Original Paper

Plasma Neutrophil Gelatinase-Associated Lipocalin Reflects Both Inflammation and Kidney Function in Patients with Myocardial Infarction

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Key Words

Neutrophil gelatinase-associated lipocalin · Estimated glomerular filtration rate · Kidney function · Inflammation · Acute kidney injury

Abstract

Background/Aims: Neutrophil gelatinase-associated lipocalin (NGAL) has emerged as a marker for acute kidney injury and cardiovascular outcome. However, the relative importance of inflammation versus kidney function on plasma NGAL levels is uncertain, making the interpretation of plasma NGAL unclear. Accordingly, we investigated the relationship between plasma NGAL, inflammation and kidney function in patients with myocardial infarction (MI).

Methods: We prospectively included 584 patients with acute ST-segment elevation MI (STEMI) treated with primary percutaneous coronary intervention (PCI) from 2006 to 2008. Blood samples were drawn immediately before PCI. Additionally, we included 42 patients who had 4 blood samples drawn before and after PCI. Plasma NGAL was measured using a time-resolved immunofluorometric assay. Cross-sectional analyses were performed in these two single-center, prospective study cohorts. **Results:** Estimated glomerular filtration rate (eGFR) was associated significantly more strongly with plasma NGAL when eGFR was abnormal compared to normal eGFR: a decrease in eGFR of 10 ml/min was associated with an increase in NGAL of 27% (18–36%) versus 4% (1–7%), respectively ($p < 0.001$). Leukocyte count and C-reactive protein were the main determinants of plasma NGAL in patients with normal eGFR, whereas eGFR was the main determinant at reduced kidney function. **Conclusions:** eGFR determines the as-

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sociation of NGAL with either inflammation or kidney function; in patients with normal eGFR, plasma NGAL reflects inflammation but when eGFR is reduced, plasma NGAL reflects kidney function, highlighting the dual perception of plasma NGAL. From a clinical perspective, eGFR may be used to guide the interpretation of elevated NGAL levels in patients with STEMI.

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Introduction

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa protein associated with the endopeptidase matrix metalloproteinase-9 (MMP-9) and stored in granules of mature neutrophils [1]. Moreover, NGAL is released by renal tubular cells upon nephrotoxic or ischemic events and is considered an early marker of tubular damage and acute kidney injury (AKI) related to poor outcome [2–4].

Recent research has focused on NGAL in cardiovascular disease. NGAL is overexpressed in atheromatous plaques [5] and correlates with characteristics of plaque instability. Through its complex formation with MMP-9, which prolongs the proteolytic activity, NGAL is also an important mediator of vascular remodeling [6–8]. Levels of plasma NGAL are associated with C-reactive protein (CRP), are elevated in coronary artery disease and even further in acute MI and are a strong predictor of outcome in these patients [9, 10]. Despite this emerging role of NGAL in cardiovascular disease, human studies of plasma NGAL are still limited. It appears as if plasma NGAL mirrors inflammation, chronic kidney disease and AKI; for this reason the interpretation of increased plasma NGAL levels remains unclear.

In the present study we measured plasma NGAL in 626 patients with ST-segment elevation myocardial infarction (STEMI) including a subgroup of 42 STEMI patients with 4 blood samples drawn before and after percutaneous coronary intervention (PCI), in order to elucidate the relationship between plasma NGAL, inflammation and kidney function in patients with myocardial infarction (MI).

Methods

Study Population

We examined plasma NGAL in patients with STEMI in two single-center, prospective study cohorts. We prospectively included 584 STEMI patients treated with primary PCI from September 2006 through December 2008 as previously described [11]. In brief, inclusion criteria were as follows: patients admitted due to a suspected STEMI with the presence of chest pain for >30 min and <12 h, and persistent ST-segment elevation ≥ 2 mm in at least 2 contiguous precordial ECG leads or ≥ 1 mm in at least two contiguous limb ECG leads. Exclusion criteria were as follows: primary PCI was not performed (no occlusion on coronary artery angiography or coronary artery bypass surgery was elected instead). A total of 1,030 patients met the inclusion criteria; however, because of the exclusion criteria a total of 735 patients were included in the study. In the present study, 151 patients were excluded because of missing values of plasma NGAL.

