

Links Between Concentrations of Serum 25-hydroxyvitamin D3 and the Numbers of Circulating Progenitor Mononuclear Cells in Patients With Metabolic Syndrome

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Abstract

Background: Evidence points to the pivotal role of vitamin D in the pathogenesis of metabolic syndrome (MetS), including deterioration of the endogenous endothelial repair system.

Objectives: This study was conducted to investigate links between serum 25-hydroxyvitamin D3 (25(OH)3D) concentrations and the numbers of circulating progenitor mononuclear cells in MetS individuals.

Methods: The cross-sectional study involved 47 patients with MetS. The circulating level of 25(OH)D3 and other biomarkers were measured at the start of the study. The number of mononuclear progenitor cells was determined using the flow cytometric technique (FCT).

Results: MetS patients from the entire group of 47 patients were divided into four cohorts depending on 25(OH)D3 levels. The groups comprised patients with 25(OH)D3 levels above 100 nmol/L (n = 10), patients with levels from 50 to 100 nmol/L (n = 12), patients with levels from 30 to 50 nmol/L (n = 14), and patients with levels below 30 nmol/L (n = 11). There were significant differences between the MetS cohorts in terms of haemoglobin A1c (HbA1c) (P = 0.038), the homeostasis model assessment for insulin resistance (HOMA-IR) (P = 0.042), triglycerides (P = 0.044), osteoprotegerin (P = 0.028), adiponectin (P = 0.018), high density lipoprotein cholesterol (HDL-C) (P = 0.036), and CD14⁺D309⁺Tie-2⁺ cells. Vitamin D deficiency in a multivariate log-linear regression model appeared to be an independent predictor of the numbers of CD14⁺D309⁺Tie-2⁺ cells (OR 1.12; 95% CI 1.06 to 1.19; P = 0.002). Osteoprotegerin, high sensitivity C-reactive protein (hs-CRP), and adiponectin have been shown to make an independent impact on the numbers of CD14⁺D309⁺Tie-2⁺ cells. Using C-statistics, we found that the use of three biomarkers (osteoprotegerin, hs-CRP, and adiponectin) can significantly improve a predictive model based on vitamin D deficiency for decreased numbers of CD14⁺D309⁺Tie-2⁺ cells.

Conclusions: We found that low levels of 25(OH)D3 were associated with depleted numbers of proangiogenic progenitor mononuclear cells in MetS patients.

Keywords: Metabolic Syndrome, Vitamin D, Cardiovascular Risk Factors, Progenitor Mononuclear Cells, Inflammation

1. Background

Metabolic syndrome (MetS) is a cluster of cardiovascular (CV) risk factors, including central obesity, glucose homeostasis, insulin resistance (IR), hypertension and atherogenic dyslipidemia (1). Recent studies have shown that patients with MetS have an almost two-fold increased risk of CV disease and CV events, asymptomatic atherosclerosis, all cause and CV death rate (2, 3).

There is evidence regarding the pivotal role of vitamin D in the pathogenesis of CV disease in dysmetabolic states (4). Serum concentrations 25(OH)D3 provide a reliable indicator of body vitamin D status. Low serum 25(OH)D3 levels have been directly linked to MetS, whereas the overall risk of MetS in the general population is probably not associated with 25(OH)D3 concentrations (5-7). Vitamin D

deficiency (defined as a serum 25(OH)D3 level below 20 ng/mL) is associated with IR, decreased insulin secretion by pancreatic beta-cells, inflammation, lower circulating adiponectin levels, and activation of the renin-angiotensin system (8, 9). In addition, abdominal obesity, hypertension, endothelial dysfunction and dyslipidemia have exhibited a close association with low levels of 25(OH)D3 (10, 11). The 25(OH)D3 serum may act through the vitamin D receptor (VDR), which mediates calcium efflux into target cells and attenuates the secretion of inflammatory cytokines via direct stimulation of transcription factor nuclear factor- κ B (NF- κ B) (12). VDR and the 1 α -hydroxylase enzyme, which catalyzes the conversion of 25(OH)D3 to 1,25-dihydroxyvitamin D (1,25(OH)2D) and is expressed on the surface of several cell types, may contribute to multiple

metabolic regulation of target cells including progenitor cells (13). Finally, oxidative stress, inflammatory cytokines, and vitamin D status mediated through several epigenetic mechanisms may change the functionality of progenitor endothelial/mononuclear cells.

The role of vitamin D in the recruitment and differentiation of progenitor mononuclear cells, which are involved in the pathophysiology of metabolic states, remains controversial (14, 15). Indeed, dysfunction of progenitor mononuclear cells may play a prominent role in the worsening of endothelium repair due to direct endothelium damage, endothelial dysfunction, microvascular inflammation, and oxidative stress (16). In contrast, endothelial function may be impaired due to altered maturation/commitment of progenitor mononuclear cells rather than as the result of a simple decrease in their production in the bone marrow. This process may be under the control of 25(OH)D3 (17). Recent studies have confirmed the relationship between 25(OH)D3 and depleted numbers of circulating endothelial progenitor cells in diabetes mellitus (18, 19). However, the relationship between the number of circulating progenitor mononuclear cells in MetS patients and 25(OH)D3 levels is not yet fully clear.

