



## Current Issues in Pharmacy and Medical Sciences

Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA

journal homepage: <http://www.curipms.umlub.pl/>



# Influence of angiotensin-converting enzyme inhibitor ramipril on indicators of tubular kidney lesion in patients with chronic glomerulonephritis

MARIIA O. DOLINNA<sup>1</sup>, OLEKSANDR O. SVYNTOZELSKYI\*<sup>1</sup>

Department of Clinical Pharmacology, Pharmacy, Pharmacotherapy, and Cosmetology Zaporizhzhia State Medical University, Ukraine

### ARTICLE INFO

Received 09 May 2019  
Accepted 12 August 2019

#### Keywords:

NGAL,  
IL-18,  
glomerulonephritis,  
diagnostics,  
ramipril.

### ABSTRACT

To research and deepen the understanding of the links between morphological tubular kidney lesion parameters and serum markers – neutrophil gelatinase-associated lipocalin (NGAL) and interleukin-18 (IL-18), in patients with chronic glomerulonephritis (CGN) with saved renal function, as well as to estimate therapeutic correction of identified changes using ACE inhibitor ramipril. The diagnosis of “chronic glomerulonephritis” was verified based on clinical, laboratory and morphological data. Patients were divided into 2 clinical groups: patients with CGN and arterial hypertension (AH) and without AH. We used the data of renal biopsies to analyze the indicators of tubular kidney lesion in patients with CGN. Levels of serum NGAL and IL-18 were measured by means of ELISA kits. Treatment of patients was carried out over 24 weeks using the ACE inhibitor ramipril. The average daily dose of ramipril for the entire treatment period for patients with AH was  $12.8 \pm 5.6$  mg, patients of the second group – without AH, were treated with ramipril at a dose of 2.5 mg. On the basis of rank correlation analysis, we demonstrated that the level of serum NGAL is directly correlated with interstitial fibrosis ( $r=0.65$ ;  $p<0.05$ ), serum IL-18 – with dystrophic changes in the epithelium of renal tubules ( $r=0.81$ ;  $p<0.05$ ).

**Conclusion.** Serum levels of NGAL and IL-18 are one of the most sensitive markers of tubular kidney lesion and have diagnostic efficiency up to 97%. A 24-week treatment with ACE inhibitor ramipril in patients with CGN with and without AH leads to a decrease in the levels of tubular kidney lesion markers.

### INTRODUCTION

Dystrophic changes in the epithelium of renal tubules and interstitial fibrosis are the basis for chronic kidney failure development [1]. Angiotensin-converting enzyme (ACE) inhibitors for efficacy and safety meet the “ideal” of antihypertensive agents [2]. Ramipril is a long-acting lipophilic drug with two major ways of excretion. It not only effectively normalizes blood pressure, but also improves the prognosis of patients with nephropathy, due to lowering the tone of efferent arterioles and intraglomerular pressure that inhibits mesangial proliferation, and by decreasing the synthesis of mesangial matrix components and the level of proteinuria [2,3]. However, ACE inhibitor therapy is used too late because the implementation of markers in routine clinical practice is limited up to the present due

to insufficient evidence base; hence the lack of information on strategies to influence on them and to justify the feasibility of studies in this area. Moreover, the clinical signs of renal failure show up only after losing more than 50% of nephrons. Therefore, the main factor for the prognosis of patients with CGN is a timely diagnostic and early treatment of tubular kidney lesion [4].

### AIM

The objective of this study was to research and deepen the understanding of the links between morphological tubular kidney lesion parameters and serum markers – neutrophil gelatinase-associated lipocalin (NGAL) and interleukin-18 (IL-18), in patients with chronic glomerulonephritis (CGN) with saved renal function, as well as to estimate therapeutic correction of identified changes by way of using the ACE inhibitor ramipril.

