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Further Evidence for Hypercoagulability in Women With Ovarian Endometriomas

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Abstract

Our previous studies have shown that platelets play a crucial role in the development of endometriosis, and women with endometriosis appear to be in a state of hypercoagulability. However, a recent study could only replicate part of our previous finding, casting doubts on this notion. We further investigated this question through a cross-sectional study by measuring additional coagulation factors in women with and without endometriosis. To this end, we conducted a cross-sectional study of 100 women with laparoscopically and pathologically diagnosed ovarian endometriomas (OMA) and another 100 women without endometriosis. The platelet count; platelet activation rate; maximum platelet aggregation rate; plasma levels of D-dimer, fibrinogen, fibrin degradation products (FDPs), plasma soluble P-selectin (sP-sel), and prothrombin fragment 1+2 (F1+2); prothrombin time; thrombin time (TT); and activated partial thromboplastin time were measured before surgery and 3 months after surgery, and their clinical data were recorded. These measurements were also performed in control patients. We found that, compared with controls, women with OMA had a significantly higher platelet activation rate and platelet aggregation rate, elevated plasma D-dimer, fibrinogen, FDPs, sP-sel, and F1+2 levels as well as shortened TT. Remarkably, TT was prolonged, and all the other coagulation measurements, except plasma fibrinogen level, were significantly reduced 3 months after surgical removal of endometriotic lesions. Thus, our study provides another piece of evidence that endometriosis is a hypercoagulable disease, and anticoagulation therapy may hold promises in treating endometriosis.

Keywords

coagulation, endometriosis, fibrin degradation product, hypercoagulability, platelet, prothrombin fragments

Introduction

Endometriosis, defined as the deposition and growth of endometrium-like tissues outside the uterine cavity, is a common disorder affecting about 6% to 10% of reproductive-age women.[1] As a major contributor to pelvic pain and infertility,[2] it is a leading cause of gynecological hospitalization in the United States[3] and likely in many other parts of the world. Despite intense research, its pathogenesis and pathophysiology still remain an enigma.[4] Consequently, its effective treatment is still a challenge,[2,5] and there is no single biomarker that has unequivocally been shown to be clinically useful in diagnosing endometriosis.[6,7]

Endometriosis has been traditionally viewed as a hormonal disease, not only due to estrogen-dependent growth and maintenance of ectopic endometrium but also due to increased local production of estrogens owing to aberrant steroidogenesis.[8] Equally important, it is also conceptualized as a pelvic inflammatory condition,[9] featuring increased production of proinflammatory cytokines and chemokines. In the past 2 decades, however, it is becoming increasingly evident that inflammation and coagulation—long regarded as 2 separate entities—are 2 major host–defense systems that interact with each other.[10,11] In fact, the 2 entities are intricately entwined: Inflammation activates the coagulation cascade, and coagulation modulates the inflammatory activity in many ways[10,11] as in cardiovascular disease.[12] Activated platelets are found to play a critical role in initiating inflammation.[13]

In the last few years, we have provided many pieces of evidence that platelets play important roles in the development of endometriosis.[14-18] We have also provided evidence that women with endometriosis appear to be in a hypercoagulable state as manifested by shortened thrombin time (TT) and activated partial thromboplastin time (aPTT) but similar prothrombin time (PT), platelet count, and international normalized ratio (INR) to those without endometriosis, suggestive of

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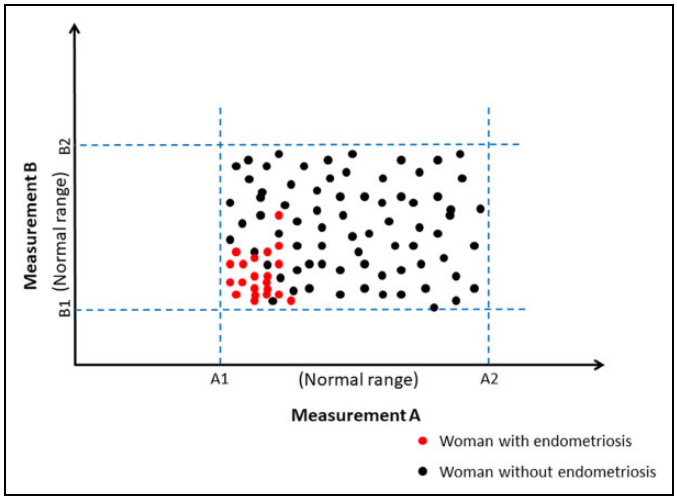

Figure 1. A hypothetical example showing the possibility of using 2 measurements, each within normal range, to discriminate women with and without endometriosis. Each red or black dot represents one data point from a woman with or without endometriosis.

the activation of the intrinsic, but not extrinsic, coagulation pathway.[19] In addition, women with endometriosis had increased plasma levels of fibrinogen and platelet activation rate.[19] One month after surgical removal of endometriotic lesions, the platelet activation rate was found to be significantly reduced but still higher than that of controls.[19] This newly found hypercoagulability provides a biologically plausible link between endometriosis and elevated risk of cardiovascular diseases[20] and raises the possibility that these coagulation parameters could be used as biomarkers of endometriosis.

However, a recent article casts some doubts on this scenario. Vigano et al found, in a cross-sectional study of 169 cases and 145 controls, that only aPTT, but not TT, INR, platelet count, neutrophil count, or platelet-to-lymphocyte ratio, was significantly different even after controlling for other confounders, although the platelet activation rate and fibrinogen levels were not evaluated.[21] In addition, the shortened aPTTs among women with endometriosis were still in the normal range, precluding the possibility of using aPTT as a biomarker for endometriosis.

