Performance of C-Reactive Protein, Procalcitonin, TAT Complex, and Factor VIII in Addition to D-Dimer in the Exclusion of Venous Thromboembolism in Primary Care Patients

Jorn S. Heerink [a,d*] **Eugenie Gemen,** [a] **Ruud Oudega,** [a,b] **Geert-Jan Geersing,** [b] **Rogier Hopstaken,** [c] **and Ron Kusters** [a,d]

Background: In primary care, D-dimer—combined with a clinical assessment—is recommended for ruling-out venous thromboembolism (VTE). However, D-dimer testing frequently yields false-positive results, notably in the elderly, and the search for novel biomarkers thus continues. We assessed the added diagnostic value of 4 promising laboratory tests.

Methods: Plasma samples from 256 primary care patients suspected of VTE were collected. We explored added value (beyond D-dimer) of C-reactive protein (CRP), procalcitonin (PCT), thrombin–antithrombin III complex (TAT-c), and factor VIII (FVIII). Diagnostic performance of these biomarkers was assessed univariably and by estimating their area under the receiver operating curve (AUC). Added diagnostic potential beyond D-dimer testing was assessed using multivariable logistic regression.

Results: Plasma samples of 237 VTE-suspected patients were available for analysis—36 patients (25%) confirmed deep vein thrombosis, 11 patients (12%) pulmonary embolism. Apart from D-dimer, only CRP, and FVIII levels appeared to be higher in patients with VTE compared to patients without VTE. The AUCs for these 3 markers were 0.76 (95% CI: 0.69–0.84) and 0.75 (95% CI: 0.68–0.83), respectively, whereas the AUC for D-dimer was 0.90 (95% CI: 0.86–0.94). Combining these biomarkers in a multivariable logistic model with D-dimer did not improve these AUCs meaningfully.

Conclusions: In our dataset, we were unable to demonstrate any added diagnostic performance beyond D-dimer testing of novel biomarkers in patients suspected of VTE in primary care. As such, D-dimer testing appears to remain the best choice in the exclusion of clinically suspected VTE in this setting.

Trial Registration: Netherlands Trial Register NL5974. (METC protocol number: 16-356/M; NL56475.041.16.)

[a]Department of Clinical Chemistry and Haematology, Jeroen Bosch Hospital, 's-Hertogenbosch, the Netherlands; [b]Julius Centre for Health Sciences and General Practice, University Medical Centre Utrecht, the Netherlands; [c]Star-shl Diagnostic Centres, Etten-Leur, the Netherlands; [d]Department of Health Technology and Services Research, Technical Medical Centre, University of Twente, Enschede, the Netherlands .

*Address correspondence to this author at: Department of Clinical Chemistry and Haematology, Jeroen Bosch Hospital, Henri Dunantstraat 1, 5223 GZ, 's-Hertogenbosch, the Netherlands. E-mail: j.heerink@jbz.nl.

Received April 2, 2021; accepted June 28, 2021.

DOI: 10.1093/jalm/jfab094

IMPACT STATEMENT

In the current study we assessed added value of 4 other promising laboratory tests in addition to D-dimer, prior to imaging. We noticed that general practitioners are in need for more scientific research with regard to a more reliable (fast) exclusion of VTE based on data from their specific (primary care) patient population. By assessing added value of several biomarkers, we will find out if a more reliable laboratory-based VTE exclusion can be reached, thus contributing to advancement of knowledge in the field of VTE.

INTRODUCTION

Deep vein thrombosis (DVT) and pulmonary embolism (PE) are 2 manifestations of venous thromboembolism (VTE), and a major cause of cardiovascular death (1). Because symptoms mimic and overlap with many common illnesses, discriminating VTE from a more innocent condition based on clinical evaluation alone is difficult (2). Moreover, recurrence rates in unprovoked VTE are about 30% in 10 years, and remnants of earlier thrombosis further complicate a correct diagnosis (3). Nevertheless, when a low D-dimer value (a fibrin degradation product) is accompanied by a low score on clinical decision rules (CDRs), it is possible to safely exclude VTE (4, 5).

A high D-dimer, however, does not necessarily confirm the presence of VTE as there is a well-known risk of yielding false-positive D-dimer results. In fact, in patients suffering from cancer but also in pregnant women, and older people (6, 7), a higher rate of false-positive D-dimer values is seen; this reduces the clinical utility of D-dimer testing to exclude VTE (8). The use of an age-dependent D-dimer increase in cutoff values has been suggested to reduce false-positives while maintaining an equivalent sensitivity and false negative rate (9, 10). However, this approach has other limitations such as the lack of standardized reporting (11, 12). In primary care, at best, half of all suspected patients can be ruled out after an adequate application of CDRs and D-dimer testing (4). The remaining suspected patients, notably due to positive D-dimer testing, are subject to imaging techniques such as ultrasonography (US) or computed tomography (CT) scans to further investigate presence of a VTE. An effective ruling-out strategy in primary care will reduce the need for US and CT scans. The latter may reduce exposure to radiation and contrast dye exposure, and also reduce healthcare costs.