2. Objectives

The objective of the study was to investigate links between 25(OH)D3 concentrations and the number of circulating progenitor mononuclear cells in MetS individuals.

3. Methods

3.1. Study Design and the Study Population

This cross-sectional study involved 47 patients with MetS who were examined within winter months (between December 2012 and February 2013, December 2013 and February 2014, and between December 2014 and February 2015) to minimize seasonal bias. The study protocol was approved by the local ethics committee review board (IRB # 3/2010) of the State Medical University of Zaporozhye (Ukraine) prior to the study commencing. The study complied with the Declaration of Helsinki and voluntary written informed consent was obtained from all patients included in this study.

We enrolled MetS subjects without known type 2 diabetes mellitus (T2DM), typical angina symptoms, or existing coronary artery disease, and for whom we did not have angiographic evidence of asymptomatic atherosclerosis obtained by contrast-enhanced multi-spiral tomography angiography, which was provided prior to taking part in the study.

MetS was diagnosed based on the national cholesterol education program adult treatment panel III criteria (20). Patients were enrolled in the MetS cohort when at least three of the following components were defined: waist circumference of ≥ 90 cm or ≥ 80 cm for men and women, respectively; HDL-C levels of < 1.03 mmol/L or < 1.3 mmol/L for men and women, respectively; triglycerides levels of ≥ 1.7 mmol/L; blood pressure at $\geq 130/85$ mmHg, current exposure to antihypertensive drugs; or fasting plasma glucose levels of ≥ 5.6 mmol/L.

3.2. Anthropometric Measurements

Anthropometric measurements including height, body weight, and waist circumference were made using standard procedures after an overnight fast following the first visit. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Systolic and diastolic blood pressure was measured using an automatic blood pressure monitor (Omron, Japan).

3.3. Current Smoking Status

Current smoking was defined as consumption of one cigarette daily for three months.

3.4. Treatment and Concomitant Medications

Subjects with MetS had been treated by the adoption of life-style and diet modifications. As a result, metformin was given to 12 patients.

3.5. Calculation of the Glomerular Filtration Rate (GFR)

The glomerular filtration rate (GFR) was calculated using the CKD-EPI formula (21).

3.6. Determining Insulin Resistance (IR)

IR was assessed by HOMA-IR (22) using the following formula:

$$\text{HOMA-IR} (\text{mmol/L} \times \mu\text{U/mL}) = \text{fasting glucose} (\text{mmol/L}) \times \text{fasting insulin} (\mu\text{U/mL}) / 22.5$$

IR was determined when the estimated HOMA-IR value was over $2.77 \text{ mmol/L} \times \mu\text{U/mL}$, as defined previously (23).

3.7. Measurement of Circulating Biomarkers

To determine circulating biomarkers, blood samples were collected at baseline in the morning (between 7 and 8 a.m.) and placed in cooled silicone test tubes to which 2 mL of 5% Trilon B solution were added. They were then centrifuged on permanent cooling at 6000 rpm for 3 minutes. Plasma was collected and refrigerated immediately to be stored at a temperature of -70°C .

Serum adiponectin, the receptor activator of nuclear factor κB ligand (RANKL), and osteoprotegerin (OPG) were

measured by high-sensitive enzyme-linked immunosorbent assays using commercial kits (R and D Systems GmbH, Wiesbaden-Nordenstadt, Germany) according to the manufacturers' recommendations. The inter-assay coefficients of variation were as follows: adiponectin 5%; RANKL 7.0%; and OPG 8.2%.

We measured hs-CRP with a commercially available standard kit (R and D Systems GmbH, Wiesbaden-Nordenstadt, Germany). The intra-assay and inter-assay coefficients of variation were < 5%.

Fasting insulin levels were measured by a double-antibody sandwich immunoassay (Elecys 1010 analyzer, F. Hoffmann-La Roche diagnostics, Mannheim, Germany). The intra-assay and inter-assay coefficients of variation were < 5%. The lower detection limit of insulin levels was 1.39 pmol/L.

Concentrations of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and HDL-C were measured by the enzymatic colorimetric method according to the standardized methodology on a Beckman Synchron LX20 chemistry analyzer.

3.8. Measurement of Plasma 25-hydroxy Vitamin D3 (25(OH)D3)

Circulating levels of 25(OH)D3 were measured using an ELISA kit (BG Medicine, Germany). Plasma 25(OH)D3 levels above 100 nmol/L are defined as the optimal vitamin D status and levels between 50 and 100 nmol/L are defined as adequate. Serum levels of 25(OH)D below 50 nmol/L suggest an inadequate vitamin D status, whereas values below 30 nmol/L indicate vitamin D deficiency.

