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THROMBOTIC BURDEN, D-DIMER LEVELS AND COMPLETE COMPRESSION
ULTRASOUND FOR DIAGNOSIS OF ACUTE SYMPTOMATIC DEEP VEIN
THROMBOSIS OF THE LOWER LIMBS

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ABSTRACT

Background: diagnostic algorithms for deep vein thrombosis (DVT) include D-dimer for its high negative predictive value, thus reducing the need for imaging. Small thrombi may be associated with low D-dimer levels, increasing false negatives. Aim: to assess the sensitivity and thus the false negative rates of standard and age-adjusted D-dimer cut offs for isolated distal DVT (IDDVT) in outpatients. Materials and Methods: We enrolled consecutive outpatients with suspected DVT of the lower limbs referring to our vascular emergency department from 2009 to 2018. Patients underwent D-dimer testing (STA, Stago, cut-off: 500 µg/L), pretest clinical probability (PTP) evaluation and complete compression ultrasonography. Follow-up was 3 months. Results: Among 3948 patients (M:1554 - 39%, median age 69), 486 proximal DVTs (12.3%) and 348 IDDVTs (8.8%) were diagnosed. Median D-dimer was higher in proximal than IDDVT (3,960 vs 1,400 µgr/L; p=0.001). The false negative rate of the standard D-dimer cut-off was 2% (95%CI: 0.8-3.2%) for proximal DVT and 14.7 % (95% CI: 11-81%) for IDDVT. The false negative rate of the age-adjusted cut-off was 4.9% (3-7%) for proximal DVT and 19.5% (95% CI: 15.4-24.7%) for IDDVT. Conclusions: Small calf thrombi are associated with low D-dimer levels, and age-adjusted D-dimer may be below the cut-off more frequently in subjects with IDDVT than standard cut-off D-dimer, although such D-dimer levels might exclude IDDVT that require treatment.

HIGHLIGHTS

- D-dimer is employed in diagnostic algorithms for DVT diagnosis for its high negative predictive value for DVT, thus reducing the need for imaging.
- Thrombotic burden may be directly associated with D-dimer levels and small thrombi may be associated with low D-dimer levels, thus reducing the yield of diagnostic algorithms.
- D-dimer levels increase with age thus reducing the specificity and age adjusted D-dimer has been proposed to increase its specificity.
- D-dimer levels correlate with the extent of DVT, and small calf thrombi are associated with low D dimer levels.
- Age-adjusted D-dimer may be below the cut-off more frequently in subjects with IDVT than standard cut-off D-dimer, thus increasing the false negative rates of D-dimer especially in case of unlikely PTP.

INTRODUCTION

D-dimer, a degradation product of cross-linked fibrin, has a high sensitivity and negative predictive value for deep vein thrombosis (DVT) diagnosis (1) and it has been employed in combination with clinical prediction rules (CPR) and compression ultrasonography (CUS), mostly to exclude DVT and /or PE and thus to reduce the need for imaging (1-4). However, most studies were performed without calf veins examination, or in outpatient populations with a predominance of proximal DVT. D-dimer assays have lower sensitivity for isolated distal (calf) DVT (IDDVT) (4). We have shown that pre-test probability with the Wells CPR has a lower diagnostic accuracy for IDDVT than for proximal DVT and that D-dimer has a better predictive negative value but alone it does not exclude IDDVT (5).

Thrombotic burden might influence the concentration of D-dimer in patients with DVT of the lower limbs. Several authors have shown that D-dimer correlates with thrombus volume both in proximal DVT and pulmonary embolism. The studies however mostly included limited patients samples (6-11) or were performed in inpatients (12). We have already demonstrated that D-dimer was correlated with thrombosis extension in patients with proximal DVT (13). Scarce data are available regarding the relation of D-dimer and IDDVT. Jennersiö et al. have shown that low concentrations of D-dimer may mirror the presence of a small thrombotic burden, such as that of IDVVT and the prevalence of IDDVT in a study population seems to have an impact on the yield of diagnostic algorithms in patients who are suspected of having an acute DVT of the lower limb (10).

Recently the PALLADIO study proposed a diagnostic algorithm (combining D-dimer, PTP and limited or extended CUS) that aimed to simplify the approach to patients with suspected DVT, thus avoiding the need for repeated serial CUS and reducing the risk of detecting IDDVTs of uncertain clinical significance (14). In fact the clinical utility of diagnosis and treating IDDVT is uncertain as several management studies showed that it is safe to not even diagnose them (15,16) and trials such as the CACTUS trial suggested that it is safe not to treat IDDVT (17) . However, DVT detectable only in the calf leg veins (i.e. IDDVT)—represents 20 to 50% of all lower limb DVTs and although

many cases spontaneously resolve, some may propagate to proximal leg veins, typically within a short space of time (18). According to the GARFIELD-VTE registry, among 2145 IDDVT, one year recurrence and all cause mortality rates were 4.8 (95% CI- confidence intervals: 3.9–5.9) and 4.6 (95% CI: 3.8–5.7) /100 person-years, respectively (18).

