

Prohepcidin, B-Type Natriuretic Peptide, and Iron Status in a Cohort of Elderly Women from the Rhine-Ruhr Area

Jana Henry^a Swaantje Casjens^a Tamara Schikowski^d Axel Stachon^b
Alfried Germing^c Ulrich Ranft^d Martin Lehnert^a Volker Harth^a
Ursula Krämer^d Thomas Brüning^a Beate Pesch^a

^aInstitute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA),

^bInstitute of Clinical Chemistry, Transfusion, and Laboratory Medicine, and ^cMedical Clinic II, Cardiology and Angiology, BG-University Hospital Bergmannsheil, Ruhr University Bochum, Bochum, and ^dEnvironmental Health Research Institute (IUF), Düsseldorf, Germany

The elderly constitute a fast growing segment of populations worldwide. Both anemia and iron overload have emerged as risk factors that are associated with a variety of adverse outcomes in older adults [1–5]. Standard laboratory parameters like serum ferritin (SF), transferrin, transferrin saturation (Tsat), serum iron (SI), Hb, MCV, and HCT are commonly used for the assessment of iron status, but rapid progress has been made in understanding the regulation of iron metabolism by the hepatic peptide hormone hepcidin [6]. Hepcidin is synthesized in response to elevated plasma iron stores, thus ensuring homeostasis. Hepcidin synthesis is also increased by infection and inflammation. Increased hepcidin concentrations are of major importance in anemia of inflammation and iron-resistant iron deficiency anemia (IDA). On the other hand hepcidin is decreased in diseases that are attended by iron overload like most hereditary hemochromatoses or iron-loading anemias. A commercial assay for the measurement of serum hepcidin was not available at the time of the SALIA (Study on the influence of

Air pollution on Lung function, Inflammation and Aging) cohort investigation. Therefore, prohepcidin, the 84-amino acid precursor of hepcidin, was determined by a commercial assay to be a potential surrogate marker for hepcidin which can be used in routine laboratory examination. Some studies have investigated the association between prohepcidin and parameters of iron status, with conflicting results [7–17]. According to our knowledge, this parameter has not yet been determined in a population of elderly women.

Among different diseases associated with iron deficiency (ID) and anemia, anemia is common in patients with heart failure [18–20]. B-type natriuretic peptide (BNP) is a reliable marker of left ventricular dysfunction. Elevated BNP concentrations are associated with a poor outcome in patients with heart failure and coronary artery disease. Studies have shown that BNP concentrations in cardiac patients [21–23] and in the general population are inversely and independently related to Hb concentrations [24]. This suggests that elevated BNP concentra-

tions in anemic patients with chronic heart diseases are partially caused by anemia. If prohepcidin is a reliable biomarker for iron status, especially for anemia, an association of BNP with prohepcidin concentrations should be expected.

The SALIA cohort was part of a comprehensive environmental health survey on the effect of air pollution on women's health in the Rhine-Ruhr area [25–29]. Here we report on parameters of iron status in 319 women aged 69–79 years measured at follow-up in 2007/2008, including prohepcidin, and their association with BNP. All participants gave their written informed consent. The study was approved by the ethics commission of Ruhr University Bochum and was conducted in accordance with the definitions of the declaration of Helsinki.

WBC, RBC, MCV, Hb, HCT, SF, SI, transferrin, BNP, C-reactive protein, alanine aminotransferase, aspartate aminotransferase, cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides were analyzed with standard laboratory methods. Serum prohepcidin concentrations were analyzed in 1 batch using an ELISA kit (EIA-4644; DRG Instruments, Ltd., Marburg, Germany). The normal range of prohepcidin was given by the manufacturer (58.9–158.1 ng/ml).

Anemia was defined as Hb <12 g/dl and HCT <36% for women according to the WHO. ID was defined as an

abnormal value from 2 or 3 laboratory tests of iron status (SF, Tsat, and MCV) [30], and IDA was defined as ID plus Hb <12 g/dl. The definition of high iron stores is inconsistent throughout the literature, but it is well accepted that SF concentrations >300 µg/l for men and >200 µg/l for women are suitable cutoff criteria for high iron stores [30, 31]. We used both thresholds for our calculations because several studies suggest 200 µg/l as the cutoff for SF in elderly women [32–34].

