Original Paper

Plasma Level of Retinol-Binding Protein 4, N-Terminal proBNP and Renal Function in Older Patients Hospitalized for Heart Failure

Marcin Majerczyk^{a, b} Piotr Choręza^c Katarzyna Mizia-Stec^d
Maria Bożentowicz-Wikarek^a Aniceta Brzozowska^e Habibullah Arabzada^a
Aleksander J. Owczarek^c Aleksandra Szybalska^f Tomasz Grodzicki^g
Andrzej Więcek^h Magdalena Olszanecka-Glinianowicz^e Jerzy Chudek^{a, i}

^aPathophysiology Unit, Department of Pathophysiology, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland; ^bDepartment of Cardiology, District Hospital in Zakopane, Zakopane, Poland; ^cDepartment of Statistics, Department of Instrumental Analysis, School of Pharmacy and Laboratory Medicine in Sosnowiec, Medical University of Silesia, Katowice, Poland; ^dFirst Department of Cardiology, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland; ^eHealth Promotion and Obesity Management Unit, Department of Pathophysiology, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland; ^fInternational Institute of Molecular and Cell Biology, Warsaw, Poland; ^gDepartment of Internal Medicine and Gerontology, Jagiellonian University Medical College, Krakow, Poland; ^hDepartment of Nephrology, Transplantation and Internal Medicine, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland; ⁱDepartment of Internal Diseases and Oncological Chemotherapy, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland

Keywords

Retinol-binding protein 4 · N-Terminal-proBNP · Heart failure · Atrial fibrillation · Hypertension · Chronic kidney disease

Abstract

Background/Aim: Elevated plasma concentration of retinol-binding protein 4 (RBP4) has recently emerged as a potential new risk factor for cardiovascular diseases, including hypertension (HT) and coronary artery disease (CAD). Limited data suggest that RBP4 promotes inflammatory damage to cardiomyocytes and participates in the development of heart failure (HF). This study aimed to analyze the relationship between concentrations of plasma RBP4 and serum N-terminal proBNP (NT-proBNP), a powerful biomarker of left ventricle dysfunction, in the older Polish population. **Methods:** The study sample consisted of 2,826 (1,487 men) participants of the PolSenior study, aged 65 years and older, including a subgroup hospitalized for HF ($n = 282$). In all subjects, plasma concentrations of RBP4, interleukin-6 (IL-6),

Jerzy Chudek, MD, PhD
Department of Internal Diseases and Oncological Chemotherapy
Medical University of Silesia, Reymonta Street 8
PL-40-029 Katowice (Poland)
E-Mail chj@poczta.fm

serum level of NT-proBNP, and hs-CRP were measured. Additionally, BMI, estimated glomerular filtration rate (eGFR), and HOMA-IR were calculated. The prevalence of HT, CAD, atrial fibrillation (AF), and medication were considered as potential confounders. **Results:** Similar RBP4 levels were found in subjects with NT-proBNP < 125 and ≥125 ng/mL, with and without AF, and in the subgroups hospitalized for HF with and without AF. Regression analysis revealed no association between $\log_{10}(\text{NT-proBNP})$ and $\log_{10}(\text{RBP4})$. Plasma levels of RBP4 were increased by HT occurrence and diuretic therapy, while diminished with regard to female gender, age, eGFR values, AF, and IL-6 levels. **Conclusion:** Our results show that RBP4 is affected by GFR but cannot be considered as an independent biomarker of heart muscle dysfunction.

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Introduction

Among the many diseases that contribute to the development of heart failure (HF), coronary artery disease (CAD) [1], hypertension (HT) [2], obesity [3], and tachyarrhythmias, especially atrial fibrillation (AF) [4, 5], play a leading role. A link between obesity-related insulin resistance (IR) and HF has also been described [6], including: alteration in glucose and free fatty acid metabolism in cardiac myocytes, induction of inflammation and oxidative stress, and promotion of overactivity of the renin-angiotensin II system [7]. Moreover, HF is associated with low-grade chronic inflammation, sympathetic nervous system hyperactivity, and elevated circulating catecholamine levels [3, 8].

