Published in final edited form as:
J Card Fail. 2011 June ; 17(6): 472–478. doi:10.1016/j.cardfail.2011.02.003.

Renal Dysfunction is a Stronger Determinant of Systemic Neutrophil Gelatinase-Associated Lipocalin Levels Than Myocardial Dysfunction in Systolic Heart Failure

Kevin Shrestha, AB, **Allen G. Borowski, RDCS**, **Richard W. Troughton, MBBS**, **James D. Thomas, MD**, **Allan L. Klein, MD**, and **W. H. Wilson Tang, MD**

[a]Department of Cardiovascular Medicine, the Cleveland Clinic, Cleveland, Ohio, U.S.A.
[b]Department of Cardiology, Christchurch School of Medicine and Health Sciences, Christchurch, New Zealand.

Abstract

Background—Neutrophil gelatinase-associated lipocalin (NGAL) is released by renal tubular cells in response to inflammation and injury. Recent studies have demonstrated that NGAL is upregulated in cardiomyocytes within the failing myocardium. However, the overall relationship between systemic NGAL levels and myocardial structure and performance has not been established.

Methods and Results—We measured systemic NGAL levels in 130 subjects with chronic systolic heart failure (HF) and comprehensive echocardiographic evaluation, as well as 69 subjects with acute decompensated systolic HF and hemodynamic evaluation. In the chronic HF cohort, higher plasma NGAL levels were modestly associated with increasing age ($r= 0.18$, $p=0.035$), higher NYHA class (rank sums, $p=0.022$) and impaired renal function (eGFR: $r= -0.53$, $p<0.0001$; cystatin C: $r= 0.60$, $p<0.0001$). Plasma NGAL levels were modestly associated with indices of diastolic dysfunction (mitral E/Ea: $r= 0.27$, $p=0.002$; LAVi, $r= 0.25$, $p=0.011$; tricuspid E/Ea: $r= 0.20$, $p=0.029$), but not after adjustment for renal function ($p>0.10$ for all). In Cox proportional hazards analysis, plasma NGAL predicted cardiac death or transplantation after adjustment for age, gender, LVEF, and mitral E/Ea (Hazard ratio 1.68, 95% confidence interval 1.08 – 2.57, $p=0.022$), but not after adjustment for renal function ($p=0.83$). In the acute HF cohort, we did not observe any relationship between NGAL and hemodynamic indices, but NGAL strongly correlated with renal function.

Conclusions—Systemic NGAL levels are largely determined by underlying impairment of renal rather than myocardial function. Our findings did not support any relationship or prognostic significance between systemic NGAL levels and indices of cardiac structure and function after adjustment for underlying renal function.

Corresponding author: W. H. Wilson Tang, MD Department of Cardiovascular Medicine Heart and Vascular Institute, Cleveland Clinic. 9500 Euclid Avenue, Desk J3-4, Cleveland, OH 44195, USA. Phone: (216) 444-2121 / Fax: (216) 445-6165 / tangw@ccf.org.

DISCLOSURES

Dr. Tang has previously received research grant support from Abbott Laboratories. All other authors declare no relationships to disclose.

Keywords

Congestive heart failure; NGAL; renal insufficiency; cardio-renal

INTRODUCTION

Neutrophil gelatinase-associated lipocalin (NGAL; also known in humans as lipocalin-2 (Lcn-2), human neutrophil lipocalin (HNL), and oncogene protein 24p3) is a 25-kDa lipocalin superfamily glycoprotein and acute phase reactant rapidly released in response to a variety of cellular stresses including ischemia and inflammation (1,2). Urinary and systemic NGAL measurements have emerged as promising and sensitive biomarkers of early acute kidney injury (AKI) in a diverse range of settings (3-5), as NGAL rapidly appears in both blood and urine (rising 10 to 100 fold respectively within hours) in response to renal tubular damage (6,7). In the setting of chronic kidney disease, NGAL expression may represent active tubular damage beyond a marker of decreased glomerular filtration alone (8,9) and predicts disease progression (10).

Due to the high incidence and poor prognosis associated with worsening renal function (WRF) in the setting of heart failure (HF), earlier reports have highlighted a role for NGAL in predicting WRF in the acute decompensated HF setting (11). Both systemic and urine NGAL also constitute sensitive markers of renal impairment in patients with chronic HF (12,13). A recent report has suggested that NGAL may be upregulated in cardiomyocytes within the failing myocardium itself, putatively in response to pro-inflammatory cytokines including interleukin-1β, as well as activators of the innate immune response, including TLR-2 and TLR-4 agonists (14). In the setting of myocarditis, the presence of cardiomyocyte expression of both NGAL and its specific receptor (24p3R) further implies cardiac-specific NGAL expression in response to pro-inflammatory cytokines (15). Hence, it is postulated that NGAL may constitute an important compensatory response to acute and chronic inflammatory and oxidative stress within the failing myocardium (16-19). The objective of this study is therefore to examine the relationship between systemic NGAL levels and myocardial structure, performance and prognostic significance in HF across the spectrum of underlying renal function.

