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Plasma midregional proadrenomedullin (MR-proADM) concentrations and their biological determinants in a reference population

https://doi.org/10.1515/cclm-2017-1044
Received November 10, 2017; accepted January 18, 2018; previously published online February 13, 2018

Abstract

Background: Midregional proadrenomedullin (MR-proADM) is emerging as a prognostic biomarker for detecting the failure of multiple organs. Establishment of scientifically robust reference intervals facilitates interpretation of laboratory test results. The objectives of this study were (i) to establish reliable reference intervals for plasma MR-proADM using a commercially available automated fluoroimmunoassay in apparently healthy individuals, and (ii) to identify biological determinants of MR-proADM concentrations.

Methods: A total of 506 questionnaire-identified apparently healthy adults were enrolled in a single-center, cross-sectional study. A final reference group (n=172) was selected after exclusion of obese individuals, those with increased values of laboratory biomarkers indicating asymptomatic myocardial injury or dysfunction, ongoing inflammation, diabetes, dyslipidemia and renal dysfunction and outliers.

Results: The 2.5th and 97.5th percentile intervals for MR-proADM values in the reference group (90% confidence interval) were 0.21 (0.19–0.23) and 0.57 (0.55–0.59) nmol/L, respectively. Although older age, higher values of HbA_{1c}, C-reactive protein, B-type natriuretic peptide and body mass index, together with a history of smoking and a decreased estimated glomerular filtration rate were significantly associated with increasing concentrations of MR-proADM in both univariate and multivariate analyses, magnitudes of these relationships were modest and did not substantially influence MR-proADM reference intervals. Sex-dependent difference in MR-proADM reference intervals was not detected [0.19 (0.16–0.22)–0.56 (0.54–0.60) nmol/L in females vs. 0.22 (0.20–0.25)–0.58 (0.57–0.63) nmol/L in males].

Conclusions: Our study successfully established robust reference intervals for MR-proADM concentrations in plasma. Considering the negligible influence of potential biological determinants on plasma MR-proADM, we recommend the adoption of single reference intervals for adult population as a whole.

Keywords: MR-proADM; proadrenomedullin; reference intervals.

Introduction

Midregional proadrenomedullin (MR-proADM) is a reliable surrogate biomarker directly reflecting blood concentrations of unstable adrenomedullin (ADM). As ADM has been identified in a variety of tissues (i.e. heart, brain, lung, kidney, blood vessels, bone, adrenal cortex and adipose tissue), it may exhibit multiple biological functions due to its potent immunomodulatory, diuretic, bactericidal and vasodilatory activities [1]. MR-proADM is emerging as a promising biomarker for detecting possible organ failure, providing prognostic information as well as enabling multidimensional risk assessment in various subsets of patients [2]. Potential clinical applications of MR-proADM measurement include diagnosis and/or risk stratification in patients with suspected or established cardiovascular disorders (i.e. heart failure, various clinical presentations of coronary artery disease and pulmonary embolism), sepsis, lower respiratory tract infections, acute kidney injury or chronic kidney disease and other non-specific complaints [2, 3].

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An automated fluoroimmunoassay is commercially available (Brahms MR-proADM Kryptor, Thermo Fisher Scientific, Hennigsdorf, Germany) [4], but detailed studies on MR-proADM reference intervals and their biological determinants in healthy individuals are still sparse. MR-proADM concentrations may vary substantially with age, renal function, inflammatory status and the presence of cardiovascular risk factors or subclinical coronary artery disease [3, 5–8]. Conflicting data exist regarding the impact of smoking status and sex on MR-proADM concentrations. Importantly, the majority of studies have been conducted in a variety of diseased populations or in unselected individuals from the general population. This emphasizes the need for a comprehensive study of MR-proADM reference values established in a well-characterized reference population. Therefore, our aim was to establish reference intervals for MR-proADM in plasma using the Kryptor system and to identify the biological determinants of concentrations of this biomarker in a well-selected, homogenous healthy population.

