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# Relationship Between Ubiquitin-Specific Peptidase 18 and Hypertension in Polish Adult Male Subjects: A Cross-Sectional Pilot Study

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**Background:** Arterial hypertension (HT) is a leading cause of cardiac hypertrophy and heart failure. Ubiquitin-specific peptidase 18 (USP18) has been recently described as a factor that prevents myocardial dysfunction. The present study measured serum USP18 levels in normotensive ( $n=29$ ), isolated diastolic hypertensive ( $n=20$ ), and systolic-diastolic hypertensive ( $n=30$ ) male participants and correlated these results with biochemical parameters that are included in routine assessments of patients with hypertension.

**Material/Methods:** Seventy-nine men, aged 24 to 82 years (mean=50.8±11.4 years), were included in the study. None of the participants had ever been treated for HT. Blood and urine parameters were assessed using routine techniques. Serum USP18 levels were measured by enzyme-linked immunosorbent assay.

**Results:** The means and 95% confidence intervals (CIs) of USP18 levels in the HT(-), iDHT(+), and HT(+) groups were 69.3 (22.1–116.5) pg/ml, 90.1 (29.0–151.3) pg/ml, and 426.7 (163.1–690.3) pg/ml, respectively. In the HT(+) group, the mean serum USP18 level was 6.2-times higher than in the HT(-) group ( $p=0.014$ ) and 4.7-times higher than in the iDHT(+) group ( $p=0.19$ ). The partial correlation analysis that was adjusted for risk factors of arteriosclerosis indicated that USP18 levels were correlated with systolic blood pressure, pulse pressure, and heart rate.

**Conclusions:** This preliminary study found that serum USP18 levels were significantly higher in drug-naive male participants with arterial hypertension compared with normotensive controls. USP18 exerts cardiovascular-protective effects. Elevations of USP18 levels may indicate a counterregulatory process that is engaged during increases in pressure in the left ventricle.

**MeSH Keywords:** **Heart Failure • Hypertension • Hypertrophy, Left Ventricular • Ventricular Remodeling**

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## Background

Arterial hypertension (HT) is a condition that is diagnosed in 30–45% of the global adult population, and this number is still growing [1]. Hypertension can lead to arterial wall remodeling and organ damage, such as kidney disease, myocardial infarction, and stroke, all of which are associated with high rates of morbidity and mortality [2]. The onset of organ damage may be asymptomatic for many years, and thus HT may go untreated.

One of the early markers of HT-related complications is the urinary albumin-to-creatinine ratio (UACR) [3]. The UACR has been suggested to indicate not only renal vascular damage but also systemic vascular damage, particularly an increase in vascular permeability and endothelial dysfunction [3–5]. Heart failure is a common complication in later stages of HT, which occurs as a result of progressive myocardial hypertrophy and remodeling [6,7].

Ubiquitin-specific peptidase 18 (USP18) is a recently identified member of the ubiquitin-specific peptidase (USP) family and considered a novel factor that is involved in HT [8]. Ubiquitin-specific peptidases have been reported to remove ubiquitin-like molecules from their substrates and regulate such cellular processes as the cell cycle, cell signaling, and the stress response. Ubiquitin-specific peptidases have also been reported to contribute to immune response regulation [9–11]. USP18 synthesis is stimulated by type I and type III interferons (IFNs), viral infection, bacterial lipopolysaccharides, tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), and genotoxic stress [10].

USP18 exerts its enzymatic function by cleaving interferon-stimulated gene 15 (ISG15) and ubiquitin from their target proteins [8,12,13]. USP18 is involved in the response to viral infection and development of several autoimmune diseases. It also contributes to the pathogenesis of some genetic diseases and has antitumor properties [14–21]. Mouse studies have shown that USP18 is also crucial for survival. The lifespan of USP18 knockout mice was significantly shorter compared with wildtype mice. Mice that lacked the USP18 gene and protein were hypersensitive to IFNs and exhibited advanced damage of the central nervous system [11].