Moreover recently, as D-dimer levels increase with age thus reducing specificity in the elderly, a progressively higher level, the so called "age-adjusted" D-dimer, has been proposed to increase the proportion of patients with negative results (19). The D-dimer age-adjusted threshold is calculated by multiplying the patient's age by 10 for patients over 50 years (e.g. 700 µg/L fibrinogen equivalent units-FEU at 70 years), while the standard threshold is used for patients 50 years and younger. So far this approach has been validated only in patients with suspected PE, but not in patients with suspected DVT and not in IDDVT (3).

The main aim of our study was to establish the sensitivity and thus the false negative rates of age-adjusted D-dimer cut offs for acute IDDVT in outpatients with suspected DVT of the lower limbs using a strategy employing D-dimer, CPR and complete compression ultrasound (C-CUS). The secondary aim was to establish diagnostic accuracy of STA Liatest D-dimer for both proximal DVT and IDDVT.

MATERIALS AND METHODS.

We conducted a prospective study in outpatients referred to our vascular emergency department for suspected symptomatic DVT of the lower limbs during business hours from July 1st 2009 to 30th April 2018. Patients were excluded if younger than 18 years, if they were receiving vitamin K antagonists or direct oral anticoagulants, or low-molecular -weight heparin or fondaparinux for more than 24h, pregnant or in puerperium, with clinical suspicion of either ipsilateral recurrent DVT, pulmonary embolism or acute superficial vein thrombosis.

Procedures

Patients underwent: a) D-dimer testing firstly; b) while waiting for the results of D-dimer, a

personal and family history was then elicited from each patient by the physician in charge who also performed a physical examination and filled the Wells CPR questionnaire and finally c) C-CUS of both lower limbs.

The study was approved by the local Ethics Committee. Written informed consent was obtained from all patients.

D-dimer

Blood samples for D-dimer testing were drawn by clean venipuncture from an antecubital vein with a 19-gauge butterfly needle and collected into 4 ml PET (polyethylene terephthalate) tubes containing 0.4 ml 0.106 M trisodium citrate (Kima, Italy). Whole blood was centrifuged at $2000\times g$ for 20 min at 20°C . Technicians performing D-dimer testing were unaware of the symptoms of the patients.

The STA Liatest® D-Di (Diagnostica Stago, Asnières, France) is an automated and rapid microlatex D-dimer assay. Special monoclonal antibody-coated latex particles agglutinate in the presence of D-dimer fibrin degradation products. The STA Liatest® D-dimer was performed on the STA Compact® coagulation analyzer as previously described (20). The results were expressed in $\mu\text{gr/L}$ (expressed in fibrinogen equivalent unit-FEU). As previously described, the cut-off value for DVT exclusion was $500 \mu\text{gr/L}$ as indicated by the manufacturer (21). Age adjusted D-dimer cut-offs were retrospectively calculated in subjects older than 50 years by multiplying age by 10 (eg: $700 \mu\text{gr/L FEU}$ at 70 years) (22).

Clinical prediction rule

The pre-test clinical probability (PTP) was assessed according to Wells and associates (23) as shown in table 1. Based on such checklist, the PTP for DVT could be estimated to be unlikely (score = 1 or less), or likely (score = 2 or more).

Complete compression ultrasonography investigation

Patients underwent a real-time B-mode and color Doppler compression ultrasonography examination of both legs by a vascular medicine physician as previously described (5).

Ultrasonography investigation was carried out with an EnVisor C HD instrument (Philips Medical

System S.p.A, Monza, Italy), with a high-resolution broadband width linear array transducer 12-5 MHz, according to the method of Schellong (24). The proximal deep veins were examined first, then the calf veins were evaluated. The following veins were scanned in the transverse plane over their entire length: posterior tibial veins, fibular veins, internal and external gastrocnemius veins, and soleal veins. DVT diagnosis was confirmed if there was lack of compression of the vein, combined with the absence of venous flow with distal compression. The anatomical extension of the DVT was recorded on standardized forms as iliofemoral, femoro-popliteal, popliteal, popliteal and calf, axial calf (posterior tibial or peroneal), muscular (gastrocnemius or soleal).

Follow-up

Patients in whom PTP was likely and/or D-dimer was positive but C-CUS was negative, were tested again after 5-7 days and if negative followed up at 3 months. Patients in whom PTP was unlikely and D-dimer negative and had a negative C-CUS and those in whom DVT was excluded at the 5-7 days evaluation were followed up at 3 months to detect VTE complications by either telephone or visit. Failure rate was the rate of thromboembolic complications at 3 month follow up for patients in whom DVT was excluded at either the initial or 5-7 day visit.

Patients were instructed to return to our vascular emergency department in case of new symptoms at the lower limbs and to the emergency department in case of respiratory symptoms. In case of suspected recurrent DVT, recurrence was diagnosed if a previously fully compressible segment (contralateral or ipsilateral) was no longer compressible or if an increase of at least 4 mm in the diameter of the residual thrombus during compression was detected (25). When thrombus diameter changed between 1.1 and 3.9 mm, or in cases of high/moderate clinical probability and normal proximal compression ultrasonography, the examination was repeated 5 to 7 days later. Failure rate included both proximal and IDVT diagnosed during follow-up as well superficial vein thrombosis (SVT) confirmed by compression ultrasound. In case of suspected pulmonary embolism, patients underwent contrast enhanced pulmonary CT or V/Q scan. All reported clinical outcomes were adjudicated by the attending physicians. Causes of death were adjudicated based on autopsy reports, if

available, or clinical reports by investigators (L.B and M.S) unaware of patients initial D-dimer levels.