Elevated plasma retinol-binding protein 4 (RBP4) level has recently emerged as a potential new risk factor for atherosclerosis [9] and cardiovascular diseases, including HT [10, 11], and CAD [12] development, the leading causes of HF in older populations [13, 14]. RBP4 is an adipokine secreted by tissues storing retinoids such as liver or adipose tissue [15].

The physiological role of RBP4 is to transport retinol into peripheral tissues. Experimental studies prove that RBP4 is involved in the pathogenesis of IR [16], type 2 diabetes (DM) [17], endothelial dysfunction [18], and smooth muscle cell proliferation via extracellular signal-regulated kinases activity, including Janus kinase [19].

At the molecular level, RBP4 augments protein synthesis and increases the size of cardiac myocytes. Also, it promotes inflammatory damage to cardiac myocytes by Toll-like receptor 4 activation and myeloid differentiation of primary response gene 88 (*MyD88*) pathways [7]. RBP4 also impairs insulin-stimulated glucose uptake in cardiac myocytes as well as decreases the expression of glucose transporter-4 [7].

Data on the role of RBP4 in patients with HF are scarce. Of note, Bobbert et al. [6] reported increased RBP4 plasma concentration proportionally to interleukin-8 (IL-8) levels in patients with inflammatory dilated cardiomyopathy (DCM) and normal kidney function. Concurrently, a group with DCM without inflammatory origin had similar plasma RBP4 levels in comparison to healthy controls. Data on the impact of HF on plasma levels of RBP4 in older subjects have not been published yet.

This study aimed to assess if there is a relationship between: HT, CAD, AF, past hospitalization for HF, plasma concentration of RBP4, and serum levels of N-terminal pro-BNP (NT-proBNP), independently of estimated glomerular filtration rate (eGFR), in the population of older Poles. To verify this hypothesis, we analyzed the relationship between the concentrations of plasma RBP4 and serum NT-proBNP in the older Polish population. In contrast to our previous study, we did not exclude from the analysis subjects with some missing data that resulted in the enlargement of the study cohort.

Fig. 1. Study flow chart.

Patients and Methods

Study Design and Setting

The PolSenior project was an extensive research project performed among the oldest groups of the Polish society (4,979 respondents over 65 years of age) [20]. Experienced visiting nurses examined each subject twice. The examination included a questionnaire survey, comprehensive geriatric assessment, body mass, height, waist circumference, and BP measurements. Finally, on a third visit, blood and urine samples were collected. The study was approved by the Bioethics Committee of the Medical University of Silesia in Katowice (KNW/0022/KB1/38/II/08/10; KNW-6,501-38/I/08), and informed written consent was obtained individually from all participants.

The current substudy was performed on available banked plasma samples stored at -70°C for up to 4 years that were left after previous assessments. In contrast to our previous study, we did not exclude from the analysis subjects with incomplete data. Finally, the study group consisted of 2,826 subjects (1,487 men, 1,339 women) (Fig. 1), including a subgroup previously hospitalized for HF ($n = 282$).

Blood Pressure Measurements

All BP measurements were performed on the patient's right arm in the seated position after at least a 5-min rest period using an automatic A&D UA787 device (A&D Medical, Tokyo, Japan) with a cuff size suitable to the subjects' arm circumference. BP measurements were performed by well-trained and experienced nurses during each visit, as described previously [21].

Biochemical Measurements

Plasma RBP4 levels (Immundiagnostik AG, Bensheim, Germany, sensitivity 0.9 pg/mL) were measured by ELISA, with mean intra-assay and inter-assay coefficients of variability $<5\%$ and $<9.8\%$, respectively, as described previously [11].

