Observer and Biological Variation of a Rapid Whole Blood D-Dimer Test

Franktien Turkstra, Edwin J. R. van Beek, Harry R. Büller

From the Academic Medical Center, University of Amsterdam, Center for Haemostasis, Thrombosis, Atherosclerosis and Inflammation Research, Amsterdam, The Netherlands

Summary

In consecutive patients with suspected venous thromboembolism the interobserver variability of the SimpliRED D-dimer test was evaluated by two observers who independently scored one plate, the between assay variation was performed simultaneously by a third independent observer, who assessed a second plate. The biological variation was studied, 1-4 hours later by an independent evaluation.

A total of 155 patients entered the study, venous thromboembolism was present in 42 patients (28%). The interobserver variability was 2/83 samples, with a kappa of 0.95 (95% confidence interval 0.88-1.0). The between assay variation was 2/98, with a kappa value of 0.96 (95% confidence interval 0.90-1.0). When testing the biological variation the observers disagreed in 2 of 69 patients (3%).

The SimpliRED D-dimer assay has a good to excellent interobserver variability, between assay variation and reproducibility.

Introduction

Despite the recent introduction of various less invasive diagnostic procedures, the diagnostic management of patients with suspected venous thromboembolism (VTE) remains complex and time consuming. In those with symptoms in the lower extremity, repeated testing with ultrasonography or similar methods is required (1). In patients with suspected pulmonary embolism the management strategy is often comprised of multiple tests varying from scintigraphy to angiography (2, 3).

Since VTE will be excluded as the cause of the symptoms in approximately 70% of patients presenting with clinically suspected VTE, an easy and reliable method for the exclusion of the disease would be an important step forward in the diagnostic management of these patients. Previously D-dimer assays have been shown promising in this respect, since the negative predictive values of a normal test have been shown to approach 100% (4-11). Initially, these assays involved ELISA methods, which required plasma preparation and were labor intensive (4-8). More recent improvements have yielded fast and acutely applicable tests which have potential for use in the clinical management of patients with suspected VTE (5-12).

One of the main requirements for any test to be used reliable in clinical practice, is that it has a high observer agreement. Furthermore, it should be relatively stable and uncompromised by heparin treatment, since this is frequently initiated during the early phase of the diagnostic work up of patients with suspected VTE.

In this study we assessed the observer variability and biological variation of a rapid whole blood D-dimer assay.

Patients and Methods

Patients

Consecutive patients, aged 18 years and older, who were referred for diagnostic work up for clinically suspected VTE to the Thrombosis Unit of the Academic Medical Center, Amsterdam, The Netherlands were eligible. Both outpatients and inpatients (admitted to the hospital for another disease in whom symptoms suggesting VTE developed during the course of their hospitalisation) were eligible. Patients with full dose of (low molecular weight) heparin for more than 24 h or those receiving long-term oral anticoagulant therapy were excluded. Patients treated with prophylactic dose of (low molecular weight) heparin were allowed to enter the study. The study was approved by the Institutional Review Board and informed consent was obtained in all participants. Fifteen patients reported in this analysis were part of a previously published study (12).

Diagnostic Tests

Deep vein thrombosis was diagnosed using serial compression ultrasonography (1). Compression ultrasonography was performed on the day of referral and one week later. If the test remained normal and during a 3-month follow-up period no symptomatic deep vein thrombosis or pulmonary embolism occurred, patients were considered not to have had thrombosis of the deep leg veins. Contrast venography was only performed in patients with suspected recurrent deep vein thrombosis or in those with high clinical suspicion and inconclusive ultrasound test results.

Perfusion (ventilation) lung scintigraphy was the initial test for diagnosing pulmonary embolism. Pulmonary angiography was performed to prove or exclude pulmonary embolism in those patients in whom a non-diagnostic lung scan result was obtained (2). Pulmonary embolism was considered excluded if the perfusion lung scan or the angiogram were normal and 3-month follow-up was uneventful.

D-dimer levels were determined using a whole blood assay (SimpliRED D-dimer, Agen Biomedical Ltd, Brisbane, Australia) with blood obtained from a fingerstick, as described previously (13). Briefly, the test uses a conjugate of monoclonal antibodies DD-3B6/22 (raised against human D-dimer) and RAT-1C3/86 (raised against red blood cells) and, if agglutination of the red blood cells is visible within 2 min, the test is scored as abnormal (positive). The first D-dimer assay testing was always performed prior to diagnostic tests for VTE.

