Unexplained pregnancy loss: a marker of basal endothelial dysfunction?

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Objective: To compare the microparticle levels of women referred for unexplained pregnancy loss with those of parous controls.
Design: Incident case-control study.
Setting: University medical center.
Patient(s): 124 women consecutively referred for unexplained pregnancy losses (two or more losses at or before 21 weeks of gestational age, or at least one later loss), and 273 parous women without pregnancy loss.
Intervention(s): Numeration of circulating microparticles by flow cytometry after differentiation of subpopulations according to the expression of membrane-specific antigens (CD51, CD144, or CD146 for endothelial, CD41 for platelet, CD45 and CD66b for leukocyte and neutrophil microparticles).
Main Outcome Measure(s): Plasma levels of microparticles.
Results: A relative hypercoagulable state assessed by thrombin generation test had been previously reported in such cases, so we hypothesized that this could be explained by an excess of procoagulant microparticles. The study women displayed statistically significantly lower platelet and higher endothelial microparticle levels than the controls. The parameters of the thrombin generation test were only correlated with the level of endothelial microparticles, with a low coefficient of Speerman's correlation (r=0.15).
Conclusion(s): The difference in microparticle levels between the patients and controls does not clearly explain the hypercoagulable state reported in the patients but could reflect chronic endothelium damage. (Fertil Steril® 2013;100:1013–7. ©2013 by American Society for Reproductive Medicine.)
Key Words: Case-control study, endothelium, microparticles, pregnancy loss, thrombin generation test

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Previous investigations of the role of genetic thrombophilic markers in unexplained pregnancy loss have yielded conflicting results (1). In a large case-control study, we found no association between unexplained pregnancy loss (early, recurrent, or late) and factor V Leiden (FVL) or prothrombin G20210A (PTG) mutations in either parent (2). To investigate the possibility of undetectable thrombophilia and interactions between different prothrombotic abnormalities, we used a global test of the hemostatic pathway in the same group enrolled in our previous study (3). Using the method described by Hemker et al. (4), we explored the potential of thrombin generation in these nonpregnant women. Especially when the pregnancy loss had occurred between weeks 9 and 12 of gestational age, the patients displayed a baseline hypercoagulable state of relative thrombomodulin resistance that was not due to either factor VIII or protein S plasma levels or to FVL or PTG mutations (3).

Circulating microparticles (MPs) are submicron vesicles released from cell membranes in response to activation or apoptosis. As both markers and effectors of the ongoing process, MPs increase in a variety of disease states, such as thrombosis and vascular dysfunction. In addition to their many other properties, MPs are well known for their procoagulant activity, which mainly depends on phosphatidylserine and tissue factor expression. We

Received February 6, 2013; revised May 30, 2013; accepted June 4, 2013; published online July 2, 2013.
E.P. has nothing to disclose. L.D.S.M. has nothing to disclose. C.B. has nothing to disclose. M.C. has nothing to disclose. F.D.G. has nothing to disclose. D.M. has nothing to disclose.
The study was supported by a grant (PHRC 2002) from the French Health Ministry.
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Fertility and Sterility® Vol. 100, No. 4, October 2013 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2013.06.008

hypothesized that the hypercoagulable state assessed in our patients at least 2 months after any recognized obstetric event could be explained by an excess of procoagulant MPs, as reported by Laude et al. (5). Therefore, we explored MPs originating from platelets (PMP), endothelium (EMP), leukocytes (LMP), and neutrophils (NMP) in the first 124 women referred for a history of unexplained pregnancy loss and in 273 controls. Both groups had been previously enrolled in a study of thrombophilic mutations (2) and assessment of the potential to generate thrombin (3).

MATERIALS AND METHODS

Study Design and Setting

Our incident case-control study compared the MP plasma levels of women referred for unexplained pregnancy loss with those of parous controls who were at least 2 months from any recognized obstetric event. The women were recruited from February 2003 to March 2008 at the University Hospital of Brest. They were seen once by an investigator for the inclusion visit, during which they underwent a medical review using a standard questionnaire and a venous blood puncture. The blood samples were always collected randomly throughout the menstrual cycle but at least 2 months after any recognized obstetric event as well as after any anticoagulation, antiplatelet, or estrogen/progesterone treatment.