Serum C-reactive protein concentrations were assessed by an automated system (Modular PPE, Roche Diagnostics GmbH, Mannheim, Germany) in a certified laboratory (intermediate precision $<5.7\%$).

Serum insulin and NT-proBNP levels were assessed by the electrochemiluminescence method using commercially available kits on a Cobas E411 analyzer (Roche Diagnostics GmbH, Mannheim, Germany) with intermediate precision <3.8 and 4.6% , respectively. Plasma IL-6 was measured by ELISA using a kit produced by R&D System (Minnesota, MN, USA) with a sensitivity of 0.04 pg/mL and mean intra-assay and inter-assay coefficients of variance <7.8 and $<7.2\%$, respectively.

Data Analysis

HT was diagnosed according to the 2013 ESH/ESC Guidelines for the management of HT (average SBP values of at least 140 mm Hg and/or average DBP values of at least 90 mm Hg based on two BP measure-

ments, or if the patient was taking antihypertensive drugs over the past 2 weeks due to an earlier diagnosis of HT) [22].

eGFR was calculated according to the short MDRD (Modification of Diet in Renal Disease) formula [23]. Chronic kidney disease (CKD) was diagnosed in subjects with eGFR <60 mL/min/1.73 m² and/or albumin-to-creatinine ratio ≥30 mg/g.

Hospitalization for HF, diagnosis of AF, and utilization of drugs recommended in HF treatment were collected from the questionnaire surveys (based on hospital discharge cards shown by participants).

Statistical Analysis

Statistical analysis was performed using STATISTICA 10.0 PL (StatSoft, Cracow, Poland) and StataSE 12.0 (StataCorp LP, TX, USA). Cases with missing data were not excluded from the analysis. No data imputation was done. Nominal and ordinal data were expressed as percentages, while interval data were expressed as a mean value ± standard deviation in the case of normal distribution, or as median (lower quartile – upper quartile) in the event of data with skewed or nonnormal distribution. Distribution of variables was evaluated by the Shapiro-Wilk test and quantile-quantile plot, and homogeneity of variance was assessed by the Levene test. The two groups were compared either with the Student *t* test in case of normal data distribution or after logarithmic normalization in case of skewed data (if appropriate), or with the Mann-Whitney U test in case of heavily skewed or nonnormal data distribution. RBP4 quartiles subgroups were compared with one-way analysis of variance in case of normal data distribution or after logarithmic normalization in case of skewed data (if appropriate) with Dunnett's post hoc test, or with nonparametric Kruskal-Wallis test with median post hoc test. To assess the relationship between RBP4 or NT-proBNP plasma levels and other variables, a stepwise backward multivariate normal and skew-*t* regression analysis was used, due to the heavily skewed distribution of some variables. Correlation between variables was measured with the Pearson linear correlation coefficient, taking appropriate requirements. Statistical significance was set at a *p* value <0.05. All tests were two-tailed.

Results

Characteristics of the Study Population

Our substudy consisted of 2,826 subjects, which makes up 56.8% of PolSenior participants aged 65 years and older (*n* = 4,979) (Fig. 1). The average age was 78 years with balanced sex distribution (1,339 women; 47.4%) (Table 1). There was a subgroup of subjects previously hospitalized for HF (*n* = 282; 10.0%) and AF (*n* = 517; 18.3%), analyzed separately. As expected, the most commonly used medications were angiotensin-converting enzyme inhibitors (ACE-I) and angiotensin II receptor blockers (ARB), and less commonly, β-blockers and diuretics, medications which are in accordance with HF guidelines [24]. Spironolactone, a mineralocorticoid receptor blocker, was used by only 8.3% of patients.

