

# Whole Blood Agglutination D – Dimer Test for the Diagnosis of Deep Vein Thrombosis

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## Abstract

Deep vein thrombosis (DVT) is a clinical syndrome the clinical diagnosis of which is unreliable and objective tests often require specialized testing facilities. The authors examined the diagnostic accuracy of the whole blood agglutination D-dimer (SimpliRED<sup>®</sup>) test in this condition. Patients who presented with acute onset of leg swelling were included in the study. Whole blood agglutination D-dimer test was performed upon admission and followed by the gold standard test, contrast venography. Forty three patients had venography proven DVT and proximal occlusion was found in 31 of them. The sensitivity and specificity of the test for DVT were 88.4 per cent and 53 per cent respectively while the positive predictive value (PPV) and the negative predictive value (NPV) were 82.6 per cent and 64.3 per cent. Interestingly, when focusing the diagnosis on proximal DVT, the sensitivity and NPV rose to 100 per cent, whereas, the specificity and PPV remained unchanged (48.3 and 67.4%). In conclusion, whole blood agglutination D-dimer test (SimpliRED<sup>®</sup>) was sensitive but not specific for DVT diagnosis. Moreover, when focusing the diagnosis on proximal DVT, the test had excellent sensitivity and NPV. This test, therefore, can be an effective screening tool for the high embolic risk proximal DVT.

**Key word :** Deep Vein Thrombosis, D-Dimer Test

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Deep vein thrombosis (DVT) is a clinical syndrome which occurs in patients with certain risks; and pulmonary embolism (PE), its complication, results in serious morbidity or mortality.

Although the incidences of DVT in Thai patients were reported to be low<sup>(1,2)</sup>, patients with PE are increasingly being detected. Therefore, DVT needs to be included in the list of differential diagnosis

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in patients with acute onset of leg swelling and once the diagnosis is established, treatment should be promptly started.

The major problem in DVT diagnosis is that the clinical signs are not specific and not reliable(3-5). Many objective tests have been developed and used as diagnostic aids; and among them, contrast venography has been a gold standard for decades despite its invasiveness and potential complications. Several non-invasive tests to identify venous occlusion have been introduced(4-6) and the currently used ones are color Doppler ultrasonography, Duplex study, impedance plethysmography and radionuclide venography. Tests which confirm active clotting process and fibrinolysis have also been developed; and currently, D-dimer, which is a cross linked fibrin degradation product has been extensively investigated(7,8). Heaton(9) and Bounameaux(10) demonstrated that D-dimer level when determined by ELISA, if higher than 200 µg/ml, had high sensitivity and high negative predictive value for the diagnosis of DVT. Nevertheless, when using the same cut off point, the Latex agglutination method failed to demonstrate the same results. They suggested that ELISA D-dimer might be used as a screening tool for DVT. This idea has not been well adopted because ELISA technique requires laboratory facilities and the results are not available at the bedside. During the past decade, whole blood agglutination D-dimer test, has been developed(11). The method is simple for it requires only a small amount of blood and hemagglutination can be perceived within 2 minutes. If this method works for DVT, it will benefit patients since diagnosis can be achieved at the bedside and prompt treatment can be given. We, therefore, conducted a study to determine the diagnostic accuracy of whole blood agglutination D-dimer in DVT.

## METHOD

### Patients

Sixty five consecutive patients in medical wards, Siriraj Hospital, Mahidol University who presented with acute unilateral or bilateral leg swelling between July 1993 and December 1994 comprised the study population. Informed consent was drawn from the patients or their surrogates after the study had been explained. The symptoms onset of more than 2 weeks or patients who had

previous trauma, surgery, sepsis, arterial embolism, recent myocardial infarction (within 2 weeks) and DIC were excluded from the study. On entering the study, whole blood agglutination D-dimer study was performed by using the patients' capillary blood obtained at the bedside. Unilateral contrast venography on the affected leg was subsequently obtained and was interpreted by one of the authors (W.C.) without the knowledge of the D-dimer test result.

