Published in final edited form as:

J Card Fail. 2010 January ; 16(1): 49–54. doi:10.1016/j.cardfail.2009.07.003.

Serum Neutrophil Gelatinase-Associated Lipocalin (NGAL) in Predicting Worsening Renal Function in Acute Decompensated Heart Failure

ARASH AGHEL, MD¹, KEVIN SHRESTHA, AB¹, WILFRIED MULLENS, MD³, ALLEN BOROWSKI, RDCS², and W. H. WILSON TANG, MD²

¹ Education Institute, Cleveland Clinic Foundation, Cleveland, OH

² Department of Cardiovascular Medicine, Heart and Vascular Institute, Cleveland Clinic Foundation, Cleveland, OH

³ Ziekenhuis Oost Limburg, Genk, Belgium

Abstract

Background—The development of worsening renal function (WRF, defined as creatinine rise ≥ 0.3 mg/dL) occurs frequently in the setting of acute decompensated heart failure (ADHF) and strongly predicts adverse clinical outcomes. Neutrophil gelatinase-associated lipocalin (NGAL) is produced by the nephron in response to tubular epithelial damage and serves as an early marker for acute renal tubular injury. We sought to determine the relationship between admission serum NGAL levels and WRF in the setting of ADHF.

Methods and Results—We measured serum NGAL levels in 91 patients admitted to the hospital with ADHF. Patients were adjudicated by independent physician into those that did or did not develop WRF over the ensuing 5 days of in-hospital treatment. In our study cohort (68% male, mean age 61 ± 15 years, mean left ventricular ejection fraction $31 \pm 14\%$), median admission serum NGAL level was 165 ng/mL (interquartile range [IQR] 108–235 ng/mL). Thirty-five patients (38%) developed WRF within the 5-day follow-up. Patients who developed WRF versus those without WRF had significantly higher median admission serum NGAL levels (194 [IQR 150–292] ng/mL vs. 128 [IQR 97–214] ng/mL, $P = .001$). High serum NGAL levels at admission were associated with greater likelihood of developing WRF (odds ratio: 1.92, 95% confidence interval 1.23–3.12, $P = .004$). In particular, admission NGAL ≥ 140 ng/mL had a 7.4-fold increase in risk of developing WRF, with a sensitivity and specificity of 86% and 54%, respectively.

Conclusions—The presence of elevated admission serum NGAL levels is associated with heightened risk of subsequent development of WRF in patients admitted with ADHF.

Keywords

Heart failure; renal failure; worsening renal function

Worsening renal function (WRF) during admissions for acute decompensated heart failure (ADHF) has been directly associated with poor prognosis. The underlying causes of WRF are multifactorial, but to some extent can be related to the underlying derangements in

cardiorenal interactions as well as adverse efforts to aggressively diurese patients in an attempt to relieve congestion. The detection of WRF is usually achieved by serial measurements of serum creatinine levels, but their rising trends may occur late in the process of end-organ dysfunction. Identifying underlying acute kidney injury early in admission may prevent the development of WRF.

Neutrophil gelatinase-associated lipocalin (NGAL) is a novel serum or urine biomarker of acute kidney injury and has been studied in the setting of post-cardiac surgery, cardiac catheterization, hemolytic uremic syndrome, and kidney transplantation.^{1–6} Expression of NGAL mRNA has been shown to be induced rapidly in renal tubules in response to acute injury.⁷ Baseline urinary measurement has also been independently associated with the diagnosis of acute renal injury.⁸ Herein, we sought to evaluate the role of serum NGAL measurements in predicting WRF in the setting of ADHF. Specifically, we aim to explore the ability of higher serum NGAL early in the treatment course to serve as an antecedent “warning sign” to subsequent renal impairment.

