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Relationship of N-Terminal fragment of Pro-B-Type Natriuretic Peptide and copeptin with erythrocytes-related parameters: A population-based study

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ABSTRACT

Background: Plasma levels of natriuretic peptides (NP) have been inversely related to hemoglobin (Hb) concentration in prior studies. However, the mechanism underlying this association remains unclear. We aimed to obtain further insights into potential mechanisms for this correlation in a cohort of healthy adults.

Methods: A population-based study was performed among 2113 healthy adults aged 25–41 years. Relationships of N-Terminal fragment of Pro-B-Type Natriuretic Peptide (NT-proBNP) or copeptin with volume-dependent (Hb, hematocrit (Hct), erythrocyte count (EC), mean corpuscular Hb concentration (MCHC)) and volume-independent (mean corpuscular volume (MCV), mean corpuscular Hb (MCH)) erythrocyte-related parameters were assessed using sex-specific multivariable linear regression analyses.

Results: The median age was 36.7 years. Median NT-proBNP (ng/L) levels were 49.5 and 20 among women and men, respectively ($p < 0.0001$). Mean (standard deviation) Hb (g/L) levels were 130.1(9.1) and 149.7(8.6) among women and men, respectively ($p < 0.0001$). Among men, multivariable adjusted β-coefficients (95% confidence interval) for NT-proBNP were −1.68 (−2.36; −1.01), $p < 0.0001$ for Hb; −0.38 (−0.57; −0.20), $p < 0.0001$ for Hct; −0.06 (−0.09; −0.04), $p < 0.0001$ for EC; −0.78 (−1.50; −0.07), $p = 0.03$ for MCHC; 0.26 (−0.04; 0.56), $p = 0.09$ for MCV; and 0.03 (−0.08; 0.14), $p = 0.61$ for MCH. For copeptin, these relationships were 1.36 (0.39; 2.32), $p = 0.006$; 0.41 (0.15; 0.68), $p = 0.002$; 0.06 (0.02; 0.09), $p = 0.002$; −0.17 (−1.19; 0.86), $p = 0.75$; −0.12 (−0.55; 0.31), $p = 0.58$ and −0.05 (−0.21; 0.10), $p = 0.52$. Similar results were observed among women.

Conclusions: We found significant relationships of NT-proBNP and copeptin with volume-dependent but not volume-independent erythrocyte-related parameters, suggesting that hemodilution may at least in part explain these associations.

1. Introduction

Plasma levels of natriuretic peptides (NP) have been established as reliable diagnostic and prognostic markers in heart failure (HF) patients and their plasma levels directly correlate with the degree of ventricular dysfunction [1–3]. NP are synthesized by atrial and ventricular cardiomyocytes, primarily as a response to increased myocardial wall stress. Accordingly, systemic effects of NP include the regulation of intravascular volume by promoting sodium and water excretion as well as systemic vasodilation [4–7].

Prior studies have suggested an inverse relationship of NP with hemoglobin (Hb) levels [8–10]. However, the mechanisms underlying these relationships are currently unknown. As prior studies were conducted in middle-aged to elderly individuals, confounding by comorbidity could be a partial explanation. In addition, given the physiologic properties of natriuretic peptides, hemodilution may also play an

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Table 1
Sex-specific baseline characteristics of the study population.

	Women (n = 1136)	Men (n = 977)
Age (years)	36.7 (31–40)	36.7 (31–40)
NT-proBNP (ng/L)	49.5 (33–80)	20 (11–32)
Copeptin (pmol/L)	2.3 (1.6–3.6)	4.0 (2.7–5.8)
Education*		
Elementary	103 (9.1)	60 (6.1)
Secondary	677 (59.6)	511 (52.3)
Superior	335 (29.5)	378 (38.7)
Alcohol intake (gr/d)	0 (0–0.8)	1.4 (0–3)
Smoking status		
Current	215 (18.9)	249 (25.5)
Past	257 (22.6)	232 (23.8)
Never	662 (58.3)	495 (50.7)
Physical activity (min/week)	120 (40–270)	190 (60–450)
BMI (kg/cm^2)	23.3 ± 3.7	25.8 ± 3.1
Body composition (%)		
Fat mass	28.8 ± 4.8	20.6 ± 5.3
Muscle mass	33.3 ± 3.6	37.6 ± 3.4
Body water	52.1 ± 4.9	57.3 ± 4.5
Water intake (L/d)	1.5 (1.5–2.0)	2.0 (1.5–2.5)
Ferritin (μg/L)	46 (28–79)	177 (113–262)
hs-CRP (mg/L)	0.9 (0.5–2.0)	0.9 (0.5–1.8)
Systolic BP (mmHg)	113.3 ± 10.1	127.3 ± 10.9

Data are mean ± standard deviation, median (interquartile range), or number (percentage), as appropriate. BMI indicates body mass index; BP, blood pressure; hs-CRP, high-sensitive C-reactive protein.
* Educational level of 49 participants was not available.

important role, as Hb is a volume-dependent parameter. Interestingly, Anand et al. previously showed that anemic HF patients had a higher prevalence of volume overload related HF symptoms and signs compared to HF patients without anemia [11], a finding that would be in line with the abovementioned hypothesis. Finally, if the hemodilution hypothesis was correct, then positive associations should be observed between Hb or hematocrit (Hct) and markers related to vasopressin metabolism, such as copeptin [12,13].