Patients were considered lost to follow-up whenever they did not answer to five telephone calls in five different days at different hours and their family physicians did not have direct news of them.

Statistical analysis

Relationships between variables were assessed using t-test for continuous variables and chi-square or Fisher's exact test for categorical variables. Multivariate analysis of variance was used to compare means among groups for normally distributed variables.

The diagnostic accuracy that is sensitivity, specificity, negative predictive value and positive predictive value of the STA Liatest® D-dimer for both DVT and IDDVT, was calculated according to the standard cut-off and age-adjusted cut-offs.

Categorical variables were expressed as frequency and percentage with 95% confidence intervals (95% CI); continuous variables were expressed as median and range. The significance level was set at ≤ 0.05 . Analysis was carried out using the SPSS software package (version 19.0; SPSS Inc. Chicago, Illinois, USA).

RESULTS

A total of 3948 outpatients were evaluated for a suspicion of acute DVT of the lower limbs. The characteristics of patients are shown in table 2.

IDDVT was diagnosed in 348 (8.8%) of subjects who were mostly female (54%) with a median age of 68 years and in 180 (48%) of whom PTP was unlikely (median: 1) with D-dimer levels below the standard cut-off in 51 (14.7%) and D-dimer below the age-adjusted cut-off in 68 patients (19.5%).

Proximal DVT was diagnosed in 486 patients (12.3%) the majority of whom were male (54%) with a median age of 74 and had a likely PTP in 80% of cases (median: 2). D- dimer levels were below the standard cut-off in only 10 (2.1%) patients with proximal DVT while in 24 patients (4.9%) D-

dimer was below the age-adjusted cut-off. There were more men among patients with DVT (either proximal or distal) than among subjects without DVT ($p=0.001$). Patients with proximal DVT were older than subjects without DVT or with IDDVT ($p=0.001$). D-dimer levels were significantly higher in subjects with proximal DVT (median: 3,600 vs. 1,400 $\mu\text{gr/L}$) than in those with IDDVT or no DVT (median: 540 $\mu\text{gr/L}$) (Table 2 and figure 1). Patients with IDDVT also had higher D-dimer levels than subjects without DVT.

Elements of the Wells score according to the presence or absence of DVT are shown in table 3.

Risk factors such as active cancer was more frequent in patients with proximal DVT than in those with no DVT or IDDVT, while immobilization or major surgery were similarly frequent in proximal and IDDVT. Hormonal therapy was more frequent in patients with IDDVT than proximal DVT. Signs such as calf swelling or pitting edema was more frequent in proximal DVT than in patients with IDDVT, while localized tenderness was similar in proximal and IDDVT.

D-dimer and pre-test clinical probability according to the presence of proximal DVT or IDDVT.

In patients proximal DVT, unlikely PTP and D-dimer below the standard cut-off were present in 6/486 (1.2%; 95% CI: 0.02-2.21%), while in subjects with IDDVT unlikely PTP and D-dimer below the cut-off were present in 30/348 (8.6%; 95% CI: 5.7-11.5%) (Table 4). In patients proximal DVT, unlikely PTP and D-dimer below the age adjusted cut-off were present in 11/486 (2.3%; 95% CI: 0.9-3.6%), while in subjects with IDDVT, unlikely PTP and D-dimer below age adjusted cut-off were present in 37/348 (10.6%; 95% CI: 7.4-13.9%) (Table 4).

The diagnostic accuracy of standard and age-adjusted of STA Lia test D-dimer cut-off are indicated in table 5. The false negative rate of the standard D-dimer cut-off for proximal DVT was 2% (95%CI: 0.8-3.2%), while it was 14.7% (95% CI: 11-18%) for IDDVT (table 5 A). The false negative rate of the age-adjusted cut-off was 4.9% (95% CI: 3-7%) for proximal DVT while it was 19.5% (95% CI: 15.4-24.7%) for IDDVT (table 5 B).

Among subjects with DVT in whom PTP was unlikely and D-dimer below the standard cut-off,

patients with proximal DVT were younger (median age 41 y vs 57 y in the no DVT group), mostly men (5/1 = 83% vs 473/833 = 56% in the no DVT group) and had femoro-popliteal DVT in 4 cases and proximal plus calf DVT in 2 cases. Patients with IDDVT were younger (median age: 47 y vs 57 y in the no DVT group), mostly men (18/12 = 60%) and with muscular DVT (21/30 = 70%).

Among patients with IDDVT, additional risk factors were present: five had had previous superficial vein thrombosis, one previous contralateral proximal DVT, two previous contralateral IDDVT, only one subject had active cancer, while five were on hormonal therapy.

D-dimer in patients with proximal DVT.

None of the subjects with ilio-femoro-popliteal DVT had D-dimer below the cut-off, while 4 (2% of all DVT), 3 (3.2%) and 3 (1.7%) of patients with femoro-popliteal, popliteal and proximal plus calf DVT had D-dimer below the standard cut-off (overall: 2.05% of all proximal DVTs; 95% CI:1.1-3.7%). When age-adjusted DVT was applied, 1 of the subjects with ilio-femoro-popliteal DVT had D-dimer below the cut-off, while 11 (5.8%), 6 (16.3%) and 6 (3.4%) of patients with femoro-popliteal, popliteal and proximal plus calf DVT had D-dimer below the cut-off, respectively, (overall: 4.9% of all proximal DVTs, 95% CI: 3.3-7.2%).

