An age-adapted approach for the use of D-dimers in the exclusion of deep venous thrombosis

Fred J.L.M. Haas,1* Roger E.G. Schutgens,2 and Douwe H. Biesma2,3

A normal D-dimer (DD) concentration for the exclusion of deep venous thrombosis (DVT) has a low specificity in older patients and compression ultrasonography is often required. Three D-dimer assays, STA Liatest, Tina-quant, and Innovance, are evaluated in symptomatic outpatients suspected for DVT with emphasis on its performance in older patients by using different cut-off levels. This study includes 466 outpatients suspected for having DVT. The diagnostic accuracy, measured as sensitivity and area under the curve of the receiver operation characteristic curve, is good for all DD assays. The specificity of the DD assays combined with a low pretest probability varies from 42.6 to 51.5%. The specificity of the three DD assays in patients ≥60 years varies, however, between 24.6 and 40.9%. Several cut-off values in different age-subgroups are studied. For patients <60 years, the most accurate cut-off value is 500 µg/L for all DD assays. For patients ≥60 years, a threshold of 750 µg/L has the best results with NPV of 100% for all assays and specificity of 48.5% (STA Liatest), 60.6% (Tina-quant), and 49.2% (Innovance), respectively. For the three assays, the number needed to test (NNT) decreases in both subgroups of patients compared to the standard algorithm. A cut-off level of 750 µg/L for patients ≥60 years improves the clinical performance of DD assays in combination with the PTP score without the loss of NPV. The NNT improves substantially with an age-adapted algorithm. Am. J. Hematol. 84:488–491, 2009. © 2009 Wiley-Liss, Inc.

Introduction

The use of the D-dimer (DD) assay as screening test for exclusion of venous thromboembolism (VTE) is well documented [1–8]. The combination of a clinical risk stratification and a DD test can exclude VTE in ~25% of the patients presenting with symptoms of VTE without the need for additional (imaging methods) investigations [9–14]. Although this strategy is widely accepted, there are some serious limitations in the use of DD assays. There is an assay-dependent variability in specificity for conditions such as thrombosis or disseminated intravascular coagulation [15]. The overall low specificity of the DD in the diagnosis of VTE limits its use in clinical practice. The specificity decreases with increasing age [16–18] and in patients with inflammation [19]. The chance of having a normal DD concentration in symptomatic cancer patients is only 9% [20]. By using the standard DD cut-off value, false-positive results are frequently found in older patients [21]. Ruling out VTE by the use of a DD in patients aged ≥75 years is limited to 10% of these patients [13]. It has also been suggested that the use of the DD assay in patients older than 80 years has little clinical value [16].

A recent study reports that the clinical usefulness of DD measurement decreases with age [22]. However, the authors consider the use of higher DD cut-off values for ruling out pulmonary embolism (PE) as unsafe. Another approach for improvement of the specificity is the use of higher cut-off values for the pretest probability (PTP) score in the exclusion of DVT [23,24]. The combination of changes in the cut-off values of the PTP and the DD assay has been suggested previously in the exclusion of PE [25]. Given the large differences in sensitivity and specificity of DD assays in the elderly [26] and the age-related discordance between DD assays [27], the choice of an appropriate DD assay for use in the elderly is also an important issue.

The objective of this study is to evaluate the use of age-adapted cut-off values for three DD assays in symptomatic outpatients suspected for having deep venous thrombosis (DVT).

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TABLE I. Demographic Data of the Study Population

<table>
<thead>
<tr>
<th></th>
<th>Current cohort</th>
<th>Original cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>466</td>
<td>812</td>
</tr>
<tr>
<td>Men</td>
<td>39%</td>
<td>36%</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>60</td>
<td>59</td>
</tr>
<tr>
<td>DVT prevalence</td>
<td>39%</td>
<td>39%</td>
</tr>
<tr>
<td>High PCP score</td>
<td>32%</td>
<td>34%</td>
</tr>
</tbody>
</table>

TABLE II. Prevalence of DVT and AUC of ROC Curve of Three D-dimer Assays

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Prevalence (%) (DVT/N)</th>
<th>STA Lia (%)</th>
<th>Tina-quant (%)</th>
<th>Innovance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>39 (182/466)</td>
<td>87.6</td>
<td>91.9</td>
<td>91.8</td>
</tr>
<tr>
<td>&lt;60</td>
<td>42 (94/224)</td>
<td>90.3</td>
<td>93.9</td>
<td>93.4</td>
</tr>
<tr>
<td>&gt;60</td>
<td>36 (85/242)</td>
<td>87.3</td>
<td>91.5</td>
<td>91.7</td>
</tr>
</tbody>
</table>

500 μg/L for patients <60 years and 750 μg/L for patients ≥60 years (Figure 2). The number needed-to-test (NNT) for this strategy is calculated and compared with the standard algorithm (Table VI).

It is known that DD values increase with age; therefore, we examined the possible relation between the DD assays and the age. There is no relation between age and Innovance (P = 0.128) and Tina-quant (P = 0.063), but there is a significant relation of STA Liatest with age (P = 0.035).

