SELECTIVE ASSOCIATION OF ENDOGENOUS OUABAIN WITH SUBCLINICAL ORGAN DAMAGE IN HYPERTENSIVE PATIENTS

PHD THESES

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Abbreviations

ABPM: ambulatory blood pressure monitoring

ACE-inhibitor: angiotensin-converting enzyme inhibitor

ACTH: adrenocorticotrop hormone

Aix: augmentation index

ARB: angiotensin receptor blocker

AT II: angiotensin II

CKD: chronic kidney disease

DM: diabetes mellitus

DUAL: patients treated with dual RAAS blockers

EDTA: ethylenediaminetetraacetic acid

EO: endogenous ouabain

ERK: extracellular signal-regulated kinase

HPLC: high performace liquid chromatography

HT: hypertension

MONO: patients treated with ACE inhibitors

Pro-BNP: pro-brain type natriuretic peptide

PWV: pulse wave velocity

RAS: renin-angiotensin-system

RIA: radio-immunoassay

Introduction

The clinical effects of digitalis have been known for more than two centuries in the treatment of congestive heart failure. The investigation and function of the sodium- potassium pump (Na/K ATP-ase) had a great role in appraisal of the pathomechanism of digitalis. It was suspected earlier that an endogen factor modifies the activity of Na/K ATP-ase in the salt-dependent hypertension and fluid-expansion conditions. In 1990s, many investigators have reported extraction of a material from blood and other tissue sources similar to ouabain, and its chemical structure is similar to digitalis. This introduction and thesis aimed to give a short overview about endogenous digitalis like factors, especially endogenous ouabain, and its role in human diseases.

1.1. Effect of endogenous ouabain

For twenty years endogenous ouabain (EO) is known as a physiologic regulator of the activity of sodium/potassium pump (Na^+/K^+ ATPase) which blocking increases intracellular calcium level by the activation of the sodium-calcium exchanger. The increased concentration of intracellular calcium causes an enhanced contraction in the heart muscle and vasoconstriction in the vascular system. Beyond the classical effects of EO, the digitalis-like factors have genomic effects inducing hypertrophy in myocardial and vascular smooth muscle cells due to the activation of numerous known intracellular signaling pathways. This activation results in hypertension and rigidity of arterial wall. The background of cardiovascular damage in diabetes mellitus and chronic kidney disease are not entirely known, the role of EO in pathogenesis of this process is also suggested. Previous studies have shown that EO is mostly produced in the adrenal cortex due to the increased level of adrenocorticotrop hormone (ACTH) and angiotensin II (AT II), and in addition to sympathetic activation and salt-dependent fluid expansion. During physical exercise, the level of EO was increased for a short time in both human and dogs; this increase could be reduced by previously receiving beta-blockers and angiotensin converting enzyme inhibitor (ACE-I). Based on this observation, the roles of sympathetic nervous system activation and renin-angiotensin-system (RAS) are suggested in the development of enhanced EO secretion. Level of EO was significantly increased in untreated patients with hypertension and normotensive offspring of hypertensive patients. The level of EO was also raised in patients with primary hyperaldosteronism,

Cushing disease, chronic renal failure, congestive heart failure compared to the controls. There is little information about correlation between the levels of plasma, urinary EO and the parameters of a complex cardiovascular examination in treated human hypertensives with comorbidities (diabetes mellitus, chronic kidney disease). Besides this, we have not found literature data about relations between levels of EO and markers of early vascular damage (arterial stiffness) nevertheless these relations are underlined by experimental data.

The level of EO is elevated in patients with untreated hypertension, but the levels of EO in treated hypertensives, and their associations with different cardiovascular parameters is not known. Endogenous ouabain enhances some intracellular signaling pathways, especially ERK 1/2 pathway, which closely associates with oxidative stress. This process may damage the vasculature, which produces atherosclerosis and high blood pressure. In the progression of worsen outcome of hypertension, diabetes mellitus and renal disease have a major role of the enhanced production of free radical, so that the measurement of this marker is important in the detection of complications of these diseases. Oxidative marker, especially the production of hydroxyl free radical modified amino acid (ortho-tyrosine) is high-quality marker of this process. The EO stimulated oxidative intracellular pathways are known, however there are no information about the correlation between the EO secretion and oxidative markers in human.