Characteristics of patients with IDDVT.

Table 6 shows the characteristics of patients with IDDVT. The majority of DVTs were confined to the muscular veins (gastrocnemial or soleal) (208; 60% of all IDDVT) and only a minority had both axial (peroneal or posterior tibial veins) and muscular (46 =13% all IDDVT). No significant difference was observed in sex distribution, age, pre-test probability, while D-dimer levels were significantly higher in subjects with both axial and muscular IDDVT than in those with either only axial or muscular DVT. D-dimer was below the standard cut-off in 15 (4.3%) of subjects with axial IDDVT, 34 (9.7%) of subjects with muscular DVT and only 2 (0.5%) of subjects with both axial and muscular DVT (overall: 14.6 % of all IDDVT; 95% CI:10.9 -18.4 %). When age-adjusted D-dimer was applied, 21 (6%) of the subjects with axial DVT , 45 (13%) and 2 (0.5%) of patients

with muscular and axial and muscular DVT had D-dimer below the cut-off (overall: 19% of all IDDVT; 95%CI: 15.3-23.7%).

VTE complications at 3 months in subjects in whom DVT was excluded

VTE complications at 3 months were reported in those subjects in whom DVT was excluded by both the initial and the 5-7 day visits. Eighty patients were lost to follow-up (2.57%; 95% CI: 2.04-3.19%).

In 1644 patients evaluated at 5-7 days, 14 VTE (2 proximal DVT, 10 IDDVT, 2 SVT) were detected (0.85%, 95% CI: 0.47-1.42%).

Overall, failure rate was 0.23% (95% CI: 0.09-0.47) with 7 VTE (1 PE fatal, 4 IDDVT, 1 SVT) detected at 3 months. If only more clinical relevant VTE such as proximal DVT and/or PE were considered, failure rate was 0.03% (95% CI: 0-1%). All cause mortality at 3 months was 0.79% (95% CI: 0.51-1.17%) with only 1 fatal PE.

DISCUSSION.

D-dimer is employed in diagnostic algorithms for DVT diagnosis, for its high negative predictive value, with the potential to reduce imaging. However clot burden may influence D-dimer levels and small thrombi, such as those of IDDVT, can be associated with low D-dimer levels, thus influencing the yield of diagnostic algorithms. Very limited data are available regarding the relationship of D-dimer and thrombus burden in IDDVT. Jennersiö et al showed that D-dimer was below the cut-off in 35% (28/81) of patients with IDDVT among 393 patients (81/393 with IDDVT) and the sensitivity of D-dimer for IDDVT was only 65% compared with 96% for proximal DVT with negative predictive values of 84 and 99%, respectively (10). In this study however the prevalence of IDDVT was 81/393 (20.6%) and this can influence the diagnostic accuracy of algorithms employing D-dimer for DVT diagnosis.

The clinical relevance of IDDVT and the need of its diagnosis and treatment is still a matter of debate. Recently the PALLADIO study proposed a diagnostic algorithm (combining D-dimer, PTP and limited or extended CUS) that aimed to simplify the approach to patients with suspected DVT.

Such an approach could limit extended whole leg ultrasonography and repeated serial CUS, thus reducing the risk of detecting IDDVTs of uncertain clinical significance (14). In addition such an approach could be applied in many settings thus reducing both costs and the need of more experienced personnel for the performance of whole leg CUS. However, in the Palladio study, limited CUS was performed only in selected patients at lower risk in comparison to patients who underwent whole leg CUS. In the whole leg CUS arm, the DVT prevalence was 49%, whereas in the limited CUS arm was 3%. Seven patients in limited CUS group had a repeat ultrasound assessment, with five DVT diagnosed in these patients. When the latter patients were included in the follow-up analysis, the 3 month incidence of VTE events in limited CUS arm increased from 1.1% to 2.3%. In a post-hoc analysis of the Palladio study, the age adjusted D-dimer was also applied with a reduction of approximately 5% of the need for imaging tests, without increasing the incidence of VTE at the 3-month follow-up. In this post-hoc analysis, nine IDDVTs, previously diagnosed in the group undergoing extended CUS, would not have been detected at the initial visit if these patients were included in group undergoing limited CUS (26).

Our study shows that D-dimer levels are correlated with the extent of DVT and they are higher in patients with proximal DVT than in patients with IDDVT, thus indicating that the **thrombus burden** is associated with D-dimer levels. D-dimer was below the standard cut-off in 6 subjects with proximal DVTs (1.2%) with unlikely PTP and in 30 subjects with calf DVTs (8.6%) with unlikely PTP. When age-adjusted D-dimer was applied retrospectively, it was below the cut-off in 11 patients (2.3%) with proximal DVT and unlikely PTP and in 37 patients with IDDVT (10.6%) and unlikely PTP, mostly muscular calf DVTs. In subjects with IDDVT with unlikely PTP and D-dimer below cut-off, either standard or age adjusted, diagnosis would have been missed by a limited CUS. In our study failure rate, that is the incidence of VTE at 3 months was 0.23% (95% CI: 0.09-0.47%), as 12 VTE were detected at repeated whole leg CUS at 7 days, while the overall incidence of VTE at 3 months in the PALLADIO study was higher (0.87%; 95% CI: 0.44–1.70%).