METHODS

Study Design and Population

We examined the determinants and prognostic significance of systemic NGAL in human systolic HF in two single-center, prospective study cohorts. All subjects gave informed consent as approved by the Cleveland Clinic Institutional Review Board. In our chronic systolic HF cohort, plasma NGAL were measured in 130 consecutive subjects 18 to 75 years of age, with a diagnosis of heart failure for at least 3 months, a left ventricular ejection fraction (LVEF) ≤35% at the time of enrollment, New York Heart Association (NYHA) functional class I-IV symptoms, and were free of significant renal, hepatic, and valvular diseases (20). Subjects were followed prospectively by telephone follow-up and chart review for adverse clinical events (all-cause mortality or cardiac transplantation), with all-cause mortality data confirmed by Social Security Death Index to 3 years of follow-up.

In the acute decompensated systolic HF cohort, baseline serum NGAL levels were measured in 69 patients admitted to the Cleveland Clinic heart failure intensive care unit for intensive medical therapy under invasive hemodynamic evaluation. In 47 of these patients, serum NGAL levels and hemodynamic indices were also measured at 24-72 hours after baseline. Eligible subjects were 18 years of age or older, had a LVEF ≤35% for at least 6 months,

elevated filling pressures defined by a pulmonary capillary wedge pressure (PCWP) ≥18 mmHg and/or a central venous pressure (CVP) >8 mmHg, NYHA functional class III-IV symptoms. Exclusion criteria included mechanical ventilation, renal replacement therapy, post-cardiac transplantation, and post tricuspid valve surgery.

Transthoracic Echocardiography

All subjects underwent comprehensive echocardiographic evaluation of cardiac structure as well as systolic and diastolic performance by an experienced sonographer. Comprehensive transthoracic echocardiography was performed using commercially available HDI 5000 (Phillips Medical Systems, N.A., Bothell, Washington) and Acuson Sequoia (Siemens Medical Solutions USA Inc., Malvern, Pennsylvania) machines for our chronic systolic HF cohort and commercially available Vingmed, System Seven (General Electric Healthcare, USA) machine for our acute systolic HF cohort. Two-dimensional and color Doppler imaging was performed in standard parasternal and apical views. Diastolic indices (including pulse-wave Doppler and tissue Doppler imaging) were acquired over ten consecutive beats using sweep speeds of 50 and 100 cm/s using previously described techniques(20,21). Classification of diastolic stage was determined according to the following modifications of the recommendations set forth by the American Society of Echocardiography (22). Normal diastolic function was defined by a left atrial volume index (LAVi) < 34 mL/m^2, septal Ea ≥ 8 cm/s and lateral Ea ≥ 10 cm/s. Presence of diastolic dysfunction was defined initially by LAVi ≥ 34 mL/m^2 and either septal Ea < 8 cm/s or lateral Ea < 10 cm/s. For subjects that met these initial diastolic dysfunction criteria, Stages I to III were subsequently determined according to the presence of the following criteria. *Stage I (impaired relaxation)* was defined by the presence of at least two of the following criteria: E/A < 0.8, mitral deceleration time (DT) > 200 ms, average E/Ea ≤ 8, and Ar - A < 0 ms. *Stage II (pseudonormal)* was defined by the presence of at least two of the following criteria: E/A 0.8-1.5, mitral DT 160-200 ms, average E/Ea 9-12, and Ar - A ≥ 30 ms. *Stage III (restrictive)* was defined by the presence of at least two of the following criteria: E/A ≥ 2, mitral DT < 160 ms, average E/Ea ≥ 13, and Ar - A ≥ 30 ms. The LV ejection fraction and cardiac volumes were measured using Simpson's biplane method. LV mass was calculated according to previously published recommendations (23). All ventricular volume and mass measurements were indexed to body surface area (BSA). Measurements were averaged over three cycles.

Systemic NGAL Levels

All samples were collected into ethylenediaminetetraacetic acid (EDTA)-plasma or serum separator vacuum collecting tubes on ice simultaneously at the time of echocardiographic and hemodynamic evaluation, processed and immediately frozen in aliquots at −80°C until analyzed. All laboratory analyses were performed with investigators blinded to cardio-renal indices and clinical outcomes data. Plasma and serum NGAL levels were measured by a research enzyme-linked immunosorbent assay (Cat. No. KIT 036, BioPorto Diagnostics, Gentofte Denmark). The minimum detection limit of the assay was 20 ng/mL. Intra-assay and inter-assay coefficients of variation (CVs) were <5% at 65 ng/mL.