Granted, just a single measurement that is well within the normal range is unlikely to be useful as biomarker to fully differentiate participants with and without a disease. However, when there are several different measurements, each having differential distributions in normal and diseased populations, these measurements, when used as a panel, could potentially be used as biomarkers, although each and every one of these measurements is still well in the normal range. Figure 1 demonstrates the case when the dimensionality is 2. Apparently, as the number of such measurements, that is, the dimensionality, increases, the ability of the biomarker panel to discriminate women with and without endometriosis should increase exponentially, although each and every individual measurement in women with endometriosis is still within the normal range.

While it is still remarkable that Vigano's group can replicate some of our findings (shortened aPTT, but not TT), their finding does raise the issue as whether women with endometriosis are truly in a hypercoagulable state as we initially reported. Shorter aPTT is one of several global coagulation tests used to assess the coagulation system and is previously reported to be risk factors for venous thromboembolism.[22] One way to settle this is to measure coagulation parameters that directly reflect the activation status of the coagulation systems.

In this study, we further evaluated TT, aPTT, and PT in women with ovarian endometriomas (OMA). In addition, we evaluated other coagulation measurements such as platelet activation rate, platelet aggregation rate, D-dimer, fibrinogen, prothrombin fragments 1+2 (F1+2), fibrin degradation products (FDPs), and soluble P-selectin levels (sP-sel). These measurements are frequently used to evaluate hemostatic conditions in people suspected to be in a hypercoagulable state. Moreover, we also measured these parameters 3 months after surgical removal of OMA, a time period long enough for patients to fully recover from surgery. We hypothesized that these coagulation parameters are significantly different between women with and without OMA, and 3 months after surgery these parameters should return back to normal levels that are compatible with that of controls. As such, they may be used as possible biomarkers for endometriosis.

Materials and Methods

Patients and Specimens

One hundred premenopausal patients with laparoscopically and histologically diagnosed OMA who were admitted to OB & GYN Hospital, Fudan University, from April 2015 to March 2016, and another 100 age-matched cycling control women without endometriosis were recruited into this study. The sample size was determined based on our previous study, which used precisely the half of the size of this study yet was able to detect difference in TT, aPTT, fibrinogen, and platelet activation rate between women with and without endometriosis. Sixty of these control women were healthy doctor or nurse volunteers or women who visited the preconception consultation clinic in our hospital and were excluded of endometriosis after systematic examinations (transvaginal ultrasound and gynecological examination), and the remaining 40 patients were patients with ovarian teratoma (n = 32) or patients with cervical intraepithelial neoplasia (CIN) III (n = 8) who underwent laparoscopic surgeries that excluded endometriosis. The patients with OMA who were subscribed gonadotropin-releasing hormone agonist, progestines, or oral contraceptive pills were previously excluded. This study was approved by the institutional ethics review board of Shanghai OB/GYN Hospital.

For all recruited participants, their family history or previous history of deep venous thrombosis or coagulation disorders was questioned but none reported. None of the recruited participants smoked or had taken any antiplatelet drug, steroid

hormones, oral contraceptives, antidiabetic, or other medications 3 months prior to the surgery. As a routine, the platelet count and blood coagulation function test including PT, TT, aPTT, INR, fibrinogen, FDPs, and D-dimer levels were measured before the surgery. In addition, total 10 mL of their peripheral blood was drawn, 5 mL was anticoagulated immediately by citric acid, 3 mL of them was fixed by paraformaldehyde, and then centrifuged to detect the platelet activation rate; 2 mL was centrifuged to detect the platelet aggregation rate; another 5 mL was anticoagulated immediately by EDTA and centrifuged and the plasma was stored at −80°C to detect the sP-sel and F1+2 levels by enzyme-linked immunosorbent assay (ELISA). All measurements were performed without knowing the group identity of the sample, thus minimizing possible bias in favor or against our hypothesis. For recruited participants, their demographic information, such as age, gravidity, parity, length of menstrual cycles, date of the last menstruation, the date on which the surgery was underwent, visual analog scale on the severity of dysmenorrhea and pelvic pain, and the amount of menses (light, if no more than 1 sanitary pad was used in each menstruation; heavy, if more than 3 pads were used; otherwise moderate) were collected. We also reviewed the medical records including clinical features, laboratory results, and pathology reports from hysterectomy.

Three months after the full recovery of the surgical injury, all the 100 patients with OMA were followed up, all their blood samples were harvested, and all the same coagulation parameters were again evaluated.

Platelet Aggregation Assay

The maximum platelet aggregation rate was determined by routine procedures.[23] Briefly, 2 mL of peripheral blood sample was anticoagulated immediately after collection by 3.2% citric acid and centrifuged at 150 *g* for 5 minutes, yielding ∼300 μL supernatant plasma (platelet-rich plasma, PRP), which were added into a test tube. The remaining blood sample was centrifuged at 1000 *g* for 15 minutes, yielding 300 μL of supernatant plasma (platelet-poor plasma, PPP), which was added into another homologous test tube. Both tubes were incubated at 37°C for 5 minutes at room temperature. Platelet aggregation in PRP was induced by 10 μL of standard adenosine diphosphate (ADP; PRECIL Medical Instrument Company, Beijing, China), and the maximum platelet aggregation rate was determined by the platelet aggregation instrument following the manufacturer's instructions (PRECIL Medical Instrument Company).