Ying et al. reported that USP18 inhibited cardiac remodeling and protected against the development of heart failure. USP18 expression was significantly higher in hearts of patients with dilated cardiomyopathy than in hearts from healthy donors [8]. In murine hearts that were exposed to an increase in afterload, the elevation of USP18 expression in cardiomyocytes was related to a decrease in left ventricular wall hypertrophy, less pronounced myocardial fibrosis, and a delayed onset of heart failure [8]. In contrast, USP18-deficient mice exhibited advanced cardiac remodeling. Ying et al. concluded that this

process was a consequence of inhibition of the transforming growth factor beta-activated kinase 1 (TAK1)-p38-c-Jun N-terminal kinase 1/2 (JNK1/2) signaling pathway [8]. To our knowledge, no other clinical or epidemiological studies have investigated USP18 as a putative biomarker or predictive factor in cardiovascular disease. Thus, the aim of the present study was to compare serum USP18 levels in normotensive male subjects, male patients with isolated diastolic HT (iDHT), and male patients with systolic-diastolic HT and assess the association between USP18 levels and basic clinical parameters in early hypertension.

## Material and Methods

### Study population

Seventy-nine adult men (mean age=50.8±11.4 years; range=24–82 years) who participated in the ProM project ([www.clinicaltrials.gov](http://www.clinicaltrials.gov); NCT03559608) were included in the study. Patients with a history or signs of acute or chronic infectious or non-infectious inflammatory disease were excluded. None of the subjects suffered from diabetes or any other clinically overt disorder that could be associated with a greater risk of cardiovascular disease. None of the patients were ever treated with blood pressure (BP)-lowering medications, statins, anti-diabetic agents, or acetylsalicylic acid. This provided a unique opportunity to analyze groups of hypertensive patients who were likely naive to treatment and otherwise healthy.

### ProM project

This cross-sectional study was performed between December 2014 and December 2017 in the Department of Nephrology, Hypertension, and Internal Medicine and Department of Pathophysiology at the University of Warmia and Mazury in Olsztyn (Poland). The primary aim of the study was to analyze the prevalence of HT and risk factors for the development of HT in an unselected group of Polish men from the region of Warmia and Mazury, which is considered one of the most underprivileged regions in Poland in terms of social and employment status. The study protocol was approved by the Ethics Committee of the Regional Chamber of Physicians, District of Warmia and Mazury, Poland. The study was conducted according to the Declaration of Helsinki and Good Clinical Practice guidelines. Each participant signed a written informed consent.

The decision to study only male individuals was based on data from the World Health Organization (<http://www.euro.who.int/en/countries/poland/data-and-statistics>; accessed May 16, 2018). These data indicate that the mean life expectancy in Poland is 8 years shorter in males (74 years) than in females (82 years). In Western Europe, this difference does not exceed

5 years (<https://www.statista.com/statistics/274514/life-expectancy-in-europe/>; accessed May 16, 2018). Moreover, the risk of cardiovascular disease in European countries, assessed by Systematic COronary Risk Evaluation (SCORE) risk charts, indicates a higher risk for cardiovascular disease and associated complications in Polish men compared with other high-risk nations in Europe [22]. Arterial HT is a key risk factor for lower life expectancy in the Polish male population. The aim of the ProM project is to investigate the prevalence of HT and promulgate interest in prevention and treatment in male inhabitants of the Warmia and Mazury region in Poland.

Arterial BP was measured according to 2013 European Society of Cardiology/European Society of Hypertension (ESC/ESH) Guidelines in 631 randomly selected subjects [2]. All of the participants completed a coded and standardized health questionnaire and underwent blood and urine tests that were performed in a certified clinical laboratory (ISO 9001: 2018). The biochemical analyses were performed using a Cobas 6000 modular analyzer (Roche, Basel, Switzerland). Serum was tested for glucose, creatinine, and lipid profile. Microalbuminuria and creatinine were measured in urine, and the UACR was calculated. The estimated glomerular filtration rate (eGFR) was calculated according to the MDRD formula.