According to earlier studies the elevated EO level due to the physical movement were blocked with administration of ACE inhibitor and β-blockers. Effect of ACE inhibitors is known, but additive effect of further AT receptor blockers on EO secretion is not identified yet in human.

The additive effect of AT II inhibitor added to the ACE inhibitor therapy (dual RAS blockade) in context to the reduction of EO secretion is not known in human. Results of mono and dual RAS blockade on urinary albumin excretion is well known in human, but the immuno-unreactive urinary albumin and its correlations with cardiovascular status were not studied earlier.

2. Aims

2.1. Levels of plasma and urinary endogenous ouabain in different study groups, and its relations with cardiovascular markers

- Aims of the study were to measure the concentration of plasma and urinary EO in only hypertensive patients and hypertensives with other diseases (type 2 diabetes mellitus, chronic renal disease). As well as we studied correlation of the measured EO levels

and diastolic dysfunction of the patients' heart, and arterial rigidity, which often correlates with these conditions.

- We also studied the relations between the concentrations of plasma, urinary ortho-tyrosine -which are relevant markers of production of hydroxyl free radicals- and EO levels.

2.2 Effects of Mono and dual RAS blockade on cardiovascular parameters, especially on levels of EO and urinary albumins

- In another study we hypothezed that EO production is reduced if the patients are treated with dual (ACE-I and AT II inhibitor) RAS blockers compared with mono RAS blockers (only ACE inhibitor, which has proved reducing effect on the EO production), and we examined their relating effect on the patients' cardiovascular parameters.
- Our aims were to look for correlations between the levels of EO, urinary albumin and cardiovascular parameters in the two groups.

3. Patients and methods

3.1 Patients

In our first cross-sectional clinical study 41 treated, adult patients were investigated, who were divided into 4 groups: (1) group of primary hypertensive, non-diabetic patients without chronic kidney disease (HT, N=10); (2) group of hypertensive patients with type 2 diabetes mellitus (HT+DM, N=11); (3) group of hypertensive patients with type 2 diabetes mellitus and chronic kidney disease (HT+DM+CKD, N=10); (4) group of hypertensive patients with chronic kidney disease (HT+CKD, N=10).

In another cross-sectional clinical study 35 adult, hypertensive patients with mild and moderate renal failure were investigated. Retrospectively, these patients received either mono- or dual blockade of the RAS. In the MONO-group patients were treated with ACE inhibitor, (N=20); in the DUAL-group patients were treated with ACE inhibitor plus ARB, (N=15) for more than 2 years (median 2.6 years).

3.2 Methods

Most of the laboratory and other cardiovascular methods were similar in the two clinical studies, thus the description of methods are summarized below in an integrated form.

3.2.1. Plasma and urinary endogenous ouabain determination

From all patients 6 ml blood was collected from cubital vein into EDTA tubes between 7-8 a.m. after at least 40 minutes strict supine position. Then tubes were centrifuged with 3000xg within 10 minutes, and plasma was separated and transferred into plastic tubes and stored at -20^{o}C until assayed. Ten milliliters of urinary samples were taken out of 24-h collected urine and were stored at -20^{o}C. Plasma and urinary EO levels were determined by radioimmunoassay (RIA) with Ouabain ^{125}I RIA kit (Biotop OY, Medipolis Center, Oulu, Finland). All samples were measured in duplicates.

Urinary catecholamine and serum pro-BNP measurements

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

Serum pro-BNP levels were measured on fully automatized (Elecsys^{TM} 2010) system (Roche Diagnostics, Mannheim, Germany), which is an electrochemiluminescent sandwich immunoassay using two polyclonal antibodies directed at the NTpBNP molecule.

O-tyrosine measurement

Briefly, from the 24-hour collected urine or freshly obtained fasting heparinized plasma samples, aliquots of 250 µL were taken and handled on ice. One hundred and twenty-five microliters of 60% trichloroacetic acid was added to the samples, vortexed, and incubated 30 minutes on ice to precipitate protein content. To remove the precipitate, samples were then centrifuged at 15,000 rpm for 10 minutes in Eppendorf tubes. The supernatant was filtered through a 0.2 µm syringe filter (Millipore, Billerica, MA, USA), and 20 µL was injected into the manual injector of the high-performance liquid chromatography (HPLC) device.