Platelet Activation Rate by FACS Assessment

Three milliliter of 3.2% citric acid anticoagulated blood were fixed by paraformaldehyde and then centrifuged at 110 *g* for 10 minutes at room temperature, and the resultant supernatant plasma was centrifuged at 1000 *g* for 15 minutes at room temperature. After platelets were isolated, 50 μL of PRP were incubated with allophycocyanin-conjugated anti-human CD61 Ab (eBioscience, San Diego, California), a marker for human platelets, and FITC-conjugated anti-human CD62p (P-sel) Ab (eBioscience), a marker for activated platelets, at room temperature but kept from light for 30 minutes. Platelets were washed with 1 mL of phosphate buffer saline and analyzed by flow cytometry cell sorting (BD FACSCalibur, San Jose, California).

Measurement of sP-sel and F1+2 by ELISA

The plasma, stored at −80°C, was melt on the ice, and concentrations of sP-sel (eBioscience) and F1+2 (MyBiosource, San Diego, California) were quantified using the human-specific ELISA kit following the manufacturer's instructions. The assay was performed in duplicate. The intra- and interassay coefficients of variation were no more than 10%.

Statistical Analysis

The comparison of distributions of continuous variables between or among 2 or more groups was made using the Wilcoxon and Kruskal tests, respectively, and the paired Wilcoxon test was used when the before–after comparison was made for the same group of participants. P values of <.05 were considered statistically significant. Multiple linear regression and Fisher linear discriminate analyses were used when appropriate. To evaluate the potential factors associated with classification capacity, a multivariate logistic regression analysis using backward elimination procedure was employed. A hierarchical cluster analysis was carried out with scaled coagulation measurement data and the Euclidean distance as the similarity metric, with the average linkage being the clustering method. The resulting dendrogram was represented as a heatmap. A multidimensional scaling analysis was performed to discriminate all recruited participants using the designated set of coagulation parameters. All computations were made with R 3.5.0 (www.r-project.org).

Results

The characteristics of the 100 patients with histologically confirmed OMA and that of the 100 control women are listed in Table 1. There was no significant difference in age, menstrual phase, parity, and gravidity between the 2 groups. Not surprisingly, however, while only 3% of controls complained dysmenorrhea, 63% of patients with OMA did (Table 1).

There was no significant difference in platelet count, mean platelet volume, INR, PT, and aPTT between the 2 groups (all Ps > .28). However, patients with OMA had significantly higher platelet activation rate ($P = 9.1 \times 10^{-12}$), maximum platelet aggregation rate ($P = .0002$), sP-sel level ($P = 9.5 \times 10^{-14}$), F1+2 level ($P = 6.6 \times 10^{-10}$), D-dimer ($P = .0004$), fibrinogen ($P = .0005$), and FDPs ($P = 7.0 \times 10^{-5}$) but lower TT levels ($P = .0097$; Figure 2). Multiple linear regression analyses controlling for age, menstrual phase, parity, and group identifier (OMA or control) indicated that the group identifier

Table 1. Characteristics of Controls and Patients With Endometriomas.

Variable	Control, n = 100	Ovarian Endometrioma, n = 100	*P* Value
Age, years			
Mean (SD)	32.0 (7.1)	33.0 (7.1)	0.31
Median (range)	31.5 (21-49)	33.0 (21-49)	
Gravidity			
0	36 (36.0%)	41 (41.0%)	0.26
1	27 (27.0%)	32 (32.0%)	
2	17 (17.0%)	17 (17.0%)	
≥3	20 (20.0%)	10 (10.0%)	
Parity			
0	48 (48.0%)	47 (47.0%)	0.67
1	44 (44.0%)	48 (48.0%)	
≥2	8 (8.0%)	5 (5.0%)	
Menstrual phase at the time of blood sampling			
Proliferative	60 (60.0%)	56 (56.0%)	0.67
Secretory	40 (40.0%)	44 (44.0%)	
Severity of dysmenorrhea			
None (VAS=0)	97 (97.0%)	37 (37.0%)	$<2.2 \times 10^{-16}$
Mild (VAS=1-3)	3 (3.0%)	40 (40.0%)	
Moderate (VAS=4-6)	0 (0.0%)	12 (12.0%)	
Severe (VAS=7-10)	0 (0.0%)	11 (11.0%)	
rASRM scores			
Mean (SD)		43.5 (28.6)	NA
Median (range)	NA	36 (4-112)	
rASRM stage			
I-II	NA	21 (21.0%)	NA
III-IV		79 (79.0%)	
Maximal diameter of the endometrioma, cm			
Mean (SD)	NA	5.7 (1.9)	NA
Median (range)		6.0 (1.0-12.0)	
Co-occurrence with adenomyosis			
No	100 (100.0%)	89 (89.0%)	NA
Yes	0 (0.0%)	11 (11.0%)	
Laterality			
Unilateral		54 (54.0%)	
Bilateral	NA	46 (46.0%)	

Abbreviations: rASRM, revised American Society for Reproductive Medicine; SD, standard deviation; VAS, visual analog scale; NA, not applicable.

was the only variable that is significantly associated with these measurements (all *P*s < .011).

