Association of biomarkers of cardiac remodeling, myocardial fibrosis and inflammation with parameters of heart function and structure in patients with arterial hypertension

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Backround and Aims. Early evaluation of cardiac remodeling may be useful in predicting heart failure in patients with arterial hypertension. The identification of biomarkers as useful clinical tools in this regard is ongoing. The aim of this study was to evaluate the association of selected cardiac biomarkers levels with parameters of cardiac structure and function in patients with arterial hypertension.

Patients and Methods. Included in the study were patients with arterial hypertension with normal left ventricular ejection fraction (LV EF) and absence of signs of heart failure. The levels of selected biomarkers: NT-proBNP, sST2, Galectin-3, GDF-15, Cystatin C, TIMP-1 and ceruloplasmin were measured and assessed together with other biochemical and echocardiographic parameters.

Results. A total of 92 patients (61% men) mean age 61.5 years were included. Mean LV EF was 64.7% and mean LV mass index was 91.7 g/m2. NT-proBNP level correlated significantly with the parameters of LV diastolic function: velocity of E wave (r=0.377, P<0.002), and with E/A ratio, (r=0.455, P<0.0001), with E lat (r=–0.354, P=0.006), E/E' ratio, r=0.393, P<0.002, with ePAP (r=0.390, P=0.014), and with age (r=0.384, P<0.0001). Statistically significant correlations for GDF-15 were as follows: with age (r=0.426, P<0.0001) and left atrial diameter (LA) (r=0.401, P<0.0001), for Cystatin C there are statistically significant correlation with age (r=0.288, P=0.006) and LA (r=0.329, P=0.004). Only sST2 level correlated significantly with parameters of cardiac structure: with LV mass (r=0.290, P<0.01) and LV mass index (r=0.307, P=0.012) and with posterior wall thickness PW (r=0.380, P<0.001). No other observed variables including Galectin-3 and TIMP-1, correlated significantly with age or echocardiographic variables. In a comparison of patients with and without left ventricular hypertrophy, statistically significant differences were found only in LA (P<0.0001) and sST2 (P=0.004). In a multivariate logistic regression, sST2 and TIMP were independent predictors of left ventricular hypertrophy.

Conclusion. NT-proBNP level as a biomarker of cardiac remodeling correlated with parameters of LV diastolic function in patients with arterial hypertension. Soluble ST2 correlated with parameters of cardiac structure. Biomarkers sST2 and TIMP-1 were associated with left ventricular hypertrophy.

An association of cardiac biomarkers with heart function and structure in arterial hypertension

The aim of the study was to evaluate the association of cardiac biomarkers levels with the parameters of cardiac structure and function in the patients with arterial hypertension. Patients with arterial hypertension, normal left ventricular ejection fraction and absence of signs of heart failure were included in the study.

Odds ratios (OR), 95% Confidence Intervals (CI) and significance levels of Wald's statistic (P) of differences in predictive values between patients with LVH (n=31) and without LVH (n=47). Only statistically significant predictors and predictors with P<0.200 are presented.

Variable	OR	95% CI	P
Model 1 – Predictive variables: NT-proBNP, GDF-15, Galectin-3, Cystatin C, sST2, and TIMP-1			
sST2	1.0033	1.0001–1.0066	0.041*
TIMP-1	1.0001	0.9999–1.0001	0.161
Model 2 – Predictive variables: NT-proBNP, GDF-15, Galectin-3, Cystatin C, sST2, and TIMP-1 + age, LV EF, LA, RV, E/A, E/E', Na, K, urea, creatinine, and eGFR			
Cystatin C	0.9998	0.9996–1.0001	0.141
sST2	1.0057	0.9992–1.0122	0.086
TIMP-1	1.0001	1.0001–1.0002	0.012
LA	1.1356	0.9710–1.3281	0.112
Age	1.0566	0.9712–1.1494	0.200

*Statistically significant predictors are marked in bold.

Biomarker of cardiac remodeling (NT-proBNP) correlated with the parameters of left ventricular diastolic function and biomarkers involved in myocardial fibrosis (sST2) correlated with parameters of cardiac structure and were associated with left ventricular hypertrophy (sST2 and TIMP-1).

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INTRODUCTION

Arterial hypertension is one of the main risk factors for the development of heart failure[1,2]. High blood pressure leads to cardiac dysfunction and subsequent heart failure through functional and structural changes in both cardiomyocytes and the extracellular matrix. Arterial hypertension is also the main cause of left ventricular hypertrophy in the general population[3,4]. In the early stages, diastolic dysfunction of the left ventricle may develop and systolic dysfunction is rarely observed. Asymptomatic diastolic left ventricular dysfunction may progress to overt heart failure with preserved ejection fraction. Arterial hypertension is also important risk factor for ischemic heart disease and myocardial infarction with the risk of developing left ventricular dysfunction, which may progress to heart failure with mildy reduced and reduced ejection fraction.

