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NGAL (neutrophil gelatinase-associated lipocalin) and L-FABP after percutaneous coronary interventions due to unstable angina in patients with normal serum creatinine

Bachorzewska-Gajewska H[1],[2]*, Poniatowski B[1], Dobrzycki S[1]

1 Department of Invasive Cardiology, Medical University, Bialystok, Poland
2 Department of Clinical Nursing, Medical University, Bialystok, Poland

* CORRESPONDING AUTHOR:
Department of Clinical Nursing,
Medical University of Bialystok,
Collegium Novum
15A Waszyngtona Str., 15-274 Bialystok,
tel.: +4885 74687 44; +4885 74507 77
e-mail: hgajewska@op.pl (Hanna Bachorzewska-Gajewska)

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ABSTRACT

Purpose: The value of NGAL (neutrophil gelatinase-associated lipocalin) and L-FABP (liver-type fatty acid binding protein) has been highlighted as a novel biomarker of detection of acute renal failure in children after cardiac surgery. Interventional cardiologists are being asked more frequently to perform percutaneous coronary intervention (PCI) and contrast nephropathy is its potentially serious complication. We aimed to prospectively assess NGAL and L-FABP in patients with normal serum creatinine undergoing PCI due to unstable angina.

Material and Methods: We measured serum NGAL, urinary NGAL and L-FABP using commercially available kits before and after 2, 4, 12, 24 and 48 hours following PCI in 25 patients.

Results: We found a significant rise in serum NGAL after 2 and 4 hours. Urinary NGAL and urinary L-FABP followed the same pattern. Both markers increased significantly after 4 hours and remained elevated up to 48 hours after PCI. Serum creatinine did not change significantly during the study period.

Conclusions: NGAL and L-FABP may represent a sensitive early biomarkers of renal impairment after PCI. Persistently increased urinary NGAL and L-FABP may suggest renotubular damage in this population.

Key words: NGAL, L-FABP, PCI, acute kidney injury

INTRODUCTION

NGAL (neutrophil gelatinase-associated lipocalin), a member of lipocalin family, was originally isolated from the supernatant of activated human neutrophils [1], however it is also expressed at a low level in other human tissues including the kidney [2]. Because of its small molecular size (25kDa) and resistance to degradation, NGAL is readily excreted and detected in urine. NGAL is highly accumulated in the human kidney cortical tubules, blood and urine after nephrotoxic and ischemic injury [3]. Thus, NGAL might represent an early, sensitive, and non-invasive urinary biomarker for ischemic and nephrotoxic renal injury [4]. Urinary NGAL might also serve as an early marker for ischemic renal injury in children after cardiopulmonary bypass [5]. Besides NGAL, various molecular biomarkers are currently under investigation to determine their value as indicators of renal injury. Among the other top candidates at present is urinary liver-type fatty acid-binding protein (L-FABP) [6,7]. Since interventional cardiologists are being asked more frequently to perform percutaneous coronary intervention-PCI on increasing number of patients, contrast nephropathy is its potentially serious complication [8]. Peak creatinine typically occurs in 3 to 5 days after contrast administration and returns to baseline values (or a new baseline) in 1 to 3 weeks [9], when patients are discharged from the hospital. Unfortunately, creatinine is an unreliable indicator during acute changes in kidney function [10]. There are well recognized inaccuracies and limitations for using serum creatinine to estimate true renal function [11]. A marked reduction in glomerular filtration rate (GFR) can appear before it is reflected by a rise in creatinine (up to 50% of kidney function has already been lost before

creatinine might change). Secondly, creatinine does not reflect kidney function during acute changes until a steady state has been achieved, which could take several days. Mishra *et al.* [5] reported that in children acute renal failure was detected 2 hours after cardiac surgery. So far there has been no prospective data on NGAL and L-FABP in patients undergoing primary angioplasty. Therefore, we tested the hypothesis that NGAL and L-FABP could represent early biomarkers of renal injury in patients with normal serum creatinine undergoing PCI.

MATERIAL AND METHODS

The study group consisted of 25 consecutive non-diabetic patients undergoing PCI due to unstable angina. We excluded patients with preexisting chronic kidney disease. All the patients gave their informed consent to participate in the study and the protocol was approved by the local Bioethics Committee. Duration of PCI ranged from 45 to 165 minutes, volume of administered contrast agent ranged from 70 to 400 ml. Iso-osmolar contrast (iodixanol) was administered in all patients. All patients were treated with statins and I-ACE. Serum creatinine was measured with the standard laboratory Jaffe method in one central laboratory. We assessed kidney function according to the simplified Modification of Diet in Renal Disease (MDRD) [12], Cockcroft-Gault [13] and bedside Jeliffe [14] formulas.

