



Fibrin monomer complex as a potential thrombosis marker related to venous thromboembolism risk in pregnant women

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Abstract

Background: Pregnancy is a risk factor for venous thromboembolism (VTE) due to increased coagulation factor activity and decreased protein S activity. However, thrombosis markers for predicting VTE in pregnancy remain controversial. This study aimed to investigate the relationship between VTE risk and thrombosis markers in pregnant women and to identify markers related to VTE risk.

Methods: Archived plasma samples from 107 pregnant women were used in this study, and the concentrations of D-dimer, fibrin monomer complex (FMC), plasmin-plasmin inhibitor complex, prothrombin time, activated partial thromboplastin time, and fibrinogen were measured. VTE risk was scored according to the Royal College of Obstetricians and Gynaecologists green-top guidelines and the patients were divided into low- or high-risk groups.

Results: The median (range) of risk score for deep vein thrombosis was 2 (0–8), and we defined the high-risk group included those with a score of ≥ 3 . D-dimer and FMC concentrations were significantly higher in the high-risk group than in the low-risk group (D-dimer 4.5 vs 2.6 $\mu\text{g/mL}$, $p = 0.008$; FMC 14.6 vs 3.4 $\mu\text{g/mL}$, $p < 0.001$). Although D-dimer concentration significantly increased with gestational age (Spearman's correlation coefficient [r_s] = 0.317, $p < 0.001$), FMC concentration did not ($r_s = -0.081$, $p = 0.409$). The area under the receiver operating characteristic curve values of D-dimer, FMC, and both D-dimer and FMC for the high-risk group were 0.656, 0.713, and 0.738, respectively.

Conclusions: FMC may be a thrombosis marker related to VTE risk in pregnancy and is potentially preferable over D-dimer concentrations.

Keywords

Pregnancy, venous thromboembolism, fibrin monomer complex, D-dimer, thrombosis markers

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Introduction

The number of patients with venous thromboembolism (VTE) is increasing annually, and pregnancy is a risk factor for VTE. Pregnant women are five times more likely to develop VTE than non-pregnant women.¹ Pregnant women with a history of recurrent VTE, medical comorbidities, surgery, and emergency caesarean sections, among others, are particularly at a high risk of VTE.^{2–5} The Royal College

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of Obstetricians and Gynaecologists (RCOG) guidelines describe and score VTE risk factors in pregnancy.⁶

Thrombin-antithrombin complex (TAT) and D-dimer concentrations are among the tests utilized clinically for predicting and diagnosing thrombosis and for diagnosing VTE in pregnancy. However, these thrombosis markers are not sufficient for VTE diagnosis during pregnancy. Reliable markers for predicting and diagnosing VTE are required to appropriately manage pregnant women.

TAT concentration is known to increase with inflammation, diabetes mellitus, inadequate phlebotomy, and pregnancy.^{7–9} D-dimer concentration also increases in various scenarios, including pregnancy, even in the absence of clinical thrombosis.⁹ The specificity of these thrombosis markers is low; therefore, these tests are not appropriate for VTE diagnosis during pregnancy.

Fibrin monomer complex (FMC) is used to monitor the proteolytic cleavage of fibrinogen by thrombin and is therefore used as a marker of thrombin activity. Thrombin cleaves fibrinopeptides A and B from fibrinogen to create soluble fibrin monomers (SFMs). These SFMs can combine with fibrinogen to form non-covalently associated soluble fibrin monomer complexes (SFMCs). In the final step, SFMCs polymerize via factor XIIIa crosslinking and a thrombus is formed. As FMC concentration reflects thrombin activity and its concentration can be detected earlier than D-dimer concentration, FMC can be used as an alternative for assessing thrombosis.¹⁰

FMC reportedly enhanced VTE detection in pregnancy in some studies, while other studies have found that it is not a useful marker. Thus, the thrombosis markers used to detect VTE in pregnant women remain controversial. Therefore, this study aimed to investigate the relationship between VTE risk and thrombosis markers in pregnant women and to identify markers related to thrombosis risk.