Materials and methods

Study design and protocol

The study was designed as a community-based, cross-sectional study and included apparently healthy Caucasian individuals aged 18–70 years, previously selected for similar projects involving measurements of cardiac troponin I (measured with a highly sensitive assay, high-sensitivity cardiac troponin I [hs-cTnI]) and galectin-3 [9–11]. The study protocol was approved by the Bioethics Committee of Nicolaus Copernicus University in Torun (Poland).

In detail, 640 potentially eligible individuals without any active or chronic inflammatory process, including infections, treatment with antibiotics, steroids, immunosuppressive agents or nonsteroidal anti-inflammatory drugs and/or pregnancy, were recruited in several workplaces in Bydgoszcz (Poland) between March and August 2013 and between July and August 2015. Prior to blood collection, all individuals signed a consent form and completed a predefined questionnaire (Supplemental Figure 1). A first screening applied to the entire population was based on the questionnaire and resulted in the exclusion of 107 subjects due to diabetes, hypertension or both. Following the further exclusion of 27 individuals for whom data were not available, 506 presumably healthy individuals were finally enrolled in the study. The second screening aimed at selecting the reference group was resultant from the following laboratory tests and corresponding cutoffs for inclusion: hs-cTnI (Abbott Architect) <15.6 ng/L in females and <34.2 ng/L in males [9], B-type natriuretic peptide (BNP) (Abbott Architect) <35 ng/L [12], C-reactive protein (CRP) <10 mg/L [13, 14], glycated hemoglobin (HbA_{1c}) <42 mmol/mol [15] and estimated glomerular filtration rate (eGFR) ≥90 mL/min/1.73 m^2 [16]. Obese subjects were also excluded with body mass index (BMI) classified according to WHO criteria [17].

The flow diagram for the selection of reference group is shown in Figure 1. We used a direct selection approach for individuals as recommended by the CLSI EP28-A3c guidelines [18]. As detailed above, the baseline presumably healthy population composed of 506 self-declared (by questionnaire) healthy subjects. Within the questionnaire-screened presumably healthy population, individuals were further categorized according to the above listed laboratory biomarker-based selection criteria. Obese individuals and those with high total cholesterol (TC) (≥6.22 mmol/L) and triglycerides (TG) (≥2.26 mmol/L) concentrations were also excluded [19]. Finally, after exclusion of four outliers (three women and one man with MR-proADM concentrations of 1.14, 1.02, 0.96 and 1.12 nmol/L, respectively), a well-selected, homogenous group of 172 individuals was used to derive MR-proADM reference intervals and further stratified to evaluate dependence of plasma MR-proADM concentrations from age and BMI.