RBP4 Concentration

The median of RBP4 plasma concentration was 41.1 (lower and upper quartile: 29.5–57.6) ng/mL (Table 1). There were no significant differences in the values between subjects with NT-proBNP <125 ng/mL and those with NT-proBNP ≥125 ng/mL (41.5 [29.6–57.2] vs. 41.0 [29.3–57.7] ng/mL, respectively; *p* = 0.69). Furthermore, plasma RBP4 concentrations in participants with a history of hospitalization for HF were similar in those without such an episode (40.2 [28.4–55.0] vs. 41.2 [29.5–57.7] ng/mL, respectively; *p* = 0.16). Also, comparable concentrations were noted in HF subgroups with or without AF (40.4 [28.6–56.4] vs. 37.9 [29.5–57.8] ng/mL; *p* = 0.12), and in the hospitalized and nonhospitalized for HF subgroups (Table 1).

In the subanalysis of RBP4 quartiles subgroups, there was no difference in the prevalence of previous hospitalization for HF, occurrence of AF, and NT-proBNP levels (Table 2). Subjects in the highest quartile had the lowest eGFR and highest prevalence of HT followed by more frequent use of β-blockers, ACE-I or ARB, and diuretics.

Table 1. Baseline characteristics of study subjects ($n = 2,826$)

	All ($n = 2,826$)	Hospitalization for heart failure		<i>p</i>
		yes ($n = 282$)	no ($n = 2,544$)	
Female gender	1,339 (47.4)	127 (45.0)	1,212 (47.6)	0.41
Age, years	78±8	79±8	78±9	<0.05
Body mass index	28.2±5.0	28.7±5.3	28.1±5.0	0.051
Atrial fibrillation	517 (18.3)	155 (55.0)	362 (14.2)	<0.001
Coronary artery disease	589 (20.8)	155 (55.0)	434 (17.1)	<0.001
Hypertension	2,076 (73.5)	229 (81.2)	1,847 (72.6)	<0.01
Diabetes	625 (22.1)	89 (31.6)	536 (21.1)	<0.001
NT-proBNP, pg/mL	222 (112–519)	403 (190–1058)	212 (107–471)	<0.001
NT-proBNP <125 pg/mL	817 (28.9)	41 (14.5)	776 (30.5)	<0.001
IL-6, pg/mL	2.2 (1.4–3.7)	2.7 (1.8–4.7)	2.2 (1.4–3.6)	<0.001
hsCRP, mg/dL	2.3 (1.1–4.8)	2.5 (1.2–5.1)	2.3 (1.1–4.7)	0.14
HOMA-IR	2.68 (1.68–4.31)	3.04 (1.84–4.80)	2.64 (1.67–4.25)	0.06
RBP4, ng/mL	41.1 (29.5–57.6)	40.2 (28.4–55.0)	41.2 (29.5–57.7)	0.17
eGFR, mL/min/1.73 m ²	75.8±20.3	69.7±21.0	76.4±20.2	<0.001
Treatment				
β-Blockers	997 (35.3)	164 (58.6)	833 (33.1)	<0.001
ACE-I or ARB	1,269 (44.9)	184 (65.3)	1,085 (42.7)	<0.001
Diuretics	547 (30.0)	124 (44.0)	423 (28.4)	<0.001
Spironolactone	236 (8.3)	57 (20.4)	179 (7.1)	<0.001

Data are presented as n (%), mean ± SD, or median (lower quartile–upper quartile). NT-proBNP, N-terminal pro-B type natriuretic peptide; IL-6, interleukin 6; hsCRP, high-sensitivity C-reactive protein; HOMA-IR, homeostatic model assessment; RBP4, retinol-binding protein 4; eGFR, estimated glomerular filtration rate; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

To assess factors affecting RBP4 serum level, the multivariate stepwise backward skew-t regression model was used with independent factors such as: female gender, age, body mass index; occurrence of: AF, CAD, HT, DM; assessment of IR and renal function according to the homeostatic model (HOMA-IR); serum levels of: NT-proBNP, IL-6, hs-CRP; treatment with: β-blockers, ACE-I, ARB, diuretics, spironolactone; and eGFR values. Plasma levels of RBP4 were diminished in the female gender, age, AF, eGFR values, and serum levels of IL-6 and increased by HT and diuretic treatment (Table 3).

