Effects of Mono- and Dual Blockade of the Renin-Angiotensin System on Markers of Cardiovascular Status in Hypertensive Patients with Mild and Moderate Renal Failure

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Key Words

Brain natriuretic peptide • Endogenous ouabain • Immuno-unreactive urinary albumin • Renin-angiotensin system blockade • Renal failure • Subclinical organ damage

Abstract

Background/Aims: Dual renin-angiotensin system (RAS) blockade has no more efficiency to decrease cardiovascular mortality than mono-blockade. Our goal was to explore differences between other cardiovascular markers in patients with RAS blockade. **Methods:** We analyzed two groups of patients treated with a long-term ACE inhibitor (MONO-group, n = 20) and an ACE inhibitor and angiotensin II receptor blocker (DUAL-group, n = 15). Ambulatory blood pressure monitoring, echocardiography, arterial stiffness and levels of catecholamine, endogenous ouabain (EO), pro-brain natriuretic peptide and more types of urinary albumin measurements were performed. **Results:** In the DUAL-group, we found significantly better cardiac parameters, but the levels of EO and urinary albumins were similar in both groups. The level of EO correlates with nighttime mean arterial blood pressure ($R = 0.556$, $p = 0.032$) and arterial β -stiffness ($R =$

0.512 , $p = 0.042$). Urinary immuno-unreactive albumin showed a relationship with diastolic dysfunction of the heart ($R = -0.508$, $p = 0.045$) diurnal index of diastolic blood pressure ($R = -0.569$, $p = 0.021$) in the MONO-group. **Conclusion:** Cardiac parameters were more prosperous in the DUAL-group, but the levels of EO did not differ between groups. The level of EO correlated with blood pressure and arterial stiffness markers in the MONO-group only. The urinary immuno-unreactive albumin may be a new marker of cardiovascular conditions.

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Introduction

Hypertension is commonly associated with other conditions such as diabetes mellitus and chronic kidney disease (CKD), especially in elderly [1]. In the pathophysiological background of these diseases, the increased renin-angiotensin system (RAS) activity plays a major role [2]. The treatment of these high-risk patients is complex, but the main goal is to reduce the grade of albuminuria and other clinical and subclinical organ damage [3]. Before

the ONTARGET study, some studies proved that the dual blockade (angiotensin-converting enzyme inhibitor and angiotensin II receptor blocker) is better for reducing proteinuria and blood pressure in hypertensive patients with diabetes mellitus and albuminuria [4]. The dual blockade has an additive effect on reduction of left ventricular hypertrophy, arterial pulse wave velocity and plasma pro-brain natriuretic peptide (pro-BNP) level compared to the mono-RAS blockade in hypertensive patients with left ventricular hypertrophy [5].

The ONTARGET study in turn suggested that the long-term dual blockade worsens the renal outcome and does not decrease the mortality in patients with high cardiovascular risk [6, 7].

Besides RAS activation, other pathophysiological abnormalities also play a role in the development of hypertension and CKD, such as increased sympathetic activation and increased levels of some endogenous vasoactive agents. Endogenous digitalis-like factors [e.g. endogenous ouabain (EO) and marinobufagenin], which are mainly formed in the adrenal cortex, have been linked to the pathophysiology of essential hypertension, congestive heart failure and end-stage renal failure [8]. Measurement of serum N-amino terminal fragment of pro-BNP has frequently been used in the risk assessment of cardiovascular events and is known as a strong predictor of cardiovascular mortality and cardiovascular events [9].

CKD and diabetic nephropathy start with a low amount of excretion of albumin (microalbuminuria) and are associated with increased risks of cardiovascular morbidity and mortality [10–12]. Early and exact detection of urinary albumin is important in patients with hypertension, diabetes mellitus and CKD to determine the appropriate therapy [13, 14]. Based on the different measurement methods, urinary albumin exists in immunoreactive and immuno-unreactive forms [15, 16]. The recently developed high-performance liquid chromatography (HPLC) assay measures the total (immunoreactive plus immuno-unreactive) urinary albumin, whereas the conventional immuno-based methods (radioimmunoassay, immunoturbidimetry, immunonephelometry) measure immunoreactive albumin only [11]. Some previously published studies have shown significant associations between the level of urinary immunoreactive albumin and markers of cardiovascular diseases, but there is little information about the level of urinary immuno-unreactive albumin [17].