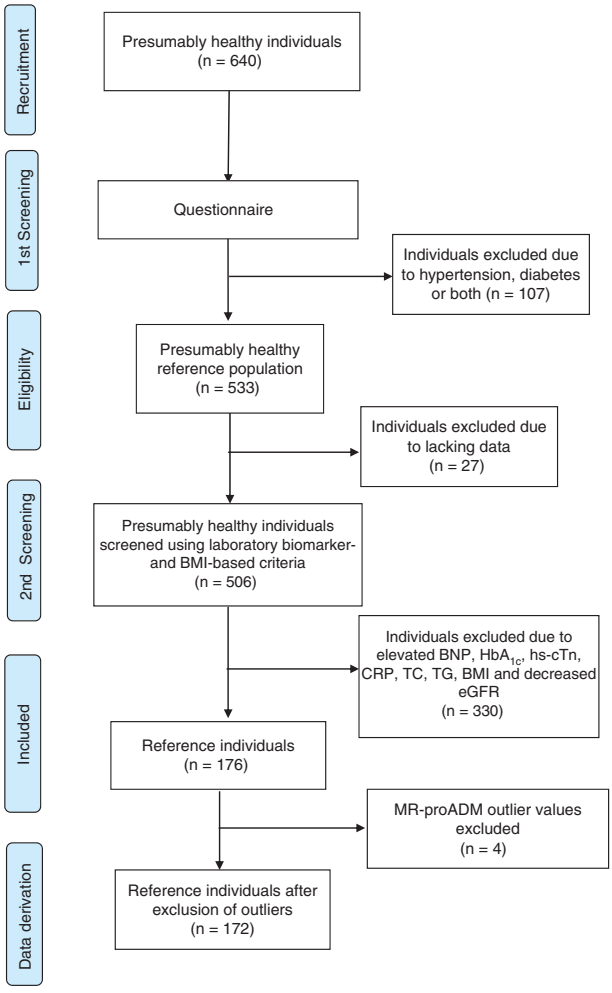

Figure 1: Flow diagram for the selection of the reference group. BMI, body mass index; BNP, B-type natriuretic peptide; CRP, C-reactive protein; HbA_{1c}, glycated hemoglobin; hs-cTnI, cardiac troponin I measured with a highly sensitive assay; TC, total cholesterol; TG, triglyceride; eGFR, estimated glomerular filtration rate; MR-proADM, midregional proadrenomedullin.

Laboratory measurements

Blood samples were taken in fasting state and collected under standardized conditions. Serum and ethylenediaminetetraacetic acid (EDTA) plasma were separated from venous blood samples by centrifugation for 10 min at 3000*g* at room temperature. Laboratory measurements were performed in serum (creatinine, basic lipid profile, hs-cTnI), whole EDTA blood HbA_{1c} and EDTA plasma BNP samples immediately following collection. All remaining plasma and serum samples were then aliquoted and stored at –70 °C until assayed for MR-proADM and CRP.

HbA_{1c}, BNP, creatinine, TC, high-density lipoprotein cholesterol (HDL-C), TG and hs-cTnI were measured on the Abbott Architect ci8200 analyzer using commercially available assays (Abbott Laboratories, Wiesbaden, Germany). Low-density lipoprotein cholesterol (LDL-C) concentrations were calculated using Friedewald's equation, except for subjects with TG >2.26 mmol/L, for whom LDL-C was measured directly. Measurements of CRP were performed on the Horiba ABX Pentra 400 analyzer (Horiba ABX, Montpellier, France). eGFR was obtained using the Chronic Kidney Disease Epidemiology Collaboration equation [20].

EDTA plasma MR-proADM concentrations were measured using the fully automated fluoroimmunoassay on the Kryptor platform, with an assay range of 0.05–100 nmol/L. The between-run CV was ≤20% in the range 0.1–0.3 nmol/L and <15% for concentrations >0.3 nmol/L [4].

Statistical analysis

Quantitative variables are expressed as medians and interquartile ranges, with categorical data as numbers and percentages. Quantitative variables following the Gaussian distribution are presented as mean values ± SD. The Shapiro-Wilk test was used to assess the normality of distribution for investigated parameters. Depending on the presence or absence of normal distribution, comparisons between two unrelated groups were performed with the Student's t-test for independent samples or the Mann-Whitney U-test, whereas the one-way analysis of variance or the Kruskal-Wallis test was applied for comparisons among more than two unrelated groups. Categorical variables were compared using the chi-square test. Spearman's rank correlation coefficient was used to test the associations between MR-proADM concentrations and other continuous variables. The impact of potential determinants on variation in MR-proADM concentrations in plasma was evaluated with multivariable regression analysis. MR-proADM concentrations were logarithmically transformed before their inclusion in this analysis to improve their adherence to normal distribution. The regression models used in our study, which included principally HbA_{1c}, BNP and eGFR, were adjusted for age and sex together with hyperlipidemia; these followed adjustments for both BMI and smoking status. A two-sided p-value <0.05 was considered statistically significant. MR-proADM reference intervals were derived using a robust method as recommended in the CLSI EP28-A3c document [18]. Ninety percent confidence intervals were calculated for reference intervals. Outlier observations were excluded from the analyzed population by the method described by Grubbs, which is a double-sided method enabling identification of the most extreme value at either side [21]. Statistical analyses were performed using Statistica 13.0 for Windows (StatSoft, Tulsa, OK, USA) and MedCalc 17.9.2 (MedCalc Software, Ostend, Belgium).

