Non-classical CD309/Tie2+ and CD14/Tie2+ Immune Phenotypes of Circulating Endothelial Progenitor Cells Predicts Development of Metabolically Unhealthy Obesity

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Introduction

Obesity is currently considered an increasing disease worldwide strongly associated with multiple metabolic abnormalities and higher cardiovascular (CV) risk [1,2]. Based on the Adult Treatment Panel-III criteria subjects with established obesity and co-existing other metabolic abnormalities including dyslipidemia, insulin resistance (IR), increased fasting glucose and impaired glucose tolerance, are referred metabolically non-healthy, whereas obese individuals without these abnormalities might be defined as metabolically healthy [3,4]. The mechanisms underlying the change in phenotype from metabolically healthy to metabolically unhealthy obesity are still unclear [5,6].

Circulating endothelial progenitor cells (EPCs) derived from bone marrow and peripheral tissues and labeled as CD34+ cells co-expressing VEGFR2 and/or CD133 [7] are actively involved into vascular repair and attenuation of endothelial function in several settings [8]. Recent pre-clinical and clinical studies have shown that low number and/or weak functionality (i.e., reduced ability to mobbing, proliferation, differentiation, adhesion, migration, incorporation into cellular structures, and consequently survival) of EPC known as “impaired phenotype” were found in type two diabetes mellitus (T2DM), metabolic syndrome (MetS), CV disease [9,10], whereas the EPCs’ dysfunction in the patients with metabolically healthy obesity is not plenty clear determined [11]. Additionally, low EPC number was known as useful predictive tool for CV risk stratification in individuals with known CV disease and subjects with T2DM and MetS [12-14]. Whether EPC number would be a tool for screening of the patients with metabolically healthy obesity at higher CV risk is not yet understood. The aim of the study: to investigate the number of circulating endothelial progenitor cells (EPCs) in patients with established metabolically healthy obesity (Met-HO).

Methods

The study was retrospectively evolved 89 patients with established abdominal obesity (47 patients with metabolically unhealthy obesity [Met-UHO] and 42 subjects with Met-HO) from the large cohort of dismetabolic patients (n=268). High-Definition Fluorescence Activated Cell Sorter methodology was performed for measurement of the number of circulating endothelial progenitor cells co-expressed CD45, CD34, CD14, CD309, and Tie-2 antigens.

Results: A significant difference between number of circulating progenitor cells labeled CD45-CD34+ and CD14+CD309+ in Met-UHO and Met-HO patients was found. In contrast, Met-UHO patients had a significantly lower level of circulating CD14+ Tie-2- cells and CD309+ Tie-2-cells compared with Met-HO individuals. In multivariate logistic regression analysis we found that HOMA-IR, hs-CRP, and number of CV risk factors were independent predictors for depletion in numerous of circulating progenitor cells with immune phenotypes CD309/Tie2+ cells and CD14/Tie2+.

Conclusion: in this investigation, we found that the lowered circulating number of CD309/Tie2+ cells / CD14/Tie2+ cells produces the well balanced discrimination on Met-HO development in Met-UHO patients than other models based on conventional CV risk factors.

Keywords: Metabolic syndrome, Metabolically healthy obesity, Metabolically unhealthy obesity, Circulating endothelial progenitor cells
obesity (47 patients with metabolically unhealthy obesity determined as MetS and 42 subjects with metabolically healthy obesity) from the large cohort of dismetabolic patients (n=268) who were examined between February 2012 and July 2016. Thirty five healthy volunteers were involved in the study as a control cohort. We have enrolled obese subjects (body mass index was more 30 kg/m²) without known CV disease including angina pectoris, previous myocardial infarction / stroke, heart failure, and asymptomatic atherosclerosis (defined by negative result of the contrast-enhanced multispiral tomography angiography). Therefore, individuals with known T2DM were not enrolled in the study. All patients have given their informed written consent for participation in the study.

