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Diagnostic biomarkers in venous thromboembolic disease

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Objective: **Venous thromboembolism (VTE) is a common disease with serious, often fatal sequelae. The optimal strategy for diagnosis of VTE remains unclear, although considerable progress has been made in this area. Several new biomarkers have showed promise for diagnosis of VTE, and more are under active investigation. We reviewed the literature for studies evaluating these diagnostic biomarkers.**
Methods: **We reviewed the English literature between 1990 and 2015, searching for papers evaluating diagnostic performance of biomarkers in VTE.**
Results: **D-dimer, a fibrin degradation product, has been thoroughly investigated, and performs well in select populations, although it is best as a rule-out test because of its generally low specificity. Soluble P-selectin, a marker of endothelial activation, has shown good diagnostic performance in several studies but has not yet been adopted widely. Others, including cellular adhesion molecules, tissue factor, circulating microparticles, and C-reactive protein, are under investigation, with varying results in a few studies.**
Conclusions: **At this time, D-dimer and P-selectin remain the most clinically valuable. New biomarkers are needed for clinical use in VTE. (J Vasc Surg: Venous and Lym Dis 2016;■:1-10.)**

Venous thromboembolism (VTE) is a common disease with an annual incidence of approximately one in 1000 in the United States.[1,2] VTE is associated with common conditions: increasing age,[2] cancer,[3] major surgery,[4] and critical illness.[5] Thus, diagnosis and treatment must be able to be expeditiously performed by nearly every medical practitioner. Accurate diagnosis is of critical importance, because VTE can often be fatal, and unnecessary use of anticoagulation carries a real risk of hemorrhagic complications. There has been a broad move toward limiting the invasiveness and expense of diagnostic tests, and several new biomarkers hold promise as effective diagnostic tools. D-dimer remains the most common clinically used biomarker, and several others are under active investigation for clinical use, including soluble P-selectin (sP-sel).

LITERATURE REVIEW

We reviewed the English language literature between 1990 and 2015. Search terms included: biomarker, D-dimer, prothrombin fragment, factor 8, soluble P-selectin, C-reactive protein, thrombin generation potential, risk assessment model, deep venous thrombosis (DVT), VTE, and pulmonary embolism. A complete listing of search terms and medical subject headings can be found in Table I. Reference lists of relevant papers were searched for further studies that our search criteria might have missed. No restrictions regarding types of patients included in the study were adopted.

CLINICAL DECISION RULES

The American College of Chest Physicians Guidelines provide a grade 2B recommendation that the choice of diagnostic test for VTE be guided first by an estimation of the patient's pretest probability.[6] Several well-validated clinical decision rules exist, the most common and well validated of which is the Wells Score (Table II). Combinations of biomarkers with the Wells Score can increase diagnostic accuracy and guide decision making regarding further diagnostic intervention.[6-9]

BIOMARKERS

P-selectin. P-selectin is an adhesion glycoprotein found in the α-granule of the platelet and in the Weibel-Palade body of the endothelium. Cell-surface P-selectin aids in leukocyte adhesion to the thrombus, and platelet P-selectin further tethers leukocytes, propagating venous thrombosis. P-selectin also triggers the release of procoagulant microparticles[10] and increases tissue factor expression on monocytes.[11]

High levels of sP-sel are associated with VTE.[12] Consequently, sP-sel has been proposed as a biomarker with better diagnostic performance than D-dimer. Studies evaluating sP-sel for clinical usefulness have been favorable (Table III). Vandy et al[8] and Ramacciotti et al[13] found sP-sel performed favorably compared with D-dimer when combined with the Wells Score. Furthermore, when combined with a Wells Score ≥2, sP-sel >90 ng/mL has

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Table I. Search terms

Search terms:
Biomarkers, biological marker, biological markers, factor eight, factor viii, F-VIII, F VIII, sP-sel, sPsel, c reactive protein, CRP, risk assessment models, inflammation markers, inflammation marker, DVT, deep vein thrombosis, venous thrombosis, venous thromboembolism, venous thrombosis, thromboembolic disease, VTE, pulmonary embolism, pulmonary embolisms, PE, pulmonary emboli
Medical Subject Headings:
Biological markers, c-reactive protein, p-selectin, Fibrin Fibrinogen Degradation Products, and supplementary concepts prothrombin fragment 2, prothrombin fragment 1, prothrombin fragment 1.2.3, prothrombin fragment 1.2, fibrin fragment D, venous thrombosis, pulmonary embolism, pulmonary thromboembolisms, and pulmonary thromboembolism

demonstrated a positive predictive value of 100%.[8,13] Levels of sP-sel of <60 ng/mL and a low probability Wells Score ruled out DVT with a sensitivity of 99% and negative predictive value of 96%.[13]

