Neutrophil Gelatinase–Associated Lipocalin as an Early Marker of Contrast-Induced Nephropathy After Elective Invasive Cardiac Procedures

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ABSTRACT

Background: Contrast-induced nephropathy (CIN) is an acute kidney injury (AKI) defined as serum creatinine (sCr) increase 48 to 72 hours after contrast administration. Because most subjects undergoing invasive cardiac procedures are discharged within 24 hours, sCr is unsuitable for CIN detection.

Hypothesis: In the present study we tested the hypothesis that neutrophil gelatinase-associated lipocalin (NGAL) is superior compared with sCr and other established nephropathy markers in early CIN diagnosis after elective invasive cardiac procedures.

Methods: Serum creatinine, urine creatinine, serum cystatin C, urine albumin, urine NGAL (uNGAL), and plasma NGAL were measured at 0, 6, 24, and 48 hours after contrast administration in 100 elective invasive cardiac procedures. Estimated glomerular filtration rate and albumin-to-creatinine ratio were calculated. Changes from baseline were considered statistically significant at $P < 0.05$ and clinically significant when > the biomarker's reference change value. Participants were divided into those with and without clinically significant uNGAL changes (uNGAL positive and negative for AKI, respectively).

Results: Thirty-three individuals were uNGAL positive for AKI. Serum cystatin C changes were statistically and clinically nonsignificant in both groups. Serum creatinine and plasma NGAL were statistically but not clinically elevated 48 hours postcatheterization in the AKI group. Except for contrast volume (higher in AKI group), groups were comparable at baseline (*P* not significant) regarding cardiovascular risk factors, coronary heart disease, coronary interventions performed, and renal biomarkers. Baseline uNGAL was significantly correlated to estimated glomerular filtration rate and albumin-to-creatinine ratio.

Conclusions: Urine NGAL is potentially superior compared with conventional nephropathy markers in early CIN diagnosis after elective invasive cardiac procedures. Definition of clinically significant uNGAL changes with reference change value is probably a valuable supplement to statistically defined significant variations.

Introduction

Contrast-induced nephropathy (CIN) after invasive cardiac procedures (cardiac catheterization, coronary angiography, or/and percutaneous coronary intervention [PCI]) is a common cause of hospital-acquired acute kidney injury (AKI).[1,2] The first 24 hours after contrast-medium administration appears to be crucial for the development of CIN.[3] However, CIN is currently defined as a ≥0.5mg/dL or ≥25% rise in serum creatinine (sCr) 48 to 72 hours after contrast exposure.[3] This is because sCr is not quite sensitive to acute renal function changes; in fact, it peaks 3 to 5 days after contrast administration and returns to baseline within 1 to 3 weeks.[4] Given that most subjects undergoing invasive cardiac procedures are typically discharged within 24 hours and only occasionally after 48 hours, sCr is rather unsuitable for CIN diagnosis in these individuals. Early detection of CIN after invasive cardiac procedures is important for the selection of patients needing extended hospitalization (>24–48 hours) for closer renal, metabolic, and fluid control.[5]

There are some additional limitations in sCr-based CIN diagnosis. Serum Cr is highly affected by age, sex, muscle mass, diet, medications, and hydration status. Besides, it is a marker of glomerular filtration rate (GFR) and not a direct marker of tubular damage (such as occurs in CIN/AKI),

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and substantial increases in sCr can be observed in cases of renal hypoperfusion (resulting in prerenal azotemia) even with structurally intact kidneys.[6–9] For the aforementioned reasons, the use of sCr for the diagnosis of CIN/AKI is now considered imperfect because nontubular injuries may be misclassified as CIN/AKI, whereas the absence of changes in sCr does not exclude tubular damage.[10]