Materials and methods

Patients and clinical samples

This study included 107 pregnant women who were admitted to the obstetrics ward and the maternal fetal intensive care unit of Tottori University Hospital in Japan between September 2019 and December 2021. All samples were collected using standard venipuncture blood collection tubes containing one-tenth volume of sodium citrate (3.2%/0.109 M) to attain a final citrate concentration of 0.32%/0.0109 M. Platelet-poor plasma was obtained after centrifugation of citrated whole blood for 15 min at 1500 g. All plasma samples were stored at -80°C and thawed at 37°C immediately prior to the assays. All samples were analysed within one month. This study was approved by the Ethics Committee of the Tottori University Faculty of Medicine (approval number: 19A056).

Risk classification based on RCOG green-top guidelines

The RCOG green-top guidelines assign 4 points for previous VTE and 3 points for surgical procedures and medical comorbidities.⁶ For those with multiple risks, the risk scores for each were summed. We performed a risk classification based on patient information at the sampling date. Surgical procedures were defined as surgeries performed during pregnancy, and medical comorbidities were defined as diseases being treated in other departments. The median deep vein thrombosis (DVT) risk score of the 107 pregnant women was 2 (range 0–8), so we defined patients with two or fewer points as the low-risk group and those with three or more points as the high-risk group (Table 1).

Measurement of coagulation/fibrinolysis markers

Prothrombin time (PT); activated partial thromboplastin time (APTT); and concentrations of FMC, D-dimer, plasmin alpha 2-plasmin inhibitor complex (PIC), and fibrinogen were measured. FMC concentration was quantitatively measured using latex immunoturbidimetry assay (LIA) using an Auto LIA FM Kit (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan). When the sample and latex test solution are allowed to act in the buffer, the complex containing the fibrin monomer in the sample aggregates due to a specific antigen-antibody reaction with the anti-fibrin monomer antibody-bound latex, resulting in turbidity. Because turbidity is proportional to the amount of FMC in the sample, the amount of FMC in the sample can be measured optically by assessing the turbidity. D-dimer and PIC concentrations were also measured using LIA. Fibrinogen concentration, APTT, and PT were measured using the coagulation time method. All measurements were performed using the CN-6000 (Sysmex, Kobe, Japan) (Table 2).

Analyses and statistics

All analyses were performed using EZR software (ver. 1.54, Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a Japanese user interface for R (ver. 4.0.3, The R Foundation for Statistical Computing, Vienna, Austria).¹¹ We compared differences in thrombosis markers using the Mann–Whitney U test. The correlation between gestational age and thrombolysis markers was calculated using Spearman's correlation coefficient. We also investigated whether D-dimer or FMC concentrations were associated with a high risk for VTE via receiver operating characteristic (ROC) curve analysis. Cases with missing values were not excluded and were treated as missing values.

Table 1. Clinical characteristics of patients.

Clinical characteristics	All (N = 107)	Score <3 (N = 68)	Score \geq 3 (N = 39)	p value
Score, median (range)	2 (0–7)	1 (0–2)	4 (3–7)	<0.001
Age, years (range)	33 (21–45)	33 (21–44)	33 (25–45)	0.277
Gestational age, weeks (range)	34.4 (8.0–41.7)	34.9 (8.0–41.7)	34 (23.9–41.1)	0.549
2BMI, kg/m (range)	25.7 (17.1–36.7)	25.7 (18.6–34.9)	25.7 (17.1–36.7)	0.363
BMI \geq 30, N (%)	21 (19.6)	10 (14.7)	11 (28.2)	0.128
Age >35, N (%)	44 (41.1)	26 (38.2)	18 (46.2)	0.541
Smoking, N (%)	4 (3.7)	0 (0.0)	4 (10.3)	0.016
Parity \geq 3, N (%)	3 (2.8)	2 (2.9)	1 (2.6)	1
Multiple pregnancy, N (%)	31 (29.0)	17 (25.0)	14 (35.9)	0.271
Medical comorbidity, N (%)	25 (23.4)	0 (0.0)	25 (64.1)	<0.001
Surgical procedure, N (%)	9 (8.4)	0 (0.0)	9 (23.1)	<0.001
Current systemic infection, N (%)	1 (0.9)	1 (1.5)	0 (0.0)	1
ART, N (%)	12 (11.2)	5 (7.4)	7 (17.9)	0.117
Caesarean section in labour, N (%)	5 (4.7)	1 (1.5)	4 (10.3)	0.056
Underwent the ultrasonography of lower limb veins, N (%)	38 (35.5)	19 (27.9)	19 (48.7)	0.037