Other Biomarker Measurements

Systemic cystatin C levels were determined by the N Latex cystatin C assay (Dade-Behring, Deerfield IL), a latex-enhanced nephelmetric immunoassay using rabbit polyclonal antibodies as previously described(24). The minimum detection limit of the assay was 0.25 ng/mL. Intra-assay and inter-assay CVs were < 1.8%. Estimated glomerular filtration rate (eGFR) was calculated based on serum creatinine levels using the standard 4-variable Modification of Diet in Renal Disease equation(25). Plasma NT-proBNP levels were assayed using a commercially available immunoassay based on electrochemiluminescence

technology (Roche Elecsys® NT-proBNP assay, Roche Diagnostics, Indianapolis IN). The minimum detection limit of the assay was 5 pg/mL. Intra-assay and inter-assay CVs were <3%.

Statistical Analysis

Continuous variables were summarized as mean ± standard deviation if normally distributed, and as median and interquartile range [IQR] if non-normally distributed. Normality was assessed by the Shapiro-Wilk W test. Spearman's rank correlation method was used as a nonparametric measure of association for correlations between NGAL levels and clinical, echocardiographic and hemodynamic indices. The Wilcoxon rank-sum or Kruskal-Wallis tests were used to compare differences in NGAL levels across categorical variables. The Wilcoxon signed-rank test was used to assess differences in NGAL levels from baseline to 24-72 hours following baseline within our acute systolic HF cohort. The Cox proportional hazards regression model was used to analyze the time to adverse event (all-cause mortality or cardiac transplantation) associated with increasing NGAL levels (with natural logarithmic transformation). The proportional hazards assumption was verified with log(time) vs. log[-log(survival)] plots. Multiplicative interaction terms were included in each Cox model to assess whether or not the interaction term was significant. Kaplan-Meier survival plots were calculated from baseline to time of all-cause mortality or cardiac transplantation. All p-values reported are from two-sided tests and a p-value <0.05 was considered statistically significant. Statistical analyses were performed using JMP 8.0.2 (SAS Institute, Cary, NC) and R version 2.10.1 (Vienna Austria).

RESULTS

Study Populations

Table 1 illustrates the baseline characteristics of our chronic and acute systolic HF cohorts. In our chronic HF study cohort, mean and median plasma NGAL levels were 93 ± 63 ng/mL and 77 [IQR: 58, 108] ng/mL, respectively. In our acute HF cohort, mean and median serum NGAL levels were higher at 151 ± 99 ng/mL and 129 [83, 196] ng/mL, respectively. Higher plasma NGAL levels were associated with advanced age (chronic HF: r= 0.18, p=0.035; acute HF: r=0.26, p=0.028) and higher plasma NT-proBNP levels (chronic HF: r=0.24, p=0.008; acute HF: r=0.38, p=0.004). In the chronic HF cohort, subjects with ischemic etiology demonstrated higher NGAL levels compared to non-ischemic etiology (84 [64-129] versus 72 [52-102] ng/mL, respectively, p=0.024). In the acute HF cohort, follow-up serum NGAL levels measured at 24-72 hours from baseline did not differ significantly from baseline levels (Wilcoxon signed-rank p=0.97). In both cohorts, systemic NGAL levels did not differ according to gender, ethnicity, history of hypertension or diabetes mellitus, or any medication use.

Systemic NGAL Levels and Renal Function

In both the chronic and acute HF cohorts, higher systemic NGAL levels were strongly associated with markers of poor renal function, including eGFR, serum creatinine, blood urea nitrogen, and cystatin C (Table 2). In chronic HF patients with relatively preserved renal function (eGFR ≥60 mL/min/1.73 m^2; n=83), plasma NGAL levels also correlated with indices of renal dysfunction including plasma cystatin C (p<0.001 for all; Figure 1).

NGAL Levels and Myocardial Structure and Performance

Table 3 presents the relationship between systemic NGAL levels and echocardiographic indices of cardiac structure and function. In both HF cohorts, systemic NGAL levels were

not related to echocardiographic indices of LV cardiac structure or LV or RV systolic function.

In the chronic HF cohort, plasma NGAL levels were modestly associated with echocardiographic indices of LV diastolic dysfunction, including the ratio of mitral E to mitral annular tissue Doppler imaging (TDI) septal Ea (mitral E/Ea) and left atrial volume index (LAVi) (Table 3), but not overall diastolic stage (rank sums p=0.21). It is interesting to note that in patients with relatively preserved renal function (eGFR ≥60 mL/min/1.73 m^2; n=83) or below-median plasma cystatin C (CysC <1.22 ng/mL; n=60), these relationships were no longer statistically significant (p>0.07 for all). Similarly, in multiple regression analysis, these relationships were not preserved following univariate adjustment for either eGFR or plasma cystatin C (p>0.10 for all).

In both HF cohorts, there were weak but statistically significant correlations between systemic NGAL levels and indices of RV diastolic function, including the ratio of tricuspid E to tricuspid annular TDI lateral Ea ratio (tricuspid E/Ea in chronic HF: r= 0.20, p=0.029; in acute HF: r=0.26, p=0.041) and hepatic vein S/D ratio (in chronic HF: r= −0.28, p=0.009; in acute HF: r=-0.45, p=0.029). However, in multiple regression analysis, these relationships were not significant after adjustment for plasma cystatin C levels (p>0.10 for all).