Three months after the surgical removal of all visible lesions by laparoscopy, these coagulation measurements, except plasma fibrinogen levels ($P = .31$), were all significantly changed (*P*s < .0019; Figure 2). Further examination revealed that while the majority of patients with OMA had reduced coagulation parameters 3 months after surgery, a few patients had slightly increased values (Figure 3). Multiple linear regression analysis of the change using age, parity, lesion size, and laterality indicated that the change was found not to be related to the lesion size or laterality (all *P*s > .10).

Compared to the controls, the changed platelet activation rate, aggregation rate, D-dimer, TT, and FDPs levels resulting from surgery were comparable (*P*s > .054; Figure 2). However, the sP-sel, F1+2, and fibrinogen levels were slightly but significantly higher than that of controls (3 *P*s < .041; Figure 2). Multiple linear regression analyses using age, parity, and menstrual phase at which the blood sample was taken gave identical results, but the R^2 were generally <.06, indicating a poor fit.

To see how well these putative biomarkers can discriminate patients with and without endometriosis, we performed a 2-way hierarchical cluster analysis of the data using the Euclidian distance and presented the cluster dendrograms as a heatmap (Figure 4A) and also projected the results of multidimensional scale onto a 2-dimenstional plane (Figure 4B). As expected, FDPs and D-dimer levels were clustered together, since the latter is one of the constituents of the FDPs. It can be seen from Figure 4 that the use of all 8 coagulation parameters could roughly classify patients with and without endometriosis, but the classification was far from perfect. In fact, using the same set of 8 variables, Fisher linear discriminate analysis yielded a false-positive rate of 7.0% and a false-negative rate of 11.6%, or the total correct classification rate of 81.4%.

Using logistic regression analysis, we identified plasma sP-sel, F1+2, and TT levels and the platelet aggregation rate as 4 potential biomarkers. Using these 4 variables, we performed another 2-way hierarchical cluster analysis and presented the results as a heatmap (Figure 5A) and also projected the results of 4-dimensional scale onto a 2-dimenstional plane (Figure 5B). It can be seen from Figure 5 that the use of just 4 coagulation parameters could roughly classify patients with and without endometriosis with similar accuracy as with the original 8 variables. In fact, using these 4 variables, Fisher linear discriminate analysis yielded a false-positive rate of 8.1% and a false-negative rate of 11.6%, or the total correct classification rate of 80.2%. In other words, the use of these 4 measurements resulted in a discrimination rate similar to that using 8 measurements.

Discussion

This study found that, compared with women without endometriosis, women with OMA had a significantly higher platelet activation rate and platelet aggregation rate, elevated plasma D-dimer, fibrinogen, FDPs, sP-sel, and F1+2 levels as well as shortened TT. Remarkably, TT was prolonged and all the other coagulation measurements, except plasma fibrinogen level, were significantly reduced 3 months after surgical removal of endometriotic lesions. These data, taken together, provide further evidence for hypercoagulability in women with endometriosis.

Coagulation pathways in humans are the most studied and the most understood. Once the coagulation cascade is initiated, F1+2 are released from prothrombin when prothrombin is converted to thrombin, and thus F1+2 can be used as a marker of coagulation activation.[24] The TT is involved in the conversion of fibrinogen to fibrin. When blood clots and fibrin nets dissolve, FDPs are released into the circulation. If the system cannot dissolve blood clots, one may experience elevated FDPs. D-dimer is one specific FDP after the interlinking of