Our goal was to compare the complex cardiovascular status (including more types of urinary albumin, urinary and plasma EO, urinary catecholamines, serum pro-BNP and some markers of cardiovascular status including the

results of echocardiography, echo-tracking and blood pressure) of the hypertensive patients with mild and moderate renal failure treated with mono- or dual RAS blockade.

Subjects and Methods

Participants

In a cross-sectional clinical study, 35 adult hypertensive patients with mild and moderate renal failure were investigated. Retrospectively, these patients received either mono- or dual therapy for blockade of the RAS blockade. In the MONO-group, patients were treated with an ACE inhibitor ($n = 20$; for more than 2 years); in the DUAL-group, patients were treated with an ACE inhibitor plus angiotensin II receptor blocker ($n = 15$; for more than 2 years; table 1). Inclusion criteria were age above 50 years, hypertension and a diagnosis of mild or moderate renal failure. Agents of RAS inhibition were unchanged for at least 1 year. All patients were on a low-sodium diet and their therapies were controlled by the physicians in our outpatient clinic. We do not have any data on the extent of target-organ damage in the patients at the time of initiation of mono- or dual blockade, except for proteinuria.

The cause of the initiation of the dual blockade was persistent albuminuria in spite of the treatment with the maximal dose of an ACE inhibitor. The drug dosage of the patients was individually determined, and a minimal effective dose of the agents that normalized blood pressure and albuminuria was used. Side effects of different RAS blockades were not found in this population.

CKD patients were diagnosed with diabetic nephropathy and nephrosclerosis. Exclusion criteria were hyper/hypothyroidism, adrenal gland adenoma, malignancy, acute infection, atrial fibrillation and pacemaker therapy. All subjects were treated for hypertension and CKD and/or type 2 diabetes mellitus in our outpatient clinic, and had no symptoms of severe congestive heart failure. None of the subjects was taking cardiac glycoside, spironolactone, prednisolone or other steroids.

The study was approved by the Ethical Committee of the Medical Faculty of the University of Pécs and all participants gave their written informed consent before taking part in the study. The study was conducted in agreement with the Declaration of Helsinki.

The ambulatory blood pressure monitoring (ABPM) was performed with ABPM-04 (Meditech Ltd., Budapest, Hungary) based on a validated oscillometric technique. During the 24-hour monitoring period, the blood pressure was registered in 15-min intervals in the daytime (8 a.m. to 10 p.m.) and 30-min intervals at nighttime (10 p.m. to 8 a.m.). The hypertensive time index was expressed as a percentage of blood pressure values that exceeded the upper level of the normal range of blood pressure during daytime (systolic/diastolic: 140/90 mm Hg) and nighttime (systolic/diastolic: 130/80 mm Hg). The results of blood pressure monitoring were analyzed by the proprietary software of ABPM-04.

Antihypertensive and insulin/antidiabetic therapies were not interrupted during the study.

There were no significant differences in serum sodium, potassium, chloride, calcium, liver enzymes (ALAT, ASAT, GGT,

Table 1. Characteristics of MONO- and DUAL-group

	MONO	DUAL
Sex, male/female	11/9	5/10
Age, years	65.7 ± 7.9	60.8 ± 6.9
Duration of hypertension, years	7.9 ± 2.3	6.1 ± 3.1
Duration of CKD, years	3.0 ± 1.3	2.7 ± 1.6
Duration of diabetes mellitus, years	4.0 ± 1.6	3.2 ± 1.7
Duration of RAS blockade before this study, years	2.6, 1.2–3.4	2.4, 1.1–3.2
Type 2 diabetes mellitus, n (%)	7 (35.5)	7 (46.6)
β-Blocker, n (%)	13 (65.0)	6 (40.0)
Calcium channel blocker, n (%)	11 (55.0)	10 (66.7)
Diuretics, n (%)	10 (50.0)	8 (53.3)
Oral antidiabetics, n (%)	6 (30.0)	3 (20.0)
Insulin, n (%)	1 (5.0)	3 (20.0)
Oral antidiabetics plus insulin, n (%)	1 (5.0)	1 (6.6)
BMI	28.1 ± 4.1	31.0 ± 5.1
HbA _{1c} , %	5.7 ± 0.3	6.3 ± 0.9*
eGFR, MDRD ml/min/1.73 m ²	55.3 ± 24.2	62.6 ± 20.6
24-hour urinary total albumin, mg/l	24.4, 17.1–60.7	24.11 ± 9.9
24-hour urinary immunoreactive albumin, mg/l	1.0, 0.2–14.0	1.0, 0.0–3.0
24-hour urinary immuno-unreactive albumin, mg/l	27.2 ± 15.6	21.1 ± 9.1
Ratio of total and immuno-unreactive urinary albumin, %	75.4 ± 32.5	89.1 ± 14.2
Urinary norepinephrine, nmol/mmol creatinine ¹	26.0 ± 14.9	24.9 ± 14.7
Urinary epinephrine, nmol/mmol creatinine ¹	7.6 ± 4.3	6.2 ± 1.8
Urinary dopamin, nmol/mmol creatinine ¹	102.6 ± 54.8	128.2 ± 53.1
Plasma EO, pmol/l	14.7 ± 8.5	14.6 ± 6.1
Urinary EO, pmol/day	99.4 ± 30.9	102.4 ± 46.4
Pro-BNP, pg/ml	176.2, 90.5–373.8	30.5, 9.8–81.1*
Intima-media thickness, mm	0.81 ± 0.14	0.70 ± 0.23