In order to investigate longitudinal changes in NGAL and estimated glomerular filtration rate (eGFR) during the hospitalization for STEMI, we also included 42 STEMI patients with 4 blood samples drawn before and after PCI who were included from February 2008 through March 2011 [12]. In- and exclusion criteria were similar although only patients with single-vessel disease and a 100% thrombotic occlusion of the left descending artery and a successful PCI, besides no previous history of MI or heart failure, were included.

All patients were included at the Gentofte University Hospital, Denmark, a high-volume PCI center with present on-site cardiac surgery performing >1,500 PCI procedures a year. Glycoprotein IIb/IIIa inhibitors were used at the discretion of the operator. Subsequent medical treatment included daily aspirin 75 mg, clopidogrel 75 mg (for 12 months), lipid-lowering drugs (statins) and β -receptor antagonists.

We defined hypertension, hypercholesterolemia and diabetes according to the use of antihypertensive, cholesterol-lowering and antidiabetic drugs, respectively, on admission. Fifty-six percent (n = 350) were

examined with conventional two-dimensional echocardiography (using Vivid 7, GE Healthcare, Horten, Norway) at a median of 2 days (IQR: 1–3) after PCI. Additionally, 98% ($n = 41$) of the patients with consecutive blood samples during the hospitalization were examined again after a median of 7 months (IQR: 7–8 months). LVEF was obtained off-line (using EchoPac, GE Healthcare) using the modified Simpson biplane method.

The local scientific ethics committee approved the study and the terms of the Declaration of Helsinki were observed. Informed consent was obtained from all participants.

Laboratory Methods

Blood samples were drawn from the femoral sheath before the PCI procedure in all patients. Additional blood samples were obtained immediately after the PCI and on the following 2 days in 42 patients. Blood was put into 4-ml EDTA containers and centrifuged within 30 min. Plasma was stored in Nunc CryoTubes at -80°C until analysis in a blinded fashion in a dedicated core laboratory. Plasma NGAL was determined using an in-house time-resolved immunofluorometric assay based on NGAL antibodies and recombinant NGAL from R&D Systems (Abingdon, UK) [13]. Samples and controls were diluted 1:100 and analyzed in duplicate. The intra-assay coefficient of variation of standards, controls, unknown samples and nonspecific background controls averaged less than 5%. The interassay coefficient of variation averaged $<9\%$ based on standards, nonspecific background controls, internal controls and repeated samples. Plasma NGAL concentrations were determined using a five-parameter standard curve fit implemented in the WorkOut 2.5 Data Analysis software (PerkinElmer Inc.). Mean values were calculated and used for statistical analyses. CRP, troponin I (TnI) and creatinine were assayed by routine laboratory methods (different TnI assays were used for the two populations, making comparisons of values impossible). TnI was measured at baseline and again after 6 and 12 h. eGFR was calculated at all times on the basis of serum creatinine, age and gender using the CKD-EPI formula.

Statistical Analysis

Plasma NGAL, CRP, TnI and leukocyte concentrations were positively skewed and therefore logarithmically transformed using the base logarithm of 2 before further analysis. Associations were evaluated using generalized linear regression models, validated with plots of residuals, fitted values and leverage. The nonlinear association between plasma NGAL and eGFR was identified and modeled using regression splines. The explanation of the variation in plasma NGAL for each variable was calculated as the mean square of a variable divided by the total mean square for all variables in the model. Changes in plasma NGAL levels were evaluated using repeated measures analysis of variance (ANOVA). In case of a violation of Mauchly's test of sphericity the Greenhouse-Geisser correction was used. Statistical calculations were performed using the SAS statistical software (SAS for Windows, release 9.2, SAS Institute Inc., Cary, N.C., USA).