Serum and urinary NGAL were evaluated before and after 2, 4, 12, 24, and 48 hours after PCI using ELISA from ANTIBODYSHOP (Gentofte, Denmark); L-FABP was assessed using commercially available kits from CMIC, Co, Ltd, Japan. Data was analyzed using Statistica 6.0. ANOVA or Kruskall-Wallis ANOVA for repeated measurements was used in statistical analysis, with $p<0.05$ considered statistically significant, when appropriate.

Table 1. **Basal clinical and biochemical characteristics in patients undergoing PCI.**

Age (years)	64.33 ± 9.98
Hypertension (n) /hypertension history (years)	20/17
Current cigarette smokers/ previous smokers	3/11
Obesity BMI>30 kg/m2	17%
BMI (kg/m2)	24.5±6.3
Ejection fraction (%)	46.83±13.92
LVIDd-left ventricular internal enddiastolic dimension (mm)	5.27±0.92
Duration of PCI (min)	62.67±23.38
Contrast volume (ml)	175.0±69.13
Hemoglobin (g/dL)	13.9 ±2.1
HbA1c (%)	4.80 ±1.18
cholesterol (mg/L)	170.70±39.89
HDL (mg/dL)	43.47±6.63
Triglycerides (mg/dL)	228.75±98.53
Hb (g/dL)	14.47±1.28
Ejection fraction (%)	46.83±13.92
MDRD equation (ml/min)	82.26±30.08
Cockcroft-Gault formula (ml/min)	65.66±27.44
Cockcroft-Gault formula weight-adjusted(ml/min)	71.89±27.70
Jeliffe formula (ml/min)	68.14±25.38

Values given as means± SD, or numbers and percentage

RESULTS

Clinical and biochemical characteristics is shown in the *Tab. 1; Tab. 2*. Mean NYHA (New York Heart Association) class was 1.8. We found a significant rise in serum NGAL after 2 and 4 hours, and in urinary NGAL after 4 and 12 hours after PCI (*Tab. 2*). Serum NGAL returned to baseline values 48 hours after PCI. Urinary NGAL and L-FABP did not return to baseline values after 48 hours following PCI. 6 patients, in whom a doubling of serum NGAL was observed, had lower baseline eGFR (all formulas, $p<0.01$), however, the time of PCI and contrast volume did not differ significantly. In all the patients doubling of L-FABP was observed. At baseline,

Table 2. **Changes in serum and urinary NGAL in patients undergoing PCI.**

	Before PCI	2 hours	4 hours	12 hours	24 hours	48 hours
Age (years)	64.33±9.98					
Serum NGAL (ng/mL)	102.77±56.13	123.61±62.05*	155.22±88.65**	145.30±85.86*	116.40±58.94	115.70±57.01
Urinary NGAL (ng/mL)	8.42(0.2-32.0)	9.38(0.2-58)	18.53(1.4-77)*	17.88(1.5-117.8)*	17.22(1.4-117.4)*	16.11(0.2-48)*
Urinary L-FABP (pg/ml)	3.76±1.52	4.86±0.84	25.90±21.93***	37.21±27.21***	33.49±26.41***	18.33±12.08**
Creatinine (mg/dL)	0.94±0.30	ND	ND	ND	0.99±0.32	1.03±0.20
Albumin (g/dl)	3.69±0.38	ND	ND	ND	ND	3.67±0.29
SBP (mm Hg)	140.0±25.55	95.0±56.07**	ND	ND	125.07±27.87	137.76±21.87
DBP (mm Hg)	81.75±13.89	60.0±34.69**	ND	ND	82.87±14.32	81.89±15.65

Data given are means ± SD, or medians (minimum, maximum)* $p<0.05$, , **$p<0.01$, ***$p<.001$ vs baseline

serum NGAL correlated with serum creatinine (r=0.47, p<0.01), eGFR (MDRD, r=-0.50, p<0.01, Cockcroft-Gault, r=-0.49, p<0.01, Cockcroft-Gault weight adjusted, r=-0.42, p<0.05, Jeliffe, r=-0.50, p<0.01), cystatin C (r=0.63, p<0.001), whereas L-FABP correlated, in univariate analysis, with HDL (r=-0.75, p<0.001), triglycerides (r=0.82, p<0.001), creatinine (r=0.81, p<0.001).

