

Validation of the STA-Liatest DDi assay for exclusion of proximal deep vein thrombosis according to the latest Clinical and Laboratory Standards Institute/Food and Drug Administration guideline: results of a multicenter management study

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Recommended strategy for venous thromboembolism (VTE) diagnosis includes the use of sensitive D-dimer (DDi) assays along with pretest probability (PTP) assessment. The Clinical and Laboratory Standards Institute (CLSI) recently issued a guideline (US FDA endorsed) on DDi in VTE exclusion. Such guideline specifies the ideal D-dimer assay characteristics and target population. Demonstrate STA-LiatestD-Di performance combined with a PTP score for proximal deep vein thrombosis (pDVT) exclusion in a CLSI compliant study. International, multicenter, prospective nonrandomized, noninterventional clinical outcome management study conducted in a standard-of-care setting. DDi was measured in DVT-suspected consecutive low/moderate PTP outpatients, without conditions possibly impacting DDi values independently of thrombosis presence (age >80, pregnancy, postoperative, cancer) using a 0.5 µg/ml (FEU) threshold for DVT exclusion. Results were used to determine test performance. One thousand two hundred and thirty-four patients (17 centers) signed informed consent. Nine hundred and eighty (mean age: 55) with valid results (494 negative DDi) completed the study (DVT prevalence: 8.7%). STA-LiatestD-Di performance exceeded CLSI/FDA requirements: sensitivity: 100% (95% CI 95.8–100%), NPV: 100% (95% CI 99.3–100%). STA-LiatestD-Di associated with PTP score showed excellent performance for pDVT exclusion, as

recently demonstrated for pulmonary embolism. The assay allows safe VTE exclusion, avoiding unnecessary imaging tests. *Blood Coagul Fibrinolysis* 29:562–566 Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

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Introduction

Venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism is a major public health problem. The estimated average annual rate of overall VTE among people of European ancestry ranges from 104 to 183 per 100 000 person-years [1]. DVT can lead to death through pulmonary embolism [2]. Objective testing for DVT, is therefore, crucial because clinical assessment alone is unreliable and the consequences of misdiagnosis are serious. Favored strategies for diagnosis of DVT combine use of pretest probability (PTP) assessment, D-dimer (DDi), and imaging techniques [3]. In patients suspected of DVT with low or

intermediate clinical probability as per PTP assessment, initial testing with DDi is the preferred approach to rule out the presence of DVT, whereas those with high PTP scores proceed to imaging testing to confirm it.

Because of the prominent role of DDi in VTE exclusion diagnosis, the Clinical and Laboratory Standards Institute (CLSI) has recently issued a guideline to describe the optimal use of DDi assays including the ideal patient target population and specify jointly with the US Food and Drug Administration (FDA)-recommended assay characteristics in terms of negative predictive value (NPV) and sensitivity [4].

The STA-LiatestD-Di (Diagnostica Stago, Asnières sur Seine, France) is a well established, rapid, automated, quantitative immune-turbidimetric assay that has been validated and FDA cleared as an aid in the diagnosis of DVT and pulmonary embolism in 2005. More recently, the assay has been cleared by the FDA for the exclusion of pulmonary embolism following its validation according to the CLSI/FDA guideline in a large multicenter management study [5].

The second arm of this multicenter management study aimed at demonstrating the clinical performance of the STA-LiatestD-Di test in the exclusion of DVT according to the same CLSI/FDA guideline. Results are reported in this article.

Study objectives

The objective of the study was to demonstrate the ability of the STA-LiatestD-Di used in conjunction with a clinical pretest probability score to exclude DVT in patients with low or moderate PTP in a 3-month follow-up.

The study was conducted for the purpose of the clearance of the assay by the US FDA for exclusion of DVT.

Methods

The study sponsored by Diagnostica Stago was an international, multicenter, prospective, nonrandomized, non-interventional clinical outcome management study (DiET

