The Immune Phenotypes of Circulating Microparticles in Patients with Type Two Diabetes Mellitus: Relevance to Serum Galectin-3 Level


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Abstract

Background: Galectin-3 (Gal-3) is a member of a family of soluble β-galactoside-binding lectins that mediates pro-fibrotic, pro-inflammatory, and pro-oxidative effects on tissues. Elevated level of Gal-3 is reported to be higher in patients with obesity, pre diabetes, and type two diabetes mellitus (T2DM). However, the interrelation of microparticles (MPs) with accelerating atherosclerosis among T2DM patients depending gal-3 level is still not clear. The aim of the study is to investigate the interrelationship between pattern of circulating MPs and serum Gal-3 in T2DM patients with asymptomatic atherosclerosis.

Methods: The study evolved a total of 103 patients with T2DM (54 subjects without documented coronary atherosclerosis and 49 patients with angiographic evidence of asymptomatic coronary atherosclerosis) who were undergone a contrast-enhanced multispiral tomography angiography prior to study entry and 35 healthy volunteers. To determine circulating biomarkers, blood samples were collected at baseline. MPs were labeled and characterized by flow cytometry.

Results: Circulating levels of MP originated apoptotic endothelial cell-derived were significantly increased in diabetic patients as compared with normal subjects, but level of activated endothelial cell-derived MPs was lower than in healthy volunteers. Gal-3 levels were correlated with normal subjects, but level of activated endothelial cell-derived were significantly increased in diabetic patients as compared with healthy volunteers.

Conclusions: Among T2DM patients an increased level of CD31+/annexin V+ MPs and Gal-3 was significantly associated with asymptomatic atherosclerosis.

Keywords: Diabetes Mellitus; Circulating microparticles; Cardiovascular risk factors; Galectin-3

Introduction

Type two diabetes mellitus (T2DM) is an important risk factor for the development and progression of cardiovascular (CV) diseases [1]. Increasing incidences of pre-diabetes and diabetes worldwide require improving methods regarding screening and CV risk stratification of the patients in general population and among subject cohorts with documented metabolic disorders. Obviously, the biomarker strategy appears to be attractive as a scoring method for identification of the subjects at high CV risk [2]. Currently there are various biomarkers with possible predictive value that might be useful in T2DM [3]. Galectin-3 (Gal-3) is a member of a family of soluble β-galactoside-binding lectins that mediates pro-fibrotic, pro-inflammatory, and pro-oxidative effects on tissues [4]. Recent clinical trials have been shown that increased circulating level of Gal-3 is a marker of cardiac fibrosis, vascular stiffening, renal dysfunction, and that it predicts incident of heart failure and cardiovascular atherothrombotic events [5-7]. Therefore, elevated level of Gal-3 is reported to be higher in patients with obesity, pre diabetes, and T2DM [8]. Although Gal-3 predicts cardiovascular outcomes in the general population and among subjects with T2DM [9], the mechanisms responsible for this effect are still not exactly understood and appear to be controversial. It has been suggested that elevated Gal-3 may contribute toward adverse outcomes through an effect on vascular remodeling and endothelial dysfunction beyond general inflammatory changes [10]. Conversely, low levels of serum Gal-3 are closely associated with insulin resistance in T2DM patients [11,12].

Accelerating atherosclerosis among T2DM patients may mediate by microparticles (MPs), which are frequently involved in controversial processes, i.e. 1) repair of vasculature, and 2) tissue injury, inflammation and thrombosis [13]. Extracellular MP are microvesicles with sizes ranging between 50 and 1000 nm released from plasma membrane of wide variety of cells by cytokine stimulation, apoptotic agents, mononuclear cooperation, coagulation, and shear stress stimuli [14]. In fact, elevated levels of platelet-, endothelial cell-, and mononuclear cell-derived MPs were found in patients with T2DM [15,16]. However, the role of MPs in T2DM patients as an inductor of development and progression of atherosclerosis remains not fully clear [17,18]. We hypothesized that imbalance between numerous of circulating MP originated from various cells due to activation or apoptosis might relate to serum Gal-3 level and thereby mediates the risk of asymptomatic atherosclerosis in T2DM subjects. The aim of the study is to investigate the interrelationship between pattern of circulating MPs and serum galectin-3 in T2DM patients with asymptomatic atherosclerosis.

