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Importance of Urinary NGAL, Serum Creatinine Standardization and Estimated Glomerular Filtration Rate in Resistant Hypertension

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A B S T R A C T

In patients with resistant hypertension (RH) we investigated the importance of urinary neutrophil gelatinase-associated lipocalin (uNGAL- a chemiluminescent microparticle immunoassay (CMIA) method became using (Abbott Diagnostics) for the measurement of NGAL in urine samples) and incidence of chronic kidney disease using the Modification of Diet in Renal Disease Study (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations in estimating glomerular filtration rate (eGFR) based on standardised serum creatinine method traceable to isotope dilution mass spectrometry (IDMS) method. It would have been difficult to predict that levels of these biomarker would perform better organ damage than traditional measurements of kidney function such as standardised serum creatinine, MDRD, or CKD-EPI equations in special population such as RH. Serum creatinine concentrations were measured in 50 patients (24M:26F, from RH Registar in Clinical Hospital Merkur) by the kinetic Jaffe method. There were no significant differences between the GFR values derived by MDRD and CKD-EPI equations in the group of patients with RH. 62% of patients have eGFR >60 mL/min/1.73 m^2, while a 38% of patients have eGFR <60 mL/min/1.73 m^2. The measurement of NGAL in urine samples of 40 patients with RH showed no difference and seems to be of no use in furthere determination of renal impairement. Higher value of uNGAL in some resistant hypertension patients could have link in the repair stage after AKI and would reveal pathways that could link AKI and CKD.

Key words: *uNGAL, serum creatinine, chronic kidney disease, resistant hypertension*

Introduction

Due to the recent international requirement for accurate and specific diagnostic tests and implementation of calibration traceability to high-order reference measurement procedures and reference materials, standardization of serum creatinine is of particular importance because of its role in assessment of renal function and the use of creatinine values for estimation of glomerular filtration rate[1–3]. The National Kidney Disease Education Program (NKDEP) recommendations reporting a numerical estimate in round numbers only for GFR values <60 mL/min per 1.73 m^2 while according to the new guideline (KDIGO) Clinical Practice Guidelines for the Evaluation and Management of Chronic Kidney Disease (CKD) glomerular filtration rate (GFR) classifi- cation scheme has remained with stages 1 through 5 (stage 3 is split into 3A and 3B) with reporting GFR values for all stages[4].

Resistant hypertension is defined as high blood pressure that remains uncontrolled despite treatment with at least three antihypertensive agents (one of which is a diuretic) at best tolerated doses[5]. Prevalence of resistant hypertension in Croatia is not known, but in USA and in Western Europe prevalence is from 9 to 13%[5,6]

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The purpose of our study was to investigate the importance of urinary neutrophil gelatinase-associated lipocalin (NGAL) and incidence of chronic kidney disease in patients with resistant hypertension using the Modification of Diet in Renal Disease Study (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD--EPI) equations in estimating glomerular filtration rate (eGFR) based on standardised serum creatinine method traceable to IDMS method.

Subjects, Materials and Methods

This study was conducted in University Hospital Merkur' among 50 patients with resistant hypertension (RH). Hypertension is defined as resistant to treatment when a therapeutic strategy that includes appropriate lifestyle measures plus diuretic and two other antihypertensive drugs belonging to different classes at adequate doses (but not necessarily including a mineralocorticoid receptor antagonist) fails to lower blood pressure (BP) values to < 140/90mmHg, respectively [7]. Prior to diagnosing a patients as having RH, we document adherence and exclude white-coat hypertension, inaccurate measurement of BP, and secondary causes of hypertension. Basic anthropometric measurements were performed on all study subjects. For blood pressure measurement we used different cuffs adjusted to the arm circumference. Office blood pressure was measured twice in the sitting position with a mercury sphygmomanometer after a resting period of 10 minutes and expressed in mmHg. To exclude WCH and to confirm the diagnosis of RH we use 24-h *ambulatory blood pressure monitoring* – ABPM Mobil-O-Graph, Hypertension Managemnet Software for Windows, version 6.02.040. We expend extreme energy to improve patient compliance and controlled adherence (few days in hospital may be necessary to check the BP effect...). We define poor or unhealthy life style in sedentary overweight smoking/drinking subjects with no exercise habits, on high-salt diet, with negative feelings about medicine. Number of antihypertensive medication was for 3–8 and most used drug classes were : diuretic in 100% (including thiazides and indapamide), angiotensin-converting enzyme (ACEI) inhibitors or angiotensin receptor blockers (ARB) in 88%, calcium antagonists in 84%, beta blockers (nebivolol or carvedilol) in 82%, central acting drugs in 64%, alfa-blockers in 26%, vasodilatators in 8%, mineralocorticoid receptor antagonists, i.e. spironolactone 25–100 mg in 24% or eplerenone (50 mg) in 4% and a direct inhibitor of renin-aliskirenin in 4%. Acute and chronic inflammation was excluded on the basis of medical history, physical examination, and routine laboratory tests, including measurement of temperature and urinalysis.