Systemic NGAL Levels and Hemodynamic Indices in Acute Decompensated Heart Failure

We observed no association between systemic NGAL levels and hemodynamic indices of intracardiac pressures or cardiac function, including pulmonary capillary wedge pressure (r= −0.04, p=0.73), mean pulmonary artery pressure (r= 0.13, p=0.28), central venous pressure (r= 0.08, p=0.49), or calculated Fick cardiac index (r= 0.14, p=0.25). Furthermore, the change in systemic NGAL levels from baseline to 24-72 hours was not associated with baseline hemodynamic indices (p>0.26 for all) or the change in hemodynamic indices from baseline to 24-72 hours (p>0.44 for all).

Prognostic Value of Systemic NGAL Levels in Chronic Systolic Heart Failure

Table 4 illustrates the age- and gender-adjusted multivariate models constructed to examine the prognostic value of systemic NGAL levels in the chronic HF cohort. Higher plasma NGAL levels predicted the composite endpoint of all-cause mortality or cardiac transplantation (HR: 1.76, 95% CI: 1.19 – 2.58, p=0.004). In multivariate analysis, plasma NGAL predicted adverse events after adjustment for age, gender, and echocardiographic indices of LV systolic and diastolic dysfunction (Table 4). However, plasma NGAL did not predict adverse events after adjustment for plasma cystatin C levels (Table 4; Figure 2) or plasma NT-proBNP levels (Table 4). In model testing for interaction between plasma NGAL and cystatin C levels, the multiplicative interaction term of plasma NGAL and cystatin C was significant within the Cox proportional hazards model for three year clinical outcomes (p=0.030). In comparison, plasma NGAL levels did not demonstrate significant interaction with age, gender, medication use, or echocardiographic indices of LV systolic or diastolic (p>0.07 for all).

DISCUSSION

We report for the first time the absence of any relationship between systemic NGAL levels and myocardial structure and performance in both acute and chronic systolic HF after adjustment for underlying renal impairment. Furthermore, we report the prognostic value of systemic NGAL levels in chronic systolic HF to remain significant following adjustment for clinical and echocardiographic indices of cardiac structure and function, but not following adjustment for cystatin C or NT-proBNP. These findings support the hypothesis that

systemic NGAL levels are largely determined by underlying impaired renal function, and demonstrate the absence of any prognostic significance or relationship between systemic NGAL levels and indices of cardiac structure and function after adjustment for underlying renal function.

Marked upregulation of NGAL in response to renal tubular cell injury has enabled the emergence of both urine and systemic NGAL as sensitive biomarkers of early acute kidney injury as well as progression of chronic kidney disease (6,7,10). Upregulation of NGAL systemically and in renal tubular epithelial cells is believed to constitute an important acute phase compensatory response, protecting against tubule cell death and enhancing re-epithelialization according to the abilities of NGAL-siderophore complexes to deliver iron into renal tubular cells, signaling antioxidant, anti-apoptotic, and cell proliferative pathways, and to scavenge excess free intracellular and extracellular iron, limiting labile iron-mediated cytotoxicity (7,31). As demonstrated in our findings, the relationship between systemic NGAL levels and indices of renal impairment remained robust in both the acute and chronic systolic HF settings. In addition, the prognostic value of systemic NGAL for prediction of long-term adverse clinical events was not significant following adjustment for cystatin C, a sensitive marker of renal impairment. These results corroborate prior reports demonstrating the relationship between elevated systemic NGAL levels and renal insufficiency, and support the utility of systemic NGAL for detecting underlying renal vulnerability that may predict subsequent WRF in the acute HF setting (11).

It is conceivable that heightened, chronic systemic NGAL expression may in addition reflect and potentially contribute to progressive adverse ventricular remodeling and fibrosis in the failing heart. As in renal tubular cells, cardiomyocyte NGAL expression may constitute an important acute phase anti-oxidant and anti-apoptotic compensatory response that reflects ongoing myocardial inflammatory and oxidative stress (14,15,32,33). In addition, while acute elevations may be protective, chronic elevations in NGAL may play a role in matrix degradation and myocardial remodeling, in addition to reduced atherosclerotic plaque stability, in heart failure disease progression (14,34,35). NGAL has been shown to form a heterodimer complex with matrix metalloproteinase-9 (MMP-9), enhancing and prolonging its proteolytic activity by supporting its allosteric activation and inhibiting its autodegradation by tissue inhibitor of metalloproteinase-1 (TIMP-1) (36,37). MMP-9 is known to be upregulated in failing hearts, and an increased ratio of MMP to TIMP activity has been implicated in left ventricular fibrosis, dilation and cardiac dysfunction (38).