A 2014 meta-analysis of sP-sel that compared 586 VTE patients with 1843 controls found significantly increased sP-sel in patients with VTE or with DVT alone and minimal heterogeneity among studies.[14] Pooled sensitivity and specificity for sP-sel were 0.57 (95% confidence interval, 0.30-0.82) and 0.73 (95% confidence interval, 0.51-0.90).[14] Interestingly, sP-sel performs well in patients with and without cancer. Moreover, a nonsignificant trend to higher sP-sel levels in proximal DVT compared with distal DVT was shown,[15] and, similarly, a nonsignificant trend was shown for increased sP-sel levels with greater extent of thrombus.[8] The introduction of a simple, point-of-care test for sP-sel would improve diagnostic accuracy when used in conjunction with the currently widely used Wells Score and D-dimer.

D-dimer. D-dimer is a product of degradation of cross-linked fibrin. Elevated levels in blood are indicative of activation of the coagulation and fibrinolytic pathways. Investigation of D-dimer as a clinically useful biomarker dates back several decades, and it is currently the most common biomarker used in diagnosis of VTE. The

Table II. Wells Score[9] for diagnosis of deep vein thrombosis (*DVT*)

Clinical features	*Score*
Active cancer (treatment ongoing or within the previous 6 months, or palliative)	1
Paralysis, paresis, or recent plaster immobilization of a limb	1
Recent bedrest >3 days, or major surgery within preceding 4 weeks	1
Localized tenderness along the deep venous system	1
Leg swelling	1
Calf swelling >3 cm compared with contralateral calf	1
Pitting edema greater in symptomatic leg	1
Collateral superficial veins	1
Previous diagnosis of DVT	1
Alternate diagnosis as or more likely	−2

American College of Chest Physicians Guidelines recommend D-dimer as the first diagnostic step in patients with a low pretest probability.[6] A recent study has shown, however, that the recommended diagnostic algorithm is not well adhered to and that D-dimer as initial screening test in low-risk patients is underused.[16]

Although D-dimer is generally a high-sensitivity test, its specificity is low, limiting its usefulness as a rule-out test. Many conditions are known risk factors for VTE and are likewise known to increase the D-dimer level, including pregnancy, infection, malignancy, and postsurgical state.[17,18] This at least partly explains the primary usefulness of D-dimer in ambulatory outpatients.

D-dimer increases diagnostic accuracy over Wells Score alone in an outpatient setting.[6,7] When combined with the Wells Score in patients with a low clinical probability, a negative D-dimer safely rules out VTE.[19] Bucek et al[20] similarly found D-dimer to be an excellent rule-out test in patients with low pretest probability, obviating the need for ultrasound examination. Wells et al,[9] in a randomized controlled trial, showed that a policy of limiting the use of ultrasound examination to outpatients with elevated D-dimer significantly decreased the use of ultrasound imaging for diagnosis. Similarly, Di Nisio et al[21] found that D-dimer performed reasonably well to rule out DVT in cancer patients with a nonhigh pretest probability, with a negative predictive value of 100%. This strategy appears safe, because only 0.4% of those patients in whom VTE had been ruled out by D-dimer had VTE diagnosed on follow-up.[19]

Whether D-dimer has reduced diagnostic efficacy in inpatients or in patients with other risk factors is unknown. A recent meta-analysis by Geersing et al[22] evaluated whether this diagnostic strategy was safe in other important subgroups of patients. They evaluated 13 studies comprising >10,000 outpatients. The combination of D-dimer with a low-probability Wells Score had a failure rate of 1.2%, below the generally accepted threshold of 2%. However, they found that the failure rate was 2.2% in patients with malignancy. Chan et al[18] evaluated the accuracy of D-dimer in pregnancy in a single-institution prospective trial of 149 pregnant women with suspected DVT. They found that D-dimer had a sensitivity and specificity of 100% and 60%, with a negative predictive value of 100% in patients with a low pretest probability of VTE. Specificity decreases in the third trimester, limiting usefulness of D-dimer in that patient population.