BMI, body mass index; ART, assisted reproductive technology.

Table 2. Reagents and methodology used for study.

Test	Reagent	Methodology
Prothrombin time/%	Thromborel S	Coagulation time
Activated partial thromboplastin time	APTT-SLA	Coagulation time
Fibrinogen	Thrombin reagent LQ	Coagulation time
Fibrin monomer complex	Auto LIA FM Kit	Latex immunoassay
D-dimer	LIASAUTO D-dimer NEO	Latex immunoassay
Plasmin alpha 2-plasmin inhibitor complex	LIASAUTO PIC	Latex immunoassay

Results

The median deep vein thrombosis (DVT) risk score of the 107 pregnant women was 2 (range 0–8). The median (range) of age and gestational age were 33 (21–45) and 34.4 (8–41.7), respectively. Other clinical characteristics of the study population (107 pregnancies) are shown in [Table 1](#). The surgical procedures were as follows: Shir-odkar operation (n = 6), uterine artery embolization (n = 1), conization (n = 1), and fetal reduction surgery (n = 1). The medical comorbidities included myelodysplastic syndrome (n = 3), gestational diabetes (n = 7), hypothyroidism (n = 5), systemic lupus erythematosus (n = 4), hyperthyroidism (n = 4), and rheumatoid arthritis (n = 2). Thirty-eight pregnant women (35.5%) underwent lower-limb echocardiography and none of them developed thrombosis. The classification of the patient's gestational age is before 16 weeks of gestation (n = 1); 16–27 weeks (n = 10); after 28 weeks (n = 96).

Distributions of FMC and D-dimer concentrations are shown in [Figure 1](#).

The median (range) concentrations of FMC and D-dimer were 4.0 μ g/mL (0.6–200 μ g/mL) and 3.3 μ g/mL (0.7–25 μ g/mL), respectively. Our hospital's standard values are less than one for D-dimer and less than seven for FMC. FMC and D-dimer concentrations were significantly higher in the high-risk group than in the low-risk group (FMC 14.6 vs 3.4 μ g/mL, $p < 0.001$; D-dimer 4.5 vs 2.6 μ g/mL, $p = 0.008$). Other coagulation/fibrinolysis markers were not significantly different between two groups ([Figure 2](#)).

The relationship between gestational age and thrombosis markers was evaluated using Spearman's correlation coefficient. D-dimer and PIC concentrations increased significantly throughout pregnancy (D-dimer; $r_s = 0.317$, $p < 0.001$, PIC; $r_s = 0.248$, $p = 0.01$); however, FMC, fibrinogen, PT, and APTT (FMC; $r_s = -0.081$, $p = 0.409$, fibrinogen; $r_s = 0.181$, $p = 0.073$, PT; $r_s = -0.031$, $p = 0.753$, APTT; $r_s = 0.063$, $p = 0.524$) were unaffected by gestational age ([Figure 3](#)).