Figure 1 shows the distribution of prohepcidin in this cohort of elderly women [median 68.2 ng/ml, interquartile range (IQR) 57.0–80.0]. The concentrations are, on average, lower than the measurements in 40 younger women provided by the manufacturer (median 85.6 ng/ml, IQR 72.9–91.5, $p < 0.0001$). This is in line with a few findings from smaller studies which suggest that prohepcidin concentrations are age- and gender-dependent [12, 13, 35–37].

The median concentrations of the standard measurements of iron status and other conditions fell within the reference range, except for cholesterol (data not shown). The prevalence rates of impaired iron status were low in this group of nonhospitalized women (table 1). On the basis of low Hb values (<12 g/dl according to the WHO), 10 (3.1%) women were classified as anemic with no further specification and 5 women (1.6%) were classified as having ID. IDA was observed in 2 women. Although lim-

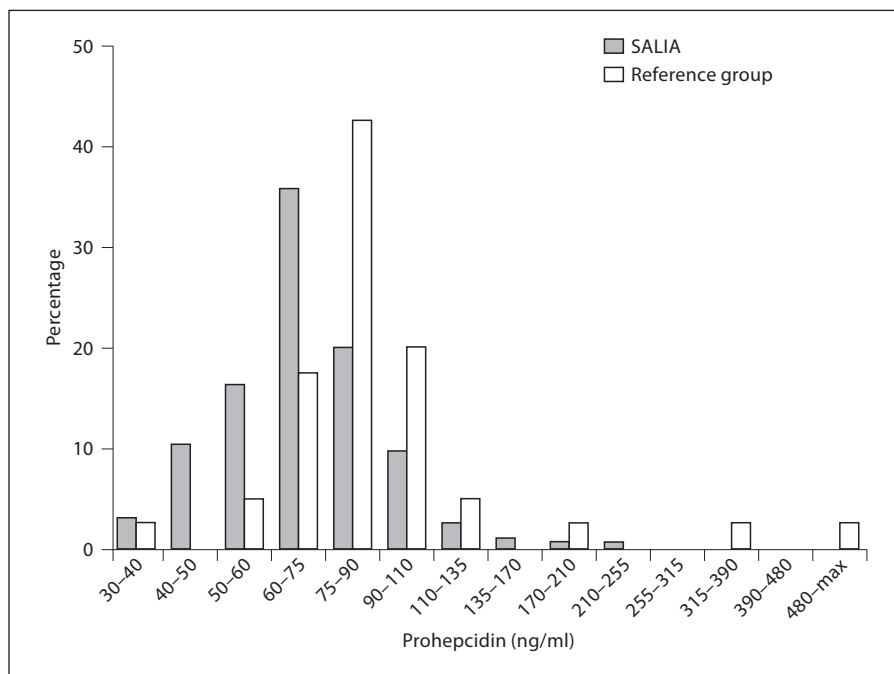


Fig. 1. Distribution of prohepcidin concentrations in 319 elderly women from the SALIA cohort and in a group of 40 women (data provided by DRG Instruments).

ited by a small number of cases, we found some evidence that ID and IDA were more common in the subgroup of women of a higher age (>73 years). The low prevalence of ID and IDA in this study is in line with other investigations in healthy white women [30, 38].

Table 1. Iron status in elderly women from the SALIA cohort

	Cutoff	All women (n = 319)	Women aged <73 years (n = 179)	Women aged ≥73 years (n = 140)
<i>Laboratory variable</i>				
SF, µg/l	<15	8 (2.5)	5 (2.8)	3 (2.1)
SI, µg/dl	<60	30 (9.4)	13 (7.3)	17 (12.1)
Transferrin, mg/dl	<200	15 (4.7)	7 (3.9)	8 (5.7)
Tsat, %	<16	30 (9.4)	15 (8.4)	15 (10.7)
Hb, g/dl	<12	10 (3.1)	2 (1.1)	8 (5.7)
HCT, %	<36	23 (7.2)	10 (5.6)	13 (9.3)
MCV, fl	<80	2 (0.6)	1 (0.6)	1 (0.7)
<i>Iron status</i>				
ID		5 (1.6)	2 (1.1)	3 (2.1)
IDA		2 (0.6)	0 (0.0)	2 (1.4)
<i>High iron stores</i>				
SF, µg/l	>200	37 (11.6)	23 (12.8)	14 (10.0)
SF, µg/l	>300	11 (3.4)	9 (5.0)	2 (1.4)

Figures in parentheses are percentages.