Risk of VTE is known to increase with age. As VTE incidence increases, it is conceivable that the diagnostic accuracy of D-dimer may decrease. Schouten et al[23] conducted a meta-analysis of studies evaluating an age-adjusted cutoff for D-dimer. They found that specificity of D-dimer decreased with age, from 57.6% in patients aged 51 to 60 years to 14.7% in patients aged >80. Age adjustment improved specificity but still demonstrated a downward trend with increasing age. Sensitivity was not affected by age-adjustment and was >97% in all groups. Another study by Schouten et al[24] evaluated the use of

Table III. Studies evaluating P-selectin in the diagnosis of venous thromboembolism (*VTE*)

Year	*Author*	*Aims*	*Sensitivity*	*Specificity*	*Results*
2015	Mosevoll[40]	Investigated sP-sel and other biomarkers in a study of 169 inpatients with suspected lower extremity DVT	Not reported	Not reported	Results showed sP-sel was significantly associated with the presence of DVT with a $P < .0001$, and was not significantly increased in patients with superficial thrombophlebitis
2015	Thaler[41]	Subanalysis of the CATS study evaluated sP-sel usefulness in prediction of VTE in patients with high-grade glioma	Not reported	Not reported	sP-sel had a hazard ratio of 2.71 ($P = .006$). Patients with low platelets and elevated sP-sel had the highest risk of developing VTE
2014	Antonpoulos[14]	A systematic review and meta-analysis of 11 studies including 586 patients with VTE or DVT only	57%	73%	Pooled sensitivity and specificity for sP-sel were 57% and 73%, respectively. Area under the receiver operating characteristic curve showed sP-sel performed well with area under the curve of .74 ($P = .05$)
2013	Vandy[8]	Prospective study sP-sel combined with Wells Score in inpatients and outpatients with suspected lower-extremity DVT	27.4% (Wells ≥2, sP-sel ≥90) 91.1% (Wells ≤2, sP-sel ≤60)	97.5% (Wells ≥2, sP-sel ≥90) 31.1% (Wells ≤2, sP-sel ≤60)	In the setting of a Wells Score ≥2, with a sPsel cutoff of ≥90, sPsel outperformed D-dimer, demonstrating sensitivity of 96% (88.7%-99%) and specificity of 31% (21.6%-42.7%). Wells Score <2 and sPsel < 60 had sensitivity and specificity of 91.1% and 31.1%, respectively
2011	Gremmel[15]	Prospective study of sP-sel for DVT at diagnosis, then at 1, 3, 6, and 12 months	Not reported	Not reported	sP-sel was significantly elevated in patients with DVT at diagnosis compared to controls 41.4 μg/L vs 33.1 μg/L ($P = .001$). At 1 month, sP-sel levels had decreased to levels comparable with controls. There was a trend toward higher sP-sel in patients with proximal DVT vs distal
2011	Ramaciotti[13]	A case-control study evaluating sP-sel with Wells Score for diagnosis of DVT	33% (Wells ≥2, sP-sel ≥90) 95% (Wells ≤2, sP-sel ≤60)	99% (Wells ≥2, sP-sel ≥90) 33% (Wells ≤2, sP-sel ≤60)	sP-sel performed and Wells Score together provided the best diagnostic performance
2008	Ay[42]	A subanalysis of CATS study, evaluating sP-sel (cutoff 53.1 ng/mL) levels in patients with a variety of malignancies	Not reported	Not reported	Results showed significant correlation of increased P-sel levels with development of VTE in cancer patients. Elevated sP-sel was an independent risk factor for development of VTE on multivariate analysis. HR for VTE development was 1.2 for each 10 ng/mL increase in sP-sel

(*Continued on next page*)

Table III. Continued.

Year	*Author*	*Aims*	*Sensitivity*	*Specificity*	*Results*
2007	Ay[43]	Case-control study evaluating sP-sel and the Thr715Pro p-selectin variant in 116 patients with recurrent VTE	29%	95%	sP-sel was elevated in patients with VTE compared with healthy controls, with an OR of 10.6 ($P < .001$) on multivariate analysis. There was no difference in the presence of the Pro715 variant in cases compared with controls
2005	Rectenwald[30]	Prospectively evaluated the diagnostic performance of sP-sel in DVT	68%	81%	sP-sel had better diagnostic accuracy than D-dimer, both as a continuous variable, and as a dichotomous variable with a cutoff of 0.68 ng/mg total protein
2003	Bucek[34]	Evaluated the diagnostic performance of sP-sel in patients with DVT	Not reported	Not reported	No statistically significant difference in sP-sel was found between healthy patients and those who developed DVT. A correlation was found between sP-sel and presence of malignancy
2003	Bozic[44]	Evaluated the diagnostic performance of sP-sel in 135 outpatients with suspected lower extremity DVT	100%	19%	sP-sel had 100% sensitivity in this study, for low, intermediate, and high pretest probability. Specificity was low, 21%, 17%, and 0% for low, intermediate, and high pre-test probability, respectively
2000	Blann[45]	Case-control study evaluating sP-sel levels for DVT	61%	60%	P-sel was elevated in DVT patients, but the data were widely scattered and offered poor accuracy
1999	Smith[33]	Investigated whether sP-sel was altered by orthopedic surgery, as well as DVT.	Not reported	Not reported	sP-sel was elevated in the presence of thrombus, but was not altered by surgery

