D-Dimer Concentrations in Normal Pregnancy: New Diagnostic Thresholds Are Needed

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Background: Pregnancy is known to increase the D-dimer concentration above the conventional normal threshold of 0.50 mg/L, leading to an increased false-positive D-dimer test when venous thromboembolism (VTE) is clinically suspected in a pregnant patient. Our aim was to determine the effect of normal pregnancy on the D-dimer concentration.

Methods: Healthy women who were seeking to become pregnant and had no preexisting condition known to increase the D-dimer concentration were identified. Quantitative D-dimer measurements (MDA turbidimetric assay) and fibrinogen assays were performed before conception, at each trimester, and at 4 weeks postpartum. Patients were excluded for fetal loss or preeclampsia.

Results: A total of 50 women were enrolled in the study, and blood samples were obtained at preconception and all trimesters from 23 women. The mean (SD) preconception D-dimer concentration was 0.43 (0.49) mg/L, and 79% of women had a D-dimer concentration < 0.50 mg/L. D-Dimer increased with each trimester such that only 22% of women in the second trimester and none (of 23) in the third trimester (95% confidence interval, 0–14%) had a D-dimer concentration < 0.50 mg/L. We found no correlation between either the D-dimer and fibrinogen concentrations or between the increases in D-dimer and fibrinogen with pregnancy.

Conclusions: Normal pregnancy causes a progressive increase in circulating D-dimer. The D-dimer test has no use in ruling out VTE in the third trimester if a cutoff of 0.50 mg/L is used. A large management study is needed to establish new thresholds for the D-dimer to rule out VTE in each trimester.

Normal pregnancy causes the maternal plasma D-dimer concentration to increase progressively from conception until delivery (1–10). This increase confounds the use of the D-dimer to rule out suspected venous thromboembolism in symptomatic pregnant patients. At approximately the beginning of the second trimester, more than one-half of pregnant women have a D-dimer concentration that exceeds 0.50 mg/L (or 1.0 fibrinogen-equivalent unit), and by the third trimester, more than 90% of women will have a D-dimer concentration > 0.50 mg/L. Authors of systematic analyses of the D-dimer as a diagnostic test for venous thromboembolism and expert opinions indicate that all patients with a D-dimer concentration > 0.50 mg/L should undergo formal imaging (11–15). Thus, in the case of suspected pulmonary embolism, if a symptomatic pregnant patient is tested for D-dimer, the threshold of 0.50 mg/L is used, and published guidelines are followed, most pregnant patients tested will then be subjected to ionizing radiation with a form of a scintillation ventilation-perfusion lung scan or a computed tomography angiography. The D-dimer test has the same limitation for evaluating a pregnant patient for possible deep venous thrombosis. Venous compression ultrasound provides excellent diagnostic sensitivity and specificity for deep venous thrombosis without ionizing radiation. However, in many clinics and smaller hospitals, venous ultrasonography is not always available, whereas a quantitative D-dimer test may be more uniformly available (16). Accordingly, the issue of adjusting the D-dimer concentration has importance in the evaluation of pregnant patients with suspected deep venous thrombosis.

The medical literature currently lacks direct evidence to determine whether the threshold (or set of thresholds broken out by trimester) of the D-dimer concentration in pregnant patients with possible venous thromboembolism should be altered. To test this question directly would require D-dimer concentrations to be measured in large numbers of pregnant women tested for venous thromboembolism. We believe that this would involve a large, protracted, and probably very expensive multicenter study. As evidence for this assertion, we point to our multicenter database of > 4653 emergency depart-
ment patients tested for a possible pulmonary embolism (17). In this database, only 154 patients were pregnant, and only 6 of the 154 were diagnosed with pulmonary embolism. We therefore submit that the first step toward redefining threshold D-dimer concentration to rule out pulmonary embolism in healthy pregnant patients requires measurement of the D-dimer concentration at preconception, during each trimester, and post partum.

The goal of the present project was to measure the magnitude of increase in D-dimer concentration induced by normal pregnancy, using an automated D-dimer assay on fresh, unfrozen plasma. No previous study has measured preconception D-dimer concentrations and repeated the measurements in the same women throughout pregnancy. This methodology allows the opportunity to explore the implications of using the conventional cutoff of 0.50 mg/L on women in each trimester and post partum and, for the purpose of illustration, how high the cutoff might need to be to maintain the specificity at the value of our patients before conception. Additionally, we measured fibrinogen concentrations. The purpose of these additional measurements was to examine whether the D-dimer increase in pregnancy was a result of fibrinogen or fibrin degradation. If the increase in D-dimer were highly correlated with increases in fibrinogen, then this finding would open the possibility of measuring fibrinogen together with D-dimer and using the fibrinogen concentration to correct for an increased D-dimer.

