Thrombosis Research 136 (2015) 427–431

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Circulating microparticles in umbilical cord blood in normal pregnancy and pregnancy with preeclampsia

Elena Campello [a], Luca Spiezia [a], Claudia M. Radu [a], Sonila Dhima [b], Silvia Visentin [b], Fabio Dalla Valle [a], Daniela Tormene [a], Barry Woodhams [c], Erich Cosmi [b], Paolo Simioni [a,*]

[a] Department of Medicine (DIMED), 5th Chair of Internal Medicine, University of Padua, Italy
[b] Department for Health of Mothers and Children, University of Padua, Italy
[c] HaemaCon Ltd, Bromley, England

ARTICLE INFO

Article history:
Received 19 April 2015
Received in revised form 20 May 2015
Accepted 27 May 2015
Available online 28 May 2015

Keywords:
Microparticles
Pre-eclampsia
Pregnancy
Venous cord blood

SUMMARY

Introduction: Placenta microthrombi being one of the prevalent recurrent histological findings in women with preeclampsia (PE), it is reasonable to think that the study of coagulation alterations in cord blood could be more informative than that observed in maternal blood. The aim of the present study was to measure different subtypes of microparticles (MP) plasma levels in the maternal peripheral blood at labour and in the venous cord blood of pregnant women with PE compared to those in a group of women without PE.
Materials and methods: Thirty-two pregnant women in labour, 16 with and 16 without PE, were enrolled. Blood samples were collected immediately after delivery from cord blood and from maternal peripheral blood. Total, cellular-derived and tissue factor- bearing MP were analyzed using flow-cytometry. Procoagulant activity of MP was assessed using the STA® Procoag PPL assay.
Results: Total MP, platelet activated-derived (P-Selectin +), leukocyte-derived and TF + MP were higher in pregnancies complicated by PE as compared with normotensive women ($p < 0.05$). Platelet-derived MP (CD61 +) levels were lower in PE than in healthy women and no difference was found in endothelial-derived MP levels between the two groups. The PPL clotting time was significantly shorter in PE compared with controls. When only venous cord blood was analysed, all MP detected were significantly higher in PE than in healthy normotensive women ($p < 0.05$).
Conclusions: MP are very likely involved in the hypercoagulable and pro-inflammatory intravascular reactions during PE. Prospective studies in a larger population are needed to define the clinical meaning of MP measurement in the PE setting.

1. Introduction

Preeclampsia (PE), a pathological condition characterized by hypertension and proteinuria, is one of the most serious causes of maternal and neonatal morbidity and mortality with a prevalence of 6-8% of pregnancies [1]. In pregnant women with PE, extensive activation of endothelial cells, leukocytes, and coagulation system have been reported [1–3]. Membrane microparticles (MP) consist of cell-derived vesicles formed from the outward blebbing of the plasma membrane and subsequent shedding into the extracellular space during apoptosis or cellular activation. MP are typically defined as 0.1–1.0 μm in size consisting of membrane proteins and cytosolic material derived from the cell from which they originate [4–6]. There is increasing evidence in the literature that the levels of circulating MP may represent a possible marker or a causative agent of PE related vascular complications [7–9]. Some of the studies showed an increase in MP sub-populations in women with PE compared to normal healthy pregnant females, including an increase in the total number of MP [10,11], an elevation of both activated and non-activated platelet-MP [10,12,13], as well as an increase in endothelial MP [14] and white blood cell MP [10,15]. Other studies revealed no differences in the total platelet MP number between normal and pregnancy complicated by PE. Moreover, it was shown that MP are present in cord blood plasma in significantly higher concentration than in mother's plasma [16]. Placenta microthrombi being one of the prevalent histological finding in women with PE [1], it is reasonable to think that the study of coagulation alterations in cord blood are more informative than that observed in maternal blood.

The aim of the present study was to evaluate different subtypes of MP plasma levels in the maternal peripheral blood at labour and in

* Corresponding author at: 5th Chair of Internal Medicine, Thrombophilia and Hemophilia Center, Department of Medicine (DIMED), University of Padua Medical School, Via Ospedale Civile 105, 35100 Padua, Italy.
E-mail address: paolo.simioni@unipd.it (P. Simioni).

http://dx.doi.org/10.1016/j.thromres.2015.05.029
0049-3848/

the venous cord blood of pregnant women with PE as compared to those of a group of healthy women at delivery.

2. Materials and Methods

2.1. Patients

All consecutive pregnant women in labour with PE, referred for delivery to the Unit of Maternal Foetal Medicine at University Hospital of Padua, between September 2013 and January 2014, were considered. A group of healthy parturient with a single, uncomplicated pregnancy, age (±3 yrs) matched with cases, who gave natural birth at term acted as control. PE was defined by high blood pressure (two separate readings taken at least 6 h apart of 140 mmHg or more in systolic blood pressure and/or 90 mmHg or more in diastolic blood pressure) and 300 mg of protein in a 24-h urine sample occurring after the 20th week of pregnancy. Exclusion criteria common to the overall study population were: *i*) age ≤18 years; *ii*) ongoing anticoagulant or antiplatelet treatment; *iii*) previous arterial or venous thrombosis; *iv*) comorbidities (i.e. cancer, diabetes, obesity, chronic hypertension, autoimmune, renal, or hepatic diseases, acute infections). Each woman received a peripheral blood sample before delivery and, subsequently, one sample of venous cord blood was obtained prior to clamping and cutting of the umbilical cord and before placental expulsion (III stage of labour). The study was performed according to the Declaration of Helsinki. Informed consent was obtained from all participants according to the University Hospital of Padua policy.