The analysis was performed using a Shimadzu Class LC-10 ADVP HPLC system (Shimadzu USA Manufacturing, Inc., Canby, OR, USA) equipped with a Shimadzu RF-10

AXL fluorescent detector (Shimadzu USA Manufacturing, Inc.). The amino acids (p-, m-, o-Tyr, Phe) were measured upon their autofluorescence, the Tyr isoforms at 275 nm excitation and 305 nm emission wavelengths, while Phe at 258 nm excitation and 288 nm emission wavelengths. The analysis was performed using a Licrospher C-18 ODS column, in an isocratic run using an aqueous solution of 1% acetic acid and 1% sodium-acetate as the mobile phase. External standard calibration and measurement of areas under the curve were used to calculate the exact concentrations of the investigated amino acids.

Urinary microalbumin measurements

Twenty-four hours collected urine samples were vortexed and centrifuged (10 min, 2500xg). 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: 3-3000 mg/l, Roche Diagnostics GmbH, Mannheim, Germany). The other portion was used for high performance liquid chromatography (HPLC) analysis straightaway or in a few days (after storage at -20°C). For HPLC measurements the FDA approved Accumin^{TM} kit (Accumin Diagnostics Inc., New York, NY, USA) was used.

Amount of immuno-unreactive albumin was calculated as the difference of HPLC and immunoturbidimetry measured urinary albumin.

Echocardiography, echo-tracking examination of arterial stiffness and ABPM

Echocardiograms were recorded with an Aloka SSD 5500 (Aloka, Aloka Co. Ltd, Tokyo) ultrasound imaging system equipped with a 3.5 MHz transducer. All patients were assessed by the same cardiologist and examined within one hour (between 7.30-8.30 a.m.) after EO samples collecting and ABPM taking off. Before echocardiography and echo-tracking all patients were supine position at least for 5 minutes. Operators were blinded to the status of the patients. The measurements of echocardiography were assessed according to the American Society of Echocardiography guidelines. Arterial stiffness parameters as the β stiffness index (β), pulse wave velocity (PWV), and augmentation index (Aix) were assessed by the characteristics of pulse wave intensity using echo-tracking system (Aloka SSD-5500, Aloka Co. Ltd, Tokyo) 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.

The ambulatory blood pressure monitoring was done with ABPM-04 (Meditech, Meditech Ltd., Budapest, Hungary) based on validated oscillometric technique. During a 24-h monitoring period the blood pressure was registered in 15 minute intervals in daytime (8 a.m. to 10 p.m.) and 30 minute intervals during nighttime (10 p.m. to 8 a.m.). The results (mean arterial blood pressure, hypertensive time index) of blood pressure monitoring were analyzed by the proprietary software of ABPM-04.

Statistical analyses

The data are expressed as mean values ± standard deviation, or as median values and interquartile range depending on the distribution. Distribution of the variables was analyzed using Kolmogorov-Smirnov test. Normally distributed variables were compared using two samples T-test, while non-normal distributed variables were compared with Mann Whitney U test. Pro-BNP levels were normalized by a logarithmic transformation in order to depict correlations with pro-BNP. Difference of parameters groups was analyzed with univariate general linear model, adjusted for age or age, HbA_{1c}, LVESD, LVESV, and ejection fraction of heart. The non-continuous parameters were analyzed by χ^2-test. The correlations between variables were analyzed using Pearson's (in cases of normally distributed variables) and Spearmann's (non-normally distributed variables) correlations. The statistical analyses were made by SPSS 13.0 (SPSS Inc, Chicago, IL, USA) and two tailed P value of <0.05 was considered statistically significant.

1.1.1. Results

In our first study the highest level of plasma endogenous ouabain (19.7 ± 9.5 pmol/l) was measured in hypertensive patients with diabetes mellitus and chronic kidney disease, which was significantly higher than in HT+CKD group (after adjusting for age). The level of urinary EO and EO clearance did not show significant difference between the groups.

