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Microparticles: Inflammatory and haemostatic biomarkers in Polycystic Ovary Syndrome

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ABSTRACT

Polycystic Ovary Syndrome (PCOS) is associated with a chronic low-grade inflammation and predisposition to hemostatic and atherosclerotic complications. This case-control study evaluated the microparticles (MPs) profile in patients with the PCOS and related these MPs to clinical and biochemical parameters. MPs derived from platelets (PMPs), leuckocytes (LMPs) and endothelial cells (EMPs) were evaluated, as well as MPs expressing tissue factor (TFMPs), by flow cytometry, comparing women with PCOS ($n = 50$) and a healthy control group ($n = 50$). PCOS women presented increased total MPs, PMPs, LMPs and EMPs levels when compared to control group (all $p < 0.05$). TFMPs was similar between the groups ($p = 0.379$). In conclusion, these MPs populations could be useful biomarkers for association with thrombosis and cardiovascular disease in PCOS women.

1. Introduction

Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder among women in reproductive age (Palomba et al., 2015; Silva et al., 2015). The etiology of PCOS is not completely understood (Dunaif, 2016; Sóter et al., 2015), but it results from environmental and genetic factors (Aytan et al., 2016; Insenser and Escobar-Morreale, 2013). The patients usually present hyperandrogenism, irregular menstruation and polycystic ovaries (Palomba et al., 2015; Aytan et al., 2016).

PCOS is an important cause of infertility due to chronic anovulation (Palomba et al., 2015). Furthermore, PCOS women present several metabolic complications, including obesity, insulin resistance (IR), type 2 diabetes mellitus (T2DM), dyslipidemia, metabolic syndrome and cardiovascular disease (Palomba et al., 2015; Silva et al., 2015; Repaci et al., 2011).

PCOS patients present unbalance between pro- and anticoagulant factors with higher atherothrombotic risk, besides increased levels of pro-inflammatory cytokines, which contribute to systemic and chronic low-grade inflammation (Palomba et al., 2015; Sóter et al., 2015; Aytan et al., 2016; Repaci et al., 2011). This subclinical inflammation also has been assumed to be the link between numerous metabolic complications, which are frequently associated with PCOS (Repaci et al., 2011). Furthermore, the inflammation probably contributes to an increased risk of cardiovascular disease in PCOS, because it is considered a hallmark of endothelial and hemostatic dysfunction (Repaci et al., 2011).

Microparticles (MPs) are extracellular vesicles with 0.1–1 μm released from the cell membrane during cell activation and apoptosis (Marques et al., 2013). MPs are important messengers in cell-cell communication and contribute to the induction of endothelial modifications, inflammation, differentiation, and angiogenesis (Marques et al., 2013). MPs membranes also carry signaling molecules, such as chemokines, cytokines, enzymes, growth factors, receptors, adhesion molecules, mRNAs and microRNA (Marques et al., 2013; Tan and Lip, 2005; VanWijk et al., 2002). The circulating MPs can affect the target cell properties by presenting

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these bioactive molecules, resulting in cell activation, phenotypic cellular modification, and the reprogramming function (Mause and Weber, 2010).

The initial step in the MPs formation is the membrane remodeling, with the development of blebs within it. This step requires an increase in intracellular calcium levels, resulting in the rearrangement and externalization of phosphatidylserine to the outer surface. Calcium-sensitive enzymes are activated and promote the cleavage of the filaments of the cytoskeleton leading to the formation of blebs on cell membrane and the release of MPs (Boulanger et al., 2006). Furthermore, MPs expose their membrane proteins provided from the specific cells that originated them, which can in turn be used to study their exact origin (Boulanger et al., 2006).

MPs levels are associated with a variety of pathological conditions, such as prothrombotic (Mooberry et al., 2006) and inflammatory disorders (Marques et al., 2012), cardiovascular (Alexandru et al., 2015; Burbano et al., 2015), autoimmune (Burbano et al., 2015) and infectious diseases (Campos et al., 2010), cancer (Goubran et al., 2015) and T2DM (Koga et al., 2005). Furthermore, MPs arising from adipose tissue can influence insulin signaling through protein kinase B pathway and expression of gluconeogenic genes (Kranendonk et al., 2014).

Few studies have evaluated the role of MPs from different cells origin in PCOS (Wills et al., 2014; Koiou et al., 2011, 2013). Because this syndrome is associated with pro-coagulant and pro-inflammatory states, we investigated MPs originated from different cell types (platelets, leukocytes, and endothelial cells) and MPs expressing TF in a case-control study. Moreover, we evaluated the association and the correlation of these MPs with clinical and laboratory parameters in PCOS women. Our data can help to clarify a possible role of MPs in PCOS and their investigation as biomarkers for this syndrome and its complications.