To evaluate the discriminative performance of FMC and D-dimer, we further applied ROC curve analysis ([Figure 4](#)). ROC analysis was performed with the high-risk group for VTE as the objective variable to compare

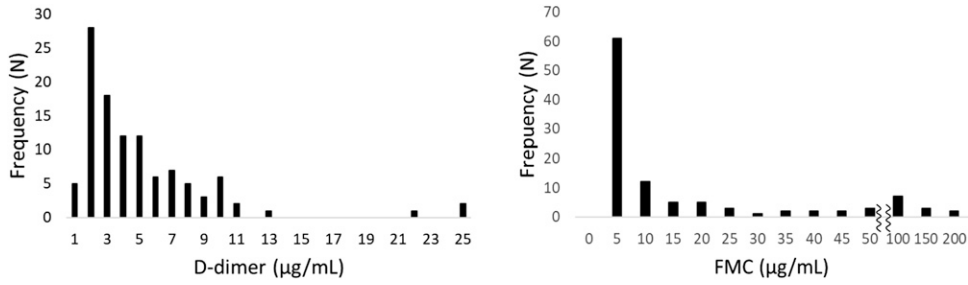


Figure 1. Distributions of fibrin monomer complex (FMC) and D-dimer concentrations in pregnant women. Reference intervals: D-dimer < 1.0 µg/mL, FMC < 7.0 µg/mL (reference intervals set by our hospital).