### Whole blood agglutination D-dimer test

Whole blood agglutination D-dimer test kits (SimpliRED<sup>®</sup>, AGEN Biomedical Ltd., Queensland, Australia) were supported from Biogenetech Co., Ltd., Thailand. Detailed biochemical reaction and development were discussed elsewhere(11). Briefly, by using chemical conjugate of specific monoclonal antibody to crosslinked fibrin degradation products (XL-FDP) which bind to the red blood cell surface, increase in patients' XL-FDP will bind to the conjugate on the red blood cell, causing visible red cell agglutination. The reaction time was 2 minutes and the reagent was adjusted to produce hemagglutination if the D-dimer level was above 250 µg/ml. The test was performed by using the patients' capillary blood obtained at the bedside. The test results were defined as positive and negative when definite agglutination and no agglutination took place. In some specimens, some cloudiness without clear cut agglutination was also observed and these findings were categorized as "equivocal" test results.

### Statistical Analysis

The sensitivity, specificity, positive predictive value and negative predictive value of Simpli RED D-dimer test were calculated 1) for all patients and 2) for patients with or without proximal DVT.

## RESULTS

Sixty five patients were included in the study. Demographic data are summarized in Table 1. The patients' mean age was  $57 \pm 8$  years. Twenty seven were male and 38 were female. Detailed descriptions of underlying diseases were also listed. It was remarkable that 16 patients had advanced malignancy and half of them had adenocarcinoma. Immobilization was also noted in 8 patients.

**Table 1. Demographic data of the studied patients (n = 65).**

Age	57 ± 8
Male : female	27 : 38
Underlying diseases	
Malignancy	16
Adenocarcinoma	8
Carcinoma of cervix	4
Other malignancies	4
Immobilization	8
Diabetes mellitus	7
Miscellaneous	21

**Table 2. Radiographic findings in the studied patients and the whole blood agglutination D-dimer results.**

	D-dimer results		
	Positive (total = 46)	Negative (total = 14)	Equivocal (total = 5)
Proximal DVT*	31	0	0
Distal DVT*	7	5	2
Without DVT	8	9	3

\* Proximal DVT denotes thrombosis of popliteal vein, femoral veins, internal and external and inferior vena cava and distal DVT includes venous occlusion below popliteal vein.

**Table 3.1 Whole blood agglutination D-dimer results in patients with DVT.**

	DVT +	DVT-
Whole blood agglutination D-dimer +	38	8
Whole blood agglutination D-dimer -	5	9
Sensitivity = 88.4%	PPV = 82.6%	
Specificity = 53%	NPV = 64.3%	

**Table 3.2 Whole blood agglutination D-dimer results in patients with proximal DVT.**

	Proximal DVT +	Proximal DVT-
Whole blood agglutination D-dimer +	31	15
Whole blood agglutination D-dimer -	0	14
Sensitivity = 100%	Specificity = 48.3%	
PPV = 67.4%	NPV = 100%	

As demonstrated in Table 2, 43 patients had venography proven DVT. Of these, 31 patients had proximal occlusion. The test results were positive in 46 patients, negative in 14 patients and equivocal in 5 patients. Two of the patients who had equivocal results had distal DVT. The data after excluding the equivocal results are tabulated in Table 1 and 2. For all patients with proximal and distal DVT (Table 1), whole blood agglutination D-dimer had a high sensitivity (88%) and low specificity (48%). The positive predictive value and negative predictive value were 82 per cent and 64 per cent respectively. When focusing the diagnosis of proximal DVT (Table 2), the sensitivity and negative predictive value increased greatly (to 100%). However, the specificity and positive predictive value remained low (48.3% and 67.4%).

## DISCUSSION

The data reported here demonstrate that; in patients with suspected DVT, whole blood agglutination D-dimer test (SimpliRED<sup>®</sup>) is sensitive but not specific. However, when focusing the diagnosis on proximal DVT, the sensitivity and negative predictive value are greatly improved. The clinical implication of these results is that high embolic risk proximal DVT can be confidently excluded in patients who have negative study results and anticoagulant therapy can be withheld. Follow-up studies by using serial noninvasive tests such as impedance plethysmography (IPG), color Doppler ultrasonography etc. are needed in such patients since distal DVT cannot be excluded and previous reports indicated proximal progression of distal DVT in approximately 20 per cent of them. Whether treatment should be promptly started in patients with a positive test depends on the degree of clinical suspicion because the positive predictive value (PPV) is low. Patients may be scheduled for confirmation study while receiving anticoagulants if not contraindicated. In addition, patients with equivocal test results in whom distal DVT may occur also require confirming tests or repeated follow-up studies.

