Increased thrombin generation measured in the presence of thrombomodulin in women with early pregnancy loss

Compared with 537 parous controls with no history of pregnancy loss, a lower thrombomodulin-related inhibition of the endogenous thrombin potential was measured in 264 cases with previous unexplained pregnancy loss, especially when losses occurred between 9 and 12 weeks of gestation. Adjusting age, protein S, factor VIII, factor V Leiden, and prothrombin G20210A did not change the results. (Fertil Steril® 2011;95:1813–5. ©2011 by American Society for Reproductive Medicine.)

Key Words: Case-control study, thrombin generation test with and without thrombomodulin, pregnancy loss, protein S, factor VIII

Prothrombotic mechanisms are evoked in unexplained pregnancy loss on the basis of a parallel drawn with the antiphospholipid syndrome. Consequently, the implication of prevalent genetic thrombophilic markers, the factor V Leiden (FVL), and prothrombin G20210A (PTG) mutations has been highly explored, leading to conflicting results (1–3). We have also addressed this issue and did not find any association between unexplained pregnancy loss (early, recurrent, or late) and FVL or PTG mutations sought from both parents (4).

In the possible event of yet undetectable thrombophilia as well as interactions between different prothrombotic abnormalities, one appropriate approach could involve using a functional and global test of the hemostatic pathway.

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Thromboelastography, which measures the effects of thrombin on clot formation but which also depends on platelets, fibrinogen, and fibrinolysis, has been explored once, suggesting a procoagulant status in women with a history of unexplained miscarriages (5).

The thrombin generation test (TGT), which is especially sensitive to prothrombotic states, is an attractive alternative to explore most of the hemostatic pathway (6). The addition of thrombomodulin (TM) ensures its sensitivity to the whole thrombin protein C pathway, including protein S, protein C itself, and disturbances in its activation (7).

In acute venous thromboembolism, publications have addressed the accuracy of TGT to predict a known thrombophilia and assess the risk of recurrence (8, 9). In unexplained pregnancy loss, more thrombin-antithrombin complexes, which are markers of the ongoing coagulation activation in vivo, have been determined in 35 patients compared with 34 controls (10). However, the assessment of the basal endogenous capacity to form thrombin has not yet been performed.

Therefore, the aim of this incident case-control study was to compare thrombin generation, which was explored in the absence and presence of TM, between women referred for a history of unexplained pregnancy loss and their female controls, who were previously enrolled for the determination of PTG and FVL mutations, from February 2003 to March 2008, at the University Hospital of Brest, France (4).

The women, aged between 18 and 45 years, were seen once by an investigator for a medical review. They underwent a venous blood sampling at least 2 months after any known obstetrical event, after any anticoagulation, antiplatelet, and estroprogestative treatment, and randomly throughout the menstrual cycle. The cases were referred for a history of unexplained pregnancy loss that was defined as two or more unexplained consecutive miscarriages with the same partner at or before 21 weeks of gestation (early pregnancy loss group: EPL group) or at least one unexplained pregnancy loss after 21 weeks of gestation (late pregnancy loss group: LPL group). The controls (no pregnancy loss and at least one living child) were recruited from the same geographical

area using electoral lists. Exclusion criteria are described in the Supplemental Material 1.

Thrombin generation was measured according to the method described by Hemker et al. (6) in a Fluoroscan Ascent fluorometer (Thermo Labsystems OY, Helsinki, Finland) equipped with a dispenser. Unlike in this method, we increased the PL concentration to approach the concentration of PL at the surface of activated platelets (see Supplemental Material 2).

The analyzed parameter was the endogenous thrombin potential (ETP) corresponding to the area under the curve, measured in the absence and presence of TM. The effect of TM on thrombin generation was expressed as the TM-related inhibition of the ETP (inhibition-r), defined as the ratio of the absolute difference in ETP determined in the absence and presence of TM to the ETP in the absence of TM. The intra- and interassay coefficients of variation of the inhibition rate were 3.8% and 4.2%, respectively. Factor VIII (FVIII) and free protein S (PS) measurements were performed using standardized assays.