The clinical benefit of diagnosing IDDVT is still a matter of controversy as most untreated IDDVTs have a benign clinical course, and only some of them may have clinically relevant thrombotic outcomes.

Moreover, several management studies have clearly established that patients with unlikely PTP and negative D-dimer have a negligible risk of VTE at 3-month follow-up. As a result, international treatment guidelines such as those issued by the American College of Chest Physicians (ACCP) recommend giving anticoagulant therapy in patients with proximal DVT or PE as well as IDDVT with severe symptoms or risk factors for extension to proximal veins (inpatients, prior history of VTE, cancer) (27). However, the real life Garfield Registry showed that active treatment of emergent IDDVT rather than risk-stratified therapy appears routine practice globally (18). Essentially, diagnosing IDDVT provides a diagnosis which itself can be therapeutic to the patient, especially in case of signs such as pain limited to the calf, and limit unnecessary further tests or treatment (e.g. antibiotics for presumed cellulitis) and defines a group of patients at higher risk of VTE progression even if anticoagulation is not proposed and of course, treatment for purposes of symptom relief may be useful.

In our study we found several patients with IDDVT in whom D-dimer was below the D-dimer standard cut-off and PTP was unlikely. We could speculate that such IDDVT could be at low risk of proximal extension and they could have been left untreated, whereas anticoagulant treatment was started in all of them.

More recently, the performance of six diagnostic strategies based on the three-level Wells scores and various cut-off levels for D-dimer, evaluated using the HemosIL D-Dimer HS 500 assay (Werfen IL, United Kingdom) assay, was validated in a multicentre study involving 1255 consecutive outpatients with non-high PTP and either DVT or PE (28). The diagnostic strategy based on the age-adjusted cut-off levels was found to perform the best in the derivation study with a better sensitivity-to-specificity ratio than the conventional strategy based on the fixed cut-off level, a higher specificity and a negative predictive value (NPV) above 99%. Such an increase in test specificity was confirmed

in the validation cohort, with the NPV remaining above 99%. Taking into account the local reimbursement rates of diagnostic tests, using this strategy led to a 6.9% reduction of diagnostic costs for PE and a 5.1% reduction for DVT, as imaging tests would be avoided in a higher percentage of patients with non high PTP. In our study the sensitivity of both standard and age-adjusted D-dimer was higher for proximal DVT than for IDDVT, while the sensitivity of the standard cut-off for both proximal and IDDVT was higher than that of age-adjusted D-dimer.

Our study is a large prospective study and it is the first to assess the yield of C-CUS in combination with PTP and D-dimer and it provides useful information to understand the likelihood of missing an IDDVT with commonly used diagnostic strategies.

Such approach may increase costs, but in our system there is no difference in costs for limited versus whole leg CUS. The latter however requires more expertise or dedicated personnel, as well as more advanced instrumentation as it can be more technically challenging.

Some limitations of our study should be taken into account. Our study was single centre and performed by dedicated experienced vascular medicine physicians. The possibility of false positive diagnosis of IDDVT, especially the muscular ones, cannot be ruled out and no inter-observer variability was assessed for IDDVT diagnosis. The age-adjusted D-dimer threshold was applied retrospectively as a post-hoc analysis of a management study with pre-defined endpoints and data collection. As a result, failure rate of this approach cannot be correctly calculated. The natural history of IDDVTs in patients without the interference of anticoagulant treatment cannot be evaluated, because an extended CUS was performed and patients were treated accordingly. Some patients were lost to follow-up, mostly because patients moved from our area, however the rate of loss was lower than 3%.

In conclusion, our results indicate that IDDVT with low thrombus burden may be missed in case the diagnostic approach is based on D-dimer and PTP, without or with limited imaging. In case age-adjusted D-dimer is employed, a higher proportion of IDDVTs with low thrombus burden can be

missed in case of limited imaging. The study results also show that D-dimer levels below the cut-off do not exclude distal DVT in outpatients; instead, it can be hypothesized that such D-dimer levels may exclude DVT that require treatment (10).

However, the clinical relevance of IDDDVT especially of muscular (gastrocnemius and/or soleal) is still uncertain and thus the relevance of its diagnosis. Further studies are required to evaluate the natural history of IDDDVT with negative D-dimer and low thrombotic burden.

