the Helsinki Declaration and all patients gave written consent.

Biomarker analysis

All blood samples were drawn between 8 and 9 a.m. and stored at -80°C until analysis. As a marker of total iron stores, ferritin was determined using a sandwich immunoassay on an Acces®2 immunoanalyzer within a Dx automated system from Beckman Coulter (Brea, CA). Functional iron availability was determined by measuring transferrin saturation (TSAT), soluble transferrin receptor (sTfR) and reticulocyte hemoglobin content (Ret-He). TSAT was calculated from serum iron and transferrin estimates obtained with standard methods on a Beckman Coulter Dx. sTfR assay was performed with an immunoassay on a BNProSpec nephelometer from Siemens (Marburg, Germany). Ret-He was performed using flow cytometric analysis with Ret-Search (II)® dye on a Sysmex XE-2100 hematology analyzer (Toa Medical, Kobe, Japan).

Serum hepcidin-25 measurements were performed by a combination of weak cation exchange chromatography and time-of-flight mass spectrometry (TOF MS) [21]. An internal standard (synthetic hepcidin-24; Peptide International Inc.) was used for quantification [22]. Peptide spectra were generated on a Microflex LT matrix-enhanced laser desorption/ionisation TOF MS platform (Bruker Daltonics). Serum hepcidin-25 concentrations were expressed as nmol/l. The lower limit of detection (LLOD) of this method was 0.5 nM; ranges for the coefficients of variation were 2.2-3.7% (intra-run) and 3.9-9.1% (inter-run). The median reference level of serum hepcidin-25 is 4.5 nM for men, 2.0 nM for premenopausal women, and 4.9 nM for postmenopausal women. The reference levels for the WCX-TOF MS method were derived from those of a competitive ELISA method [23], based on the regression line between the data of both methods on the same samples [21].

Serum NGAL levels were measured on the Triage® NGAL test (Alere Inc. San Diego, CA, USA), an immunoassay in a single-use plastic cartridge that contains a fluorescently labeled monoclonal antibody against NGAL labeled with a fluorescent dye and NGAL. Measurements of NGAL concentration in the range from 60 to 1300 ng/ml. Calibration information is relayed to the meter via a lot-specific EPROM chip [4].

Assessment of changes in hemoglobin levels in time

Two study groups received similar dosages of ESA treatment during the study period (together the ESA group). After two weeks the ESA group was split into the ESA-Hb-stable and the ESA-Hb-rise group. In the ESA-Hb-stable group the Hb levels were kept at baseline level using phlebotomies. To assess the necessity of a phlebotomy, a preceding increase in Hb was required, for which the Hb levels were measured every 2 weeks, which makes comparison of single time-point measurements unreliable. We therefore assessed the Hb response by calculating the area under the curve for Hb change in time (Hb AUC) in the groups. Furthermore, the Hb AUC is more informative to asses the Hb response to ESA treatment and its clinical significance on end points [24]. The Hb AUC was calculated by linear trapezoidal integration, in which the total area under the Hb change versus time curve is obtained by summation of each individual area between two consecutive time points, as extensively described elsewhere [24]. The Hb AUC was based on monthly Hb measurements in all patients.

Statistical analysis

Data are presented as means ± standard deviation (SD) for normally distributed variables and median with inter-quartile ranges (IQR) for non-normally distributed variables. Normality of data was evaluated using the Kolmogorov-Smirnov test. Non-normally distributed variables were log transformed, after which normality was tested again. Differences between groups were compared with the unpaired student's t-test, Mann-Whitney U test or χ^2–test where appropriate. Paired data were compared with the paired student's t-test using a Bonferroni adjusted alpha level. Pearson correlation or Spearman's rho were used for bivariate

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Table 1: Baseline characteristics

Characteristic	ESA, n=37	No ESA, n=19	p- value
Age, years	76 [70-81]	72 [66-77]	0.52
Male sex, n (%)	23 (62.2)	14 (73.7)	0.39
BMI (kg/m^2)	26.1 ± 4.2	27.4 ± 4.2	0.68
Diabetes, n (%)	12 (32.4)	7 (36.8)	0.74
Hypertension, n (%)	27 (80.0)	16 (84.2)	0.35
NGAL (ng/mL)	189 [133-265]	238 [129-313]	0.86
NT-proBNP (pg/mL)	1400 [744-2631]	1680 [659-2610]	0.81
LVEF (%)	42.1 ± 9.1	44.1 ± 12.3	0.22
hs-CRP (mg/L)	5.4 [1.3-10.9]	4.3 [1.7-6.9]	0.55
RAS inhibitor, n (%)	35 (94.6)	19 (100.0)	0.30
Hemoglobin (g/dL)	11.7 ± 0.95	11.8 ± 0.79	0.74
Hematocrit (%)	0.35 ± 0.031	0.35 ± 0.026	0.96
Ferritin (ng/mL)	130 [75-209]	128 [76-164]	0.43
Transferrin saturation (%)	20 [15-25]	20 [18-29]	0.76
Erythropoietin (IU/L)	13 [9-16]	15 [5-17]	0.95