Results

In the total population, plasma NGAL levels at admission (median, IQR) were similar in patients with blood samples evaluated only at baseline compared to patients with serial blood samples taken: $123.6\text{ }\mu\text{g/l}$ ($94.7\text{--}170.5\text{ }\mu\text{g/l}$) versus $128.6\text{ }\mu\text{g/l}$ ($101.6\text{--}163.4\text{ }\mu\text{g/l}$) ($p = 0.58$), respectively. Baseline characteristics of the total study population are presented in table 1 according to eGFR at admission. Notably, patients with decreased renal function (eGFR $<60\text{ ml/min}$) had higher NGAL levels than patients with normal renal function (eGFR $\geq 60\text{ ml/min}$); however, they were also older, more likely to have cardiogenic shock and had higher levels of peak TnI, CRP and leukocytes.

NGAL and Kidney Function

Plasma NGAL and eGFR were significantly associated at admission ($r = -0.48$, $p < 0.001$) (fig. 1a). Importantly, this association appeared to be nonlinear. Indeed this was confirmed using regression splines ($P_{\text{nonlinear}} < 0.001$) (fig. 1b); eGFR was associated more strongly with plasma NGAL when eGFR was reduced ($\leq 60\text{ ml/min}$) compared to when eGFR was in the normal range ($>60\text{ ml/min}$). Considering this nonlinear relationship, eGFR correlated strongly

Table 1. Baseline characteristics

Variables	eGFR ≥ 60 ml/min (n = 460)	eGFR < 60 ml/min (n = 166)	p value
Age, years	60 $\pm$ 11	71 $\pm$ 12	<0.001
Male sex	77%	65%	0.002
Hypertension	27%	46%	<0.001
Diabetes	9%	11%	0.25
Current smoking	56%	34%	<0.001
Hypercholesterolemia	17%	17%	0.54
Previous MI	4%	7%	0.17
Body mass index	26.5 $\pm$ 4.3	26.8 $\pm$ 5.0	0.56
Systolic blood pressure, mm Hg	135 $\pm$ 24	128 $\pm$ 9	0.015
Peak TnI, μ g/l	115 (32–241)	160 (37–338)	0.031
CRP, mg/l	3 (1–8)	5 (2–14)	0.001
Leukocyte count, 10^9 /l	12.1 (9.8–15.2)	13.2 (10.8–16.2)	0.007
Serum creatinine, μ mol/l	81 (72–94)	123 (109–149)	<0.001
eGFR, ml/min	84 $\pm$ 15	43 $\pm$ 14	<0.001
LVEF ^a	46 $\pm$ 9%	44 $\pm$ 9%	0.053
Cardiogenic shock at admission	8%	18%	<0.001
Symptom-to-balloon time, min	195 (129–307)	195 (135–315)	0.63
Multivessel disease	23%	30%	0.05
LAD lesion	51%	51%	0.97
Glycoprotein IIb/IIIa inhibitor	28%	28%	0.50
Plasma NGAL, μ g/l	112 (88–147)	167 (123–291)	<0.001

Dichotomous variables are presented as percentage, Gaussian values distributed as mean $\pm$ standard deviation and non-Gaussian values distributed as median (IQR). LAD = Left anterior descending coronary artery. ^aAvailable in 56% (n = 350).

Fig. 1. Nonlinear association between plasma NGAL and eGFR. **a** Plasma NGAL according to categories of eGFR. Values are given as median (center line), 25th and 75th percentiles (box), 10th and 90th percentiles (top and bottom). **b** The association between log2-NGAL and eGFR and the nonlinear regression line.

with plasma NGAL in patients with reduced eGFR ($r = -0.58$, $p < 0.001$), whereas the correlation was significantly weaker in patients with normal eGFR ($r = -0.13$, $p = 0.008$).