Methods

Study Population

Between September 2006 and March 2008, hospital admissions were screened using electronic medical records for the primary diagnosis of ADHF within 12 hours of admission. Eligible patients were then evaluated at the bedside for the presence of clinical evidence of congestive heart failure (shortness of breath, orthopnea, paroxysmal nocturnal dyspnea, fatigue), plus physical examination findings of fluid retention (including but not limited to presence of rales, jugular venous distention, or peripheral edema). Patients with congenital heart disease, critical aortic stenosis, end-stage renal disease on dialysis, known exposure to nephrotoxic agents (ie, contrast dye), known urinary tract infection, or expected hospital stay less than 24 hours were excluded.

Study Design

This is a single-center prospective cohort study approved by the Cleveland Clinic Institutional Review Board, and all subjects provided written consent. Treatment for ADHF was based on standard of care and was entirely independent of the study. After informed consent, blood samples were drawn for study purposes on hospital day 1 and day 3. Subject demographics and clinical history, relevant comorbidities, and documented left ventricular ejection fraction within the past 3 months were collected upon enrollment. Changes in blood urea nitrogen and creatinine were monitored during their hospital stay for 5 days or until hospital discharge (whichever came first). Glomerular filtration rate was calculated using the standard 4-variable Modification of Diet in Renal Disease equation.⁹ WRF was defined as rise in serum creatinine ≥ 0.3 mg/dL from admission as used in prior studies.^{10–12}

Serum NGAL Measurements

Samples for NGAL assay were obtained at enrollment (admission) and at 48 to 72 hours after enrollment (day 3), and were immediately processed, aliquoted, and stored at -80°F until sample analysis. NGAL was measured by a research enzyme-linked immunosorbent assay (Cat. No. KIT 036, BioPorto Diagnostics, Gentofte Denmark). A 2000-fold dilution was used to bring concentrations of each sample within the 100-fold sensitivity range of the assay, 10 to 1000 pg/mL. All diluted samples fell within the range of the calibration curve.

Statistical Analysis

Continuous variables were summarized as mean $\pm$ standard deviation if normally distributed. Non-normally distributed continuous variables were summarized as median and

inter-quartile range (IQR). Categorical variables were summarized as proportions and frequencies. 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 serum NGAL, creatinine, and estimated glomerular filtration rate (eGFR). The serum levels of NGAL and all other admission clinical characteristics were compared between patients who developed WRF and those who did not using the Student *t*-test for normally distributed variables and the Wilcoxon rank sum test for non-normally distributed variables. Proportions for admission clinical characteristics were compared between patients who developed WRF and those who did not using contingency table analysis. Odds ratios for the prediction of WRF were calculated through logistic regression analysis using NGAL, blood urea nitrogen, creatinine, and eGFR modeled as continuous variables and evaluated according to the likelihood ratio test. The Cox proportional hazards model was used to assess the clinical risks of increasing continuous standardized increments of admission NGAL. The proportional hazards assumption was verified with log[time] versus log(-log[survival]) plots. All *P* values reported are from 2-sided tests and a *P* value < .05 was considered statistically significant. All statistical analyses were performed using JMP 5.1, SAS 9.1.3 (SAS Institute, Cary, NC) and PASS (Kaysville, UT). The authors had full access to the data and take responsibility for data integrity. All authors have read and agree to the manuscript as written.

Results

Subject Population

Clinical characteristics of patients are summarized in Table 1. The median values of serum NGAL were 165 [IQR 108–235] ng/mL at admission and 177 [IQR 111–272] ng/mL at day 3. Overall, there was a modest correlation between admission NGAL and admission serum creatinine ($r = 0.57$, $P < .0001$) and eGFR (Spearman's $r = -0.60$, $P < .0001$).