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REFERENCES

1. Chopard R, Albertsen IE, Piazza G. Diagnosis and Treatment of Lower Extremity Venous Thromboembolism: A Review. *JAMA*. 2020;324(17):1765-1776
2. Wells PS, Iezzoni R, Reilly A, Forgie MA. Diagnosis of Venous Thromboembolism: 20 Years of Progress. *Ann Intern Med*. 2018;168(2):131-140
3. Bates SM, Jaeschke R, Stevens SM, Goodacre S, Wells PS, Stevenson MD, et al. Diagnosis of DVT: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest*. 2012;141(2 Suppl):e351S-e418S
4. Lim W, Le Gal G, Bates SM, Righini M, Haramati LB, Lang E, et al. American Society of Hematology 2018 guidelines for management of venous thromboembolism: diagnosis of venous thromboembolism. *Blood Adv*. 2018;2(22):3226-3256
5. Sartori M, Cosmi B, Legnani C, Favaretto E, Valdres L, Guazzaloca G et al . The Wells rule and D-dimer for the diagnosis of isolated distal deep vein thrombosis. *J Thromb Haemost*. 2012;10(11):2264-2269
6. Keller K, Beule J, Balzer JO, Dippold W. D-Dimer and thrombus burden in acute pulmonary embolism. *Am J Emerg Med*. 2018;36(9):1613-1618
7. Mousa AY, Broce M, De Wit D, Baskharoun M, Abu-Halimah S, Yacoub M et al. Appropriate Use of Venous Imaging and Analysis of the D-Dimer/Clinical Probability Testing Paradigm in the Diagnosis and Location of Deep Venous Thrombosis. *Ann Vasc Surg*. 2018;50:21-29
8. Kurklinsky AK, Kalsi H, Wysokinski WE, Mauck KF, Bhagra A, Havyer RD, et al. Fibrin d-dimer concentration, deep vein thrombosis symptom duration, and venous thrombus volume. *Angiology*. 2011;62(3):253-256
9. Hochuli M, Duester S, Frauchiger B. Quantitative d-dimer levels and the extent of venous

- thromboembolism in CT angiography and lower limb ultrasonography. *Vasa*. 2007;36(4):267-274
10. Jennersjö CM, Fagerberg IH, Karlander SG, Lindahl TL. Normal D-dimer concentration is a common finding in symptomatic outpatients with distal deep vein thrombosis. *Blood Coagul Fibrinolysis*. 2005;16(7):517-523
11. Goldin Y, Berliner S, Rogowski O, Paslowski O, Serov J, Halpern P, et al. Correlated expression of D-dimer concentrations with thrombotic burden in acute pulmonary embolism. *Blood Coagul Fibrinolysis*. 2008;19(2):153-158
12. Chi G, Goldhaber SZ, Hull RD, Hernandez AF, Kerneis M, Al Khalfan F, et al. Thrombus Burden of Deep Vein Thrombosis and Its Association with Thromboprophylaxis and DDimer Measurement: Insights from the APEX Trial. *Thromb Haemost*. 2017;117(12): 2389- 2395
13. Sartori M, Favaretto E, Cini M, Legnani C, Palareti G, Cosmi B. D-Dimer, FVIII And Thrombotic Burden In The Acute Phase Of Deep Vein Thrombosis In Relation To The Risk Of Post-Thrombotic Syndrome. *Thrombosis Research* 2014; 134 (2): 320-5.
14. Ageno W, Camporese G, Riva N, Iotti M, Bucherini E, Righini M et al. Analysis of an algorithm incorporating limited and whole-leg assessment of the deep venous system in symptomatic outpatients with suspected deep-vein thrombosis (PALLADIO): a prospective, multicentre, cohort study. *Lancet Haematol*. 2015;2(11):e474-e480
15. Bernardi E, Camporese G, Büller HR, et al. Serial 2-Point Ultrasonography Plus D-Dimer vs Whole-Leg Color-Coded Doppler Ultrasonography for Diagnosing Suspected Symptomatic Deep Vein Thrombosis: A Randomized Controlled Trial. *JAMA*. 2008;300(14):1653–1659. doi:10.1001/jama.300.14.1653
16. Robert-Ebadi H, Righini M Management of distal deep vein thrombosis. *Thromb Res* 2017; 149:48–55
17. Righini M, Galanaud JP, Guennevez H et al. Anticoagulant therapy for symptomatic calf

- deep vein thrombosis (CACTUS): a randomised double-blind, placebo-controlled trial. *Lancet Haematol* 2016 3(12) :e556–e562
18. Schellong SM, Goldhaber SZ, Weitz JI, Ageno W, Bounameaux H, Turpie AGG, et al. Isolated Distal Deep Vein Thrombosis: Perspectives from the GARFIELD-VTE Registry [published correction appears in *Thromb Haemost.* 2019 ;119(10):e1]. *Thromb Haemost.* 2019;119(10):1675-1685
19. Nybo M, Hvas AM. Age-adjusted D-dimer cut-off in the diagnostic strategy for deep vein thrombosis: a systematic review. *Scand J Clin Lab Invest.* 2017;77(8):568-573
20. Escoffre-Barbe M, Oger E, Leroyer C, Grimaux M, Le Moigne E, Nonent M, et al. Evaluation of a new rapid D-dimer assay for clinically suspected deep venous thrombosis (Liatest D-dimer). *Am J Clin Pathol.* 1998;109(6):748-753
21. Sidelmann JJ, Gram J, Larsen A, Overgaard K, Jespersen J. Analytical and clinical validation of a new point-of-care testing system for determination of D-Dimer in human blood. *Thromb Res.* 2010;126(6):524-530
22. Douma RA, Tan M, Schutgens REG, Bates SM, Perrier A, Legnani C, et al. Using an agedependent D-dimer cut-off value increases the number of older patients in whom deep vein thrombosis can be safely excluded. *Haematologica* 2012;97(10):1507-1513
23. Wells PS, Owen C, Doucette S, Fergusson D, Tran H. Does this patient have deep vein thrombosis?. *JAMA.* 2006;295(2):199-207
24. Schellong SM, Schwarz T, Halbritter K, Beyer J, Siegert G, Oettler W et al. Complete compression ultrasonography of the leg veins as a single test for the diagnosis of deep vein thrombosis. *Thromb Haemost.* 2003;89(2):228-234
25. Prandoni P, Cogo A, Bernardi E, Villalta S, Polistena P, Simioni P, et al. A simple ultrasound approach for detection of recurrent proximal-vein thrombosis. *Circulation.* 1993;88(4 Pt 1): 1730-1735
26. Riva N, Camporese G, Iotti M, Bucherini E, Righini M, Kamphuisen PW, et al. Age adjusted

D-dimer to rule out deep vein thrombosis: findings from the PALLADIO algorithm.