Novel biomarkers of renal tubular damage aim to facilitate the early differential diagnosis between intrinsic AKI and prerenal azotemia and help the stratification of patients at risk. In contrast to conventional markers, such as sCr or serum cystatin C (sCysC), they do not reflect kidney function but rather structural damage of the kidney cells. Of many promising molecules, neutrophil gelatinase–associated lipocalin (NGAL) has generated considerable attention.[11] It is a 25-kDa protein, a member of the lipocalin family, and was originally isolated from the supernatant of activated human neutrophils.[12] As a low-molecular-weight protein (LMWP) and due to its resistance to degradation, plasma NGAL (pNGAL) is readily excreted and can be detected in urine. Filtered NGAL is normally reabsorbed by megalin-faciliated endocytosis in the proximal tubules.[13] Consequently, the proximal tubular injury occurring in CIN/AKI reduces reabsorption and increases NGAL concentration in urine. Moreover, NGAL is produced in renal tubules in response to nephrotoxic or ischemic stimuli and it is secreted in urine.[14,15] Thus, urine NGAL (uNGAL) might serve as an early marker of CIN/AKI after invasive cardiac procedures. However, standard reference values have not been defined, as its concentration can be elevated in patients with chronic kidney disease and/or albuminuria.[16,17] These limitations can be bypassed by defining clinically significant uNGAL changes taking into account baseline renal function and calculating the reference change value (RCV) based on the biological and analytical variation of uNGAL measurement.[18]

Building on the previous reports, the present study sought to assess the role of NGAL as an early marker of CIN/AKI after elective invasive cardiac procedures, compared with traditional markers (sCr, sCysC, estimated GFR [eGFR], and urine albumin-to-Cr ratio [ACR]). The effect of baseline renal function on uNGAL levels and the incidence of uNGAL RCV-based tubular damage were also examined.

Methods

Participants and Study Design

All adults (age >18 years) undergoing elective invasive cardiac procedures at our institution over a 6-month period were enrolled. Written informed consent was obtained from all, and the study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of our institution. Serial morning spot urine and blood samples were collected at baseline (prior to cardiac procedure) and at 6, 24, and 48 hours after contrast administration.

Biochemical Analyses

Serum and urine Cr were measured with a modified Jaffe method on an ARCHITECT ci16200 analyzer (Abbott, Chicago, IL). The analytical coefficient of variation (CV_a) of the assay in our laboratory is 2.8%.

Serum CysC was measured with a turbidimetric assay (Sentinel, Milan, Italy) on the same analyzer. The CV_a of the assay in our laboratory is 2.0%.

Estimated GFR was calculated with the Modification of Diet in Renal Disease (MDRD) formula (eGFR_MDRD $= 175 \times sCr^{-1.154} \times age^{-0.203}$ [$\times$ 0.742 if female]) and the CysC-based formula (eGFR_CysC $= 71/sCysC^{1.28}$).

Urine albumin was measured with a turbidimetric assay on the same analyzer and ACR was calculated.

Urine NGAL was also measured on the same analyzer using a 2-step sandwich immunoassay with chemiluminescent signal detection. This assay utilizes high-affinity mouse anti-NGAL antibodies generated toward distinct, nonoverlapping NGAL epitopes. The functional sensitivity of the assay is 2 ng/mL with a range that extends up to 1500 ng/mL. The CV_a of the assay in our laboratory is 4.5%.

Plasma NGAL was measured with a manual enzyme-linked immunosorbent assay (Bioporto, Gentofte, Denmark).

Statistical Analysis

Data were analyzed using SPSS statistical software, version 19 (IBM Corp, Armonk, NY). Continuous variables are presented as mean ± SD and categorical variables as n (%). The *t* test (paired or unpaired, as appropriate) was used to evaluate differences in continuous variables among or between groups once normality was demonstrated (Kolmogorov-Smirnov or Shapiro-Wilk test); otherwise, a nonparametric test (Wilcoxon or Mann-Whitney, respectively) was used. Differences in categorical variables were analyzed using the χ^2 test (or Fisher exact test, if applicable). A general linear model for repeated measures or a nonparametric test (2-way ANOVA) was used to identify differences within serial measurements of the continuous variables at the 4 time points. Correlations between continuous variables were determined using the Pearson *r* coefficient. Differences were considered statistically significant for a 2-sided *P* value <0.05.