The most available imaging tool for the recognition of structural and functional cardiac changes is echocardiography. In patients with arterial hypertension, echocardiography is sufficiently sensitive for the evaluation of left ventricular hypertrophy and functional changes[5,6].

Early recognition of the structural and functional changes in the heart may also be assessed using biomarkers of cardiovascular remodeling. Several biomarkers of cardiac remodeling and myocardial fibrosis have been tested in the different clinical settings, including patients with arterial hypertension with evidence of left ventricular hypertrophy, arterial hypertension and metabolic syndrome, and arterial hypertension and heart failure with normal or preserved ejection fraction. For example, N-terminal pro-brain natriuretic peptide (NT-proBNP) has been tested in the prediction of cardiovascular events in patients with hypertension and left ventricular hypertrophy, for the screening of left ventricular hypertrophy and in the detection of diastolic dysfunction in asymptomatic patients[7-11]. There are futher several novel biomarkers of cardiac remodeling and myofibrosis, which have been evaluated in arterial hypertension. For example, soluble receptor sST2 (soluble supression of tumorigenicity) is a member of the interleukin-1 receptor family, which is involved in the pathophysiology of cardiac hypertrophy and myofibrosis. The levels of sST were studied in patients with arterial hypertension in association with left ventricular hypertrophy and in detection of heart failure in patients with arterial hypertension and normal ejection fraction[12,13]. Soluble ST2 levels have been also evaluated as a potential marker of left ventricular hypertrophy in patients with essential hypertension and those with heart failure and preserved ejection fraction together with other new biomarkers of cardiac remodeling, e.g.: Galectin-3 (Gal-3), growth differentiation factor 15 (GDF-15), matrix metalloproteinase-3 (MMP-3) and tissue inhibitor of metalloproteinase-1 (TIMP-1) (ref.[14,15]). Galectin-3 is a protein produced by macrophages and as a member of the galectin family is involved in fibrotic processes[16]. Growth differentiation factor 15 is a cytokine and its blood concentration may change in response to hypoxia, ischemia, oxidative stress, myocardial pressure and volume overload and inflammation[17]. Matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinase-1 (TIMP-1) play a role in extracellular matrix remodeling[18,19]. It has been proposed, that inflammation may play a role in the development of arterial hypertension and hypertension mediated organ damage[20]. Several biomarkers of inflammation have thus been evaluated in arterial hypertension. For example, levels of Cystatin C, a marker of inflammation and also a biomarker of kidney function, were associated with hypertension in women and were higher in patients with uncontrolled hypertension[21,22]. Ceruloplasmin is a glycoprotein and acute phase reactant. Its levels were higher in patients with hypertension compared to healthy controls, including high sensitive C-reactive protein[23].

We hypothesised, that concentrations of biomarkers of cardiac remodeling, myofibrosis and inflammation may be associated with the severity of structural and functional impairment in arterial hypertension as assessed by echocardiography, and thus may be useful in the prediction of the risk heart failure development in these patients.

PATIENTS AND METHODS

Consecutive patients with established essential arterial hypertension followed at a tertiary care clinic and who had normal left ventricular (LV) ejection fraction and absence of signs of heart failure were included in the study between 1st January and 30th June 2015. Patients with history of heart failure, reduced LV ejection fraction and secondary cause of hypertension were not included. Blood samples were collected in the morning in fasting patients. The level of biomarkers N-terminal pro-brain natriuretic peptide (NT-proBNP), soluble supression of tumorigenicity (sST2), Galectin-3 (Gal 3), growth differentiation factor 15 (GDF-15), Cystatin C, tissue inhibitor of metalloproteinase-1 (TIMP-1) and ceruloplasmin was analyzed, and office blood pressure recordings and echocardiographic study were realized on the day of clinic visit.

NT-proBNP concentration was measured using by a validated, commercially available sandwich electrochemiluminescence immuno-assay on Cobas e411 analyzer (Roche Diagnostics, Mannheim, Germany). The concentration of sST2, Gal 3, GDF-15, Cystatin C and TIMP-1 in serum was measured by RayBio™ Custom Quantibody Array (Raybiotech, Inc., Norcross, GA, USA). Cerulopasmin level was measured by turbidimetric immunoassay on AU 400 analyser (Olympus Life and Material Science Europa GmbH, Hamburg, Germany). Other biochemical parameters including electrolytes, liver function tests, parameters of kidney function, lipids, albumin and hemoglobin were measured on the analyser Unicel DxC 800 (Beckman Coulter company, Germany).