2. Material and methods

2.1. Subjects

We evaluated 50 women with PCOS (aged from 14 to 41 years) and 50 women without the syndrome (20–43 years). The PCOS group was recruited at Hospital Borges da Costa, UFMG (Belo Horizonte, Minas Gerais, Brazil), in the period 2011–2013. The control group was recruited among employees and students from UFMG in the same period, and consisted of healthy women with regular menstrual cycles and no signs of hyperandrogenism or micropolycystic ovary.

PCOS diagnosis was performed according to the European Society of Human Reproduction/Embryology and the American Society for Reproductive Medicine criteria (ESHRE/ASRM) (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004), considering the presence of at least two of three criteria: (1) oligo/amenorrhea and anovulation; (2) clinical or laboratory hyperandrogenism and (3) ultrasonography showing micropolycystic ovaries, as defined by the presence of 12 or more follicles in the ovary each measuring 2–9 mm in diameter and/or increased ovarian volume (>10 mL).

Exclusion criteria for both groups were the presence of diabetes mellitus, autoimmune, adrenal, kidney and liver disease, thyroid disorders, cancer, acute inflammatory disease, orthopedic implant, hyperprolactinemia, hypogonadism, and pregnancy. Current or recent (past 3 months) users of the following medications were also excluded: steroidal and non-steroidal anti-inflammatory medications, anabolic steroids, isotretinoin, cyclosporine, antiretroviral, insulin and oral contraceptives. Individuals with C-reactive protein (CRP) > 10 mg/dL were also excluded.

2.2. Clinical and laboratorial evaluation

Venous blood samples were obtained after 12 h fasting using tubes with sodium citrate or anticoagulant-free (Vacuette®). The samples were centrifuged at 1500 xg for 20 min at 4 °C to obtain the plasma or serum. Aliquots were immediately processed or stored at −80 °C until the use.

The serum samples were used for measure fasting glucose, CRP and lipid profile using Vitros kits (Johnson and Johnson®). Insulin and testosterone levels were also measured in serum samples using Abbott Architect®. All procedures were conducted according to the manufacturer's instructions.

Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg at the time of interview, or regular use of antihypertensive medication (Chobanian et al., 2003). Hirsutism in PCOS group was assessed according to the modified Ferriman–Gallwey scale (Api et al., 2009) for a single observer to avoid subjectivity. The visual scoring of hair density on the upper lip, chin, chest, upper back, lower back, upper abdomen, lower abdomen, arms, and thighs were classified visually on a scale of zero to four. A total score was calculated.

We considered dyslipidemic women as those currently using lipid lowering medication or with altered lipid profile, according to the III Brazilian Guidelines on Dyslipidemia and Atherosclerosis Prevention (total cholesterol > 240 mg/dL, LDL-cholesterol > 160 mg/dL, HDL-cholesterol < 40 mg/dL, or triglycerides > 201 mg/dL) (Santos and De Cardiologia., 2001). Body Mass Index (BMI) was measured by weight in kilograms divided by the square of the height in meters (kg/m2) (Obesity, 2000). The waist circumference (WC) was measured between the lowest ribs and the iliac crest, as recommended by World Health Organization and International Diabetes Federation (Wen-Ya et al., 2013).

The Lipid Accumulation Product (LAP) index was calculated by using the formula: [(waist circumference cm − 58) × (triglycerides mg/dL)] (Lwow et al., 2016). The Homeostatic Model Assessment (HOMA) for IR was calculated using the following the formula: [insulin (mU/L) x glucose (mM/L)]/22.5 (Tang et al., 2015).

2.3. MPs flow cytometry assay

The purification of the MPs was performed according to Campos et al. (2010) (Campos et al., 2010). In order to obtain platelet-free plasma, the plasma samples were centrifuged at 15,300 xg for five minutes diluted 1:3 in phosphate buffered saline (PBS) containing heparin, and again centrifuged at 15,300 xg for 90 min at 15 °C. The subsequent MPs pellet was resuspended in 1X annexin V binding buffer (BD Pharmingen®).

MPs were isolated in a LSR Fortessa cytometer (BD Biosciences®) and gated on basis of their forward (FSC) and side (SSC) scatter distribution of synthetic 0.7–0.9 μm SPHEROTM Amino Fluorescent Particles (Spherotech®). The presence of phosphatidylserine residues on the MP surfaces was assessed for their positive staining with monoclonal antibodies against annexin V (BD Pharmingen®) labeled with fluorescein isothiocyanate (FITC).