We observed high iron stores more frequently in women below 73 years of age, independently of the 2 thresholds. Using the more commonly applied gender-specific cutoff for SF (>200 µg/l), 11.6% of the SALIA women showed elevated iron stores which corresponds with the observations of 12.2% in the Framingham Heart Study cohort [30] and of 16.6% in healthy elderly Parisian women [39]. Using the higher cutoff (>300 µg/l) the prevalence was 3.4% in the SALIA cohort, 6% in the Framingham cohort, and 3.7% in elderly Danish women [38].

Table 2 describes selected associations between iron status measures and other laboratory variables using Spearman's correlation coefficients (r_s). No associations were found between prohepcidin and parameters of iron status and BNP, while BNP correlated inversely with standard parameters of iron status (Hb, HCT, SF, and SI). There was no correlation of BNP with risk factors for coronary artery disease like elevated triglycerides, cholesterol, and low-density lipoprotein cholesterol or lowered high-density lipoprotein cholesterol concentrations.

Data on the role of prohepcidin with regard to iron status are inconsistent. Some studies found an association between prohepcidin and parameters of iron status [7–11], while other studies did not [12–17]. The majority of these studies were limited by small numbers. According to our knowledge, SALIA is one of the largest study populations with prohepcidin analysis. We observed no association between prohepcidin and any marker of iron

Table 2. Spearman's correlation coefficients with 95% confidence limits between iron status measures and other laboratory variables in elderly women from the SALIA cohort

Marker	Prohepcidin	SF	SI	Transferrin	Tsat	Hb	HCT	MCV
Prohepcidin	1.00							
SF	0.06 (-0.05, 0.17)	1.00						
SI	-0.02 (-0.13, 0.09)	0.24* (0.13, 0.34)	1.00					
Transferrin	0.07 (-0.04, 0.18)	-0.33* (-0.43, -0.23)	0.08 (-0.03, 0.19)	1.00				
Tsat	-0.07 (-0.17, 0.05)	0.36* (0.26, 0.45)	0.85* (0.81, 0.88)	-0.40* (-0.49, -0.30)	1.00			
Hb	0.10 (-0.01, 0.21)	0.13 (0.02, 0.24)	0.40* (0.30, 0.49)	0.18* (0.08, 0.29)	0.25* (0.15, 0.35)	1.00		
HCT	0.09 (-0.02, 0.20)	0.09 (-0.02, 0.20)	0.35* (0.25, 0.44)	0.20* (0.09, 0.30)	0.20* (0.09, 0.30)	0.97* (0.96, 0.98)	1.00	
MCV	-0.08 (-0.19, 0.03)	0.31* (0.21, 0.40)	0.36* (0.26, 0.45)	-0.05 (-0.16, 0.06)	0.35* (0.24, 0.44)	0.07 (-0.04, 0.18)	0.03 (-0.08, 0.14)	1.00
BNP	-0.05 (-0.16, 0.06)	-0.13 (-0.24, -0.02)	-0.11 (-0.22, -0.0005)	0.01 (-0.10, 0.12)	-0.10 (-0.21, 0.01)	-0.19* (-0.30, -0.08)	-0.17 (-0.27, -0.06)	-0.08 (-0.19, 0.03)

* $p < 0.001$.

status in nonhospitalized elderly women. The assumed association of prohepcidin as a reliable marker for iron status with BNP could not be confirmed in our study. This supports the conclusion that prohepcidin is likely not a suitable surrogate marker of the active form of the peptide hepcidin in healthy populations. Recent studies report that hepcidin concentrations in serum are much lower than prohepcidin concentrations are [40, 41]; thus, fluctuations in hepcidin levels might be associated with minor changes in prohepcidin concentrations. It is important to note that the ELISA assay might not be sufficiently specific for prohepcidin. It has been suggested that the antibody also recognizes the proregion of hepcidin [42]. Currently, a commercial immunoassay for human serum hepcidin is being developed [43] and will be investigated using stored SALIA samples.

In conclusion, a low prevalence of ID and anemia was observed in the SALIA cohort of healthy elderly women. Prohepcidin was not associated with the standard parameters of iron metabolism and BNP. We confirmed an expected negative correlation between iron status measures and BNP.

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