Methods

The study evolved a total of 103 patients with T2DM (54 subjects without documented coronary atherosclerosis and 49 patients with angiographic evidence of asymptomatic coronary atherosclerosis) who were undergone a contrast-enhanced multispiral tomography angiography prior to study entry and 35 healthy volunteers who...
were examined in three of our centers (City Hospital # 6, Regional Center of Cardiovascular Diseases, and Regional Zaporozhye Hospital located in Zaporozhye, Ukraine) between February 2013 and November 2014. Patients with typical anginal signs and symptoms, subjects with clinical evidences of pre-existing coronary artery disease, i.e. myocardial infarction / acute coronary syndrome, heart failure, atrial fibrillation, any atherothrombotic events, as well as patients with declined glomerular filtration rate < 60 mL/min/1.73 m², and candidates for insulin therapy were excluded from the study.

All the patients have given their voluntary informed written consent for participation in the study. The study was approved by the local ethics committee of State Medical University, Zaporozhye, Ukraine. The study was performed in conformance with the Declaration of Helsinki.

**Study design: retrospective cohort study**

T2DM was diagnosed with revised criteria provided by American Diabetes Association when source documents were reviewed [19]. When one or more of the following components were found (glycated hemoglobin [HbA1c] ≥ 6.5%; fasting plasma glucose ≥ 7 mmol/L; 2-h plasma glucose ≥ 11.1 mmol/L during an oral glucose tolerance test; a random plasma glucose ≥ 11.1 mmol/L; exposure of insulin or oral antidiabetic drugs; a previous diagnosis of T2DM) T2DM was determined.

Current smoking was defined as consumption of one cigarette daily for three months. Anthropometric measurements were made using standard procedures.

No untreated subjects were enrolled. Patients with T2DM were treated with life-style modification, diet and orally taken antidiabetic drugs except sulfonylurea derivates and glitazones. Metformin in monotherapy or in combination with glinides and / or gliptines was given in individually optimized daily doses to be achieving full or partly full control for T2DM. Therefore, insulin was not used in enrolled patients.

**Methods for visualization of coronary arteries**

Contrast-enhanced multiphasial computed tomography angiography has been performed for all the patients with T2DM prior to their inclusion in the study on Optima CT660 scanner (GE Healthcare, USA) using non-ionic contrast Omnipaque (Amersham Health, Ireland) [20]. Obtained results were interpreted by cardiologist and one of sub-investigator independently each other before study entry. Atherosclerosis was determined when plaques in at least of one coronary artery were visualized.

**Cardiovascular Risk Calculation**

A 10-year cardiovascular risk for study patients was calculated using the Framingham General Cardiovascular Risk Score (2008) by on-line calculator and interpreted using contemporary approaches [21].

**Calculation of glomerular filtration rate**

Glomerular filtration rate (GFR) was calculated with CKD-EPI formula [22].

**Measurement of circulating biomarkers**

To determine circulating biomarkers, blood samples were collected at baseline in the morning (at 7-8 a.m.) into cooled silicone test tubes wherein 2 mL of 5% Trilinol 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. Circulating Gal-3 level was determined by ELISA method (Bender Med Systems GmbH, Vienna, Austria). High-sensitive C-reactive protein (hs-CRP) was measured by commercially available standard kit (R&D Systems GmbH, Wiesbaden-Nordenstadt, Germany). The inter-assay coefficients of variation were as follows: Gal-3: 4.6%; hs-CRP: 4.4%.