Materials and Methods
This study was approved by the Institutional Review Board at Carolinas Medical Center. Written informed consent was obtained from all patients. We enrolled healthy women who indicated their desire to become pregnant at a private obstetrics office. The exclusion criteria were designed to rule out any possible cause of increased D-dimer: family or personal history of thromboembolic disease; bleeding disorders, including disseminated intravascular thrombocytopenia (determined by laboratory tests); obvious morbid obesity (>40 kg/m²); varicose veins; any personal history of malignancy; liver disease (abnormal liver function tests); renal disease (abnormal creatinine); autoimmune diseases such as systemic lupus erythematosus, HIV, or immune thrombocytopenic purpura; pancreatitis (abnormal amylase and lipase concentrations with abdominal pain); a condition requiring daily injections (insulin-dependent diabetes mellitus); injury requiring hospitalization or an emergency room visit within 4 weeks; surgery within the previous 4 weeks; recent systemic sepsis syndrome (American College of Chest Physicians criteria); current infection with fever >38 °C (upper respiratory infection or organ infection, including pyelonephritis, pneumonia, soft tissue, or bone infection); active menstruation; strenuous exercise within 12 h (i.e., marathon running); and current use of oral contraceptives containing estrogen compounds.

Blood was drawn by a qualified phlebotomist into a (blue-top) tube containing sodium citrate dihydrate to yield a final concentration of citrate in plasma of 0.11 mmol/L (3.2%). Two of these tubes were drawn and transported on ice. Both tubes were immediately centrifuged at 2500g for 15 min, and the plasma fraction was separated from the red cell mass. The plasma D-dimer concentration was measured on unfrozen plasma within 4 h of the blood draw in the hospital laboratory by a commercial D-dimer assay (MDA immunoturbidimetric Assay; Organon Teknika). The fibrinogen concentration was measured with a US Food and Drug Administration-cleared device in the hospital laboratory. The second tube of citrated blood was centrifuged within 1 h at 2500g for 15 min, and the plasma portion was stored at ~70 °F (−57 °C) in the investigator’s laboratory, but no analyses are reported here from that fraction. D-Dimer concentrations are reported as mg/L, which is equivalent to ~2 fibrinogen-equivalent units/mL.

Patients submitted blood samples at preconception, during each trimester of pregnancy at routine follow-up visits (12, 24, and 36 weeks), and then at a 4-week postpartum checkup. Thus, patients with complete data submitted five blood specimens. We planned in advance to exclude any patients who developed preeclampsia (defined as blood pressure >140/90 mmHg and proteinuria >300 mg/24 h), abruptio placenta, active thromboembolic disease, amniotic fluid embolism, or intrauterine growth restriction of the fetus.

Statistical Analysis
The D-dimer and fibrinogen concentrations during pregnancy were compared by graphical scatter plot. The Pearson correlation coefficient was used to explore the relationship between D-dimer and fibrinogen concentrations. Confidence intervals (CIs) were computed from the exact binomial formula (Stats Direct, Ver. 2.3.3).

Sample Size
The goal of this study was to determine, in pregnancy, the magnitude of increase in the cutoff for the D-dimer required to maintain the same “specificity” observed for the conventional cutoff of 0.50 mg/L at preconception. Here the term specificity refers to the fact that all patients in this cohort were healthy and free of thrombosis and therefore were all true negatives. We emphasize that the derived cutoffs are for hypothetical purposes only and are not meant to be used in clinical practice without further research. From previous reports in the literature in which D-dimer was measured in healthy persons with similar exclusion criteria, we expected that 85% of healthy women would have a D-dimer concentration <0.50 mg/L (18). We used the inferential approach described by Arkin and Wachtel (19) to set the sample size to narrow the 95% confidence limits around this 80% proportion to less than approximately ±15% at all trimesters. This mandated that we collect blood samples throughout the third trimester
on a minimum 22 patients. We expected that ~60% of women would become pregnant within 2 years, and we anticipated a subsequent 15% loss to follow-up and exclusion rate. We therefore enrolled 50 patients with the expectation that ~25 would provide blood samples throughout all three trimesters.