Nevertheless, statistically significant changes in renal biomarkers may not be of clinical significance. In general, changes in measured biomarkers are considered clinically significant (with 95% probability) when they are greater than the combined analytical (CV_a) and biological (CV_i) variation of the measured variable. This total variation (named RCV) is calculated with the following formula: $RCV = 2^{1/2} \times 1.96 \times (CV_a^2 + CV_i^2)^{1/2}$, where CV_a is the analytical variation of the biomarker in the laboratory and CV_i is its within-subjects biological variation.[19] For sCr, sCysC, and uNGAL, the CV_a in our laboratory is 2.8%, 2.0%, and 4.5%, respectively, and the CV_i is 5.95%, 5.0%, and 84%, respectively.[20,21] This means that changes from baseline in sCr, sCysC, and uNGAL must be greater than 18.1%, 14.9%, and 233%, respectively (which is their RCV) to be considered clinically significant (with 95% probability). Based on uNGAL changes from baseline, our population was divided in 2 groups: those with clinically significant changes (>233%), characterized as "uNGAL positive for AKI," and those with clinically nonsignificant changes (<233%), characterized as "uNGAL negative for AKI."

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Table 1. Baseline Characteristics of the Study Population

	uNGAL Positive for AKI, n = 33	uNGAL Negative for AKI, n = 67	*P* Value	Total Population, N = 100
Clinical Parameters				
Age, y, mean (SD)	64.3 (7.7)	63.3 (9.8)	0.598	63.6 (9.2)
Sex, M/F, n	25/8	55/12	0.457	80/20
Arterial hypertension, %	69.7	67.8	0.851	68.0
DM, %	30.3	23.4	0.462	26.0
Dyslipidemia, %	63.6	51.7	0.266	56.0
Smoking, %	39.4	42.9	0.500	41.0
BMI, kg/m², mean (SD)	28.2 (3.4)	29.6 (5.0)	0.264	29.0 (4.4)
Angiographic parameters				
Contrast volume, mL, mean (SD)	427 (257)	300 (178)	0.005	343 (215)
Significant CHD, %	72.8	74.6	0.734	73.0
PCI, %	60.6	55.2	0.439	57.0
Renal biomarkers				
uNGAL, ng/mL, mean (SD)	15.16 (9.98)	17.20 (12.84)	0.419	16.53 (11.81)
Range	3.10–50.13	6.10–66.50	—	3.10–66.50
sCr, mg/dL, mean (SD)	0.92 (0.18)	0.96 (0.26)	0.393	0.95 (0.23)
Range	0.67–1.46	0.62–2.35	—	0.62–2.35
sCysC, mg/L, mean (SD)	0.95 (0.24)	0.94 (0.23)	0.814	0.94 (0.23)
Range	0.65–1.65	0.61–1.81	—	0.61–1.81
pNGAL, ng/mL, mean (SD)	137.67 (68.28)	109.32 (52.93)	0.025	118.68 (58.12)
Range	28.24–301.45	33.78–262.07	—	28.24–301.45
eGFR_MDRD, mL/min/m², mean (SD)	86.8 (18.8)	85.1 (21.0)	0.704	85.7 (20.3)
Range	50.3–125.3	30.2–145.1	—	30.2–145.1
eGFR_cysC, mL/min/m², mean (SD)	81.1 (20.8)	82.4 (21.4)	0.776	82.0 (21.2)
Range	37.4–123.2	33.2–133.7	—	33.2–133.7
ACR, mg/g, mean (SD)	42.0 (129.2)	28.5 (61.7)	0.479	33.0 (94.0)
Range	3.3–739.9	2.3–419.6	—	2.3–739.9

Abbreviations: ACR, albumin-to-creatinine ratio; AKI, acute kidney injury; BMI, body mass index; CHD, coronary heart disease; CysC, cystatin C; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; F, female; M, male; MDRD, Modification of Diet in Renal Disease; PCI, percutaneous coronary intervention; pNGAL, plasma neutrophil gelatinase–associated lipocalin; sCr, serum creatinine; sCysC, serum cystatin C; SD, standard deviation; uNGAL, urine neutrophil gelatinase–associated lipocalin.

Results

A total of 100 subjects (80% males) were included in the analysis. Their baseline characteristics are presented in Table 1. Clinically (and statistically) significant increases in uNGAL from baseline ("uNGAL positive for AKI") were noted in 33 patients (33.0%). The remaining 67 patients (67%) had only minor changes in uNGAL ("uNGAL negative for AKI"), as shown in Table 2. With the exception of the administered contrast volume (significantly higher in the AKI group), the 2 groups were comparable at baseline (*P* value not significant) with regard to the prevalence of cardiovascular risk factors (age, sex, arterial hypertension, diabetes mellitus, dyslipidemia, smoking, obesity) and significant coronary heart disease, the number of PCIs performed, as well as the levels of all renal biomarkers (Table 1).