Echocardiographic study was performed on the clinic visit day by a broadband transducer with a transmitting frequency from 1.7 to 4.0 MHz on commercially available equipment (Vivid 7, GE, USA). The size of cardiac chambers was measured by two dimensional echocardiography. The left ventricular mass and relative wall thickness were used as parameters of cardiac structure. The left ventricular mass (LVM) was calculated by standard formula from interventricular septal (IVS) thickness in diastole, posterior wall (PW) thickness in diastole and left ventricular internal diameter (LVID) in diastole: LVM = 1.04 [(LVID + PW + IVS)^3 – LVID^3] – 13.6. Left ventricular mass index (LVMi) was calculated as LVM divided by patient's body sufrace area (BSA). Relative wall thickness (RWT) was calculated by a standard formula: RWT = (IVS + PW)/ LVID. Left ventricular ejection fraction was calculated by Simpson's method [24]. Normal values of LVMi in females were < 96 g/m^2 and < 116 g/m^2 in males and normal values for RWT were < 0.43 for both genders[25].

The left ventricular diastolic function was assessed by the pulse wave Doppler (PWD) and by the tissue Doppler imaging (TDI). The mitral early filling velocity (E wave) and the atrial contraction velocity (A wave) were measured and E/A ratio was calculated. The mitral annular diastolic velocity (E') was obtained and the mean of septal E' and lateral E' was calculated, and E/E' ratio was calculated[26]. The estimated systolic pulmonary artery pressure (ePAP) was obtained by continuous wave (CW) Doppler of tricuspid regurgitation velocity plus estimated right atrial pressure (RAP) (ref.[27]).

The study was conducted in the compliance with the Declaration of Helsinki. The approval fot the study protocol was granted by the Local Board Ethics Committee and the informed consent for the study and for the publication was obtained from all participants.

STATISTICAL ANALYSIS

All quantitative variables are described as means and standard deviations or medians and ranges as appropriate. To determine the relationship between novel biomarkers, echocardiographic and biochemical parameters and the age of patients Pearson's correlation coefficient and in the case of skewed variables the non-parametric Spearman's correlation coefficient was calculated.

To investigate in detail the novel biomarkers, including NT-proBNP, GDF-15, Galectin-3, Cystatin C, sST2, and TIMP-1 multivariate regression analysis using the "whole model" technique for each individual biomarker separately was performed. For dependent variables we used demographic (age), echocardiographic (LV EF, LA, RV, E/A, E/E', IVS, PW, RWT, LAi, LVMi), and biochemical (Na, K, urea, creatinine, ALT, AST, bilirubin, Hgb, ceruloplasmin, eGFR).

Our patients were divided into two groups according to the presence of left ventricular hypertrophy (LVH). To determine the statistically significant difference between patients with and without left ventricular hypertrophy in novel biomarkers and biochemical and echocardiographic parameters and again the age of patients the parametric Student's t-test or non-parametric Mann-Whitney U-test (for non-normally distributed variables) were calculated.

To evaluate the discriminating power of each individual biomarker separately between patients with and without LVH Receiver operating characteristic curve (ROC) analysis was performed.

Lastly, to evaluate the effect of individual clinical, biochemical, echocardiographic factors as well as novel biomarkers on developing LVH, a multivariate logistic regression model for the two groups (patients with LVH vs. without LVH) was constructed. Candidate predictor variables included in the logistic regression model were as follows: novel biomarkers (NT-proBNP, GDF-15, Galectin-3, Cystatin C, sST2, and TIMP-1), demographic (age), biochemical (Na, K, urea, creatinine, ALT, AST, bilirubin, Hgb, ceruloplasmin, eGFR). The echocardiographic parameters which were used to divide the patients into two groups were excluded from the analysis.

Statistica version 14.0.0.15 (TIBCO Software Inc., CA USA) and IBM SPSS Statistics version 29.0.0.0 (IBM Corporation, IL USA) were used for statistical analysis. A p-value less than 0.05 was considered to be statistically significant.

RESULTS

A total number of 92 patients, 56 males (61%) and 36 females (39%) mean age 61.5 years met the criteria for the study. All patients were treated for arterial hypertension and the subjects had significant commorbidities: 73% patients had dyslipidaemia, 29% of subjects had diabetes mellitus and 15% had history of ischemic heart disease. Patients' characteristics are shown in the Table 1. The mean number of drugs for the treatment of arterial hypertension was 2.34. The most commonly prescribed antihypertensive drugs were betablockers and thiazide diuretics in 52 (57%) patients, angiotensin converting enzyme inhibitors were used by 42 (46%) patients, angiotensin receptor blockers were prescribed in 43 (47%) subjects, calcium channel blockers were used by 42 (46%) patients and spironolactone was prescribed in 16 (17%) of patients. Other medications included acetylosalicylic acid (32%), a statin (64%), oral antidiabetics (21%), and alopurinol (14%). The mean LV EF was 64.7%, mean LV

Table 1. Laboratory and Echocardiographic parameters – means and standard deviations (SD), medians and ranges (Min – Max) of patients, n=92, males 56 (61%), females 36 (39%).