The study protocol complies with the Declaration of Helsinki as well as with local institutional guidelines, and was approved by the local ethics committees (by ethic committee of the Hospital Merkur).

Serum creatinine concentrations and GFR estimations (from standardized creatinine, with MDRD and CKD-EPI equations[3] were determined in 50 patients (24M:26F, from RH Registar in Clinical Hospital Merkur) at Department of Medical Biochemistry and Laboratory Medicine, accredited according to ISO 15189[8]. Venous blood was collected under controlled pre-analytical conditions into vacutainer tubes (Becton Dickinson) without additives. Serum was prepared 30 min after blood collection by centrifugation at 1800 x g. and anlysed on the same day. Serum creatinine concentrations were measured by the kinetic Jaffe method (Beckman Coulter OSR6178) traceable to the isotope dilution mass spectrometry (IDMS) method and National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 967 calibrator. All measurements were performed on the Beckman Coulter AU 680 multiparametric chemistry analyzer (Beckman Coulter Inc., USA). Estimates of within laboratory precision were provided by internal quality control data using pool serum samples and commercial control sera for normal (ODC0003 Beckman Coulter control level1) and pathological (ODC-0004 Beckman Coulter control level 2) concentrations. Trueness estimates were based on the long-term results of external quality assessment (EQA) obtained by participation in the National EQA Scheme organized by Croatian Society of Medical Biochemistry and Laboratory Medicine and International EQA schemes for general and special medical biochemistry organized by Labquality – WHO Collaborating Centre for Education and Training in Laboratory Quality Assurance, Helsinki, Finland[9]. Estimation of measurement uncertainties was done on the basis of the »Guide to the Expression of Uncertainty in Measurement«[10]. The obtained expanded measurement uncertainties (k=2) were in normal concentration range 5,6 and in pathological concentration

TABLE 1
CLINICAL AND METABOLIC CHARACTERISTICS OF 50 PATIENTS

Variable	Value
Age (years)	64 (25–89)
Duration of hypertension (years)	11 (1–45)
Body mass index (kg/m^2)	34 (25–47)
Systolic blood pressure (mmHg)	170 (145–220)
Diastolic blood pressure (mmHg)	98 (90–120)
LDL cholesterol (mmol/L)	3.8±0.6
HDL cholesterol (mmol/L)	1.1±0.4
Triglycerides (mmol/L)	1.91 (1.4–4.1)
Serum creatinine (μmol/L)	112±14
eGFR (mL/min/1.73 m^2)	51±16
Urinary NGAL (ug/L)	47.2 (9.5–444.6)
Erythrocytes (x10^{12}/L)	4.31±1.5
Haemoglobin (g/L)	132±17
AKI	9 patients

eGFR – estimated glomerular filtration rate, AKI – acute kidney injury in the past, NGAL – neutrophil gelatinase-associated lipocalin; Values are expressed as mean ± standard deviation; Data are presented as number

range 5,2% indicating that measurement of less than 8.2% for total analytical error was achieved[3].

A chemiluminescent microparticle immunoassay (CMIA) method became commercially available, using the automated platform ARCHITECT (Abbott Diagnostics)[11] for the measurement of NGAL in urine samples of 40 patients with RH (28 with eGFR<60 and 12 with eGFR <60 mL/min per 1.73 m^2, uNGAL – reference interval <132 μg/L).

Data are expressed as means ±SD for normally distributed values, as median with range for non-normally distributed values, and percentage using the Statistics for Windows program. To investigate the relation between urinary NGAL with renal function parameters data were also stratified in different groups of eGFR and information of acute kidney injury (AKI) in the past. Level of statistical significance was chosen to be $\alpha=0.05$. Statistical analysis was performed by statistical package STATA/IC ver.11.1.

Results

The characteristics of the study subjects with resistant hypertension are listed in Table 1. Mean/median values of cholesterol, triglycerides, erythrocytes and haemoglobin levels as well as urine analysis were performed for 50 patients. 60% of patients with resistant hypertension were overweight and obese (12% BMI >40 kg/m^2). Active urine sediment (erythrocytes) was found in 20% and albuminuria in 26 % of patients.