3.9. Assay of Circulating Mononuclear Progenitor Cell Subsets

The FCT was used to consistently identify circulating cell subsets, which depend on the expression of CD45, CD34, CD14, Tie-2, and CD309 (VEGFR2), using the high-definition fluorescence activated cell sorter (HD-FACS) methodology (24). Accordingly, the cells were labeled on the basis of their forward scatter characteristic (FSC) and side scatter characteristic (SSC) profiles. Using standardized and calibrated instruments, sensitivity and fluorescence and light scatter resolution were determined according to the standard protocol (25).

The cells were directly stained and analyzed for phenotypic expression of surface proteins using anti-human monoclonal antibodies, including anti-CD45 FITS (BD biosciences, San Jose, CA, USA), anti-CD34 FITS (BD Biosciences), anti-VEGFR-2 known as anti-CD309 (BD Biosciences), anti-Tie2 (BD Biosciences) and anti-CD14 (BD Biosciences). The fluorescence minus one technique was used to provide

negative controls and to establish positive stain boundaries. After lysis of erythrocytes with UTILIZE wash solution, the samples were centrifuged at $200 \times g$ for 15 minutes. The samples were then washed twice with phosphate buffer solution (PBS) and fixed immediately. Double- and triple-positive events were determined using Boolean principles (and, not, or, etc.).

3.10. Determination of Circulating Progenitor Mononuclear Cells of Endothelial Origin

Circulating mononuclear progenitor cells were defined as CD34/D309 (VEGFR2) positive cells lacking CD45 expression. From each tube 500,000 events were analyzed. For CD14⁺ populations, co-expression with Tie-2 and/or VEGFR-2 was determined using quadrant analysis. Standardized cell counts were presented as a percentage of the total of the white blood cell count, identified as the total number of all CD45⁺ cells. The fluorescein isothiocyanate (FITC)-labeled isotype control was analyzed with the same gate and window settings. Angiogenic phenotype for mononuclear progenitor cells was determined as CD14⁺D309⁺ (VEGFR2/KDR), CD14⁺Tie-2⁺ and D309⁺ (VEGFR2/KDR) Tie-2⁺ antigen presentation. The reproducibility of mononuclear progenitor cell measurements was 3.5% using the standard protocol.

3.11. Statistical Analysis

Statistical analysis of the results was carried in SPSS for Windows (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp, USA). The data were presented as mean (M) and standard deviation (\pm SD) or a 95% confidence interval (CI), as well as median and in the 25% - 75% interquartile range (IQR). The sample size ($n = 47$) was determined statistically by power analysis methods, taking into account an anticipated effect size (f_2) equal to 0.25, a desired probability level of 0.05, a statistical power level of 0.8, and 3 predictors. To compare the main parameters of the patient cohorts, a two-tailed Student t-test or the Mann-Whitney U test were used. To compare categorical variables between groups, a Chi2 test (χ^2) and Fisher exact test were used. Predictors of depleted numbers of progenitor mononuclear cells in MetS patients were examined in univariate and multivariate log-linear regression analysis. We calculated the observed power for multiple regression, given the observed probability level, the number of predictors, the observed R^2 , and the sample size ($n = 47$). C-statistics, integrated discrimination indices (IDI), and net-reclassification improvement (NRI) were used for prediction performance analyses. A two-tailed probability value of < 0.05 was considered as significant.

4. Results

The mean age of the 47 MetS patients (30 males, 63.3%) was 48.34 years. The baseline characteristics of the patients are listed in Table 1. Regarding CV risk factors, 68% of the patients were hypertensive, 55.3% of subjects exhibited dyslipidemia, and 34% were smokers. The mean GFR was 92.5 mL/minute/1.73 m². Regarding 25(OH)D3 levels, MetS patients from entire group were divided into four cohorts comprising patients with 25(OH)D3 levels above 100 nmol/L (n = 10), patients with levels between 50 and 100 nmol/L (n = 12), patients with levels between 30 and 50 nmol/L (n=14), and patients with levels below 30 nmol/L (n = 11), defined as adequate vitamin D status, inadequate vitamin D status, insufficient vitamin D and vitamin D deficiency, respectively. The mean plasma levels of 25(OH)D3 in each MetS cohort are presented in Figure 2.

The patients with vitamin D deficiency appear to be have a higher age (P = 0.046) and frequently-defined dyslipidemia (P = 0.044) (Table 1). Therefore, there was a sufficient difference between cohorts in terms of HbA1c (P = 0.038), HOMA-IR (P = 0.042), triglycerides (P = 0.044), osteoprotegerin (P = 0.028), adiponectin (P = 0.018), HDL-C (P = 0.036), and CD14⁺D309⁺Tie-2⁺ cells (Table 2).

