BASIC INVESTIGATIONS

Role of the Peripheral Intravenous Catheter in False-positive D-dimer Testing

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Abstract. **Objective:** To determine whether inserting a peripheral intravenous catheter (IV) can significantly increase the circulating D-dimer concentration. **Methods:** Twenty healthy young adult volunteers underwent cannulation of an antecubital vein with a 20-gauge Teflon IV. Time 0 venous blood was drawn during IV insertion. The IV was saline-locked and left in place for 90 minutes, at which time a second venipuncture was performed in a contralateral antecubital vein (+90 min). A qualitative D-dimer assay [erythrocyte-agglutination assay, SimpliRED (SRDD)] and a quantitative spectrophotometric assay [enzyme-linked immunosorbent assay (EIA), Dimertest Gold] were performed on all samples. Time 0 means (±SD) were compared with +90 min means by paired t-test, and SRDD pairs were compared with McNemar's test. **Results:** Time 0 initial venipuncture blood samples yielded a mean D-dimer concentration of 15 ± 24 ng/mL, with 2/20 SRDD tests read as positive (95% CI = 1% to 32%). At +90 min, the D-dimer concentration was 33 ± 21 ng/mL (p = 0.04 vs time 0), with 5/20 SRDD tests read as positive (95% CI = 9% to 49%, p = 0.248). **Conclusions:** Insertion of an IV increased the circulating D-dimer concentration (determined by EIA), but did not lead to a significant increase in false-positive conversion of the SRDD. An effort should be made to perform D-dimer testing on "first-stick" blood to optimize specificity. However, a strongly positive D-dimer reaction cannot be ascribed to the presence of an IV. **Key words:** D-dimer; diagnosis; deep venous thrombosis; pulmonary embolism; thromboembolism; decision making; diagnostic tests; respiratory system. ACADEMIC EMERGENCY MEDICINE 2001; 8:103–106

IN THE past decade, numerous studies have demonstrated the utility of plasma D-dimer analysis in the rapid exclusion of thromboembolic disease (see recent meta-analysis[1] and references therein). Most studies have demonstrated that the D-dimer assay provides high test sensitivity, but the overall diagnostic performance of the D-dimer has been limited in part by low test specificity.[1–4] In unpublished pilot work derived from a published study of pulmonary embolism in outpatients,[5] we discovered an apparently higher rate of false-positive D-dimer tests among subjects who had intravenous catheters (IVs) placed compared with patients who did not have IVs. We postulated that an IV could elevate circulating D-dimer levels and cause false positive D-dimer results. This study was undertaken to measure plasma D-dimer concentrations in healthy volunteers before and after IV placement.

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METHODS

Study Design. This was a prospective investigation of D-dimer concentrations in healthy volunteers undergoing IV insertion. The study protocol was approved by the Institutional Review Board of Carolinas Medical Center. Written informed consent was obtained from all subjects.

Study Setting and Population. Healthy subjects were recruited from emergency department (ED) staff working a weekday shift. Subjects were excluded for any process that might increase the D-dimer concentrations,[2,6] including age >50 years, history of malignancy, liver failure, thrombosis, connective tissue disease, injury or surgery within 4 weeks, current infection (e.g., upper respiratory infection), strenuous exercise within 12 hours, pregnancy, childbirth within 4 weeks or active menstruation, or current use of oral contraceptives containing estrogen compounds.

Study Protocol. A 1.5-inch, 20-gauge Teflon IV (Insyte, Becton Dickinson, Sandy, UT) was placed into an antecubital vein and 10 mL of blood was withdrawn through the catheter. This blood sample was designated as the "time 0" sample. Three mL of blood was transferred to labeled, glass tubes containing 0.5 mL of 0.11-mM sodium citrate (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). The blood and citrate solution were mixed thoroughly by inverting the tubes five times, and the tubes were stored on ice. All blood specimens were stored in duplicate in precoded sample tubes to ensure blinding during D-dimer analysis. The indwelling catheter was filled with saline, sealed, and left in place. During the subsequent 90 minutes, subjects were free to ambulate as needed, but otherwise maintained a comfortable seated position.

Ninety minutes after IV insertion, all subjects underwent a second venipuncture with a 20-gauge needle and withdrawal of 10 mL of blood from the contralateral antecubital vein. This blood sample (identified as the "+90 min" sample) was treated in identical fashion to the time 0 blood sample. After the +90 min blood sample was obtained, the IV was withdrawn and the catheter was inspected for evidence of thrombus formation.