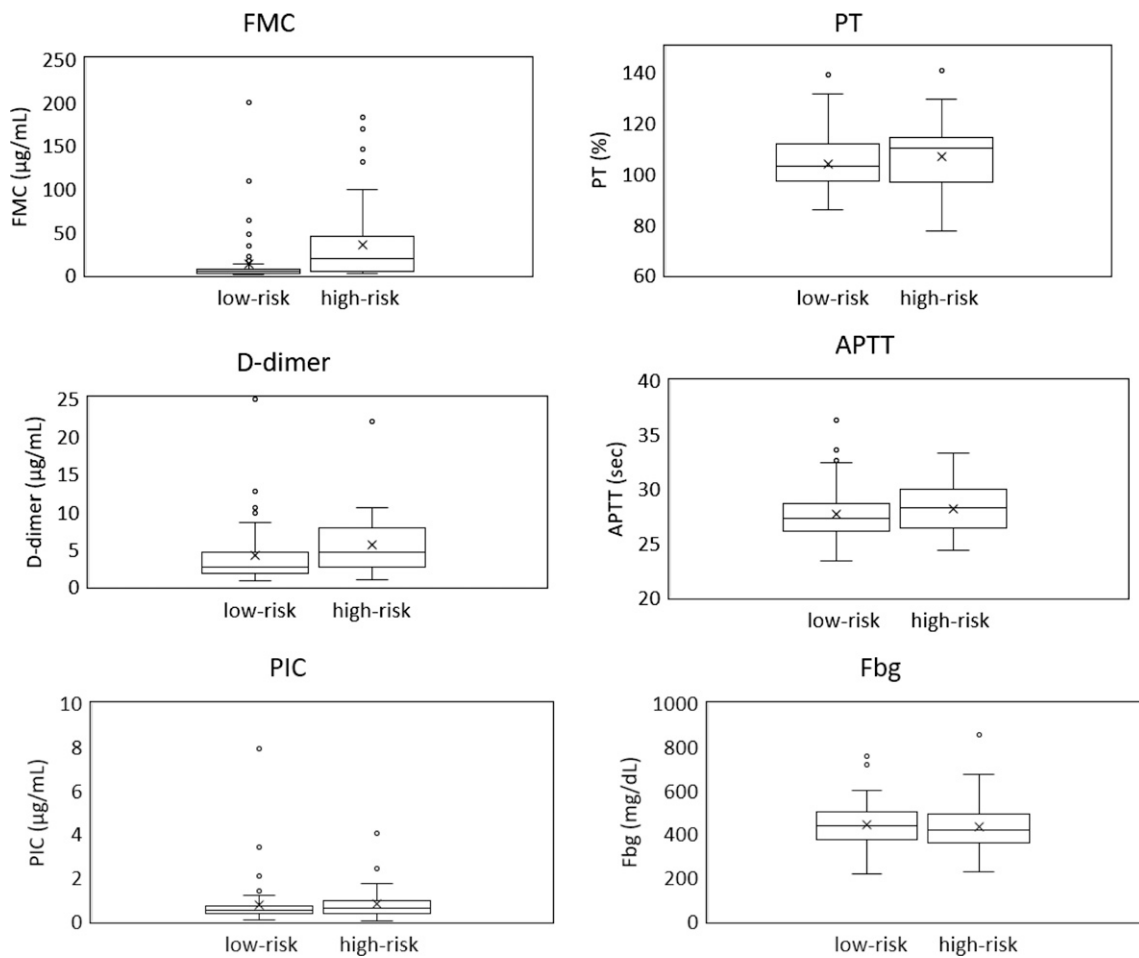


Figure 2. Comparisons of thrombosis markers between high-risk and low-risk groups. Reference intervals: FMC < 7.0 µg/mL, D-dimer < 1.0 µg/mL, PIC < 0.8 µg/mL, PT 70~130%, APTT 23.3~38.2sec, Fbg 200~400 mg/dL (reference intervals set by our hospital).

D-dimer and FMC. The area under the curve, cut-off value, sensitivity, and specificity of D-dimer and FMC were 0.656 (95%CI; 0.546-0.766), 4.4 µg/mL, 0.590, and 0.721 and 0.713 (95%CI; 0.608-0.818), 5.8 µg/mL, 0.692,

and 0.750, respectively. We also performed multivariate ROC analysis by combining D-dimer and FMC concentrations. The area under the curve of the multivariate ROC analysis was 0.738 (95%CI; 0.641-0.834).

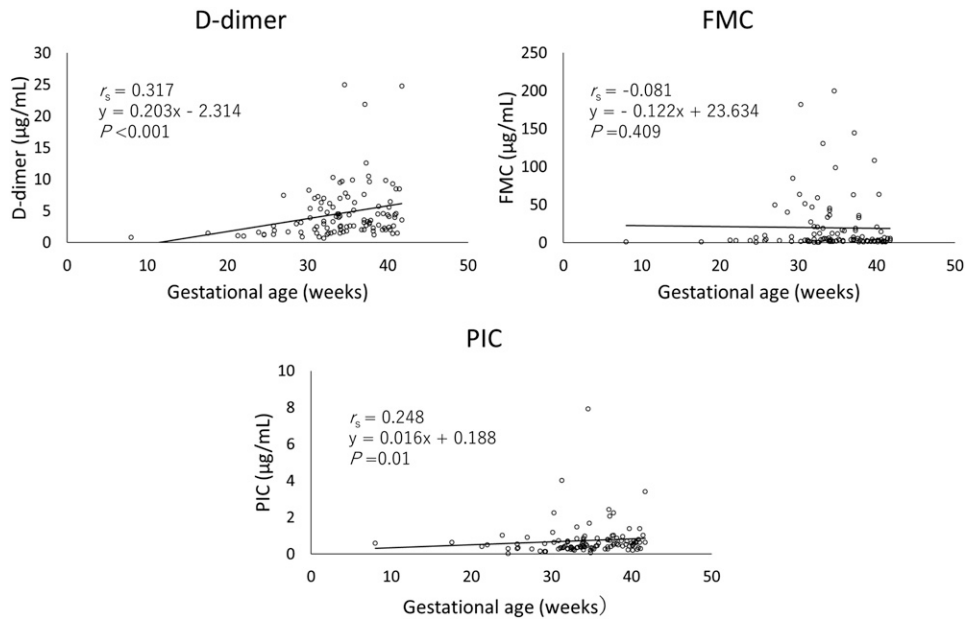


Figure 3. The relationship between gestational age and thrombosis markers.