**Results**

Over the course of 12 months (July 2001–June 2002), 50 women were enrolled. The mean (SD) age was 31 (6) years. The majority of women were Caucasian (82%), and the majority had never been pregnant (44% nulligravida) or had been pregnant once before (35% gravida 1). Two patients became pregnant but were excluded from analyses in accordance with the study protocol, one for miscarriage and another for preeclampsia; these patients are not included in any subsequent analyses, including measurements obtained at preconception. Thirty-two of the 48 remaining women became pregnant within 2 years and submitted the second blood sample representing the first-trimester measurement. Twenty-three of these 32 patients submitted the fourth (third-trimester) sample and were known to have delivered healthy infants, but only 18 of these 23 women returned to submit a postpartum blood sample. None of the nine patients who submitted a first-trimester blood sample but failed to submit a third-trimester specimen was known to have suffered fetal loss. From chart review, we determined that the reasons that 38 (79%) had a D-dimer concentration

The mean D-dimer concentrations measured at preconception, during each trimester, and at the postpartum measurement are shown in Fig. 1. The mean preconception D-dimer concentration was 0.43 (0.49) mg/L, and 38 of 48 women (79%) had a D-dimer concentration <0.50 mg/L, indicating that the conventional cutoff for an abnormal D-dimer occurred at the 79th percentile in healthy women seeking to become pregnant. As expected, the percentage of women who had a normal D-dimer, based on the conventional 0.50 mg/L cutoff of a normal D-dimer concentration, decreased during each trimester (Table 1). In the first trimester, 16 of 32 (50%; 95% CI, 32–68%) women had a normal D-dimer. The number decreased to 7 of 31 (22%; 95% CI, 11–39%) in the second trimester, and by the third trimester, 0 of 23 (95% CI, 0–14%) women had a normal D-dimer. The upper limit of the 95% CI indicates that clinicians can be highly confident that normal pregnancy will cause a positive D-dimer by the third trimester if the conventional cutoff of 0.50 mg/L is used to define abnormal.

We then determined the percentage of women who had a preconception D-dimer concentration that was normal, using the conventional 0.50 mg/L cutoff. Of 48 women who submitted a preconception blood specimen, 38 (79%) had a D-dimer concentration <0.50 mg/L. Because it is obvious from the foregoing data that the conventional cutoff of 0.50 mg/L will cause a high number of false-positive D-dimer results, we used the 79th percentile as a hypothetical reference point to estimate adjustment upward of the D-dimer concentration during pregnancy. For example, in the third trimester, 18 of 23 (78%; 95% CI, 58–90%) women had a D-dimer concentration <1.481 mg/L, and the lower and upper 95% confidence limits were 1.220 and 2.710 mg/L.

The mean increase in D-dimer that was imparted by pregnancy in healthy women, measured by subtracting the preconception D-dimer concentration from the D-dimer concentration measured during gestation or postpartum, is shown in Table 2. The major point of Table 2 is to illustrate that by the third trimester, normal pregnancy causes the D-dimer concentration to increase by an mean absolute magnitude of 0.69 mg/L and that 16 of 23 (70%; 95% CI, 49–84%) women had an increase in D-dimer that exceeded 0.50 mg/L. Stated another way, by the third trimester, it can be expected that a normal pregnancy usually contributes enough of an increase to maternal circulating D-dimer concentrations that the increase alone usually is more than the conventional threshold for an abnormal D-dimer.

A vertical point plot of the fibrinogen concentration for each time point is shown in Fig. 2. Fibrinogen increased successively with each trimester. We used first-order regression analysis to determine whether the increase in fibrinogen was correlated with the increase in D-dimer.
Discussion

In this study, we examined the magnitude of increases in the D-dimer and fibrinogen induced by normal pregnancy in a healthy woman. It is well known that pregnant women have higher D-dimer concentrations than age-matched nonpregnant women. However, the present study is novel inasmuch as we measured D-dimer in fresh plasma before conception and throughout pregnancy in a cohort of women. We also imposed very strict criteria to exclude any extraneous causes of an increased D-dimer.