Results

Characteristics of the studied population

Table 1 summarizes baseline characteristics of the study population. The questionnaire-screened presumably healthy individuals had a higher median age than the

Table 1: Baseline characteristics of the study population.

Variable	Presumably healthy population (n = 506)	Reference group (n = 172)	p-Value
Age, years	40 (32–51)	35 (30–41)	<0.0001
Sex, females	227 (55%)	86 (50%)	0.240
BMI, kg/m²	24.9 (22.0–27.9)	23.7 (21.4–25.8)	<0.0001
MR-proADM, nmol/L	0.41 (0.35–0.48)	0.39 (0.33–0.45)	0.0014
hs-cTnI, ng/L	2.4 (1.7–3.2)	2.1 (1.4–3.0)	0.110
BNP, ng/L	15 (10–24)	11 (10–18)	0.0002
HbA_{1c}, mmol/mol	35.5 (32.2–38.8)	35.5 (32.0–37.7)	0.022
eGFR, mL/min/1.73 m²	94 (86–103)	99 (94–109)	<0.0001
TC, mmol/L	5.12 (4.49–5.95)	4.83 (4.31–5.35)	<0.0001
LDL-C, mmol/L	3.10 (2.50–3.80)	2.76 (2.30–3.36)	0.0008
HDL-C, mmol/L	1.45 (1.21–1.76)	1.47 (1.23–1.73)	0.893
TG, mmol/L	1.07 (0.78–1.62)	0.88 (0.68–1.20)	<0.0001
CRP, mg/L	0.53 (0.20–1.73)	0.32 (0.11–0.77)	<0.0001
Current or former smoker	141 (33%)	61 (38%)	0.06

Quantitative variables are expressed as medians and 1st–3rd quartile ranges and categorical data as numbers and percentages. BMI, body mass index; MR-proADM, midregional proadrenomedullin; hs-cTnI, cardiac troponin I measured with a highly sensitive assay; BNP, B-type natriuretic peptide; HbA_{1c}, glycated hemoglobin; eGFR, estimated glomerular filtration rate; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; CRP, C-reactive protein.

Table 2: Spearman correlation coefficients between midregional proadrenomedullin and age, body mass index and laboratory parameters in the presumably healthy population (n = 506).

Variable	R_S	p-Value
Age, years	0.343	<0.0001
BMI, kg/m^2	0.394	<0.0001
hs-cTnI, ng/L	0.012	0.906
BNP, ng/L	0.164	0.0006
HbA_{1c}, mmol/mol	0.038	0.403
eGFR, mL/min/1.73 m^2	−0.257	<0.0001
TC, mmol/L	0.148	0.005
LDL-C, mmol/L	0.130	0.012
HDL-C, mmol/L	−0.140	0.001
TG, mmol/L	0.288	<0.0001
CRP, mg/L	0.289	<0.0001

R_S, Spearman correlation coefficient; BMI, body mass index; hs-cTnI, cardiac troponin I measured with a highly sensitive assay; BNP, B-type natriuretic peptide; HbA_{1c}, glycated hemoglobin; eGFR, estimated glomerular filtration rate; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; CRP, C-reactive protein.

reference individuals and significantly higher MR-proADM concentrations. Additionally, comparisons of the investigated parameters revealed significantly higher values of BMI, BNP, HbA_{1c}, TC, LDL-C, TG and CRP and lower eGFR values in the presumably healthy subjects. Although the distribution of sexes was comparable in both investigated groups, rates of smokers tended to be higher in the reference group; however, a p-value did not reach the threshold of statistical significance. MR-proADM concentrations in plasma of reference group (n = 172) followed a normal distribution (Supplemental Figure 2).