It has been proposed to add different biomarkers to D-dimer for a more effective use of the diagnostic algorithm. For example, inflammation markers may be useful in discriminating between thrombosis and diseases such as pneumonia or erysipelas (13). Meanwhile, coagulation factors such as thrombin- or fibrin-related markers may provide more information on the condition of the thrombotic process than the degradation products of the thrombus alone (14). One of these markers is the so-called thrombin–antithrombin III or TAT complex (TAT-c), which is generated due to thrombin inhibition by antithrombin, thereby reflecting the functional state of the coagulation system (15). At the initial phase of the coagulation cascade, persistently elevated levels of factor VIII (FVIII) activity, a cofactor for factor IXa, proved to be an independent risk factor for VTE and recurrent thrombosis in particular (16).

In the current study, we set out to prospectively evaluate the contribution of measuring the

inflammation biomarkers C-reactive protein (CRP) and procalcitonin (PCT) as well as the coagulation factors TAT-c and FVIII in addition to D-dimer for VTE diagnosis in a population of VTE-suspected primary care patients, prior to imaging and initiation of anticoagulant therapy.

MATERIALS AND METHODS

Study Design

We used prospectively collected data from the EVA study (Evaluation of biomarkers in VTE) on patients with suspected DVT and/or PE visiting their general practitioner (GP) in the Netherlands (17). The study was performed under the tenets of the Helsinki declaration and local laws and regulations; the study protocol of the EVA study has been registered in the Netherlands Trial Register (NL5974) after approval by the Medical Ethics Committee of the University Medical Centre Utrecht, the Netherlands. All patients provided written informed consent prior to study participation.

Study Population

In short, patients with suspected DVT or PE were first assessed by their GP for risk estimation (low or high) using a CDR based upon the guideline of the Dutch College of GPs (18). Those with a high CDR score were referred to the hospital for imaging. Exclusion criteria were (*a*) age below 18; (*b*) ongoing anticoagulant treatment (vitamin K antagonists, nonvitamin K oral anticoagulants, and/or low molecular-weight heparin) for other causes than VTE; or (*c*) a life expectancy less than 3 months. The inclusion period was September 26, 2016 - January 10, 2019. Patients with a low CDR score [for DVT, we used the Oudega rule (≤3), for PE, we used the Wells rule (≤4)] received a venipuncture from the anterior cubital vein (4, 19). A D-dimer test was performed in a laboratory facility for primary care prior to the initiation of any anticoagulant treatment, and patients were referred in case of a positive test result.

Patient Samples

In addition, an extra blood sample (4 mL) was collected in a lithium-heparin tube (LH PST^{TM} II), 2 citrate tubes (9NC 0.105 M buffer, Na_3 citrate), all from Becton Dickinson, NJ, USA. After incubation, these additional tubes were centrifuged and plasma was aliquoted (centrifugation settings: 2000 *g*; 10 minutes; 21 °C). Aliquots were stored until analysis at -70 °C.

Measurements of Biomarkers

After thawing, sample processing was performed in the Jeroen Bosch Hospital, where D-dimer, CRP, PCT, and FVIII levels were measured. Also, TAT-c levels were measured in the University Medical Centre of Utrecht. All measurements were done in citrate plasma, except for CRP and PCT, which were performed in lithium-heparin plasma.

Immuno-turbidimetric assays based on a latex-coated antibody–antigen (=analyte) reaction were used for D-dimer (STA-Liatest®D-Di PLUS, Stago Diagnostica) and CRP testing (ADVIA®Chemistry, Siemens Healthcare), and a two-site chemiluminometric (sandwich) magnetic immunoassay was used for PCT testing (ADVIA®Centaur, Siemens). A chromogenic assay using factor-deficient plasma and an ELISA were used for FVIII (TriniCHROM®FVIII:C, Stago) and TAT-c measurements (Affinity Biologicals), respectively.

Technicians who performed the measurements were blinded to the patients' earlier diagnostic test results.

Clinical Outcome

In accordance with earlier studies in the field of diagnosing VTE in primary care, GPs were asked whether a DVT or PE was diagnosed during a period of 3 months after the initial risk assessment

by the GP and subsequent D-dimer testing. In this way, also patients that were initially not referred for imaging based on their D-dimer result were monitored.

Data Analysis

Diagnostic variables. Patient characteristics and results of biomarker measurements were analyzed by descriptive statistical analysis and stratified for the presence of VTE. Significance of any difference between these diagnostic variables and patients with or without a final VTE, was analyzed using chi square or univariate regression analysis for dichotomous and numeric variables, respectively. Differences are presented as *P* values and odds ratios (ORs) with their respective 95% CIs. *P* values below 0.05 were considered statistically significant.

Additional biomarker analysis. ROC curves were generated from biomarker results demonstrating a statistically significant difference between the VTE and non-VTE population. Their corresponding AUCs (areas under the curve) were calculated. Also, multivariate (binary logistic) regression analyses were performed to test the contribution of measuring combinations of D-dimer and biomarkers for the presence of VTE. Hereto, the additional biomarkers under evaluation in this study were iteratively added to a multivariable logistic model and their added discriminative information was evaluated in ROC space.