### Blood pressure measurement

Office blood pressure (BP) and heart rate (HR) were measured by pulse detection of both arms using an Omron M3 device (Kyoto, Japan). After 10 min of rest in a sitting position, we repeated office BP measurements on the arm with the higher systolic blood pressure (SBP) value twice, and the mean of 2 measurements was used for further analyses. Mean arterial pressure (MAP) was calculated as the sum of two thirds of diastolic blood pressure (DBP) and one third of SBP. Pulse pressure (PP) was calculated as the difference between SBP and DBP. Three groups could be distinguished among the study participants according to 2013 ESH/ECS Guidelines: HT(–) (normotensive men, SBP <140 mmHg, DBP <90 mmHg,  $n=29$  [36.7%]), iDHT(+) (isolated diastolic hypertensive men, SBP <140 mmHg, DBP  $\geq 90$  mmHg,  $n=20$  [25.3%]), HT(+) (systolic-diastolic hypertensive men, SBP  $\geq 140$  mmHg, DBP  $\geq 90$  mmHg,  $n=30$  [38.0%]).

### USP18 measurement

Blood samples were collected by qualified medical staff. Approximately 2 ml of each serum sample was frozen at  $-80^{\circ}\text{C}$ . The samples were then thawed at  $-20^{\circ}\text{C}$  for 24 h,  $6^{\circ}\text{C}$  for the next 24 h, and then at room temperature for 30 min immediately before mixing by a vortex shaker. Serum USP18 levels were assayed using an enzyme-linked immunosorbent assay (ELISA) kit for human USP18 (Wuhan Fine Biotech Co., Ltd., Wuhan, Hubei, China) by a technician with more than 10 years

of experience in ELISA procedures. The technician was blinded to the clinical data. To verify reliability of the ELISA method, USP18 measurements were repeated twice for each sample with a coefficient of variation (CV) of 0.84%. The CV was calculated as the ratio of the standard deviation to the mean multiplied by 100.

### Statistical analysis

The data distribution was first checked using the Kolmogorov-Smirnov test. The data were then analyzed using SPSS 23 software (IBM, New York, USA). Two-tailed values of  $p < 0.05$  were considered statistically significant. To compare differences between 2 groups, unpaired Student's *t*-test with Bonferroni correction was used for data with a normal distribution, the results of which are expressed as means  $\pm$  standard deviations. For data with a non-normal distribution, the nonparametric Mann-Whitney U test for unpaired samples was used, the results of which are presented as medians and interquartile ranges. To compare differences between 3 groups, one-way analysis of variance was used for data with a normal distribution, and the nonparametric Kruskal-Wallis test for unpaired samples was used for data with a non-normal distribution. *p* values were not calculated for the BP parameters, which were used to define the groups. Systolic blood pressure in the iDHT(+) and HT(–) groups did not contribute to this division; in this case, *p* values were calculated.

To determine associations between clinical parameters and USP18 levels, Pearson and Spearman correlation coefficients and partial correlations that were adjusted for risk factors of arteriosclerosis (i.e., age, Body Mass Index [BMI], total cholesterol, triglycerides, and glucose) were calculated. Analysis of covariance was performed using univariate and multivariate linear analyses. Age, BMI, total cholesterol, triglycerides, and glucose were used as confounding variables.

Non-adjusted and adjusted *p* values are presented in the Results section, Tables 1–5, and Figure 1. The graphical representation of data variability is illustrated by error bars as a 95% confidence interval (CI) of the measured values.

## Results

Demographic, anthropometric, and biochemical parameters in the study groups are summarized in Table 6 ( $n=30$  for HT[+] group,  $n=20$  for iDHT[+] group,  $n=29$  for HT[–] group). Table 1 shows the comparisons between groups in terms of the clinical parameters. Of the 79 participants, only 7 (8.86%) declared awareness of their having HT that remained untreated, and 72 (91.14%) had never been diagnosed with HT.