MetS was diagnosed based on the National Cholesterol Education Program Adult Treatment Panel III criteria [15]. Patients were enrolled in the MetS cohort when at least three of the following components were defined: waist circumference ≥90 cm or ≥80 cm in men and women respectively; high density lipoprotein (HDL) cholesterol <1.03 mmol/L or <1.3 mmol/L in men and women respectively; triglycerides ≥1.7 mmol/L; blood pressure ≥130/85 mmHg or current exposure of antihypertensive drugs; fasting plasma glucose ≥5.6 mmol/L. Participants who had 2 criteria and less of MetS were classified as metabolically healthy obese patients. Those who had 2 or more criteria of MetS were classified as metabolically abnormal and were not considered candidates for this study. Flow chart with including / excluding criteria of the study is reported (Figure 1).

Smoking status

Current smoking was defined as consumption of one cigarette daily for three months [16].

Anthropometric measurements

Anthropometric measurements (weight, height, body mass, body mass index, waist circumference, and waist-to-hip ratio) were made using standard procedures [17,18]. Height and weight were measured by professional health staff with the participants standing without shoes and heavy outer garments with a wall-mounted stadiometer (OMRON, Japan). Body mass index (BMI) was calculated as weight (kg) divided by height squared (m²). Waist circumference was measured at the level midway between the lower rib margin and the iliac crest with participants in a standing position without heavy outer garments and with emptied pockets, breathing out gently. Hip circumference was recorded as the maximum circumference over the buttocks.

Transthoracic B-mode echocardiography

Transthoracic B-mode echocardiography was performed according to a conventional procedure on ACUSON scanner (SIEMENS, Germany) using phased probe with modulated frequency of 2.5-5 MHz. Left ventricular (LV) mass was estimated using formula recommended American Society of Echocardiography’s Guidelines and Standards Committee and the Chamber Quantification Writing Group [19]. LV hypertrophy (LVH) was defined as a LV mass / body surface area (BSA) ≥ 96 g/m², for women, and ≥ 116 g/m², for men [20].

Calculation of glomerular filtration rate

Glomerular filtration rate (GFR) was calculated with CKD-EPI formula [21].

Measurement of circulating biomarkers

To determine circulating biomarkers, blood samples were

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Figure 1: Flow chart with including / excluding criteria of the study

Abbreviations: MetS, metabolic syndrome; PCOS, polycystic ovary syndrome; NCEP, National Cholesterol Education Program; CV, cardiovascular.
collected at baseline in the morning (at 7-8 a.m.) after at least 10 h fasting into cooled silicone test tubes wherein 2 mL of 5% Trilon B solution were added. Then they were centrifuged upon permanent cooling at 6,000 rpm for 3 minutes. Plasma was collected and refrigerated immediately to be stored at a temperature -70°C.

High-sensitive C-reactive protein (hs-CRP) and adiponectin were measured by commercially available standard kit (R&D Systems GmbH, Wiesbaden-Nordenstadt, Germany). The intra-assay and inter-assay coefficients of variation were <5%. The lower detection limit of insulin level was 1.39 pmol/L.

Fasting insulin level was measured by a double-antibody sandwich immunoassay (Elecsys 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 level was 1.39 pmol/L.

Insulin resistance was assessed by the homeostasis model assessment for insulin resistance (HOMA-IR) [22] using the following formula:

\[ \text{HOMA-IR} = \frac{\text{fasting insulin (µU/mL)}}{\text{fasting glucose (mmol/L)}} \times 22.5 \]

IR was arbitrarily defined as a homeostasis model assessment-IR index (HOMA-IR) value above the 75th percentile of normal glucose tolerance equal 2.45 mmol/L × µU/mL.

Hemoglobin A1c (HbA1c) were determined by high-pressure liquid chromatography method. Concentrations of total cholesterol (TC), cholesterol of high-density lipoproteins (HDL-C), triglycerides (TG), and low-density lipoproteins (LDL-C) were measured by direct enzymatic method (Roche P800 analyzer, Basel, Switzerland).

Quality control was assessed daily for all determinations.