In order to test the hypothesis that hemodilution may at least in part explain the inverse relationship between NP and Hb, we assessed the association of plasma levels of N-Terminal fragment of Pro-B-Type Natriuretic Peptide (NT-proBNP) and copeptin with several volume-dependent and volume-independent erythrocyte-related parameters in a large population-based sample of young and healthy individuals.

2. Materials and methods

The 'genetic and phenotypic determinants of blood pressure and other cardiovascular risk factors' (GAPP) study is an ongoing prospective population-based cohort study. Our detailed study methods were published earlier [14]. Between 2010 and 2013, all inhabitants of the Principality of Liechtenstein aged between 25 and 41 years were invited to participate in GAPP and 2170 were successfully enrolled in the study. Our main exclusion criteria were prevalent cardiovascular disease, chronic kidney disease, a body mass index (BMI) >35 kg/m^2, medically treated diabetes mellitus, or any other severe illness. In the current analyses, individuals with treated hypertension (n = 35) were excluded. A total of 2113 participants (98.3%) who had valid determinations of Hb, NT-proBNP, and copeptin were finally included for our analysis. The local ethics committee approved our study protocol and all participants provided written informed consent.

Table 2
Sex-specific erythrocytes-related baseline characteristics of the study population.

	Women (n = 1136)	Men (n = 977)
Hemoglobin (g/L)	130 ± 9.1	149.7 ± 8.6
Erythrocyte count (10^{12}/L)	4.5 ± 0.3	5.1 ± 0.31
Hematocrit (%)	38.7 ± 2.5	43.6 ± 2.3
MCHC (g/L)	336.1 ± 8.8	343.4 ± 8.8
MCV (fL)	87.1 ± 4.3	86.2 ± 3.7
MCH (10^{-12}/g)	29.3 ± 1.7	29.6 ± 1.4
Anemia [number; (%)]	93 (8.2)	15 (1.5)

Data are mean ± standard deviation or number (percentage), as appropriate. MCH indicates mean corpuscular hemoglobin; MCV, mean corpuscular volume; and MCHC, mean corpuscular hemoglobin concentration.

2.1. Assessment of study variables

Baseline examination included standardized assessment of personal, medical, lifestyle, and nutritional factors by questionnaires. The individual physical activity questionnaire (IPAQ) was used for the evaluation of the subjects' physical activity [14]. Weight and height were measured in a standardized manner and BMI was calculated by dividing weight in kilograms by height in meters squared [14]. Smoking status of the participants was self-reported and classified as current, former, or never smoking. Conventional office blood pressure (BP) was obtained in triplicate in a quiet environment after at least 5 min rest in a sitting position [14]. Bioelectrical impedance analysis was used to assess fat and muscle mass as well as body water content of all participants using standardized methodology and a validated device (BIA ego fit, 2010, Germany) [14].

2.2. Blood and urinary samples

A fasting venous blood sample was obtained from every subject by a trained study nurse using a minimally traumatic venipuncture. All samples were immediately centrifuged. Plasma levels of NT-proBNP, iron, ferritin, sodium, creatinine, and high-sensitive C-reactive protein (hs-CRP) were analyzed on a Roche Cobas 6000 analyzer (F. Hoffmann – La Roche, Switzerland) using fresh blood samples [14]. Hb and complete blood count were obtained using the sodium–lauryl–sulfate Hb method (Sysmex XE 5000) [14]. Plasma copeptin was directly assayed using an immunoluminometric test (BRAHMS GmbH, Germany) [14]. Hct (%) was assessed using the erythrocyte pulse height detection method of whole erythrocyte count (EC) volume in whole blood. The following indices were calculated according to the following formulas: Mean corpuscular volume (MCV): 10 × Hct (%)/EC (10^{12}/L); mean corpuscular Hb (MCH): 10 × Hb (g/L)/EC (10^{12}/L); mean corpuscular Hb concentration (MCHC) (g/L): 100 × Hb (g/L)/HC (%). Females and males with Hb levels below 120 and 130 g/l, respectively, where considered to have anemia.

Plasma copeptin levels were measured using a Brahms Luminometry analyzer using fresh blood samples. For the estimation of the glomerular filtration rate (eGFR), we used the creatinine-based chronic kidney disease epidemiology collaboration (CKD-EPI) formula [15]. Urinary sodium was measured from fresh and fasting spot urine samples using a Roche Cobas 6000 analyzer (F. Hoffmann – La Roche, Switzerland). Sodium urinary excretion was estimated using the previously validated formula by Kawasaki et al. [16]. The classification of the erythrocytes-related parameters on volume-dependent or volume-independent variables was done according to the units of measurement in which they were expressed [17], considering as volume-dependent those that had a volume unit on its denominator (the variable is then relative to a given volume).

2.3. Statistical analysis

Baseline characteristics were stratified according to sex. Distribution of continuous variables was assessed using skewness, kurtosis, and visual inspection of the histogram. Baseline characteristics of continuous variables were presented as means (standard deviation) or medians (interquartile range) and compared using analysis of variance or Kruskal Wallis tests, as appropriate. Categorical variables were presented as counts (percentages) and compared using chi-square tests.