Biological determinants of MR-proADM concentrations in plasma

In univariate analysis, plasma MR-proADM concentrations were correlated with most investigated variables, except for hs-cTnI and HbA_{1c} (Table 2). The highest Spearman correlation coefficients were shown for BMI, age and CRP. To further examine relationships between log-transformed MR-proADM concentrations and independent covariates, found to be significantly related to MR-proADM in univariate analysis, we developed multiple linear regression models. Models were initially adjusted for age, sex and hyperlipidemia, followed by adjustments for BMI and smoking. eGFR and CRP were significantly related to log-transformed MR-proADM concentrations after adjustment for age, sex and the presence of hyperlipidemia (Table 3), and remained significantly associated with log-transformed MR-proADM concentrations after adjustment for BMI and smoking habit. In the fully adjusted model, in addition to eGFR and CRP, also HbA_{1c}, BNP, BMI and smoking habit were correlated with log-transformed MR-proADM concentrations. Age significantly contributed to increased MR-proADM concentrations in all investigated

Table 3: Impact of selected variables on log-transformed MR-proADM concentration in multiple regression analysis in the presumably healthy population (n = 506).

Regression models (all including HbA_{1c}, BNP, eGFR)	Model characteristics
Model adjusted for age and sex[a]	$R^2 = 0.19$ Significant determinants of log-transformed MR-proADM concentration: age (β = 0.007; p < 0.0001), sex (β = 0.099; p = 0.0001), eGFR (β = −0.003; p = 0.001) and CRP (β = 0.008; p < 0.0001) Lack of impact: BNP and HbA_{1c}
Model adjusted for age, sex and the presence hyperlipidemia[a]	$R^2 = 0.20$ Significant determinants of log-transformed MR-proADM concentration: age (β = 0.007; p < 0.0001), sex (β = 0.096; p = 0.0002), eGFR (β = −0.003; p = 0.001) and CRP (β = 0.008; p < 0.0001) Lack of impact: BNP, HbA_{1c} and the presence of hyperlipidemia
Model adjusted for age, sex, hyperlipidemia, BMI and smoking status[a]	$R^2 = 0.29$ Significant determinants of log-transformed MR-proADM concentration: age (β = 0.005; p = 0.0002), HbA_{1c} (β = −0.006; p = 0.045), BNP (β = 0.001, p = 0.036), eGFR (β = − 0.003; p = 0.001), CRP (β = 0.008; p < 0.0001), BMI (β = 0.020; p < 0.0001) and smoking (β = 0.092; p = 0.002) Lack of impact: sex and the presence of hyperlipidemia

The presence hyperlipidemia was defined as coefficient values refer to a 1 unit increase of the investigated variables, if not otherwise stated. Males and females were coded as 1 and 0, respectively. Current or former smokers and non-smokers were coded as 1 and 0, respectively. BMI, body mass index; BNP, B-type natriuretic peptide; CRP, C-reactive protein, eGFR, estimated glomerular filtration rate; HbA_{1c}, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; [a]p < 0.0001.

regression models, whereas the effect of sex lost its statistical significance in the fully adjusted model.

Reference intervals for MR-proADM concentrations in plasma

Reference intervals for plasma MR-proADM concentrations established in this study, also categorized according to sex as well as to age, are shown in Table 4. The derived limits were only slightly higher in males and older individuals. A visual inspection of continuously displayed 2.5th and 97.5th percentile reference intervals for plasma MR-proADM, however, did not show any evident association with age (Figure 2A), with a modest though constant rise with increasing BMI (Figure 2B). However, the MR-proADM reference interval derived from individuals with BMI <25 kg/m^2 (n = 112) was 0.20–0.55 nmol/L, therefore comparable to that obtained for all reference group (0.21–0.57 nmol/L).

Table 4: Reference limits (with 90% confidence intervals) for midregional proadrenomedullin concentrations in plasma.