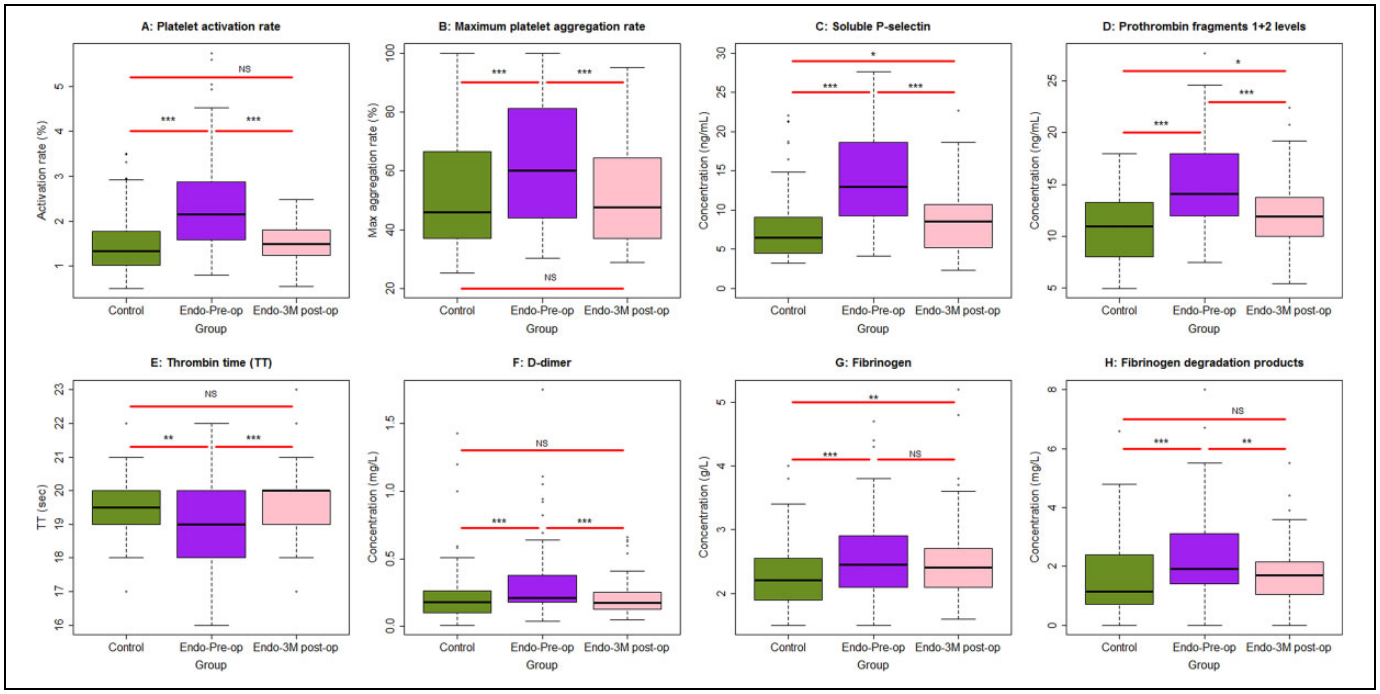

Figure 2. Data summary by boxplots. The percentage of activated platelets (A), maximum platelet aggregation rate (B), soluble P-selectin levels (C), prothrombin fragments 1+2 levels (D), thrombin time (TT) (E), D-dimer levels (F), fibrinogen levels (G), and levels of fibrin degradation products (H) in the peripheral blood from women without endometriosis (control), women with ovarian endometriomas before the surgery (Endo-pre-OP) and 3 months after the surgery (Endo-3 M post-OP). The statistical significance levels of the between-group difference in the designated groups are shown. Levels of statistical significance are *$P < .05$; **$P < .01$; ***$P < .001$; NS: $P > .05$. OP indicates operation; NS, nonsignificant.

fibrin monomer and factor XIII (FXIII). When present in peripheral blood, D-dimer indicates the simultaneous activation of coagulation and fibrinolysis pathways.[24] Therefore, increased FDPs and D-dimer levels reflect the activation of coagulation and the fibrinolytic system and as such can be used as indicators of coagulation activation. Given our findings of shortened TT, increased platelet activation rate and platelet aggregation rate, and elevated plasma D-dimer, fibrinogen, FDPs, sP-sel, and F1+2 levels in the peripheral blood from women with endometriosis, the evidence for a hypercoagulable state is unmistakable. As such, this provides a biologically plausible link between endometriosis and elevated risk of cardiovascular diseases.[20] Moreover, the return of these coagulation parameters (except fibrinogen) to normal levels 3 months after surgical removal of endometriotic lesions indicates that the aberration in these coagulation parameters results from the presence of lesions. When lesions are removed and the patients are fully recovered, the root cause for the aberration is eliminated, thus explaining the return to normal levels.

Our study has several limitations. First, the inclusion of patients with teratoma and CIN III as controls may have diluted the signal-to-noise ratio, making the detection of the difference in aPTT between women with and without endometriosis more difficult. Second, failure to quantify the progression stage of endometriotic lesions, as we did in animal models,[18,25] precludes an in-depth analysis as why the discriminating power of the putative biomarker panel is less than perfect.

It should be noted that the coagulation cascade activated in women with endometriosis is actually consistent with the notion that endometriotic lesions are wounds that undergo repeated tissue injury and repair.[18,26] Indeed, cyclic bleeding signifies endothelial damage as well as tissue damage, resulting in platelet activation and aggregation. Of course, increased vascular permeability due to increased angiogenesis and inducers of platelet-activating factors (PAFs) released by endometriotic lesions[27] may also result in platelet aggregation in lesions. The activated platelets would further release von Willebrand factor (vWF), ADP, serotonin, PAF, thromboxin A2 (TXA_2), and platelet factor 4 (also known as CXCL4), causing further platelet aggregation and perhaps perpetuating the coagulation activation.

This may explain as why in women with endometriosis vWF concentration is elevated[28] and possibly elevated expression of CXCL4 variant[29] and of P2X purinoceptor 3[30] in endometriotic lesions. This also raises the possibility that ADP receptors such as P2Ys, PAF receptors, thromboxane receptors, and CXCL4 receptor CXCR3 may also be involved in the development of endometriosis. In addition, other procoagulation factors and thrombin generation markers, such as FV, FVIII, FIX, and FXI,[31,32] microparticles,[33] and perhaps also urinary metabolites of TXA_2[34] may also display aberrations in women with endometriosis. Indeed, circulating microparticles have been reported recently to be elevated in women with deep endometriosis.[35] Finally, the hypercoagulability seemingly resulting