Risk factors such abdominal obesity (waist circumference in men >102 cm, women >88 cm) was detected in 86% of patients and asymptomatic organ damage-chronic kidney disease with eGFR 30–60 mL/min/1.73 m^2 in 36%.

In the RH patients creatinine values in the early stages of CKD fallen within population based reference intervals, as shown in Table 2. MDRD = 60 mL/min per 1.73 m^2 (stages 1 and 2 of CKD) have 62 % of patients (17 males and 14 females). 38 % of patients have CKD stage 3

TABLE 2
LEVELS OF uNGAL AND eGFR VALUES DERIVED BY MDRD AND CKD-EPI EQUATIONS

		eGFR mL/min/1.73 m^2		
		=60	<60	p
Age (yrs)	Mean±SD	71.25±12.08	55.79±12.75	<0.05
	Median	71	56.5	
	Range	49–89	25–82	
Creatinine (IDMS) μmol/L	Mean±SD	76.82±23.37	250.44±140.11	0.02
	Median	78	221	
	Range	44–111	87–482	
MDRD Sex (n= 24; 48%) (n= 26; 52%)	Male	17	7	0.044
	Female	14	12	
CKD-EPI Sex (n= 24; 48%) (n= 26; 52%)	Male	16	8	
	Female	15	11	
uNGAL (μg/L) n=40	Mean±SD	75.69±119.62	35.91±62.91	0.189
	Median	43.6	24.12	
	Range	5.1–444.6	6.4–350.9	
uNGAL in AKI n=7	Mean±SD	136.2±88.2	188.4±48.3	0.44
	Median	53.2	58.6	
	Range	40.3–444.6	26.2–350.9	
CHRONIC KIDNEY DISEASE		MDRD	CKD-EPI	
Stage 1 and 2 (sex, n)	Male	17	16	1.0
	Female	14	15	
Stage 3a and 3b (sex, n)	Male	3	4	
	Female	8	7	
Stage 4 (sex, n)	Male	3	3	
	Female	4	4	
Stage 5 (sex, n)	Male	1	1	
	Female	0	0	

Data are presented as number and (%); RH – resistant hypertension, IDMS – isotope dilution mass spectrometry, eGFR – estimated glomerular filtration rate, MDRD – Modification of Diet in Renal Disease Study, CKD-EPI – Chronic Kidney Disease Epidemiology Collaboration, AKI – acute kidney injury in the past, NGAL – neutrophil gelatinase-associated lipocalin.

(22%, 3 males and 8 females), stage 4 (14%, 3 males and 4 females) and stage 5 (2%, one man). CKD-EPI = 90 mL/min per 1.73 m^2 (stage 1of CKD) have 11 patients (6M:5F), stage 2 have 20 patients (9M:11F), stage 3a was only in female (5), stage 3b in 5patients (3M:2F), stage 4 in 8 patients (3M:5F) and in one man was stage 5. There were no significant differences between the eGFR values derived by MDRD and CKD-EPI in patients with RH ($p>0.05$). Creatinine values and patients number were significant different between eGFR groups (Table 2). Number of RH patients with eGRF<60 mL/min per 1.73 m^2 was significantly lower ($p<0.05$), they were significantly younger ($p<0.05$) and creatinin values were significantly over in them ($p=0.02$).

Association of uNGAL with parameters of renal function is performed in 40 RH patients: 12 patients with eGFR<60 and in 28 patients with eGFR=60 mL/min per 1.73 m^2. Preliminal data of uNGAL were 35,91 (6,4–350,9) ug/L in RH patients with eGFR<60 and 75,69 (5,1–444,6) ug/L in RH patients with eGFR=60 mL/min per 1.73 m^2. There were no significant differences between levels of uNGAL and eGFR values in patients with RH ($p=0.189$). Patients with RH who had AKI in the past (it was found in 7 patients with eGFR<60 and 2 with eGFR=60 mL/min per 1.73 m^2) had not differences between levels of uNGAL ($p=0.44$).

Preliminal data of uNGAL and AKI in the past were 136.2 (40.3–446.6) ug/L in RH patients with eGFR<60 mL/min per 1.73 m^2 and 188.4 (26.2–350.9) ug/L in patients with eGFR=60 mL/min per 1.73 m^2 (uNGAL – reference interval <132 μg/L) shown in Table 2.