Cell-specific monoclonal antibodies were used to identify the source of the MPs. CD41-PECY7 (eBioscience®), CD45-APC (eBioscience®), CD51/61-PE (BD Pharmingen™) and CD142-PE (BD Pharmingen™) were used to label platelet-derived MPs (PMPs), leukocyte-derived MPs (LMPs), endothelium cell-derived MPs (EMPs) and MPs that express TF (TFMPs), respectively. The antibodies were used in concentrations according to manufacturer's instructions. The specific monoclonal antibody was corrected for isotype-matched control antibodies. FACSDIVA 6.2 software (BD®) was used for data acquisition and the analysis was performed using the FlowJo® software (Tree Star).

Table 1
Characterization of PCOS and control groups.

Variables		PCOS n = 50	Control n = 50	p
Age (years)		30.62 ± 4.88	29.58 ± 7.75	0.214
BMI (kg/m2)		29.59 ± 5.72	24.06 ± 4.41	<0.001*
WC (cm)		97.00 (17.50)	81.00 (19.50)	<0.001″
Testosterone (mmol/L)		52.41 ± 35.61	30.74 ± 14.14	<0.001*
Ferriman-Gallweyscale		10.00 (10.00)	–	–
Fasting Glucose (mmol/L)		87.76 ± 9.14	84.00 ± 14.73	0.265
Insulin (uUI/mL)		12.30 (18.00)	7.50 (3.60)	<0.001*
HOMA-IR		2.70 (3.40)	1.57 (0.8)	<0.001*
LAP		52.23 (51.20)	13.00 (19.30)	<0.001*
Total Cholesterol		189.24 ± 35.74	175.71 ± 32.44	0.005*
HDL-c (mmol/L)		48.41 ± 12.46	56.79 ± 13.26	<0.001*
LDL-c (mmol/L)		113.87 ± 31.24	99.63 ± 28.10	0.001*
VLDL-c (mmol/L)		20.00 (17.00)	17.00 (9.00)	0.006*
Triglycerides (mmol/L)		109.00 (89.00)	82.50 (40.00)	0.002*
CRP (mg/dL)		5.00 (9.00)	4.00 (2.00)	0.166
Dyslipidemia	Yes	10.3%	6.1%	0.308
	No	89.7%	93.9%	
Hypertension	Yes	4.1%	1.0%	0.209
	No	95.9%	99.0%	

BMI (Body Mass Index), WC (Waist Circumference), PG (Postload Glucose), HOMA (Homeostasis Model Assessment), LAP (Lipid Accumulation Product), HDL (High Density Lipoprotein), LDL-c (Low Density Lipoprotein), VLDL-c (Very Low Density Lipoprotein), CRP (C-reactive Protein). Normal variables are shown as mean ± Standard Deviation. No normal variables are shown as median (Interquartile Range). Student *t*-test for normal variables and Mann-Whitney test for non-normal variables. Categorical variableswere analyzed by Chi-square test and its results are shown as percentage. Significant*: $p < 0.05$.

To determine the absolute MPs plasma levels, the cytometer was set to operate with a high setting flow for 60 s for each sample. The MPs/μL of plasma was calculated as described by Campos et al. (2010): MPs/μL = (N x 400)/(100 × 60), in which N is equal to the number of events, 400 is the total volume of sample into the tube prior to analysis, 60 is the volume of the analyzed sample, and 100 is the original volume of MPs suspension. This formula was validated using Trucount tubes (BD PharmingenTM) in five samples, randomly selected.

2.4. Statistical analyses and power calculation

Statistical analyses were performed using Statistical Package of the Social Sciences (SPSS; 13.0 version). The Shapiro-Wilk test was used to determine the normality of all the variables analyzed in this study. Normal data are presented as mean and standard deviation, while non-normal variables are presented as median and interquartile range (25th–75th percentiles). We performed the Student *t*-test for normal variables and the Mann-Whitney test for non-normal variables. For categorical variables, we used the chi-square test. Linear correlation was assessed using the Spearman rank correlation method. We consider the correlation strength was weak ($0 < r \leq 0.35$), moderate ($0.36 \geq r \leq 0.67$) or strong ($r \geq 0.68$), as proposed by Taylor (1990). For all analyses, we considered $p < 0.05$ statistically significant.

The study size was determined using the free software Java Applets for Power and Sample Size (The University of Iowa) and statistical parameters obtained from published studies of similar design (Wills et al., 2014; Koiou et al., 2011, 2013). Sample size calculation indicated that 50 cases and 50 controls would allow the detection of differences of at least 0.7 standard deviation in biomarker levels between the two groups with 90% statistical power and 95% confidence.