Blood sampling for measurement of circulating endothelial progenitor cells and mononuclear progenitor cells

Blood samples were received from peripheral vein in blood collection tubes. Each sample contains 75 μL into 1mL PBS containing 5 μM EDTA (10 μL of 0.5 M stock) To prevent clotting, samples were mixed immediately. Peripheral blood mononuclear cells were removed using density gradient centrifugation with Ficoll-Paque (Miltenyi Biotec Inc., Germany). After layer 35 mL of diluted cell suspension over 15 mL of Ficoll-Paque in a 50 mL conical tube all blood samples with anticoagulants (EDTA) have centrifuged at 400×g for 30–40 minutes at 20°C in a swinging-bucket rotor without brake. The upper layer leaving the mononuclear cell population, co-expression with Tie-2 and/or CD309+ was determined using quadrant analysis. Cell fluorescence was measured immediately after staining, and data were analyzed with the use of Cell Quest software (FACS Calibur, Becton Dickinson). Units of all measured components are absolute cell counts obtained after the measurement of 500,000 events. Figure 2 represents a sample of FACS results for CD45+ CD34+, CD309+/antiTie2-, CD14+/CD309+, CD14+/antiTie2- EPCs.

Statistical Analysis

Statistical analysis of the results obtained was performed in SPSS system for Windows, Version 22 (SPSS Inc, Chicago, IL, USA). The data were presented as mean (M) and standard deviation (±SD) or 95% confidence interval (CI); as well as median (Me) and 25%-75% interquartile range (IQR). To compare the main parameters of patient cohorts, two-tailed Student t-test or Shapiro–Wilk U-test were used. To compare categorical variables between groups, Chi2 test (χ2) and Fisher F exact test were used. Univariate and multivariate linear regression models were used to determine a relation between circulating number of progenitor cells and other biomarkers. Predictors of changes in the progenitor cells in abdominal obesity patients were examined in multivariable regression analysis. C-statistics, integrated discrimination improvement (IDI) and net-reclassification improvement (NRI) were utilized for prediction performance analyses. A two-tailed probability value of <0.05 was considered as significant.

Results

The demographic and anthropometric characteristics, the prevalence of CV risk factors are reported in Table 1. There was
not a significant difference between healthy volunteers and entire cohort, as well as between subjects with metabolically unhealthy obesity (Met-UHO) and metabolically healthy obesity (Met-HO) in age, sex, adherence to smoking, and haemodynamic performances. Abdominal obesity subjects exhibited higher BMI, WHR, HOMA-IR and lower adiponectin, as well as they had increased frequency of LVH, hypertension and IR presentation. Met-UHO patients had higher HOMA-IR than those with Met-HO, while BMI, systolic and diastolic blood pressure, heart rate were similar in both cohorts. Additionally, hypertension, IR and dyslipidemia presentation were found frequently in Met-UHO patients compared to Met-HO individuals.

Healthy volunteers and abdominal obesity individuals from...
entire cohort had similar level of GFR, hemoglobin, and creatinine. Consequently, abdominal obesity individuals had higher level of fasting glucose, HbA1c, total cholesterol, LDL cholesterol, triglycerides, uric acid, hs-CRP and lower adiponectin (Table 2). No difference was seen in GFR, hemoglobin, fasting glucose, creatinine, and lipids’ level between Met-UHO and Met-HO patients. However, Met-UHO patients had higher HbA1c, uric acid, and lower hs-CRP than those with Met-HO.

Healthy volunteers had significantly increased level of progenitor cells labeled CD45-/CD34+, CD309+/CD14+ and CD14+/CD309+ than those with abdominal obesity (Figure 3). However, there was not found a significant difference between number of circulating progenitor cells labeled CD45-/CD34+ and CD45+/CD309+ in Met-UHO and Met-HO patients. In contrast, Met-UHO patients had a significantly lower level of circulating CD14+/Tie2+ cells and CD309+/Tie2+ cells compared with Met-HO individuals.