To evaluate the interobserver variability, one plate was inspected at the same time by two observers. After two minutes both independently recorded their interpretation of the test outcome and the results were compared afterwards. The between assay variation was tested from the same fingerstick, using a second assay plate. The second test was performed at the same time as the first test, and analyzed by a third independent observer. Biological variation was

Correspondence to: Dr. F. Turkstra, Academic Medical Center, University of Amsterdam, Center for Haemostasis, Thrombosis, Atherosclerosis and Inflammation, Research, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands – Tel.: +31 20 5665976; FAX Number: +31 20 6968833

Table 1 Analysis of interobserver variation of a whole blood D-dimer assay in 83 patients with clinically suspected venous thromboembolism

		First observer		
		Agglutination present	Agglutination absent	Total
Second observer	Agglutination present	48	1	49
	Agglutination absent	1	33	34
Total		49	34	83

Agreement 81/83 = 98%, kappa = 0.95; 95% confidence interval 0.88-1.00.

Table 2 Analysis of between assay variation of a whole blood D-dimer assay in 98 patients with clinically suspected venous thromboembolism

		First observer		
		Agglutination present	Agglutination absent	Total
Second observer	Agglutination present	56	2	58
	Agglutination absent	0	40	40
Total		56	42	98

Agreement 96/98 = 98%, kappa = 0.96; 95% confidence interval 0.90-1.00.

evaluated by repeated fingersticking and testing, one to four hours after performing the initial test.

If possible all three tests were performed in one patient. In case there were not enough observers available, selected types of testing were performed (i.e. starting with between assay variation, then interobserver variation).

Results

Patients

A total of 151 consecutive patients with a mean age of 58 years with suspected VTE entered the study. Of those patients, 66% were female and 27% were inpatients. The diagnosis was refuted or proven in 109 and 42 patients, respectively. Hence, the prevalence of VTE in this cohort was 28%. Of the 42 patients with VTE 40 had an abnormal test result. One patient in whom pulmonary embolism was proven angiographically, had symptoms for less than 24 h. The test became subsequently positive upon repeated testing 4 h later. The other patient with proven deep vein thrombosis, had complaints since 4 weeks. Of the 42 patients with VTE 3 were detected during follow-up, all had a positive D-dimer test result initially.

Interobserver Variation

The assessment of the interobserver variation, i.e. the inspection of the same plate by two observers, was performed in the samples of 83 patients. The observed agreement was 98%, for a kappa value of 0.95 (95% confidence interval of 0.88-1.00; Table 1). The observers disagreed in 2 patients, in whom VTE was subsequently excluded.

Between Assay Variation

The variation between two assays obtained from the same fingerstick was determined in 98 patients. In 96 patients (98%) the plates showed the same results, with a kappa of 0.96 (95% confidence interval 0.90-1.00; Table 2). In the two instances of between assay variation, the patients did not have VTE.

Biological Variation

The biological variation of plasma D-dimer levels was assessed in 69 patients of whom 25 had VTE. As described above, one patient with proven pulmonary embolism showed a positive test result only after 4 h. In patients in whom venous thromboembolism was excluded, variation was present in one patient in whom a negative result converted to an abnormal test after 4 h. Hence, the plasma D-dimer levels varied in 3% of patients, leading to conflicting results over a one to four-hour period.

In 24 patients heparin therapy was started within 24 h before the first D-dimer test or in between the first and second D-dimer tests. All except one patient had a positive first D-dimer test, all 24 patients had a positive second D-dimer test result.

Discussion

SimpliRED D-dimer is a whole blood bed-side test, which previously showed potential clinical utility in the exclusion of VTE in symptomatic patients (10-12). In the present study, venous thromboembolism was adequately excluded in 58 of 151 patients presenting with suspected VTE, but was apparently missed in 2 of 40 patients with proven venous thrombosis. The reasons for false negative findings in these two patients may have been related to the duration of their symptoms. In one patient this was relatively short (less than 24 h), whereas this was much longer in the other patient (1 month). It is conceivable that either insufficient D-dimer have been produced if patients are seen (too) early, whereas the D-dimer levels may have regressed to below the threshold for an abnormal test in more chronic venous thromboembolism. Our observation is in agreement with findings of a higher sensitivity in patients with shorter duration of symptoms in other studies (14, 15).