NGAL Levels in Worsening Renal Function

In our study cohort, 35 patients (38%) developed WRF within the 5-day follow-up. Of these patients, 46% had this occur by day 3. Figure 1 demonstrates serum NGAL levels at admission and at day 3 with WRF diagnoses superimposed on each histogram. Patients who developed WRF versus those without WRF had significantly higher admission serum NGAL levels (194 [IQR 150–292] ng/mL vs. 128 [IQR 97–214] ng/mL, $P = .001$) and day 3 NGAL levels (221 [IQR 171–298] ng/mL vs. 145 [IQR 100–219] ng/mL, $P = .001$). Figure 2 demonstrates serum NGAL levels at admission and at day 3 stratified by occurrence of WRF. In patients with admission serum creatinine levels within the normal range (≤ 1.4 mg/dL), those who developed WRF versus those without WRF also had significantly higher admission serum NGAL levels (176 [IQR 143–238] ng/mL vs. 107 [IQR 81–145] ng/mL, $P = .002$, $n = 46$) and day 3 NGAL levels (214 [IQR 170–313] ng/mL vs. 112 [IQR 83–148] ng/mL, $P = .001$, $n = 42$).

Comparing Renal Indices in Predicting Worsening Renal Function

Table 2 shows the logistic regression analysis associated with WRF according to different admission characteristics. High admission serum NGAL levels were associated with greater likelihood of subsequently developing WRF (odds ratio [OR]: 1.92, 95% CI: 1.23–3.12, $P = .004$). After adjusting for admission serum creatinine, admission NGAL levels were still associated with development of WRF (OR: 1.84, 95% CI: 1.08–3.26, $P = .02$). In contrast, admission serum creatinine was not associated with WRF in this model. By receiver operating characteristic curve analysis, admission serum NGAL ≥ 140 ng/mL had a sensitivity and specificity of 86% and 54%, respectively, to predict the development of WRF (area under the curve = 0.70, 95% CI = 0.585–0.803, $P = .004$, Figure 3). Based on the given

sensitivity and specificity, the positive and negative likelihood ratios were calculated as 1.85 and 0.27, respectively. Furthermore, the negative predictive value of admission serum NGAL < 140 ng/mL for developing WRF was 86%, and the risk of development of WRF in those with admission serum NGAL $\geq$ 140 ng/mL compared with < 140 ng/mL was more than 7-fold (OR: 7.44, 95% CI = 2.69–24.37, $P < .0001$).

Admission Serum NGAL Levels and Short-term Follow-up

Over a mean follow-up of 17 ± 8 months, there were 12 deaths in our study population. In Cox proportional hazard analysis, admission serum NGAL $\geq$ 215 ng/mL (optimal receiver operating characteristic cutoff) conferred a higher hazard ratio for death (HR: 3.39, 95% CI: 1.08–11.45, $P = .037$). Higher admission serum NGAL modeled as a continuous variable trended toward conferring a higher hazard ratio for death (HR: 1.64, 95% CI: 0.97–2.70, $P = .063$).

Discussion

There are several key findings in this hypothesis-generating study that support a relationship between renal tubular injury and the development of renal impairment in the setting of ADHF. We confirmed the previously reported relationship between serum NGAL levels and estimated glomerular filtration rate in the setting of chronic heart failure.¹³ More importantly, we demonstrated for the first time the ability of serum NGAL levels measured early during hospital admission to predict the subsequent development of WRF. Importantly, this is particularly true in those with preserved renal function on admission. Taken together, our findings support that the presence of underlying acute tubular injury, detected by elevated admission NGAL measurements, may be directly associated with development of WRF during hospital stay in the setting of ADHF. Therefore, a rise in NGAL levels may warrant considerations of renal-sparing therapeutic strategies to reduce the risk of subsequent WRF.