The univariate linear regression was used to determine the link between plasma levels of 25(OH)D3, the numbers of progenitor mononuclear cells, CV risk factors, and other biomarkers. The data showed that plasma levels of 25(OH)D3 are positively associated with the numbers of CD14⁺ D309⁺Tie-2⁺ cells (r = 0.41, P = 0.001) and the numbers of CD14⁺Tie-2⁺ cells (r = 0.28, P = 0.001). There is an inverse relationship between 25(OH)D3 levels and BMI (r = -0.44, P = 0.001), osteoprotegerin (r = -0.36, P = 0.001), HbA1c (r = -0.35, P = 0.003), age (r = -0.35, P = 0.001), dyslipidemia (r = -0.34, P = 0.001), hs-CRP (r = -0.33, P = 0.001), triglycerides (r = -0.32, P = 0.001), Framingham risk score (r = -0.31, P = 0.001), HOMA-IR (r = 0.30, P = 0.003), and adiponectin (r = -0.29, P = 0.001).

In multivariate linear regression analyses, plasma levels of 25(OH)D3 were associated with the numbers of CD14⁺ D309⁺Tie-2⁺ cells (r = 0.406, P = 0.001), osteoprotegerin (r = -0.34, P = 0.001), BMI (r = -0.41, P = 0.001), age (r = -0.35, P = 0.001), dyslipidemia (r = -0.31, P = 0.001), hs-CRP (r = -0.32, P = 0.001), and adiponectin (r = -0.29, P = 0.002). After adjustment for BMI and age, plasma levels of 25(OH)D3 were associated with the numbers of CD14⁺ D309⁺Tie-2⁺ cells (r = 0.38, P = 0.001), osteoprotegerin (r = -0.34, P = 0.003), hs-CRP (r = -0.31, P = 0.001) and adiponectin (r = -0.28, P = 0.002).

The log linear regression models exhibited a graduated effect of vitamin D status on the depletion of the numbers of CD14⁺D309⁺ Tie-2⁺ cells (Table 3). In the univari-

ate regression model, decreased numbers of CD14⁺D309⁺ Tie-2⁺ cells had a close relationship with vitamin D deficiency or insufficient vitamin D (odds ratio (OR) 1.16; 95% CI 1.05 to 1.23; P = 0.001 and OR 1.06; 95% CI 1.01 to 1.12; P = 0.001, respectively) rather than adequate vitamin D status (OR 1.02; 95% CI 0.98 to 1.05; P = 0.46). In addition, osteoprotegerin, hs-CRP, and adiponectin exhibit a significant dependent variable effect. Interestingly, vitamin D deficiency in the multivariate log-regression model appeared to be an independent predictor of depleted numbers of CD14⁺D309⁺ Tie-2⁺ cells (OR 1.12; 95% CI 1.06 to 1.19; P = 0.002), whereas other statuses of vitamin D were not found to be predictors. In contrast, osteoprotegerin, hs-CRP, and adiponectin had an independent impact on the numbers of CD14⁺D309⁺ Tie-2⁺ cells.

Using C-statistics for models with plasma levels of 25(OH)D3 below 30 nmol/L and circulating biomarkers (osteoprotegerin, hs-CRP, and adiponectin) as continuous variables, we found that adding inflammatory biomarkers to the base model (vitamin D deficiency) improved relative IDI by 7.2% for decreased numbers of CD14⁺ D309⁺Tie-2⁺ cells (Table 4). When we used another model based on entering continuous variables, osteoprotegerin, hs-CRP, and adiponectin did not significantly improve the predictive model based on the vitamin D deficiency status. In the patient study population for category-free NRI, 3% of events (P = 0.16) and 4% of non-events (P = 0.12) were correctly reclassified by the addition of circulating inflammatory biomarkers to the base model.

5. Discussion

The findings of the study show that vitamin D deficiency was a statistically significant predictor for decreased numbers of proangiogenic progenitor mononuclear cells, labelled as CD14⁺ D309⁺Tie-2⁺ cells in MetS patients without known CV disease. From current knowledge, the depletion of proangiogenic subsets of circulating progenitor mononuclear cells is a marker of asymptomatic atherosclerosis and CV events (26, 27). There is large body of evidence regarding a close inverse association between serum levels of inflammatory biomarkers (osteoprotegerin, hs-CRP, and adiponectin) and numbers/functionality of proangiogenic progenitor mononuclear cells in dysmetabolic patients with known CV disease (28). In diabetics, oxidative stress components, osteoprotegerin, hs-CRP, and adiponectin may enhance the epigenetic mechanisms of cell function regulation, leading to methylation-related changes to the gene promoter region and DNA damage to target cells (29, 30). These pathways remain triggers of angiogenic progenitor mononuclear cell dysfunction (31, 32). They are considered as