The main finding of this study indicates that the interobserver variation, between assay variation and biological variation of this whole blood D-dimer test evaluated in patients with clinically suspected VTE is good to excellent, yielding kappa values of greater than 0.95. Although several other D-dimer tests have been evaluated for

their potential clinical use in patients with suspected venous thromboembolism, little is known about their observer variability and reproducibility. Observer variation in a latex based D-dimer assay was tested only once, concordance was present in 24 of 32 samples (16). The interobserver variation in 4 ELISA D-dimer assays varied between 2 and 17% (17). The between assay variation was tested in 5 ELISA based assays (17, 18) as well as in an imunofiltration D-dimer assay (5, 6). The test-outcome varied in this respect between 12 and 26% in three of these studies (5, 6, 17) and was found to be excellent in one, when D-dimer measurements of the left and right leg were performed (18). Thus, although 2% to 3% disagreement was observed in our study, the disagreement was much higher in most other studies, including those with ELISA based D-dimer assays. However, as for most visual interpretation tests, new observers do have a learning curve with the SimpliRED D-dimer assay, especially in patients with trace positive test results.

A short duration of heparin therapy did not appear to influence the test outcome: none of the 23 patients with initial positive test became negative during the time interval of testing during heparin therapy. This is in accordance with observations from others (19-22) and is to be expected since the half-life of D-dimers is approximately 8 hours (15). The clinical consequence of this finding is that D-dimer assays can be interpreted reliably during the first hours of heparinization.

Before a new diagnostic test can be widely advocated for the use in patient care, three types of studies should have been performed with good outcome (23). Firstly, the observation variation, assay variation and biological variation should be determined and be minimal. If possible, reasons for variation should be identified. Secondly, the sensitivity and specificity, as compared to the gold standard for the diagnosis of the disease, should be determined. This D-dimer assay now has proven to be adequate in both these aspects. Finally, the test should be tested in a clinical management trial, in which the decision about whether the patient will not be further investigated is based on a normal D-dimer test result should be prospectively determined. This final step still needs to be completed.

Acknowledgements

Dr. Harry Büller is an established investigator for the Dutch Heart Foundation.