CATS, Vienna Cancer and Thrombosis Study; *DVT,* deep vein thrombosis; *HR,* hazard ratio; *OR,* odds ratio; *sP-sel,* soluble P-selectin.

an age-adjusted cutoff for D-dimer. In 1374 outpatients, they found that both an age-adjusted cutoff of age times 10 μg/L, in patients aged >50 years, and a fixed cutoff value of 750 μg/L improved diagnostic efficiency of D-dimer.

There remains some question concerning the accuracy of the various types of D-dimer assay and whether these perform equally well.[25] On the one hand, latex quantitative assays provide rapid results, in as few as 15 minutes. Microplate enzyme-linked immunosorbent assay (ELISA), on the other hand, carries the disadvantage of requiring several hours to return results.[25] Latex and whole-blood assays are qualitative assays with less inter-rater reliability.[25]

Several papers have investigated these questions. Stein et al[26] published a review of studies evaluating the variability of the different assays. They found significant variability in diagnostic performance. ELISA and rapid-quantitative ELISA performed best in their analysis (Table IV). Di Nisio et al[25] published a systematic review of 217 papers evaluating D-dimer for DVT, calculating the sensitivity and specificity of a variety of different assays. There was a trade-off in the various assays between sensitivity and specificity. Another meta-analysis, by Goodacre et al[27] evaluated 97 studies, finding overall sensitivity and specificity of D-dimer of 90.5% and 54.7%; however, they found significant variation, as well, by type of assay performed.[27]

Other biomarkers. Several other biomarkers are under investigation for use in VTE diagnosis. These studies are presented in Table V.

Circulating procoagulant microparticles, derived from platelets and leukocytes and rich in tissue factor and P-selectin glycoprotein ligand-1, have been evaluated as novel biomarkers. Elevated levels of microparticles are associated with the presence of inflammatory pathologies, malignancy, and VTE.[28,29] However, they have generally not been found useful in VTE diagnosis.[13,30-32]

Table IV. Diagnostic performance of D-dimer assays[a]