NT-proBNP Concentration

The values fluctuated in a wide interquartile range from 112 to 519 pg/mL with a median of 222 pg/mL (Table 1), and higher values were found in participants previously hospitalized for HF than in nonhospitalized participants (403 [190–1058] vs. 212 [107–471] pg/mL; $p < 0.001$). Also higher NT-proBNP levels were observed in AF patients (343 [135–1,014] and 503 [225–1,166] pg/mL; $p < 0.05$) in the nonhospitalized and previously hospitalized due to HF subgroups.

To assess factors affecting NT-proBNP serum levels, the multivariate stepwise backward skew-t regression models (including alternatively DM and HOMA-IR) were used with plasma RBP4 levels and independent factors as used and stated previously. Serum levels of NT-proBNP were diminished by female gender, HOMA-IR (but not DM), and eGFR values and increased by age, AF, CAD, serum levels of IL-6, and hsCRP (in the model with HOMA-IR only), as well as β-blockers and spironolactone treatment (Table 2).

Table 2. Comparison of plasma RBP4 quartile subgroups

	RBP4 quartiles				p
	Q1	Q2	Q3	Q4	
RBP4, ng/mL	23.9 (20.6–26.9)	35.2 (32.0–38.1)	47.9 (44.4–52.3)	75.0 (64.5–94.4)	–
Female gender	337 (47.6)	338 (47.8)	337 (47.8)	327 (46.3)	0.93
Age, years	78.8±8.5	78.1±8.5	77.7±8.3	78.0±8.5	0.06
Body mass index	27.9±5.2	28.1±4.7	28.2±4.9	28.5±5.4	0.14
Atrial fibrillation	138 (19.5)	125 (17.7)	132 (18.7)	122 (17.3)	0.70
Coronary artery disease	144 (20.3)	134 (18.9)	156 (22.1)	155 (21.9)	0.41
Hypertension	490 (69.2)	516 (73.0)	514 (72.9)	556 (78.7) [#]	<0.001
Diabetes	136 (19.2)	166 (23.5)	165 (23.4)	158 (22.4)	0.17
History of hospitalization					
for heart failure	77 (10.9)	72 (10.2)	68 (9.7)	65 (9.2)	0.77
NT-proBNP, pg/mL	231 (117–547)	219 (109–535)	204 (106–448)	242 (114–549)	0.31
IL-6, pg/mL	2.4 (1.6–4.2)	2.3 (1.6–3.6)	2.1 (1.4–3.4)**	2.2 (1.3–3.6)*	<0.01
hsCRP, mg/dL	2.3 (1.1–5.0)	2.3 (1.0–5.0)	2.1 (1.0–4.2)*	2.5 (1.2–5.0)	<0.05
HOMA-IR	2.56 (1.61–4.15)	2.58 (1.59–4.25)	2.84 (1.75–4.42)**	2.75 (1.74–4.43)	<0.05
eGFR, ml/min/1.73 m ²	81.2±19.8	76.3±18.6 [#]	74.5±20.1 [#]	71.1±21.5 [#]	<0.001
Treatment					
β-Blockers	231 (32.6)	232 (32.8)	243 (34.5)	291 (41.2)**	<0.01
ACE-I or ARB	303 (42.8)	301 (42.6)	307 (43.6)	360 (51.0)**	<0.01
Diuretics	165 (23.3)	188 (26.6)	215 (30.5)**	279 (39.5) [#]	<0.001
Spironolactone	53 (7.5)	58 (8.2)	53 (7.5)	72 (10.2)	0.22