Qualitative D-dimer (Erythrocyte Agglutination) Assay. The SimpliRED D-dimer (SRDD) (Agen Biomedical Ltd., Brisbane Australia) test is a qualitative autologous whole-blood erythrocyte agglutination assay (EAA) that uses a bispecific monoclonal antibody formed by conjugation of antibodies against human D-dimer (DD-3B6/22) and red blood cell surface (RAT-1C3/86). In the presence of elevated D-dimer (>200 μg/L), this reagent induces visible red cell agglutination, signifying a positive test. All blood samples underwent qualitative analysis by SRDD assay immediately after each venipuncture. Ten μL of whole blood was mixed with one drop of test reagent in the test well and compared with a negative control sample per manufacturer instructions. After 2 minutes of mixing, the samples were independently read by the two authors. To help visualize the presence of agglutination, the examiners held the plastic test kit in front of a radiographic view box and gently rocked the blood sample while looking for agglutination. Examiners graded tests as positive for any visible agglutination, and were unaware of each other's readings. It was decided a priori that tests with interobserver disagreement would be considered positive tests.

Quantitative D-dimer Assay (Enzyme-linked Immunosorbent Assay). Batch quantitative immunoassay using monoclonal antibody DD-3B6 was performed in duplicate on all blood samples. The assay procedure was begun 30 minutes following the +90 min venipuncture. Dimertest Gold EIA (Agen Biomedical Ltd., Acacia Ridge, Australia) instructions were performed according to package insert instructions. Enzyme-linked immunosorbent assay (EIA) wells were read on a microplate spectrophotometer (Dynatech MA5000, Chantilly, VA). Study sample D-dimer concentrations were interpolated from the standard curve using linear regression.

Data Analysis. D-dimer concentrations are reported as means ±SD. D-dimer concentrations at time 0 were compared with those for +90 min using a paired t-test. To determine whether the insertion of an IV caused a significant increase false-positive rate on SRDD testing, discordant pairs were compared using McNemar's test. $P < 0.05$ was considered significant. Frequency of agreement between the two observers for the qualitative D-dimer test was examined with the Cohen kappa statistic.[7]

We estimated that a sample size of 19 subjects would be required, based on the assumption that baseline D-dimer concentrations would be approximately 270 ± 250 ng/L in normals (Dimertest Gold insert, Agen Inc.), and we sought to show a change of +230 ng/L with $\alpha = 0.05$ and $\beta = 0.20$ using a paired t-test.

RESULTS

Twenty subjects were enrolled (13 men) of mean age (±SD) 29 (±4) years (range 25–42 years). No subject had a history of any chronic illness, nor was any subject taking any medicine. All IV insertions were successful on the first attempt with no visible subcutaneous hematoma formation noted in any of the 20 subjects. An interpretable SRDD was obtained in all subjects. The SRDD testing was performed within 30 minutes of blood withdrawal at time 0 and +90 min. Table 1 summarizes the study results. Time 0 blood samples yielded a mean D-dimer concentration of 15 ± 24 ng/mL. At time 0, two of 20 SSRD tests were read as positive with concordant readings for all 20 samples by both readers. Ninety-minute post-IV (+90 min) plasma samples yielded a D-dimer concentration of 33 ± 21 ng/mL. At +90 min, five of 20 (25%) SRDD tests were read as positive. Two of the five positive SRDD tests were also positive at time 0, such that three additional patients converted from a negative SRDD at time 0 to a positive SRDD at +90 min. At +90 min, the readers disagreed on the interpretation of one of 20 SRDD tests. Thus, out of a total of 40 observations, the readers agreed in 39 cases (97.5%), yielding $\kappa = 0.90$. The difference be-

tween pre- and post-IV mean D-dimer concentrations was +17 ± 32 ng/mL and reached statistical significance by paired t-test (p = 0.04). The pre- and post-IV change (2/20 vs 5/20) in the proportion of SRDD assays read as positive was not significant by McNemar's test (p = 0.248). After IV removal, no catheter contained visible thrombus within the catheter lumen.

DISCUSSION

Two major reasons have been forwarded to explain false-positive D-dimer readings when the test is used to screen for thromboembolic diseases. First, some D-dimer assays cross-react with products of fibrinogen degradation.[8,9] Second, and probably more important, in the clinical setting, serum D-dimer concentrations may be elevated secondary to processes other than thromboembolism that cause fibrin deposition, including inflammatory, infectious, neoplastic, and traumatic insults. This study examined the role of a common source of minor trauma in the clinical setting, IV insertion.