Subgroup	n	2.5th Percentile, nmol/L	97.5th Percentile, nmol/L
Overall	172	0.21 (0.19–0.23)	0.57 (0.55–0.59)
Males	86	0.22 (0.20–0.25)	0.58 (0.57–0.63)
Females	86	0.19 (0.16–0.22)	0.56 (0.54–0.60)
<40 years	120	0.19 (0.17–0.22)	0.57 (0.55–0.60)
≥40 years	52	0.24 (0.20–0.28)	0.60 (0.57–0.64)

Outlier results

We reassessed the concentrations of MR-proADM in all four outliers and obtained exactly the same results as those initially reported in the manuscript. Therefore, we do not consider them to be analytical outliers. We attempted to contact all four persons with outlier concentrations. Six months after completion of the study, one participant suffered from hypothyreosis which is now well controlled. This subject had not experienced any other adverse events at the 24 month follow-up. Unfortunately, we were unable to contact the three other persons presenting outlier concentrations.

Discussion

Establishment of robust reference intervals facilitates the interpretation of laboratory test results. In our cross-sectional study, we have successfully established reliable reference intervals for MR-proADM concentrations in plasma, using predefined selection criteria to identify a healthy reference group. In order to achieve this goal, we applied a health questionnaire combined with laboratory biomarkers screening. Although HbA_{1c}, CRP and BMI, together with a history of smoking and eGFR, remained significantly associated with concentrations of MR-proADM, magnitudes of these relationships were modest and did not substantially influence reference intervals for MR-proADM in plasma. Notably, the reference values for MR-proADM in our study were comparable between females and males.

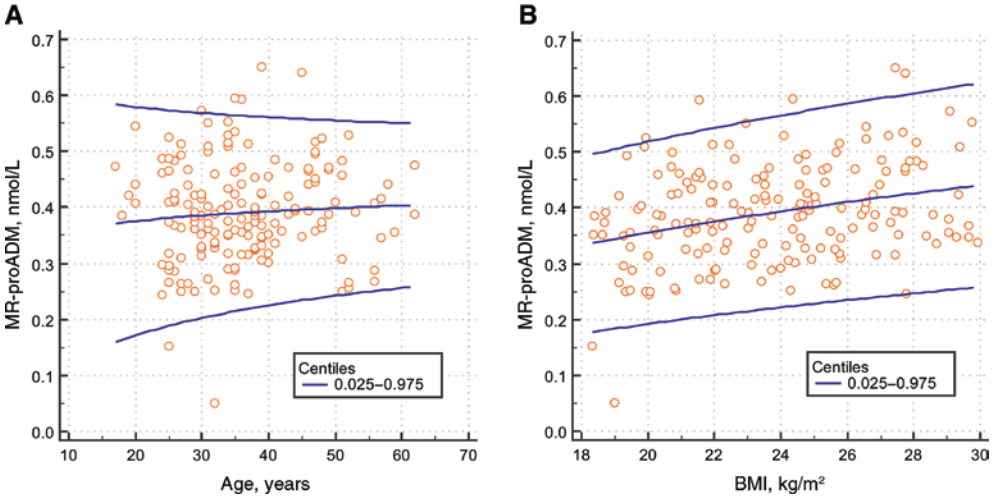

Figure 2: Age- (A) and body mass index (B)-related midregional proadrenomedullin concentrations in plasma and reference intervals in the reference group (n = 172).
BMI, body mass index; MR-proADM, midregional proadrenomedullin.

MR-proADM plasma concentrations measured in our reference group are comparable to those originally provided by Caruhel et al. [4] and then reported by Brahms in the assay package insert (median: 0.39 nmol/L; 97.5th percentile: 0.55 nmol/L), but slightly lower than those reported by Smith et al. (median: 0.41 nmol/L; 97.5th percentile: 0.64 nmol/L and 0.66 in men and women, respectively) [8]. However, this study preferentially used clinical and less tightened laboratory-based exclusion criteria (e.g. eGFR <60 mL/min/1.73 m^2 and CRP >20 mg/L), whereas the selection of our reference individuals was based on more stringent criteria, which may account for slightly lower MR-proADM plasma concentrations in the reference group and consequently in derived reference intervals.