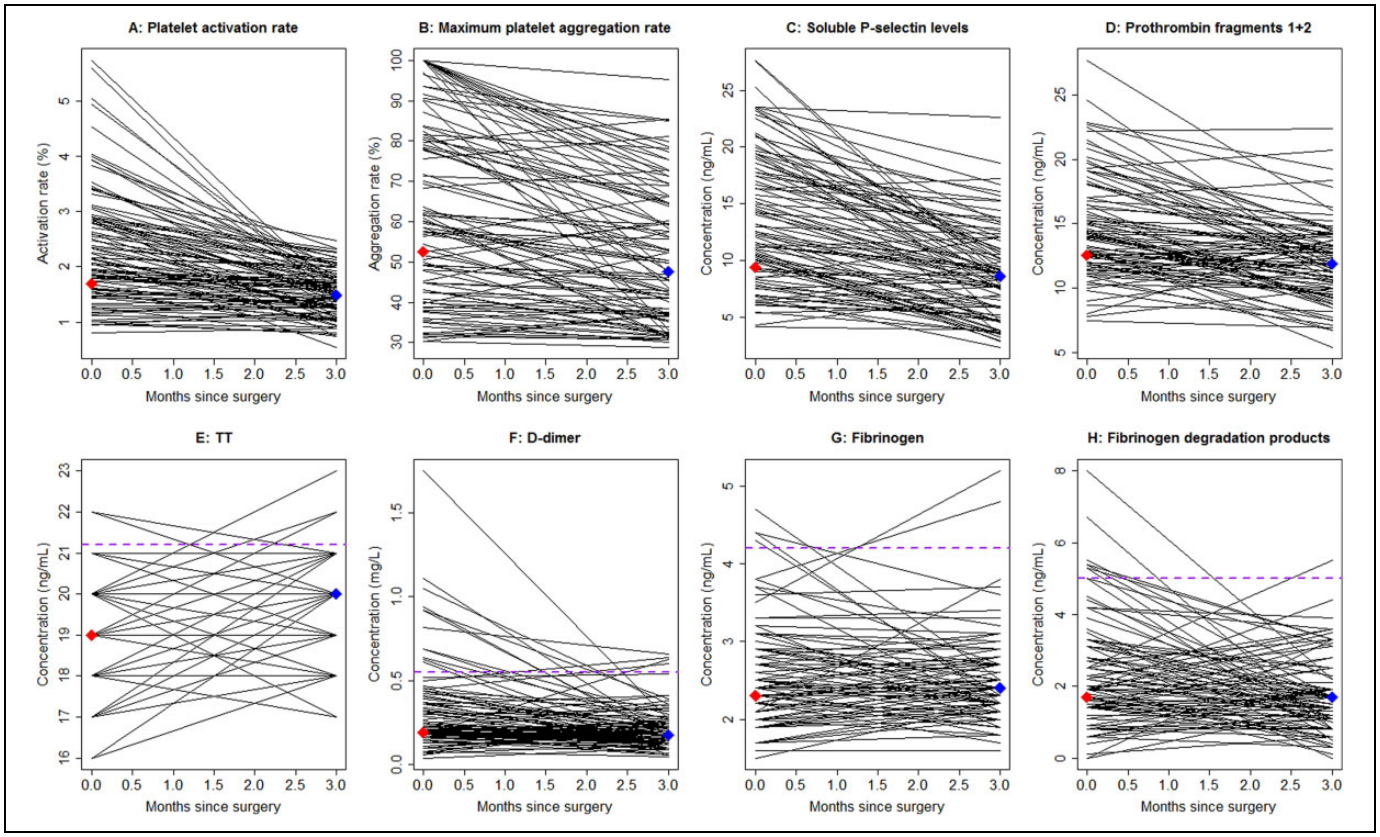

Figure 3. Changes in various coagulation measurements before and 3 months after surgery in the peripheral blood from women with ovarian endometriomas. (A) The percentage of activated platelets, (B) maximum platelet aggregation rate, (C) soluble P-selectin levels, (D) prothrombin fragments 1+2 levels, (E) thrombin time (TT), (F) D-dimer levels, (G) fibrinogen levels, and (H) levels of fibrin degradation products. Each line in the plot represents one data point from one patient; however, in cases when there are limited data readings (as in TT), one line shown in the figure may be data from several patients. The dashed line in some plots represents the boundary of the normal range (as the upper bound of the normal range in TT). The red and blue diamonds represent, respectively, the median values of the measurements before and 3 months after the surgery.

from endometriosis suggests that anticoagulation or antiplatelet therapy could be promising as already shown in animal models of endometriosis.[15,16,25]

Although our study did not replicate our previous finding that aPTT is shortened in women with endometriosis,[19] we were able to replicate other findings reported previously, that is, shortened TT, elevated plasma fibrinogen levels, and increased platelet activation rate.[19] In addition, we replicated our previous finding that the platelet activation rate was significantly reduced after surgical removal of endometriotic lesions. In conjunction with the higher platelet activation rate and platelet aggregation rate, and elevated plasma D-dimer, fibrinogen, FDPs, sP-sel, and F1+2 levels, our data provide a stronger piece of evidence for a hypercoagulable state in women with endometriosis.

The failure to replicate the finding of shorter aPTT in[21] and in our own previous study[19] may be simply due to the difference in patient population (and perhaps ethnicity) and the composition of the control group. Indeed, all the controls in our previous study were healthy and apparently free of any diseases, gynecological or otherwise.[19] This should clearly distinguish any significant difference in TT or aPTT between women with endometriosis and controls. In contrast, 40% of the control patients in this study had either teratoma or CIN III. If some of these patients were also hypercoagulable, then detection of the difference would have had been more difficult than that in.[19] Similarly, the control group in[21] consisted of patients who received a surgery due to various gynecological conditions except endometriosis. It is possible that some of these patients may also have a hypercoagulable tendency, and if this is the case, it would have been difficult to detect the difference in coagulation measurements between women with and without endometriosis.