Because the three assays are compared in all age subgroups and one assay shows a significant relation with age, we calculated the relation of the differences between the DD assays and age. There is no relation of the differences between the assays with age, delta (Innovance–STA Liatest) P = 0.80, delta (Innovance–Tina-quant) P = 0.63, and delta (Tina-quant–STA Liatest) P = 0.52, so we may assume that the assays behave similar in the age subgroups.

Discussion

This study demonstrates the clinical performance of three DD assays in the diagnostic workup of DVT with special interest for the influence of age. The specificity of the three DD assays declines with the age of the patients from 44.6% (STA Liatest), 53.1% (Tina-quant), and 46.1% (Innovance) in patients <60 years to 11.1% (STA Liatest), 27.6% (Tina-quant), and 6.7% (Innovance), respectively, in patients ≥80 years. After combining the results of the DD assays with a PTP score <2, the performance for all assays shows a slight improvement. The low specificity of the DD assays in the elderly is, however, disappointing for its use in daily practice.

Our study confirms previous studies with regard to the increase in false-positive DD results in the elderly with regard to VTE [16]. Diagnostic accuracy can be expressed as sensitivity and specificity or as the AUC of the ROC curve [28]. These parameters do not provide equal information. Sensitivity and specificity are calculated for a specific cut-off value, whereas the ROC curve is calculated for the whole spectrum of sensitivity and specificity. Sensitivity, specificity, and AUC of the ROC curve are dependent of the spectrum of the disease in the group, but are independent of prevalence of the disease. Therefore, reporting only one value for sensitivity and specificity is an oversimplification of accuracy [29]. The discrepancy between the low specificity and high AUC of the ROC curve in the older patients is a strong indication for using a higher cut-off value with increasing age.
TABLE V. Performance of Three D-dimer Assays for Different Ages and Cut-Off Values, Combined With a Low PTP Value (<2)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Cut-off</th>
<th>STA Lia (%)</th>
<th>Sens</th>
<th>Spec</th>
<th>NPV</th>
<th>Sens</th>
<th>Spec</th>
<th>NPV</th>
<th>Sens</th>
<th>Spec</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>500</td>
<td>97.4 (93.7)</td>
<td>43.1 (28.7)</td>
<td>98.3 (88.9)</td>
<td>100.0 (94.9)</td>
<td>51.5 (43.5)</td>
<td>100.0 (94.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>500</td>
<td>95.7 (78.0)</td>
<td>50.0 (23.5)</td>
<td>97.2 (85.2)</td>
<td>100.0 (85.6)</td>
<td>62.0 (49.7)</td>
<td>100.0 (82.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥60</td>
<td>500</td>
<td>91.3 (73.2)</td>
<td>96.3 (87.5)</td>
<td>87.5 (84.5)</td>
<td>100.0 (100.0)</td>
<td>60.6 (42.8)</td>
<td>100.0 (83.8)</td>
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</tbody>
</table>

For the selected cut-off values, the 95% lower level of confidence are given in parenthesis.

In this study, sensitivity, specificity, and NPV for the three assays in combination with a low PTP are recalculated for increasing cut-off values and subgroups of age. The specificity increases from 34.8% (STA LiaTest), 40.9% (Tina-quant), and 24.6% (Innovance) to 48.5% (STA LiaTest), 60.6% (Tina-quant), and 49.2% (Innovance), respectively, using a DD cut-off value of 750 µg/L in patients ≥60 years with a low PTP; the sensitivity and NPV remains 100% for the three assays. The improvement of the clinical performance is found in all three tests analyzed.

In the proposed strategy, the NNT values for both subgroups of patients decrease compared to the standard algorithm, which support the efficacy of this strategy.

The use of different age-dependent cut-off values for the DD assays has been proposed previously and is still a matter of debate in the diagnosis of VTE. Harper et al. suggested an age-dependent cut-off value of 500 µg/L for patients <60 years and 1000 µg/L for those aged ≥60 years, using the Vidas D-dimer assay[16]. Righini et al. encouraged the use of higher cut-off levels than 500 µg/L for patients aged more than 60 or even more than 70, because of an unacceptable rate of false-negative results [30].

Most arguments for an age-dependent DD cut-off level are based on general frailty and acquired risk factors such as cancer, hospitalization, trauma, or neurological disease [18]. Furthermore, plasma fibrinolytic potential decreases with age, resulting in elevated DD levels [31]. Several enzymes, such as plasmin, elastase, and cathepsin-G, cleave the crosslinked fibrin compound and form different fibrin degradation products (FDP) [32]. Not all DD assays are specific for the plasmin degradation products, some DD assays also measure FDP formed by activity of elastase in case of inflammation [33]. In the recently developed DD assays, monoclonal antibodies with high specificity for the plasmin degradation products are used. In our study, the new Innovance D-dimer assay was compared with the earlier developed Tina-quant and STA LiaTest DD assays with specific interest in its performance in the elderly. No relation between age and the Innovance or Tina-quant D-dimer assays could be found and in contrast to the results from Barro et al. [27], there was not an age related discordance of the used assay. To our opinion, there is no restriction or limitation in the use of these DD assays in the elderly and the age-adapted cut-off values can be used for all three assays.