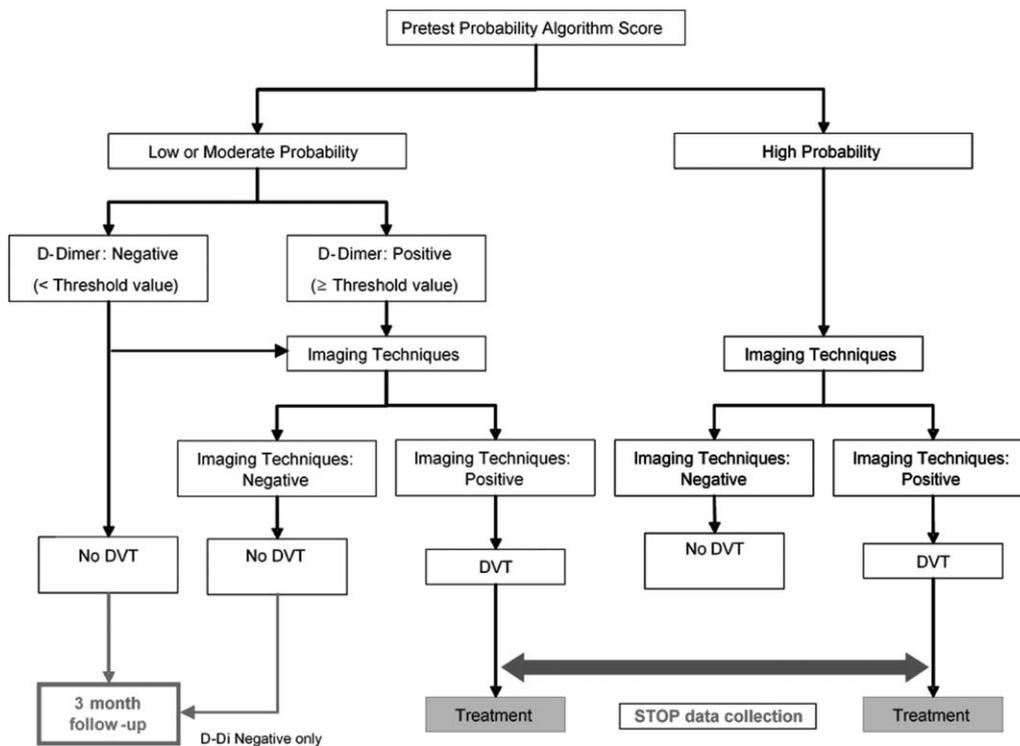
study, NCT01221805) conducted in a standard-of-care setting in consecutive, ambulatory outpatients suspected of having DVT. Study protocol included a 3-month follow-up for patients with a low or moderate PTP and a negative (i.e. below exclusion threshold 0.5 µg/ml) DDi assay result.

Each investigational site used imaging procedures (compression ultrasonography or venography) according to standard of care and diagnosis was formulated according to local practice.

The study population was recruited from prospective, consecutive, ambulatory outpatients (presenting at the emergency unit or outpatient clinic) suspected of deep venous thrombosis (DVT). Detailed inclusion and exclusion criteria, ethics, STA-LiatestD-Di assay, and statistical methods have been detailed elsewhere [5]. Noteworthy, patients aged greater than 80 years, pregnant women, postoperative patients, and patients with cancer were excluded from the study, as well as patients on anticoagulant therapy at curative or prophylaxis dose started 24h or more before the DDi level is measured.

Eligible patients were assessed for DVT using the Wells score [6,7] and then followed the patient’s management algorithm depicted in Fig. 1. Briefly, patients with low or intermediate PTP were assigned to DDi test whereas patients with high PTP were referred to imaging

Fig. 1



Deep vein thrombosis clinical algorithm.

Table 1 Main included patients' characteristics

Age (SD)	55.0 years (15.8)
Sex	Female: 59.4% Male: 40.6%
Ethnicity	Caucasian: 81.8% Black: 7.7% Hispanic: 1.8%

SD, standard deviation.

techniques. Patients with low or intermediate PTP and a DDi level below the predefined exclusion threshold (0.50 mg/ml FEUs) were considered as not having DVT and were followed up after 3 months. Patients with low or intermediate PTP and a DDi level above the exclusion cut-off were referred to imaging techniques.

Results

Participants

Ten US centers and seven out-of-US (OUS) centers (two in France, two in Italy, two in Spain and one in Canada) enrolled patients in the study.