Baseline uNGAL was significantly negatively correlated to eGFR_MDRD and eGFR_CysC ($r = -0.604$ and $r = -0.575$, respectively; Figure 1) and significantly positively correlated to ACR ($r = 0.496$).

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Table 2. Contrast-Induced Changes in Renal Biomarkers Over Time

		uNGAL Positive for AKI, n = 33		uNGAL Negative for AKI, n = 67		
		Mean (SD)	P_1 Value[a]	Mean (SD)	P_1 Value[a]	P_2 Value[b]
uNGAL, ng/mL	Baseline	15.16 (9.98)	—	17.20 (12.84)	—	0.419
	6 hours	43.18 (24.44)	0.022	18.02 (12.42)	0.942	<0.001
	24 hours	72.54 (62.16)	<0.001	19.35 (14.82)	0.616	<0.001
	48 hours	62.60 (50.66)	<0.001	17.64 (15.15)	0.985	<0.001
		P_3 Value < 0.001[c]		P_3 Value = 0.780[c]		
sCr, mg/dL	Baseline	0.92 (0.18)	—	0.96 (0.26)	—	0.393
	6 hours	0.93 (0.22)	0.979	0.88 (0.23)	0.109	0.241
	24 hours	1.01 (0.22)	0.160	0.93 (0.22)	0.761	0.078
	48 hours	1.04 (0.22)	0.049	0.95 (0.23)	0.982	0.055
		P_3 Value = 0.049[c]		P_3 Value = 0.196[c]		
sCysC, mg/L	Baseline	0.95 (0.24)	—	0.94 (0.23)	—	0.814
	6 hours	0.96 (0.24)	1.000	0.89 (0.21)	0.369	0.151
	24 hours	0.99 (0.25)	0.824	0.91 (0.22)	0.845	0.104
	48 hours	1.03 (0.26)	0.418	0.93 (0.23)	0.944	0.063
		P_3 Value = 0.517[c]		P_3 Value = 0.527[c]		
pNGAL, ng/mL	Baseline	137.67 (68.28)	—	109.32 (52.93)	—	0.025
	6 hours	152.57 (66.73)	0.712	107.57 (60.29)	0.997	0.001
	24 hours	171.64 (69.31)	0.120	123.48 (66.63)	0.414	0.001
	48 hours	178.86 (71.66)	0.047	123.07 (68.48)	0.476	0.001
		P_3 Value = 0.072[c]		P_3 Value = 0.299[c]		

Abbreviations: AKI, acute kidney injury; pNGAL, plasma neutrophil gelatinase–associated lipocalin; sCr, serum creatinine; sCysC, serum cystatin C; SD, standard deviation; uNGAL, urine neutrophil gelatinase–associated lipocalin.
[a]Comparison between each time point and baseline. [b]Comparison between biomarker-positive and -negative subjects at each time point. [c]Comparison within repeated measurements.

Serial changes of NGAL, sCr, and sCysC during the 48-hour follow-up are presented in Table 2 and Figure 2. Urine NGAL showed significant statistical and clinical elevations as early as 6 hours postcatheterization in the uNGAL positive for AKI group with only minor changes in the uNGAL negative for AKI group. Serum CysC did not show any significant statistical or clinical change at any time point of the follow-up in either of the 2 groups. Serum Cr and pNGAL were statistically significantly elevated in the AKI group, but only 48 hours postcatheterization. However, these changes were not clinically significant because they were lower than the threshold of RCV.

Discussion

To our knowledge, this is one of the few studies in the literature assessing the role of NGAL as an early marker of CIN after invasive cardiac procedures compared with established markers of nephropathy. The main findings of this study denote that no CIN cases could be diagnosed early based on sCr changes, whereas uNGAL emerges as superior in the early-CIN diagnosis within 24 hours after elective invasive cardiac procedures. Thus, uNGAL is useful for the selection of patients needing extended hospitalization (>24–48 hours) for closer renal follow-up. The use of RCV for the definition of clinically significant uNGAL changes is probably a valuable supplement to the statistically defined significant variations. Moreover, baseline renal function (eGFR, ACR) seems to greatly influence uNGAL levels.

