Hemostatic Disorders in Women

Written by Toni Rizzo

Increasing awareness of the high prevalence of hemostatic disorders in women has led to an increase in clinical research on the mechanisms, diagnosis, and treatment of bleeding disorders in women. Rezan A. Kadir, MD, The Royal Free Hospital, London, United Kingdom, discussed emerging areas of research on hemostatic disorders in women and their potential clinical applications.

RECURRENT MISCARRIAGE

The role of coagulation factors in blastocyst implantation and early placental development has been a subject of research for some time. Hemostasis is necessary to prevent bleeding at the site of trophoblast invasion; however, the minimal level of coagulation factors needed to achieve this goal are unknown. The only coagulation factor deficiencies that have been implicated in recurrent miscarriage and placental abruption are those of Factor XIII (FXIII) and fibrinogen. In addition to their roles in hemostasis, FXIII and fibrinogen function are adhesive proteins that anchor cytotrophoblastic cells to the decidual cells.

Recent systematic literature review of FXIII deficiency in women, found that miscarriages occurred in 127 (66%) of 192 pregnancies in 63 women, with a recurrent miscarriage rate of 25% [Sharief L. 2013 In press]. In 136 pregnancies in women receiving no prophylaxis, 124 (91%) resulted in a miscarriage. The 12 pregnancies that reached viability stage (24 weeks of gestation and beyond) were complicated with antepartum or postpartum hemorrhage. Among the 45 pregnancies in women who received prophylaxis, only 3 (7%) resulted in a miscarriage but there were significant antepartum hemorrhage in 2 (5%). These results demonstrate that FXIII is needed to maintain a pregnancy. However, it is unknown what is the minimal necessary level of FXIII for this purpose.

This has raised a question whether FXIII level is altered in women with unexplained recurrent miscarriage and whether borderline level of FXIII is also a risk factor for a miscarriage. A recent study reported no difference in the mean FXIII-A and -B antigen levels in women with recurrent miscarriages (n=264) compared with controls (n=264; FXIII-A, 97.9% vs 96.0%; OR, 1.004; 95% CI, 0.996 to 1.012; p=0.310; FXIII-B, 95.4% vs 93.2%; OR, 1.006; 95% CI, 0.997 to 1.015; p=0.200) [Pasquier E et al. *J Thromb Haemost* 2012]. A meta-analysis of FXIII polymorphisms and recurrent miscarriage also found no link between the two [Sotiriadis A et al. *Obstet Gynecol* 2007]. Prof. Kadir's group studied FXIII activity levels in patients at a recurrent miscarriage clinic. Preliminary data showed no difference in mean FXIII levels between patients with at least three previous miscarriages (n=68) and controls with at least one living child and no miscarriage (n=62). However, a significant difference was observed in FXIII distribution, with FXIII levels <70% in 12% of the miscarriage group versus 2% of controls (p=0.0037; Figure 1). Further studies are needed to establish a link between FXIII and recurrent miscarriage.

An increased risk of miscarriage among women with mild von Willebrand's disease (VWD) has been questioned by many clinicians. There are no studies in the literature to address this. In a study of women with mild VWD, threatened miscarriage occurred in 33% of 84 pregnancies [Kadir RA et al. *Br J Obstet Gynaecol* 1998]. The overall miscarriage rate was 21%. These rates are similar to those of the general population. A Dutch study reported a mean of 1.9 live births in women with moderate or severe VWD, which is comparable to the general Dutch population [De Wee EM et al. *Thromb Haemost* 2011]. Preliminary data among our patients with recurrent miscarriage, showed a higher mean von Willebrand factor (VWF) and FVIII levels compared with the control group. Based on the limited preliminary data, Prof. Kadir concluded that there is no evidence to support a link between mild VWD and miscarriage. High rates of excessive bleeding associated with childbirth or miscarriage were observed in women with VWD in the Dutch study (52%) and a US study (77%) [Byams VR et al. *Haemophilia* 2011].

PRENATAL DIAGNOSIS OF HEMOPHILIA

Prenatal diagnosis of hemophilia started in the late 1970s with umbilical cord blood sampling to test FVIII and FIX levels. In the 1980s, genetic analysis of chorionic villous biopsies was implemented. By the 2000s, prenatal diagnosis of fetal gender by noninvasive testing of free fetal DNA in maternal plasma become possible, with an accuracy rate of about 100% at 12 weeks gestation, and it is now used to avoid invasive testing in pregnancies with a female fetus. Prof. Kadir and colleagues developed a noninvasive technique for specific prenatal diagnosis of hemophilia using quantitative digital polymerase chain reaction (PCR) to detect dosage imbalance between mutant and wild-type alleles in the plasma of pregnant carriers of hemophilia with a male fetus [Tsui NBY et al. *Blood* 2011]. This technique correctly identified fetal hemophilia status in all 12 plasma samples tested (Table1).

