The Sensitivity and Specificity of a Red Blood Cell Agglutination D-Dimer Assay for Venous Thromboembolism When Performed on Venous Blood

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Background: Studies evaluating the accuracy of the SimpliRED D-dimer assay for venous thromboembolism (VTE) have used a capillary fingerstick blood sample, which requires the test to be performed immediately at the bedside. Initial studies showed a sensitivity for VTE of 90% to 95% when the assay was performed by a finite number of experienced health care workers. However, because of the test’s subjectivity, misinterpretation of the result is possible when performed by inexperienced staff. Recent reports by other investigators indicated a low sensitivity of this assay for VTE and noted a reduction in sensitivity (84%) for pulmonary embolism.

Objective: To determine the sensitivity and specificity of the D-dimer test performed in the laboratory by experienced technologists on venous whole-blood samples in routine collection tubes. If D-dimer testing results accurately detect VTE when performed in this manner, concerns about the sensitivity of this assay would be solved.

Methods: One hundred forty-eight consecutive patients with suspected VTE underwent D-dimer testing at the bedside using a fingerstick sample and venous blood collected into a plain tube. Venous blood was also collected into tubes containing tri-potassium EDTA, sodium citrate, or a combination of lithium and heparin for D-dimer testing in the laboratory. In addition, the EDTA tube was refrigerated overnight at 4°C for retesting at approximately 24 hours. The presence or absence of VTE was determined by means of objective results of testing and a 3-month follow-up.

Results: Thirty-four subjects (23%) had confirmed VTE (25 with deep vein thrombosis; 9 with pulmonary embolism). All laboratory venous blood D-dimer results showed sensitivities of 97%, specificities of 61% to 64%, and negative predictive values of 99%, compared with 88%, 71%, and 95%, respectively, when the results were obtained by means of fingerstick at the bedside.

Conclusions: The SimpliRED D-dimer assay performed in the laboratory on venous blood collected into any of 3 routine laboratory tubes, is sensitive and moderately specific for VTE. Based on this study, immediate bedside testing (particularly by inexperienced personnel) under suboptimal conditions is unnecessary. Furthermore, the high sensitivity of refrigerated EDTA samples allows specimens to be stored or transported (on ice at 4°C) for testing for 24 hours after collection.

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D-DIMER is a fibrin degradation product formed by the enzymatic activity of plasmin on cross-linked fibrin polymers. Plasma levels can be measured and are useful in patients who present with symptoms of pulmonary embolism (PE) or deep vein thrombosis (DVT), because negative test results (below a predefined cut point) rule out the likelihood of these diseases.

The SimpliRED D-dimer assay (Agen, Inc, Brisbane, Australia) is a unique whole-blood assay that uses a bispecific antibody to human red blood cells and D dimer. In a whole-blood sample, in the presence of elevated levels of D dimer (>0.2 g/mL), this antibody will cause visible agglutination of red blood cells. When performed by experienced personnel, this assay has a high sensitivity (89%-94%), moderate specificity (66%-77%), and a high negative predictive value (96%-98%) for DVT and PE. A high sensitivity of a D dimer for DVT and PE is critical, because the consequences of missing the diagnosis can be fatal or nonfatal recurrence. However, as most patients (approximately 80%) with suspected DVT or PE do not have the disease, the specificity of a D-dimer assay is also important to ensure that a sufficient number of these patients have a negative result to make the test useful. In our institution, a limited number of nurses and technologists perform the assay, and the result is a high sensitivity and moderate specificity.
PATIENTS AND METHODS

Consecutive patients with suspected DVT or PE who were referred to the thromboembolism service at the McMaster Division of the Hamilton Health Sciences Corporation, Hamilton, Ontario, were enrolled from June 1, 1997, through November 30, 1998, and the last 3-month follow-up occurred in February 1999. Patients were excluded from the study if (1) they were receiving ongoing warfarin sodium therapy or they had received more than 24 hours of unfractiopanor or low-molecular-weight heparin therapy; (2) they were pregnant; or (3) they had undergone major surgery in the past 3 days. Consent was obtained from all patients after the study was explained, and all patients underwent assessment by a physician specializing in thromboembolism. The study was approved by the institutional review board of the hospital.

Patients with suspected DVT received a diagnosis of DVT on the basis of a positive finding on ultrasound (CUS) or venography. No DVT was indicated if findings on serial CUS, impedance plethysmography (IPG),7 or venography were negative and if the patients remained free of VTE within the 3 months of follow-up.

Patients with suspected PE underwent ventilation perfusion (VQ) lung scanning, and further testing was performed according to the scan results.6 Patients received a diagnosis of PE on the basis of a positive finding on a pulmonary angiogram, a high-probability lung scan, or a non-diagnostic VQ lung scan and a positive test result for DVT. No PE was indicated if they had a normal finding on a pulmonary angiogram or VQ lung scan or nondiagnostic VQ lung scan and negative results of serial CUS, serial IPG, or venography and if they remained free of VTE within 3 months of follow-up.