Data are presented as *n* (%), mean ± SD, or median (lower quartile–upper quartile). * *p* < 0.05, ** *p* < 0.01, [#] *p* < 0.001 vs. first group. RBP4, retinol-binding protein 4; NT-proBNP, N-terminal pro-B type natriuretic peptide; IL-6, interleukin-6; hsCRP, high-sensitivity C-reactive protein; HOMA-IR, homeostatic model assessment; eGFR, estimated glomerular filtration rate; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

Association between NT-proBNP, RBP4, eGFR, and Other Factors

In our study subjects, both NT-proBNP and RBP4 levels were inversely related to eGFR values (Fig. 2, 3). Another factor negatively affecting both examined marker levels was female gender (Table 3).

Regression analysis between log₁₀(NT-proBNP) and log₁₀(RBP4) did not reveal a relationship between these two factors (*r* = −0.001; *p* = 0.98) (Fig. 4).

Discussion

Little is known about the role of RBP4 in the development of left ventricle dysfunction (LVD) and other cardiovascular diseases. Experimental studies suggest a cardiac prohypertrophic effect of RBP4 [12, 13], which may lead to HF. In addition, an increased serum RBP4 was shown in patients with HF before implantation of a left ventricular assist device (LVAD) [8], but not in patients with DCM and low levels of inflammatory markers [6]. These data suggest that RBP4 is a potential biomarker of LVD. However, our data failed to prove any differences in RBP4 between subjects with NT-proBNP <125 ng/mL (assuming the lack of HF) and ≥125 ng/mL, as well as those with and without a history of hospitalization for HF. Moreover, there was no relation between concentrations of RBP4 and NT-proBNP, the most sensitive biomarker of LVD [25]. Nonetheless, we observed a weak negative effect of AF on RBP4 levels in multivariate regression analysis only.

Table 3. Results of the multivariate stepwise backward skew-t regressions for factors affecting plasma RBP4 and serum levels of NT-proBNP

	$\log_{10}(\text{RBP4}) \times 100$					$\log_{10}(\text{NT-proBNP}) \times 100$ (with HOMA-IR)					$\log_{10}(\text{NT-proBNP}) \times 100$ (with diabetes)				
	β	SE (β)	$\pm 95\%$ CI	z	p	β	SE (β)	$\pm 95\%$ CI	z	p	β	SE (β)	$\pm 95\%$ CI	z	p
Female	-2.26	0.80	-3.84 to -0.69	-2.82	<0.01	-4.35	1.54	-7.37 to -1.33	-2.82	<0.01	-4.89	1.55	-7.94 to -1.84	-3.15	<0.01
Age (years)	-0.23	0.05	-0.33 to -0.12	-4.42	<0.001	2.12	0.1	1.92 to 2.31	21.41	<0.001	2.23	0.10	2.04 to 2.42	22.81	<0.001
Hypertension	2.33	0.92	0.52 to 4.13	2.53	<0.05	-	-	-	-	-	-	-	-	-	-
Atrial fibrillation	-2.8	1.04	-4.84 to -0.76	-2.69	<0.01	14.43	2.15	10.21 to 18.65	6.71	<0.001	14.88	2.16	10.64 to 19.11	6.88	<0.001
Coronary artery disease	-	-	-	-	-	9.38	2.03	5.40 to 13.36	4.62	<0.001	8.22	2.05	4.21 to 12.23	4.01	<0.001
$\log_{10}\text{IL-6}$	-5.66	1.32	-8.25 to -3.07	-4.28	<0.001	21.8	2.86	16.19 to 27.40	7.62	<0.001	23.95	2.54	18.97 to 28.92	9.44	<0.001
eGFR (ml/min/1.73 m ²)	-0.22	0.02	-0.26 to -0.18	-10.20	<0.001	-0.35	0.04	-0.43 to -0.27	-8.37	<0.001	-0.32	0.04	-0.40 to -0.24	-7.70	<0.001
$\log_{10}\text{hsCRP}$	-	-	-	-	-	4.46	1.83	0.87 to 8.04	2.44	<0.05	-	-	-	-	-
$\log_{10}\text{HOMA-IR}$	-	-	-	-	-	-16.5	2.26	-20.92 to -12.08	-7.31	<0.001	-	-	-	-	-
Diabetes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diuretics	3.97	0.92	2.16 to 5.77	4.31	<0.001	-	-	-	-	-	-	-	-	-	-
β -Blockers	-	-	-	-	-	16.91	1.71	13.56 to 20.26	9.9	<0.001	15.83	1.72	12.45 to 19.20	9.20	<0.001
Spirololactone	-	-	-	-	-	8.43	2.94	2.68 to 14.19	2.87	<0.01	7.62	2.96	1.81 to 13.42	2.57	<0.05
Intercept	185.5	5.1	175.4 to 195.6	36.1	<0.001	65.61	10.14	45.73 to 85.48	6.47	<0.001	48.61	9.88	29.23 to 67.98	4.92	<0.001