	DVT				*Pulmonary embolism*			
Analysis	*Sensitivity (95% CL)*	*Specificity (95% CL)*	*Positive likelihood ratio (95% CL)*	*Negative likelihood ratio (95% CL)*	*Sensitivity (95% CL)*	*Specificity (95% CL)*	*Positive likelihood ratio (95% CL)*	*Negative likelihood ratio (95% CL)*
Tier 1[b]								
ELISA	**0.96**[c] **(0.91, 1.00)**	0.38 (0.28, 0.48)	1.55 (1.32, 1.81)	**0.12**[d] **(0.04, 0.33)**	**0.95**[e] **(0.85, 1.00)**	0.44 (0.34, 0.54)	1.68 (1.44, 1.95)	**0.13**[d] **(0.03, 0.58)**
Quantitative rapid ELISA	**0.96**[c] **(0.90, 1.00)**	0.44 (0.32, 0.55)	1.70 (1.39, 2.09)	**0.09**[f] **(0.02, 0.41)**	**0.95**[e] **(0.83, 1.00)**	0.39 (0.28, 0.51)	1.56 (1.32, 1.83)	**0.13**[d] **(0.02, 0.84)**
Semiquantitative rapid ELISA	0.89 (0.81, 0.98)	0.39 (0.28, 0.50)	1.47 (1.21, 1.78)	0.27 (0.12, 0.60)	**0.93**[e] **(0.79, 1.00)**	0.36 (0.23, 0.50)	1.45 (1.20, 1.76)	**0.20**[d] **(0.04, 0.96)**
Qualitative rapid ELISA	**0.93**[c] **(0.84, 1.00)**	0.47 (0.30, 0.63)	1.75 (1.28, 2.39)	**0.15**[d] **(0.04, 0.56)**	0.93 (0.74, 1.00)	**0.68**[e] **(0.50, 0.87)**	2.92 (1.77, 4.79)	**0.11**[d] **(0.01, 0.93)**
Quantitative latex	0.85 (0.74, 0.95)	**0.66**[c] **(0.55, 0.78)**	2.49 (1.77, 3.51)	0.24 (0.12, 0.45)	0.89 (0.81, 0.98)	0.45 (0.36, 0.53)	1.62 (1.43, 1.84)	0.24 (0.13, 0.45)
Semiquantitative latex	0.78 (0.67, 0.89)	**0.66**[c] **(0.56, 0.76)**	2.30 (1.69, 3.13)	0.33 (0.21, 0.54)	0.92 (0.79, 1.00)	0.45 (0.31, 0.59)	1.68 (1.35, 2.09)	**0.17**[d] **(0.04, 0.78)**
Whole blood	0.87 (0.68, 1.00)	**0.83**[c] **(0.65, 1.00)**	4.97 (1.84, 13.42)	**0.16**[d] **(0.04, 0.65)**	0.78 (0.64, 0.92)	**0.74**[e] **(0.60, 0.88)**	2.93 (1.89, 4.52)	0.31 (0.18, 0.51)
Tier 2[g]								
ELISA	**0.95**[h] **(0.91, 0.99)**	0.40 (0.32, 0.49)	1.60 (1.39, 1.83)	**0.12**[d] **(0.05, 0.29)**	**0.96**[i] **(0.88, 1.00)**	0.51 (0.44, 0.59)	1.97 (1.72, 2.26)	**0.08**[f] **(0.01, 0.43)**
Quantitative rapid ELISA	**0.96**[h] **(0.90, 1.00)**	0.44 (0.34, 0.54)	1.71 (1.43, 2.05)	**0.10**[d] **(0.03, 0.36)**	**0.97**[i] **(0.87, 1.00)**	0.41 (0.30, 0.51)	1.64 (1.40, 1.91)	**0.07**[f] **(0.00, 1.55)**
Semiquantitative rapid ELISA	0.90 (0.83, 0.98)	0.39 (0.29, 0.50)	1.48 (1.24, 1.78)	0.25 (0.12, 0.55)	0.93 (0.79, 1.00)	0.40 (0.27, 0.54)	1.55 (1.25, 1.93)	**0.18**[d] **(0.04, 0.94)**
Qualitative rapid ELISA	**0.93** **(0.87, 0.99)**	0.46 (0.35, 0.57)	1.73 (1.40, 2.13)	**0.15**[d] **(0.07, 0.37)**	0.91 (0.68, 1.00)	**0.70**[i] **(0.47, 0.93)**	3.01 (1.52, 5.96)	**0.13**[d] **(0.01, 1.28)**
Quantitative latex	0.86 (0.78, 0.94)	**0.61**[h] **(0.51, 0.71)**	2.20 (1.70, 2.84)	0.23 (0.13, 0.41)	0.89 (0.80, 0.99)	0.47 (0.38, 0.57)	1.69 (1.44, 1.99)	0.23 (0.11, 0.48)
Semiquantitative latex	0.79 (0.69, 0.88)	**0.66**[h] **(0.57, 0.75)**	2.33 (1.75, 3.11)	0.32 (0.20, 0.51)	0.80 (0.65, 0.94)	0.56 (0.42, 0.70)	1.81 (1.35, 2.42)	0.36 (0.20, 0.67)
Whole blood	0.86 (0.80, 0.93)	**0.67**[h] **(0.61, 0.73)**	2.62 (2.17, 3.16)	**0.20**[d] **(0.13, 0.32)**	0.83 (0.74, 0.92)	**0.64**[i] **(0.55, 0.73)**	2.32 (1.87, 2.88)	0.27 (0.17, 0.42)

CL, Confidence limit; *DVT*, deep vein thrombosis; *ELISA*, enzyme-linked immunosorbent assay.

[a]The values in boldface represent statistically superior values.

[b]DVT: Overall differences in sensitivity and specificity: $P = .020$ and $P < .001$, respectively (Wald test). Pulmonary embolism: Overall differences in sensitivity and specificity: $P > .2$ and $P < .001$, respectively (Wald test).

[c]DVT: Tier 1—Sensitivity: The ELISA is superior to the quantitative latex agglutination and semiquantitative latex agglutination assays ($P = .039$ and $P = .002$, respectively), the quantitative rapid ELISA is superior to the quantitative latex agglutination and semiquantitative latex agglutination assays ($P = .039$ and $P = .002$, respectively), and the qualitative rapid ELISA is superior to the semiquantitative latex agglutination assay ($P = .026$). Specificity: The quantitative latex agglutination, semiquantitative latex agglutination, and whole-blood agglutination assays are superior to the ELISA ($P < .001$, $P < .001$, and $P < .001$, respectively), quantitative rapid ELISA ($P = .004$, $P = .002$, and $P < .001$, respectively), semiquantitative rapid ELISA ($P = .0040$, $P < .001$, and $P < .001$, respectively), and qualitative rapid ELISA ($P = .048$, $P = .038$, and $P = .003$, respectively).