**Table 1.** Comparison of anthropometric and biochemical parameters between the study groups\*.

	Mean±SD Median (IQR)			p <sup>1</sup>			p <sup>2</sup>
	HT(-) n=29	iDHT(+) n=20	HT(+) n=30	HT(+) vs. HT(-)	iDHT(+) vs. HT(-)	HT(+) vs. iDHT(+)	
Age [years]	49.3±12.4 48.5 (40.3–63.8)	50.25±9.6 53.0 (45.5–57.0)	52.9±11.3 51.0 (43.5–57.5)	>0.99	>0.99	>0.99	0.93
BMI [kg/m <sup>2</sup> ]	25.4±3.1 25.5 (24.3–28.5)	28.0±3.0 29.2 (24.8–31.0)	28.3±3.8 28.7 (25.1–31.2)	0.0093	>0.99	0.061	0.0060
Triglycerides [mg/dl]	122±78 103 (72–176)	147±115 112.0 (93–208)	145±92 128 (73–165)	0.53	0.26	0.61	0.52
Total cholesterol [mg/dl]	207.3±41.1 216 (184–241)	207.3±36.2 201 (173–233)	211.0±42.4 215 (189–250)	0.81	0.46	0.42	0.68
Glucose [mg/dl]	101.7±17.2 100 (92–106)	96.8±11.1 91 (87–101)	100.1±17.0 98 (92–106)	0.90	0.074	0.076	0.14
Creatinine [mg/dl]	0.986±0.14 1.0 (0.90–1.10)	0.945±0.16 0.90 (0.80–1.00)	0.987±0.14 1.0 (0.90–1.10)	0.75	0.051	0.077	0.11
eGFR [ml/min]	83.5±13.1 82.3 (71.6–93.1)	87.5 ±16.4 87.0 (77.1–103.5)	81.7±13.1 80.1 (74.4–91.5)	>0.99	0.18	0.41	0.16
UACR [mg/g]	3.07±3.72 1.84 (1.19–2.51)	2.34±1.34 2.05 (0.97–3.67)	3.41±8.24 1.94 (1.20–3.08)	0.70	0.80	0.88	0.92
SBP [mmHg]	124.8±7.4 125.5 (119–132)	134.3±4.0 132.3 (128–137)	156.2±14.2 153 (145–162)	–	0.0073	–	–
DBP [mmHg]	80.9±5.9 82.3 (76–85)	93.4±2.4 93.3 (92.3–95.1)	98.5±10.5 97.5 (94–104)	–	–	–	–
MAP [mmHg]	95.5±5.3 96 (94–100)	107.0±2.0 106 (104–108)	117.7±10.7 115 (112–121)	–	–	–	–
PP [mmHg]	43.9±7.8 42.8 (37.5–49.8)	40.9±5.0 37.8 (35.0–41.1)	57.8±10.7 53.0 (48.0–62.0)	<0.0012	0.0051	<0.0011	<0.0010
HR [beats/min]	69.8±9.9 71 (63–77)	76.6±10.8 76 (71–80)	76.7±12.8 74 (65–87)	0.16	0.071	>0.99	0.039
USP18 [pg/ml]	69.3±124.0 22.3 (0.0–77.0)	90.1±130.6 44.6 (0.0–120.6)	426.7±706 80.4 (20.3–317)	0.030	0.58	0.19	0.088

\* Respecting the values distribution (Table 6), p<sup>1</sup> values were calculated by the Mann-Whitney U test for data with a non-normal distribution or by unpaired Student's t-test with Bonferroni correction for data with a normal distribution; p<sup>2</sup> values were calculated for 3 independent groups of participants by non-parametric Kruskal Wallis test for data with a non-normal distribution or by one-way analysis of variance for data with a normal distribution. p values were not calculated for the blood pressure parameters, which defined the groups. Systolic blood pressure in iDHT(+) and HT(-) did not contribute to this division; in this case, p value was calculated; n – patients number; SD – standard deviation; IQR – interquartile range.