Of the initial 1234 patients (878, i.e. 71.2% included in Emergency Departments and 356, i.e. 28.8% included in Outpatient Clinics) who signed the informed consent, 15 were excluded from the study and 1219 underwent PTP assessment. One thousand and sixty-one patients showed a low or intermediate PTP and 158 a high PTP. Thirty

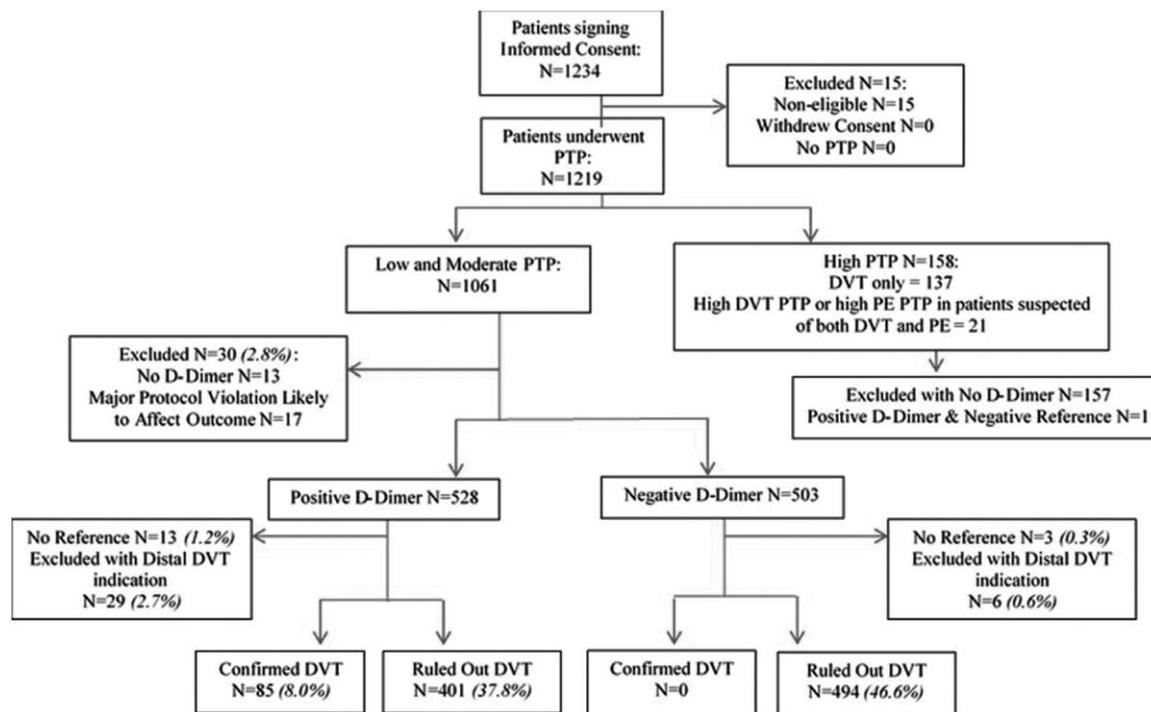
patients with low or intermediate PTP were excluded from the study (no valid DDi test: 13; major protocol violation likely to affect the outcome: 17). One thousand and thirty-one patients had a valid DDi test: 528 DDi test results were positive (DDi above the DVT exclusion threshold) and 503 had a negative DDi test result (DDi below the DVT exclusion threshold). Among the 528 positive DDi patients, 42 were excluded from the study (13 had no reference imaging; 29 were diagnosed with distal DVT). DVT was confirmed in 85 patients and ruled out in 401 patients. Among the 503 negative DDi patients, 9 were excluded from the study (3 had no reference – no imaging or lost to follow-up; 6 were diagnosed with distal DVT). Main patient characteristics are depicted (Table 1). In total, DVT diagnosis was ruled out in 494 patients with a negative DDi test result and no false negative result occurred. No DVT or pulmonary embolism was observed during the 3-month follow-up period in patients with a negative DDi result (Fig. 2).

Eighty-five patients (8.7%) of patients with low or moderate PTP had confirmed DVT; all were with positive DD.

STA-LiatestD-Di assay performance

STA-LiatestD-Di assay performance for exclusion of DVT was evaluated combining results obtained in patients with low or moderate PTP, as recommended by the CLSI guideline [4] and are depicted in Table 2. It

Fig. 2



Study flowchart.

Table 2 STA-LiatestD-Di assay performance for deep vein thrombosis exclusion

	STA-LiatestD-Di (%)	Requirements [4]	
		CLSI (%)	FDA (%)
Sensitivity			
Point estimate	100.0	≥97.0	≥95.0
95% lower limit of CI	95.8	≥90.0	≥90.0
95% upper limit of CI	100.0	NA	NA
NPV			
Point estimate	100.0	≥98.0	≥97.0
95% lower limit of CI	99.3	≥95.0	≥95.0
95% upper limit of CI	100.0	NA	NA

CI, confidence interval; CLSI, Clinical and Laboratory Standards Institute; FDA, Food and drug Administration; NA, not applicable; NPV, negative predictive value.

should be noted that the assay performance exceeded the sensitivity and NPV requirements of both CLSI and the US FDA. In addition, specificity was 55.2% (95% CI 51.9–58.5%), positive predictive value was 17.5% (95% CI 14.2–21.2%), and positive likelihood ratio (PLR) was 2.23 (95% CI 2.08–2.40). In our study, sensitivity is 100%, that is, number of false negative cases is zero. Confidence interval includes division by the number of false negatives, and for that reason, negative likelihood ratio, that is, $(1 - \text{sensitivity})/\text{specificity}$ could not be calculated.