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) [23] using the following formula:

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

Concentrations of total cholesterol (TC), cholesterol of high-density lipoproteins (LDL-C), and cholesterol of high-density lipoproteins (HDL-C) were measured by enzymatic colorimetric method according standardized methodology on Beckman Synchrone LX20 chemistry analyzer.

Direct Enzymatic HbA1c Assay was used for glycated hemoglobin A1c (HbA1c) measurements on Beckman Synchrone LX20 chemistry analyzer.

**Determination of MP populations**

Circulating MPs were isolated from 5 ml of venous citrated blood drawn from the fistula-free arm. To prevent contamination of samples platelet-free plasma (PPP) was separated from whole blood. PPP was centrifugated at 20,500 × rpm for 30 min. MP pellets were washed with DMEM (supplemented with 10 µg/mL polymyxin B, 100 U of streptomycin, and 100 U/ml penicillin) and centrifuged again (20,500 rpm for 30 min). The obtained supernatant was extracted, and MP pellets were re-suspended into the remaining 200 µL of supernatant. PPP, MPs, and supernatant were diluted five-, 10-, and five-fold in PBS, respectively. Only 100 µL of supernatant was prepared for further analysis through incubation with different fluorochrome-labeled antibodies or their respective isotypic immunoglobulins (Beckman Coulter).

MPs were labeled and characterized by flow cytometry technique per HD-FACS (High-Definition Fluorescence Activated Cell Sorter) methodology independently after supernatant diluted without freeze [24].

CD14+ was used as a more specific marker of platelets, and CD64+ was considered a more specific marker of monocytes. CD31 antigen was determined as essential marker for endothelial cells, platelets, and leukocytes. CD144+ was used to identify a pure population of endothelial cells. CD31+/annexin V+ and CD144+/CD31+/annexin V+ MPs were defined as apoptotic endothelial cell-derived MPs, and MPs labeled for CD105+ or CD62E+ were determined as MPs produced due to activation of endothelial cells [25]. We used anti–CD31 [platelet endothelial cell adhesion molecule [PECAM]-1]-phycocerythrin (PE; 20 µl/test), anti–CD41a-PC5 (10 µl/test), anti–CD144– [vascular endothelial [VE]-cadherin]-allophycocyanin [APC] (10 µl/test), anti–CD64–FITC (20 µl/test), anti–CD105–FITC (20 µl/test), and anti–CD62E– [E-selectin]-FITC (20 µl/test) antibodies obtained from Beckman Coulter. MPs that expressed phosphatidylserine were labeled using fluorescein-conjugated Annexin V solution (20 µl/test; BD Biosciences, USA) in the presence of CaCl2 (5 mM) according to the recommendation of the supplier.

The samples were incubated in the dark for 15 min at room temperature according to the manufacturer's instructions. It was performed the analysis of area, height, and width forward scatter (FSC) and side scatter (SSC) parameters as well as side scatter width (SSC-W). The gate for MPs was defined by size, using 0.5 and 1.0 µm beads (Sigma, St Louis, MO, USA). For each sample, 500 thousand events have been analyzed. Compensation tubes were used with similar reagents as were used in the sample tubes. Data were constructed as numerous of MPs depending on marker presentation (positive or negative) and determination of MP populations.

Calculation of the number of MPs per liter plasma was based upon the particle count per unit time, the flow rate of the flow cytometer, and the net dilution during sample preparation of the analyzed MP suspension. MP-exposed antigen concentrations were calculated in each sample by multiplying the total concentration of positive MPs by the mean fluorescence intensity of the antigen exposure of the total positive MP population.

Statistical Analysis

Continuous variables were expressed as mean (M) and standard deviation (± SD) or 95% confidence interval (CI); as well as median (Me) and 25%-75% interquartile range (IQR). Categorical variables are given as number (n) and percentage (%). To compare the main parameters of patient cohorts, two-tailed Student t-test or Mann-Whitney U-test were used. To compare categorical variables between groups, Chi² test (χ²) and Fisher F exact test were used. Predictors for asymptomatic atherosclerosis were determined by univariate and multivariate log regression analysis. In the Cox regression model the significance of odds ratios was tested on the basis of Wald statistics. A two-tailed probability value of < 0.05 was considered as significant.