Mean ± standard deviation or median [interquartile range] are shown. Abbreviations: BMI, body mass index; NGAL, neutrophil gelatinase-associated lipocalin; NTproBNP, N-terminal pro-brain natriuretic peptide; LVEF, left ventricular ejection fraction; hs-CRP, high sensitivity C-reactive protein; RAS inhibitor, renin-angiotensin-system inhibitor

correlations (resp. normally and non-normally distributed variables). The one-way ANOVA was used for multiple group comparisons. Multivariable linear regression models with stepwise forward selection process were performed. Differences were considered significant when P<0.05, two-sided. For statistical analyses the Statistical Package for Social Sciences (IBM, Chicago, Illinois, USA) version 18 for Mac was used.

Results

Baseline characteristics
The study population of the EPOCARES study comprised of 62 patients, of whom 5 patients withdrew their informed consent and 1 patient was withdrawn because of malignancy. Baseline characteristics of the remaining 56 patients are displayed in Table 1, divided by ESA group and no-ESA group. All patients had CKD, CHF and were anemic, as shown by the decreased eCrCl, left ventricular ejection fraction (LVEF), Hb-levels and the higher NT-proBNP levels. Despite oral iron supplementation, TSAT levels were low in some patients (< 20% in 26 of the patients or < 15% in 8 patients). However, in the 37 patients that received ESA, Ret-He did not decrease, indicating that there was no iron-restricted erythropoiesis. At baseline, there were no differences between the ESA group and the no-ESA group.

The NGAL levels in the EPOCARES cohort were increased in comparison to healthy age- and sex-matched volunteers (207 [132-287] ng/mL vs. 77 [60-116] ng/mL, n=25; p<0.001). When divided according to baseline NGAL levels below and above the median (high ≥ 207 ng/mL vs. low < 207 ng/mL), patients with high NGAL values had hi gher cystatin C, NT-proBNP and interleukin-6 and lower eCrCl, serum iron and endogenous EPO levels, as shown in Table 2.

Univariate correlations of baseline NGAL and multiple regression analysis
Table 3. shows the univariate correlations with serum NGAL levels at baseline. Serum NGAL levels correlated positively with urea nitrogen, cystatin C levels and microalbuminuria

Table 2: Baseline variables according to serum NGAL levels below or above median

Characteristic	Low NGAL, n=28	High NGAL, n=28	p- value
Diabetes, n (%)	10 (35.7)	9 (32.1)	0.50
Hypertension, n (%)	19 (67.9)	24 (85.7)	0.10
eCrCl (ml/min)	44 ± 16	30 ± 10	**<0.001**
Cystatin C (mg/L)	1.45 [1.10-1.68]	2.40 [1.87-2.87]	**<0.001**
NT-proBNP (pg/mL)	1280 [531-2139]	1742 [894-5115]	**0.020**
hs-CRP (mg/L)	5.4 [1.3-10.9]	4.3 [1.7-6.9]	0.21
Interleukin-6 (pg/mL)	2.8 [1.7-4.7]	4.1 [2.3-7.2]	**0.044**
Hemoglobin (g/dL)	11.8 ± 0.86	11.8 ± 0.94	0.74
Serum iron (µmol/L)	12 [10-16]	10 [8-14]	**0.027**
Ferritin (ng/mL)	133 [78-216]	117 [75-167]	0.53
Transferrin saturation (%)	22 [18-26]	18 [14-25]	0.19
sTfR (mg/L)	1.28 ± 0.36	1.48 ± 0.55	0.11
Ret-He (fmol)	1.90 ± 0.14	1.88 ± 0.14	0.11
Hepcidin-25 (nM)	5.4 [3.2-7.4]	7.0 [4.4-8.8]	0.14
Erythropoietin (IU/L)	15 [12-19]	10 [6-15]	**0.005**