2.2. Blood Sampling and Conventional Coagulation Parameters

Nine mL of venous blood was drawn from the antecubital vein with a light tourniquet, using a butterfly device with 21-gauge needle without venostasis. Blood was collected directly into syringes pre-filled with 1 mL of sodium citrate 109 mol/L. White blood cells and platelet count (r.v. 4.5-10.0 $x10^9$/L and 150–450 $x10^9$/L, respectively) were measured on the Sysmex Counter XE-2100 (Dasit Spa, Milan, Italy). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were automatically measured according to the standard methods. Platelet-poor plasma (PPP) was prepared within 1 hour of blood collection by double centrifugation (2 x 15 minutes at 2500 g) at room temperature. Prothrombin time (PT/INR), activated partial thromboplastin time (aPTT), D-Dimer, protein C (PC, chromogenic and coagulometric assays) and protein S (PS) were measured in all samples according to the methods described elsewhere [17,18]. Aliquots (1.5 ml) of PPP were immediately frozen and then stored at −80 °C until use. Samples, analyzed only after a single freeze-thaw cycle, were thawed by incubation for 5 minutes in a water bath at 37 °C immediately before assay.

2.3. MP Assessment and Characterization

MP were identified by size and annexin V- fluorescein isothiocyanate (FITC) (Bender MedSystems GmbH, Vienna, Austria) labelling. The MP gate was established using a blend of mono-dispersed fluorescent beads of three diameters (0.5, 0.9 and 3 μm) (Megamix, BioCytex, Diagnostica Stago, France). MP were analyzed on a Cytomics FC500 flow cytometer (Beckman Coulter, Miami Florida). To measure the different populations, the MP were co-labelled with antibodies against cell-type specific antigens and annexin V, as previously described [19,20]. Thirty microliters (μL) of freshly thawed PPP were incubated for 15 minutes at room temperature in the dark with 3 μL of monoclonal antibodies against cell-type specific antigens and 3 μL of annexin V-FITC. Platelet-derived MP were identified using CD61-PE (phycoerythrin) and activated platelet-derived using CD62P-PE (P-Selectin +) - both from Beckman Coulter, Miami, Florida; endothelial-derived MP using CD62E-PC5 (phycoerythrin-cyanin 5.1) (Beckman Coulter, Miami, Florida); leukocyte-derived MP using CD45-PC5 (BioLegend Europe, The Netherlands) and Tissue factor-bearing (TF + MP) with CD142-PE, (clone HTF-1, BD, Biosciences, Milan, Italy). The samples were diluted in 500 μL of annexin V kit binding buffer (Bender MedSystems GmbH, Vienna, Austria) before analysis. Thirty μL of counting beads with an established concentration (Flow Count TM Fluorospheres, Beckman Coulter, Miami, Florida) were added to each sample in order to calculate MP as absolute numbers per μL of PPP.

2.4. MP Procoagulant Activity

Procoagulant activity of the MP was measured using the STA® Procoag PPL assay (Diagnostica Stago, Asnieres, France). The assay measures the clotting time in a system dependent on the total plasma phase procoagulant phospholipid content of the sample [21]. It differs from solid phase assays in that there is no pre-selection of annexin-V bound activity. The assay is performed using phospholipid depleted substrate plasma to eliminate the influence of any coagulation factors upstream. Factor Xa, in the presence of calcium, triggers the coagulation cascade and a shortening clotting time of the sample indicates an increased concentration of procoagulant phospholipids – a shorter clotting time indicating increased PPL activity. This activity linearly correlates with the functional activity of MP present in the sample [22].

2.5. Statistical Analysis

Statistical analysis was performed using the PASW Statistics 17.0.2 (SPSS Inc.) for Windows. The demographic characteristics of patients were presented as means ± standard deviations. The study groups were compared with Student-t-test. Data of the flow cytometry and PPL were not normally distributed and therefore presented as median ad interquartile range (IQR) and analyzed with Mann–Whitney U-tests for differences between two groups. Frequencies were provided for all nominal values and differences were calculated using Chi-square test. Pearson's correlation analysis was used to detect significant correlations between MP numbers and other parameters. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Patients

Out of twenty-five pregnant women diagnosed with PE consecutively referred for delivery to the Unit of Maternal Foetal Medicine at University Hospital of Padua, 16 were enrolled in the study. Five were excluded because blood sample collection ($n = 3$) and/or informed consent ($n = 2$) were not available; 2 were under antiplatelet therapy treatment; 1 had an acute infection and 1 had experienced a venous thrombotic event. Sixteen healthy pregnant women during labor referred for delivery to the same Center, during the same period of time, acted as controls. Baseline characteristics of the study population are reported in Table 1. No differences in age, parity, and BMI between parturient women with and without PE were observed. As expected, both systolic and diastolic blood pressure was significantly higher in patients with PE than in normotensive pregnancies ($p < 0.01$). Birth weight and gestational age at delivery were significantly lower in the PE women compared with the normotensive parturient women ($p < 0.05$ for both comparisons). Most PE patients were delivered by cesarean section while most of the non-PE subjects had normal vaginal delivery (Table 1).

3.2. Coagulation Parameters

Preeclamptic women had slightly higher levels of PC activity (both chromogenic and coagulometric) and slightly lower platelet count than normotensive parturient subjects ($p < 0.05$ in all comparisons). No statistically significant differences were observed in INR, aPTT, PS