J Thromb Haemost. 2018;16(2):271-278

27. Stevens SM, Woller SC, Baumann Kreuziger L, Bounameaux H, Doerschug K, Geersing GJ, Huisman MV, Kearon C, King CS, Knighton AJ, Lake E, Murin S, Vintch JRE, Wells PS, Moores LK, Antithrombotic Therapy for VTE Disease: Second Update of the CHEST Guideline and Expert Panel Report – Executive Summary, *CHEST* (2021), doi: <https://doi.org/10.1016/j.chest.2021.07.056>.

28. De Pooter N, Brionne-François M, Smahi M, Abecassis L, Toulon P. Age-adjusted D-dimer cut-off levels to rule out venous thromboembolism in patients with non-high pre-test probability: Clinical performance and cost-effectiveness analysis. J Thromb Haemost. 2021;19(5):1271-1282

Table 1: Wells Score Criteria and calculation of pre-test probability of DVT

| Items | Points if present |
|--|-------------------|
| Active cancer (treatment ongoing or within previous 6 months or palliative) | 1 |
| Paralysis, paresis, or recent plaster immobilisation of the lower extremities | 1 |
| Recently bedridden for more than 3 days or major surgery, within 4 weeks | 1 |
| Localized tenderness along the distribution of the deep venous system | 1 |
| Entire leg swollen | 1 |
| Calf swelling at least 3 cm larger than that on the asymptomatic side (measured 10 cm below tibial tuberosity) | 1 |
| Pitting edema confined to the symptomatic leg | 1 |
| Collateral superficial veins (non varicose) | 1 |
| Previously documented deep-vein thrombosis | 1 |
| Alternative diagnosis as likely as or more likely than DVT | -2 |

PTP unlikely :score = 1 or less or likely (score = 2 or more)

Table 2: Characteristics of enrolled patients*

| | All patients N=3948 | No DVT N=3114 78.9% | Proximal DVT N= 486 12.3% | IDDTV N=348 8.8% | P |
|---|----------------------------|----------------------------|---------------------------------|-------------------------|--------|
| M/F M % | 1554/2394 39% (38-41) | 1130/1984 36% (34-38) | 264/222 54% (50-58) | 160/188 46% (41-51) | — |
| Age median, range age < 50 y (%) | 69 18-103 | 68 18-102 | 74 19-103 79 (16) | 68 18-97 | 0.0001 |
| Wells'score ≥2 | 998 25% (24-27) | 413 13% (12-14) | 390 80% (77-84) | 180 52% (46-57) | — |
| Pre-test clinical probability score median, range | 0; -2-6 | -1; -2-5 | 2.0 ; -2- 6 | 1; -2- 5 | 0.0001 |
| D-dimer µgr/L median, range | 700 20-73,200 | 540 20-23,260 | 3,960 170-73,200 | 1,400 30-60,000 | 0.001 |
| D-dimer below 500 µgr/L | 1501 37.6% (37-40) | 1435 46% (44-48) | 10 2.1% (1-3) | 51 15% (11-18) | — |
| D-dimer below age adjusted cut-off | 1947 49% (48-51) | 1855 60% (58-61) | 24 4.9% (3-7) | 68 19% (15-24) | — |
| Right/left/ bilateral lower limb (%) | 1841/1987/160 [46/49/5] | 1445/1531/138 [46/49/5] | 220/254/12 [46/53/2] | 158/178/12 [46/51/3] | ns |

* figures in parentheses indicate 95% confidence intervals

Table 3 Elements of the Wells' score and other characteristics according to the presence or absence of DVT *

| | No DVT N=3114 | Proximal DVT N= 486 | IDDTV N=348 |
|--|------------------------|------------------------|-----------------------|
| Active cancer (treatment ongoing or within previous 6 months or palliative) | 56 -1.8% (1-2) | 30 - 6.1% (4-8) | 11 - 3.3% (1-5) |
| Paralysis, paresis, or recent plaster immobilisation of the lower extremities | 65 - 2.1% (2-3) | 17 - 3.5% (2-5) | 17 - 5% (3-7) |
| Recently bedridden for more than 3 days or major surgery, within 4 weeks | 74 - 2.4% (2-3) | 38 - 7.8% (5-10) | 23 - 6.6% (4-9) |
| Localized tenderness along the distribution of the deep venous system | 607- 19.5% (18-21) | 192 - 39.8% (35-44) | 152- 43.6% (38-49) |
| Entire leg swollen | 96 - 3.1% (2-4) | 74 - 15.2% (12-18) | 11 - 3.3% (1-5) |
| Calf swelling at least 3 cm larger than that on the asymptomatic side (measured 10 cm below tibial tuberosity) | 168 - 5.3% (5-6) | 115 - 23.8% (20-27) | 17 - 5.5% (3-7) |
| Pitting edema confined to the symptomatic leg | 544 - 17.5% (16-18) | 162 - 33.3% (29-38) | 63 - 18.2% (14-22) |
| Collateral superficial veins (non varicose) | 0 | 5 - 1% | 0 |
| Alternative diagnosis as likely as or more likely than DVT | 24- 0.8% | 8-1.6% | 10-3% |
| Family/personal history of VTE | 152-4.9% (4-6) | 73- 15% (12-18) | 38-11% (8-14) |
| Hormonal therapy | 156- 5% (4-6) | 27 - 5.6% (4-8) | 48 - 13.7% (10-17) |
| Known thrombophilia | 12 - 0.4% (0-1) | 7 - 1.2% (2-3) | 7 - 2.0% (4-3) |