These results seem to be in agreement with the current literature. Liebetrau et al recently reported that uNGAL is a biomarker for predicting contrast-induced AKI when measured 24 hours after PCI.[22] Padhy et al found that even pNGAL and sCysC may act as early markers of contrast-induced AKI in patients undergoing PCI.[23] However, AKI diagnosis was sCr-based in this study; also, RCV was not used for the definition of clinically significant biomarker changes, but specific cutoff values were determined instead.

The reported incidence of CIN varies widely in the literature, depending not only on the specific population and the baseline risk factors, but also on the definition of this clinical event.[1,3,4,24] In the general population,

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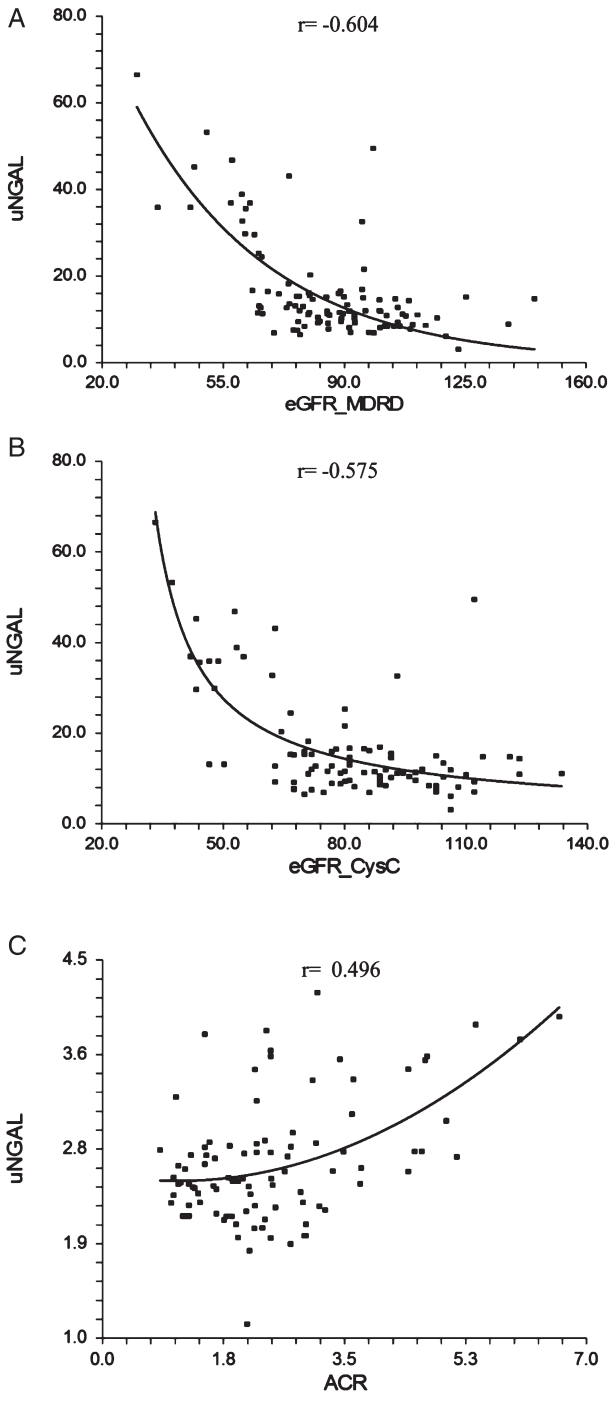

Figure 1. Correlation of baseline uNGAL with (A) eGFR_MDRD and (B) eGFR_CysC-based formula and (C) ACR. Abbreviations: ACR, albumin-to-creatinine ratio; CysC, cystatin C; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; uNGAL, urine neutrophil gelatinase–associated lipocalin.

the incidence of CIN is reported to be 0.6% to 2.3%.[3] However, in several patient subsets it is significantly higher (up to 20%).[3] This is especially true for patients with cardiovascular pathology.[3] Ozcan et al recently reported a 9.3% incidence of CIN after elective PCI in nondiabetic individuals with metabolic syndrome, compared with 4.9% in the control group (nondiabetic subjects without metabolic syndrome).[24]