#### \* Corresponding author

e-mail: [asw1@ukr.net](mailto:asw1@ukr.net)

**MATERIAL AND METHODS**

Eighty-one patients with CGN were enrolled in the study (average age was 37.6±1.3 years). The diagnosis of “chronic glomerulonephritis” was verified based on clinical, laboratory and morphological data. Patients were divided into 2 clinical groups: patients with CGN and arterial hypertension (AH) and without AH. The first group included 49 patients with CGN and AH, 34 (69%) men, 15 (31%) women (the difference the pool of male and female patients in the sample can be explained by the natural distribution of the disease in population and did not influence the results), who had an average age of 36.3±2.3 years, disease duration 87.1±9.8 months. Nephrotic syndrome with proteinuria (PU) above 3 g/l was observed in 8% of all patients. Urinary syndrome with low PU and different severity of erythrocyturia was detected in 86% of all patients, PU above 1 g/l – in 6%. The average daily level of PU was 1.4±0.1 g/day. The second clinical group – 32 patients with CGN without AH, there were 20 (63%) men and 12 (37%) women, the average age was 38.7±7.5 years, disease duration – 47.1±6.8 months. The urinary syndrome was manifested by low PU and different severity of erythrocyturia. The average daily level of PU was 0.41±0.04 g/day. In 66% of all patients, lab picture was defined as remission. The control group consisted of 20 healthy individuals, there were 10 (50%) men and 10 (50%) women with average age 40.1±2.4 years. They were examined to clarify the standards of level markers. Treatment of patients was carried out over 24 weeks using the ACE inhibitor ramipril. The starting dose for patients with CGN and AH was 10 mg once daily, for patients with CGN without AH – 2,5 mg 1 time per day. All patients did not receive permanent antihypertensive therapy or it was canceled 48 hours prior to inclusion into the study. If there was no blood pressure reduction of at least 10% compared with baseline during the first two weeks of treatment, the study protocol anticipated an increase in the dose of the drug to 20 mg per day. The dose was increased for 8 patients (6 men and 2 women). The average daily dose of ramipril for the entire treatment period for patients with AH was 12.8±5.6 mg. Patients of the second group – without AH, were treated with ramipril at a dose of 2.5 mg without further titration. We used the following morphological parameters to assess tubular kidney lesion: dystrophic and necrotic changes in the epithelium of renal tubules, thickening and/ or splitting of the tubular basement membrane, presence of cellular infiltration, interstitial fibrosis (IF) [2]. The level of serum NGAL was determined using ELISA kits (BioVendor, Czech Republic), serum IL-18 – Bender Medsystems (Austria), all rates were obtained automatically and calculated in ng/ml (NGAL) and pg/ml (IL-18). We evaluated the accuracy of renal morphological changes diagnostics on serum levels of NGAL and IL-18 compared to kidney biopsy by calculating operating performance tests, which include: diagnostic sensitivity (Se), diagnostic specificity (Sp), and diagnostic efficiency (DE). Operating characteristics are determined by the test conditions: diagnostics are performed by two methods, one of which is the standard (its result is considered to be the most accurate), and another method,

the one to be checked for accuracy; both of diagnostic methods are used independently; each of the methods yields positive or negative results.

**Statistics**

All statistical analyses were performed in SPSS for Windows v.7 17.0 (SPSS Inc., USA). The study data were presented as mean and ± standard deviation, serum levels of markers – as median and 25 %-75% confidence interval (CI). A calculated difference of p<0.05 was considered significant. Chi-square test was performed for quality comparisons of group characteristics, as well as for the frequency of occurrence indicators. The degree of correlation between pairs of independent traits, expressed in a quantitative scale, was made using Pearson correlation coefficient (r) or p. Spearman rank correlation (R), depending on the nature of the distribution of variables. The probability of correlation was evaluated by comparing the calculated coefficients with the critical ones.

**RESULTS**

The results of the basic laboratory parameters of studied patients are presented in Table 1. P-values of these parameters were not significant.