Results

General characteristic of subjects participating in the study was reported in Table 1. The mean age for T2DM patients and healthy volunteers was 48.41 years and 46.12 years (P = 0.68) respectively. Therefore, 64.1% of T2DM patients and 65.7% of healthy volunteers were men (P = 0.86). There was a significant difference between healthy volunteers and entire cohort of T2DM patients in BMI, waist circumference, cardiovascular risk factors (hypertension, dyslipidemia, adherence to smoking), Framingham risk score, and lipid abnormalities. HOMA-IR, Hba1c, fasting blood glucose, insulin, hs-CRP, TG, and Gal-3 were higher in T2DM patients when compared with healthy volunteers (P < 0.005 for all cases).

T2DM patients with asymptomatic coronary atherosclerosis demonstrated significantly higher BMI, waist circumference, circulating levels of LDL-C, hs-CRP, Gal-3, and lower level of HDL-C in comparison with those who had no coronary atherosclerotic lesions.

There were no significant differences between healthy volunteers and T2DM patients in circulating numbers of MPs labeled as CD41a+, CD64+, CD144+, CD144+/CD31+, Annexin V+, CD144+/CD144+, and 1.0 µm beads (Sigma, St Louis, MO, USA).

Abbreviations: IQR – inter quartile range; BMI - Body mass index, T2DM – type two diabetes mellitus, TG – triglycerides, BP – blood pressure, BMI - Body mass index, GFR – glomerular filtration rate, HDL-C - high-density lipoprotein cholesterol, LDL-C - Low-density lipoprotein cholesterol, hs-CRP – high sensitive C reactive protein, Gal-3 – galectin-3.
annexin V+, and CD414+/CD31+/annexin V+ (Table 2). The lower number of MPs with immune phenotypes CD62E+, CD105E+ and higher numbers of CD31+/annexin V+ MPs were reported in T2DM patients when compared with healthy volunteers. Therefore, we found an increased level of circulating CD41a+ MPs, CD144+/CD31+ MPs, CD31+/annexin V+ MPs, and decreased level of CD62E+ MPs in T2DM patients with asymptomatic coronary atherosclerosis in comparison with those who had no asymptomatic atherosclerosis.

Among T2DM patients with asymptomatic atherosclerosis Gal-3 levels were correlated positively with CD31+/annexin V+ MPs (r = 0.38, P < 0.001), BMI (r = 0.32, P < 0.05), age (r = 0.30, P < 0.01), the total number of diseased vessels (r = 0.29, P < 0.001), CD144+/CD31+ MPs (r = 0.22, P < 0.001), hs-CRP (r = 0.22, P < 0.05), CD41a+ MPs (r = 0.22, P < 0.001), male sex (r = 0.21, P < 0.05), waist circumference (r = 0.21, P < 0.001), HOMA-IR (r = 0.19, P < 0.001), LDL-C (r = 0.18, P < 0.05) and negatively associated with eGFR (r = -0.32, P < 0.05), CD62E+ MPs (r = -0.36, P < 0.001).

T2DM patients without coronary atherosclerosis Gal-3 levels were correlated positively with BMI (r = 0.28, P < 0.05), age (r = 0.22, P < 0.01), CD31+/annexin V+ MPs (r = 0.21, P < 0.01), male sex (r = 0.20, P < 0.05), HOMA-IR (r = 0.16, P < 0.001), LDL-C (r = 0.16, P < 0.05) and negatively associated CD62E+ MPs (r = -0.24, P < 0.01). In contrast, there were not found association between MP counts and Gal-3 level in healthy volunteers, while Gal-3 was positively associated with age (r = 0.24, P < 0.001).