Despite these postulates of a role for NGAL as a potential marker of myocardial inflammatory and oxidative stress and adverse progressive ventricular remodeling, we did not observe any relationship in the acute or chronic systolic HF settings between systemic NGAL levels and echocardiographic indices of cardiac structure or systolic function, and only a limited relationship between NGAL and indices of left and right ventricular diastolic function which was not significant after adjustment for underlying renal function. Furthermore, in our acute systolic HF setting, systemic NGAL levels were not related to invasive hemodynamic measures including pulmonary capillary wedge pressure, mean pulmonary artery pressure, central venous pressure and cardiac index.

Our findings do not yet rule out the importance of local myocardial expression of NGAL which may not be reflected in systemic NGAL levels, and the consequences of local autocrine or paracrine NGAL signaling secondary to cardiomyocyte expression may warrant future investigations. We also did not have serial data within our chronic systolic HF cohort to assess the relationship between changes in systemic NGAL levels with changes in indices of myocardial and renal function as well as impact of drug therapy. Urine specimens also were not collected for either our chronic or acute systolic HF cohorts. We therefore were not

able to compare urine versus systemic NGAL levels in relation to renal and cardiac determinants as well as prognostic significance. In addition, the relatively low sample size and event rate in our chronic heart failure cohort limited multivariable adjustment in Cox proportional hazards analyses. Nevertheless, our observations imply that systemic NGAL levels are predominantly determined by impairment of renal rather than myocardial function in the HF setting. Any interpretation that systemic NGAL levels may reflect underlying myocardial responses or remodeling in patients with heart failure is therefore not supported by our observations, and at present NGAL should be considered a marker of renal insufficiency.

CONCLUSION

Consistent with prior reports, elevated systemic NGAL levels are associated with renal insufficiency in both acute and chronic systolic HF. We also observed associations between elevated systemic NGAL levels and poorer prognosis in the chronic systolic HF setting. However, we did not observe any statistically significant relationship between systemic NGAL levels and indices of myocardial structure and performance after adjustment for underlying renal function. Taken together, our observations imply that systemic NGAL levels are predominantly determined by impairment of renal rather than myocardial function in the HF setting.

Acknowledgments

Financial Disclosure/Support: This work was also supported in part by grants from the American Society of Echocardiography, the National Institutes of Health Clinical and Translational Science Award (CTSA UL1-RR024989), and the National Space Biomedical Research Institute (NASA NCC 9-58).

REFERENCES

1. Xu S, Venge P. Lipocalins as biochemical markers of disease. Biochim Biophys Acta. Oct 18; 2000 1482(1-2):298–307. [PubMed: 11058770]
2. Kjeldsen L, Johnsen AH, Sengelov H, Borregaard N. Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. J Biol Chem. May 15; 1993 268(14): 10425–32. [PubMed: 7683678]
3. Mishra J, Ma Q, Prada A, Mitsnefes M, Zahedi K, Yang J, et al. Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. J Am Soc Nephrol. Oct; 2003 14(10):2534–43. [PubMed: 14514731]
4. Mishra J, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, Kelly C, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet. Apr 2-8; 2005 365(9466):1231–8. [PubMed: 15811456]
5. Nickolas TL, O'Rourke MJ, Yang J, Sise ME, Canetta PA, Barasch N, et al. Sensitivity and specificity of a single emergency department measurement of urinary neutrophil gelatinase-associated lipocalin for diagnosing acute kidney injury. Ann Intern Med. Jun 3; 2008 148(11):810–9. [PubMed: 18519927]
6. Schmidt-Ott KM, Mori K, Li JY, Kalandadze A, Cohen DJ, Devarajan P, et al. Dual action of neutrophil gelatinase-associated lipocalin. J Am Soc Nephrol. Feb; 2007 18(2):407–13. [PubMed: 17229907]
7. Mori K, Lee HT, Rapoport D, Drexler IR, Foster K, Yang J, et al. Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. J Clin Invest. Mar; 2005 115(3):610–21. [PubMed: 15711640]
8. Mori K, Nakao K. Neutrophil gelatinase-associated lipocalin as the real-time indicator of active kidney damage. Kidney Int. May; 2007 71(10):967–70. [PubMed: 17342180]