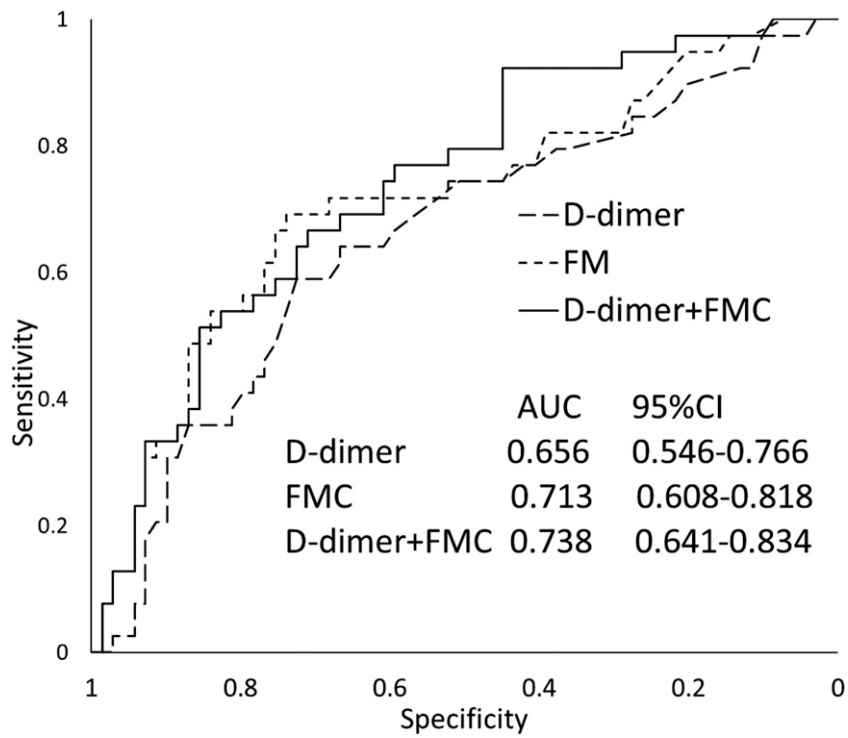


Figure 4. Discriminative performance of D-dimer, FMC, and both for the high-risk group.

Discussion

This study demonstrated that D-dimer concentrations in the high-risk group were significantly higher than those in the low-risk group and this marker was significantly elevated during pregnancy. Although the reference value of D-dimer is often $< 1 \mu\text{g/mL}$, only four patients in our study had concentrations within the reference range. The median D-dimer concentration in this cohort was $3.3 \mu\text{g/mL}$, and D-dimer concentrations were beyond the reference range in $> 95\%$ of the patients who were at a relatively high risk. Furthermore, several studies including healthy pregnant women reported an increase in D-dimer concentrations during late pregnancy.^{12,13} The RCOG guidelines also advise that D-dimer testing should not be performed in the investigation of acute VTE in pregnancy;¹⁴ therefore, D-dimer is not recommended for managing VTE in pregnancy.

The present study also demonstrated that FMC concentration in the high-risk group was significantly higher than that in the low-risk group. Additionally, it was demonstrated that in contrast to PIC and D-dimer concentrations, FMC concentration was unaffected by gestational age. Although the reference value of FMC used in our hospital is $< 7 \mu\text{g/mL}$, sixty-eight patients (63.5%) in our study had concentrations within the reference range. Compared to DD, most patients were within the standard values. These findings suggest that FMC is a thrombosis marker that is preferable over PIC and D-dimer. There are three types of antibodies that recognize SF and FMC. IF-43 reacts with most of SF and J2-23 reacts with most of SF and FM. The F405 we used also reacts with SF, FM, fibrin degradation products (FMDP) activated by plasmin on SF and their complexes. Ieko et al.¹⁵ reported there was a significant difference between SF and FMC measurements in DIC patients, but not in hypercoagulable states. From this, we believe that the results of this study will not change even if other measurement reagents are used.