Age- and sex adjusted univariate regression analysis has shown that BMI, LDL-C, Gal-3, hs-CRP, MPs phenotyped CD41a+, CD62E+, CD31+/annexin V+ were determined as predictors for asymptomatic atherosclerosis in T2DM patients (Table 3). However, after including in multivariate regression model all variables with p value < 0.2, we found that BMI, Gal-3, and CD31+/annexin V+ MPs remained independent predictors for asymptomatic atherosclerosis.

Multivariate Cox regression analysis for T2DM patients showed similar effect of Gal-3 and CD31+/annexin V+ MPs on risk of asymptomatic atherosclerosis (Table 4).

### Discussion

The results of our study demonstrate that increased level of CD31+/annexin V+ MPs and Gal-3 was significantly associated with asymptomatic atherosclerosis. In contrast, MPs derived from platelets and leukocytes did not demonstrate an association with asymptomatic atherosclerosis. We suggest that elevated Gal-3 level probably might

### Table 2: Numbers of microparticles in participants of the study

<table>
<thead>
<tr>
<th>Immune phenotype of MPs</th>
<th>Healthy volunteers (n = 35)</th>
<th>Entire cohort of enrolled T2DM patients (n = 103)</th>
<th>T2DM patients without coronary atherosclerosis (n = 54)</th>
<th>T2DM patients with asymptomatic coronary atherosclerosis (n = 49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD41a+ MPs, n/µL</td>
<td>23 (19-28)</td>
<td>25 (16-33)</td>
<td>23 (17-30)</td>
<td>29 (19-38)#</td>
</tr>
<tr>
<td>CD64+ MPs, n/µL</td>
<td>3.9 (3.5-4.6)</td>
<td>4.2 (3.2-5.1)</td>
<td>4.1 (3.3-4.9)</td>
<td>4.4 (3.4-5.0)</td>
</tr>
<tr>
<td>CD144+ MPs, n/µL</td>
<td>0.29 (0.22-0.36)</td>
<td>0.33 (0.24-0.39)</td>
<td>0.30 (0.25-0.38)</td>
<td>0.35 (0.26-0.42)</td>
</tr>
<tr>
<td>CD144+/CD31+ MPs, n/µL</td>
<td>0.87 (0.72-1.35)</td>
<td>0.92 (0.36-1.32)</td>
<td>0.88 (0.39-1.28)</td>
<td>0.96 (0.35-1.41)#</td>
</tr>
<tr>
<td>CD62E+ MPs, n/µL</td>
<td>1.35 (0.95-1.68)</td>
<td>1.03 (0.86-1.13)*</td>
<td>1.10 (0.89-1.17)</td>
<td>0.98 (0.79-1.10)#</td>
</tr>
<tr>
<td>CD105E+ MPs, n/µL</td>
<td>2.32 (1.92-2.56)</td>
<td>2.24 (1.85-2.41)*</td>
<td>2.29 (1.92-2.60)</td>
<td>2.18 (1.86-2.50)</td>
</tr>
<tr>
<td>Annexin V+ MPs, n/µL</td>
<td>4655.5 (3724-6237)</td>
<td>5495 (3988-6957)</td>
<td>5371 (3855-6792)</td>
<td>5673 (3952-7099)</td>
</tr>
<tr>
<td>CD144+/annexin V+ MPs, n/µL</td>
<td>0.95 (0.11-1.78)</td>
<td>1.15 (0.13-2.31)</td>
<td>1.11 (0.12-2.23)</td>
<td>1.19 (0.16-2.37)</td>
</tr>
<tr>
<td>CD144+/CD31+/annexin V+ MPs, n/µL</td>
<td>0.82 (0.27-1.55)</td>
<td>1.01 (0.29-1.70)</td>
<td>0.95 (0.37-1.56)</td>
<td>1.12 (0.39-1.81)</td>
</tr>
<tr>
<td>CD31+/annexin V+ MPs, n/µL</td>
<td>0.154 (0.03-0.21)</td>
<td>0.316 (0.261-0.374)*</td>
<td>0.298 (0.255-0.341)</td>
<td>0.337 (0.280-0.395)#</td>
</tr>
</tbody>
</table>