Discussion

Reporting of eGFR rate is of most importance in recognition of early stages of CKD characterised with slightly or moderately reduced eGFR and serum creatinine values usually within the population based reference intervals. Ceriotti F, et al. recommended »common« reference intervals for global application that could be used for creatinine measurement in all laboratories using methods producing traceable results to IDMS[12]. Department of Medical Biochemistry and Laboratory Medicine, University Hospital Merkur, Zagreb, Croatia as the Reference Center of the Ministry of Health of Croatia for the production of reference values in the field of general medical biochemistry approved the applicability of common reference intervals for serum creatinine concentrations to the Croatian population[13]. Common reference intervals for creatinine concentrations in serum, adapted from Ceriotti F, et al. in healthy adult population ranged from 64 to104 μmol/L in males and from 49 to 90 μmol/L in females[12]. We don't know the prevalence of CKD in patients with RH, and the prevalence of RH in CKD (I–IV stages) patients is also underestimated[14]. Obesity as well as chronic kidney disease could be the reason of resistance and 60% of patients in this study were overweight and obese.

The new guideline (KDIGO) Clinical Practice Guidelines for the Evaluation and Management of CKD recommended the CKD-EPI equations for estimating glomerular filtration rate (eGFR) with every serum creatinine result for all clinical laboratories[4]. This equation is characterised by a better analytical performance for estimating GFR and gives more satisfactory results even for eGFR in ranges between 60 and 90 mL/min per 1.73 m^2. According to our preliminary results presented in Table 2 in the group of patients with RH creatinine values in the early stages of CKD fallen within population based reference intervals, but there were no significant differences between the eGFR values derived by MDRD and CKD-EPI equations. So we measured urinary NGAL (uNGAL) – molecular marker for ischemia and compared data. Acute kidney injury (AKI) is being increasingly shown to be risk factor for chronic kidney disease (CKD), but little is known about possible mechanisms. Data from animal models suggested that persistent inflammation and immune responses late after AKI could contribute to the pathogenesis of CKD, late upregulation of NGAL could be a useful marker for sustained renal injury after AKI and hypertension-related gene changes could underlie mechanisms for persistent renal and vascular injury after AKI[15,16]. Reference values of NGAL are rather arbitrary, since they have emerged from groups of healthy people that participated in a clinical study as a control group. The kit insert of the CMIA assay reports a value of 132 ng/mL as the 95th percentile of NGAL values, measured in 196 blood donors[10].

Limitations regarding NGAL measurement include storage conditions (NGAL is stable in urine if stored at 4 °C for up to 7 days and plasma or urine samples are stable if stored for a long time at –80 °C), presence of haemolysis, and production of NGAL by neutrophils in urinary tract infections. In order to limit this phenomenon, urine should be centrifugated to remove neutrophils in cases of urinary tract infection.

In this study significant differences were found only in the levels of uNGAL among patients who had AKI in the past (7 patients. with eGFR <60 and 2 >60 mL/min per 1.73 m^2), therefore persistent inflammation and immune responses late after AKI could contribute to the pathogenesis of CKD in human medicine?

In conclusion, there were no significant differences between the eGFR values derived by MDRD and CKD--EPI in patients with RH. Both eguations for estimation of eGFR are equal, and uNGAL seems to be of no use in further determination of renal impairment in RH patients.

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ZNAČENJE RAZINE NGAL-A I PROCJENE BUBREŽNE FUNKCIJE U BOLESNIKA S REZISTENTNOM HIPERTENZIJOM

SAŽETAK

U radu se istražuje moguća povezanost razine urinarnog neutrophil gelatinase-associated lipocalin (NGAL, CMIA, Abbott) i učestalost kronične bubrežne bolesti u bolesnika s rezistentnom hipertenzijom (RH) služeći se dvjema metodama: Modification of Diet in Renal Disease Study (MDRD) i Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formulama u određivanju veličine glomerularne filtracije (GFR) bazirane na standardiziranom kreatininu. U populaciji 50 bolesnika (24M:26F) s RH (iz Registra RH KB Merkur, odobreno od Etičkog povjerenstva) odredili smo koncentraciju serumskog kreatinina, eGFR je određen primjenom MDRD i CKD-EPI, a značajne razlike između metoda nije nađeno. Prvi i drugi stupanj KBB (MDRD=60) imalo je 62% bolesnika (17M:14F). 38% imalo je treći (22% – 3M:8F), četvrti (14% – 3M:4F) i peti (2%) stupanj KBB. Razina uNGAL-a određena je u 40 bolesnika. Prosječna razina uNGAL-a bila je unutar referentnih intervala i ne korelira sa stupnjem bubrežnog oštećenja. Povišena razina uNGAL-a utvrđena je samo kod dijela bolesnika s RH koji su imali u prošlosti akutnu ozljedu bubrega (AKI) čime možda možemo povezati mehanizme perzistentne bubrežne ozljede nakon AKI i eventualno predvidjeti razvoj KBB.