Importantly, this nonlinear association between eGFR and plasma NGAL persisted after multivariable adjustment; a decrease in eGFR of 10 ml/min was associated with an increase in NGAL of 27% (18–36%) in patients with reduced eGFR versus 4% (1–7%) in those with

Table 2. Associations between NGAL and covariates stratified by eGFR

Variables	Contribution to plasma NGAL, %	Multivariable	
		β	p value
<i>eGFR >60 ml/min</i>			
Leukocyte count (per doubling) ^a	58	68% (48–91)	<0.001
CRP (per doubling) ^a	11	4% (2–6)	<0.001
Male gender ^b	6	13% (3–24)	0.008
eGFR (decrease per 10 ml/min) ^c	4	4% (1–7)	0.027
<i>eGFR ≤60 ml/min</i>			
eGFR (decrease per 10 ml/min) ^c	48	27% (18–36)	<0.001
Leukocyte count (per doubling) ^a	14	46% (21–78)	<0.001
Glycoprotein IIb/IIIa inhibitor treatment ^b	11	–25% (–38 to –10)	0.002
Male gender ^b	6	24% (4–47)	0.016

Variables included in the multivariable model are as follows: age, gender, hypertension, diabetes, hypercholesterolemia, smoking, previous MI, body mass index, CRP, leukocyte count, eGFR, symptom-to-balloon time, multivessel disease, left anterior descending coronary artery lesion and glycoprotein inhibitor.

^a β -Coefficient indicates increment in plasma NGAL per doubling of the variable. ^b β -Coefficient indicates increment (positive or negative) in plasma NGAL if variable is present. ^c β -Coefficient indicates increment in plasma NGAL per decrease of 10 ml/min in eGFR.

Fig. 2. Plasma NGAL during hospitalization for STEMI stratified according to categories of eGFR. Values are shown as geometric mean calculated on the log scale and back-transformed for ease of interpretation.

normal eGFR ($p < 0.001$). Accordingly, associations between NGAL and other covariates were stratified according to eGFR (table 2). As seen, in patients with eGFR in the normal range, the leukocyte count was the main determinant of plasma NGAL, whereas eGFR contributed little. By contrast, eGFR was the main determinant of plasma NGAL in patients with reduced eGFR.

Out of the 626 patients, 4 consecutive blood samples were obtained in 42 patients thus allowing for serial measurements of plasma NGAL levels. Of these 42 patients, 4 had reduced eGFR (≤ 60 ml/min) at admission. In agreement with the previous results, plasma NGAL was significantly higher in these 4 patients compared to the patients with normal eGFR. Importantly this difference in plasma NGAL remained throughout the hospitalization (repeated measures ANOVA, $F = 5.723$, $p = 0.022$) with the mean plasma NGAL being 49% higher (6–109%) in patients with reduced eGFR (fig. 2).

Table 3. Association between
NGAL change and eGFR change

NGAL change	eGFR change	
	β^a	p value
Univariable	–1.32 (–2.38 to –0.27)	0.014
Model 1	–2.24 (–3.27 to –1.22)	<0.001
Model 2	–2.30 (–3.34 to –1.25)	<0.001
Model 3	–2.35 (–3.37 to –1.34)	<0.001

Model 1 = Admission NGAL and eGFR; model 2 = model 1 + age and gender; model 3 = model 2 + use of glycoprotein inhibitor, TnI, CRP and leukocyte count. ^a β -Coefficient indicates increment in eGFR per 10 $\mu\text{g/l}$ increase in plasma NGAL during the observation period (total of 4 blood samples).

Changes in Plasma Levels of NGAL

In the 42 patients with serial measurements of NGAL, eGFR decreased during the hospitalization (from 92 ± 22 to 83 ± 20 ml/min, $p < 0.001$). Even in the group with normal eGFR at admission ($n = 38$) eGFR decreased during the hospitalization (97 ± 14 to 87 ± 17 ml/min, $p < 0.001$) indicating renal impairment and possibly AKI. Interestingly, even in these 38 patients with normal eGFR at admission, during the hospitalization, the correlation between eGFR and plasma NGAL became progressively stronger, suggesting that in the days following an MI, plasma NGAL increasingly reflects eGFR, thus demonstrating a distinct shift from mirroring inflammation towards kidney function (fig. 3).