Sample size calculation. As this was an exploratory analysis of novel biomarkers, we did not perform a sample size calculation a priori.

Statistical analyses were performed using the Statistical Package for the Social Sciences Software® (PASW v.22, IBM, Somers, NY, USA). ROC curves were generated using EP Evaluator® (v.11D, Data Innovations, South Burlington, VT, USA).

RESULTS

A total of 256 consecutive patients with clinically suspected VTE and a low CDR score were screened, of whom 14 patients were excluded (13 patients: withdrawal of informed consent; one patient died soon after his first GP visit). Blood samples from 5 of the 242 included patients contained insufficient blood for processing, so samples from 237 patients (144 DVT suspicions and 93 PE suspicions) were used in the current study (Fig. 1).

Diagnostic Variables

Patient demographic, clinical, and biomarker characteristics and the presence of VTE are listed in Table 1. VTE was finally diagnosed in 47 patients (20%); 36 and 11 patients (25% and 12%) suffered from a DVT and PE, respectively. Mean age of the patients was 55.5 ± 16.1 and 87 (36.7%) were men. Median D-dimer level was 490 [250, 1275] ng/mL. The inflammation markers CRP and PCT demonstrated median levels of 5 [1, 15] mg/L and 0.01 [0.009, 0.03] ng/mL, respectively. The coagulation factors TAT-c and FVIII demonstrated median levels of 35 [23, 71] ng/mL and 176 [124, 248]%, respectively.

D-dimer, CRP, and FVIII levels in patients who developed a VTE were significantly higher than in patients without VTE. A total of 87% of patients suffering from an inflammatory condition were patients without a recorded (concurrent) VTE diagnosis. In Table 2, an overview of prevalence of all known inflammatory conditions in the study population is presented.

Analyses of Additional Biomarkers

Since D-dimer, CRP, and FVIII levels showed a statistically significant difference between VTE and non-VTE populations, the predictive value of these biomarkers was examined by plotting ROC curves

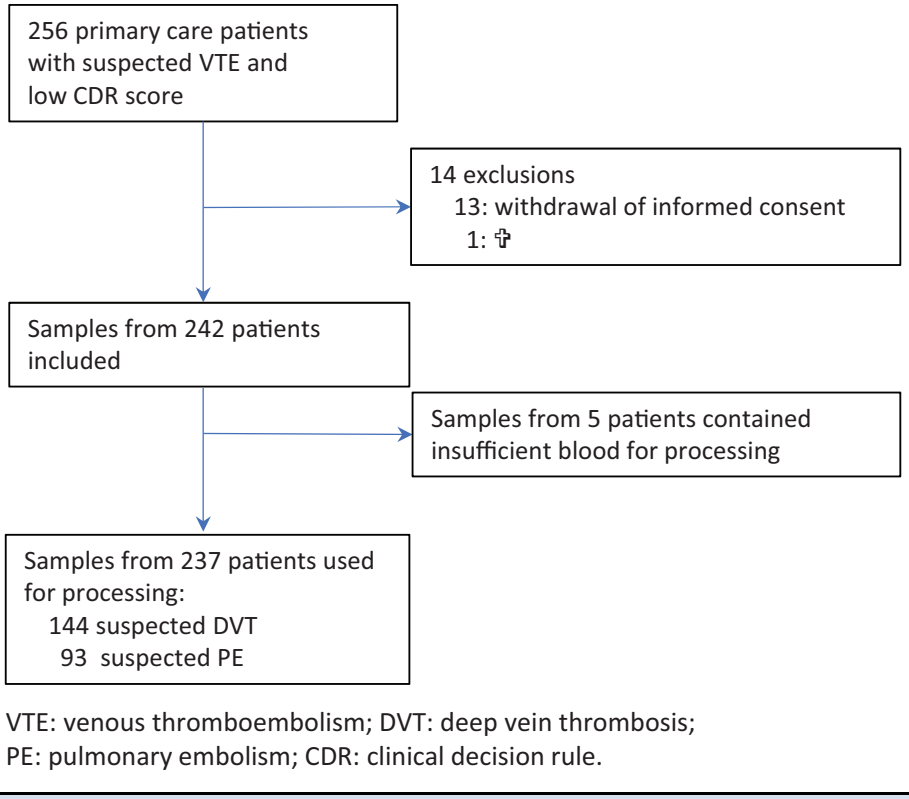


Fig. 1. Flow diagram of study design. VTE: venous thromboembolism; DVT: deep vein thrombosis; PE: pulmonary embolism; CDR: clinical decision rule.

for the presence of VTE (Fig. 2). The AUC of these individual biomarkers with their corresponding CIs were 0.90 (0.86–0.94), 0.76 (0.69–0.84), and 0.75 (0.68–0.83), respectively.