3. Results

The PCOS and the control groups were matched for age ($p = 0.214$). PCOS women had increased HOMA-IR, LAP index, testosterone, insulin, total cholesterol, LDL-c, VLDL-c and triglycerides, as well as lower HDL-c levels when compared to the control group (all $p < 0.05$). Moreover, BMI and WC were higher in PCOS than control groups ($p < 0.001$). Fasting glucose, and CRP levels were not different between the groups ($p > 0.05$). Although more patients with PCOS presented dyslipidemia and hypertension when compared to the control group, this difference was not significant ($p = 0.308$ and $p = 0.209$, respectively) (Table 1).

A higher total number of MPs was observed in women with PCOS (57.69 ± 37.71 MPs/μL) than in the control group (28.22 ± 15.88 MPs/μL, $p < 0.001$) (Fig. 1A). We observed higher levels of circulating PMPs [24.37 (22.08)], LMPs [22.87 (21.85)], and EMPs [27.33 (21.78)] in PCOS when compared to control group [15.90 (11.45); 4.53 (3.77); 18.50 (13.57), respectively] (all $p < 0.05$, values express as MPs/μL) (Fig. 1B, C and 1D). However, the groups did not display different number of TFMPs [1.70 (1.68) for PCOS; 1.93 (1.12) for control; $p = 0.379$] (Fig. 1E).

Correlation analysis showed a strong and positive correlation between PMPs and EMPs. A moderate positive correlation was observed for PMPs and LMPs, LMPs and EMPs, which were significant. No correlation involving TFMPs were observed ($p > 0.05$) (Table 2).

We also evaluated the correlation between MPs and clinical and laboratory variables in PCOS group. The only variables that showed significant correlation with MPs populations analyzed were shown in Fig. 2. WC, LAP index, VLDL-c and triglycerides levels showed a significant positive correlation ($p < 0.05$) with PMPs (Fig. 2A, 2B, 2E, 2F), LMPs (Fig. 2G, 2H, 2I, 2J) and EMPs (Fig. 2K, 2L, 2O, 2P). Total cholesterol (TC) levels presented positive significant correlation with PMPs and EMPs (Fig. 2C and 2M), besides an inverse and weak correlation with TFMPs (Fig. 2Q). Therefore, LDL-c showed positive correlation with PMPs and EMPs (Fig. 2D and 2N).

4. Discussion

This study evaluated the relationship between PCOS and MPs, with special emphasis on the metabolic features of the syndrome. We observed that total MPs (annexin-positive) levels were increased in women with PCOS when compared to the control group, as well as PMPs, LMPs and EMPs, which suggests their