In order to further investigate the relationship between changes in NGAL and eGFR, we analyzed whether changes in NGAL during the hospitalization were associated with changes in eGFR during the admission (table 3). As seen, even in multivariable models, increasing NGAL remained independently associated with decreasing eGFR. The association between a change in NGAL and a change in eGFR was heavily influenced by 3 patients. As a sensitivity analysis, we tried excluding these 3 patients; importantly the association persisted after the exclusion of these outliers [β : -1.01 (-1.91 to -0.10), $p = 0.029$].

NGAL Levels, CRP and Cardiac Function

Plasma NGAL levels at admission were associated with CRP ($p < 0.001$) both in patients with normal and reduced eGFR (fig. 4). However, after adjusting for confounding factors NGAL was only associated with CRP in patients with normal renal function (table 2).

Recent data suggest that the association between plasma NGAL and cardiovascular outcome could be explained by NGAL upregulation in cardiomyocytes in the failing myocardium in response to proinflammatory cytokines [14]. Due to our finding of an association between plasma NGAL and inflammation, we investigated a possible association between NGAL and cardiac function estimated by LVEF which was available in 56% ($n = 350$). In these patients, plasma NGAL was not significantly associated with LVEF ($p = 0.11$) (fig. 5).

Furthermore, we also analyzed whether NGAL levels during the hospitalization ($n = 42$) were altered in patients with low LVEF ($<35\%$); however, NGAL levels were similar in patients with low and high LVEF (repeated measures ANOVA, $F = 0.827$, $p = 0.45$). Finally, we investigated whether NGAL was associated with LVEF after a median of 7 months following the MI ($n = 42$); however, again we did not find a significant association (repeated measures ANOVA, $F = 1.242$, $p = 0.27$).

Lindberg et al.: Plasma Neutrophil Gelatinase-Associated Lipocalin Reflects Both Inflammation and Kidney Function in Patients with Myocardial Infarction

Fig. 3. Correlation between NGAL and eGFR before and after PCI. The relationship between plasma NGAL and eGFR in the 4 blood samples obtained during the hospitalization for STEMI is shown.

Fig. 4. Association between plasma NGAL and CRP. Plasma NGAL according to categories of CRP stratified according to eGFR >60 ml/min (black) and eGFR ≤60 ml/min (grey). Median (center line), 25th and 75th percentiles (box), and 10th and 90th percentiles (top and bottom) are shown.

Discussion

We have recently shown that eGFR and plasma NGAL are associated in a nonlinear fashion in the general population; when eGFR is reduced (≤ 60 ml/min) eGFR is the strongest determinant of plasma NGAL. By contrast, when eGFR is in the normal range (> 60 ml/min) eGFR contributes little to plasma NGAL [15].

Fig. 5. Association between LVEF and NGAL. Mean and 95% CI calculated on the log scale and back-transformed for ease of interpretation are shown.

The main finding of the present study is that we were able to confirm this finding in an independent population, extending it to patients with MI. Furthermore, we show that even in patients with normal eGFR, plasma NGAL was associated progressively more strongly with eGFR following the MI, demonstrating a distinct shift from inflammation towards kidney function, highlighting the dual perception of plasma NGAL. By contrast, we did not find an association with LVEF.

NGAL is expressed in granulocytes, epithelial cells, hepatocytes and renal tubular cells and released during injury [16, 17]. NGAL is now viewed as a multifunctional glycoprotein with various biological characteristics including bacteriostatic properties and nephron-inducing activity [18]. NGAL also acts as a scavenger in inflamed areas, modulating inflammation through interaction with the chemotactic peptide fMLP [19, 20]. Several studies have demonstrated that increased levels of NGAL in urine, serum and plasma precede classic markers of kidney damage such as creatinine and β_2 -microglobulin levels. NGAL has therefore become a novel biomarker of AKI [4, 21–23] and according to guidelines from the Acute Dialysis Quality Initiative NGAL is likely to become integrated in clinical practice [24].