9. Bolignano D, Lacquaniti A, Coppolino G, Campo S, Arena A, Buemi M. Neutrophil gelatinase-associated lipocalin reflects the severity of renal impairment in subjects affected by chronic kidney disease. Kidney Blood Press Res. 2008; 31(4):255–8. [PubMed: 18600028]
10. Bolignano D, Lacquaniti A, Coppolino G, Donato V, Campo S, Fazio MR, et al. Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. Clin J Am Soc Nephrol. Feb; 2009 4(2):337–44. [PubMed: 19176795]
11. Aghel A, Shrestha K, Mullens W, Borowski A, Tang WH. Serum neutrophil gelatinase-associated lipocalin (NGAL) in predicting worsening renal function in acute decompensated heart failure. J Card Fail. Jan; 2010 16(1):49–54. [PubMed: 20123318]
12. Damman K, van Veldhuisen DJ, Navis G, Voors AA, Hillege HL. Urinary neutrophil gelatinase associated lipocalin (NGAL), a marker of tubular damage, is increased in patients with chronic heart failure. Eur J Heart Fail. Oct; 2008 10(10):997–1000. [PubMed: 18804416]
13. Poniatowski B, Malyszko J, Bachorzewska-Gajewska H, Malyszko JS, Dobrzycki S. Serum neutrophil gelatinase-associated lipocalin as a marker of renal function in patients with chronic heart failure and coronary artery disease. Kidney Blood Press Res. 2009; 32(2):77–80. [PubMed: 19287180]
14. Yndestad A, Landro L, Ueland T, Dahl CP, Flo TH, Vinge LE, et al. Increased systemic and myocardial expression of neutrophil gelatinase-associated lipocalin in clinical and experimental heart failure. Eur Heart J. May; 2009 30(10):1229–36. [PubMed: 19329498]
15. Ding L, Hanawa H, Ota Y, Hasegawa G, Hao K, Asami F, et al. Lipocalin-2/neutrophil gelatinase-B associated lipocalin is strongly induced in hearts of rats with autoimmune myocarditis and in human myocarditis. Circ J. 2010; 74(3):523–30. [PubMed: 20057160]
16. Bahmani P, Halabian R, Rouhbakhsh M, Roushandeh AM, Masroori N, Ebrahimi M, et al. Neutrophil Gelatinase-Associated Lipocalin induces the expression of heme oxygenase-1 and superoxide dismutase (1, 2). Cell Stress Chaperones. Nov 11.2009
17. Roudkenar MH, Halabian R, Ghasemipour Z, Roushandeh AM, Rouhbakhsh M, Nekogoftar M, et al. Neutrophil gelatinase-associated lipocalin acts as a protective factor against H(2)O(2) toxicity. Arch Med Res. Aug; 2008 39(6):560–6. [PubMed: 18662586]
18. Ding L, Hanawa H, Ota Y, Hasegawa G, Hao K, Asami F, et al. Lipocalin-2/Neutrophil Gelatinase-B Associated Lipocalin is Strongly Induced in Hearts of Rats With Autoimmune Myocarditis and in Human Myocarditis. Circ J. Jan 7.2010
19. Tong Z, Wu X, Ovcharenko D, Zhu J, Chen CS, Kehrer JP. Neutrophil gelatinase-associated lipocalin as a survival factor. Biochem J. Oct 15; 2005 391(Pt 2):441–8. [PubMed: 16060857]
20. Troughton RW, Prior DL, Pereira JJ, Martin M, Fogarty A, Morehead A, et al. Plasma B-type natriuretic peptide levels in systolic heart failure: importance of left ventricular diastolic function and right ventricular systolic function. J Am Coll Cardiol. Feb 4; 2004 43(3):416–22. [PubMed: 15013124]
21. Troughton RW, Prior DL, Frampton CM, Nash PJ, Pereira JJ, Martin M, et al. Usefulness of tissue doppler and color M-mode indexes of left ventricular diastolic function in predicting outcomes in systolic left ventricular heart failure (from the ADEPT study). Am J Cardiol. Jul 15; 2005 96(2): 257–62. [PubMed: 16018853]
22. Nagueh SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. J Am Soc Echocardiogr. Feb; 2009 22(2):107–33. [PubMed: 19187853]
23. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr. Dec; 2005 18(12):1440–63. [PubMed: 16376782]
24. Tang WH, Van Lente F, Shrestha K, Troughton RW, Francis GS, Tong W, et al. Impact of myocardial function on cystatin C measurements in chronic systolic heart failure. J Card Fail. Jun; 2008 14(5):394–9. [PubMed: 18514931]

25. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med. Mar 16; 1999 130(6):461–70. [PubMed: 10075613]
26. Tang WH, Shrestha K, Van Lente F, Troughton RW, Martin MG, Borowski AG, et al. Usefulness of C-reactive protein and left ventricular diastolic performance for prognosis in patients with left ventricular systolic heart failure. Am J Cardiol. Feb 1; 2008 101(3):370–3. [PubMed: 18237602]
27. Tang WH, Tong W, Troughton RW, Martin MG, Shrestha K, Borowski A, et al. Prognostic value and echocardiographic determinants of plasma myeloperoxidase levels in chronic heart failure. J Am Coll Cardiol. Jun 19; 2007 49(24):2364–70. [PubMed: 17572253]
28. Schmidt-Ott KM, Mori K, Kalandadze A, Li JY, Paragas N, Nicholas T, et al. Neutrophil gelatinase-associated lipocalin-mediated iron traffic in kidney epithelia. Curr Opin Nephrol Hypertens. Jul; 2006 15(4):442–9. [PubMed: 16775460]
29. Li X, Hassoun HT, Santora R, Rabb H. Organ crosstalk: the role of the kidney. Curr Opin Crit Care. Dec; 2009 15(6):481–7. [PubMed: 19851101]
30. Grigoryev DN, Liu M, Hassoun HT, Cheadle C, Barnes KC, Rabb H. The local and systemic inflammatory transcriptome after acute kidney injury. J Am Soc Nephrol. Mar; 2008 19(3):547–58. [PubMed: 18235097]
31. Haase M, Bellomo R, Haase-Fielitz A. Novel biomarkers, oxidative stress, and the role of labile iron toxicity in cardiopulmonary bypass-associated acute kidney injury. J Am Coll Cardiol. May 11; 55(19):2024–33. [PubMed: 20447525]
32. Roudkenar MH, Kuwahara Y, Baba T, Roushandeh AM, Ebishima S, Abe S, et al. Oxidative stress induced lipocalin 2 gene expression: addressing its expression under the harmful conditions. J Radiat Res (Tokyo). Jan; 2007 48(1):39–44. [PubMed: 17229997]
33. Aigner F, Maier HT, Schwelberger HG, Wallnofer EA, Amberger A, Obrist P, et al. Lipocalin-2 regulates the inflammatory response during ischemia and reperfusion of the transplanted heart. Am J Transplant. Apr; 2007 7(4):779–88. [PubMed: 17391123]
34. Bu DX, Hemdahl AL, Gabrielsen A, Fuxe J, Zhu C, Eriksson P, et al. Induction of neutrophil gelatinase-associated lipocalin in vascular injury via activation of nuclear factor-kappaB. Am J Pathol. Dec; 2006 169(6):2245–53. [PubMed: 17148685]
35. Hemdahl AL, Gabrielsen A, Zhu C, Eriksson P, Hedin U, Kastrup J, et al. Expression of neutrophil gelatinase-associated lipocalin in atherosclerosis and myocardial infarction. Arterioscler Thromb Vasc Biol. Jan; 2006 26(1):136–42. [PubMed: 16254208]
36. Tschesche H, Zolzer V, Triebel S, Bartsch S. The human neutrophil lipocalin supports the allosteric activation of matrix metalloproteinases. Eur J Biochem. Apr; 2001 268(7):1918–28. [PubMed: 11277914]
37. Yan L, Borregaard N, Kjeldsen L, Moses MA. The high molecular weight urinary matrix metalloproteinase (MMP) activity is a complex of gelatinase B/MMP-9 and neutrophil gelatinase-associated lipocalin (NGAL). Modulation of MMP-9 activity by NGAL. J Biol Chem. Oct 5; 2001 276(40):37258–65. [PubMed: 11486009]
38. Yan AT, Yan RT, Spinale FG, Afzal R, Gunasinghe HR, Arnold M, et al. Plasma matrix metalloproteinase-9 level is correlated with left ventricular volumes and ejection fraction in patients with heart failure. J Card Fail. Sep; 2006 12(7):514–9. [PubMed: 16952784]

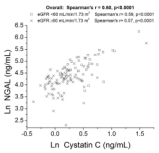

Figure 1.

Correlation between plasma NGAL and plasma CysC levels within the chronic HF cohort (n=130) stratified according to subjects with relatively preserved renal function (eGFR ≥60 mL/min/1.73 m^2; n=83) versus without (eGFR <60 mL/min/1.73 m^2; n=39). eGFR, estimated glomerular filtration rate; NGAL, neutrophil gelatinase-associated lipocalin; CysC, cystatin C.

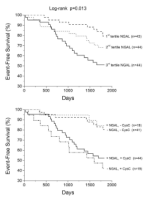

Figure 2.
Kaplan-Meier analysis of all-cause mortality or cardiac transplantation in chronic HF. (A) Patients stratified according plasma NGAL tertiles (first tertile: <64 ng/mL; second tertile: 64-98 ng/mL; third tertile: ≥98 ng/mL). (B) Patients stratified according to above (+) versus below (−) median plasma CysC (1.22 ng/mL) and median plasma NGAL (77 ng/mL) levels. NGAL, neutrophil gelatinase-associated lipocalin; CysC, cystatin C.

Table 1

Baseline Subject Characteristics for Chronic HF (n=130) and Acute HF (n=69) Cohorts

Variable	Chronic HF (n=130)	Acute HF (n=69)
Demographics:		
Age (years)	57 ± 13	56 ± 13
Male gender, n (%)	98 (75%)	54 (78%)
BMI (kg/m^2)	28 ± 5	28 ± 6
African American, n (%)	22 (17%)	10 (15%)
Caucasian, n (%)	108 (83%)	58 (85%)
Heart failure history:		
Ischemic etiology, n (%)	57 (44%)	37 (54%)
NYHA class III or IV, n (%)	43 (33%)	55 (95%)
Co-morbidities:		
Hypertension, n (%)	70 (55%)	29 (42%)
Diabetes mellitus, n (%)	37 (29%)	25 (36%)
Echocardiographic indices:		
LV mass index (g/m^2)	157 ± 46	187 ± 58
LVEDVi (mL/m^2)	110 ± 35	112 ± 45
LVEF (%)	26 ± 6	28 ± 9
Diastolic stage III, n (%)	34 (54%)	43 (90%)
Medications:		
ACE inhibitors and/or ARBs, n (%)	119 (94%)	20 (30%)
Beta-blockers, n (%)	83 (65%)	31 (47%)
Spironolactone, n (%)	32 (27%)	19 (29%)
Loop diuretics, n (%)	98 (77%)	31 (47%)
Digoxin, n (%)	74 (62%)	21 (32%)
Laboratory data:		
NT-proBNP (pg/mL)	1241 [540, 3292]	4510 [2177, 8539]
Cystatin C (ng/mL)	1.22 [1.03, 1.60]	1.61 [1.19, 2.55]
eGFR (mL/min/1.73m^2)	72 ± 25	71 ± 42
NGAL (ng/mL)	77 [58, 108]	129 [83, 196]