Parameter	Mean ± SD	Median (Min–Max)
Laboratory parameters		
Na [mmol/L]	138.4 ± 2.26	138 (132–144)
K [mmol/L]	4.1 ± 0.47	4.1 (3.0–5.6)
urea [mmol/L]	5.1 ± 1.68	4.9 (1.9–10.3)
creatinine [μmol/L]	86.2 ± 24.36	80 (45–204)
eGFR [ml/sec]	1.2 ± 0.29	1.1 (0.5–2.0)
urea [μmol/L]	352.1 ± 68.5	353 (156–517)
bilirubin [μmol/L]	13.4 ± 5.47	12.2 (3.4–31.9)
ALT* [μkat/L]	0.6 ± 0.35	0.5 (0.2–2.4)
AST* [μkat/L]	0.4 ± 0.22	0.3 (0.1–1.5)
chol-total [mmol/L]	4.8 ± 1.19	4.8 (2.4–8.2)
HDL-chol [mmol/L]	1.3 ± 0.4	1.2 (0.6–3.7)
LDL-chol [mmol/L]	2.8 ± 0.99	2.8 (0.9–5.5)
TG [mmol/L]	1.7 ± 1.02	1.5 (0.3–6.1)
TP [g/L]	72.4 ± 6.08	73.0 (28.6–85.0)
albumin [g/L]	42.8 ± 1.60	43.5 (40.0–44.0)
Hgb [g/L]	145.5 ± 11.27	146.0 (124.0–171.0)
glucose [mmol/L]	6.4 ± 1.46	6.0 (3.9–12.0)
GDF-15* [ng/L]	1608.1 ± 1351	1133.6 (433.7–8478.0)
Galectin-3* [ng/L]	1689.4 ± 2323	503.1 (19.2–9903.0)
Cystatin C [ng/L]	18802.6 ± 5717	17283.6 (9046.9–32866.3)
sST2 [ng/L]	298.9 ± 178	258.3 (41.4–817.7)
TIMP-1 [μg/L]	299456.0 ± 106527	276400.1 (140685.8–589331.0)
NT-proBNP* [pg/mL]	20.9 ± 7.78	11.0 (1.0–197.0)
Echocardiographic parameters		
LV EF* [%]	64.7 ± 5.3	65 (45–75)
EDD [mm]	49.2 ± 4.2	49 (40–58)
ESD [mm]	32.8 ± 4.4	33 (25–45)
LA [mm]	38.9 ± 6.2	38 (29–73)
RV [mm]	27.4 ± 3.1	28 (21–35)
E [cm/sec]	67.1 ± 16.5	65 (38–117)
A [cm/sec]	65.5 ± 17.6	63 (3–116)
E/A	1.1 ± 0.46	1.0 (0.5–3.1)
E sept [cm/sec]	10.6 ± 2.3	11 (7–17)
E lat [cm/sec]	10.9 ± 2.5	11 (5–17)
E/E’	7.1 ± 3.5	6.3 (2.8–21.6)
ACT RVOT [ms]	119.1 ± 17.7	116 (83–160)
ePAP [mmHg]	15.3 ± 2.4	15 (11–20)
IVS [mm]	10.7 ± 1.63	11 (7–15)
PW [mm]	9.8 ± 1.29	10 (7–13)
LVM [g]	189.9 ± 52.2	184 (113–357)
LVMi [g/m^2]	91.7 ± 15.8	90.8 (15.8–154.1)
RWT	0.4 ± 0.05	0.4 (0.3–0.5)
LAi [mm/m^2]	19 ± 2.7	18.8 (14.3–31.1)

*Variables that do not fulfill conditions of a normal distribution were marked with an asterisk.
Laboratory parameters: Na, sodium; K, potassium; eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; chol, total cholesterol; HDL-chol, high density lipoprotein cholesterol; LDL-chol, low density lipoprotein cholesterol; TG, triglycerides; TP, total protein; Hgb, hemoglobin; GDF-15, growth differentiation factor; sST2, soluble receptor “suppression of tumorigenicity 2”; TIMP-1, tissue type inhibitor of matrix metalloproteinases; NT-proBNP, N-terminal pro-brain natriuretic peptide; Cp, ceruloplasmin.
Echocardiographic parameters: LV EF, left ventricle ejection fraction; EDD, enddiastolic diameter of left ventricle; ESD, endsystolic diameter of left ventricle; LA, left atrial diameter; RV, right ventricle diameter; E, velocity of flow at early transmitral diastolic filling of left ventricle; A, velocity of flow at atrial contraction; E/E’, E velocity divided by the mean velocity of mitral annulus as assessed by tissue Doppler imaging; ACT RVOT, acceleration time at right ventricular output tract; ePAP, estimated systolic pulmonary artery pressure; IVS, interventricular septum thickness; PW, posterior wall thickness; LVM, left ventricular mass; LVMi, left ventricular mass index; RWT, relative wall thickness; Lai, left atrial index.

mass was 189.9 g, LV mass index was 91.7 g/m^2, the mean RWT was 0.40 and E/A and E/E' ratios were 1.1 and 7.1 respectively.