The biological determinants of MR-proADM plasma concentrations found in our study are similar to those previously reported [5, 8]. In contrast to our project, however, prior studies included unselected subjects. In participants of the Malmö Diet and Cancer study (n = 5258), Smith et al. [8] demonstrated increasing values of age, BMI, body fat percentage, TC, LDL, TG, HbA_{1c}, CRP, N-terminal pro-B-type natriuretic peptide (NT-proBNP), cystatin C and alcohol intake, together with current smoking, as independent determinants of MR-proADM plasma concentrations. However, a large majority of these parameters were only weakly associated with MR-proADM plasma concentrations. The strongest independent association was shown for cystatin C, which accounted for 18% of MR-proADM variability in the multivariate model [8]. In their study involving 1002 elderly individuals from the general community, Eggers et al. [5] found that MR-proADM was independently related to current smoking, renal dysfunction, obesity, lower left-ventricular ejection fraction and higher concentrations of NT-proBNP and CRP.

Despite the recognized potential for the clinical application of this biomarker, detailed mechanisms explaining how biological determinants may influence MR-proADM concentrations are not fully understood. The association of MR-proADM with renal function could reflect the clearance of MR-proADM molecule from circulation by renal emunctory [8]. In patients with chronic kidney disease, Dieplinger et al. [22] demonstrated a strong correlation between MR-proADM concentrations and the glomerular filtration rate measured by iohexol clearance (–0.82; $p < 0.001$). The relationship between MR-proADM and both BMI and HbA_{1c} may in turn reflect the influence of overweight/obesity or metabolic syndrome on ADM synthesis. In line with this assumption, increased expression of ADM was observed in the adipose tissue of obese vs. lean women [23]. Moreover, ADM may also directly inhibit insulin secretion [24] and lead to insulin resistance [25]. Additionally, an accumulating body of evidence indicates that inflammation triggers ADM synthesis [26, 27], which may explain the positive correlation between CRP and MR-proADM found in our study. Finally, we can also speculate that smoking may cause subclinical arthrosclerosis resulting in higher MR-proADM concentrations.

Elevated MR-proADM concentrations may reflect the failure of multiple organs, which translates into potentially broad clinical indications for MR-proADM testing [2]. However, this biomarker remains non-specific, and therefore, interpretation of a result needs to be integrated with careful clinical assessment and other examinations. Our study was conducted with a rigorous methodology and a direct approach. Its strength lies in the selection and definition of a healthy reference group, the application to this well-defined cohort of standardized laboratory measurements and clinically validated cutoffs, the employment of appropriate statistical methods and the identification of major biological determinants of plasma MR-proADM concentrations. Some limitations of our study should be mentioned. First, due to the nature of our single center study and identification of 2.3% of the already-prescreened population as statistical outliers, our findings warrant verification in larger studies with a clinical follow-up. The presence of outlier values may potentially affect the prognostic power of MR-proADM assays. Second, our study protocol did not include cardiac imaging examinations. However, the cutoff value for BNP used in our study was previously shown to successfully exclude patients with both heart failure and left ventricular dysfunction [12]. Third, we did not assess cystatin C, insulin resistance and abdominal adipose tissue, other potential determinants of MR-proADM concentrations in blood. Fourth, the measurement of MR-proADM was conducted on samples that were initially stored in a frozen state. However, this does not substantially affect the accuracy of our results due to the validated stability of MR-proADM [28].

In conclusion, our study successfully established reliable reference intervals for MR-proADM concentrations in plasma. Considering the negligible influence of potential biological determinants on MR-proADM concentrations, we recommend the adoption of single reference intervals for adult population as a whole.

Acknowledgments: MR-proADM reagents to carry out the study were a generous gift of Brahms GmbH, Germany. The technical contribution of Claudia Michelazzo and Simona Borille is acknowledged.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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Supplemental Material: The online version of this article offers supplementary material (https://doi.org/10.1515/cclm-2017-1044).