Mean ± standard deviation or median [interquartile range] are shown. Abbreviations: NGAL, neutrophil gelatinase-associated lipocalin; eCrCl, estimated creatinine clearance by Cockroft Gault; NTproBNP, N-terminal pro-brain natriuretic peptide; hs-CRP, high sensitivity C-reactive protein; sTfR, soluble transferrin receptor; Ret-He, reticulocyte hemoglobin content

and negatively with eCrCl. Furthermore, there was a negative correlation between baseline NGAL levels and endogenous EPO levels (Fig 1A). This correlation between endogenous EPO levels and serum NGAL remained significant after adjustment for eCrCl and cystatin C (adjusted r= -0.358, p=0.008), as for microalbuminuria (adjusted r= -0.338, p=0.020) and for Hb-levels (adjusted r= -0.400, p=0.003). There was no significant association between serum NGAL levels and age, Hb levels or with parameters for iron stores and iron availability such as ferritin, TSAT, sTfR and Ret-He. Nor did we find a correlation with hepcidin-25 levels (Fig. 1B) or inflammation.

After multiple regression analysis, stepwise including variables with univariate correlation with NGAL with p<0.1, cystatin C (β=0.804, p<0.001), endogenous EPO levels (β= -0.249, p=0.004) and urea (β= 0.325, p=0.016) were the predictors for baseline serum NGAL levels (explaining 74.0% of NGAL variations).

Acute response to ESA treatment
Table 4. shows the effect of 2 weeks ESA treatment versus no treatment on the variables. Hb levels, reticulocytes and sTfR

Table 3: Univariate correlations with serum NGAL levels at baseline

Variable	NGAL	
	r	p-value
Age	- 0.184	0.18
Hemoglobin	- 0.112	0.41
NT-proBNP	0.219	0.11
Urea nitrogen	0.725	< 0.001
eCrCl	- 0.571	< 0.001
Cystatin C	0.767	< 0.001
Microalbuminuria	0.515	< 0.001
CRP	0.133	0.33
hs-CRP	0.123	0.37
Interleukin-6	0.194	0.16
Erythropoietin	- 0.395	**0.003**
Transferrin	- 0.387	**0.003**
Ferritin	- 0.110	0.42
TSAT	- 0.136	0.32
Soluble transferrin receptor	0.148	0.28
Reticulocyte hemoglobin content	- 0.119	0.39
Hepcidin-25	0.147	0.28

Abbreviations: NGAL, neutrophil gelatinase-associated lipocalin; NTproBNP, N-terminal pro-brain natriuretic peptide; eCrCl, estimated creatinine clearance by Cockroft Gault; CRP, C-reactive protein; hs-CRP, high sensitivity CRP; TSAT, transferrin saturation
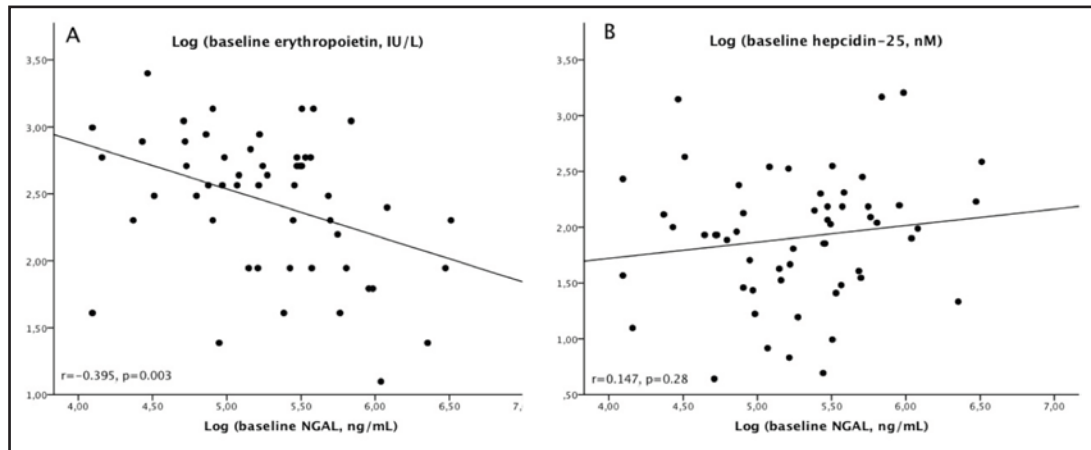
Fig. 1. The correlation between log-transformed baseline serum NGAL levels and baseline log-transformed erythropoietin levels (A) and baseline log-transformed hepcidin-25 levels (B). NGAL levels positively correlate with baseline erythropoietin levels and show no correlation with hepcidin-25 levels. Abbreviations: NGAL, neutrophil gelatinase associated lipocalin.