The patients were divided into two groups according to the presence of left ventricular hypertrophy. Left ventricular hypertrophy (LVH) was defined by LVMi > 95 g/m^2 in females or > 115 g/m^2 in males and RWT > 0.42 for both genders. The number in the first group – patients with LVH (n=31) and for the second group – patients without LVH (n=47), 14 patients were not classified. The unclassified patients did not meet both criteria for LVH (LVMi and RWT), only one of them (LVMi or RWT).

Comparison of patients with and without LVH

The differences in all normally distributed observed variables (Na, K, urea, creatinine, eGFR, urea, bilirubin, chol-total, HDL-chol, LDL-chol, TG, TP, albumin, glucose, Cystatin C, sST2, TIMP-1, Cp, EDD, ASD, LA, RV, E, A, E/A, E sept, E lat, E/E', ACT RVOT, ePAP, IVS, PW, LVM, LVMi, RWT and LAi) between the patients with and without LVH were compared using an independent sample t-test. Statistically significant differences were found only in LA (P<0.0001) and sST2 (P=0.004). For non-normally distributed variables (ALT, AST, GDF-15, Galectin-3, NT-proBNP and LV EF) the nonparametric Mann-Whitney U-test was calculated. For none of these variables was any statistically significant difference found.

Correlation of novel biomarkers with echocardiographic and biochemical parameters and age of patients

Correlation coefficients at significant level 0.05 for our sample size, i.e., for our 92 patients, that yield 80% power must be greater than or equal to 0.288, so only those correlation coefficients, that are greater than this value, are really statistically significant (Spearman correlation coefficients r are marked with asterisk).

Using this criterion NT-proBNP levels correlated significantly with the parameters of LV diastolic function: velocity of E wave (r*=0.377, P<0.002), and with E/A ratio, (r*=0.455, P<0.0001), with E lat (r*=−0.354, P=0.006), E/E' ratio, r*=0.393, P<0.002, with ePAP (r*=0.390, P=0.014), and with age (r*=0.384, P<0.0001). Statistically significant correlations for GDF-15 were as follows: with age (r*=0.426, P<0.0001) and LA (r*=0.401, P<0.0001), for Cystatin C they are statistically significant correlation with age (r=0.288, P=0.006) and LA (r=0.329, P=0.004). Only sST2 level correlated significantly with parameters of cardiac structure: with LV mass (r=0.290, P<0.01) and LV mass index (r=0.307, P=0.012) and with posterior wall thickness PW (r=0.380, P<0.001). No other observed variables, or Galectin-3 or TIMP-1, correlated significantly with age or echocardiographic variables.

The statistically significant correlations with biochemical parameters are as follows: NT-proBNP with urea (r*=0.352, P=0.001), creatinine (r*=0.384, P<0.0001), Hgb (r*=−0.355, P=0.001) and eGFR (r*=−0.415, P<0.0001); GDF-15 with K (r*=0.390, P<0.0001), with creatinine (r*=0.343, P=0.001), Hgb (r*=−0.330, P=0.002), and with TG (r*=0.301, P=0.009); Cystatin C with urea (r=0.343, P=0.001), creatinine (r=0.390, P<0.0001), and eGFR (r=−0.423, P<0.0001); sST2 with ALT (r*=0.326, P=0.002) and AST (r*=0.363, P=0.001); TIMP-1 with K (r=0.314, P=0.003); no statistically significant correlation between Galectin-3 and some of the biochemical parameters was found.

Estimation of discriminating power of novel biomarkers

The Receiver operating characteristic (ROC) curve analysis was used to estimate the discriminating power between two groups of patients (with and without LVH) for each novel biomarker separately. The areas under the ROC curve with 95% CI were calculated. Accuracy of novel biomarkers discriminating power is rated using the traditional academic point system[28].

The results are as follows: NT-proBNP (AUC 0.588, CI 0.445 – 0.725, Accuracy: FAIL), GDF-15 (AUC 0.575, CI 0.445 – 0.706, Accuracy: FAIL), Galectin-3 (AUC 0.532, CI 0.400 – 0.664, Accuracy: FAIL), Cystatin C (AUC 0.504, CI 0.361 – 0.627, Accuracy: FAIL), sST2 (AUC 0.606, CI 0.475 – 0.738, Accuracy: POOR) and TIMP-1 (AUC 0.649, CI 0.524 – 0.774, Accuracy: POOR). As the discriminating power to distinguish between our two groups of patients of all biomarkers FAIL or are POOR, no sensitivity, specificity, positive and negative predictive values were estimated.

Estimation of significant biochemical and echocardiographic parameters influencing novel biomarkers

We successively used all novel biomarkers (NT-proBNP, GDF-15, Gelactin-3, cystatin C, sST2, and TIMP-1) as output variables to develop individual regression models. As input variables all predictive variables listed in section STATISTICAL ANALYSIS were used; "whole model" technique was performed. Models for NT-proBNP, GDF-15, Cystatin C and sST2 were significant and adequately interpolated the data (all P<0.05). The coefficients of determination (R^2) and significant parameters as well as their unstandardized (B) and standardized coefficients (Beta) for all models are shown in Table 2.