As expected, we found that pregnancy increased the D-dimer concentration in a stepwise fashion from preconception to the third trimester, as Fig. 1 illustrates. The mean D-dimer concentrations (0.43 mg/L at preconception and 0.58, 0.83, and 1.16 mg/L in the first, second, and third trimesters, respectively) indicate an ~39% relative increase in D-dimer concentration for each trimester compared with the previous measurement. These results agree with previous studies that used commercially available D-dimer assays on frozen plasma from healthy women with normal pregnancies. In the third trimester, Nolan et al. (1) found a mean D-dimer concentration of 1.75 mg/L, using the Asserchrom D-Di ELISA assay, and van Wersch and Ubachs (9) and Bellart et al. (8) reported mean D-dimer concentrations of 1.2 and 1.0 mg/L, respectively, using an ELISA from Boehringer-Mannheim. Although the D-dimer is known to remain relatively stable in frozen plasma for long periods, some decay does occur (20). Our data also agree with two previous studies that examined D-dimer concentrations on fresh plasma from pregnant patients. Giavarina et al. (5) used a turbidimetric assay (Liatest) and found mean D-dimer concentrations of 0.88 mg/L in the early third trimester and 1.09 mg/L in the late third trimester. Morse (2) measured D-dimers in a group of 34 nonpregnant women and 48 other women throughout pregnancy, using the IL Test™. Morse found a 36% increase in D-dimer in the pregnant women at 16 weeks of gestation compared with the nonpregnant women and a 36% increase in D-dimer from 26 weeks to 36 weeks of gestation. Because the IL Test uses a capture antibody different from that in the MDA assay, which we used in this study, our absolute D-dimer concentrations cannot be directly compared with those reported by Morse.

Our data are the first to allow computation of the magnitude of increase in the maternal D-dimer concentration conferred by normal pregnancy. The mean increase in D-dimer concentration from preconception to the third trimester of pregnancy was 0.69 mg/L, an absolute increase in D-dimer that exceeds the conventional threshold for a normal D-dimer concentration. As a
result, none of 23 patients (95% CI, 0–14%) for whom a third-trimester blood specimen was available had a D-dimer concentration <0.5 mg/L. Thus, it can be expected that normal pregnancy should cause a positive D-dimer test, and the data show the need to adjust the conventional 0.50 mg/L threshold upward when using the D-dimer to evaluate a pregnant patient for possible venous thromboembolism. Accordingly, as a hypothetical exercise, we determined the percentage of our patients who had a D-dimer concentration <0.50 mg/L before pregnancy and found this to be 79% (i.e., the specificity in this healthy population was 79%). We then determined the D-dimer concentration at each trimester that corresponded to the 79th percentile during each trimester. The 79th percentile occurred at D-dimer concentrations of 0.75, 1.00, and 1.50 mg/L for the first, second, and third trimesters of pregnancy, respectively. We reemphasize that the cutoff values of 0.75, 1.00, and 1.50 mg/L are not valid in any way for clinical practice, but we submit that these thresholds could be evaluated in a prospective research study in which pregnant patients with possible venous thromboembolism are evaluated by a validated diagnostic algorithm.

As a secondary objective, we questioned whether the increase in D-dimer concentration might be stoichiometrically related to a simultaneous increase in circulating fibrinogen in normal pregnancy. This question was based on the hypothesis that most of the D-dimer increase in pregnancy is a consequence of increased circulating fibrinogen, as opposed to fibrin, the product of fibrinolysis within a thrombus. This hypothesis, if true, would open the possibility for a clinician to request a D-dimer and a fibrinogen measurement simultaneously in a pregnant patient and to compare the D-dimer concentration with the fibrinogen concentration. If D-dimer were increased but the fibrinogen concentration lower than expected, this might suggest the presence of a pathologic thrombus. Accordingly, we performed first-order regression between the increase in D-dimer concentration as a function of increase in fibrinogen concentration in each woman. We found no significant relationship between these variables, indicating that a simultaneous measurement of fibrinogen and D-dimer would not be a practical way to determine whether an increased D-dimer can be explained by increased fibrinogen in a pregnant patient.

In conclusion, our results indicate that normal pregnancy causes the mean D-dimer concentration to increase to >0.50 mg/L in all trimesters and that by the third trimester, it can be expected that the D-dimer concentration will almost always be abnormally high. Our findings therefore suggest that the D-dimer has no practical diagnostic use in ruling out venous thromboembolism in the third trimester if the threshold for abnormal (0.50 mg/L) is used. These data show the need for more research to determine how much to increase the threshold for abnormal D-dimer in pregnant patients.