**Table 2.** Simple correlation analysis between USP18 and BMI, UACR, serum and blood pressure parameters.

	USP18	
	<i>r</i>	<i>p</i>
BMI*	0.31	0.0061
Triglyceride**	0.42	0.0018
Total cholesterol	-0.36	0.0013
Creatinine	-0.053	0.65
eGFR	0.0060	0.96
UACR	-0.072	0.53
SBP	0.27	0.016
DBP	0.26	0.023

\* Pearson's correlation analysis; \*\* Spearman's correlation analysis.

In the HT(+) group, mean serum USP18 levels were significantly higher than in the HT(-) group (Figure 1, Table 1). The difference between median USP18 levels in the HT(-) and HT(+) groups was also statistically significant (22.3 pg/ml in HT[-] group and 80.4 pg/ml in HT[+] group). Systolic blood pressure in the iDHT(+) group was <140 mmHg. Systolic blood pressure was significantly higher in the iDHT(+) group than in the HT(-) group ( $p<0.001$ ). Pulse pressure was significantly higher in the HT(+) group than in both the HT(-) and iDHT(+) groups (Table 1). The UACR did not differ between groups. In each group, the UACR was below the threshold for microalbuminuria (<30 mg/g; Table 1). No correlation was found between USP18 levels and the UACR ( $r=-0.072$ ,  $p=0.53$ ), serum creatinine ( $r=-0.053$ ,  $p=0.65$ ), and eGFR ( $r=0.006$ ,  $p=0.96$ ). USP18 levels were significantly correlated with BMI ( $r=0.309$ ,  $p=0.006$ ), triglycerides ( $r=0.423$ ,  $p<0.001$ ), total cholesterol ( $r=-0.356$ ,  $p=0.001$ ), SBP ( $r=0.269$ ,  $p=0.016$ ), and DBP ( $r=0.255$ ,  $p=0.023$ ; Table 2). No other significant correlations were found between USP18 levels and the clinical parameters.

**Table 3.** Correlation between USP18 and blood pressure parameters.

	Pearson correlation		Partial correlation*	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
SBP	0.33	0.0040	0.314	0.0081
DBP	0.11	0.38	0.14	0.24
MAP	0.23	0.057	0.234	0.051
PP	0.39	0.0013	0.37	0.0023
HR	0.25	0.035	0.24	0.043

\* Partial correlations were adjusted by risk factors of arteriosclerosis (age, BMI, serum total cholesterol, triglyceride and glucose).

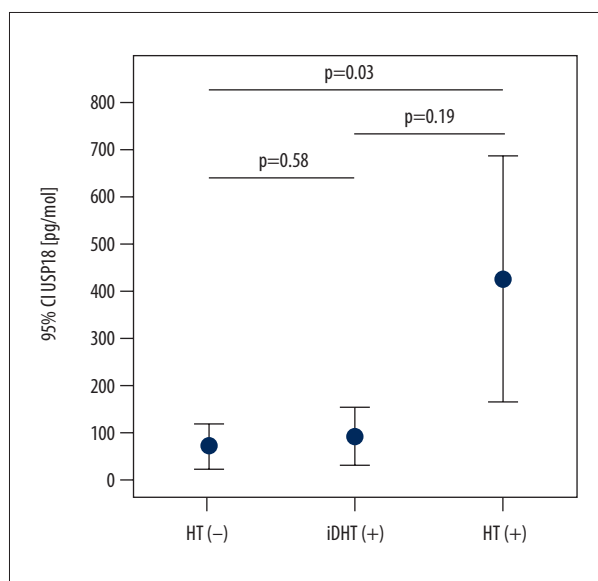
**Table 4.** Univariate linear analysis between USP18 and BMI, UACR, serum and blood pressure parameters.