Abbreviations: BMI, body mass index; NYHA, New York Heart Association; LV, left ventricular; LVEDVi, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; NT-proBNP, amino-terminal pro-B-type natriuretic peptide; eGFR, estimated glomerular filtration rate; NGAL, neutrophil gelatinase-associated lipocalin.

Table 2

Univariate Correlations between Systemic NGAL Levels and Indices of Renal Impairment for Chronic HF (n=130) and Acute HF (n=69) Cohorts.

	Chronic HF (n=130)		Acute HF (n=69)	
Variable	**r**	**p**	**r**	**p**
Estimated GFR	−0.53	<0.0001	−0.51	<0.0001
Creatinine	0.52	<0.0001	0.51	<0.0001
Blood urea nitrogen	0.42	<0.0001	0.45	<0.001
Cystatin C	0.60	<0.0001	0.65	<0.0001

Abbreviations: GFR, glomerular filtration rate; HF, heart failure.

Table 3

Univariate Correlations between Systemic NGAL Levels and Echocardiographic Characteristics for Chronic HF (n=130) and Acute HF (n=69) Cohorts.

	Chronic HF (n=130)		Acute HF (n=69)	
Variable	**r**	**p**	**r**	**p**
LV Structure:				
LV mass index (g/m^2)	0.00	0.994	0.06	0.657
LVEDVi (mL/m^2)	−0.01	0.938	0.02	0.873
LV Systolic Function:				
LVEF (%)	0.04	0.705	0.02	0.895
LV Diastolic Function:				
Mitral E/A	0.17	0.061	−0.02	0.891
Mitral DT (ms)	−0.12	0.203	0.26	0.034
Pulmonary vein S/D	−0.17	0.060	−0.22	0.123
Mitral E/Ea	0.27	0.002	0.14	0.265
LAVi (mL/m^2)	0.25	0.011	0.19	0.115
RV Systolic Function:				
RV S wave	−0.16	0.068	−0.09	0.452
RV Diastolic Function:				
Tricuspid E/A	0.12	0.205	−0.12	0.406
Tricuspid DT (ms)	−0.04	0.695	0.08	0.671
Hepatic vein S/D	−0.28	0.009	−0.45	0.029
Tricuspid E/Ea	0.20	0.029	0.26	0.041
RAVi (mL/m^2)	0.14	0.135	0.12	0.338

Abbreviations: LV, left ventricular; LVEDVi, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; DT, deceleration time; LAVi, left atrial volume index; RV, right ventricular; RAVi, right atrial volume index; HF, heart failure.

Table 4

Age- and Gender-Adjusted Multivariate Cox Proportional Hazards Analyses of Time to Adverse Clinical Events in Chronic HF subjects (n=130).

	3 year Death/Transplant (27 events)	
Variable	**HR (95% CI)**	**p-value**
Univariable Model		
NGAL (ng/mL)*	1.76 (1.19 – 2.58)	0.004
Multivariable Model 1		
NGAL (ng/mL)*	1.68 (1.08 – 2.57)	0.022
LVEF (%)*	1.15 (0.71 – 1.88)	0.578
Mitral E/Ea*	1.21 (0.82 – 1.64)	0.309
Multivariable Model 2		
NGAL (ng/mL)*	1.06 (0.66 – 1.84)	0.831
Cystatin C (ng/mL)*	1.69 (1.06 – 2.59)	0.027
Multivariable Model 3		
NGAL (ng/mL)*	1.40 (0.92 – 2.21)	0.117
NT-proBNP (pg/mL)*	1.59 (1.03 – 2.48)	0.038

Abbreviations: NGAL, neutrophil gelatinase-associated lipocalin; LVEF, left ventricular ejection fraction; NT-proBNP, amino-terminal pro-B-type natriuretic peptide.

*All multivariable models adjusted for age and gender. Abbreviations: NGAL, cystatin C and NT-proBNP are natural logarithmic transformed. Hazard ratios per 1 SD increment (1 SD Ln NGAL = 0.58 ng/mL, 1 SD Age = 13.4 years; 1 SD LVEF = 5.9%; 1 SD E/septal Ea = 12.0; 1 SD Ln Cystatin C = 0.34 ng/mL; 1 SD Ln NT-proBNP = 1.27 pg/mL).