Logistic regression

In order to evaluate the effect of individual predictive factors of developing left ventricular hypertrophy, in the last part of our study two multivariate logistic regression models for two respondent groups – patients with LVH vs. the group of patients without LVH were developed.

1. Patients with LVH vs. Patients without LVH: only novel biomarkers – NT-proBNP, GDF-15, Galectin-3, Cystatin C, sST2, and TIMP-1 were used as predictive variables.
2. Patients with LVH vs. Patients without LVH: to the novel biomarkers, using "the best model" technique, the next predictive variables were added: age, LV EF, LA, RV, E/A, E/E', Na, K, urea, creatinine, and eGFR.

The basic logistic regression characteristics of both models, which are summarized in Table 3, shows that the first model is not statistically significant, both models interpolate data adequately, the classification ability of the

Table 2. Multivariate linear regression analysis of demographic (age), echocardiographic (LV EF, LA, RV, E/a, E/e', IVS, PW, RWT, LAi, LVMi), and biochemical (Na, K, Urea, Creatinine, Bilirubin, Hemoglobin, eGFR) parameters associated with novel biomarkers in hypertension patients (n=92). Only statistically significant parameters are shown.

Model	B	SE	Beta coefficient	t	*P*
Dependent variable: NT-proBNP, R^2 = 0.766, *P*=0.0085					
Age	0.509	0.243	0.409	2.098	0.043
E/A	16.054	5.330	0.481	3.012	0.005
Dependent variable: GDF-15, R^2 = 0.874, *P*=0.0016					
IVS	373.36	159.00	0.557	2.348	0.027
LVMi	−25.16	11.58	−0.533	2.172	0.039
Hgb	−41.02	15.19	−0.365	−2.700	0.012
Dependent variable: Cystatin C, R^2 = 0.793, *P*<0.0001					
LA	277.45	96.39	0.3235	2.8783	0.063
E/E'	−593.11	205.11	−0.3422	−2.8917	0.006
Creatinine	93.75	32.44	0.4260	2.8803	0.006
Dependent variable: sST2, R^2 = 0.779, *P*=0.0018					
Creatinine	2.56	1.058	0.418	2.417	0.021
AST	299.58	97.065	0.441	3.086	0.004

R^2 expresses the percentage variation of the matrix of the dependent variable explained by the independent variables.
Beta coefficient expresses the relative contribution of each independent variable in the prediction of the dependent variable; *P* represents the statistical significance of each independent variable.
sST2, soluble receptor "suppression of tumorigenicity 2"; GDF-15, growth differentiation factor; TIMP1, tissue type inhibitor of matrix metalloproteinases; LV EF, left ventricle ejection fraction; LA, left atrial diameter; RV, right ventricle diameter; E/E', E velocity divided by the mean velocity of mitral annulus as assessed by tissue Doppler imaging; E/A, E velocity divided by the velocity of flow at atrial contraction; LVMi, left ventricular mass index; Lai, left atrial index.

first models is only 64.8%, while the second model is up to 83.0%. The discrimination power measured using ROC analysis was FAIR [x1] for the first model and GOOD for the second model. The regression coefficients with *P*-value less or equal 0.200 for both models are presented in Table 4.

Interpretation of regression coefficients

PATIENTS WITH LVH vs. PATIENTS WITHOUT LVH

Model 1: predictive input variables – only novel biomarkers: NT-proBNP, GDF-15, Gelactin-3, Cystatin C, sST2, and TIMP-1 (first part of Table 4):

The chance of developing LVH increased in the following cases: sST2 (OR 1.0033; CI 1.0001–1.0066) – 1.0 ng/L increase in sST2 increases the patient's chance of developing LVH by 0.33%; TIMP-1 (OR 1.0001; CI 0.9999–1.0001) – 1.0 µg/L increase in TIMP-1 increases the patient's chance of developing LVH by 0.01%.

Model 2: predictive input variables – NT-proBNP, GDF-15, Gelactin-3, Cystatin C, sST2, and TIMP-1 + age, LV EF, LA, RV, E/A, E/E', Na, K, urea, creatinine, and eGFR (second part of Table 4):

The chance of developing LVH increased in the following cases**:** Cystatin C (OR 0.9998; CI 0.9996–1.0001) – 1.0 ng/L increase in Cystatin C decreases the patient's chance of developing LVH by 0.02%; sST2 (OR 1.0057; CI 0.9992–1.0122) – 1.0 ng/L increase in sST2 increases the patient's chance of developing LVH by 0.57%; TIMP-1 (OR 1.0001; CI 1.0001–1.0002) – 1.0 µg/L increase in TIMP-1 increases the patient's chance of developing LVH by 0.01%; LA (OR 1.1356; CI 0.9710–1.3281) – 1.0 mm increase in LA increases the patient's chance of developing LVH by 13.56%; and Age (OR 1.0566; CI 0.9712–1.1494) – if the patient gets 1 year older the chance of the patient developing LVH increases by 5.66%.