**Table 1.** The basic laboratory parameters of patients with CGN (M±S)

The indicator	Patients with CGN and AH (n=49)	Patients with CGN without AH (n=32)
Hemoglobin, g/l	136.5±2.0	138.5±0.5
Blood albumin, g/l	42.8±0.7	44.1±0.2
Blood cholesterol, mmol/l	5.6±0.3	4.9±0.4
Blood creatinine, mmol/l	101.9±3.5	97.2±2.2
Blood urea, mmol/l	5.8±0.2	5.9±0.3
Glomerular filtration rate (CKD-EPI), ml/min/1,73/m <sup>2</sup>	88.7±2.2	91.3±2.3

S – standard deviation

According to the results of renal microscopy, 88% of both groups of patients had mesangial proliferative glomerulonephritis, 7% – membranous nephropathy, 5% – membranous proliferative glomerulonephritis.

We investigated serum levels of NGAL and IL-18 in patients with CGN and in the control group. Results are presented in Table 2.

**Table 2.** Average serum levels of markers in patients with CGN and in the control group (Md: 25%-75%)

Biomarkers	Patients with CGN and AH (n=49)	Patients with CGN without AH (n=32)	Control group (n=20)
Serum NGAL, ng/ml	6.2: 4.2-74	4.7: 3.8-7.4	2.8: 0.9-3.4
Serum IL-18, pg/ml	103.2: 79.1-127.5	42.1: 23.7-92.3	36.2: 18.1-39.8

Md – Median

We determined serum NGAL and IL-18 as markers of tubular kidney lesion, so a rank correlation analysis between serum levels of biomarkers and indicators of tubular kidney lesion in patients with CGN was conducted. Results are presented in Table 3.

**Table 3.** Results of a rank correlation analysis between serum NGAL, IL-18 and indicators of tubular kidney lesion in patients with CGN

The indicator of tubular kidney lesion	Serum NGAL	Serum IL-18
Presence of cellular infiltration	r = + 0.15; p>0.05	r = - 0.03; p>0.05
Necrotic changes in the epithelium of renal tubules	r = + 0.27; p<0.05	r = + 0.34; p<0.05
Dystrophic changes in the epithelium of renal tubules	r = + 0.35; p<0.05	r = + 0.81; p<0.05
Thickening and/or splitting of the tubular basement membrane	r = + 0.42; p<0.05	r = + 0.16; p>0.05
Interstitial fibrosis	r = + 0.65; p<0.05	r = + 0.61; p<0.05

r – Pearson’s correlation coefficient

We discovered that the level of serum NGAL directly correlated with necrotic and dystrophic changes in the epithelium of renal tubules, thickening and/or splitting of the tubular basement membrane. We then statistically confirmed a noticeable direct link between serum NGAL and IF. As to serum IL-18, it directly correlated with necrotic changes in the epithelium of renal tubules, as well as with IF and dystrophic changes in the epithelium of renal tubules. Subsequently, diagnostics of IF was carried out by two methods in 81 patients with CGN: the first method – renal biopsy, which is considered to be diagnostic standard, the second – definition of serum NGAL. Accordingly, we determined Se for IF definition of serum NGAL, which was 90.6%, Sp – 100%, DE – 95.3%. So this method is highly sensitive and specific, with the efficiency up to 95,3 %. Statistic analysis led to the conclusion that raising serum NGAL above 5 ng/ml indicated a high probability of having IF in patients with CGN. We determined Se for dystrophic changes in the epithelium of renal tubules definition of serum IL-18, which was 93.1%, Sp – 100%, DE – 96.6%. Hence this method is highly sensitive and specific, with the efficiency up to 96.6%. Analysis of the constructed nonlinear model led to the conclusion that the level of serum IL-18 above 600 pg/ml indicated dystrophic changes in the epithelium of renal tubules in patients with CGN.

Dynamics of average values of the basic laboratory parameters in patients with CGN before and after ramipril treatment is presented in Table 4.