[d]Negative likelihood ratios of 0.1 to 0.2 generate moderate shifts in pretest to post-test probability.

[e]Pulmonary embolism: Tier 1—Sensitivity: The ELISA, quantitative rapid ELISA, and semiquantitative rapid ELISA are superior to the whole-blood agglutination assay ($P = .020$, $P = .016$, and $P = .047$, respectively). Specificity: the qualitative rapid ELISA is superior to the ELISA, quantitative rapid ELISA, semiquantitative rapid ELISA, quantitative latex agglutination assay, and semiquantitative latex agglutination assay ($P = .004$, $P = .002$, $P = .001$, $P = .005$, and $P = .019$, respectively); the whole-blood agglutination assay is superior to the ELISA, quantitative rapid ELISA, semiquantitative rapid ELISA, quantitative latex agglutination assay, and semiquantitative latex agglutination assay ($P < .001$, $P < .001$, $P < .001$, $P < .001$, and $P = .001$, respectively).

[f]Negative likelihood ratios <0.1 generate large and often conclusive changes from pretest to post-test probability.

[g]DVT: Overall differences in sensitivity and specificity: $P = .005$ and $P < .001$, respectively (Wald test). Pulmonary embolism: Overall differences in sensitivity and specificity: $P = .071$ and $P < .001$, respectively (Wald test).

[h]DVT: Tier 2—Sensitivity: The ELISA is superior to the quantitative latex, semiquantitative latex, and whole-blood agglutination assays ($P = .040$, $P = .001$, and $P = .020$, respectively); the quantitative rapid ELISA is superior to the quantitative latex agglutination, semiquantitative latex agglutination, and whole-blood agglutination assays ($P = .037$, $P = .001$, and $P = .022$, respectively); and the qualitative rapid ELISA is superior to the semiquantitative latex agglutination assay ($P = .010$). Specificity: The quantitative latex agglutination, semiquantitative latex agglutination, and whole-blood agglutination assays are superior to the ELISA ($P = .001$, $P < .001$, and $P < .001$, respectively); quantitative rapid ELISA ($P = .012$, $P < .001$, and $P < .001$, respectively); semiquantitative rapid ELISA ($P = .002$, $P = .002$, and $P < .001$, respectively); and qualitative rapid ELISA ($P = .047$, $P = .004$, and $P = .001$, respectively).

[i]Pulmonary embolism: Tier 2—Sensitivity: The ELISA and quantitative rapid ELISA are superior to the semiquantitative latex agglutination assay ($P = .024$ and $P = .031$, respectively) and whole-blood agglutination assay ($P = .016$ and $P = .021$, respectively). Specificity: The quantitative rapid ELISA is superior to the quantitative rapid ELISA, semiquantitative rapid ELISA, and quantitative latex agglutination assay ($P = .012$, $P = .015$, and $P = .040$, respectively); the whole-blood agglutination assay is superior to the ELISA, quantitative rapid ELISA, semiquantitative rapid ELISA, and quantitative latex agglutination assay ($P = .018$, $P < .001$, $P = .001$, and $P = .004$, respectively).

Reproduced with permission from: Stein PD, Hull RD, Patel KC, Olson RE, Ghali WA, Brant R, et al. D-dimer for the exclusion of acute venous thrombosis and pulmonary embolism: a systematic review. Ann Intern Med 2004;140:589-602.

Table V. Other biomarkers for venous thromboembolism (*VTE*)