Data are presented as means ± standard deviation or as median and interquartile range; and frequency (%) in cases of categorical variables. ¹ Measurement in morning urine. * p < 0.05 vs. MONO-group.

LDH), serum triglyceride, HDL, LDL cholesterol, C-reactive protein, or hematological parameters (red blood cell count, white blood cell, platelet) between groups (data not shown).

Patients' morning fresh urine was assessed by routine examination, including pH, glucose, protein, pus, acetone, urobilinogen and creatinine, sodium, and potassium. Urinary creatinine was measured as a part of routine laboratory procedure by buffered kinetic Jaffe reaction (IDMS-calibrated) without deproteinization (Cobas Integra 400; Roche, Germany). The assessments of fresh urines did not show any abnormalities and there were no significant differences between groups in the results of routine fresh urinary examination (data not shown). The patients also collected 24-hour urine to measure the level of EO and microalbumin.

Plasma and Urinary EO Determination

All patients had a 6-ml morning blood draw from the cubital vein in EDTA tubes between 7:00 and 8:00 a.m. after being in a strict supine position for 40 min. The blood samples were centrifuged at 3,000 g for 10 min, and plasma was separated and transferred into plastic tubes and stored at –20°C until assayed. Ten-milliliter urinary samples were taken out of the 24-hour collected

urine and stored at –20°C. The plasma and urinary samples were assayed after collecting all samples; the average storage period was 2 months. Plasma and urinary EO levels were determined by radioimmunoassay with Ouabain ¹²⁵I RIA kit (Biotop OY, Medipolis Center, Oulu, Finland), as previously described [18]. All samples were measured in duplicate.

Urinary Catecholamine and Serum pro-BNP Measurements

The morning urinary catecholamines (epinephrine, norepinephrine and dopamine) were measured by the Bio-Rad Clinical HPLC System (Bio-Rad Laboratories Inc., France). Briefly, the acidified urinary samples were prepared by Analytical Micro-Guard™ Cartridges and 20 µl of prepared samples were analyzed by HPLC with the Model 1340C Electrochemical Detector (at 0.55 mV). Urinary catecholamines/creatinine (assessed by Jaffe methods) ratios were calculated.

Serum pro-BNP levels were measured on a fully automated (Elecys™ 2010) system (Roche Diagnostics, Mannheim, Germany), which is an electrochemiluminescent sandwich immunoassay using two polyclonal antibodies directed at the N-TpBNP molecule. The intra-assay and interassay variabilities were 1.3–4.2 and 1.8–4.6%, respectively [19].

Urinary Microalbumin Measurements

The 24-hour urine samples were vortexed and centrifuged (10 min, 2,500 g). Supernatants were divided into two portions. One portion was used to detect urinary albumin concentration by immunoturbidimetry using a Roche/Hitachi 812 Modular P Analyser (sensitivity: 3 mg/l, intra-assay precision: 1.3 and 1.7%, inter-assay precision 4.3 and 2.6%, respectively, linearity: 3–3,000 mg/l; Roche Diagnostics GmbH, Mannheim, Germany). The other portion was used for HPLC analysis straightaway or in a few days (after storage at -20°C). For HPLC measurements, the FDA-approved AccuminTM kit [Accumin Diagnostics Inc., New York, N.Y., USA; sensitivity (quantitation limit): 3 mg/l, linearity: 3–2,000 mg/l, interassay and intra-assay precision (percentage coefficient of variation): 5.8 and 2.5% respectively], was used.