The univariate linear regression analysis between numerous of progenitor cells with immune phenotypes determined CD309+/Tie2+ and CD14/Tie2+ CV risk factors, hemodynamic performances, and other biomarkers was performed. In Met-UHO patients the number of CD309+/Tie2+ cells in peripheral blood inversely related to BMI (r = -0.39, P = 0.001), HOMA-IR (r = -0.35, P = 0.003), hs-CRP (r = -0.34, P = 0.001), number of CV risk factors (r = -0.32, P = 0.001), LDL cholesterol (r = -0.30, P = 0.002), and serum uric acid (r = -0.30, P = 0.001), LV hypertrophy (r = -0.24, P = 0.012), whereas in Met-HO individuals HOMA-IR (r = -0.32, P = 0.001) and number of CV risk factors (r = -0.31, P = 0.001) were seen a significant relation to CD14+/Tie2+ cells’ number. Therefore, the number of CD309+/Tie2+ cells positively related to level of adiponectin in Met-UHO patients (r = 0.33, P = 0.001). In contrast, there was not a significant association between number of CD309+/Tie2+ cells and adiponectin in Met-HO individuals.

The circulating number of CD309+/Tie2+ cells negatively related to BMI (r = -0.42, P = 0.001), HOMA-IR (r = -0.36, P = 0.001), number of CV risk factors (r = -0.34, P = 0.001), hs-CRP (r = -0.34, P = 0.001), LDL cholesterol (r = -0.30, P = 0.002), serum uric acid (r = -0.27, P < 0.05), LV hypertrophy (r = -0.26, P = 0.01) and positively associated with adiponectin (r = 0.36, P = 0.001) in Met-UHO patients. In contrast, in Met-HO cohort number of CD14+/Tie2+ cells significantly related to HOMA-IR (r = -0.34, P = 0.001) and number of CV risk factors (r = -0.32, P = 0.001).

Multivariate linear regression analysis adjusted to BMI has shown that HOMA-IR (r = -0.32, P = 0.001) and r = -0.33, P = 0.001), number of CV risk factors (r = -0.29, P = 0.001 and r = -0.32, P = 0.001), and LDL cholesterol (r = -0.26, P = 0.001 and r = -0.29, P = 0.003) have related well to the number of CD309+/Tie2+ cells and CD14+/Tie2+ cells respectively in Met-UHO patients. In Met-HO individuals the number of circulating CD309+/Tie2+ cells and CD14+/Tie2+ cells related significantly to HOMA-IR (r = -0.23, P = 0.001 and r = -0.26, P < 0.001) respectively.

In multivariate logistic regression analysis we found that HOMA-IR, hs-CRP, and number of CV risk factors were independent predictors for depletion in numerous of circulating progenitor cells with immune phenotypes CD309+/Tie2+ and CD14/Tie2+ (Table 3). The comparison of Met-UHO predictive models based on several biomarkers including hs-CRP, number of conventional CV risk factors, HOMA-IR and circulating number of CD309+/Tie2+ cells and CD14+/Tie2+ cells is reported in Table 4. As one can see, the lowered circulating number of CD309+/Tie2+ cells / CD14+/Tie2+ cells produces the well balanced discrimination on Met-UHO development in Met-HO patients than other models.

Discussion

In this investigations, we found that number of circulating EPCs with non-classical immune phenotypes labeled CD309+/Tie2+ cells and CD14/Tie2+ might be a predictor of Met-UHO development in Met-HO individuals. In contrast, EPCs with classical phenotypes CD45-/CD34+/CD309+ did not associate with Met-UHO. Obese patients present a low number of EPCs labeled as CD45-/CD34+ and CD133+CD309+, and high levels of pro-inflammatory adipocytokines, although this association is correct for Met-UHO, whereas individuals with Met-HO may not be distinguished in circulating EPC number with classical phenotypes. It is an intriguing situation, because recent clinical studies have shown that decreased number of circulating classic