Table 4: Effects of 2 weeks ESA treatment versus no treatment

Characteristic	ESA (n=37)			No ESA (n=19)		
	t=0	t= 2 weeks	p-value	t=0	t= 2 weeks	p-value
Hemoglobin (g/dL)	11.7 ± 0.95	12.0 ± 1.12	**0.018**	11.8 ± 0.79	11.7 ± 0.86	0.50
Reticulocytes (x10^{12}/L)	0.046 ± 0.015	0.064 ± 0.018	**<0.001**	0.044 ± 0.016	0.047 ± 0.017	0.30
sTfR (mg/L)	1.39 ± 0.48	1.77 ± 0.53	**<0.001**	1.35 ± 0.47	1.44 ± 0.49	0.12
Ret-He (fmol)	1.90 ± 0.15	1.82 ± 0.23	0.24	1.88 ± 0.12	1.84 ± 0.15	0.30
eCrCl (ml/min)	35 ± 12	35 ± 11	0.40	39 ± 20	39 ± 18	0.87
Cystatin C (mg/l)	2.0 ± 0.83	1.9 ± 0.76	0.78	2.0 ± 0.77	2.0 ± 0.72	0.64
hs-CRP (mg/L)	5.4 [1.3-10.9]	2.2 [1.1-4.8]	0.08	4.3 [1.7-6.9]	6.6 [2.1-14.2]	0.34
Interleukin-6 (pg/mL)	3.7 [1.8-4.9]	3.7 [2.2-5.8]	0.28	3.2 [2.3-7.0]	3.3 [2.0-5.8]	0.93

Mean ± standard deviation or median [interquartile range] are shown. Abbreviations: sTfR, soluble transferrin receptor; Ret-He, reticulocyte hemoglobin content; eCrCl, estimated creatinine clearance by Cockroft Gault; hs-CRP, high sensitivity CRP; NTproBNP, N-terminal pro-brain natriuretic peptide

significantly increased in the ESA group compared to baseline. Individual NGAL levels at baseline and after two weeks are shown in Figure 2. NGAL levels significantly decreased after two weeks in the ESA group (p= 0.02) whereas there was no change in the no-ESA group (p=0.62).

The magnitude of decrease in NGAL levels after two weeks ESA treatment correlated positively with baseline endogenous EPO levels (r=0.431, p=0.01, Fig. 3A) and Ret-He (r=0.396, p=0.02, Fig. 3B) and negatively with baseline serum NGAL levels (r= -0.615, p<0.001). There was no correlation between the magnitude in decrease in NGAL levels and baseline eCrCl, cystatin C, baseline Hb-levels or the magnitude in increase of reticulocyte count and sTfR.