Because hemophilia mutations are heterogeneous, a real-time PCR assay is required for each individual case, making the approach practically challenging. Thus, in our ongoing research we focus on prenatal diagnosis of the

intron 22 inversion, which is responsible for 50% of severe hemophilia cases. Using the same principal of dosage imbalance, the aim of this ongoing study is to detect whether the male fetuses inherited the affected or normal maternal X chromosome, thus indirectly determining if the fetus has intron 22 inversion. The development of noninvasive prenatal diagnosis makes prenatal gene therapy possible in early pregnancy when the fetus is immune tolerant.

POSTPARTUM HEMORRHAGE

The risk of postpartum hemorrhage (PPH) in women with bleeding disorders can be reduced through careful management of labor and delivery and hemostatic assessment and treatment. Factors to consider in risk assessment include the type and severity of the disorder, and coagulation factor levels in the third trimester; low third-trimester factor levels have been shown to be associated with a risk of PPH in women with VWD and hemophilia A and B [Kadir RA et al. *Br J Obstet Gynaecol* 1997]. In other factor deficiency states such as FXI deficiency, factor levels do not correlate well with the bleeding risk. In such cases the bleeding history and score, and possibly global hemostatic tests can help risk assessment.

Table 1. Noninvasive Detection of Fetal Hemophilia Mutations in Maternal Plasma by Digital RMD

		Digital PCR result				
Plasma sample	Fetal hemophilia status	Total wells	Mutant count	Wild-type count	Fetal %	SPRT Classification
H9	Affected	4590	1022	801	14.8	Mutant
H26a	Unaffected	9180	1590	1710	3.8	Wild-type
H26b	Unaffected	4590	590	650	6.8	Wild-type
H15a	Affected	4590	573	435	10.5	Mutant
H15b	Affected	4590	2506	1956	10.7	Mutant
H17	Unaffected	4590	329	342	14.0	Wild-type
H30a	Unaffected	4590	611	780	18.2	Wild-type
H30b	Unaffected	4590	1839	2017	11.4	Wild-type
H25a	Affected	9180	1160	1108	4.6	Mutant
H25b	Affected	4590	223	166	15.0	Mutant
H12a	Affected	9180	511	464	9.0	Mutant
H12b	Affected	4590	293	230	25.1	Mutant

A recent study in women with PPH and no bleeding disorders found that coagulation defects are the second highest risk factor for intractable PPH (PPH not responding to obstetric measures; OR, 16.2; 95% CI, 3.2 to 83.5) [Luo FY et al. *Zhonghua Fu Chan Ke Za Zhi* 2012]. A study of hemostatic assessment in women with PPH found that women with severe PPH had significantly lower fibrinogen, FV, antithrombin activity, prolonged prothrombin time (PT), and higher D-dimer and thrombin-antithrombin (TAT) complex [Charbit B et al. *J Thromb Haemost* 2007]. On multivariate analysis, fibrinogen concentration at the point of enrollment was the only parameter independently associated with severe PPH. For each 1-g/L decrease in fibrinogen, the risk of severe PPH was 2.69-times higher. A review of 456 deliveries with bleeding losses >1500 mL showed that fibrinogen levels correlated with blood loss ($r=-0.48$; $p<0.001$) [de Lloyd L et al. *Int J Obstet Anesth* 2011].

Fibrinogen transfusion therapy has not been studied in women with PPH. There is no consensus on the threshold for a fibrinogen transfusion, nor is the best way to replace fibrinogen understood. The ongoing FIB-PPH trial is investigating the early use of fibrinogen concentrate in women with PPH [Wikkelsoe AJ et al. *Trials* 2012].

Other treatments for PPH include antifibrinolytic agents and recombinant FVIIa. The antifibrinolytic tranexamic acid has been shown to reduce blood loss and decrease the need for invasive interventions in women with PPH [Ducloy-Bouthors AS et al. *Crit Care* 2011]. The World Maternal Antifibrinolytic [WOMAN] trial is currently evaluating tranexamic acid for the treatment of PPH [Shakur H et al. *Trials* 2010].

Data from the North European Registry (2000 to 2004) show that treatment of PPH with recombinant FVIIa (n=128) resulted in 80% improvement in blood loss; there were 4 cases of venous thromboembolism (VTE), 1 myocardial infarction, and 5 deaths (none due to VTE) [Alfirevicz Z et al. *Obstet Gynecol* 2007]. Similar results were seen in the Australian and New Zealand Registry (2002 to 2008), with 76% improvement, 2 cases of VTE, and 9 deaths [Phillips LE et al. *Anesth Analg* 2009]. Both studies showed reduced hysterectomy rate after administration of rFVIIa and no death thought to be directly related to rFVIIa.

A consensus document based on a systematic review of the literature developed by an international expert panel recommends (grade C-IV evidence) the following for appropriate and effective management of PPH; the use of tranexamic acid in view of increased postpartum fibrinolysis, refilling substrates with the aim of maintaining fibrinogen level >2 g/L and platelets >80, and optimizing red cell/plasma/platelet ratio during transfusion, with the use of rFVIIa as a last resort prior to hysterectomy in patients with persistent bleeding [Kadir RA, Davies J. *J Thromb Haemost* 2013].

In conclusion, major progress has been made in the treatment of women with inherited bleeding disorders. Research is ongoing to gain a better understanding of local endometrial and systemic hemostasis in early pregnancy development, pregnancy outcomes, and PPH.