The amount of immuno-unreactive albumin was calculated as the difference between HPLC and immunoturbidimetry measured urinary albumin. In this study, we did not calculate with the albumin/creatinine ratio because the concentration of albumin was measured from 24-hour collected urine.

Echocardiography and Echo-Tracking Examination

Echocardiograms were recorded with an Aloka SSD 5500 (Aloka Co. Ltd., Tokyo, Japan) ultrasound imaging system equipped with a 3.5-MHz transducer. All patients were assessed by the same cardiologist and examined within 1 h (between 7:30 and 8:30 a.m.) after the collection of EO samples and ABPM. Before echocardiography and echo-tracking, all patients were in a supine position for at least for 5 min. Operators were blinded to the status of the patients. Left ventricle (LV) internal diameter, septal and posterior wall thickness were measured at end-diastole and end-systole, according to the American Society of Echocardiography guidelines [20]. Left ventricular mass was calculated at end-diastole by using the Devereux correction according to the American Society of Echocardiography cube left ventricular formula. Individual values for LV mass were indexed by body surface area. LV end-diastolic and end-systolic volumes were obtained by using the Teicholz formula. Stroke volume was calculated as the difference between end-diastolic and end-systolic volumes. The ejection fraction was calculated as the ratio of stroke volume to LV end-diastolic volume. Individual values were indexed by body surface area. The stroke index was multiplied by the heart rate to obtain the cardiac index. Blood pressure was recorded at the time of echocardiography. LV diastolic dysfunction was defined using transmitral Doppler parameters. Transmitral Doppler peak early diastolic E-wave velocity (cm/s), peak late A-wave diastolic velocity (cm/s), E/A ratio and deceleration time (ms) were recorded [20]. The atrial areas (mm^2) were measured by the product of two atrial diameters.

Arterial stiffness parameters as the β -stiffness index, pulse wave velocity and augmentation index were assessed by the characteristics of pulse wave intensity using an echo-tracking system (Aloka SSD-5500) with a 10-MHz linear array probe. We used the right common carotid arterial diameter change waveforms to obtain pressure waveforms noninvasively. The peak and bottom values of a diameter change waveform were calibrated using systolic and diastolic pressure measured with a cuff-type manometer applied to the upper arm, and the diameter change waveform was used as a blood pressure waveform [21].

Intima-Media Thickness Measurement

Intima-media thickness was measured in the supine position by a real-time ultrasound scanner equipped with a 10-MHz linear transducer (ALOKA SSD 4000). The measurement of intima-media thickness was done in B mode on the carotid artery under the bifurcation of the carotid artery in an anterolateral longitudinal view.

Statistical Analyses

The data are expressed as mean values $\pm$ standard deviation, or as median values and interquartile range depending on the distribution. Distribution of the variables was analyzed using a Kolmogorov-Smirnov test. Normally distributed variables were compared using a two-sample t test, while non-normal distributed variables were compared with a Mann-Whitney U test. Parameter differences between the groups were analyzed with a univariate general linear model, adjusted for HbA_{1c} , LVESD, LVESV and ejection fraction of the heart. Noncontinuous parameters were analyzed by a χ^2 test. The correlations between variables were analyzed using Pearson's (in cases of normally distributed variables) and Spearman's (non-normally distributed variables) correlations. The statistical analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, Ill., USA) and two-tailed $p < 0.05$ was considered statistically significant.

Results

The main characteristics of the MONO- and DUAL-groups are presented in table 1. The level of pro-BNP was significantly higher in the MONO-group and HbA_{1c} in the DUAL-group, but after adjusting for parameters which were significantly different between the two groups (HbA_{1c} , LVESD, LVESV and ejection fraction), the difference was not significant. After adjusting for HbA_{1c} only, the level of pro-BNP remained significant ($p = 0.045$).

The level of pro-BNP correlated with almost all parameters of echocardiography listed in table 2 ($R = 0.341$ – 0.524) in both groups (data not shown).

Sodium and the potassium/creatinine ratio were measured in the morning fresh urine, but no differences between the groups were found ($p > 0.05$).

The cardiovascular parameters of the two groups are shown in table 2.

We found no differences between the two groups regarding blood pressure and arterial stiffness parameters. The parameters of echocardiography showed some differences, but after adjusting for HbA_{1c} , only the ejection fraction remained significantly higher in the DUAL-group than in the MONO-group.