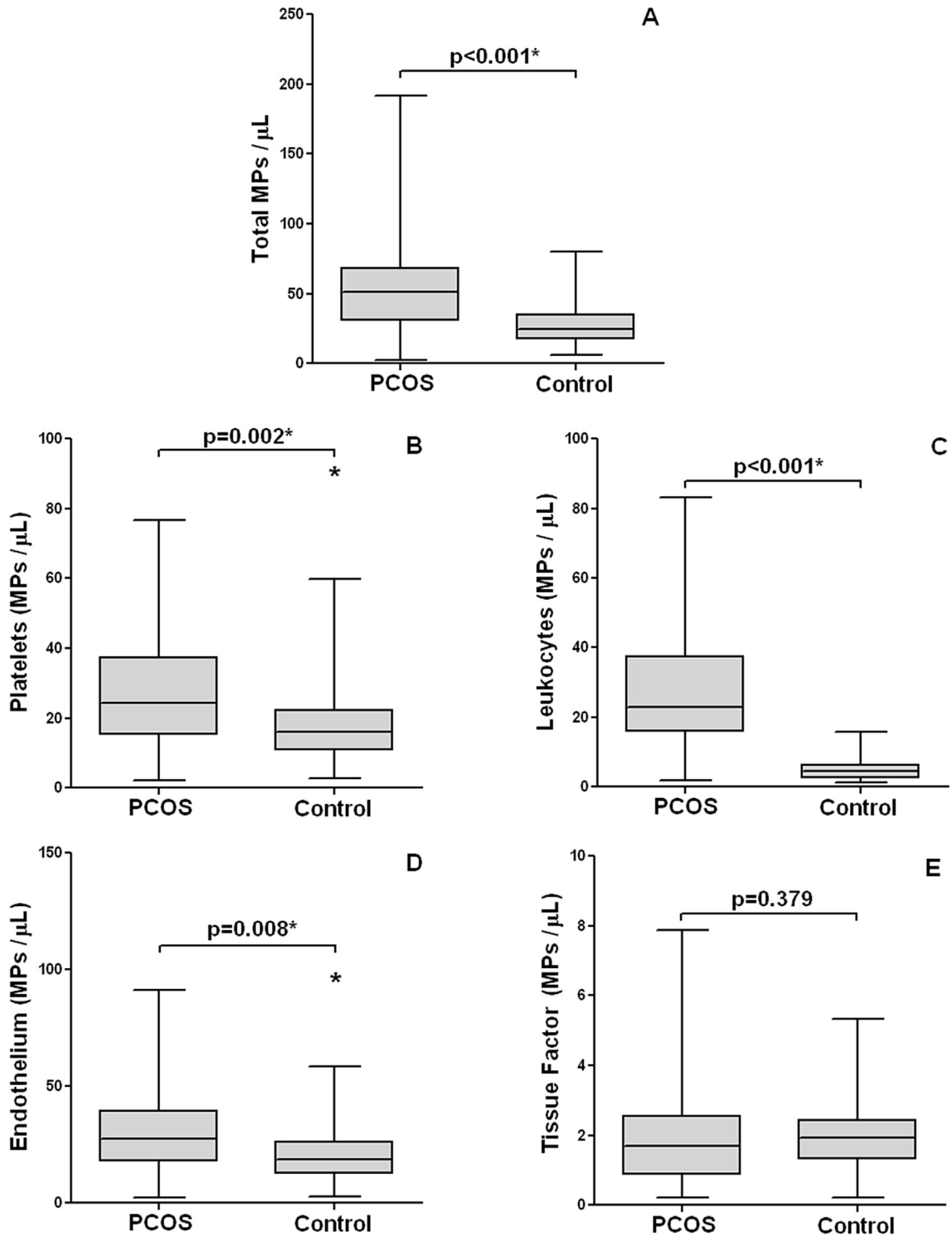

Fig. 1. Distribution of MPs levels between PCOS and control groups. Distribution of MPs counts/µL between groups (control and PCOS). A) Total MPs (annexin positive); B) Platelets-derived MPs (annexin and CD41 positive); C) Leukocytes-derived MPs (annexin and CD45 positive); D) Endothelium cell-derived MPs (annexin and CD51/61 positive); E) Distribution of MPs that express TF (annexin and CD142 positive). A: T Student test, B – E: Mann–Whitney *U* test. Significant*: $p < 0.05$.

potentialities as biomarkers in PCOS associated to inflammation and coagulation disorders. However, TFMPs levels did not differ between groups.

BMI and WC was significantly higher in patients with PCOS when compared to control women. This was expected, considering that this syndrome is characterized by a greater predisposition to obesity (Palomba et al., 2015; Silva et al., 2015). The case group also showed higher testosterone levels, since laboratory hyperandrogenism is a diagnostic criteria for PCOS (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Moreover, according to the Ferriman-Gallwey scale, PCOS women showed a mean score that characterizes hirsutism (≥8)

Table 2
Correlation between MPs levels in PCOS women.

	Platelets MPs	Leukocytes MPs	Endothelium MPs	TF MPs
Platelets MPs		r = 0.487 p < 0.001*	r = 0.991 p < 0.001*	r = 0.011 p = 0.940
Leukocytes MPs	r = 0.487 p < 0.001*		r = 0.482 p < 0.001*	r = 0.252 p = 0.078
Endothelium MPs	r = 0.991 p < 0.001*	r = 0.482 p < 0.001*		r = 0.004 p = 0.978
TF MPs	r = 0.011 p = 0.940	r = 0.252 p = 0.078	r = 0.004 p = 0.978	

Spearman correlation test. Significant*: $p < 0.05$.

(Api et al., 2009). The women with PCOS had higher insulin levels and HOMA-IR, although the levels of fasting glucose did not show difference between groups. We also observed a significant difference in LAP index in PCOS group compared to the controls, and these data corroborate with the fact that PCOS increases the risk for metabolic syndrome (Palomba et al., 2015; Silva et al., 2015).

A higher number of women with dyslipidemia and hypertension were observed among PCOS group, but there were no significant difference, probably due to the small number of women presenting these metabolic disorders in both groups. However, the lipid profile of women with PCOS is suggestive of increased cardiovascular risk, since the levels of pro-atherogenic lipoproteins (LDL-c and VLDL-c) are increased in women with PCOS, when compared with the control group, as well as total cholesterol levels and triglycerides. In contrast, HDL-c levels – the anti-atherogenic lipoprotein - are decreased in women with PCOS. Although higher CRP levels were observed in PCOS group, no significant difference was observed between the groups, which could be explained by the systemic low-grade inflammation in this syndrome that is not enough to significantly raise CRP values. We also considered CRP levels above 10 mg/dL as exclusion criteria in order to avoid the confounding effect of individuals with infectious and inflammatory diseases.