To compare patients and controls, the Student's *t* test and χ^2-test were used for normal parameters and categorical variables, respectively. The odds ratio (OR) was calculated using logistical regression. $P<.05$ was considered statistically significant. The OR were adjusted with respect to [1] predefined variables (FVL and PTG mutations, FVIII and PS) and [2] potential confounding variables ($P<.2$). The interactions among inhibition-r, FVIII, PS, FVL, and PTG were studied using a backward stepwise logistic regression.

The study was supported by a grant (PHRC) from the French Health Office. The study was approved by the local Ethics Committee, the CPP of Brest University Hospital. The thrombin generation, PS, and FVIII measurements were available for the first 264 cases and 537 controls previously enrolled in the study by Pasquier et al. (4). Their main characteristics are summarized in Table 1.

In the absence of TM, ETP was similar between cases and controls. However, in the presence of TM, ETP was higher in cases compared with in controls ($P=.001$; Table 1). The mean inhibition-r was 38.6% (±14.4) vs. 42.3% (±15) for cases and controls, respectively ($P=.001$; OR, 0.18; 95% confidence interval [CI], [0.07–0.5]). After adjustment for age, these results remained similar.

With regard to FVL or PTG mutations, no statistically significant difference was observed between cases and controls as reported elsewhere (4). As expected, the cases or controls with FVL or PTG mutations had significantly lower inhibition-r (31.2% ± 12.4%). Excluding these women did not modify the results of the inhibition-r comparison between cases and controls (OR, 0.15; 95% CI, [0.05–0.45]). The FVIII and PS levels were significantly higher in cases than in controls ($P=.025$ and $P<.001$, respectively; Table 1). In both cases and controls, the FVIII and PS levels were significantly correlated to the inhibition-r, inversely and positively, respectively. Using a backward stepwise logistic regression, we did not find any significant impact of FVIII, PS, and FVL or PTG mutations on the inhibition-r difference observed between cases and controls. At the last iteration the remaining significant variables were inhibition-r (OR, 0.07; 95% CI, [0.03–0.2]) and PS (OR, 1.02; 95% CI, [1.01–1.03]).

We conducted an analysis according to the gestation time of the cases. The 230 women of the EPL group (median of three losses,

TABLE 1

Comparison of cases and controls with regard to main characteristics, thrombophilic markers and thrombin generation parameters, and inhibition-r after stratification according to the gestation time and to the number of losses.

Characteristic	Controls	Cases	*P* value	OR (95% CI)
n	537	264		
Age (SD)	34.6 (4.1)	32.6 (4.9)	<.001	0.9 (0.86–0.93)
Body mass index, kg/cm^2 (SD)	22.9 (4.2)	23 (4.8)	.6	1.01 (0.9–1.04)
Venous thromboembolism, %	2.8	3.4	.3	1.7 (0.7–4)
Free protein S, % (SD)	85 (18)	90 (19)	<.001	1.03 (1.01–1.04)
Factor VIII, % (SD)	98 (30)	103 (32)	.025	1.007 (1.002–1.012)
ETP without TM, μM × mn (SD)	2.09 (0.29)	2.11 (0.29)	.3	1.3 (0.8–2.1)
ETP with TM, μM × mn (SD)	1.21 (0.37)	1.3 (0.38)	.001	1.9 (1.3–2.8)
Inhibition-r, % (SD)	42.3 (15)	38.6 (14.4)	.001	0.18 (0.07–0.5)
Group and subgroup analysis	**n**	**Inhibition-r (%)**		**Comparison with controls, OR (95% CI)**
EPL group	230	38.9 (14.2)		0.2 (0.07–0.6)
LPL group	47	39.6 (16.7)		0.3 (0.04–2.2)
MGT ≤9 WA	144	39 (13.8)		0.2 (0.06–0.74)
9< MGT ≤12 WA	61	37.7 (14.8)		0.13 (0.02–0.76)
MGT >12 WA[a]	25	41.8 (16)		0.8 (0.05–12.7)
At least a loss >12 WA[a]	83	40.4 (14.2)		0.4 (0.08–2.2)
No loss >12 WA[a]	147	38.6 (13.9)		0.18 (0.05–0.7)
≥3 EPL	151	39.1 (14)		0.23 (0.07–0.8)
LPL or EPL and MGT>12 WA	67	40.8 (14.8)		0.5 (0.08–3)

Note: Inhibition-r = TM-related inhibition rate of the ETP; MGT = mean gestation time; WA = weeks of amenorrhea.
[a] Among women of the EPL group.

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