DISCUSSION

This is the first study to prospectively examine novel markers of acute renal injury in patients with normal serum creatinine undergoing PCI. None of our patients fulfills criteria of contrast nephropathy. It is of interest, that a rise in serum NGAL was observed as early as 2 hours after PCI and lasted up to 4 hours. Urine NGAL increased after 4 hours and remained significantly elevated in comparison to baseline even 48 hours after PCI without changes in serum creatinine.

An earlier NGAL rise in serum than in urine may be due to the fact that NGAL was released into the circulation probably secondary to inflammatory activation of neutrophils initiated by PCI. Moreover, since NGAL is increased in atherosclerotic plaques [15], it might also be released into the circulation during the PCI. In addition, our patients were well hydrated and without diuretics. Patients with ischemic heart disease often present renal dysfunction of some degree due to concomitant diabetes, hypertension, congestive heart failure, despite normal serum creatinine. Of interest is a significant fall in both systolic and diastolic blood pressure after the procedure. It might affect kidney perfusion, resulting in transient kidney ischemia.

In post-ischemic kidney, NGAL is markedly upregulated in proximal tubules and distal nephron segment [4]. In the former it co-localizes at least partly with proliferating epithelial cells [4]. Nephrotoxic injury after cisplatin administration resulted in similar pattern of NGAL changes [16]. Recently, NGAL was identified as iron-transporting protein during nephrogenesis [17]. Iron is crucial for cell growth and development. Presumably, iron is also critical for renal regeneration following injury. NGAL could potentially recycle iron into the viable cells, thus stimulating growth and development. NGAL may also serve as a storage place for iron released from damaged tubule cells. By removing iron from the site of injury, NGAL may limit iron-mediated cytotoxicity. On the other hand, L-FABP followed the same pattern as urinary NGAL. It increased significantly as early as 4 hours after the procedure and remained significantly elevated even after 48 hour. So far there has been limited data on L-FABP in patients undergoing PCI. Kato *et al.,* studied changes in creatinine, cystatin C, α,β microglobulins, NAG and L-FABP in 87 patients undergoing elective catheterization with or without PCI [18]. They found a rise in L-FABP only 1 day after elective PCI in 41 patients with mild chronic kidney disease (ie. eGFR 89-60 ml/min), but after 1 and 2 days after elective PCI in 31 patients with moderate chronic kidney disease defined as eGFR 59-30 ml/min (modified MDRD equation in Japanese). In our population mean creatinine clearance according to Cockcroft-Gault formula [13] was 65.66±27.44 ml/min, according to MDRD equasion [12] was 82.26±30.08 ml/min, and according to Jeliffe formula [14] was 68.14± 25.38 ml/min. Majority of our patients represented mild impairment of kidney function despite normal serum creatinine. In another study a rise in urinary L-FABP levels was observed in patients with contrast-induced nephropathy on the next day and 2 days after angiography [19]. After 14 days, serum creatinine returned to the baseline level, but urinary L-FABP level remained still high. However, in the only report on inflammatory markers in patients undergoing primary angioplasty, a decrease NGAL and malondialdehyde-marker of oxidative stress was found in samples taken 1.5, 3 and 24 hours after verified reperfusion by angiography [20]. First samples were taken at baseline with the occluded coronary vessel from arterial site during PCI, subsequent samples were taken from peripheral vein. Different methodology might be the explanation for the results obtained. On the other hand, in the recent study patients with acute post-myocardial infarction heart failure had significantly elevated serum NGAL associated with adverse outcomes (median of follow–up was 27 months) [21]. Value of NGAL in our population was also significantly higher than values reported previously for healthy volunteers [22].

The value of our study is its prospective design, simultaneous measurement of urinary, serum NGAL, and urinary L-FABP (up to 48 hours after the procedure) in low-risk population. These patients, both diabetic and non-diabetic, may particularly benefit more from early detection of the contrast-induced nephropathy (CIN) because they are generally given less attention with regards to CIN. They may develop CIN unnoticed in comparison to those with already elevated creatinine at baseline. The limitations are, that this study is single-centre study on a relatively small population.

CONCLUSIONS

NGAL and L-FABP may represent sensitive early biomarkers of renal impairment after PCI. Persistently increased urinary NGAL and L-FABP may suggest renotubular damage in this population. Our findings may have important implications for the clinical management of patients undergoing PCI. The "window of opportunity" is narrow in contrast nephropathy and time to introduce proper treatment after initiating insult is limited, particularly when patients are discharged early from the hospital. Lack of biomarkers delays our ability to administer effective therapy, however new and promising biomarkers are on the horizon.

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