We observed a decrease in such indicators as blood creatinine and PU by 8.2% and 69%, respectively, (p<0.05) in patients with CGN and AH. In the group of patients with CGN without AH, there was a reduction of PU and blood creatinine to 92% and 9.9% respectively (p<0.05). Beyond the aforementioned, we statistically confirmed that treatment

**Table 4.** Dynamics of average values of the basic laboratory parameters in patients with CGN before and after ramipril treatment (M±S)

The indicator	Patients with CGN and AH (n=49)		Patients with CGN without AH (n=32)	
	before ramipril treatment	after ramipril treatment	before ramipril treatment	after ramipril treatment
Proteinuria, g/l	1.41±0.1	0.43±0.03	0.40±0.04	0.032±0.001
	p<0.05		p<0.05	
Blood creatinine, mmol/l	101.9±38	93.5±2.1	97.2±6.2	87.6±3.1
	p<0.05		p<0.05	
Glomerular filtration rate (CKD-EPI), ml/min/1.73 m <sup>2</sup>	80.0±3.9	80.7±2.6	87.5±3.9	90.4±3.0
	p>0.05		p>0.05	

S – standard deviation

with ramipril in patients with CGN led to a lower average of levels of tubular kidney lesion markers. The dynamics of markers changes before and after ramipril treatment is presented in Table 5.

We observed a decrease in such indicators as NGAL serum up to 38.3%, IL-18 serum – 21.5% (p<0.05) in patients with CGN and AH. In the group of patients with CGN without AH there was also a statistically significant observed reduction in markers: serum NGAL levels – up to 36.6%, IL-18 serum – 29.4 % (p<0.05).

**Table 5.** Dynamics of average levels of tubular kidney lesion markers in patients with CGN before and after ramipril treatment (Md: 25%-75%)

The indicator	Patients with CGN and AH (n=49)		Patients with CGN without AH (n=32)	
	before ramipril treatment	after ramipril treatment	before ramipril treatment	after ramipril treatment
Serum NGAL, ng/ml	6.2: 4.2-7.4	3.9: 2.2-5.4	4.7: 3.8-7.4	2.3: 1.8-5.1
	p<0.05		p<0.05	
Serum IL-18, pg/ml	704.2: 648.6-1201.0	581.6: 295.6-722.8	160.6: 76.5-613.6	110.3: 72.1-295.6
	p<0.05		p<0.05	

Md – Median

Thus, levels of tubular kidney lesion markers reduced under the influence of 24-week ramipril treatment confirmed the nephroprotective effect of the ACE inhibitor, ramipril.

## DISCUSSION

The question of which damage mechanism is the main in the development of nephrosclerosis (glomerular or tubulointerstitial) remains discursive. For today, it is recognized that tubulointerstitial fibrosis is the pathomorphological basis for the progression of kidney failure [5,6]. The main markers of kidney damage in CKD are albuminuria, serum creatinine, and GFR. However, these indicators, reflecting a certain degree of change in the glomerular apparatus and do not carry information about the tubular kidney lesion.

Nowadays, measurement of NGAL and IL-18 levels is recognized as providing, to some extent, the ability to detect tubular kidney lesion [7-9]. However, we were the first who used the correlation analysis method to demonstrate the exact link between serum NGAL and indicators of tubular kidney lesion. In support of our results, an inverse relationship between the levels of markers and GFR, and the direct relationship with the level of PU in patients with CGN was found. Bolignano *et al.* (2009, 2018) in their study also determined the links and considered NGAL as a strong and independent marker for progression of CGN [10,11]. Moreover, the correlation analysis we carried out showed that there is a link between serum IL-18 and necrotic, dystrophic changes in the epithelium of renal tubules, as well as thickening and/or splitting of the tubular basement membrane, IF in the patients with CGN. These results somewhat contradict the modern literature data, where urine IL-18 is the most indicative marker of the tubular lesion itself [4,12], but our research is based on morphological data. In our study, the highest levels of blood IL-18 in patients with CGN and AH were detected, thus confirming the results of limited studies

of IL-18 as an indicator of the level of the activity of the diseases with tubular component [13,14]. Our work statistically proved that there is a direct relationship between serum IL-18 marker and PU in patients with CGN. Furthermore, our results indicate that serum NGAL and IL-18 are the most common markers for tubular kidney lesion. What is more, according to prospective randomized trials (REIN, MICRO-HOPE, AIPRI, AASK, etc.), a decrease in PU with RAAS suppressing drugs leads to a significant decline in the rate of CKD progression, which might suggest the existence of nephroprotective qualities that need to be studied more closely to establish exact pharmacodynamic mechanisms.