The IPG and CUS were performed and interpreted as previously described9,10. CUS was reported as diagnostic of DVT if 2 contiguous proximal deep venous segments were noncompressible. Venography was performed using the technique of Rabino and Paulin,1, and findings were interpreted as positive if an intraluminal filling defect was visible in at least 2 views. Lack of filling of vessels was considered to be nondiagnostic. The VQ lung scans and pulmonary angiography were performed as previously described.12 The results of the lung scan were classified as normal, high probability (segmental or greater, or a perfusion defect with normal ventilation and visible on 2 views), or nondiagnostic (segmental defects with matched ventilation defects, or subsegmental perfusion defects with or without matched ventilation defects). Diagnostic criteria for positive angiographic findings consisted of a constant intraluminal filling defect seen in multiple views or a sharp cutoff in a vessel greater than 2.5 mm in diameter.

A fingerstick D-dimer assay was performed (by a research assistant) at the bedside using the method described by John et al.13 Subsequently, with the use of a sterile technique, venous blood was obtained in 4 tubes containing the following additives: none (plain), 0.105M buffered 3.2% sodium citrate (blue top), a combination of lithium and heparin (green top), and tri-potassium EDTA (purple top) (Becton Dickinson, Mississauga, Ontario). The D-dimer assay was repeated by a second research nurse or assistant at the bedside in the plain tube. The remaining 3 tubes were sent to the laboratory for testing. A laboratory technologist performed the D-dimer test within 4 hours of collection on all 3 remaining tubes using whole blood (samples kept at room temperature before testing). In addition, the EDTA tube was placed in a refrigerator (at 40°C) overnight and retested the following morning; this tube is referred to as the 24-hour EDTA sample. The D-dimer results from the EDTA tubes tested within 4 hours are referred to as the 4-hour EDTA samples. All 4 laboratory personnel (including M.R.) performing D-dimer assays were unaware of the results of objective tests for VTE and the capillary fingerstick and plain-tube D-dimer test results already performed at the bedside.

We decided a priori to perform the study in 2 parts. In the first part, 50 patients would be enrolled, and if the results were poor, we planned to terminate the study, whereas if the results looked promising, we would continue enrollment. Of the first 50 patients, 10 had confirmed VTE, and the 24-hour EDTA sample showed a sensitivity of 100% for VTE compared with a sensitivity of 70% for bedside fingerstick testing. Based on these results and on our objective demonstration that laboratory testing had a high sensitivity, we estimated that an additional 100 patients would need to be enrolled. By enrolling 150 patients, and with an expected prevalence of VTE of 20% (30 patients) if the observed sensitivity of the 24-EDTA samples (and other samples) was 93% (28 of 30 patients), the sensitivity of laboratory testing would be sufficiently high and the 95% confidence interval (CI) would be sufficiently narrow that we would satisfy our objective.

We calculated descriptive statistics with means and SDs using SPSS software (Version 8.0; SPSS Inc, Chicago, Ill.). Sensitivities, specificities, and negative predictive values were calculated for each collection method. We compared the capillary fingerstick sensitivity and the most sensitive laboratory test result using the Fisher exact test. The 95% CIs of proportions were calculated using CIA software (Version 1.1; ISO Data Centre, Saclay, France).

This D-dimer assay is one of a few that has undergone extensive clinical testing; it has been evaluated in published studies enrolling more than 1500 patients with suspected venous thromboembolism (VTE).1-4 However, concerns have been raised that the subjectivity of the assay reduces its accuracy. In the initial studies when the assay was performed by selected personnel, it showed a sensitivity of 90% to 95% for VTE.2,3 In a subsequent, larger multicenter study of patients with suspected PE that used multiple personnel to perform and interpret the assay, we observed a sensitivity of only 84%.4 Moreover, in the hands of other investigators, the sensitivity for VTE has been reported to be as low as 65%.5 Based on our clinical experience, we reasoned that these reports of the low sensitivities were probably the result of inexperienced, busy health care workers who performed the assay under suboptimal conditions.

To overcome these problems, 2 options are available. First, performance and interpretation of the assay should be limited to a finite number of experienced personnel working under optimum conditions. Second, an objective technique should be incorporated to read the assay; this technique is currently not available.

To explore the first solution, we tested the hypothesis that this D-dimer assay has as high a sensitivity for VTE when performed by a relatively small group of ex-
performed, well-trained laboratory technologists as it has when performed by a small group of well-trained research personnel. To facilitate laboratory testing, we collected samples of venous whole blood into 3 routine blood collection tubes (containing sodium citrate, EDTA, or a combination of lithium and heparin). We also wanted to estimate the accuracy of the assay when the blood specimen was stored overnight, to explore whether laboratories could send the blood sample within 24 hours to another laboratory that has the assay.