### Table 3: Age- and sex adjusted univariate and multivariate regression analysis for asymptomatic atherosclerosis

<table>
<thead>
<tr>
<th>Variable</th>
<th>B coefficient</th>
<th>P value</th>
<th>B coefficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.23</td>
<td>0.034</td>
<td>0.18</td>
<td>0.022</td>
</tr>
<tr>
<td>Gal-3</td>
<td>0.48</td>
<td>0.001</td>
<td>0.43</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.28</td>
<td>0.046</td>
<td>0.13</td>
<td>0.42</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>0.28</td>
<td>0.001</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>CD41a+ MPs</td>
<td>0.011</td>
<td>0.042</td>
<td>0.010</td>
<td>0.078</td>
</tr>
<tr>
<td>CD62E+ MPs</td>
<td>-0.38</td>
<td>0.001</td>
<td>-0.15</td>
<td>0.012</td>
</tr>
<tr>
<td>CD31+/annexin V+ MPs</td>
<td>0.32</td>
<td>0.026</td>
<td>0.26</td>
<td>0.012</td>
</tr>
</tbody>
</table>

### Table 4: Multivariate Cox regression analysis for T2DM patients: known asymptomatic atherosclerosis versus no atherosclerotic lesions

<table>
<thead>
<tr>
<th>Variables</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI per 5.0 kg/m²</td>
<td>1.02</td>
<td>1.00 – 1.05</td>
<td>6.37</td>
</tr>
<tr>
<td>Gal-3 per 2.5 ng/mL</td>
<td>1.06</td>
<td>1.02 – 1.10</td>
<td>12.5</td>
</tr>
<tr>
<td>CD31+/annexin V+ MPs per 0.1 n/µL</td>
<td>1.05</td>
<td>1.03 – 1.08</td>
<td>11.4</td>
</tr>
</tbody>
</table>

### Abbreviations
- BMI: Body Mass Index
- hs-CRP: High Sensitive C-Reactive Protein
- LDL-C: Low-Density Lipoprotein Cholesterol
- MP: Microparticles

### Notes
- Data are presented as median and 25-75% IQR. P-value is a comparison of mean or median variables between both cohorts (ANOVA test).
- Significant difference between healthy subjects and entire cohort of T2DM patients; # - significant difference between T2DM patients with and without asymptomatic atherosclerosis.

### Abbreviations
- IQR: Inter Quartile Range; MP: Microparticles.
reflect predominantly immune phenotype of circulating MPs, which mediates an injury of vasculature and induces endothelial dysfunction [26,27]. Indeed, Gal-3 attenuates organ fibrosis and inflammation depending on the levels of advanced glycation endproducts [advanced lipoxidation endproducts, of which it is a scavenging receptor] [12]. Moreover, Gal-3 acting as β-galactoside-binding lectin promotes maladaptive changes in the endothelium that lead to its dysfunction and contribute to the vascular pathology of T2DM [28]. Whether this effect is cause to secretion of apoptotic-induced endothelial cell-derived MPs or opposite they directly injure the endothelium is still not clear. However, more documentation at the molecular level to determine an interrelationship between Gal-3 and the immune phenotype of MPs is required.