Patients

All women enrolled in the study were aged between 18 and 45 years, were from the West Brittany area, and had been consecutively referred for a history of unexplained pregnancy loss by obstetricians in the area either in private and/or public practice who participate in our reliable obstetric network (2). Pregnancy loss was defined as two or more unexplained consecutive miscarriages at or before 21 weeks of gestational age (EPL group), or at least one unexplained stillbirth after 21 weeks (LPL group).

The exclusion criteria consisted of maternal or paternal carrier of a structural chromosomal rearrangement, maternal persistent antiphospholipid antibodies, or any anatomic abnormality likely to be responsible for pregnancy loss. A standard evaluation comprised maternal testing for lupus anticoagulant, determination of immunoglobulin G and M antibodies against cardiolipin and β2-glycoprotein-1, a hysterography or hysteroscopy, and maternal and paternal cytogenetic analysis. Additionally concerning late pregnancy losses, any recognized (maternal or fetal) cause of fetal loss, comprising preeclampsia and placental abruption, was considered as an exclusion criterion; a systematic histopathologic examination of the fetus and the placenta was performed to improve the detection of infection, placental abruption, placenta membranacea, or circumvallate placenta, which are among the well-known causes of fetal loss. It is worth noting that a premature rupture of membranes before 20 weeks of gestation and a history suggesting cervical incompetency or fetal infection were considered to be exclusion criteria.

Controls

The controls were enrolled from the same geographical area, among women aged between 18 and 45 years, using the electoral registers. Women were potentially eligible if they had given birth to at least one living child. Exclusion criteria included previous pregnancy loss and preclinical miscarriages (2, 3). For both patients and controls, a history of venous thromboembolism (idiopathic or not), the use of assisted reproduction, or polycystic ovary syndrome were not considered to be exclusion criteria.

Samples

For the MP numeration (6), we obtained venous blood samples by venipuncture with 0.129 mol/L sodium citrate tubes. Blood samples were centrifuged at 1,500 $\times$ g for 15 minutes within 2 hours of venipuncture. The plasma was then recovered and centrifuged at 13,000 $\times$ g for 2 minutes.

Reagents

Biocytex supplied the phycoerythrin (PE) conjugated monoclonal antibodies against CD146. Beckman-Coulter Immunotech provided the fluorescein isothiocyanate (FITC) conjugated annexin V to label the total MPs, the PE-conjugated and FITC-conjugated isotype controls to define the background noise of the labeling, the PE-conjugated monoclonal antibodies against CD144, and the FITC-conjugated monoclonal antibodies against CD45, CD66b, and CD51 to label the MP subpopulations.

Numeration of Circulating Microparticles by Flow Cytometry

The membrane MP subpopulations were differentiated in platelet-free plasma according to the expression of membrane-specific antigens. Numeration of EMP was performed using anti-CD51, anti-CD144, or anti-CD146 labeling; numeration of PMP, LMP, and NMP was performed using anti-CD41, anti-CD45, and anti-CD66b labeling, respectively. Moreover, regardless of their cellular origin, annexin V binding was used to numerate the phosphatidylserine-expressing circulating MPs. After labeling and dilution, samples were analyzed by flow cytometry on an EPICS XL (Beckman Coulter). The MPs present in plasma were analyzed according to their parameters of size and fluorescence. Using 0.8-μm latex beads, we defined MP as vesicles <1 μm in diameter and positively them labeled with specific monoclonal antibodies (compared with IgG isotype-matched controls) or annexin V. The absolute values of MPs were calculated using Flowcount beads and expressed as MPs per microliter (μL) of plasma.

Thrombin Generation Test

Thrombin generation was performed in the whole plasma according to the method described by Hemker et al. (4) in a Fluoroscan Ascent fluorometer (Thermo Labsystems OY) equipped with a dispenser, as previously reported elsewhere (3). The analyzed parameter was the endogenous thrombin