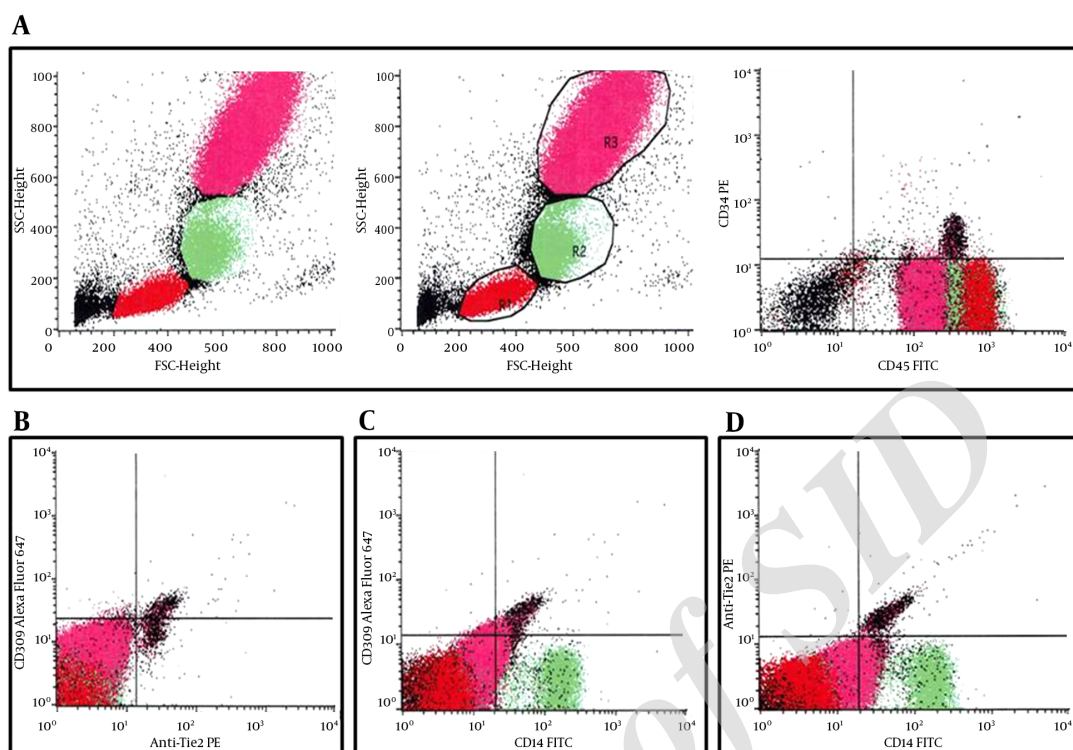


Figure 1. The Sample of Flow Cytometry Represents CD45-CD34⁺ cells (A), CD309⁺ anti-Tie2⁺ cells (B), CD309⁺ CD14⁺ cells (C), and CD14⁺ anti-Tie2⁺ cells (D)

Table 1. Characteristics of Metabolic Syndrome (MetS) Patients Included in the Study^{a,b,c}

Variables	Entire Cohort of MetS Patients (n = 47)	Plasma Levels of 25(OH)D3				P Value
		> 100 nmol/L (n = 10)	50 to 100 nmol/L (n = 12)	30 to 50 nmol/L (n = 14)	< 30 nmol/L (n = 11)	
Age, y	48.30 ± 3.94	46.40 ± 1.88	46.90 ± 2.26	47.10 ± 3.00	49.80 ± 2.55	0.046
Males, No. (%)	30 (63.8)	6 (60.0)	8 (66.7)	9 (64.2)	7 (63.6)	0.74
BMI, kg/m ²	28.2 (16.7-31.0)	27.1 (16.2-29.6)	28.5 (17.1-30.3)	28.9 (17.5-31.8)	29.5 (19.9-32.1)	0.22
Waist circumference, cm	92 (69-105)	93 (71-104)	92 (68-106)	92 (69-103)	94 (71-106)	0.88
Hypertension, No. (%)	32 (68.0)	7 (70.0)	8 (66.7)	10 (71.4)	7 (63.6)	0.78
Dyslipidemia, n (%)	26 (55.3)	4 (40.0)	7 (58.3)	9 (62.3)	6 (54.5)	0.044
Smoker, No. (%)	16 (34.0)	4 (40.0)	4 (33.3)	5 (35.7)	5 (45.5)	0.42
Framingham risk score	8.09 ± 2.12	7.88 ± 1.95	7.96 ± 2.03	8.07 ± 2.11	8.12 ± 1.80	0.44
Systolic BP, mm Hg	137 ± 4	137 ± 4	135 ± 4	137 ± 5	136 ± 5	0.92
Diastolic BP, mm Hg	87 ± 5	88 ± 5	88 ± 4	87 ± 7	87 ± 5	0.92
Heart rate, beats per minute	71 ± 6	71 ± 6	70 ± 5	71 ± 5	72 ± 5	0.94
GFR, mL/min/1.73 m ²	92.5 (83.1-107.4)	92.5 (83.1-107.4)	101.2 (88.3-109.2)	94.5 (85.0-106.1)	90.3 (82.3-102.7)	0.52

Abbreviations: ANOVA, analysis of variance; BMI, body mass index; BP, blood pressure; GFR, glomerular filtration rate; IQR, interquartile range.