Endogenous Ouabain

We also wanted to analyze the correlation of levels of EO and urinary immuno-unreactive albumin, which

Table 2. Cardiovascular parameters of MONO- and DUAL-group

Parameter	MONO	DUAL
<i>ABPM, mm HG</i>		
24-hour mean		
Systolic blood pressure	127.2 ± 15.0	130.4 ± 19.9
Diastolic blood pressure	70.5 ± 9.4	73.6 ± 8.5
Arterial blood pressure	88.7 ± 10.3	92.2 ± 11.6
Pulse pressure	56.5 ± 10.3	56.0 ± 13.0
Daytime mean		
Systolic blood pressure	129.3 ± 14.6	132.3 ± 19.2
Diastolic blood pressure	73.1 ± 9.6	77.0 ± 8.8
Arterial blood pressure	91.8 ± 10.0	95.5 ± 11.5
Pulse pressure	55.8 ± 10.7	54.7 ± 12.6
Nighttime mean		
Systolic blood pressure	120.8 ± 13.9	127.4 ± 22.5
Diastolic blood pressure	63.8 ± 8.4	68.1 ± 8.8
Arterial blood pressure	82.5 ± 9.4	87.7 ± 12.5
Pulse pressure	56.6 ± 10.5	59.0 ± 16.4
<i>Echocardiography</i>		
LVEDD, mm	32.7 ± 5.2	29.2 ± 3.2 ^a
LVEDD, mm	48.7 ± 5.3	46.7 ± 3.6
LVESV, ml	39.5 ± 19.9	27.1 ± 8.9 ^a
LVEDV, ml	125.1 ± 41.7	108.7 ± 25.5
LVEDVI, ml/m ²	67.0 ± 20.5	57.4 ± 13.6
LVMI, g/m ²	153.6 ± 47.4	138.8 ± 24.7
Ejection fraction, %	56.6 ± 4.5	60.6 ± 4.3 ^{a,*}
E/A	0.87 ± 0.34	0.91 ± 0.30
SI, ml/m ²	45.8 ± 11.8	43.1 ± 10.4
CI, l/min/m ²	3.14 ± 0.88	2.95 ± 0.54
<i>Echo-tracking</i>		
Carotid PWV, m/s	7.3 ± 1.6	6.9 ± 1.4
Carotid Aix, %	23.7 ± 12.3	16.4 ± 9.4
Carotid β-stiffness	13.0 ± 8.6	10.8 ± 4.0

Data are presented as means ± standard deviation.

^a p < 0.05 vs. MONO-group.

* p < 0.05 vs. MONO-group after adjusting for HbA_{1c}.

LVEDD = Left ventricular end-systolic diameter; LVEDD = left ventricular end-diastolic diameter; LVESV = left ventricular end-systolic volume; LVEDV = left ventricular end-diastolic volume; LVEDVI = left ventricular end-diastolic volume index; LVMI = left ventricular mass index; E/A = the ratio between the early and late peak flow velocity; SI = stroke index; CI = cardiac index; Aix = augmentation index.

were not investigated previously in the context of mono- and dual RAS blockade in hypertensives.

The level of plasma EO correlated with nighttime systolic (R = 0.512, p = 0.046), mean arterial blood pressure (R = 0.541, p = 0.038) and nighttime hypertensive time index of mean arterial pressure (fig. 1a), and urinary EO was associated with arterial stiffness parameters, such as

carotid artery pulse wave velocity (R = 0.621, p = 0.005) and β-stiffness (fig. 1b) in the MONO-group. These correlations were not found in the DUAL-group.

Urinary Albumins

Urinary immunoreactive albumin did not show any relationship with cardiovascular parameters or hormones in either the MONO- or DUAL-group. In the MONO-group, total urinary albumin was significantly associated with some blood pressure parameters, such as the 24-hour and daytime hypertensive time index of diastolic blood pressure (R = 0.611, p = 0.034 and R = 0.654, p = 0.026, respectively) and diastolic dysfunction (E/A) of the heart (R = -0.467, p = 0.049). Immuno-unreactive albumin strongly correlated with diastolic dysfunction (E/A) of the heart and the diurnal index of diastolic blood pressure (fig. 2). In the DUAL-group, total urinary albumin and immuno-unreactive albumin correlated only with the plasma level of EO (fig. 3).