According to most definitions, slight sCr changes at 48 to 72 hours after exposure to a contrast agent establish CIN diagnosis.[3,25] However, sCr-based CIN diagnosis turns out to be rather difficult. The laboratory analytical variation and the within-subjects biological variation are the most important sources of variation in its measurement, frequently leading to misdiagnosis. To keep the total variation minimal and to achieve a uniform measurement between laboratories, the analytical variation must be <50% of the biological variation.[26] As previously reported, this goal was achieved in our laboratory. Even at this case, with a 18.1% RCV for sCr, only changes >18.1% in serial sCr measurements were able to be discriminated. During the 48-hour follow-up, none of the participants exhibited such a sCr change (sCr-based incidence of CIN, 0%). Meantime, a significant proportion of the same population (33.3%) exhibited clinically significant elevations of uNGAL, which is a tubular damage–specific biomarker (uNGAL-based incidence of CIN, 33%). This is probably because we actually diagnosed a "subclinical acute kidney injury," previously described as "biomarker-positive/sCr-negative kidney injury" representing the first stage of AKI and characterized by biomarker positivity only.[5,27–29] Our data support the idea of a "mild tubular injury" because peak uNGAL levels found in the present study (72.54 ± 62.16 ng/mL) are much lower than those observed in severe AKI cases (1113.4 ± 88.8 ng/mL in a study with subjects undergoing cardiac surgery).[30]

The correlation between baseline renal function (eGFR, ACR) and uNGAL levels found in the present study practically means that subjects with lower eGFR or/and higher ACR exhibit higher baseline uNGAL levels; and, therefore, greater uNGAL increase is required after contrast administration to be characterized as CIN/AKI. In other words, the presence of chronic kidney disease and/or albuminuria may increase the threshold for the detection of CIN/AKI using NGAL. McIlroy et al have previously reported that the relationship between uNGAL and AKI after cardiac surgery varies with baseline renal function, with optimal discriminatory performance in patients with normal preoperative function.[31] In intact kidneys, albumin is filtered through the glomerulus and is reabsorbed by megalin-cubulin receptor–mediated endocytosis.[32,33] In the presence of proteinuria, competition for the same receptor between albumin, uNGAL (produced by neutrophils or/and kidney epithelial cells), and other LMWPs could account for increased uNGAL levels, independently of tubular injury.[17,34] Other LMWPs absorbed by receptor-mediated endocytosis include some of the newly discovered biomarkers of AKI, such as urine cystatin C (uCysC), liver fatty-acid binding protein, α1-microglobulin, and β2-microglobulin.[35–39] Nejat et al have shown that repeated protein loading could induce transient albuminuria, leading to increased uCysC levels that decreased again when albuminuria returned to baseline.[17] Nishida et al found that the extent of proteinuria is correlated with uNGAL levels in children with chronic renal diseases.[40] These observations enhance the hypothesis that albuminuria decreases the absorption of LMWPs by competition for a common