The EO clearance in HT group (31.2%) was significantly higher in the HT (14.3%) and HT+DM (11.5%) groups. There were no differences between groups in daytime, nighttime systolic, diastolic, mean arterial blood pressure and their hypertensive time index as well as diurnal index. According to the mean blood pressures of the groups, the patients were well-

treated in this study. The mean parameters of echocardiography and echo-tracking arterial stiffness were not shown differences between groups.

Plasma level of EO showed several significant correlations mainly with parameters of the nighttime blood pressure as nighttime systolic (p=0.014), diastolic (p=0.029), mean arterial blood pressure (p=0.009) and their hypertensive time indicis in the whole population. Relationship between these parameters may due to the increased nighttime/morning sympathetic tone because the levels of urinary catecholamines (norepinephrine) correlated with EO level (R=0.396, p=0.011). In the analysis of data of whole population we found some significant association between the level of urinary EO and carotid artery PWV (p=0.023) and β-stiffness (p=0.009).

The nighttime mean arterial blood pressure independently correlated with the level of plasma endogenous ouabain (p=0.004), while independent predictor of the β-stiffness of carotid artery was the urinary endogenous ouabain (p=0.011).
Fractional excretion of EO was higher in HT+DM+CKD and HT+CKD groups than other groups, and grade of fractional excretion showed significant correlation with nighttime mean arterial blood pressure (R=-0.361, p=0.029) as well as the end-systolic (R=0.345, p=0.029) and diastolic (R=0.356, p=0.024) diameters of the heart.
The fractional excretion of EO also showed close relations with pulse wave velocity and β-stiffness of carotid artery in the HT+DM group.
In the HT+DM+CKD group the plasma EO and diastolic dysfunction of the heart (R=-0.665, p=0.036), as well as some nighttime blood pressure parameters were associated.
The urinary level of o-tyr is not differed in groups, but the level of plasma o-tyr was significantly higher the in the HT+DM+CKD and HT+CKD groups than HT group (p=0.028 and p=0.04, respectively). We have not found correlations between plasma o-tyr and markers of cardiovascular disease in the whole population or in the groups. Levels of o-tyr did not show any correlations with levels of EO. Level of o-tyr correlated with the markers of renal function only.

In our second study we have found that the long-term dual RAS blockade did not reduce more the levels of urinary and plasma EO, compared to the mono RAS blockade. In the cardiovascular parameters the groups were not different, except of some echocardiographic parameters (ejection fraction of heart, end-systolic diameter and volume).

We have found difference between the pro-BNP levels of the groups only.

The plasma level of EO correlated with some nighttime blood pressure values, as systolic (R=0.512, p=0.049) or mean arterial blood pressure (R=0.541, p=0.038) in the MONO-group. Also, the urinary EO level associated with pulse wave velocity (R=0.621, p=0.005) and β-stiffness (R=0.681, p=0.001) of carotid artery in the MONO-group. In the DUAL-group, these correlations were not found.

Regarding the urinary albumins we have explored that the immuno-unreactive urinary albumin correlated with diastolic dysfunction marker of the heart (R=-0.492, p=0.028), diurnal index of diastolic blood pressure (R=-0.540, p=0.014) in the MONO-group; however the immuno-unreactive albumin correlated with only the level of plasma EO (p=0.014) in the DUAL-group.

1.1.2. Discussion

Association of EO levels and blood pressure parameters have known for a long time in untreated hypertensives, but the relationships of EO levels with the parameters of a complex cardiovascular measurement in treated patients with hypertension are not studied.

In our study the samples collection, echocardiography, and echo-tracking measurements were completed in a short time (between 7.30 - 8.30 a.m.). Thus, we tried to eliminate the inaccuracy from the circadian variation of EO (which is not known), and so the levels of plasma EO strongly mirror the patient's current (night and early morning) cardiovascular status. The pathophysiological abnormalities of this period especially nighttime and morning hypertension are major risk factors for acute cardiovascular diseases.

In our first study the level of EO was highest in HT+DM+CKD group. This result is in agreement with results of earlier investigations which suggest that more comorbidities potentially associate with higher level of EO.