DISCUSSION

There are several important findings in our study. First, NT-proBNP level was not significantly higher in patients with arterial hypertension and left ventricular hypertrophy compared to subjects without LVH. And, NT-proBNP level did not correlate with LV mass index and relative wall thickness. Our findings are comparable with the results of the study published by Yasumoto et al.[10]. Yasumoto et al. showed, that atrial natriuretic peptide (ANP), but not B-natriuretic peptide (BNP) correlated significantly with LV mass index as assessed by echocardiography in untreated patients with arterial hypertension.

Our findings differ from those of Hildebrandt et al.[11]. The authors have shown, that NT-proBNP correlated with LV mass index as assessed by magnetic resonance imaging in the patients with arterial hypertension and electrocardiographic LV hypertrophy and preserved left ventricular ejection fraction. The explanation of that different result may be the difference of the patients population investigated in our study and in the study by Hildebrandt. First, we included unselected consecutive patients with arterial hypertension regardless the evidence of LV hypertrophy and the identification of LV hypertrophy was one of the study results. In the study of Hildebrandt, only patients with evidence of LV hypertrophy were included. Another explanation may be the level of NT-proBNP in our study. All but one patients in our study had NT-proBNP level in

Table 3. Overview of logistic regression models and results of statistical evaluation criteria.

Model:	Patients with LVH (n=31) vs. patients without LVH (n=47)	
Predictive variables:	NT-proBNP, GDF-15, Gelactin-3, Cystatin C, sST2, and TIMP-1	NT-proBNP, GDF-15, Gelactin-3, Cystatin C, sST2, and TIMP-1 + age, LV EF, LA, RV, E/A, E/E', Na, K, urea, creatinine, and eGFR
Omnibus test – 2LL*	0.146	0.046
Hosmer-Lemeshow test for goodness of fit**	0.583	0.628
Nagelkerke R^2***	0.169	0.529
% of correctly classified	64.8	83.0
AUC†	0.704	0.887
Sensitivity††	43.33%	80.00%
Specificity†††	80.48%	85.71%

*Omnibus test – 2LL of model coefficients gives an indication of whether the model with the independent variables fits the data better than the baseline model ("intercept only" model). If the test was significant the "final model" fits better than the baseline model.
**Hosmer-Lemeshow test compares the actual result for each respondent with the outcome predicted with the model. If this test was non-significant the observed and expected counts should be similar and the model fits the data.
***Nagelkerke R^2 indicates the improvement in fit of the model with predictors over the baseline model (0–0.1 poor improvement, 0.1–0.3 modest improvement, 0.3–0.5 moderate and more than 0.5 strong improvement).
†AUC (Area under the ROC curve) is a measure of the accuracy of the model, which depends on how well the model separates the groups being tested.
††Sensitivity is the power to identify positives.
†††Specificity is the power to identify negatives.

Table 4. Odds ratios (OR), 95% Confidence Intervals (CI) and significance levels of Wald's statistic (*P*) of differences in predictive values between patients with LVH (n=31) and without LVH (n=47). Only statistically significant predictors and predictors with $P<0.200$ are presented.

Variable	OR	95% CI	*P*
Model 1 – Predictive variables: NT-proBNP, GDF-15, Gelactin-3, Cystatin C, sST2, and TIMP-1			
sST2	1.0033	1.0001–1.0066	**0.041***
TIMP-1	1.0001	0.9999–1.0001	0.161
Model 2 – Predictive variables: NT-proBNP, GDF-15, Gelactin-3, Cystatin C, sST2, and TIMP-1 + age, LV EF, LA, RV, E/A, E/E', Na, K, urea, creatinine, and eGFR			
Cystatin C	0.9998	0.9996–1.0001	0.141
sST2	1.0057	0.9992–1.0122	0.086
TIMP-1	1.0001	1.0001–1.0002	**0.012**
LA	1.1356	0.9710–1.3281	0.112
Age	1.0566	0.9712–1.1494	0.200

*Statistically significant predictors are marked in bold.

normal range (< 125 pg/mL) and the mean NT-proBNP was in normal range both in patients with and without LV hypertrophy.

In the presence of LV hypertrophy, natriuretic peptides are strongly predictive for cardiovascular events. It has been shown in the study by Hildebrand and also in the study by Olsen[7]. In the study by Olsen and co-authors, NT-proBNP concentration above median was associated with increased risk of cardiovascular events including cardiovascular death, myocardial infarction, and stroke, especialy in patients without diabetes and cardiovascular disease. The association of biomarker levels with the risk of cardiovascular events was not objective of our study.

NT-proBNP is probably not optimal biomarker for the detection of left ventricular hypertrophy in the general population. It has been shown by Vasan and co-authors, that natriuretic peptides were not optimal in the screening of LV hypertrophy and LV systolic dysfunction in the community-based prospective study[8]. Unlike the study by Vasan, another research published by Coutinho et al. have shown that higher concentrations of natriuretic peptides were associated with left ventricular mass index in black siblings of hypertensive subjects[29].