In addition, while it is known that a short aPTT is associated with hemostatic activation as evidenced by elevations in F1+2, thrombin–antithrombin complexes, D-dimers, and FVIII coagulant activity—all markers for increased thrombin generation in plasma,[36] we should bear in mind that aPTT is frequently used in conjunction with PT to evaluate how quickly blood clotting takes place and is not used alone. The aPTT measures the overall speed at which blood clots by means of 2 consecutive series of biochemical reactions known as the intrinsic pathway (also called "contact activation pathway")[37] and the

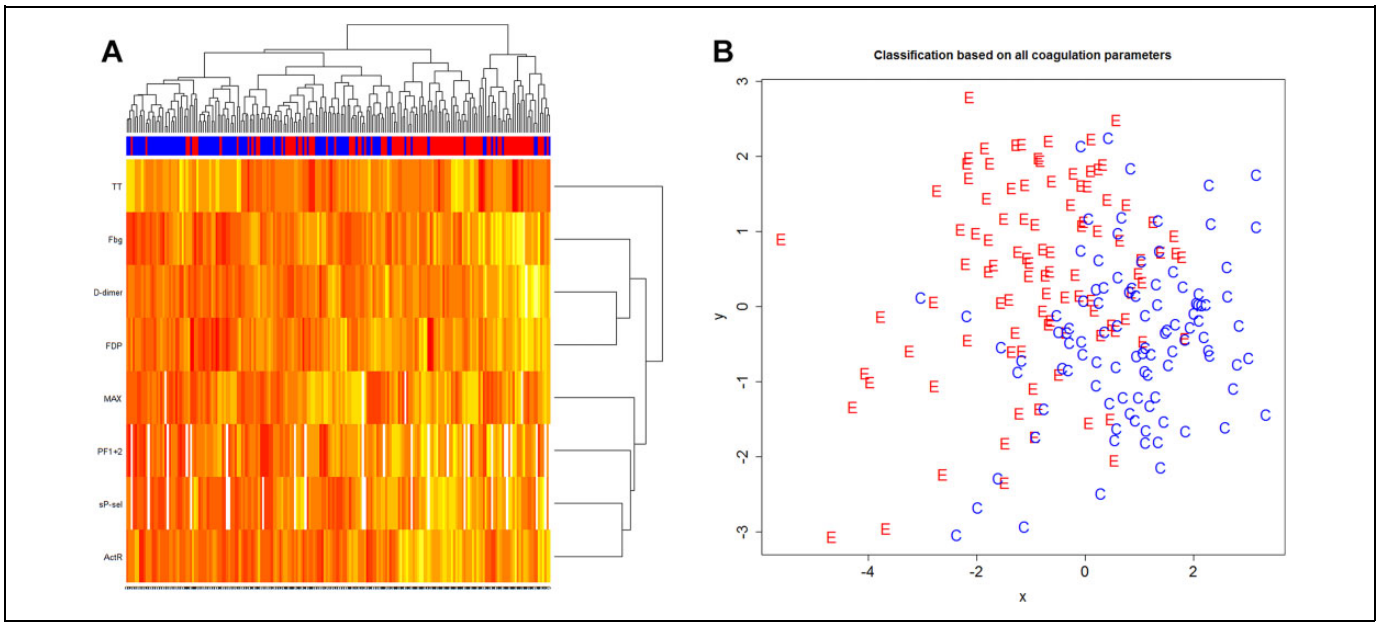

Figure 4. A, Hierarchical clustering heatmap of coagulation measurements and all recruited participants. The heatmap was organized by clustering both participants (by columns) and measured coagulation parameters (by rows). Within the heatmap, the red color represents the minimal values, while the white color represents the maximal values. The labels on the bottom panel represent which group the participants came from: E (endometriosis) and C (control). The group label is also depicted by coloration in the uppermost panel, with red representing E and blue, C. The labels in the left are the names of the coagulation measurements. B, Classification of recruited participants by multi-dimensional scaling using the abovementioned 8 measurements. E and C stand, respectively, for women from the endometriosis and the control group. TT indicates thrombin time; Fbg, fibrinogen; FDP, fibrin degradation products; MAX, maximum platelet aggregation rate; PF1+2, prothrombin fragments 1+2; sP-sel, soluble P-selectin; ActR, platelet activation rate.