The clinical performance of the new Innovance D-dimer assay is in good agreement with the results of a recently published study, comparing the new Innovance D-dimer assays with three other DD assays [34].

A limitation of our study might be the subcohort of patients. The original and the current cohort do, however, not differ in the clinical characteristics and the main outcome parameters. The remaining frozen plasma samples can be considered as a random selection.

In conclusion, the use of age-dependent cut-off values improves the clinical usefulness of the DD assay in the exclusion of DVT. A cut-off value of 750 µg/L for patients ≥60 years results in a substantial increase of the specificity of all three tested DD assays with preservation of its high sensitivity and NPV. In both subgroups of patients, the NNT values are lower than the overall group. The safety of the proposed algorithm using higher cut off values for DD assays in the elderly has to be confirmed in a prospective management study.

Patients and Methods

Patients. In this retrospective study, symptomatic outpatients suspected of having DVT are included. These patients have been participants in a multicenter management study investigating the use of the D-dimer assay in combination with the PTP score in the exclusion of DVT. In brief, patients with a PTP score <3 and a normal D-dimer concentration had no further investigations; patients with a score ≥3 and a normal D-dimer concentration underwent a single B-mode compression ultrasonography (CUS) and patients with an abnormal D-dimer concentration had repeated CUS. All patients without further investigation or normal CUS result were followed for 3 months [11].

TABLE VI. The Number Needed to Test and 95% CI for the Standard and Proposed Algorithms

<table>
<thead>
<tr>
<th>Algorithm</th>
<th>Age (years)</th>
<th>Cut-off</th>
<th>STA Lia (%)</th>
<th>Sens</th>
<th>Spec</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>All</td>
<td>500</td>
<td>3.0 (2.5–3.4)</td>
<td>2.5 (2.1–2.8)</td>
<td>3.0 (2.6–3.3)</td>
<td></td>
</tr>
<tr>
<td>Proposed</td>
<td>&lt;60</td>
<td>500</td>
<td>2.7 (2.2–3.2)</td>
<td>2.2 (1.7–2.6)</td>
<td>2.3 (1.8–2.7)</td>
<td></td>
</tr>
<tr>
<td>≥60</td>
<td>750</td>
<td>2.5 (2.0–3.1)</td>
<td>2.0 (1.6–2.4)</td>
<td>2.4 (1.9–3.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Number of false-negative DD results (n): a = 1, b = 1.
**Methods.** Spare plasma samples have been collected from all patients included in the management study and in aliquots frozen at −70 °C. The D-dimer fragment stored in this temperature is stable over time [35,36]. The clinical performance of five different assays in these patients have been published previously [5]. We present here the results of the Tita-quan [5] D-dimer assay (Roche, Mannheim, Germany) and the STA Liatest D-dimer assay (Diagnostica Stago, Asnière, France) in combination with the results of the new Innovance D-dimer assay (Dade Behring Marburg GmbH, a Siemens Company, Germany). The Tita-quan assay results are obtained during the management study with fresh plasma. The results of the STA-liatest and Innovance assays are from batch mode measurements with the stored samples. The frozen samples are thawed in a water bath of 37 °C and centrifuged. The Innovance D-dimer assay is a particle-enhanced, immunoturbidimetric assay, using polystyrene particles coated with a single monoclonal antibody (BDS) and can be measured with a different kind of analyzers [37]. In this study, the results of the Innovance D-dimer assay are obtained with the BCS analyzer (Dade Behring Marburg), according to the instructions of the manufacturer. The D-dimer values of the three assays are reported as fibrinogen equivalent unit (FEU) and a value of the DD < 500 μg/L FEU is considered to be negative. For all the assays, the performance is calculated for the cutoff values of 500, 750, and 1000 μg/L, for all patients and the patient subgroups with the age from <60 to <80 and ≥ 80 years old.

We use the dichotomized PTP score according to Wells that classifies patients into unlikely (<2) or likely (>2) for having DVT [14].

**Statistics.** All calculations are performed on the same set of samples for the three assays and nearly all calculations are performed with SPSS 15 (SPSS, Chicago, IL). The correlation between DD results and age is calculated with the exact binominal method. All series of data are calculated for the cut-off values of 500, 750, and 1000 μg/L, for all patients and the patient subgroups with the age from <60 to <80 and ≥ 80 years old.

The level of agreement of the three DD assays is calculated by the level of agreement, if the agreement is moderate to good, the kappa is 0.40 to 0.75, if the agreement is moderate to poor, the kappa value will be between 0.40 and 0.75. The ROC curve is used to calculate the AUC as a measure of the diagnostic accuracy. The AUC of the ROC curve is a suitable parameter to evaluate the diagnostic accuracy. The AUC of the ROC curve is a suitable parameter to evaluate the diagnostic accuracy. The AUC of the ROC curve is a suitable parameter to evaluate the diagnostic accuracy.

**References**