When comparing the results with those of earlier D-dimer tests<sup>(9,10)</sup>, namely ELISA and Latex agglutination techniques, the test reported here proved to be more useful although the main results of these studies are comparable. The reason is, as described above, that whole blood agglutina-

tion D-dimer can effectively rule out high risk DVT. Moreover, this test is easy to use, may be performed at the bedside and gives prompt results. We may use this dimer test as an alternative way for DVT screening and diagnosis<sup>(12)</sup>.

When compared with other noninvasive leg tests, whole blood agglutinative D-dimer screens the presence of clot and clot lysis in a simple way while others demonstrate venous occlusion. Despite its low specificity compared with gold standard contrast venography or noninvasive tests such as color Doppler ultrasonography and Duplex studies, it is simple and can be used for screening purposes. Physicians areas where noninvasive leg tests are not available may use this method. Moreover, the simplicity of this test renders it more practical for bedside purposes, whereas, color Doppler ultrasound, Duplex and IPG need skillful personnel. In addition, one may use a combination of D-dimer with one of the noninvasive leg tests which proved to work well in the study of Heijboer<sup>(13)</sup> et al. In this study, they combined the study of plasma D-dimer level (ELISA) with a non-invasive leg study (IPG or compression ultrasonography) and compared this approach with serial non-invasive leg tests (IPG or compression ultrasono-

graphy). Better diagnostic accuracy and cost saving were demonstrated in the combined group. Work on combination of diagnostic tests is in process in order to better understand clinical uses and cost effectiveness in Thai people.

Limitations of this test depend on the nature of coagulation and fibrinolysis<sup>(14,15)</sup>. Many disease states which pose high DVT risks such as trauma, major operation and sepsis already have high D-dimer levels at the beginning and may give false positive test results. On the other hand, the duration in which the D-dimer level is high enough to make the test positive has not been established. Some patients who come late in the course of the disease may have negative results. However, we did not find such a case in our study. In addition, these results can be applied only on specific types and manufacturers because of the nature of reagents and cut off point.

In conclusion, whole blood agglutination D-dimer is a very sensitive tool for diagnosis of proximal DVT and may be used as a screening study. Negative results rule out proximal DVT and immediate anticoagulation treatment can be withheld.

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## การวินิจฉัยภาวะลิ่มเลือดอุดตันในหลอดเลือดดำชั้นลึก (deep vein thrombosis) ด้วยการตรวจ Whole blood agglutination D-dimer

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ผู้รายงานได้ทำการศึกษาความแม่นยำของการตรวจพบสาร D-dimer ในเลือดโดยปฏิกิริยา hemagglutination (whole blood agglutination D-dimer test, SimplicRED<sup>®</sup>) ในการวินิจฉัยภาวะลิ่มเลือดอุดตันในหลอดเลือดดำชั้นลึก เทียบกับการตรวจมาตรฐานด้วยการฉีดสารทึบแสง ในผู้ป่วยที่มีอาการขาบวมเจ็บพอง พบว่าผู้ป่วย 65 รายที่เข้ารับการศึกษามี 43 รายที่มีภาวะลิ่มเลือดอุดตันในหลอดเลือดดำชั้นลึก และในจำนวนนี้มี 31 รายที่มีการอุดตันในหลอดเลือดดำส่วนต้นขา (proximal vein) ความไว ความจำเพาะ ค่า positive predictive value (PPV) และ negative predictive value (NPV) ของการตรวจในภาวะลิ่มเลือดอุดตันในหลอดเลือดดำชั้นลึก มี 88.4% 53% 82.6% และ 64.3% ตามลำดับ เมื่อพิจารณาเฉพาะการอุดตันในหลอดเลือดดำส่วนต้นขา พบว่าความไวและ NPV เพิ่มขึ้นเป็น 100% ส่วนค่าความจำเพาะและ PPV ไม่เปลี่ยนแปลง (48.3% และ 67.4%) สรุปได้ว่าการวินิจฉัยภาวะลิ่มเลือดอุดตันในหลอดเลือดดำ โดยเฉพาะอย่างยิ่งการอุดตันในหลอดเลือดดำส่วนต้น การตรวจพบสาร D-dimer โดยปฏิกิริยา whole blood agglutination มีความไวและ NPV สูง จึงสามารถนำมาใช้ในการตรวจคัดกรองผู้ป่วยได้

**คำสำคัญ :** การวินิจฉัยภาวะลิ่มเลือดอุดตันในหลอดเลือดดำชั้นลึก, ภาวะขาบวม, การตรวจพบสาร D-dimer ในเลือด

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