<table>
<thead>
<tr>
<th>Variables</th>
<th>Healthy volunteers (n=35)</th>
<th>Entire patient cohort (n=89)</th>
<th>P value between healthy volunteers and entire patient cohort</th>
<th>Subjects with metabolically unhealthy obesity (n=47)</th>
<th>Subjects metabolically healthy obesity (n=42)</th>
<th>P value between subgroups with obesity</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR, ml/min/1.73 m²</td>
<td>112.4 (102.2 - 123.4)</td>
<td>109.5 (101.2-117.5)</td>
<td>0.11</td>
<td>107.3 (98.7 - 114.1)</td>
<td>112.2 (100.1-118.3)</td>
<td>0.12</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>136.3 (129.8 - 147.2)</td>
<td>134.9 (126.7 - 143.9)</td>
<td>0.86</td>
<td>135.4 (128.5 - 142.1)</td>
<td>134.8 (127.3 - 144.8)</td>
<td>0.68</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>4.24 (3.60-4.91)</td>
<td>5.18 (4.51-5.92)</td>
<td>0.012</td>
<td>5.47 (4.43-5.92)</td>
<td>4.92 (4.1-5.80)</td>
<td>0.18</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>4.78 (4.21-5.15)</td>
<td>5.47 (4.82-5.90)</td>
<td>0.042</td>
<td>5.78 (5.42-6.16)</td>
<td>4.93 (4.54-5.48)</td>
<td>0.022</td>
</tr>
<tr>
<td>Creatinine, µmol/L</td>
<td>65.4 (58.2-81.2)</td>
<td>70.3 (60.7-83.56)</td>
<td>0.74</td>
<td>70.5 (58.7-85.7)</td>
<td>70.1 (60.2-84.5)</td>
<td>0.66</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.56 (3.25-4.88)</td>
<td>5.45 (4.46-6.15)</td>
<td>0.044</td>
<td>5.60 (4.71-6.52)</td>
<td>5.33 (4.32-6.17)</td>
<td>0.12</td>
</tr>
<tr>
<td>HDL Cholesterol, mmol/L</td>
<td>1.03 (0.98 - 1.08)</td>
<td>0.93 (0.90-1.12)</td>
<td>0.046</td>
<td>0.92 (0.88 - 1.13)</td>
<td>0.95 (0.9 - 1.15)</td>
<td>0.22</td>
</tr>
<tr>
<td>LDL Cholesterol, mmol/L</td>
<td>2.77 (2.33 - 3.10)</td>
<td>3.54 (3.34-3.66)</td>
<td>0.012</td>
<td>3.63 (3.21 - 3.70)</td>
<td>3.41 (3.30-3.65)</td>
<td>0.46</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.67 (1.31 - 1.94)</td>
<td>2.27 (2.14 - 2.55)</td>
<td>0.014</td>
<td>2.31 (2.13 - 2.59)</td>
<td>2.24 (2.08 - 2.43)</td>
<td>0.52</td>
</tr>
<tr>
<td>Serum uric acid, µmol/L</td>
<td>295 (210 - 367)</td>
<td>341 (266 - 462)</td>
<td>0.012</td>
<td>345 (253 - 456)</td>
<td>337 (224 - 448)</td>
<td>0.01</td>
</tr>
<tr>
<td>hs-CRP, mg/L</td>
<td>3.27 (0.5 - 33.3)</td>
<td>5.15 (2.09-8.03)</td>
<td>0.001</td>
<td>7.10 (6.25-8.20)</td>
<td>3.04 (1.12-5.42)</td>
<td>0.044</td>
</tr>
<tr>
<td>Adiponectin, mg / L</td>
<td>13.65 (10.12-24.93)</td>
<td>10.12 (6.88-14.95)</td>
<td>0.001</td>
<td>8.36 (5.11-16.77)</td>
<td>11.25 (7.41-16.17)</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Table 2: The biomarkers in the study population
Note: The values correspond to medians and IQR of 25%-75%. Abbreviations: GFR - glomerular filtration rate; HbA1c - glycated hemoglobin, HDL - high-density lipoprotein; LDL - Low-density lipoprotein.
Table 3: The independent predictors of lowered circulating progenitor cell number: The results of BMI-adjusted multivariate logistic regression analysis

<table>
<thead>
<tr>
<th>Factors</th>
<th>CD14&lt;sup&gt;+&lt;/sup&gt; Tie2&lt;sup&gt;+&lt;/sup&gt; cells</th>
<th>CD309&lt;sup&gt;+&lt;/sup&gt;Tie2&lt;sup&gt;+&lt;/sup&gt; cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P Value</td>
</tr>
<tr>
<td>hs-CRP, per 4.50 mg/L</td>
<td>1.03 (1.01-1.06)</td>
<td>0.024</td>
</tr>
<tr>
<td>Number of CV risk factors, per 1 factor</td>
<td>1.02 (1.00-1.05)</td>
<td>0.046</td>
</tr>
<tr>
<td>HOMA-IR, per 0.65 mmol/L × µU/mL</td>
<td>1.06 (1.02-1.09)</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; IQR, inter quartile range; BMI, Body mass index; hs-CRP, high sensitive C reactive protein; OR, odds ratio; HOMA-IR, homeostatic model assessment of insulin resistance index; IR, insulin resistance.