References

1. Lensing AWW, Hirsh J, Büller HR. Diagnosis of venous thrombosis. In: Hemostasis and Thrombosis, basic principles and clinical practice. Colman RW, Hirsh J, Marder VJ, Salzman EW (eds). 3rd edition. Philadelphia: JP Lippincott, 1994; 1297-321.
2. Stein PD, Hull RD, Pineo G. Strategy that includes serial noninvasive leg tests for diagnosis of thromboembolic disease in patients with suspected acute pulmonary embolism based on data from PIOPED. Arch Intern Med 1995; 155: 2101-4.
3. Oudkerk M, van Beek EJR, van Putten WL, Büller HR. Cost-effectiveness analysis of various strategies in the diagnostic management of pulmonary embolism. Arch Intern Med 1993; 153: 947-54.
4. van Beek EJR, Schenk BE, Michel BC, van den Ende B, Brandjes DPM, van der Heide YT, Bossuyt PMM, Büller HR. The role of plasma D-dimer concentration in the exclusion of pulmonary embolism. Br J Haematol 1996; 92: 725-32.
5. Dale S, Gogstad GO, Brosstad F, Godal HC, Holtlund J, Mork E, et al. Comparison of three D-dimer assays for the diagnosis of DVT: ELISA, Latex and an immunofiltration assay (Nycocard D-dimer). Thromb Haemost 1994; 71: 270-4.
6. Elias A, Huc B, Chale JJ, Nguyen F, Cambus JP, Boccalon H, Boneu B. D-dimer test and diagnosis of deep vein thrombosis: a comparative study of 7 assays. Thromb Haemost 1996; 76: 518-22.
7. Janssens MCH, Heebels AE, de Metz M, Verbruggen H, Wollersheim H, Janssen S, et al. Reliability of five rapid D-dimer assays compared to ELISA in the exclusion of deep vein thrombosis. Thromb Haemost 1997; 77: 262-6.
8. Becker DM, Philbrick JT, Bachhuber TL, Humpries JE. D-dimer testing and acute venous thromboembolism. Arch Intern Med 1996; 156: 939-46.
9. de Moerloose P, Desmarais S, Bounameaux H, Reber G, Perrier A, Dupuy G, Pittet JL. Contribution of a new, rapid, individual and quantitative automated D-dimer ELISA to exclude pulmonary embolism. Thromb Haemost 1996; 75: 11-3.
10. Ginsberg JS, Wells PS, Brill-Edwards P, Donovan D, Panju A, van Beek EJR, Patel A. Application of a novel and rapid whole blood assay for D-dimer in patients with clinically suspected pulmonary embolism. Thromb Haemost 1995; 73: 35-8.
11. Wells PS, Brill-Edwards P, Stevens P, Panju A, Patel A, Douketis J, et al. A novel and rapid whole-blood assay for D-dimer in patients with clinically suspected deep vein thrombosis. Circulation 1995; 91: 2184-7.
12. Turkstra F, van Beek EJR, ten Cate JW, Büller HR. Reliable rapid blood test for the exclusion of venous thromboembolism in symptomatic outpatients. Thromb Haemost 1996; 76: 9-11.
13. John MA, Elms MJ, O'Reilly EJ, Rylatt DB, Bundesen PG, Hillyard CJ. The SimpliRED D-dimer test: a novel assay for the detection of crosslinked fibrin degradation products in whole blood. Thromb Res 1990; 58: 273-81.
14. D'Angelo A, D'Alessandro G, Tomassini L, Pittet JL, Dupuy G, Crippa L. Evaluation of a new rapid quantitative D-dimer assay in patients with clinically suspected deep vein thrombosis. Thromb Haemost 1996; 75: 412-6.
15. Speiser W, Mallek R, Koppensteiner R, Stumpflen A, Kapiotis S, Minar E, et al. D-dimer and TAT measurement in patients with deep venous thrombosis: utility in diagnosis and judgement of anticoagulant treatment effectiveness. Thromb Haemost 1990; 64: 196-201.
16. Heaton DC, Billings JD, Hickton CM. Assessment of D dimer assays for the diagnosis of deep vein thrombosis. J Lab Clin Med 1987; 110: 588-91.
17. Van Beek EJR, van den Ende B, Berckmans RJ, van der Heide YT, Brandjes DPM, Sturk A, ten Cate JW. A comparative analysis of D-dimer assays in patients with clinically suspected pulmonary embolism. Thromb Haemost 1993; 70: 408-13.
18. Bounameaux H, Schneider P-A, Reber G, de Moerloose P, Krahenbuhl B. Measurement of plasma D-dimer for diagnosis of deep venous thrombosis. AJCP 1989; 91: 82-5.
19. Amelsberg A, Zurborn K-H, Gartner U, Kiehne KH, Preuße A-K, Bruhn HD. Influence of heparin treatment on biochemical markers of activation of the coagulation system. Thromb Res 1992; 66: 121-31.
20. Demers C, Ginsberg JS, Johnston M, Brill-Edwards P, Panju A. D-dimer and thrombin-antithrombin III complexes in patients with clinically suspected pulmonary embolism. Thromb Haemost 1992; 67: 408-12.
21. Estivals M, Pelzer H, Sie P, Pichon J, Boccalon H, Boneu B. Prothrombin fragment 1+2, thrombin-antithrombin III complexes and D-dimers in acute deep venous thrombosis: effect of heparin treatment. Br J Haematol 1991; 78: 421-4.
22. Minnema MC, ten Cate H, van Beek EJR, van den Ende A, Hack CE, Brandjes DPM. Effects of heparin therapy on fibrinolysis in patients with pulmonary embolism. Thromb Haemost 1997; 77: 1164-7.
23. Sacket DL, Haynes RB, Guyatt GH, Tugwell P. The selection of diagnostic tests. In: Clinical epidemiology, a basic science for clinical medicine. Sacket DL, Haynes RB, Guyatt GH, Tugwell P. 2nd edition. Boston, Toronto, London: Little, Brown and Company,1991; 51-68.

Received June 23, 1997 Accepted after revision August 19, 1997