The addition of CRP to D-dimer and FVIII to D-dimer in a two-biomarker prediction model for VTE based on multivariable regression was not significant for CRP and FVIII in contrast to D-dimer itself. The ROCs of these combinations of biomarkers are presented in Fig. 3, together with the ROC of the separate D-dimer measurements. All AUCs are comparable to the AUC of individual D-dimer measurements (0.90). This suggests that no combination of biomarkers is more discriminating than D-dimer measurement alone.

DISCUSSION

In the current study, our analyses suggest that a combination of D-dimer and other biomarkers (CRP, PCT, TAT-c, and/or FVIII) do not significantly improve discriminatory power of D-dimer in excluding a VTE in a population of primary care patients. By itself, this is not unexpected as the AUC for diagnosing VTE with D-dimer already is 0.90 (95% CI: 0.86–0.94), which implies already excellent discriminative power. This good diagnostic performance of D-dimer is well documented for VTE and is hereby confirmed in our study population. Although other biomarkers were discriminative for VTE detection -notably FVIII measurements- adding one of the other parameters

Table 1. Association between each diagnostic predictor and the presence or absence of VTE ($n = 237$).

Diagnostic variable	VTE ($n = 47$)		No VTE ($n = 190$)		*P* value	Odds ratio	95% CI
Age in years: mean, (±SD)	60.3	(12.9)	54.3	(16.6)	0.02	1.025	1.003–1.047
Sex					0.35	1.36	0.71–2.61
Male	20	42.6%	67	35.3%			
Female	27	57.4%	123	64.7%			
DVT suspicion	36	25.0%	108	75.0%			
Oudega CDR score: median [IQR]	2.5	[2–4]	2	[1–3]	0.01	1.57	1.106–2.223
Presence of known inflammatory condition[L1]	4	0.11%	15	10.4%	0.67	0.78	0.240–2.507
PE suspicion	11	11.8%	82	88.2%			
Wells CDR score: median [IQR]	1.5	[1–3]	1	[0–3]	0.14	1.41	0.895–2.221
Presence of known inflammatory condition[L1]	0	0.0%	11	13.4%	NA[1]	NA[1]	NA[1]
Biomarkers: median [IQR]							
D-dimer (ng/mL)	2371	[1320–3680]	355	[210–750]	<0.01	1.001	1.001–1.002
						36.88[2]	8.681–156.6
C-reactive protein (mg/L)	16	[7–44]	3	[1–10]	<0.01	1.013	1.004–1.022
						6.638[2]	2.935–15.01
Procalcitonin (ng/mL)	0.01	[0.009–0.04]	0.01	[0.009–0.03]	0.91	0.772	0.011–56.23
						1.543[2]	0.786–3.030
TAT complex (ng/mL)	45	[32–76]	34	[22–70]	0.78	1.000	1.000–1.000
						2.025[2]	1.047–3.919
Factor VIII (%)	248	[174–290]	158	[117–238]	<0.01	1.011	1.016–1.015
						3.632[2]	1.766–7.471

DVT: deep vein thrombosis; PE: pulmonary embolism; CI: confidence interval; IQR: interquartile range; NA: not applicable.
L1: Conditions recorded: erysipelas, arthritis, thrombophlebitis, cellulitis, tendinitis (suspected DVT) or upper airway infection, (pleuro)pneumonia, bronchitis (suspected PE).
[1] Cannot be calculated due to the absence of inflammatory cases in the PE population.
[2] After transforming biomarkers in dichotomous variables using median values.

did not meaningfully improve discriminatory ability to distinguish VTE from non-VTE patients. In the paragraphs that follow, we will outline the most relevant considerations per biomarker. After that, we will conclude with a general discussion section.

C-reactive Protein

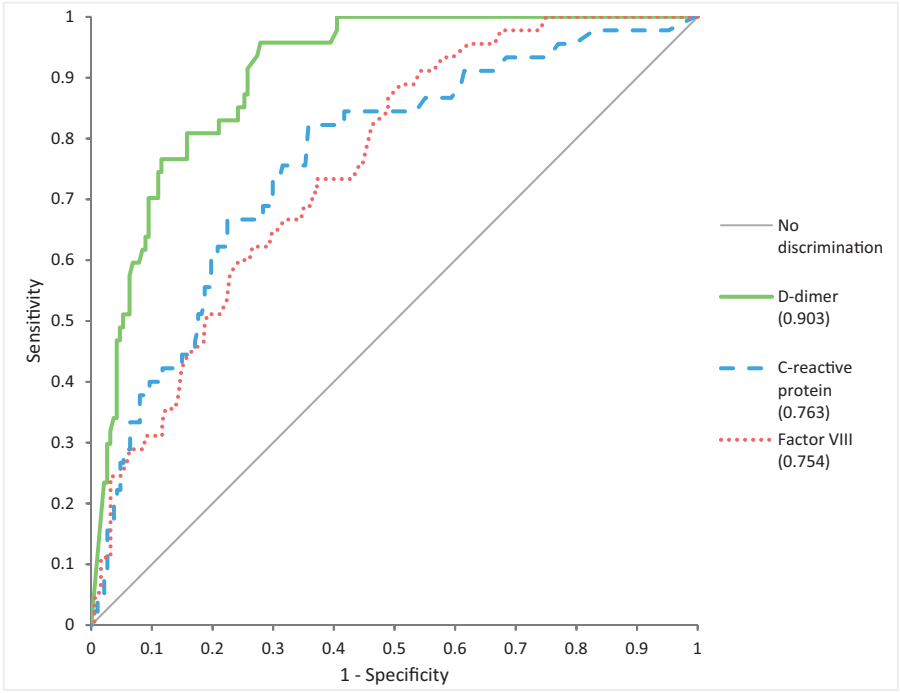
The discriminatory value in the assessment of VTE of the inflammation marker CRP as such has been investigated earlier and performance results do not outperform or add value to D-dimer (20, 21). These results are in accordance with recent studies showing that CRP does not add much to D-dimer in VTE assessment (22, 23), which we confirmed in a primary care population. Low CRP values were found in both VTE and non-VTE populations. What we consider to be the most likely explanation for this low CRP values, is the fact that all included patients visited a GP and frequently suffer from mild complaints, some of