According to our results, total MPs levels were increased in women with PCOS when compared to control group. MPs are heterogeneous and biologically active, providing a bridge between inflammation and coagulation (Foley and Conway, 2016). Mice that received MPs had increased thrombin generation, elevated thrombin–antithrombin levels, and a drop in platelet count. Therefore, elevated circulating MPs levels, parallel to D-dimers and P-selectin in deep vein thrombosis, are a risk factor for coronary artery disease and atherothrombosis in humans (Foley and Conway, 2016; Chirinos et al., 2005). MPs can trigger complement activation, facilitate leukocyte adhesion and trafficking, induce cytokine release, and modulate endothelial cell nitric oxide (Foley and Conway, 2016; Mause et al., 2005).

When the MPs was characterized according to cell origin, we observed that PMPs were significantly increased in women with PCOS, when compared to controls. PMPs have diverse properties in innate immunity, inflammation and thrombosis (Kapur et al., 2015). Considering that platelets play a central role in primary hemostasis (Puddu et al., 2010; Varon and Shai, 2015), we could hypothesize that PMPs can result from platelet aggregation in PCOS. PMPs can also act in secondary hemostasis because they exhibit procoagulant properties, partly because of membrane phosphatidylserine, which provides a surface for assembly of the prothrombinase complex that catalyzes the conversion of prothrombin (Factor II) to thrombin (Factor IIa) (Zhou et al., 2014; Morel et al., 2006). Moreover, PMPs seem to have the capacity to capture and incorporate TF in order to promote coagulation (Del Conde et al., 2005). In fact, PCOS is associated with increased thrombin generation and thrombotic complications (Glintborg et al., 2015) and increased levels of PMPs have been also reported in other diseases associated with hemostatic disorders, such as rheumatoid arthritis, cancer and arterial thrombosis (Mezouar et al., 2014). PMPs also can stimulate the release of cytokines and change the endothelial reactivity (Varon and Shai, 2015), which could explain its strong correlation with LMPs and EMPs.

LMPs may originate from neutrophils, monocytes/macrophages, and lymphocytes (Wojta, 2015; Angelillo-Scherrer, 2012). In our study, we demonstrated higher levels of these MPs in the PCOS group, which reflects leukocyte activation and the systemic low-grade inflammation observed in PCOS. LMPs circulate at a high level in the bloodstream of patients with high atherothrombotic risk (Angelillo-Scherrer, 2012). In inflammatory diseases, as arthritis, LMPs are increased in the blood and other fluids, where they stimulate cell release of proinflammatory cytokines. Indeed, LMPs trigger complement activation, facilitate leukocyte adhesion and modulate endothelial cell release of nitric oxide and prostacyclin (PGI2). They may deliver arachidonic acid to endothelial cells that upregulate leukocyte adhesion molecules (intercellular adhesion molecule-1 - ICAM, and vascular adhesion molecule-1 - VCAM) and promote leukocyte-leukocyte interactions (Mause et al., 2005; Miller et al., 2016). Therefore, LMPs can modify the endothelial function (Mesri and Altieri, 1998), take part in coagulation and platelet activation and promote the recruitment of inflammatory cells in the vascular wall, which are all necessary processes for the progression of the atherosclerotic lesion (Wojta, 2015). Consequently, the correlation between LMPs-PMPs-EMPs can be expected, suggesting that LMPs could be involved in hemostasis and inflammation and may be useful biomarkers for predicting atherosclerotic risk in PCOS.

Higher EMPs levels in women with PCOS than in controls suggest an increased endothelial activation. These MPs are elevated in numerous cardiovascular-related diseases, such as systemic lupus erythematosus and rheumatoid arthritis (McCarthy et al., 2016). EMPs may affect vascular function and contribute to endothelial dysfunction by suppressing the production of nitric oxide and PGI2, reducing vasodilatation (Densmore et al., 2006) and promoting oxidative stress (Densmore et al., 2006; Brodsky et al., 2004). It has been shown that *in vitro* treatment of mouse and human vessels with EMPs suspension causes increased vascular permeability (Brodsky et al., 2004), which is related to the recruitment of inflammatory cells. Moreover, recently it was proved that under inflammatory conditions, EMPs cause muscle calcification of the blood vessel, contributing to vascular stiffening (Buendia et al., 2015). Accordingly, EMPs can contribute to the development of atherosclerosis in PCOS patients (Buendia et al., 2015). As an early detection of damage in endothelial function is important for the initiation of an anti-inflammatory protective treatment, preventing major vascular damage, EMPs can potentially be an important biomarker in this prevention (Helbing et al., 2014; Mesri and Altieri, 1998).