One hundred fifty-three patients were enrolled. For technical reasons, D-dimer assay results were unavailable for 2 patients, and only the results of bedside testing were available for 3 patients; results of these latter 3 patients are included in the final analysis. Three patients died before their 3-month follow-up and were excluded from the final analysis. For the 148 patients whose results were included in all analyses, mean (SD) age was 58 (16) years. Ninety-four patients (64%) were female. Of the 30 patients presenting with suspected PE, 9 had diagnostic findings positive for the disease. Of 118 with suspected DVT, 25 had positive diagnostic findings.

Of the 3 patients who died, none were considered to have VTE. One was an 86-year-old woman who had negative findings of initial objective tests for DVT and negative results of the D-dimer assay. Four weeks later, she died at her nursing home after a clinically diagnosed stroke; results of her D-dimer assay by means of capillary fingerstick sampling were negative, but results of laboratory findings were positive. Three patients died 5 days after enrollment. At initial assessment, he had negative findings on CUS, and all results of D-dimer assay were positive. Three days later, he was admitted to another hospital with hematuria, urinary retention, and obstructive renal failure. Ventricular fibrillation developed shortly after cystoscopy, and he could not be resuscitated. An autopsy was not performed.

The Table summarizes the sensitivities, specificities, and negative predictive values and the corresponding 95% CI for all test formats. The median time for testing of the 24-hour EDTA tube after initial bedside testing was 22.6 hours (SD, ±8.5 hours). The sensitivity of the capillary fingerstick D-dimer assay was 88% (95% CI, 73%-97%); specificity, 71% (95% CI, 63%-79%). Although the point estimates of the sensitivities all favored laboratory testing compared with bedside testing, the differences between the sensitivities of the capillary fingerstick bedside method and of the other D-dimer assay methods are not statistically significant (P = .36).

The results of this study confirm that the SimpliRED D-dimer assay performed in the laboratory on venous blood samples collected in laboratory tubes containing one of the 3 anticoagulants has a high sensitivity and negative predictive value for VTE. These results have 2 important practical implications. First, samples can be tested in the laboratory by experienced technologists under optimal conditions without compromising sensitivity. Second, if the assay is not available in one institution, samples can be transported within 24 hours (refrigerated or on ice) to another site that has the test, if an EDTA-containing tube is used.

The observed sensitivity for the laboratory tubes of 97% (95% CI, 85%-99%) is consistent with the 94% sensitivity seen in the initial studies with D-dimer testing performed at the bedside by selected, trained personnel. Furthermore, for each of the laboratory tubes, the lower limit of the 95% CI is 85%, which is higher than the observed sensitivity for this assay (performed at the bedside) in a recent large multicenter trial (84%). This finding is consistent with our hypothesis that the fall in sensitivity is due to performance of the assay by less experienced personnel, perhaps under suboptimal conditions.

Several factors could influence the accuracy of the laboratory D-dimer results, including the expertise of the observer and, for laboratory testing, the additives in the tubes. In almost all medical centers, laboratory technologists have more experience at reading red blood cell agglutination (also an end point when crossmatching of red blood cells is performed) than nonlaboratory staff, which likely maintain the accuracy when the test is performed on blood samples taken from laboratory tubes. Although we were initially concerned that dilution of blood by citrate (and to a lesser extent, heparin) would

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dilute the D dimer and decrease the sensitivity of the assay, we did not find this.

To minimize bias in D-dimer interpretation, laboratory staff were unaware of the results of objective tests for VTE and the capillary fingerstick and plain-tube D-dimer assay results. Laboratory samples were all batch tested, often by one technician, before storage of the EDTA tube overnight. We did not specify an order of tube testing, and it was not practical to blind the technologist to the SimpliRED D-dimer assay results for each of the individual laboratory tubes. The results of the first laboratory tube could have biased interpretation of agglutination for the other tubes. In some instances, laboratory staff who tested the 24-hour EDTA sample tested the 4-hour EDTA sample from the same patient. Therefore, we cannot discount the possibility of biased assessment of the 24-hour EDTA results.

Although the point estimates of sensitivities favor laboratory testing, this study was not designed to, and does not have sufficient power to, establish superiority of laboratory testing compared with fingerstick testing at the bedside.

### CONCLUSIONS

This study shows that the SimpliRED D-dimer assay can be performed on venous blood from routine laboratory samples without compromising accuracy. This finding is important because it means that the assay should be performed on venous whole-blood samples by laboratory staff, in centers where trained personnel are not available to perform bedside fingerstick testing. In addition, the accuracy of the 24-hour EDTA results means that centers that do not have access to D-dimer testing can refrigerate and transport samples, within 24 hours, to a laboratory that has this D-dimer assay.

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### REFERENCES