Table 2. Prevalence of known inflammatory conditions in the study population ($n = 237$).

Diagnostic variable	Condition	Number of patients
DVT suspicion	erysipelas	8
	arthritis	2
	thrombophlebitis	5
	cellulitis	2
	tendinitis	2
PE suspicion	upper airway infection	9
	(pleuro)pneumonia	1
	bronchitis	1

DVT: deep vein thrombosis; PE: pulmonary embolism.

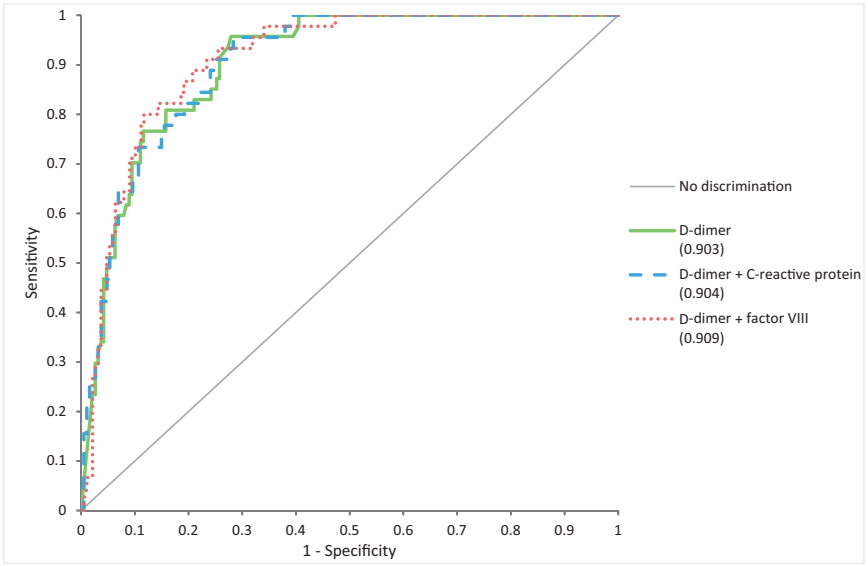
(mild) inflammatory and some of other origin. At the same time, a clot can also be accompanied with an inflammatory process, although the low CRP values suggest that in our population this inflammatory component is often being overshadowed by the thrombotic component. These two phenomena could provide an explanation for the finding that CRP is not contributory to D-dimer in our study. As a consequence, it is questionable whether simultaneous D-dimer and CRP testing in all VTE-suspected primary care patients is useful. After all, an elevation of both D-dimer and CRP levels may falsely suggest that D-dimer is increased due to an inflammation, which is, as we



	AUC	95% CI	SE
D-dimer	0.903	0.863 to 0.943	0.0204
C-reactive protein	0.761	0.685 to 0.837	0.0388
Factor VIII	0.754	0.682 to 0.825	0.0364

AUC: Area Under the Curve; CI: confidence interval; SE: Standard Error.

Fig. 2. Receiver operating characteristic curves of predicting a venous thromboembolism for individual biomarker measurements (D-dimer, C-reactive protein, factor VIII). AUC: area under the curve.



	AUC	95% CI	SE
D-dimer	0.903	0.863 to 0.943	0.0204
D-dimer + C-reactive protein	0.904	0.864 to 0.945	0.0205
D-dimer + factor VIII	0.909	0.869 to 0.949	0.0203

AUC: Area Under the Curve; CI: confidence interval; SE: Standard Error.

Fig. 3. Receiver operating characteristic curves of predicting a venous thromboembolism for D-dimer and combined biomarker measurements using multivariate regression. AUC: area under the curve.

demonstrated, often not the case. In line with this is a study on PE-suspected patients presented to the emergency department, which demonstrated that a tandem measurement of a positive D-dimer and CRP does not reduce the need for imaging (24). Recently, in a study on primary care patients with suspected PE, an alternative use of CRP was proposed. The authors suggested that, once a PE has been excluded based upon the D-dimer value, CRP (cutoff 10 mg/L) may contribute in further excluding clinically relevant object disease (CROD) (13). However, not all CRODs could be excluded in this way. So, also in patients with suspected PE, we would argue for much restraint with respect to ordering and interpreting a CRP test in addition to a D-dimer test, and would, if ordered, recommend only to interpret the test within the context of a negative D-dimer.