Discussion and conclusion

Symptoms suggestive of DVT are unfortunately nonspecific, making challenging its clinical diagnosis. The Wells score is one of the most documented clinical decision tool for patient risk stratification. On the other hand, DDi is an effective tool for DVT diagnosis exclusion. The combined use of Wells score and DDi is the recommended approach for DVT exclusion as it improves diagnosis efficiency compared with the sole use of either Wells score or DDi [4,8]. However, DDi assays are heterogeneous and not all DDi assays perform equally. For this reason, the CLSI recently issued a guideline endorsed by the US FDA describing the minimal test performances for FDA clearance of DDi assays applying for a VTE exclusion claim [4]. These performances have to be demonstrated in a management study.

The purpose of the DiET study was to demonstrate the ability of the STA-LiatestD-Di assay to comply with the CLSI/FDA requirement for VTE exclusion. The DiET study consisted into two arms: the first one focused on pulmonary embolism exclusion; results were recently published in this journal [5]. The results of the second arm of the study focusing on pDVT exclusion are presented in this article.

Sensitivity and NPV obtained for the STA-LiatestD-Di assay exceed the most stringent CLSI/FDA requirements for DVT exclusion with a sensitivity of 100% and a NPV of 100%. With these performances, the US FDA cleared the device end of 2016. Altogether with the previous

clearance for pulmonary embolism exclusion obtained in September 2014, the STA-LiatestD-Di is now approved for both pulmonary embolism and DVT exclusion, when used in combination with a PTP score.

No false negative was observed in the study. However, three patients have no reference (i.e. no imaging or lost to follow-up). In a worst case scenario, these cases could be considered as false negative and result in a failure rate of 0.6% (95% CI 0–2%). Moreover, six patients were diagnosed with distal DVT and excluded from the study. Indeed, patients with distal DVT will have a normal DDi 35% of the time and the test cannot be used to avoid ultrasound evaluation. All patients with suspected distal DVT require ultrasound evaluation. DDi used in concert with lower extremity ultrasound evaluation may nevertheless be helpful [4]. Maximizing the failure rate by considering the three patients with no reference and the six patients with distal DVT as a whole as false negatives in a worst case scenario, the failure rate, although raising up to 1.7% (95% CI 1–3%), would not compromise the test diagnosis performance.

The present study has some limitations, some of them because of the fact that it was designed to comply with regulatory requirements in order to obtain clearance by the US FDA. There were different imaging techniques used depending on the one in force for the routine practice at each center. The impact of heterogeneous imaging techniques should, however, be mitigated as the CLSI does not recommend any specific method. Second, 13 of the 528 patients with positive DDi did not undergo imaging. This was because of the fact of an alternative diagnosis made before the DDi test was performed. Indeed, one of the possible situation leading to a low or moderate probability as determined with the Wells' score is that an alternative diagnosis is considered. Third, three patients with a negative DDi had no reference, that is, no follow-up: all three were recorded as lost to follow-up patients. These patients were initially planned to be followed up, but the follow-up was not completed. Finally, depending on local practices, it may have happened that patients with a negative DDi undergo imaging. In that particular case, negative DDi result was confirmed by negative imaging. This practice did, however, not influence the study results.

In conclusion, the DVT DiET study arm demonstrated the excellent performances of the STA-LiatestD-Di assay for pDVT exclusion, when used in combination with the Wells' score. This allowed clearance of the assay by the US FDA for this indication. Altogether with the previous clearance obtained for the exclusion of pulmonary embolism, the STA-LiatestD-Di has demonstrated its ability to exclude VTE when used in combination with the Wells' score. More generally, this study confirms the relevance of the recommended VTE diagnosis

algorithm including patients' stratification using a validated PTP score and a sensitive DDi assay for the exclusion of VTE. This approach maximizes the diagnosis performance while limiting imaging and associated costs to a selected subgroup of patients.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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