Table 4: Statistics for model fit for the prediction of transformation of Met-HO to Met-UHO Abbreviations: AUC, area under the curve; 95% CI, 95% confidence interval; NRI, net reclassification index; IDI, integrated discrimination index; HOMA-IR, homeostatic model assessment of insulin resistance index; EPCs, endothelial progenitor cells.

<table>
<thead>
<tr>
<th>Predictive Models</th>
<th>AUC</th>
<th>NRI</th>
<th>IDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Based Model: Met-HO</td>
<td>0.58 (0.52 - 0.63)</td>
<td>-</td>
<td>Reference</td>
</tr>
<tr>
<td>Based Model + hs-CRP</td>
<td>0.60 (0.54 - 0.65)</td>
<td>0.48</td>
<td>0.31</td>
</tr>
<tr>
<td>Based Model + number of CV risk factors</td>
<td>0.63 (0.52 - 0.70)</td>
<td>0.21</td>
<td>0.34</td>
</tr>
<tr>
<td>Based Model + HOMA-IR</td>
<td>0.70 (0.58 - 0.81)</td>
<td>0.06</td>
<td>0.38</td>
</tr>
<tr>
<td>Based Model + decreased number of CD309/Tie2&lt;sup&gt;+&lt;/sup&gt; and CD14/Tie2&lt;sup&gt;+&lt;/sup&gt; cells</td>
<td>0.73 (0.62 - 0.83)</td>
<td>0.12</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Figure 3: The number of circulating endothelial cell-derived and mononuclear cell-derived progenitors (cells /µL) in abdominal obesity patients and healthy volunteers.

Abbreviations: Met-UHO, metabolically unhealthy obesity; Met-HO, metabolically healthy obesity.
phenotype EPCs in patients with Met-UHO may predict MetS and T2DM development [25-27]. Moreover, there is a large body of evidence regarding being of positive correlations between adipokynes (i.e. adiponectin, omentin, visfatin), adipose tissue content and depletion of EPCs [14,28,29]. It has suggested that the lowered number and weak activity of EPCs associate with the poor regeneration processes in the vessels worsening angiogenesis and endothelial function [9,10]. Whether the effect of adipocytes and adipoptysis on entire progenitor cell populations is not fully clear and it is under scientific discussion.

The identification of biomarkers to predict a transformation of Met-HO to Met-UHO is promised, while the conventionally used biomarkers (i.e., i.e. fasting plasma glucose, 2 h plasma glucose, HbA1c, BMI, homeostasis model assessment of β-cell function, homeostasis model assessment of insulin sensitivity, fasting insulin, serum visfatin, omentin, and oxidative stress components) are considered to play a leading role to progression of Met-UHO into T2DM [30,31]. In contrast, EPCs are positively related to markers of adiposity in abdominal obesity, when not complicated by T2DM. Additionally, circulating number of EPCs with classic phenotype is inversely correlated with carotid intima-media thickness, confirming their protective role also in abdominal obesity [32]. In the study we first investigated the interrelation between circulating number of non-classical EPCs and Met-UHO to Met-HO. Our results clarified that the deficiency of CD309/Tie2+ cells and CD14/Tie2+ EPCs might be identified in patients with Met-HO as an early marker of further metabolic complications served as causes to transformation of Met-HO to Met-UHO. Interestingly, that in Met-UHO there was not found sufficient association between number of CD309/Tie2+ cells and CD14/Tie2+ EPCs and other markers of obesity apart from BMI and IR. Indeed, factors that contribute to the continuing risk of Met-HO-to-Met-UHO transformation affect endothelial repair and progenitor cell health, which may closely bound with IR [33].