Another finding in our study is, that NT-proBNP level correlates with the parameters of LV diastolic function: E/A ratio as assessed by Pulsed Wave Doppler echocar-

diography (PWD), and E/E' ratio as assessed by PWD and tissue Doppler imaging. Our study confirmes the results of the recent study published by Dhungana et al.[9]. The objective of study of Dhungana was the association of NT-proBNP with LV diastolic function in the patients with arterial hypertension. The authors have shown, that NT-proBNP levels were significantly higher in the patients with arterial hypertension compared to healthy controls, and there were significant correlations between NT-proBNP and LV diastolic function parameters as assessed by echocardiography. NT-proBNP concentrations were significantly higher in all grades of diastolic dysfunction as defined by E/E' ratio in the patients with arterial hypertension.

The association of B-natriuretic peptide BNP (as measured by plasma NT-proBNP) with the parameters of diastolic dysfunction may be explained by its role in the pathophysiology of cardiac dysfunction and heart failure. The BNP production is stimulated mainly by an increased wall stress in cardiomyocytes, and by the effectors of activated renin-angiotensin-aldosteron system (RAAS) – angiotensin II and aldosteron. BNP promotes diuresis, natriuresis and vasodilatation and this hormonal system is compensatory to increased RAAS activity in the circulation and tissues. Thus BNP (and NT-proBNP) is considered to be an optimal biomarker of heart failure[29,30]. We have shown in our study in concert to other findings, that NT-proBNP may be a good biomarker of diastolic dysfunction in arterial hypertension.

The second important finding of our study is that biomarker of myocardial remodeling and fibrosis sST2 is associated with the presence of LV hypertrophy in the patients with arterial hypertension. The mean sST2 level is significantly higher in the patients with LV hypertrophy and correlates with the echocardiographic parameters of LV hypertrophy (interventricular septal thickness, posterior wall thickness, LV mass and LV mass index and relative wall thickness). Our results have been confirmed by a recent study published by Wei and co-authors[12]. The authors showed, that sST2 levels were significantly higher in patients with arterial hypertension and LV hypertrophy compared to patients without LV hypertrophy, and sST2 levels correlated significantly with LV mass index. In another study, sST2 was used for the detection of heart failure in subjects with arterial hypertension and normal LV ejection fraction[13,14]. Soluble ST2 had additional role when assessed together with NT-proBNP in the diagnosis of heart failure with normal ejection fraction in hypertensive subjects, but was not better in the evaluation of diastolic dysfunction than NT-proBNP.

The association of sST2 with the LV hypertrophy has not been yet completely explained. Soluble ST2 is a receptor for interleukin-33 (IL-33) and is expressed in the situations associated with mechanical stress of the cardiomyocytes. It has been shown, that sST2 decreased the cardioprotective effect of IL-33 with consequent increased extent of myocardial damage after myocardial infarction and promoted myocardial fibrosis, apoptosis, inflammation and cardiac remodeling[32]. The role of sST2 in aterial hypertension is a subject of ongoing investigations. Soluble ST2 levels correlated with the systolic blood pressure and with the use of antihypertensiv drugs in the Framingham Heart Study[33]. The association of sST2 with cardiac geometry may be explained by its role in the pro-inflammatory status. This hypothesis was the objective of study published by Celic et al.[34]. The authors have shown, that soluble ST2 concentrations were independently associated with LV mass index in the patients with metabolic syndrome (94% of subjects had arterial hypertension).

We have shown that TIMP-1 – a potential biomarker of extracellular matrix remodeling – is associated with the presence of left ventricular hypertrophy. There is a limited information on the role of matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs) in arterial hypertension[35]. In the Framingham Offspring Study, higher TIMP-1 levels were associated with higher risk of having hypertension[36]. In a study by Tan and co-authors, higher TIMP-1 and also MMP-9 were associated with increased large artery stifness in hypertensive patients[37]. Our findings are comparable to results of a study published by Ahmed. Higher TIMP-1 and also MMP-9 levels were associated with left ventricular hypertrophy[38].

In our study we failed to show any association of other biomarker of cardiac remodeling, myofibrosis and inflammation with the parameters of cardiac structure and function in the patients with arterial hypertension, normal LVEF and absence of heart failure symptoms. We did not find any association of Cystatin-C, biomarker of inflammation and kidney function, and ceruloplasmin with the parameters of cardiac structure and function in the patients with arterial hypertension.

CONCLUSION

We conclude that the NT-proBNP level as a biomarker of cardiac remodelling correlates with the parameters of LV diastolic function in patients with arterial hypertension and normal LV EF and absence of heart failure signs. Of other biomarkers, the sST2 level as a marker of myocardial fibrosis and inflammation correlates with parameters of cardiac structure. Biomarkers ST2 and TIMP-1 are associated with the presence of left ventricular hypertrophy.

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