Year	*Author*	*Biomarker*	*Aims*	*Sensitivity*	*Specificity*	*Results*
2015	Rabinovich[39]	CRP ICAM-1 IL-6 IL-10	Subanalysis of the SOX Trial, a randomized controlled trial of first acute proximal DVT, investigated association of inflammatory markers with extent and severity of DVT at diagnosis and at 1 month	Not reported	Not reported	Elevated CRP and IL-6 were associated with extent and worsened severity (by Villata Score) of DVT at diagnosis. Elevated baseline CRP was also associated with a more severe DVT at 1 month
2015	Malatino[35]	Albumin, % of total protein Protein C Protein S Antithrombin III	Single-center study conducted in hospitalized medical patients, to evaluate biomarkers in diagnosis of DVT	73% Not reported 65% Not reported	79% Not reported 94% Not reported	Protein S was a good predictor of VTE, with area under the ROC curve of 0.821. Accuracy of protein S was increased by combination with albumin
2014	Mosevoll[40]	43 individual biomarkers were investigated, including: ICAM-1, VCAM-1, MMP, chemokines, and growth factors	Investigated whether any of 43 potential biomarkers had utility in diagnosis of lower extremity DVT	Not reported	Not reported	Of 43 biomarkers investigated, sP-sel, VCAM-1, MMP-8 and hepatocyte growth factor were significantly elevated in patients with DVT
2014	Thaler[41]	Prothrombin 1+2 Factor VIII activity Peak thrombin generation CRP Fibrinogen	Subanalysis of CATS study evaluating biomarkers in patients with high-grade glioma patients after neurosurgical intervention	Not reported	Not reported	Prothrombin 1+2, factor VIII activity, D-dimer, and sP-sel were associated with risk for VTE
2013	Zwicker[28]	Microparticles	Compared incidence of VTE in cancer patients with high vs low serum levels of TF-bearing microparticles	Not reported	Not reported	High levels of TF-bearing microparticles were associated with increased risk of VTE in cancer patients (HR, 6.7), and LMWH reduced this risk to levels comparable with those patients with lower microparticle levels
2012	Thaler[32]	Microparticles	Prospective observational study investigating whether TF-bearing microparticles are associated with VTE and mortality in cancer patients	Not reported	Not reported	There was no statistically significant correlation between TF microparticle activity and the development of VTE
2011	Ramacciotti[13]	Microparticles CRP	Case-control study evaluating microparticles (total, leukocyte-derived, platelet-derived, and TF-positive microparticles) for diagnosis of DVT	Not reported	Not reported	CRP and platelet-derived microparticles were able to differentiate patients with DVT from healthy controls (2.1 μg/mL vs 0.8 μg/mL)
2011	Tiedje[46]	Fibrinogen	Subanalysis of CATS study evaluating fibrinogen for prediction of VTE	Not reported	Not reported	Fibrinogen level was not associated with the development of VTE on multivariate analysis (HR, 1.05)
2011	Thaler[31]	Microparticles	An analysis of banked plasma samples from the CATS study, investigating association between TF-microparticles and VTE	Not reported	Not reported	TF-microparticle levels were elevated in cancer patients, however there was no correlation between TF-microparticle elevation and development of VTE

(*Continued on next page*)

Table V. Continued.

Year	*Author*	*Biomarker*	*Aims*	*Sensitivity*	*Specificity*	*Results*
2012	Kaya[47]	sCD40L	Evaluated sCD40L levels in PE patients	Not reported	Not reported	In 65 PE patients, compared with 29 healthy controls, sCD40L levels were significantly elevated in patients with PE
2011	Kanz[36]	CRP	Subanalysis of CATS study evaluating CRP for prediction of the development of VTE and mortality	Not reported	Not reported	CRP was associated with VTE development in univariate analysis (HR, 1.2), but this association was lost on multivariate analysis (HR, 1.0)
2010	Elf[48]	APC-PCI	To evaluate the use of APC-PCI in the diagnosis of DVT compared with D-dimer	74%	80%	In a post hoc analysis of 350 plasma samples, APC-PCI had a lower sensitivity and negative predictive value than D-dimer. Specificity and positive predictive value were higher. Specificity did not decrease with increasing pretest probability
2009	Ay[49]	Prothrombin 1+2	Subanalysis of CATS study evaluating prothrombin 1+2 and D-dimer in cancer patients	Not reported	Not reported	Prothrombin 1+2 was elevated in patients who developed VTE with a HR of 1.8 and 2.1 on Univariate and multivariate analysis, respectively. Patients with elevated D-dimer and prothrombin 1+2 had a strong association with development of VTE on Kaplan-Meier analysis (15.2%)
2009	Vormittag[50]	Factor VIII Level	Subanalysis of CATS study evaluating factor VIII levels for prediction of VTE in cancer patients	Not reported	Not reported	Risk of VTE development in cancer patients increased continuously; VTE risk increased 120% for each 20% increase in factor VIII level. Risk associated with elevated factor VIII decreases with age
2009	Zwicker[51]	Microparticles	Case control study evaluated TF-microparticles in cancer patients with VTE			Elevated TF-microparticles were associated with VTE, with an OR of 3.72 ($P = .01$); 34.8% of cancer patients without VTE and with elevated TF-microparticles developed VTE ≤1 year, none of the patients with low TF-microparticles developed VTE (OR, 7.0; $P = .02$)
2008	Khorana[52]	Plasma TF	Prospective observational study evaluating circulating TF for prediction of VTE in cancer patients	Not reported	Not reported	Found an significant association between development of VTE and elevated circulating TF in cancer patients
2005	Rectenwald[30]	Microparticles	Prospectively evaluated the diagnostic performance of total microparticles in patients with DVT	50%	67%	Total microparticles did not perform as well as sP-sel or D-dimer as continuous or as dichotomous values

(*Continued on next page*)

Table V. Continued.