Our results indicate a small but statistically significant rise in serum D-dimer mean concentration, and a trend toward an increase in the false-positive rate of the qualitative SRDD assay. These findings support the hypothesis that an IV can decrease the specificity of the D-dimer assay. However, both the pre- and the post-IV D-dimer levels were very low, probably because we studied only young, healthy subjects who had no known reason to have elevated D-dimer concentrations. Moreover, the methods of storing the blood samples were specifically designed to prevent in-vitro fibrin deposition (e.g., storage on ice with citrate anticoagulation). The D-dimer concentrations were lower than was anticipated based on data from the D-dimer manufacturer. However, the D-dimer levels of our study group agree reasonably well with the 75 ng/mL mean concentration documented by Whitaker et al. in a study of 25 healthy blood donors (age unknown).[10] The present data also indicate that the increase in D-dimer concentration induced by peripheral IV insertion is relatively trivial compared with other non-thromboembolic etiologies. For example, Goldhaber et al. reported the D-dimer concentration to be 2208 ± 2236 ng/mL in 128 hospitalized patients with suspected pulmonary embolism but with normal pulmonary angiograms.[11]

The post-IV D-dimer concentration was well below the positive threshold for all rapid D-dimer assays, and for all quantititive D-dimer assays.[1] Nonetheless, the SRDD was read as postive in 7/40 (17.5%) study samples. Quantitative assay of the same blood samples revealed D-dimer levels well below the 200 ng/mL threshold required for significant erythrocyte agglutination conversion as reported by the manufacturer in all seven cases (SimpliRED insert). Therefore, all SRDD conversions in this study were actual false-positive results (i.e., erythrocyte agglutination occurred in the absence of elevated D-dimers). Our study yielded a SRDD specificity of 90% and 75% for pre- and post-IV subsets, respectively. This trend toward a decrease in specificity of the SRDD assay probably did not stem from the subjective nature of its interpretation given that interobserver disagreement for SRDD testing occurred in only a single case (2.5%).

TABLE 1. Summary of the Study Results

	Pre-IV Insertion (Time 0)	Post-IV Insertion (Time +90 Minutes)	p-value
D-dimer concentration (ng/mL)	15 ± 24	33 ± 21	0.04*
SRDD false-positive tests (95% CI)	2/20 (1–32%)	5/20 (10–44%)	0.248†

*Paired t-test.
†McNemar's test.

LIMITATIONS AND FUTURE QUESTIONS

Limitations of this study include the small sample size, which precluded the demonstration of a significant increase in false-positive testing with the qualitative SRDD by McNemar's test. This study also was unable to distinguish whether the modest elevation in D-dimer concentration that we measured after IV placement might have been the result of the minor vascular injury induced by simple venipuncture rather than a result of the Teflon IV. The timing of the second blood sample could represent a limitation. We chose the 90-minute time frame based on a common-sense approach. First, we assume that for most patients who have symptoms consistent with pulmonary embolism, and in whom an IV is inserted, this procedure will occur soon after ED sign-in. Second, based on previous experience,[5,12] we believe that the emergency physician is most likely to decide to order a D-dimer in the second hour after an IV is placed, because this is when routine testing, such as chest radiography and 12-lead electrocardiography, is completed, and the suspicion for pulmonary embolism increases when those studies show no alternative diagnosis. We measured only the D-dimer concentration at +90 min, and at this time point, the data suggest that the D-dimer concentration was increasing. With serial D-dimer measurements performed over a longer time range (perhaps 24 hours), a larger increase might be observed. With

this paradigm, a control group, who had no IV inserted but who underwent serial venipunctures, would be required to elucidate whether multiple venipunctures could increase the D-dimer concentration. It also remains possible that multiple venipunctures, multiple catheters, or large-bore peripheral catheters, and central venous lines could produce more intravascular thrombosis and higher D-dimer elevation than was observed with a single, peripheral, 20-gauge catheter.

Finally, we studied only healthy subjects with no hypercoagulable conditions. As a future question, it will be important to measure baseline blood D-dimer concentrations in subjects with known reason for fibrin deposition (e.g., pregnancy or malignancy) and then insert an IV and measure the D-dimer again. It remains possible that a synergistic interaction will occur whereby the IV produces larger relative increases in D-dimer concentration in subjects who have a hypercoagulable state and are therefore predisposed to fibrin deposition and subsequent D-dimer formation.

CONCLUSIONS

Insertion of a peripheral intravenous catheter produced a marginally increased circulating D-dimer concentration as measured by EIA, and showed a trend toward the development of false-positive testing with a qualitative erythrocyte agglutination D-dimer assay. However, the post-IV D-dimer level was well below the threshold to produce a positive test for commercially available D-dimer assays. These findings suggest that clinicians and nurses should attempt to perform D-dimer testing on blood obtained from an initial venipuncture; however, a strongly positive D-dimer reaction cannot be ascribed to the presence of a peripheral IV.

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