None of the patients in this study developed DVT, even though this study included relatively high-risk pregnancies. Onishi et al.¹³ reported the case of a patient who developed DVT with their FMC concentration increasing significantly up to $32.4 \mu\text{g/mL}$. Subsequently, DVT improved and the FMC concentration gradually normalized. Since FMC remained relatively stable throughout pregnancy compared to D-dimer and PIC, FMC could be a specific thrombosis marker. There have been consistent reports on the usefulness of FMC measurement in pregnant women.^{13,16} FMC measurements potentially help in identifying pregnant women who could be at higher risk for perinatal complications.

This study suggests that it is better to assess FMC and D-dimer together than to consider either one of them in isolation. In practice, D-dimer is often used, whereas FMC is rarely used for managing VTE in pregnant women. The usefulness of a combination of thrombosis markers is yet to be investigated.

TAT measurements are often used to manage VTE in pregnant women. TAT is a good marker of coagulation activation since it is formed during the inactivation of thrombin by its inhibitor, antithrombin.^{7,17,18} However, there is a report that TAT is not useful in predicting the thrombosis risk in pregnant women because it does not correlate with a risk stratification assessed by clinical criteria.⁷ TAT concentration is non-specifically elevated during pregnancy, particularly during late pregnancy.^{13,19} Therefore, TAT is not suitable for detection of DVT in pregnancy. FMC, reflecting fibrin formation, is induced when thrombin generation is high enough to form complexes and is thus more specific to fibrin formation than TAT.^{18,20,21}

PIC is occasionally used as a marker of thrombosis. PIC is a fibrinolytic marker and an increase in PIC indicates fibrinolytic activity by plasmin production, which increases with endogenous fibrinolytic activation in pathological conditions in which a thrombus is formed *in vivo*. However, this study showed that PIC levels are also elevated significantly during pregnancy. Thus, PIC is not a good marker for VTE detection during pregnancy. It remains unknown why these markers increase significantly during pregnancy, whereas FMC concentrations reflecting fibrin formation remain relatively stable.

This study had some limitations. First, it was a single-center study with a relatively small number of participants. Second, none of the participants in this study developed VTE. Thus, we could not estimate the sensitivity or set a cut-off value for FMC for diagnosing VTE in pregnancy. Third, the study was retrospective and VTE surveillance was at the obstetrician's discretion. A prospective study is required to determine a cut-off value for FMC, which would clarify the significance of FMC in VTE management in pregnant women.

In conclusion, we found that FMC was significantly higher in the high-risk group than in the low-risk group and was unaffected by gestational age. We also showed that the combination of FMC with D-dimer might be more associated with a high risk for thrombosis than each marker alone. These findings suggest that FMC is an effective marker for predicting thrombosis during pregnancy. Despite some limitations, this is the first report evaluating thrombosis markers using score of RCOG guidelines. Although our study could not set a cut-off value for diagnosing VTE, the FMC cut-off value determined in this study may be useful for initiating treatment in patients at high risk of VTE. Further studies with larger patient populations, including more cases of DVT, are required to confirm these findings.

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Declaration of conflicting interests

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Ethical approval

This study was approved by the Ethics Committee of Tottori University Faculty of Medicine (approval number: 19A056).

Guarantor

T.I.

Contributorship

T.I. and Y.H. designed and performed the study; T.I. wrote the first draft of the manuscript; H.N., N.K., T.H., N.Y., and H.I. acquired and analysed the data; T.F. and T.M. supervised the study and reviewed and edited the manuscript.