	USP18		
	<i>R</i> beta	<i>R</i> <sup>2</sup>	<i>p</i>
Age	0.049	0.0022	0.67
BMI	0.0022	<0.0014	0.99
Triglyceride	0.234	0.055	0.038
Total cholesterol	0.19	0.034	0.10
Glucose	-0.013	<0.0011	0.91
Creatinine	-0.032	0.0012	0.78
eGFR	0.00	<0.0012	1.0
UACR	0.069	0.0054	0.55
SBP	0.36	0.13	0.0013
DBP	0.17	0.028	0.14
MAP	0.27	0.073	0.016
PP	0.39	0.16	<0.0012
HR	0.32	0.11	0.0044

**Table 5.** Multivariate analysis between USP18 and selected parameters\*.

	USP18			
	CI 95% lower limit	CI 95% upper limit	R beta standardized	p
Age	-8.95	9.73	0.0094	0.93
Total cholesterol	-1.58	3.73	0.090	0.42
Creatinine	-752.63	628.83	-0.019	0.86
UACR	-17.17	21.96	0.028	0.81
PP	5.33	23.50	0.34	0.0022
HR	1.30	19.018	0.25	0.025

\* CI 95% – 95% confidence interval.



**Figure 1.** Comparison of 95% confidence intervals (CI) of USP18 levels in the normotensive (HT[–]) group, isolated diastolic hypertensive (iDHT[+]) group, and systolic-diastolic hypertensive (HT[+]) group. The *p* values were calculated using the Mann-Whitney U test.

After adjusting for risk factors for atherosclerosis (i.e., age, BMI, total cholesterol, triglycerides, and glucose), USP18 levels were significantly correlated with SBP ( $p=0.008$ ), PP ( $p=0.002$ ), and HR ( $p=0.043$ ) in the whole study cohort (Table 3). Univariate and multivariate analyses confirmed associations between parameters (Table 4). The *p* values and lower and upper limits of 95% CIs in the multivariate analysis indicated that HR and PP were independently related predictors of high USP18 levels in hypertensive men (Table 5).

## Discussion

Arterial HT increases afterload and leads to myocardial hypertrophy. If left untreated, it ultimately leads to congestive heart failure [6,7]. In the long term, pressure overload induces the mitogen-activated protein kinase (MAPK)-dependent signaling pathway, modifies the structure of contractile proteins, and causes a pattern of concentric myocardial hypertrophy. Concentric hypertrophy and heart failure with a preserved ejection fraction may lead to eccentric hypertrophy and progressive cardiomyocyte damage, resulting in cardiac dilation and heart failure with a reduced ejection fraction [23].

Ying et al. reported that USP18 is a novel factor that is involved in the development of cardiac hypertrophy. Under conditions of chronic increases in afterload, USP18 inhibited the development of congestive heart failure in both an experimental mouse model and humans by blocking MAPKs within the TAK1-p38-JNK1/2 axis [8]. Based on these data, we hypothesized that high USP18 levels may reflect an ongoing counterregulatory process in response to an increase in afterload in an effort to maintain proper cardiomyocyte structure and compensate for their contractile function. Therefore, an increase in serum USP18 levels may be an early marker of cardiac remodeling.

The present results suggested that an increase in USP18 levels in the HT(+) group may be a biochemical signature of early cardiomyocyte remodeling. The HT(+) group was characterized by significantly higher serum USP18 levels compared with the HT(–) group. The 3 groups in the present study did not differ in age or other demographic, anthropometric, or biochemical parameters, with the exception of BMI. Serum USP18 levels were not associated with BMI (Table 4). Only the difference in BP emerged as a determinant of higher USP18 levels in the HT(+) group compared with the HT(–) and iDHT(+) groups. This implies that iDHT at this stage of the disease does not evoke cardiac remodeling. This is consistent with previous studies that found that cardiac hypertrophy was more prevalent in individuals