Traditional explanations regarding the mechanisms of WRF in the setting of ADHF include overzealous diuresis, leading to reduced renal perfusion, and low cardiac output heart failure resulting in acute tubular injury. These can lead to excessive neurohormonal activation and altered tubuloglomerular feedback. In addition, more recent studies have demonstrated the association between venous congestion rather than low cardiac output with WRF in ADHF.¹⁴ Clearly, no single mechanism can explain this complex pathophysiologic interaction between the failing heart and the impaired kidneys, especially when confounded with an iatrogenic objective to relieve congestion without any reliable physiologic guidance. Given the heterogeneity of patients who develop WRF, the use of more sensitive and early markers of kidney function such as NGAL may enable us to define different subtypes of patients currently falling under the broad concept of WRF. Previous studies have identified an early rise in NGAL to be associated with acute renal injury in a broad range of patients admitted from the emergency department.⁸ Based on our findings, identifying a higher risk cohort of patients vulnerable to renal compromise may allow future studies to test the hypothesis that different therapeutic strategies guided by biomarkers such as NGAL may potentially contribute to lower rates of WRF.

The shortcoming of creatinine for monitoring renal function in the acute setting is well known. Creatinine lacks the sensitivity to detect minor changes in GFR and its increase is delayed by the need for accumulation after a reduction in GFR. Thus, the use of NGAL to detect early pathophysiologic processes previously undetectable by conventional markers is of significant clinical importance. This suggests that ongoing or prior renal tubular injury detected by elevated admission NGAL measurements is the “driving factor” for WRF during hospitalization. However, even patients with normal admission serum creatinine but

elevated admission NGAL were more likely to have a >0.3 mg/dL rise in serum creatinine during hospital stay. This may suggest that interventions to treat congestion during hospitalization (ie, diuresis) take a larger toll on patients with tubular injury at the time of admission. This may be the mechanism by which admission NGAL may predict subsequent WRF.

It is important to recognize that the timing of biomarker measurement has important impact on the ability of NGAL to predict the development of WRF, since the latter was also time-dependent. As illustrated in prior studies, the incidence of developing WRF increases over time, while reaching a plateau at 5 days of admission.¹² This was also observed in our study cohort, in which more than half of our patients developed WRF by day 3. Because we only examined serum NGAL at 2 specified time points (admission and day 3), we cannot determine the optimal time range that NGAL reliably predicts the development of WRF, especially if a large majority of the “events” observed were within the first few days of admission.

Study Limitations

Despite the prospective nature of the study and the careful description of renal function based on daily laboratory assessment of blood urea nitrogen/creatinine, inability to use fluid input/output and daily weight because of inconsistent documentation remains a study limitation. In the same token, data related to treatment modalities used during hospitalization, especially dosage of diuretics, are lacking in our study. The definition of WRF relies on absolute rise in serum creatinine, as arbitrarily defined in several other major epidemiologic studies. Nevertheless, our study cohort is representative of the typical patient population admitted for ADHF, with a 1 in 3 incidence of WRF during hospitalization similar to previous studies.

Additionally, determining the “true” baseline creatinine in a heterogeneous HF population without formal renal function testing is challenging and documentation of clinical stability at the time of “baseline” draw is often lacking. Furthermore, a subset of our subjects enrolled in the study did not have a prior laboratory analysis performed within a reasonable time frame before their admission. We have arbitrarily chosen a definition of WRF used in previous studies on defining WRF in ADHF, and results may differ with other definitions.

It has been recognized that different intrinsic renal conditions (such as urinary tract infections or immune diseases) can also lead to an increase in NGAL levels. Despite our attempts to exclude these patients by clinical history, they may have confounded our study population. Finally, we did not have renal hemodynamic measurements or more precise measurements of glomerular filtration rates to directly associate rising NGAL levels to renal compromise.

Conclusion

The presence of renal tubular injury (indicated by elevated serum NGAL levels) is associated with heightened risk of subsequent development of WRF in patients admitted with ADHF.

Acknowledgments

Supported in part by the American Society of Echocardiography Sonographers' Grant (A.B.) and the National Institute of Health CTSA Award (UL1-RR024989). Dr. Tang has received research support from Abbott Laboratories and is a consultant for Medtronic Inc and Merck & Co.