It is necessary to note that BMI, age and male sex contribute serum Gal-3 in T2DM patients. It is not unexpected, but similar effect of elevated Gal-3 and CD31+/annexin V+ MPs on the risk of asymptomatic atherosclerosis in Cox model appears to be surprising. This finding requires more explanation in future studies. However, Gal-3 may correspond with CD31+/annexin V+ MPs toward diabetic vasculopathy development, endothelial dysfunction and vascular dysintegrity. Importantly, the results of the study support the assumption regarding Gal-3 effect that realizes beyond pro-inflammatory conditions. We suggest that an ability of Gal-3 mediates proliferation and macrophage chemotaxis may explain the effect of this molecule on secretion of various microvesicles by endothelial cells. In fact, Gal-3 release is increased in human monocytes and macrophages, and this process involving exosomes and regulated by reactive oxygen species/NADPH oxidase activity [29]. Therefore, serum Gal-3 level correlates with NADPH oxidase activity in peripheral blood mononuclear cells [29], which play a pivotal role in vascular injury, proliferative response, and atherosclerosis [30-32]. Accordingly, Gal-3 interferes key underlying mechanisms involved in atherosclerosis etiology, development, and plaque evolution, such as infiltration of circulating cells, oxidative stress and low-intense pro-inflammatory activation.

It is not clear whether elevated Gal-3 is direct destroyed factor for endothelium or is its response to prevent endothelial dysfunction and vascular dysintegrity in resulting apoptotic-induced over secretion of CD31+/annexin V+ MPs. The fact that there are less CD144+ MPs than CD144+/Annexin V+ MPs and CD31+/annexin V+ MPs could mean that apoptotic cells produce more MPs than non apoptotic cells. Because insulin resistance might associate with phagocytic NADPH oxidase activation, the highest cardiovascular risk and decreased Gal-3 levels in patients with metabolic syndrome and T2DM [11,12,33], we suggest that the results of our investigation support a hypothesis regarding an important role of the impaired immune phenotypes of circulating MPs in development of metabolic diseases [34,35]. We see in the future the perspectives for large clinical investigations to confirm or reject this hypothesis. Thus, Gal-3 has unique features for early verification of innate pathogenetic mechanisms regarding vasculature injury and repair in T2DM patients. All these novel findings doubtless might explain predictive value of Gal-3 elevation in patient populations with and without CV diseases.

In conclusion, among T2DM patients an increased level of CD31+/annexin V+ MPs and Gal-3 was significantly associated with asymptomatic atherosclerosis.

Study limitations

This study has some limitations. A preparation of isolates in samples is the most sophisticated step for further examination. Venous citrated blood drawn from the fistula-free arm was performed obligatorily. We believe that these risks are systemic, and to minimize them, we refused to freeze the blood samples before measurement of MPs. Therefore, measurement of CD31 and CD144 is mandatory for identification of endothelial cells, platelets, and leukocytes. Lack of consensual requirements regarding identification of MPs originated from various cells leads to controversial methods provided to its measurement. All these might explain a difference between identified numbers of circulating MPs when various antigens were used. In our study less CD144+ than CD144+/Annexin V+ for all groups, and less CD144+/CD31+ than CD144+/CD31+/Annexin V+ for the T2DM group were found. These findings are required more explanation and scrutinizes. Additionally, relative small sample size may limit the significance of the present study. The authors believe that a greater cohort of patients with more incidences detected is desirable to improve the credibility of the study.

Acknowledgments

We thank all patients for their participation in the investigation, staff of the Regional Zaporozhye Hospital (Ukraine), and the doctors, nurses, and administrative staff in Regional Center of Cardiovascular Diseases (Zaporozhye, Ukraine) and City Hospital # 6 (Zaporozhye, Ukraine), general practices, and site-managed organizations that assisted with the study.

Authors’ Contributions

Alexander E Berezin initiated the hypothesis and designed the study protocol, contributed to collect, analyze and interpret the data, including results of contrast-enhanced multipurpose computed tomography angiography, performed statistical analysis, wrote the manuscript and approved final version of the paper. Alexander A. Kremzer contributed to enroll the patients; interpreted data of contrast-enhanced multipurpose computed tomography angiography, collected and analyzed the data reviewed the source documents. Tatyana A. Berezina contributed to enroll the patients in the study and collect the data. Martovitskaya YuV contributed in performing of cytometry and interpretation of the obtained results.

References