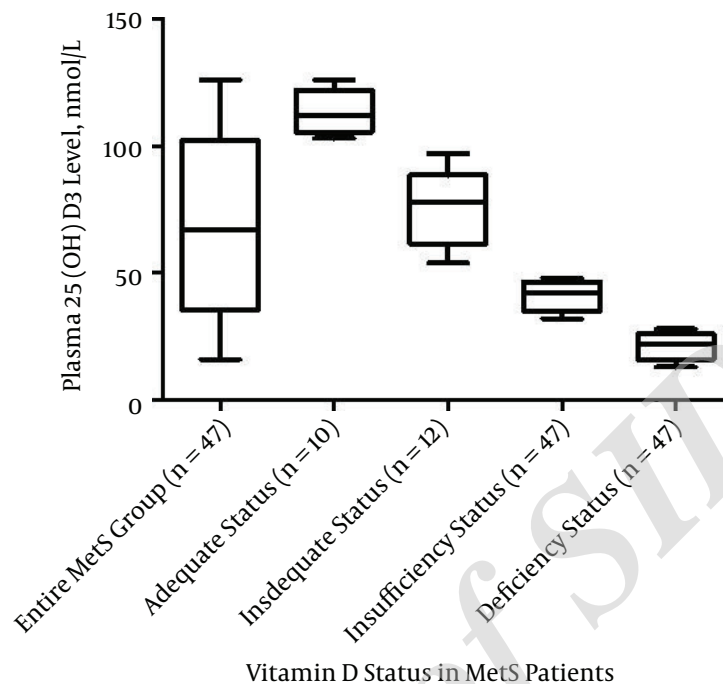
^a Values are expressed as mean ± SE, 95% CI, median and 25-75% IQR.

^b Categorical variables are expressed as numbers (n) and percentages (%).

^c P-value is a comparison of mean or median variables between both cohorts (ANOVA test).

clues in forming metabolic memory, which mediates manifestation and progression of CV disease in diabetes (33). 25(OH)D3 may regulate the functionality of angiogenic progenitor mononuclear cells and attenuate their repar-

ative capability (34, 35). Taken together, the findings suggest that vitamin D deficiency could coordinate the altered repair ability of progenitor mononuclear cells and inflammation in MetS.

Figure 2. The Mean Plasma Levels of 25(OH)D3 in MetS Cohorts

Values are expressed as median and IQR, and were compared using ANOVA. The line within the box represents the median value; the top and bottom lines of the box reflect the 25th and 75th percentiles respectively; and the top and bottom vertical lines outside the boxes represent the 10th and 90th percentiles, respectively. Abbreviations: ANOVA, analysis of variance; IQR, interquartile range.

We speculated that 25(OH)D3 could be involved in the pathogenesis of MetS and CV complications, affecting the molecular mechanisms of the proliferation, differentiation, and function of angiogenic progenitor mononuclear cells. We also noticed that vitamin D status was closely associated with age, BMI, dyslipidemia, and HOMA-IR, as well as the serum levels of inflammatory biomarkers (osteoprotegerin, hs-CRP, and adiponectin). Interestingly, after adjustment for BMI and age, plasma levels of 25(OH)D3 continued to be linked to the numbers of angiogenic progenitor mononuclear cells and inflammatory biomarkers.

Recent studies have shown that 25(OH)D3 plays a pivotal role in the formation of capillary-like structures, which are considered a definite step in angiogenesis de novo (36, 37). Because mononuclear progenitor cells participate in maintaining endothelial integrity and vascular homeostasis, 25(OH)D3 deficiency may contribute to vascular dysfunction in MetS through altered differentiation of mononuclear progenitors into mature endothelial cells (38). The results of our study showed that deficiency of 25(OH)D3 may be associated with impaired progenitor mononuclear patterns in MetS patients by enhancing IR development and low-grade inflammation.

Probable glucose toxicity via the epigenetic effect may contribute to IR in mononuclear progenitor cells (39). Indeed, a negative impact of 25(OH)D3 as an inhibitor of neo-vascularization and cell migration on the pattern of angiogenic progenitor mononuclear cells may occur when up-regulated inflammatory cascade cytokines are present. It is difficult to explain why 25(OH)D3 deficiency can lead to controversial effects among these settings. It has been reported that most metabolically healthy obese individuals do not exhibit a high risk of diabetes development where IR is present (40). In addition, the numbers of angiogenic progenitor mononuclear cells appear to be increased in the MetS patient population (41). In contrast, MetS individuals have shown decreased levels of progenitor mononuclear cells with angiogenic immune phenotypes (42), which independently predict CV and atherosclerotic disease progression (43, 44). This may suggest that vitamin D deficiency in MetS subjects could lead to an exacerbation of inflammation through changes to the endothelium repair system, i.e., angiogenic progenitor mononuclear cells. The causative relationship between vitamin D deficiency and depleted numbers of circulating progenitor mononuclear cells with angiogenic immune pheno-

Table 2. Circulating Biomarkers and Endothelial-Derived Microparticles in MetS Patients^{a,b,c}