Response to ESA treatment after 26 weeks

After two weeks the ESA group split into the ESA-Hb-stable and the ESA-Hb-rise group. Based on the single time point Hb levels after 26 weeks, the Hb increased significantly compared to baseline in the ESA-Hb-rise group (resp. 13.3 ± 1.40 vs 11.8 ± 1.08, p< 0.001) and the ESA-Hb-stable group (resp. 12.6 ± 0.74 vs 11.7 ± 0.84 g/dL, p=0.001). The Hb AUC, depicting the cumulative Hb change over time and a better reflection of the overall exposure to higher Hb level than a comparison at 1 time point, was significantly higher in the ESA-Hb-rise group compared to the ESA-Hb-stable group (resp. 19.8 ± 17.2 vs 5.9 ± 9.6, p= 0.009).
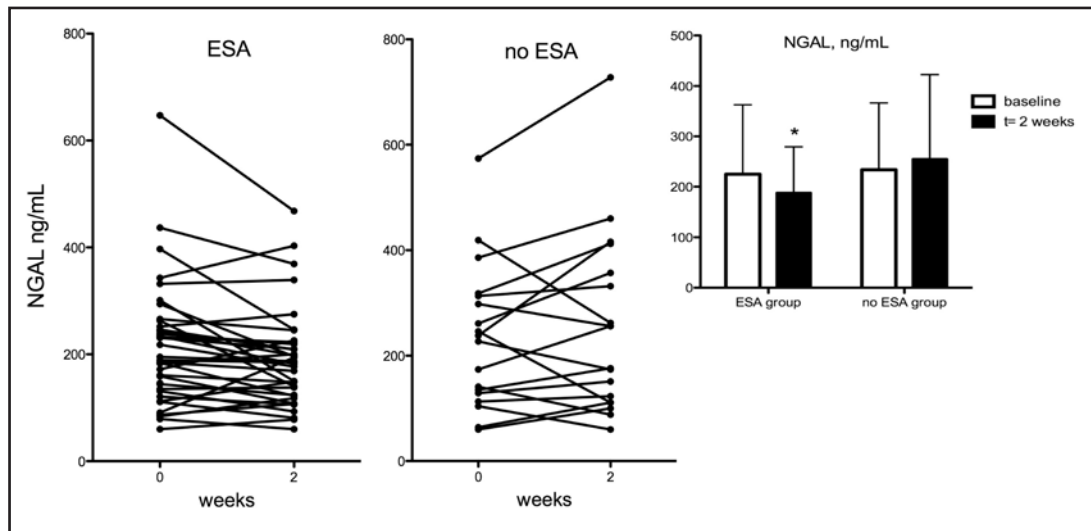
Fig. 2. Serum NGAL levels at baseline and after two weeks of ESA treatment; individual serum NGAL levels at baseline and after two weeks of ESA treatment (n=37) versus no ESA treatment (n=19). Two weeks of ESA treatment decreased log-transformed serum NGAL levels in patients with combined CHF and CKD, as depicted in the bar graph. Abbreviations; NGAL, neutrophil gelatinase associated lipocalin; ESA, erythropoiesis-stimulating agent. Error bars represent SD; * p<0.05.
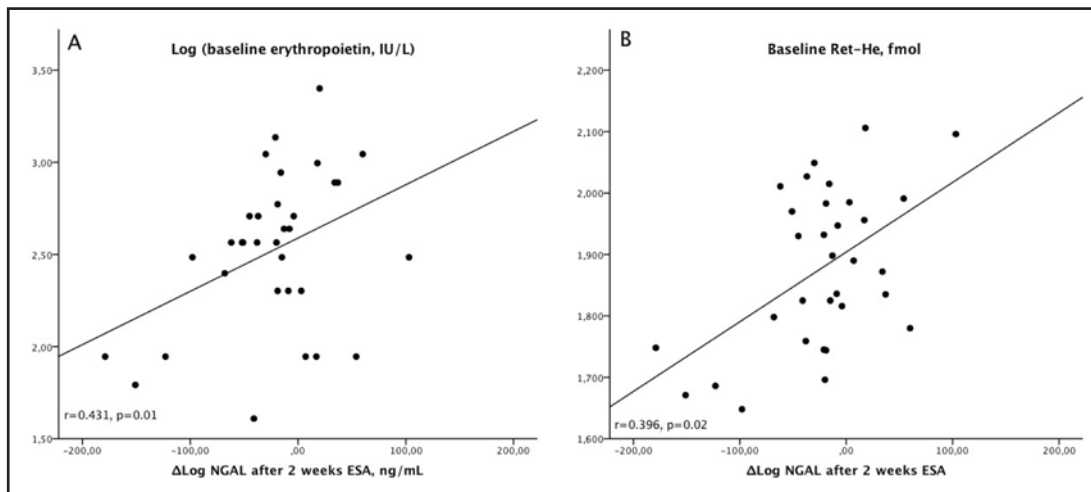

Fig. 3. The correlation between the magnitude in log-transformed serum NGAL decrease after two weeks of ESA treatment and baseline log-transformed erythropoietin levels (A) and baseline reticulocyte hemoglobin content levels (B). Abbreviations: NGAL, neutrophil gelatinase associated lipocalin; ESA, erythropoiesis-stimulating agent; Ret-He, reticulocyte hemoglobin content.

Furthermore, the Hb AUC in the control group (-2.2 ± 9.4) did not differ from the ESA-Hb-stable group (p= 0.99).

NGAL values after 26 weeks showed no significant changes compared to baseline levels in all three the study groups (ESA-Hb-rise, 168 [144-259] vs 195 [146-266] ng/mL, p= 0.27; ESA-Hb-stable 152 [128-211] vs 187 [126-280] ng/mL, p= 0.97; control group 244 [115-319] vs 238 [129-313], p= 0.99). Nor were there any significant changes in cystatin C or eCrCl values after 26 weeks compared to baseline (data not shown).