In more than 5,000 participants from the general population, the neutrophil leukocyte count and hsCRP were the main determinants of plasma NGAL. By contrast, eGFR was mainly associated with plasma NGAL when eGFR was reduced [15]. Accordingly, we speculate that in healthy humans, plasma NGAL mainly reflects inflammation, whereas the kidney only contributes to plasma NGAL in the setting of chronic kidney disease or AKI.

The present study confirms the association with inflammation and chronic kidney disease. Our finding that NGAL was associated progressively more strongly with eGFR following the acute event demonstrates a distinct shift from mirroring inflammation towards kidney function, highlighting the dual perception of plasma NGAL. It seems plausible that the shift from being associated with inflammation to kidney function is caused by underlying subclinical AKI, which is common in acute hospitalized patients, as NGAL predicts AKI 24–72 h before creatinine increases in critically ill patients undergoing cardiac surgery [3, 4, 25].

It appears that plasma levels of NGAL reflect the sum contribution from several sources mainly neutrophil leukocytes and renal tubular cells, while the prognostic/diagnostic value of NGAL seems to depend on the specific clinical setting. However, as both synthesis and secretion of NGAL are highly induced in the proximal tubules following AKI, urinary NGAL

may be a more specific marker of AKI [3, 26]. Indeed, Parikh et al. [25] showed that urine NGAL but not plasma NGAL was associated with postoperative AKI in children undergoing cardiac surgery. Unfortunately, urine samples were not available in the present study.

NGAL has been suggested to play a role in atherosclerosis due to the binding to MMP-9 which is involved in the degradation of extracellular matrix in atherosclerotic plaques leading to plaque rupture [5, 14, 27–29]. Hemdahl et al. [5] showed that increased NGAL expression in atherosclerotic plaques co-localized with MMP-9 in areas with high proteolytic activity. Along this line, an increased expression of NGAL was observed in cardiomyocytes in patients with heart failure, where the NGAL-MMP-9 complex may be involved in myocardial remodeling [14]. Elevated levels of plasma NGAL have been associated with poor prognosis and are a strong predictor of outcome in patients with acute MI and heart failure [30, 31]. We have also reported this in the 584 patients with STEMI, although it still remains speculative whether the prognostic role of NGAL is due to the relationship with inflammation and/or AKI [11]. Due to the reported independent association between plasma NGAL and cardiovascular outcome, even after adjusting for eGFR and CRP, it has been hypothesized that plasma NGAL through cardiac remodeling may be associated with cardiac dysfunction. However, in patients with chronic or acute heart failure, Shrestha et al. [32] did not find an association between plasma NGAL and LVEF. Our findings confirm this, suggesting another pathophysiological mechanism than myocardial damage caused by increased inflammation.

Study Limitations and Strengths

The present findings are based on observational data and thus cannot prove causality. Our data are based only on measurements of plasma NGAL. It would have strengthened the conclusions if urinary NGAL had been available. Finally, we used eGFR as a surrogate marker of kidney function, knowing that eGFR has limitations when used in patients with unstable creatinine such as acute hospitalized patients with MI [33].

Conclusion

We show that in patients with STEMI, eGFR determines the association of NGAL with either inflammation or kidney function; in patients with normal eGFR, plasma NGAL reflects inflammation, whereas when eGFR is reduced, plasma NGAL reflects kidney function. Moreover, our data demonstrate a dynamic shift from NGAL mirroring inflammation before treatment with PCI towards NGAL mirroring kidney function after PCI in patients with STEMI, thus showing the dual properties of NGAL as a marker of both inflammation and AKI.

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Disclosure Statement

None.

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