In our opinion, hypertensive therapy with ramipril is promising due to antihypertensive activity, as well as to the possible organoprotective effect, which is realized in reversion or stabilization of kidney lesion. It should be emphasized that marker levels decrease even with the use of 2.5 mg of ramipril in the patients with CGN without AH, which demonstrates the absence of a dose-dependent effect of ramipril.


## CONCLUSIONS

Overall there is a direct correlation between serum NGAL and IF, serum IL-18 and dystrophic changes in the epithelium of renal tubules in patients with CGN. As a result, serum levels of NGAL and IL-18 are the most sensitive markers of tubular kidney lesion with diagnostic efficiency up to 97%. According to our study results, a 24-week treatment with ACE inhibitor ramipril in patients with CGN with and without AH will reduce the levels of tubular kidney lesion markers. This outcome confirms the nephroprotective effect of the drug.

## ORCID iDs

Oleksandr Svyntozelskyi

 <https://orcid.org/0000-0002-5761-1131>

Mariia Dolinna  <https://orcid.org/0000-0002-8080-2198>

## REFERENCES

1. Coppo R, Cattaran D. The new oxford clinic-pathological classification of IgA nephropathy. *Sec Biol Med Sci*. 2010;1:241-8.
2. Devarajan P. The use of targeted biomarkers for chronic kidney disease. *Adv Chronic Kidney Dis*. 2010;17:469-79.
3. Presta P, Minutolo R. Renin-angiotensin system inhibitors reduce the progression of mesangioproliferative glomerulonephritis: 10-year follow-up. *Eur J Intern Med*. 2011;22:90-4.
4. Wright JT, Bakris G, Greene T. African American study of kidney disease and hypertension study group. Effect of blood pressure lowering and antihypertensive drug class on progression of hypertensive kidney disease: results from the AASK trial. *JAMA*. 2013;288:2421-31.
5. López-Novoa J, Rodríguez-Peña A, Ortiz A, Martínez-Salgado C, López Hernández F. Etiopathology of chronic tubular, glomerular and renovascular nephropathies: clinical implications. *Nephrology (Saint-Petersburg)*. 2013;17(2):9-38.
6. Okon K, Sulowicz W, Smolenski O. Interstitial, tubular and vascular factors in progression of primary glomerulonephritis. *Pol J Pathol*. 2017;58(2):73-8.
7. Nickolas TL, Barasch J, Devarajan P. Biomarkers in acute and chronic kidney disease. *Curr Opin Nephrol Hypertens*. 2018;43(22):113-27.
8. Schmidt-Ott KM. Neutrophil gelatinase-associated lipocalin as a biomarker of acute kidney injury – where do we stand today? *Nephrol Dial Transplant*. 2011;26(3):762-4.
9. Ding H, He Y, Li K, Yang J, Li X, Lu, et al. Urinary neutrophil gelatinase-associated lipocalin is an early biomarker for renal tubulointerstitial injury in IgA nephropathy. *Clin Immunol*. 2017; 42:227-34.
10. Bolognani D, Coppolino G, Campo S. Urinary neutrophil gelatinase-associated lipocalin is associated with severity of renal disease in proteinuric patients. *Nephrol Dial Transplant*. 2008;23:414-6.
11. Bolognani D, Lacquaniti A, Coppolino G. Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. *Clin J Am Soc Nephrol*. 2009;4(2):44-237.
12. Huang F, Horikoshi S, Kurusu A. Urinary levels of interleukin-18 and disease activity in patients with IgA nephropathy. *J Clin Lab Anal*. 2011;15(1):30-4.
13. Beili S, Ni Z, Cao L, Zhou M. Serum IL-18 is closely associated with renal tubulointerstitial injury and predicts renal prognosis in IgA Nephropathy. *Mediators Inflamm*. 2012;2:1-9.
14. Shimizu C, Fujita T, Fuke Y, Ito K, Satomura A, Matsomoto K, Soma M. High circulating levels of the Interleukin-18 binding protein indicate the severity of glomerular involvement in systemic lupus erythematosus. *Mod Rheumatol*. 2012;22(1):73-9.