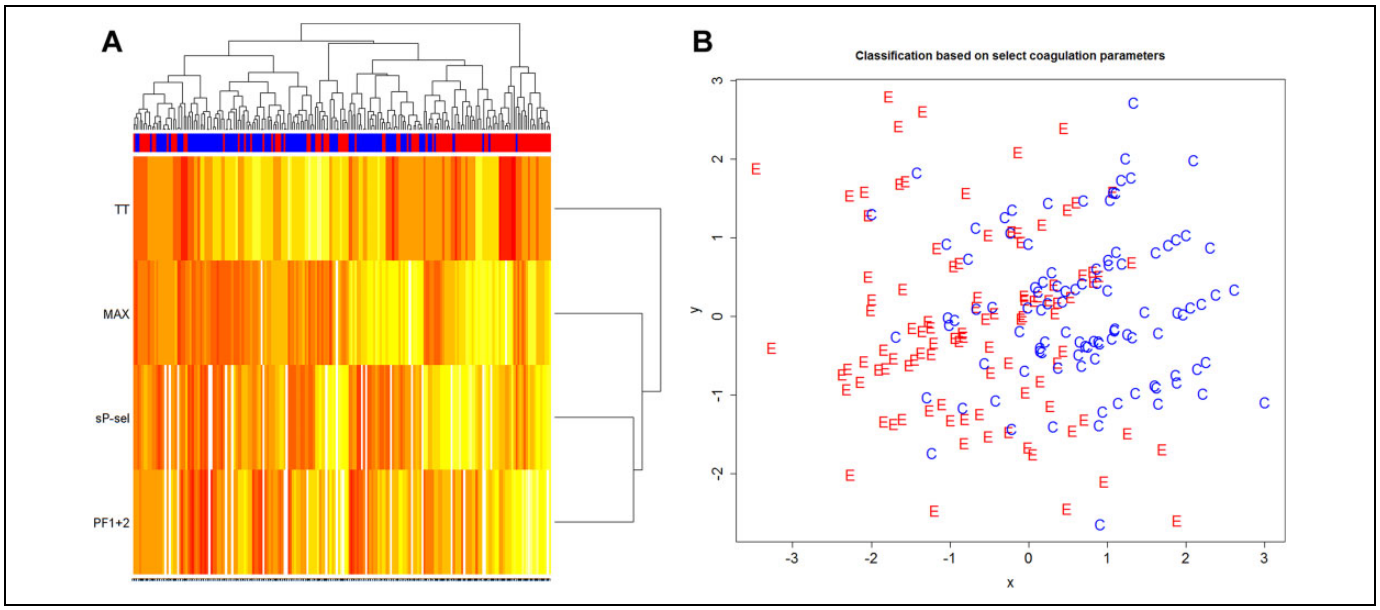

Figure 5. A, Hierarchical clustering heatmap of coagulation measurements and all recruited participants. The heatmap was organized by clustering both participants (by columns) and measured coagulation parameters (by rows). Within the heatmap, the red color represents the minimal values, while the white color represents the maximal values. The labels on the bottom panel represent which group the participant came from: E (endometriosis) and C (control). The group label is also depicted by coloration in the uppermost panel, with red representing E and blue, C. The labels in the left are the names of the coagulation measurements. B, Classification of recruited participants by multidimensional scaling using the abovementioned 4 measurements. E and C stand, respectively, for women from the endometriosis and the control group. TT indicates thrombin time; MAX, maximum platelet aggregation rate; PF1+2, prothrombin fragments 1+2; sP-sel, soluble P-selectin.

common coagulation pathways. If Vigano et al[21] also measured fibrinogen, D-dimer, F1+2, FDPs, and perhaps other procoagulation factors, they might have found similar results as we did in this study.

While the results of using the coagulation parameters to classify patients with and without endometriosis appear to be appealing as well as promising, they are not very satisfactory from a practical standpoint (Figures 4 and 5). Granted, an 80% accuracy is good for a non-invasive test but that was based on the training data set and not an independent data set. For a more realistic case when women without endometriosis could have other gynecological conditions, the discriminating power using these putative biomarkers is likely to be lower. This suggests several points. First, despite each variable having somewhat distinct distributions for the 2 populations (diseased and not), as in our case, the discriminating power may still be far from optimal even when all variables are used as a panel. Second, it is very likely that coagulation may well be just one aspect of endometriosis, and as such, using all coagulation parameters may still not capture the distinct features that separate women with endometriosis from those without. What other features should be is at this moment unclear and would require future investigations. Third, if women without endometrisis do have other disorders that may also present hypercoagulability, the discriminating power will surely decrease. Finally, it is quite plausible that the less-than-desirable discriminating power of the panel is attributable to the fact that all patients with OMA were treated as equal (ie, they all have OMA), but in actuality, different patients most likely had OMA lesions in quite different developmental stage toward fibrosis.[38] Moreover, some patients with OMA may also have had comorbidity, such as uterine fibroids, deep endometriosis, and adenomyosis, let alone other nongynecological disorders. As we have shown previously, endometriotic lesions in different developmental stages have entirely different molecular and cellular aberrations[17,18] and so do different subtypes of endometriosis, such as OMA and deep endometriosis.[39] Treating all patients with endometriosis as if they were cast in the same mold and failure to account for the difference in disease development stage and comorbidity can also seriously reduce the discriminating power. This may account for, perhaps to a large extent, the abject failure in the identification of biomarkers for endometriosis.[6,7]

Nonetheless, our results indicate that one possible additional requirement for an ideal biomarker for endometriosis is that the biomarker value should be similar to those without endometriosis well after endometriotic lesions are completely removed. This would require that the biomarker is indeed the result of endometriosis. One possible candidate of course is the coagulation measurements investigated in this study.

In summary, our study provides more convincing evidence that women with OMA are in a hypercoagulable state, as manifested by higher platelet activation rate and platelet aggregation rate, elevated plasma D-dimer, fibrinogen, FDPs, sP-sel, and F1+2 levels, as well as shortened TT in their peripheral blood. In addition, nearly all these coagulation parameters return back to the normal levels 3 months after surgical removal of endometriotic lesions. As such, our study provides a biologically plausible link between endometriosis and elevated risk of cardiovascular diseases. In addition, it implicates many accessories that are very likely to be involved in the development of endometriosis, but so far have not been even suspected. Moreover, other procoagulation factors could be potential candidates for biomarkers of endometriosis. Finally, the hypercoagulability seemingly resulting from endometriosis suggests that anticoagulation or antiplatelet therapy could be a promising therapeutics, as has been shown in animal models of endometriosis.[15,16,25]

Authors' Note

Ding Ding and Xishi Liu contributed equally to this work.

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