β , slope of linear regression model; SE(β), standard error of β coefficient; RBP4, retinol-binding protein 4; NT-proBNP, N-terminal pro-B type natriuretic peptide; IL-6, interleukin-6; hsCRP, high-sensitivity C-reactive protein; HOMA-IR, homeostatic model assessment; eGFR, estimated glomerular filtration rate.

Kidney function appears to be the strongest confounder in RBP4 studies. Hemodynamic-related improvement of renal function after LVAD implantation, in the already mentioned cohort of patients with advanced HF, was followed by normalization of initially increased serum RBP4 levels [8]. The authors have also observed an improvement in glucose tolerance, cytokine signalling, and liver function in this cohort, which may somehow explain the decrease in serum RBP4 levels after LVAD implantation. Zhang et al. [26] showed a rapid decrease in RBP4 concentration only 1 day after kidney transplantation. The dominant role of the kidneys in RBP4 catabolism and elimination from circulation is consistent with our findings where renal function measurement by eGFR was a strong factor affecting plasma RBP4 concentrations. Therefore, we speculate that RBP4 is not a biomarker of LVD but that this adipokine may simply accumulate in the circulation in advanced HF, as a consequence of kidney function deterioration clinically manifested by cardiorenal syndrome. This hypothesis is supported by studies reporting elevated plasma RBP4 levels in CKD [27–29], and its normalization after kidney transplantation [30, 31], as well as the results of the multivariate regression analysis in the presented study. Further evidence can be seen with normal RBP4 levels in patients with

Fig. 2. Linear regression model with 95% confidence interval (dashed lines) and 95% confidence ellipse between $\log_{10}(\text{RBP4})$ and eGFR.

Fig. 3. Quadratic regression model with 95% confidence interval (dashed lines) between $\log_{10}(\text{NT-proBNP})$ and eGFR.

Fig. 4. Linear regression with 95% confidence interval (dashed lines) between $\log_{10}(\text{NT-proBNP})$ and $\log_{10}(\text{RBP4})$.

DCM (ejection fraction: $36 \pm 14\%$) but normal eGFR (93.4 ± 15.7 mL/min) and a low level of inflammatory markers [6].

There are hypotheses explaining the RBP4 elevation in CKD. A study performed in normal-weight, nondiabetic CKD subjects proved that RBP4 plasma elevation could be an effect of passive accumulation of transthyretin (TTR) – the protein that carries RBP4 bound to retinol in circulation, and prevents its filtration in the kidneys [30]. It has been shown that fenretinide (a synthetic retinoid that disrupts binding of RBP4 to TTR) enhances renal excretion and leads to decreased RBP4 concentration levels in mice [31]. Jing et al. [32] suggested that altered hepatic synthesis and secretion of RBP4 and retinol or disturbed retinoid homeostasis were caused by uremic toxins.