Variables	Entire cohort of MetS patients (n = 47)	Plasma levels of 25(OH)D3				P value
		> 100 nmol/L (n = 10)	50 to 100 nmol/L (n = 12)	30 to 50 nmol/L (n = 14)	< 30 nmol/L (n = 11)	
HbA1c, %	6.82 (4.61 - 5.37)	6.75 (4.58 - 5.30)	6.79 (4.60 - 5.34)	6.83 (4.57 - 5.35)	6.87 (4.72 - 5.41)	0.038
Fasting blood glucose, mmol/L	5.46 (4.23 - 4.76)	5.44 (4.24 - 4.72)	5.45 (4.21 - 4.77)	5.46 (4.26 - 4.80)	5.47 (4.25 - 4.71)	0.22
Insulin, μ U/mL	14.2 (12.5 - 15.7)	14.0 (12.3 - 15.2)	14.2 (12.1 - 15.0)	14.3 (12.0 - 15.8)	14.3 (12.9 - 16.1)	0.49
HOMA1R, mmol/L \times μ U/mL	3.45 (3.22 - 3.78)	3.38 (3.19 - 3.72)	3.41 (3.20 - 3.71)	3.47 (3.20 - 3.75)	3.48 (3.29 - 3.80)	0.042
Creatinine, μ mol/L	72.3 (56.1 - 86.9)	72.3 (56.1 - 86.9)	70.5 (53.7 - 83.2)	72.9 (56.0 - 86.5)	74.1 (59.2 - 91.4)	0.66
Total cholesterol, mmol/L	5.3 (4.5 - 5.9)	5.2 (4.4 - 5.7)	5.3 (4.5 - 5.7)	5.3 (4.6 - 6.0)	5.4 (4.5 - 6.1)	0.72
LDL-C, mmol/L	3.48 (3.30 - 4.07)	3.45 (3.29 - 4.04)	3.46 (3.30 - 4.06)	3.48 (3.28 - 4.10)	3.48 (3.29 - 4.08)	0.62
HDL-C, mmol/L	1.01 (0.90 - 1.13)	1.09 (1.01 - 1.15)	1.06 (0.92 - 1.12)	1.01 (0.90 - 1.10)	1.01 (0.88 - 1.06)	0.036
TG, mmol/L	1.77 (1.62 - 1.95)	1.47 (1.32 - 1.63)	1.56 (1.44 - 1.68)	1.78 (1.50 - 2.13)	1.99 (1.53 - 2.19)	0.044
hs-CRP, mg/L	7.87 (4.92 - 9.43)	7.65 (4.90 - 9.15)	7.80 (5.03 - 9.44)	7.88 (4.99 - 9.73)	7.90 (5.11 - 10.25)	0.22
sRANKL, pg/mL	24.10 (14.7 - 36.9)	19.70 (13.82 - 30.10)	21.40 (15.1 - 32.7)	22.90 (15.3 - 33.4)	27.60 (15.90 - 37.15)	0.18
Osteoprotegerin, pg/mL	718.5 (572.1 - 846.2)	693.2 (551.1 - 811.6)	703.9 (565.1 - 822.6)	715.2 (580.2 - 850.1)	727.6 (583.5 - 870.1)	0.028
Adiponectin, mg/L	13.61 (9.74 - 22.35)	11.55 (8.87 - 14.92)	12.87 (9.15 - 16.62)	13.25 (9.80 - 17.75)	17.90 (9.97 - 22.76)	0.018
CD45 ⁺ CD34 ⁺ , cells/ μ L	0.060 (0.055 - 0.066)	0.062 (0.056 - 0.068)	0.061 (0.054 - 0.068)	0.060 (0.053 - 0.066)	0.057 (0.052 - 0.064)	0.06
CD14 ⁺ CD309 ⁺ , cells/ μ L	0.335 (0.257 - 0.418)	0.359 (0.270 - 0.425)	0.341 (0.263 - 0.416)	0.335 (0.234 - 0.402)	0.329 (0.257 - 0.418)	0.10
CD14 ⁺ Tie-2 ⁺ , cells/ μ L	0.031 (0.021 - 0.040)	0.036 (0.028 - 0.044)	0.033 (0.024 - 0.042)	0.028 (0.019 - 0.038)	0.028 (0.019 - 0.036)	0.07
D309 ⁺ Tie-2 ⁺ , cells/ μ L	0.022 (0.017 - 0.032)	0.027 (0.021 - 0.032)	0.025 (0.019 - 0.030)	0.022 (0.016 - 0.028)	0.021 (0.014 - 0.027)	0.12
CD14 ⁺ D309 ⁺ Tie-2 ⁺ , cells/ μ L	0.032 (0.025 - 0.040)	0.039 (0.032 - 0.047)	0.035 (0.028 - 0.044)	0.030 (0.022 - 0.041)	0.028 (0.016 - 0.033)	0.026

Abbreviations: ANOVA, analysis of variance; hs-CRP, high sensitive C reactive protein; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; sRANKL, serum receptor activator of NF- κ B ligand; TG, triglycerides.