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Fig. 1.
Serum neutrophil gelatinase-associated lipocalin (NGAL) levels at admission and at day 3.

Fig. 2. Serum neutrophil gelatinase-associated lipocalin (NGAL) levels at admission and at day 3 stratified by occurrence of worsening renal function (WRF).

Fig. 3. Predictive value of admission neutrophil gelatinase-associated lipocalin (NGAL), estimated glomerular filtration rate (eGFR), and blood urea nitrogen (BUN) for worsening renal function (WRF).

Table 1

Admission Clinical Characteristics

Variable	Worsening Renal Function		<i>P</i> Value
	Yes (n = 35)	No (n = 56)	
Variable			
Age (y)	65 ± 16	58 ± 14	.011
Male gender (%)	21 (60%)	41 (73%)	.188
African American (%)	11 (31%)	10 (18%)	.135
Diabetes (%)	21 (64%)	23 (41%)	.040
History of hypertension (%)	24 (69%)	28 (50%)	.082
Ischemic etiology (%)	19 (54%)	26 (46%)	.466
NYHA Class III or IV at presentation (%)	28 (85%)	49 (92%)	.263
LV ejection fraction (%)	34 ± 15	30 ± 12	.397
Admission creatinine (mg/dL)	1.8 ± 0.9	1.5 ± 0.6	.117
Admission creatinine ≥1.5 mg/dL, n (%)	19 (56%)	25 (45%)	.301
Admission BUN (mg/dL)	42 ± 23	38 ± 25	.310
Admission eGFR (mL/min/1.73 m ²)	54 ± 41	64 ± 34	.086
Admission ACE inhibitors/ARBs (%)	13 (37%)	23 (41%)	.709
Admission β-blockers (%)	21 (60%)	34 (61%)	.946
Admission aldosterone antagonist (%)	6 (17%)	17 (30%)	.158
Admission loop diuretics (%)	24 (69%)	39 (70%)	.914

LV, left ventricular; NYHA, New York Heart Association; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blockers.

Values in mean ± standard deviation; or number (percent).

Table 2Logistic Regression Analysis for Prediction of WRF (Creatinine Rise ≥ 0.3 mg/dL)

Variable	Odds ratio (95% CI)	P Value
Admission NGAL (ng/mL)	1.92 (1.23–3.12)	.004
Follow-up NGAL (ng/mL)	2.18 (1.36–3.71)	.001
Changes in NGAL (ng/mL)	1.29 (0.84–2.05)	.251
Multiple logistic regression model 1		
Admission NGAL (ng/mL)	1.61 (0.99–2.70)	.051
Age (y)	1.44 (0.86–2.52)	.162
DM	1.87 (0.73–4.87)	.191
Multiple logistic regression model 2		
Admission NGAL (ng/mL)	1.97 (1.17–3.48)	.01
Admission eGFR (mL·min ⁻¹ ·1.73 m ²) [*]	1.06 (0.59–1.80)	.825
Multiple logistic regression model 3		
Admission NGAL (ng/mL)	2.07 (1.24–3.62)	.004
Admission BUN (mg/dL)	0.85 (0.49–1.40)	.539
Multiple logistic regression model 4		
Admission NGAL (ng/mL)	1.84 (1.08–3.26)	.023
Admission creatinine (mg/dL)	1.07 (0.62–1.85)	.795

WRF, worsening renal function; NGAL, neutrophil gelatinase-associated lipocalin; eGFR, estimated glomerular filtration rate; BUN, blood urea nitrogen.

^{*} Odds ratio per 1-SD increment (1 SD for admission NGAL = 84.43 ng/mL; 1-SD for follow-up NGAL = 92.91 ng/mL; 1-SD for Δ NGAL = 62.25 ng/mL; 1-SD for age = 15.1 y; 1-SD for admission eGFR = 36.7 mL·min⁻¹·1.73 m²; 1-SD for admission creatinine = 0.74 mg/dL).