Another potential confounder affecting the association between RBP4 and NT-proBNP is the high prevalence (44.9%) of ACE-I and ARB usage in our study group. This supposition is supported by the results obtained by Gao et al. [7] showing stimulation of adipose tissue *rbp4* expression by angiotensin II. However, we failed to find a significant effect of ACE-I/ARB uses in multivariate analysis, and showed the highest prevalence of ACE-I and ARB use in the quartile with the highest plasma RBP4 levels.

Furthermore, bearing in mind the study of Bobbert et al. [6] in patients with dilated inflammatory cardiomyopathy, we included markers of systemic inflammation (hsCRP, IL-6) as cofounders modulating the association between RBP4 and NT-proBNP. By implementing the sophisticated multivariate stepwise backward skew-t regression model, we found the opposite association between IL-6 and RBP4 and between IL-6 and NT-proBNP. Nevertheless, there was an inverse relation between IL-6 and RBP4, inconsistent with the previous study

[33], but supported by experimental studies showing *rbp4* downregulation in human adipocytes by IL-1 β [34] and in macrophages by TNF α [35]. Moreover, Bobbert et al. [6] showed an upregulation of *rbp4* mRNA expression in adipocytes after incubation with IL-8; however, that cytokine was not measured in our study. These conflicting results necessitate further studies.

The impact of RBP4 on the development of IR is multifaceted. Reduction of membrane density of glucose transporter type 4 causes overexpression of *rbp4* in adipocytes. Increased RBP4 levels suppress insulin action on metabolic pathways in the liver, muscles, and adipocytes by the impaired tyrosine phosphorylation in substrate 1 of the insulin receptor [31]. In the liver, RBP4 stimulates hepatic gluconeogenesis by activation of phosphoenolpyruvate carboxykinase [31] and inhibition of phosphatidylinositol 3-kinase [36].

It is worth emphasizing that HOMA-IR values in our subjects were elevated. However, we could not find a correlation between plasma concentration of RBP4 and log₁₀HOMA-IR values, as well as the occurrence of DM. In a population-based ARIC study, a weak association between circulating RBP4 levels and HOMA-IR was found only among women [37]. However, the ARIC population was middle aged (45–64 years old). There are also findings of Cho et al. [38], who reported increased levels of RBP4 in subjects with impaired glucose tolerance and DM in a middle-aged population, though the difference was quite small and no measure of dispersion, except range, was provided. The inconsistency with our data may be explained by the difficult role of glucose uptake by adipocytes in the regulation of *rbp4* expression [39] related to changes in adipocyte proteome with ageing, recently demonstrated in an animal model [40].

The impact of age on RBP4 levels requires a separate discussion. Gavi et al. [41] showed, that in contrast to younger subjects, in subjects >60 years old, changes in insulin sensitivity and percentage of trunk fat did not explain RBP4 level variability. Our results have also shown that changes in HOMA-IR as well as eGFR values did not explain the observed decrease in circulating RBP4 levels with age. It has been suggested that the factor responsible for the decrease in RBP4 levels with age in women is lower 17 β -estradiol levels and changes of gonadotropin secretion [42, 43]. However, this does not explain the changes of RBP4 in men. It cannot be excluded that changes in the body composition, especially sarcopenia play an important role in the decrease in RBP4 levels. Thus, further studies are necessary to explain the impact of age on RBP4 levels.

Limitations

This study has some limitations, mostly related to the lack of a subgroup with severe HF, and missing validation of our data by a Western blot analysis, as recommended [44]. Secondly, we did not evaluate left ventricle performance echocardiographically, which is difficult to apply in population-based studies performed in participants' place of living. We used history of hospitalization for HF as a measure of HF, and NT-proBNP level for HF exclusion.

Conclusion

Our results show that RBP4 is affected by GFR but cannot be considered as an independent biomarker of heart muscle dysfunction.

Disclosure Statement

The authors declare that they have no conflicts of interest.

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