Discussion

Our first main finding was that numerous markers/parameters of the cardiovascular system did not show any difference between long-term mono- and dual RAS blockade in hypertensive patients with comorbidities. These results are in agreement with the previous results of the ON-TARGET study [6]. On the other hand, left ventricular diameters and function (ejection fraction) were significantly better in the DUAL-group than in the MONO-group, and the level of pro-BNP was significantly lower in the DUAL-group, which also shows an improved cardiac prognosis. The difference of levels in pro-BNP remained significant after adjusting for HbA_{1c}, but ceased after adjusting for HbA_{1c} and cardiac parameters. The association of the level of pro-BNP with parameters of echocardiography and the differences between the groups underline the hypothesis that the main cause of the level difference of pro-BNP may be the altered cardiac conditions.

The differences between echocardiographic parameters could not be explained by different sympathetic activity and its suppressions because the excreted levels of catecholamine and β-blocker therapy were similar.

The levels of plasma and urinary EO were similar in the two groups. Our level of plasma EO was similar to the one of the other study using the same method [18].

It is known that the production of EO can be decreased by an ACE inhibitor [8], but there are no data on the effect of dual blockade. According to the results of this cross-

Fig. 1. Correlations between plasma EO and nighttime hypertensive time index of mean arterial blood pressure (MAP) (a) as well as between urinary EO and carotid artery β -stiffness (b) in the MONO-group.

Fig. 2. Correlations between urinary immuno-unreactive albumin and E/A (a) and diurnal index of diastolic blood pressure (b) in the MONO-group.

Fig. 3. Significant correlations between plasma EO level and urinary total (a) and immuno-unreactive albumin (b) in the DUAL-group.

sectional study, the level of EO was not lower with dual blockade compared to the MONO-group.

In the MONO-group, the levels of EO correlated with blood pressure and arterial stiffness but, with the exception of albuminuria, we have not found associations with the levels of EO in the DUAL-group. A previous study did not find an association between the endogenous digitalis-like substance and immunoreactive albumin excretion in hypertensive diabetic patients [22].

EO blockage of the sodium/potassium pump (Na^+/K^+ -ATPase) consecutively increases the intracellular calcium level through the activation of the sodium-calcium

exchanger, which produces vasoconstriction and enhances heart contraction. In addition to this classical effect, EO binds to Na^+/K^+ -ATPase, which initiates several intracellular pathways, leading to myocardial and vascular smooth muscle cell hypertrophy [8]. EO also causes collagen overproduction by increased fibroblast activity in the heart muscle [23].

Our observation may suggest that although the dual blockade did not lead to lower levels of EO, it may decrease the intracellular pathway activity which leads to ventricular remodeling. In our opinion, dual blockade has a long-term effect on cardiac remodeling, which is

underlined by better cardiac parameters of echocardiography.

Our other main findings were that the level of urinary albumin was higher when assessed by HPLC than immunoturbidimetry, and the cardiovascular parameters (blood pressure parameters, diastolic dysfunction of the heart) correlated with the grade of albuminuria determined by HPLC in the MONO-group. In the DUAL-group, however, EO was the only one which correlated with the urinary total and immune-unreactive albumins. None of these parameters correlated with immunoreactive urinary albumin.

In our study, the urinary level of albumin was in the near-normal albuminuric range, which shows that the therapy was appropriate and the greater part of total excreted urinary albumin remained immuno-unreactive. The exact nature and formation of immuno-unreactive albumin is not known. A study proposed that the immuno-unreactive albumin contain modified/split albumin, which is held together by noncovalent intrachain bridges [15, 24]. A particular analysis of the immuno-unreactive albumin was made by Nakayama et al. [25] who detected that the main component of this fraction was modified albumin and contained a small amount of transferrin and other molecules in patients with cardiovascular disease.

It is also known that the reabsorbed albumin is degraded in the tubular cells of the kidneys and that the

peptides of degradation are excreted to both the apical and basolateral sides of the cells and appear in the urine [26]. According to our previous results, as well as those of others, less than 15% of the proteins in HPLC albumin peak may be not albumin [25, 27]. Our correlations with urinary immuno-unreactive albumin, but not with immunoreactive albumin (which is frequently used by physicians) suggest that the examination of this fraction of urinary albumin may be better in clinical practice.

The limitations of our study are its cross-sectional nature and that we did not have the complete basal data of the patients.

In summary, we found that dual blockade of RAS compared to the mono-blockade in multimorbid patients with low albuminuria resulted in similar cardiovascular conditions, except for some echocardiographic parameters and pro-BNP. The level of EO, a new marker of cardiovascular status, did not differ between the two groups and it had correlations with nighttime blood pressure and arterial stiffness in the MONO-group only.

Disclosure Statement

The manuscript has been seen and approved by all authors and is not under consideration for publication elsewhere in a similar form, in any language, except in abstract form.

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