Procalcitonin

The diagnostic value of the inflammation marker PCT has been demonstrated for the differentiation of PE versus pneumonia and DVT versus erysipelas (25, 26). However, no studies were carried out in which combined D-dimer and PCT tests were used for the purpose of a pure VTE vs non-VTE discrimination, as done in the current study. In fact, in our primary care study population, both VTE and non-VTE patients had low median PCT values, yielding no discriminatory effect of additional PCT testing. In patients with suspected DVT, this may be explained by the phenomenon that usually patients suffering from fulminant inflammatory conditions can be clearly distinguished from those with a minor thrombosis and are therefore not eligible for D-dimer testing.

TAT Complex

Factors in the clot-forming part of the coagulation system such as thrombin- or fibrin-related markers play a central role in thrombus formation: a complex process that also interacts with other (patho)physiological processes such as inflammation and immunity (27). Consequently, it has been suggested that coagulation factors such as TAT-c could be useful for the diagnostic work-up of thrombosis (14). TAT-c levels have indeed been shown to be higher in patients with DVT and PE, and might be used in the exclusion of either of these diagnoses (28). However, due to the short half-life of TAT-c, TAT-c levels increase rapidly and diminish relatively soon after the onset of VTE. This could explain the results from a study from Bozic et al., which demonstrated that TAT-c measurements could not substitute or supplement D-dimer testing in diagnostic work-up of DVT, neither in patients with low nor in those with high pretest probability (29). Our observations are in line with those results and confirm that TAT-c appears to be not contributory to D-dimer testing in patients with suspected VTE in primary care.

Factor VIII

Although numerous studies have been clearly demonstrated that high FVIII levels constitute a prevalent, independent, dose-dependent risk factor for DVT and PE, we are not aware of any studies that combine D-dimer and FVIII testing in VTE assessment (16). We can confirm that differences were seen between VTE and non-VTE patients, but demonstrated that FVIII is not contributory to D-dimer.

General Discussion

Nowadays, in primary care, access to instant measurement of (multiple) biomarkers, including D-dimer, is increasingly being improved because of the availability of multianalyte (capillary) point-of-care devices (17, 30). Although studies on other biomarkers than D-dimer have already been performed in hospital settings, i.e., in the outpatient or emergency room department, our study focuses on a primary care population which is distinctive in prevalence, case-mix, and physician experience in VTE (31).

While many previous hospital studies are (retrospective) case control studies, we decreased the risk of selection bias by a prospective study design, and the inclusion of only low-risk VTE-suspected primary care patients. Besides, in contrast to various other studies we chose to perform the blood drawings and measurements directly following the GP's consultation. In this way, by performing the study before further examinations such as ultrasound or CT scanning, a potential diagnostic-based selection bias was avoided and we were assured that any anticoagulant treatment had not been initiated at that point, thus avoiding potential measurement interferences.

Undoubtedly, performing a prospective study on a low prevalent disease, such as VTE in primary care, entails that numbers of patients with VTE in this study were limited. This limits us to draw firm conclusions on DVT and PE as separate conditions. Prochaska et al. demonstrated an age-dependent diagnostic performance of D-dimer and CRP for DVT (21). Our study lacks statistical power to investigate this approach through an age-dependent subgroup analysis.

Since this study once again confirms the strength of dedicated use of D-dimer testing in primary care, our results do not give rise to measurement of the investigated biomarkers in the GP's office for supplementing D-dimer testing in diagnostic work-up of VTE.

If no other candidate biomarkers become available, further improvement might be expected from refinement of the interpretation of the D-dimer measurement itself. Differentiated cutoffs -such as age-dependent or clinical pretest probability adjusted interpretations of D-dimer- are expected to make a substantial contribution in this regard (12, 32).

In addition, since neural networks have recently been proved to exclude DVT without the need for ultrasound (33), cutoff values might be further differentiated by such networks using a set of readily available clinical parameters. Instead, using D-dimer on a continuous scale rather than using various cutoffs might also improve the utility of D-dimer testing as it allows to incorporate the diagnostic value of D-dimer over the full range of measurements (34); a machine learning-based probability model including both clinical input parameters and the exact D-dimer value might be introduced to assess the likelihood of safely ruling-out a VTE.

Finally, we would like to point out that standardization of D-dimer unit-reporting and large-scale implementation of harmonization of D-dimer testing is a key issue to this day (8, 12, 35, 36). Doing so will in itself contribute to a higher reliability of D-dimer testing which will, in turn, reduce the need for using (unnecessary) imaging techniques.

Conclusion

In conclusion, we were not able to demonstrate that a D-dimer test was outperformed by the addition of other biomarkers and therefore appears to remain the best choice in the exclusion of clinically suspected VTE in primary care.

Ethical Approval

The medical ethics committee of University Medical Centre of Utrecht approved this study (METC protocol number: 16-356/M; NL56475.041.16).