The level of plasma EO in healthy individuals was 9-12 pmol/L, assessed previously by the same method as we used in hypertensives. In our study the average level of plasma EO was 14.7 ± 5.7 pmol/L, nevertheless our patients were treated with RAAS blockers and/or β-blockers. In our study the levels of EO was lower than in some studies in literature using other immunoassay methods which are not comparable with our results because of using different antibody and methods.

According to our results the level of endogenous ouabain associated with nighttime blood pressures suggesting possible role of endogenous ouabain in the pathogenesis of impaired diurnal rhythm of blood pressure and nighttime blood pressure.

Fractional excretion of EO was significantly higher in groups including patients with chronic kidney diseases than others. The EO fractional excretion is less than 2% in healthy men and in our patients with hypertension its levels were 11.5 – 31.2 %. This observation may underline the role of kidney in regulation of EO level.

Endogenous ouabain may therefore take part in the development of hypertension through increased sympathetic tone, direct prohypertrophic effects on myocytes of heart and arteries. These processes can lead to cardiac hypertrophy in vivo. This thesis is emphasized with our results in which the level of EO associates with urinary catecholamine level.

In our study we have found association between left ventricular diastolic dysfunction (E/A) and level of plasma EO in HT+DM+CKD group. Similar correlation was proved within patients with end stage kidney disease suggesting that the prolonged elevated level of plasma EO may have a role in the development of ventricular hypertrophy.

Long-time effects of endogenous digitalis like factors contribute to the arterial rigidity, which explains the correlation of EO levels and arterial stiffness markers in our study.

Changed excretion of EO play role in the formation of arterial stiffness, because of the independent predictor of carotid artery β-stiffness was urinary EO in the whole population. This could be a new component between the decreased renal function and arterial stiffness.

According to our results, the level of EO is a useful marker for the prediction of the severity of subclinical organ damage in patients with hypertension.

In the literature some studies found significant correlations between oxidative stress markers and cardiovascular parameters. However, it also known that several medications (e.g. AT II receptor blocker, alpha and beta-blocker) decrease the levels of oxidative markers in hypertensive patients. The endogenous digitalis like factors regulate the intracellular free radical production so we supposed that EO levels and hydroxyl free radical material (o-tyr) also correlates with together. In our investigation we have not found any correlation between the levels plasma and urinary o-tyr and EO neither in the whole population, nor in the groups.

In a previous study the level of urinary o-tyr was higher in the patients with diabetes mellitus and kidney disease than in healthy controls. In this study the relations between o-tyr level and cardiovascular markers were not assessed. In our analysis of data we did not find direct relationship between the levels plasma and urinary o-tyr and blood pressure as well as echocardiographic, stiffness markers of patients neither in the whole population, nor in the

groups. Plasma o-tyr showed correlation with renal function only, which underlines our earlier hypothesis that the main regulator of o-tyr may be the kidney. We could not detect a direct correlation between level of EO and marker of oxidative stress. The explanation of this missing association may be due to renal excretion of o-tyr and to the treatment of the patients and the presence of diabetes (in some groups).

The therapy with RAS blocking drugs have been used for some decades to reduce the blood pressure, grade of albuminuria and other clinical and subclinical organ damage. Several multicenter, randomized studies proved that beneficial effects of dual RAS blockade on cardiovascular morbidity, mortality and severity of the subclinical organ damage opposite to the mono RAS blockade. ***In our second study*** we also compare the results of long-term mono and dual RAS blockade on cardiovascular markers, especially focus on immuno-unreactive urinary albumin and levels of EO. Level of plasma and urinary EO (as a new marker in the assessment of cardiovascular status) were similar in the two groups. Level of EO correlated with nighttime blood pressure as well arterial stiffness parameters in the MONO-group only.
We have found no difference between the two groups in regarding blood pressure and arterial stiffness parameters. Left ventricular status and ejection fraction significantly better in the DUAL-group than in the MONO-group, the level of pro-BNP was significantly lower in the dual-group, which can refer a enhanced cardiac outcome. The differences between echocardiographic parameters could be not explained by different sympathetic activity and its suppressions because the excreted levels of catecholamine and β-blocker therapy were similar. Our observation may suggest that although the dual blockade did not lead to lower levels of EO, but may decrease the intracellular pathway activity which leads to ventricular remodelling. One may suppose, that dual RAS blockade is more effective, than mono blockade to preserve lower stiffness of arteries, but according to the literature dual RAS blockade compared to the mono blockade does not decrease the risk of cardiovascular mortality.