We hypothesize that at early stage of abdominal obesity IR of progenitor cells originated from peripheral blood mononuclears might be a cause of weak functionality of EPCs leading to poor reparative ability and shaping endothelial dysfunction. Later at development of Met-UHO the broad spectrum of white adipose tissue related pro-inflammatory cytokines (predominantly interleukin-6, tumor necrosis factor-alpha, leptin) via NF-kB activation might stimulate and sustain mobbing and differentiation of EPCs [34]. Thus, non-classic phenotype EPCs may mitigate the vascular reparation and attenuate the endothelial function, but shaping deficiency of EPCs mediates worsening angiopoetic ability of progenitor cells disrupting the balance between endothelial damage and repair [35]. Consequently all these might associate with higher risk of future CV disease [36].

We suggest that the use of CD309/Tie2+ cells and CD14/Tie2+ EPC measurement might be a biomarker of higher risk of Met-UHO, as well as additional risk factor of CV events. However, the large clinical investigations are required to dearly explain the role of EPCs with non-classical phenotypes in development of abdominal obesity.

Conclusions

A number of circulating EPCs with non-classical immune phenotypes labeled CD309/Tie2+ cells and CD14/Tie2+ might discuss as a key mediator between abdominal obesity and vascular injury determining transformation of Met-UHO into Met-HO. Although there is the commonly used clinically validated biomarkers to identify CV risk in abdominal obesity, discovery of the novel biomarkers of endothelial reparation could improve our scoring of the Met-HO and Met-UHO patients requiring personalized CV care. The matter of metabolic modification of Met-HO into Met-UHO is currently uncertain and requires more investigations in future.

Study limitations

This study has some limitations. The first limitation is low number of the patient involved in the investigation. Another limitation affects the methods of isolation and determination of EPCs. Although HD-FACS methodology is widely used, this method is not standardized. Therefore, there are some overlaps between centrifugation velocity and further determination of EMPs that might reflect some obstacles for results' interpretation. The authors suppose that these restrictions might have no significant impact on the data interpretation.

Acknowledgement

We thank all patients for their participation in the investigation, staff of the Regional Zaporozhie Hospital (Ukraine) and the doctors, nurses, and administrative staff in City hospital #6 (Zaporozhie, Ukraine), City Hospital #10 (Zaporozhie, Ukraine), Private Clinic “Vita Center” (Zaporozhie, Ukraine), Regional Center of Cardiovascular Diseases (Zaporozhie, Ukraine) general practices, and site-managed organizations that assisted the study.

Abbreviations

AUC - Area Under the Curve 
BMI - Body Mass Index 
CI - Confidence Interval 
CV - Cardiovascular 
EPCs - Endothelial Progenitor Cells 
PSC - Forward Scatter Characteristic 
GFR - Glomerular Filtration Rate 
HD-FACS - High-Definition Fluorescence Activated Cell Sorter 
HDL-C - High-Density Lipoprotein Cholesterol 
hs-CRP - High Sensitive C-reactive Protein 
IDI - Integrated Discrimination Index 
LDL-C - Low-density Lipoprotein Cholesterol 
Met-HO - Metabolically Healthy Obesity 
MetS - Metabolic Syndrome 
Met-UHO - Metabolically Unhealthy Obesity 
NCEP - National Cholesterol Education Program 
NRI - Net Reclassification Index 
OR – Odds Ratio 
PCOS - Polycystic Ovary Syndrome 
SSC - Side Scatter Characteristic
Authors’ Contributions

Alexander Berezin initiated the hypothesis and designed the study protocol, contributed to collect, analyze and interpret the data, performed statistical analysis, wrote the manuscript and approved final version of the paper. Alexander Kremzer enrolled the patients; collected and analyzed the data reviewed the source documents. Tatyana Berezina contributed to enroll the patients in the study and collected the data. Yulia Martovitskaya took in performing of cytofluometry and interpreted of the obtained results. All authors read the manuscript before submitting and agree with final version of the paper.

References