It is noteworthy that CD51/61 complex is a traditional marker not only for endothelial cells (Chou et al., 2010), but it is also found in monocytes/macrophages, platelets, some B cells (Zola et al., 2007), as well as osteoclastoma and melanoma cells (Karosi et al., 2006). Nevertheless, Jimenez et al. (2003) observed that CD51/61 is the main antigen expressed in EMPs in inflammatory processes and endothelium injury. Moreover, other study that evaluated inflammatory diseases observed that CD51/61 only was able to quantify EMPs (Sabatier et al., 2002). These results corroborates with our choice to use this marker to determine EMPs. However, additional monoclonal antibodies (e.g. CD31, CD105) could be added in further studies in order to confirm the endothelial damage in PCOS, and the correlation of EMPs with other variables, including other MPs source.

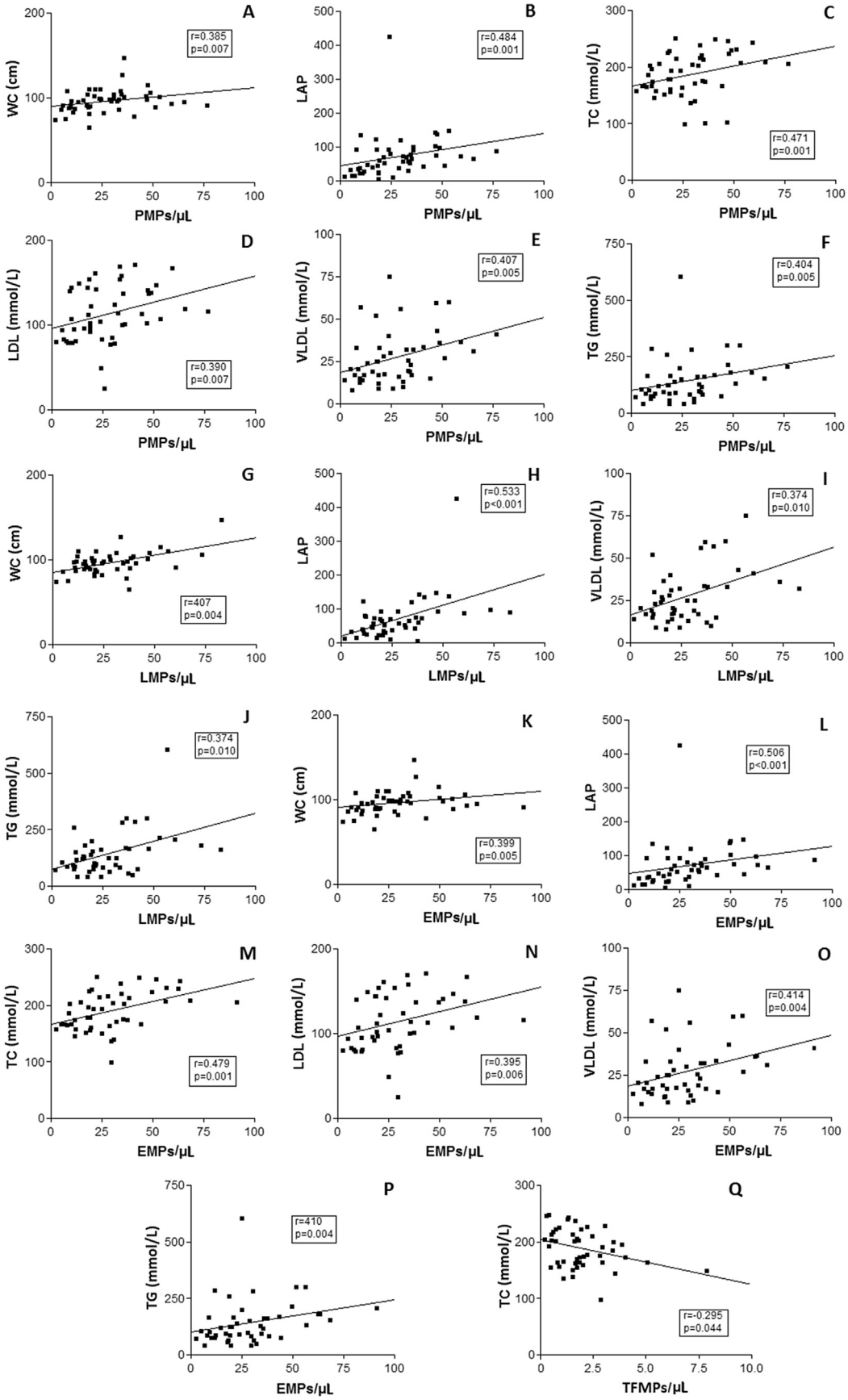

Fig. 2. Correlation between MPs levels and clinical/biochemical variables in PCOS women. PMPs - Platelets-derived MPs; LMPs - leukocytes-derived MPs; EMPs - endothelium cells-derived MPs; TFMPs - MPs expressing TF;WC -Waist Circumference; LAP - Lipid Accumulation Product; TC – total cholesterol; VLDL -Very Low Density Lipoprotein; LDL - Low Density Lipoprotein; TG – triglycerides. Spearman correlation test. Significant: $p < 0.05$.