* figures in parentheses indicate 95% confidence intervals

Table 4

Unlikely pre-test clinical probability and standard or age-adjusted D-dimer cut-off according to the absence or presence of DVT

| | No DVT N=3114 | Proximal DVT N=486 | IDDVVT N=348 |
|----------------------|-------------------|--------------------|-------------------|
| Wells'score <=1 | D-dimer < cut-off | D-dimer < cut-off | D-dimer < cut-off |
| Standard cut-off | 1306 (42%) | 6 (1.2%) | 30 (8.6%) |
| Age-adjusted cut-off | 1681 (54%) | 11 (2.3%) | 37 (10.6%) |

Table 5

A) The diagnostic accuracy of STA Lia test D-dimer standard cut-off for proximal DVT and IDDVT.

| Standard D-dimer cut off | Prox DVT | IDDVT | All DVTs |
|---------------------------|--|---|--|
| Sensitivity | 476/486 = 97.9 % (95% CI: 96.7-99.2%) | 297/348 = 85.3% (95% CI: 81.6-89.1) | 773/834 = 92.7% (95% CI: 91-94.5%) |
| Specificity | 1435/3114= 46% (95% CI: 44.3-47.8%) | 1435/3114 = 46% (95% CI: 44.3-47.8%) | 1435/3114=46% (95% CI = 44.3-47.8%) |
| Positive predictive value | 476/2155 = 22% (95% CI: 20.3-23.8) | 297/1976:15% (95% CI: 13.5-16.5) | 773/2452 = 31% (95% CI: 29.7-33.4%) |
| Negative predictive value | 1435/1445 = 99.4% (95% CI: 99-99.8%) | 1435/1492 = 96.2% (95% CI: 95-97%) | 1435/1497 = 95.9% (95% CI:94.8-97%) |

Table 5

B) The diagnostic accuracy of the STA Lia test D-dimer age-adjusted cut-off for proximal DVT and IDDVT.

| Standard D-dimer cut off | Prox DVT | IDDVT | All DVTs |
|---------------------------|---|---|---|
| Sensitivity | 462/486 = 95% (95% CI: 93.1-97%) | 280/348 = 80.5% (95% CI: 76.3-84.6%) | 742/834 = 89% (95% CI: 86.8-92%) |
| Specificity | 1855/3114 = 59% (95% CI: 57.8-61.3%) | 1855/3114 = 59% (95% CI: 57.8-61.3%) | 1855/3114 = 59% (95% CI: 57.8-61.3%) |
| Positive predictive value | 462/1721 = 26.8% (95% CI: 24.8-28.9%) | 280/1539 = 18% (95% CI: 16.3-20%) | 742/2001 = 37% (95% CI: 35-39.2%) |
| Negative predictive value | 1855/1879 = 98.7% (95% CI: 98.2-99.2%) | 1855/1923=96.5% (95% CI: 95.6-97%) | 1885/1953 = 96.5% (95% CI: 95.7-97.3%) |

Table 6 Characteristics of patients with (calf) IDVT

| | Axial DVT n=94 (27%) | Muscular DVT n=208 (60%) | Axial and muscular DVT n=46 (13%) | P |
|---|----------------------------|--------------------------------|--|--------|
| M/F M % | 39/55 41 | 98/110 47 | 23/23 50 | 0.55 |
| Age median, range | 68 25-90 | 68 18-97 | 64 38-97 | 0.79 |
| Wells'score ≤1 | 53 (56%) | 104 (50%) | 23 (50%) | 0.57 |
| Wells'score ≥2 | 41 (43%) | 104 (40%) | 23 (50%) | -- |
| Pre-test clinical probability score median, range | 2; -2 / 4 | 2; -2/4 | 1;-1/ 5 | 0.32 |
| D-dimer µgr/L median, range | 1,270 0,17-9,360 | 1,290 0,03-60,400 | 3,260 0,38-20.000 | 0.0001 |
| D-dimer below standard cut-off | 15 (4.3%) | 34 (9.7%) | 2 (0.5%) | 0.1 |
| D-dimer below age- adjusted cut-off | 21 (6%) | 45 (13%) | 2 (0.5%) | 0.42 |
| Right/left/ bilateral (%) | 44/48/2 (47/51/2) | 100/103/5 (48/49/2) | 14/27/5 (30/59/11) | 0.019 |

Figure 1: D-dimer levels according to the absence or presence of proximal or distal DVT, Horizontal bars indicate means with 95% confidence intervals