Exact detection of urinary albumin is important for the appropriate treatment in several diseases. Based on the different measurement methods, urinary albumin exists in immunoreactive and immuno-unreactive form. 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. In our other study we measured the level of both type urinary albumin and their correlations in patients with use of mono and dual RAS blockers. Some previously published studies show 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.

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

In our study the urinary level of albumin was in the near normalbuminuric range which shows that the therapy of patients 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.

LIST OF PHD THESIS

1. Measurement of EO levels is useful markers of the damage of cardiovascular status in treated hypertensives, and in hypertensives with comorbidity.

2. Level of plasma EO is higher in hypertensives with comorbidity (diabetes mellitus and renal failure)

3. Level of EO is correlated with arterial stiffness parameters.

4. Level of EO correlates with nighttime blood pressure, possibly across by increased sympathetic activity.

5. The hydroxyl free radical marker, o-tyr is not associated with levels of EO in patients with treated hypertension.

6. Kidney is the main regulator of the plasma level of o-tyr.

7. ARBs with ACE-inhibitors (DUAL RAS blockade) do not decrease more the levels of plasma and urinary of EO than ACE-inhibitors alone.

LIST OF PUBLICATIONS

Cumulative impact factor: full papers and letters: 19.003, abstracts: 80.611

Cumulative impact factor of publications used in this thesis: full papers: 3.676, letter: 6.614

This thesis is based on the following publications:

I. Full papers

1. Selective association of endogenous ouabain with subclinical organ damage in treated hypertensive patients.

Nagy G, Gaszner B, Lányi E, Markó L, Fehér E, Cseh J, Kõszegi T, Betlehem J, Sulyok E, Cziráki A, Wittmann I. *J Hum Hypertens*. 2011;25(2): 122-129. **IF 2010: 2,176**

2. Effect of mono and dual blockade of the renin-angiotensin system on the markers of cardiovascular status in hypertensive patients with mild and moderate renal failure

Gábor Nagy, István A. Szijártó, Balázs Gaszner, Éva Lányi, Lajos Markó, Ákos Mérei, Gergő A. Molnár, Kinga Németh, József Betlehem, István Wittmann

Kidney and Blood Pressure Research 2011;34(3): 150-157. **IF 2010: 1,500**

3. Az endogén ouabain összefügg a hipertóniás betegek kardiovaszkuláris állapotával

Nagy G, Gaszner B, Lányi E, Markó L, Fehér E, Cseh J, Kőszegi T, Betlehem J, Sulyok E, Cziráki A, Wittmann I.

Magyar Belorvosi Archívum 2010;63(6):435-442.

II. Letter

1. Nighttime activity influences the evaluation of ambulatory blood pressure monitoring. In response to Agarwal R, Light RP, Bills JE, Hummel LA. Hypertension. 2009;54:646-651.

Nagy G, Nagy CB *Hypertension* 2009;54 (6) e139. **IF 2009: 6,614**

III. Abstracts

1. Endogén ouabain és a szubklinikai szervkárosodások kapcsolata hipertóniás vesebetegekben

Nagy Gábor, Gaszner Balázs, Mérei Ákos, Lányi Éva, Markó Lajos, Cseh Judit, Sulyok Endre, Betlehem József, Cziráki Attila, Wittmann István

Place of presentation: Magyar Nephrologiai Társaság Nagygyűlése 2009. szeptember 17-19. Siófok

Hypertonia és Nephrologia, 2009.13 (S1) 39-39.

2. Hidroxil szabadgyök-termelés hipertóniás veseelégtelen betegekben

Nagy Gábor, Gaszner Balázs, Lányi Éva, Markó Lajos, Molnár A. Gergő, Betlehem József, Wittmann István

Place of presentation: Magyar Nephrologiai Társaság Nagygyűlése 2010. október 21-23. Szeged

Hypertonia és Nephrologia, 2010.14 (S1) 27-27.