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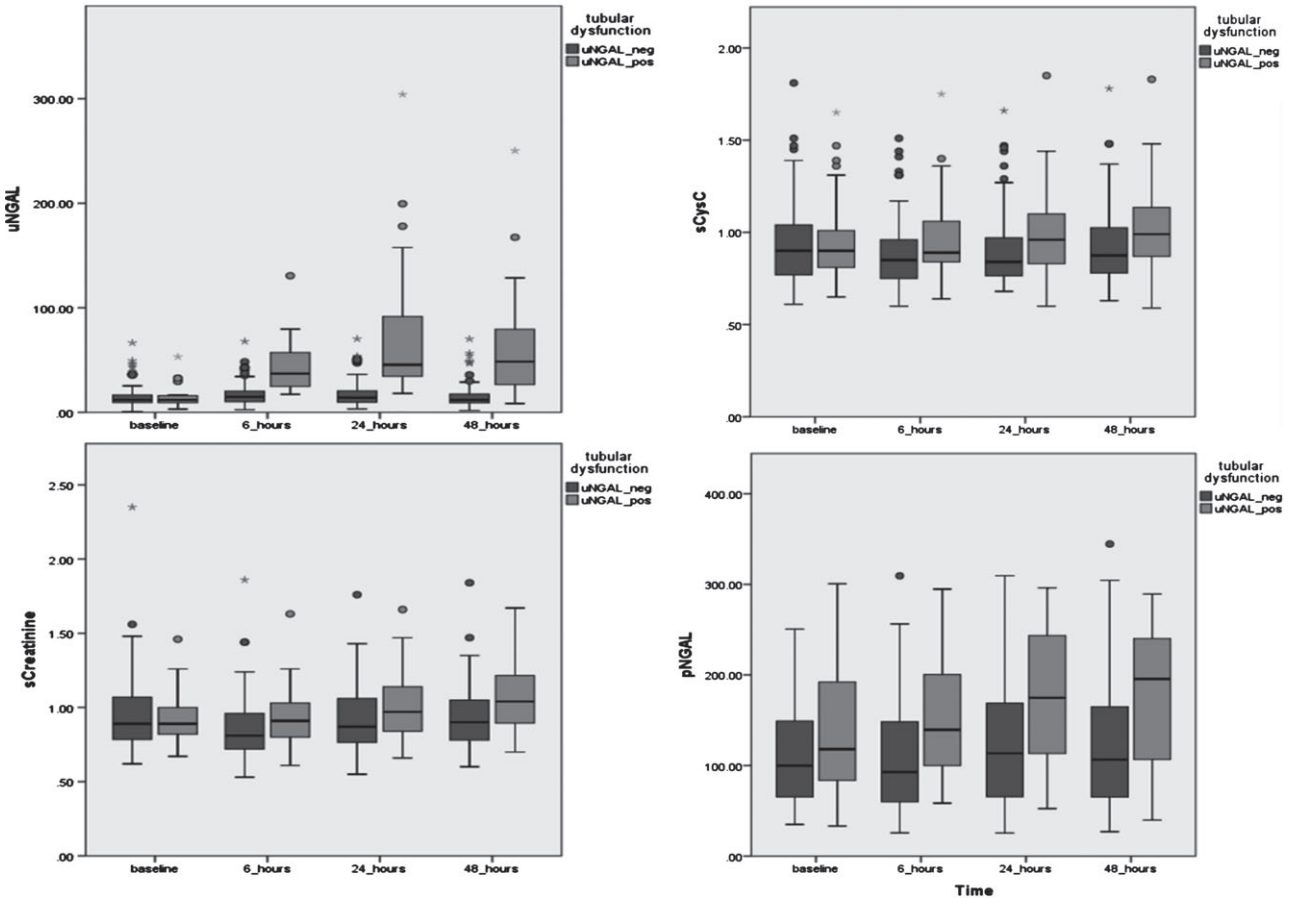

Figure 2. Serial changes of (top to bottom) uNGAL, sCr, sCysC, and pNGAL over time. Abbreviations: pNGAL, plasma neutrophil gelatinase–associated lipocalin; sCr, serum creatinine; sCysC, serum cystatin C; uNGAL, urine neutrophil gelatinase–associated lipocalin.

transport mechanism. For these reasons, a standard NGAL cutoff value would probably be problematic. Instead, the interpretation of uNGAL changes from baseline in the base of RCV is probably a better approach.

Study Limitations

It is important to consider potential limitations. First, this was a narrow-scale study with a rather limited number of participants. Larger-scale studies are necessary to confirm our results. Second, the CV_i used for RCV calculation is the one reported in literature and has been calculated in apparently healthy individuals. However, RCV derived from "healthy CV_i" may be inappropriate for monitoring patients in certain diseases.[41] Although it is difficult to measure biological variation in the setting of specific diseases, it has been reported that, for the majority of markers that are routinely measured in clinical laboratories, only minor differences in CV_i are present between healthy subjects and populations with chronic diseases.[41] In the present study, patients with acute coronary syndrome (ACS) were excluded and only stable patients undergoing elective procedures were enrolled. Thus, the use of "healthy CV_i" has probably minimal impact on the accuracy of the results. However, the exclusion of patients with ACS is another limitation of the study. Future studies on ACS patients are necessary to examine the role of NGAL as a marker of CIN.

Conclusion

Urine NGAL emerges as potentially superior compared with established markers of nephropathy in the early diagnosis of CIN/AKI after elective invasive cardiac procedures and the selection of patients needing extended hospitalization (>24–48 hours) for closer renal and fluid follow-up. The use of RCV for the definition of clinically significant uNGAL changes is probably a valuable supplement to the statistically defined significant variations. The definition of standard cutoff values for uNGAL is rather improper given that its levels are highly dependent on eGFR and ACR. Future larger-scale studies are necessary to confirm our results and extend them to ACS patients.

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