Nonstandard Abbreviations: VTE, venous thromboembolism; CRP, C-reactive protein; PCT, procalcitonin; TAT complex, thrombin–antithrombin III complex; FVIII, factor VIII; AUC, area under the (receiver operating) curve; DVT, deep vein thrombosis; PE, pulmonary embolism; CDR, clinical decision rule; US, ultrasonography; CT, computed tomography; GP, general practitioner; OR, odds ratio.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

R. Oudega and R. Kusters conceived the study and were involved in protocol development, gaining ethical approval, and patient recruitment. E. Gemen did the processing of the samples. J.S. Heerink was involved in data analysis and wrote the first draft of the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:* **Employment or Leadership:** None declared. **Consultant or Advisory Role**: None declared. **Stock Ownership**: None declared. **Honoraria**: None declared. **Research Funding**: This research was supported by the Jeroen Bosch Hospital, the Julius Centre for Health Sciences and General Practice, and Nano-Ditech Corporation. **Expert Testimony**: None declared. **Patents**: None declared.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, preparation of manuscript, or final approval of manuscript.

Acknowledgments: We would like to thank Mirrian Hilbink (epidemiologist) for her support on statistics, and Afzal Kariman and Rolf Urbanus for their support on test measurements.

REFERENCES

1. Naess IA, Christiansen SC, Romundstad P, Cannegieter SC, Rosendaal FR, Hammerstrom J. Incidence and mortality of venous thrombosis: a population-based study. J Thromb Haemost 2007;5:692–9.
2. Oudega R, Moons KG, Hoes AW. Limited value of patient history and physical examination in diagnosing deep vein thrombosis in primary care. Fam Pract 2005;22:86–91.
3. Geersing GJ, Hendriksen JMT, Zuithoff NPA, Roes KC,