^aValues are expressed as mean \pm SE, 95% CI, median and 25 - 75% IQR.

^bCategorical variables are expressed as numbers (n) and percentages (%).

^cP-value is a comparison of mean or median variables between both cohorts (ANOVA test).

Table 3. The Impact of Vitamin D Status and Inflammatory Cytokines on the Numbers of CD14⁺D309⁺Tie-2⁺ Cells; The Results of Univariate and Multivariate Age- and BMI-Adjusted Log-Linear Regression Analysis

Variables	Dependent Variable: Numbers of CD14 ⁺ D309 ⁺ Tie-2 ⁺ Cells					
	Univariate Log-Linear Regression			Multivariate Log-Linear Regression		
	OR	95% CI	P Value	OR	95% CI	P Value
25(OH)D3 < 30 nmol/L vs \geq 30 nmol/L	1.16	1.05 - 1.23	0.001	1.12	1.06 - 1.19	0.002
25(OH)D3 < 50 nmol/L vs \geq 50 nmol/L	1.06	1.01 - 1.12	0.001	1.04	0.99 - 1.07	0.10
25(OH)D3 < 100 nmol/L vs \geq 100 nmol/L	1.02	0.98 - 1.05	0.46		Not entered	
OPG per 225 pg/mL	1.12	1.06 - 1.19	0.001	1.10	1.04 - 1.15	0.003
HbA1c	1.03	1.00 - 1.06	0.28		Not entered	
hs-CRP per 2.5 mg/L	1.08	1.05 - 1.13	0.003	1.05	1.02 - 1.09	0.001
Adiponectin per 1.5 mg/L	1.05	1.02 - 1.09	0.001	1.03	1.01 - 1.06	0.046
sRANKL per 2.5 pg/mL	1.04	1.02 - 1.07	0.001	1.02	1.00 - 1.04	0.054
Dyslipidemia	1.02	0.98 - 1.05	0.68		Not entered	

Abbreviations: BMI, body mass index; CI, confidence interval; hs-CRP, high-sensitive C-reactive protein; OPG, osteoprotegerin; OR, odds ratio; sRANKL, serum receptor activator of NF- κ B ligand.

types requires more investigation in the future.

In conclusion, we found that low levels of 25(OH)D3 may be associated with depleted numbers of proangiogenic progenitor mononuclear cells in MetS patients. Further research is required to explain the link between vi-

tamin D status and the numbers of circulating angiopoietic progenitor mononuclear cells in MetS patients without known CV disease.

Table 4. C-Statistics for Models With Plasma Levels of 25(OH)D3 < 30 nmol/L With hs-CRP, OPG, and Adiponectin as Continuous Variables^a

Models	Dependent Continuous Variable: Numerous of CD14 ⁺ D309 ⁺ Tie-2 ⁺ Cells				
	AUC (95% CI)	ΔAUC	P	IDI ± SE	Relative IDI (%)
Model 1 (base model: plasma levels of 25(OH)D3 < 30 nmol/L)	0.676	-	-	-	-
Model 1 + OPG vs Model 1	0.690	0.014	0.46	0.02 ± 0.010	2.2
Model 1 + hs-CRP vs Model 1	0.685	0.009	0.74	0.01 ± 0.008	1.7
Model 1 + OPG + hs-CRP vs Model 1	0.689	0.013	0.48	0.02 ± 0.009	1.9
Model 1 + adiponectin vs Model 1	0.682	0.006	0.78	0.01 ± 0.006	1.6
Model 1 + adiponectin + OPG vs Model 1	0.694	0.018	0.12	0.02 ± 0.008	2.9
Model 1 + hs-CRP + OPG + adiponectin	0.720	0.044	< 0.05	0.03 ± 0.011	7.2

Abbreviations: AUC, area under curve; hs-CRP, high sensitive C-reactive protein; OPG, osteoprotegerin; SE, standard error.

^aRelative IDI calculated as the ratio of IDI over the discrimination slope of the model without plasma levels of 25(OH)D3 < 30 nmol/L.

5.1. Study Limitations

This study has some limitations. Due to the small size of the study population we cannot exclude the possibility that other immune phenotypes of progenitor mononuclear cells might be affected by vitamin D status. Despite the statistically significant findings regarding the prediction of biomarkers, it seems that the results become clinically relevant after adjustments for age and BMI. The predictive role of age and BMI needs to be carefully explored. However, the authors believe that these restrictions should have no significant impact on the interpretation of the study data.

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Footnotes

Authors' Contribution: Alexander E Berezin initiated the hypothesis and designed the study protocol, helped collect, analyze and interpret the data, performed statistical analysis, and wrote the manuscript. Alexander A. Kremzer helped enroll the patients, collected and analyzed the data, and reviewed the source documents. Yulia V. Martovitskaya carried out flow cytometry and analyzed the results of examination. Tatyana A. Berezina helped enroll the patients in the study and helped collect the data.

Conflicts of Interest: None declared.

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