TFMPs are also important in procoagulant disorders because they activate the extrinsic pathway of coagulation. Platelets seem to have the capacity to capture and incorporate TF or TF-rich vesicles to promote coagulation. TF-enriched MPs, that display P-selectin glycoprotein ligand-1, can interact with P-selectin expressed by activated platelets, leading to fusion of the PMPs surface, acquisition of TF, with consequent augmentation of the TF-VIIa proteolytic activity (Foley and Conway, 2016; Del Conde et al., 2005). In addition, MPs can induce monocytes to release the procoagulant TF, contributing to the procoagulant properties of MPs (Lin et al., 2015). However, we did not observe a significant difference in TFMPs between the PCOS and the control group and the TMPs levels in both groups were lower when compared to other MPs from a different cell origin. We suppose that TF is expressed in a minor quantity by cells and therefore it was not possible to detect minimal differences of TFMPs levels between the groups.

Curiously, LDL-c, VLDL-c, total cholesterol and triglycerides were positively correlated with PMPs and EMPs in PCOS women. LMPs are also correlated to these parameters, except LDL-c and total cholesterol. WC also was positively correlated with PMPs, EMPs and LMPs in this group (Fig. 2). It is noteworthy because visceral fat – that is frequently augmented in PCOS women - is more lipolytic than subcutaneous adipose tissue (Klöting et al., 2007), releasing free fatty acids in higher quantities, which contributes to dyslipidemia. Furthermore, the accumulation of macrophages in abdominal adipose tissue is higher when compared to subcutaneous fat, which is associated with the secretion of inflammatory adipokines, as IL-6 and TNF-α (Papaetis et al., 2015). Another important finding in this context is that oxidized LDL (oxLDL) promotes platelet activation and recruitment of inflammatory cells in dyslipidemic disorders (Magwenzi et al., 2015; Stellos et al., 2012). The mechanism for oxLDL-mediated platelet hyperactivity requires generation of reactive oxygen species (Magwenzi et al., 2015), and oxidative stress is an important mechanism involved in PCOS (Murri et al., 2016). We suggest that PMPs, EMPs and LMPs levels, in synergism with lipid profile disturbance, may be predictors of cardiovascular risk in PCOS (Wojta, 2015; Miller et al., 2016; Santilli et al., 2016; Chiva-Blanch et al., 2016). Contrary to expected, total cholesterol was inversely correlated with TFPMs, but this correlation was weak and can reflect the limitation to quantify TF expressed in MPs.

The LAP index also correlated with PMPs, LMPs and EMPs. Since this index is a good predictor of metabolic syndrome, we suggest that MPs levels arising from these three source cells (platelets, leukocytes and endothelium) may also be related to metabolic syndrome, at least in PCOS patients.

Some previous studies also evaluated the MPs in PCOS (Wills et al., 2014; Koiou et al., 2011, 2013). Accordingly our results, Wills et al. (2014) demonstrated that total MPs annexin-positive was significantly higher in women with PCOS and Koiou et al. (Koiou et al., 2011) showed that PMPs levels were higher in women with PCOS compared to the control group. Koiou et al. in another study (Koiou et al., 2013), considering only overweight and obese women, also found PMPs levels higher in the PCOS group. Wills et al. (2014) demonstrated that PMPs levels predominated in relation to others sources (EMPs and monocytes-derived MPs), which were uncommon, but not significant difference was observed in this case-control study. Contrary to our results, Wills et al. (2014) did not observe elevated EMPs levels in PCOS women. This disagreement can be due to the heterogeneous populations studied, different PCOS diagnostic criteria, or small sample size in some studies. Therefore, more studies in other populations should be performed in order to expand the external validity of the present results.

5. Conclusions

We propose that MPs play an important role in the pathogenesis of PCOS and could be a biomarker of non-reproductive complications associated to the syndrome. The phosphatidylserine present in the membrane of MPs probably contributes to haemostatic complications, as well as other molecules carried by PMPs and LMPs, arising from their original cells. PMPs, LMPs and EMPs are involved in inflammation and may be useful biomarkers for predicting cardiovascular risk and metabolic syndrome in PCOS. In conclusion, these data may contribute to understand the pathogenesis of PCOS and its complications, such as metabolic, inflammatory and hemostatic disorders.

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