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Discussion

The main finding of the present study is that in combined CKD and CHF elevated serum NGAL levels inversely correlate with baseline EPO levels, independent of renal function. Concurrently low dose ESA treatment induced a moderate decrease in serum NGAL levels. However, there was no long-term effect of 26 weeks ESA treatment on NGAL levels. Lastly, although our study confirms that baseline serum NGAL levels are elevated in mildly anemic patients with combined CKD and CHF, NGAL levels did not correlate with parameters of iron metabolism.

Recent reports show that decreased serum NGAL levels in hemodialysis patients correlate with higher (total) hepcidin levels and lower TSAT [11, 12], suggesting that low serum NGAL levels are associated with reduced iron utilization. Both CKD and CHF are chronic inflammatory conditions in which iron metabolism is disturbed. Therefore, we further explored the findings of Bolignano et al [12] and Malyszko et al [11], in a cohort of oral iron supplemented, mildly anemic patients with combined chronic CHF and CKD. In this cohort we found, unexpectedly, no association between serum NGAL levels and parameters for iron availability, as assessed by hepcidin-25 levels (determined by mass spectrometry), reticulocyte hemoglobin content (Ret-He), soluble transferrin receptor (sTfR) and TSAT. In addition, we detected a moderate association between serum NGAL levels and inflammation, as estimated by hs-CRP and IL-6, However, it should be noted that the median hs-CRP levels in this stable, low–inflammatory cohort were only mildly elevated.

An important finding of our study is that serum NGAL levels inversely correlated with endogenous EPO levels, independent of renal function, and that NGAL levels, contrary to our hypothesis, acutely decreased in response to low-dose ESA treatment, albeit that the response was modest and variable. To our knowledge, this is the first study that demonstrates an effect of low-dose ESA treatment on serum NGAL levels. A stronger decrease in NGAL levels correlated with higher baseline EPO levels and Ret-He. However, this effect was no longer present after 6 months treatment, regardless of maintained Hb-levels by phlebotomies or increased Hb-levels.

It is unlikely that in our study NGAL levels reflected iron metabolism as baseline NGAL levels and the ESA induced decrease in NGAL were not associated with markers of iron metabolism such as hepcidin-25. A possible explanation for our findings is that in our population serum NGAL is a marker of on-going tubular damage. This finding is in agreement with studies showing that NGAL predominantly reflects tubular damage in acute conditions [25, 26]. A comparable association was already explored about ten years ago for IgA nephropathy; urinary N-acetyl-β-D-glucosaminidase (NAG), a marker of tubular injury, correlated with EPO levels [27]. Also, several animal studies show a direct cytoprotective effect of ESA on intrinsic renal cells [28, 29]. In a rat model of ischemic injury, low doses of darbepoietin, not resulting in increases in hematocrit levels, significantly reduced glomerulosclerosis and tubulointerstitial damage, and rarefaction of peritubular capillaries was prevented [28]. It should be pointed out that, although initial studies suggested a beneficial effect of fix-dose ESA administration on renal function [28, 30], this was not confirmed in later studies that used variable doses of ESA to reach preset hemoglobin targets [31, 32]. However, in order to corroborate a role for NGAL as tubular marker in our study, urinary NGAL levels and other urinary markers should be performed, which was not the case in this study [33].

The present study admittedly has some limitations. First, the study size is relatively small which is due to the complexity of the study design. Studying univariate correlations is of limited value is a small cohort. However, we believe that the lack of association between serum NGAL levels and iron metabolism is unambiguous, due to its assessment by multiple parameters for iron metabolism, including hepcidin-25 levels. However, we cannot exclude the possibility that the lack of univariate association between NGAL and inflammation is due to lack of power.

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Conclusions

Serum NGAL levels in combined CHF/CKD do not reflect iron homeostasis, as assessed by hepcidin-25 levels, Ret-He, sTfR and TSAT and therefore may reflect the tubular damage. Short-term low-dose ESA treatment discreetly decreases serum NGAL levels, which might possibly reflect an effect of ESA on tubular damage. Furthermore, baseline EPO levels correlate with serum NGAL levels, independent of eCrCl or Hb-levels. Further research is required to investigate these acute effects of low-dose ESA treatment on serum NGAL levels in CHF, CKD or the combination of both.

Conflict of Interest

The authors of this manuscript state that they have no conflicts of interest.

Acknowledgements

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