**Table 6.** Anthropometric and biochemical parameters of the whole study population\*.

	Distribution Kolmogorov-Smirnov test	All participants n=79	
		Mean±SD	Median (IQR)
Age [years]	Normal	50.9±11.3	50 (43; 58)
BMI [kg/m <sup>2</sup> ]	Normal	27.1±3.6	27.4 (24.6; 30.1)
Triglyceride [mg/dl]	Non-normal	137.0±93.2	109 (78; 161)
Total cholesterol [mg/dl]	Non-normal	209.0±40.0	212 (183; 241)
Glucose [mg/dl]	Non-normal	99.8±15.7	99.00 (90.75; 105.25)
Creatinine [mg/dl]	Non-normal	0.98±0.15	1.00 (0.90; 1.025)
eGFR [ml/min]	Normal	83.7±14.02	84.6 (74.9; 92.4)
UACR [mg/g]	Non-normal	3.37±5.6	1.89 (1.18; 3.19)
SBP [mmHg]	Non-normal	139.1±93.2	136.5 (128.5; 149.5)
DBP [mmHg]	Normal	90.7±10.7	91.0 (83.0; 95.5)
MAP [mmHg]	Non-normal	106.9±12.1	106.5 (97.8; 113.5)
PP [mmHg]	Non-normal	48.4±11.3	46 (41; 54)
HR [beats/min]	Normal	74.1±11.6	74 (65; 80)
USP18 [pg/ml]	Non-normal	210.3±473.3	37.72 (0; 163.22)

\* n – patients number; SD – standard deviation; IQR – interquartile range.

with systolic hypertension than in individuals with diastolic hypertension [24–26]. Nevertheless, iDHT patients are up to 23 times more likely than normotensive individuals to develop systolic-diastolic hypertension during the next 10 years [27,28]. The UACR is generally accepted as a marker of early vascular damage, particularly endothelial damage, that is not necessarily limited to the kidneys. The UACR in the present study was within the normal range and not different between any of the groups [3,4]. Unlike USP18 levels, the UACR was not correlated with BP parameters. Such a trend was detectable only for PP ( $r=0.20$ ,  $p=0.077$ ). This observation suggests that elevations of USP18 levels may occur earlier than increases in the UACR.

Clinical studies of the relationship between cardiovascular and BP parameters and USP18 levels in humans are lacking. Associations between PP (which indirectly reflects vascular stiffness) and different cytokines or markers of cardiac damage have been previously reported. For example, Yamada et al. described an independent correlation between PP and TNF $\alpha$  in elderly hypertensive patients. Sasaki et al. reported an association between PP and N-terminal pro-B-type natriuretic peptide (NT-pro-BNP) [29,30]. Several BP parameters (including PP) were correlated with NT-pro-BNP in patients with peripheral vascular disease [31]. Such an observation was also recently confirmed in the large, population-based Atherosclerosis Risks in Communities study [32]. Sympathetic overactivity, reflected by an increase in heart rate, is one of the most important determinants of cardiac injury, reflected by NT-pro-BNP or cardiac troponins [33].

One limitation of the present study was that we did not measure markers of inflammation; thus, we were unable to analyze possible links between markers of inflammation and USP18 levels. Such an association, however, is unlikely because of our very careful patient selection and clinical assessments. One advantage of the present study was that none of the subjects had ever received antihypertensive medications, and the subjects did not have immunological or cardiovascular disease.

## Conclusions

In summary, serum USP18 levels were significantly higher in patients with early-stage systolic-diastolic arterial HT than in normotensive subjects and were also higher than in patients with iDHT. Our findings suggest that elevations of USP18 levels may represent subclinical cardiomyocyte remodeling. Given the correlations we found between USP18 levels and HR and PP, further studies should assess possible relationships between USP18 levels and other markers of cardiomyocyte damage, echocardiographic parameters, common carotid artery intima-media thickness, and pulse wave velocity (i.e., parameters that reflect the degree of atherosclerosis and arterial stiffness).

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## Conflicts of interest

None.