Oudega R, Takada T, et al. Effect of tailoring anticoagulant treatment duration by applying a recurrence risk prediction model in patients with venous thromboembolism compared to usual care: a randomized controlled trial. PLoS Med 2020;17: e1003142.
4. Oudega R, Moons KG, Hoes AW. Ruling out deep venous thrombosis in primary care. A simple diagnostic algorithm including D-dimer testing. Thromb Haemost 2005;94:200–5.
5. van Belle A, Buller HR, Huisman MV, Huisman PM, Kaasjager K, Kamphuisen PW, et al.; Christopher Study Investigators. Effectiveness of managing suspected pulmonary embolism using an algorithm combining clinical probability, D-dimer testing, and computed tomography. JAMA 2006;295:172–9.
6. Schouten HJ, Geersing GJ, Oudega R, van Delden JJ, Moons KG, Koek HL. Accuracy of the Wells clinical prediction rule for pulmonary embolism in older ambulatory adults. J Am Geriatr Soc 2014;62:2136–41.
7. Schouten HJ, Koek HL, Oudega R, van Delden JJ, Moons KG, Geersing GJ. Validation of the Oudega diagnostic decision rule for diagnosing deep vein thrombosis in frail older out-of-hospital patients. Fam Pract 2015;32:120–5.
8. Favresse J, Lippi G, Roy PM, Chatelain B, Jacqmin H, Ten Cate H, Mullier F. D-dimer: preanalytical, analytical, postanalytical variables, and clinical applications. Crit Rev Clin Lab Sci 2018;55:548–77.
9. Douma RA, Le Gal G, Sohne M, Righini M, Kamphuisen PW, Perrier A, et al. Potential of an age adjusted D-dimer cut-off value to improve the exclusion of pulmonary embolism in older patients: a retrospective analysis of three large cohorts. Bmj 2010;340:c1475.
10. Schouten HJ, Koek HL, Oudega R, Geersing GJ, Janssen KJ, van Delden JJ, Moons KG. Validation of two age dependent D-dimer cut-off values for exclusion of deep vein thrombosis in suspected elderly patients in primary care: retrospective, cross sectional, diagnostic analysis. BMJ 2012;344:e2985.
11. Goodwin AJ, Higgins RA, Moser KA, Smock KJ, Chandler WL, Kottke-Marchant K, et al. Issues surrounding age-adjusted D-dimer cutoffs that practicing physicians need to know when evaluating patients with suspected pulmonary embolism. Ann Intern Med 2017;166:361–3.
12. Solberg R, Glass G. Adjusting D-dimer cutoffs: brief literature summary and issues in clinical use. Am J Emerg Med 2018;36:2105–7. 2018.
13. Lucassen WA, Kuijs-Augustijn M, Erkens PM, Geersing GJ, Buller HR, van Weert HC. The additional value of the CRP test in patients in whom the primary care physician excluded pulmonary embolism. Eur J Gen Pract 2013;19: 143–9.
14. Wada H, Sakuragawa N. Are fibrin-related markers useful for the diagnosis of thrombosis? Semin Thromb Hemost 2008;34:33–8.
15. Lee SY, Niikura T, Iwakura T, Sakai Y, Kuroda R, Kurosaka M. Thrombin-antithrombin III complex tests. J Orthop Surg 2017;25:170840616684501.
16. Jenkins PV, Rawley O, Smith OP, O'Donnell JS. Elevated factor VIII levels and risk of venous thrombosis. Br J Haematol 2012;157:653–63.
17. Heerink JS, Gemen E, Oudega R, Hopstaken R, Geersing GJ, Kusters R. Analytical performance and user-friendliness of five novel point-of-care D-dimer assays. Scand J Clin Lab Invest 2020;27:1–8.
18. NHG-werkgroep Diepe veneuze trombose en longembolie. NHG-Standaard Diepe veneuze trombose en longembolie (tweede partiële herziening). Huisarts Wet 2017;60:460.
19. Geersing GJ, Erkens PM, Lucassen WA, Buller HR, Cate HT, Hoes AW, et al. Safe exclusion of pulmonary embolism using the Wells rule and qualitative D-dimer testing in primary care: prospective cohort study. BMJ 2012;345:e6564.
20. Aujesky D, Hayoz D, Yersin B, Perrier A, Barghouth G, Schnyder P, et al. Exclusion of pulmonary embolism using C-reactive protein and D-dimer. A prospective comparison. Thromb Haemost 2003;90:1198–203.
21. Prochaska JH, Frank B, Nagler M, Lamparter H, Weißer G, Schulz A, et al. Age-related diagnostic value of D-dimer testing and the role of inflammation in patients with suspected deep vein thrombosis. Sci Rep 2017;7:4591.
22. Crop MJ, Siemes C, Berendes P, van der Straaten F, Willemsen S, Levin MD. Influence of C-reactive protein levels and age on the value of D-dimer in diagnosing pulmonary embolism. Eur J Haematol 2014;92:147–55.
23. Paparoupa M, Spineli L, Framke T, Ho H, Schuppert F, Gillissen A. Pulmonary embolism in pneumonia: still a diagnostic challenge? Results of a case-control study in 100 patients. Dis Markers 2016;2016:8682506.
24. Mitchell AM, Nordenholz KE, Kline JA. Tandem measurement of D-dimer and myeloperoxidase or C-reactive protein to effectively screen for pulmonary embolism in the emergency department. Acad Emerg Med 2008;15:800–5.
25. Kokturk N, Kanbay A, Bukan N, Ekim N. The value of serum procalcitonin in differential diagnosis of pulmonary embolism and community-acquired pneumonia. Clin Appl Thromb Hemost 2011;17:519–25.
26. Rast AC, Knobel D, Faessler L, Kutz A, Felder S, Laukemann S, et al. Use of procalcitonin, C-reactive protein and white blood cell count to distinguish between lower limb erysipelas and deep vein thrombosis in the emergency department: a prospective observational study. J Dermatol 2015;42:778–85.
27. Branchford BR, Carpenter SL. The role of inflammation in venous thromboembolism. Front Pediatr 2018;6:142.
28. LaCapra S, Arkel YS, Ku DH, Gibson D, Lake C, Lam X. The use of thrombus precursor protein, D-dimer, prothrombin fragment 1.2, and thrombin antithrombin in the exclusion of proximal deep vein thrombosis and pulmonary embolism. Blood Coagul Fibrinolysis 2000;11: 371–7.
29. Bozic M, Blinc A, Stegnar M. D-dimer, other markers of haemostasis activation and soluble adhesion molecules in patients with different clinical probabilities of deep vein thrombosis. Thromb Res 2002;108:107–14.

30. Tang R, Yang H, Choi JR, Gong Y, You M, Wen T, et al. Capillary blood for point-of-care testing. Crit Rev Clin Lab Sci 2017;54:294–308.
31. van Maanen R, Rutten FH, Klok FA, Huisman MV, Blom JW, Moons KGM, Geersing GJ. Validation and impact of a simplified clinical decision rule for diagnosing pulmonary embolism in primary care: design of the PECAN prospective diagnostic cohort management study. BMJ Open 2019;9:e031639.
32. Weitz JI, Fredenburgh JC, Eikelboom JW. A test in context: D-dimer. J Am Coll Cardiol 2017;70:2411–20.
33. Willan J, Katz H, Keeling D. The use of artificial neural network analysis can improve the risk-stratification of patients presenting with suspected deep vein thrombosis. Br J Haematol 2019;185:289–96.
34. Geersing GJ, Kraaijpoel N, Buller HR, van Doorn S, van Es N, Le Gal G, et al. Ruling out pulmonary embolism across different subgroups of patients and healthcare settings: protocol for a systematic review and individual patient data meta-analysis (IPDMA). Diagn Progn Res 2018;2:10.
35. Linkins LA, Lapner TS. Review of D-dimer testing: good, bad, and ugly. Int J Lab Hematol 2017;1: 98–103.
36. Mullier F, Vanpee D, Jamart J, Dubuc E, Bailly N, Douxfils J, et al. Comparison of five D-dimer reagents and application of an age-adjusted cut-off for the diagnosis of venous thromboembolism in emergency department. Blood Coagul Fibrinolysis 2014;25:309–15.