Year	*Author*	*Biomarker*	*Aims*	*Sensitivity*	*Specificity*	*Results*
2003	Bucek[34]	ICAM-1 VCAM-1 E-selectin	Evaluated the diagnostic performance of ICAM-1 and VCAM-1 in patients with DVT	Not reported	Not reported	No statistically significant difference in ICAM-1, VCAM-1, or E-selectin was found between healthy patients and those who developed DVT
2003	Bozic[44]	Prothrombin 1+2 TAT-c VCAM-1	Evaluated the diagnostic performance of F1+2 and TAT in 135 outpatients with suspected lower extremity DVT	100% 100% 100%	11% 18% 0	F1+2, VCAM-1 and TAT were elevated in patients with DVT; F1+2 was higher in patients with proximal DVT compared with distal. D-dimer outperformed all other assays in the study; VCAM-1 had the lowest AUC
1999	Smith[33]	ICAM-1 VCAM-1 E-selectin L-selectin TAT-c TF Thrombomodulin vWF	Investigated whether thrombus biomarkers were altered by orthopedic surgery, as well as DVT	Not reported	Not reported	TAT, vWF and thrombomodulin were elevated after orthopedic surgery. VCAM-1 was also elevated in the presence of DVT and was not elevated after surgery
1998	Taheri[53]	sTNF receptor	Pilot study evaluated sTNF-receptor by ELISA for diagnosis of VTE	Not reported	Not reported	sTNF levels were elevated in patients with VTE, but diagnostic performance was not investigated
1989	Thomas[54]	CRP	Evaluated CRP and liquid crystal thermography in diagnosis of DVT	100%	52%	In this small cohort of patients, CRP performed well as a sensitive test for the presence of DVT

APC-PCI, Activated protein C-protein C inhibitor complex; *AUC,* area under the curve; *CATS,* Vienna Cancer and Thrombosis Study; *CRP,* C reactive protein; *DVT,* deep vein thrombosis; *ELISA,* enzyme linked immunosorbent assay; *HR,* hazard ratio; *ICAM-1,* intercellular adhesion molecule 1; *IL,* interleukin; *LMWH,* low-molecular-weight heparin; *MMP,* matrix metalloproteinase; *OR,* odds ratio; *PE,* pulmonary embolism; *ROC,* receiver operating characteristic; *sCD40L,* soluble CD40 ligand; *SOX,* Compression Stockings to Prevent the Post-Thrombotic Syndrome After Symptomatic Proximal Deep Venous Thrombosis; *sP-sel,* soluble P-selectin; *sTNF,* soluble tumor necrosis factor; *TAT-c,* thrombin-antithrombin complex; *TF,* tissue factor; *VCAM-1,* vascular cell adhesion molecule 1; *vWF,* von Willebrand factor.

Although sP-sel has shown promise as a biomarker, levels of other soluble adhesion molecules, such as intercellular adhesion molecule 1, vascular cell adhesion molecule 1, E-selectin, and L-selectin have not been shown to correlate as well with the presence of DVT.[33,34] Serum albumin and protein S have been shown to increase the accuracy of the Wells Score.[35] C-reactive protein is very sensitive, but generally lacks enough diagnostic accuracy to justify its use.[13,36] Mean platelet volume combined with D-dimer increased specificity but reduced sensitivity.[37] Finally, galactin-3 binding protein and galactin-3 are being investigated for DVT.[38]

CONCLUSIONS

The diagnostic intervention with the best performance remains duplex compression ultrasound imaging. However, this operator-dependent test is not available 24 hours a day in most hospitals. Consequently, the development of rapid, inexpensive serum biomarkers capable of both ruling in and ruling out VTE is paramount. At this time, only D-dimer and sP-sel have shown clinical usefulness. D-dimer used as a rule-out study has been shown to be safe and to obviate the need for further testing with ultrasound imaging or more invasive diagnostic interventions, although it suffers from low specificity. Combination with sP-sel as a rule-in test would increase diagnostic accuracy.

AUTHOR CONTRIBUTIONS

Conception and design: BJ, AO, TW
Analysis and interpretation: BJ, AO, TW
Data collection: BJ
Writing the article: BJ, AO
Critical revision of the article: TW
Final approval of the article: TW
Statistical analysis: BJ
Obtained funding: TW
Overall responsibility: BJ

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