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References

1. Heit JA, Kobbervig CE, James AH, et al. Trends in the incidence of venous thromboembolism during pregnancy or postpartum: a 30-year population-based study. *Ann Intern Med.* 2005;143:697–706.
2. Jacobsen AF, Skjeldestad FE and Sandset PM. Ante- and postnatal risk factors of venous thrombosis: a hospital-based case-control study. *J Thromb Haemost.* 2008;6:905–912.
3. Kane EV, Calderwood C, Dobbie R, et al. A population-based study of venous thrombosis in pregnancy in Scotland 1980–2005. *Eur J Obstet Gynecol Reprod Biol.* 2013;169:223–229.
4. Liu S, Rouleau J, Joseph KS, et al. Epidemiology of pregnancy-associated venous thromboembolism: a population-based study in Canada. *J Obstet Gynaecol Can.* 2009;31:611–620.
5. Macklon NS and Greer IA. Venous thromboembolic disease in obstetrics and gynaecology: the Scottish experience. *Scott Med J.* 1996;41:83–86.
6. Royal College of Obstetricians and Gynaecologists. *Reducing the risk of venous thromboembolism during pregnancy and the puerperium*; 2015. RCOG Green-top Guideline No 37a.
7. Bombeli T, Raddatz-Mueller P and Fehr J. Coagulation activation markers do not correlate with the clinical risk of thrombosis in pregnant women. *Am J Obstet Gynecol.* 2001; 184:382–389.
8. Von Felten A. Prevention of venous thromboembolism with heparin in risk patients during pregnancy: monitoring by measuring thrombin-antithrombin III complex. *Thromb Haemost.* 1992;49:837–842.
9. Shizuka R, Amagai H, Kojima J, et al. A trial of setting of the reference intervals of thrombin antithrombin III complex and fibrin/fibrinogen degradation products in pregnant women. *Rinsho Byori.* 1994;42:1194–1198.
10. Refaai MA, Riley P, Mardovina T, et al. The Clinical Significance of Fibrin Monomers. *Thromb Haemost.* 2018;118: 1856–1866.
11. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant.* 2013;48:452–458.
12. Gutiérrez García I, Pérez Cañadas P, Martínez Uriarte J, et al. D-dimer during pregnancy: establishing trimester-specific reference intervals. *Scand J Clin Lab Invest.* 2018;78: 439–442.
13. Onishi H, Kaniyu K, Iwashita M, et al. Fibrin monomer complex in normal pregnant women: a potential thrombotic marker in pregnancy. *Ann Clin Biochem.* 2007;44: 449–454.
14. Royal College of Obstetricians and Gynecologists. *Thromboembolic disease in pregnancy and the puerperium: acute management*; 2015. RCOG Green-top Guideline No37b.
15. Ieko M, Nakabayashi T, Tarumi T, et al. Soluble fibrin monomer degradation products as a potentially useful marker for hypercoagulable states with accelerated fibrinolysis. *Clin Chim Acta.* 2007;386:38–45.
16. Grossman KB, Arya R, Peixoto AB, et al. Maternal and pregnancy characteristics affect plasma fibrin monomer complexes and D-dimer reference ranges for venous thromboembolism in pregnancy. *Am J Obstet Gynecol.* 2016; 215:466.e1-466.e8.
17. Abou-Nassar K, Kahn SR, Kovacs MJ, TIPPS investigators, et al. The effect of dalteparin on coagulation activation during pregnancy in women with thrombophilia: a randomised trial. *Thromb Haemost.* 2007;98:163–171.
18. Van Wersch JW and Ubachs JM. Blood coagulation and fibrinolysis during normal pregnancy. *Eur J Clin Chem Clin Biochem.* 1991;29:45–50.
19. Reinthaller A, Mursch-Edlmayr G and Tatra G. Thrombin-antithrombin III complex levels in normal pregnancy with hypertensive disorders and after delivery. *Br J Obstet Gynaecol.* 1990;97:506–510.
20. Karlsson O, Sporrang T, Hillarp A, et al. Prospective longitudinal study of thromboelastography and standard hemostatic laboratory tests in healthy women during normal pregnancy. *Anesth Analg.* 2012;115:890–898.
21. Hellgren M. Hemostasis during normal pregnancy and puerperium. *Semin Thromb Hemost.* 2003;29:125–130.