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REVIEW

Disease management

The current role and future prospects of D-dimer biomarker

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D-dimers have been discovered as by-products of fibrinolysis. In situations where the fundamental pathology is associated with increased thrombolytic activity, D-dimer assays could serve an integral role in the clinical workup, and have an already established role in the diagnosis of clinical disorders of venous thromboembolism, and disseminated intravascular coagulation. However, there is growing literature suggesting that this is not the only clinical scenario where D-dimers may be of significance. They may also become an important biomarker in coronary and carotid artery atherosclerosis and aortic diseases. Being a non-invasive and quick means of diagnosis, D-dimers are a cost-effective tool used for diagnosing diseases. With the future being steered in the direction of preventive cardiology, it is imperative for clinicians to understand how to effectively utilize biomarkers in order to diagnose disorders. In this context, we review D-dimer's origin, current clinical utility, and potential future applications.

Keywords Biomarkers • Acute coronary syndrome • Aortic disorders

Introduction

D-dimers are by-products derived from the cleavage of cross-linked, insoluble fibrin molecules. The fibrinogen molecule consists of two outer D-domains and a central E-domain. Ultrastructurally, each molecule is a dimer, consisting of three polypeptide chains termed alpha, beta, and gamma, which are held together by two disulfide bonds. Each dimer in turn is then held by three disulfide bonds to its other half.[1]

As endovascular thrombosis evolves, plasmin cleaves cross-linked insoluble fibrin monomers to yield by-products known as fibrin degradation products (FDPs).[2] Amongst these products, the remnant composed of two adjacent bonded D-domains from cross-linked fibrils are what are now known as 'D-dimers'.[3] D-dimer found in blood samples is non-covalently bonded to E-fragments, forming a stable D-dimer/E-complex (*Figure 1*).[3,4] To detect serum D-dimer antigen, assays have been developed that utilize antibodies reacting with epitopes specifically present on the D-dimer molecule.[5] These epitopes are generated as a result of factor XIII's cross linkage of fibrin polymers, and are not found on other FDPs. D-dimer assays are not uniform since the commercial kits use different antibodies.[6]

The blood sample is drawn into a citrated tube, keeping a recommended ratio of blood to citrate greater than ~9:1. This sample is then centrifuged and depending upon the lab, a selected measurement assay is performed. A variety of assay systems, both qualitative and quantitative assays are used to measure D-dimer levels.[4] Each method of measurement has a differing, inherent sensitivity, and specificity.

From a clinical perspective, it is well known that serum D-dimer levels correlate with the extent of the total thrombolytic activity in the body. However, new data suggest that this biomarker if used in appropriate clinical settings can inform clinicians of both diagnostic and prognostic information.

Methods

PubMed (Medline) was used as comprehensive literature research tool to locate studies, review articles and trials, which assessed the molecular origin of D-dimers and its relation to various disease pathologies including venous thromboembolism (VTE), pulmonary emboli (PE), aortic aneurysm (AA), coronary artery disease (CAD), etc. The database was searched for using terms 'D-dimer OR fibrin degradation products'

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AND 'origin OR molecular origin' AND 'deep venous thrombosis (DVT)' AND 'pulmonary emboli' AND 'ischaemic heart disease OR CAD OR acute myocardial infarction' AND 'aortic diseases OR aortic dissection OR aortic aneurysm'. The filters applied were (i) English language and (ii) human subjects. Approximately 350 articles were yielded from the search. Criteria for studies to be eligible was the following:

(1) Studies had information regarding molecular origins of D-dimer, methods of measurement, and extractable information regarding baseline D-dimer levels.
(2) Information on major adverse cardiovascular event (MACE) could be extracted.
(3) Studies contained extractable data on disease pathologies notably aortic disorders, DVT, Pulmonary emboli, and peripheral artery disease (PAD).

Studies were excluded from review process for the following reasons:

(1) Data quantifying D-dimer levels could not be extracted.
(2) Did not use D-dimer measurement as a predictor of disease outcomes.
(3) No follow-up available on disease pathology's outcomes.

After reviewing study abstracts, ~80 studies were assessed to be relevant by the authors, excluding the rest. The shortlisted studies and their respective references were manually reviewed further and assessed for relevant data including study type, journal of publication, year of publication, patient characteristics, primary and secondary outcomes, and statistical analysis. Data were extracted paying close attention to the following information:

- Study details including type of study, author, journal type, and year of publication.
- Total patient sample included in study and patient variables.
- Disease pathology under investigation in respective study.
- D-dimer assays, methods of measurement, and their quantification.
- Study outcomes measures.
- Statistical analysis and results.

Relevant data were summarized and then assimilated detailing pertinent information with respect to disease type in the ensuing review text.

Venous thromboembolism

The clinical role of serum D-dimer is integral to the diagnosis of VTE. Elevated serum D-dimers raise clinical suspicion of active VTE (i.e. deep vein thrombosis and/or PE). Wells *et al.*[7] studied D-dimer assays in clinically appropriate patients to diagnose DVT; the results documented strong correlation between D-dimer assays and VTE diagnosis. De Bastos *et al.*[8] retrospectively evaluated 335 patient records and found D-dimer assays had 100% sensitivity when symptom duration was <15 days; over 15 days, D-dimer sensitivity fell to ~50% for the diagnosis of VTE. Thus to retain clinical value, D-dimer assays must be collected shortly after symptom onset.

Multiple studies have validated the role of D-dimer assays to safely rule out DVT.[9] However, D-dimers retain their value only when used in appropriate patient population. D-dimer assays may be elevated in patients who have non-VTE diagnoses, such as underlying inflammatory disease, chronic thrombotic activity, the elderly and in hospitalized patient populations.[10] In such circumstances, D-dimer assays may lose their specificity and no longer serve to guide the clinician regarding VTE activity.

A similar algorithm exists for the diagnosis of PE. D-dimer values, in appropriately risk-stratified population, may be a diagnostic test for acute pulmonary embolism.[11] Patients at risk can be stratified according to validated scoring systems such as the Wells score[12] or the revised Geneva score[13] (*Table 1*). The Wells score is the more commonly used; it classifies patients into *PE likely* (i.e. high-risk group) and *PE unlikely* (i.e. low–medium risk group). In the *PE unlikely* group, workup should include a serum D-dimer quantitative assay; if negative, it is sufficient to rule out PE and no further imaging is required. If positive then one should proceed to diagnostic imaging

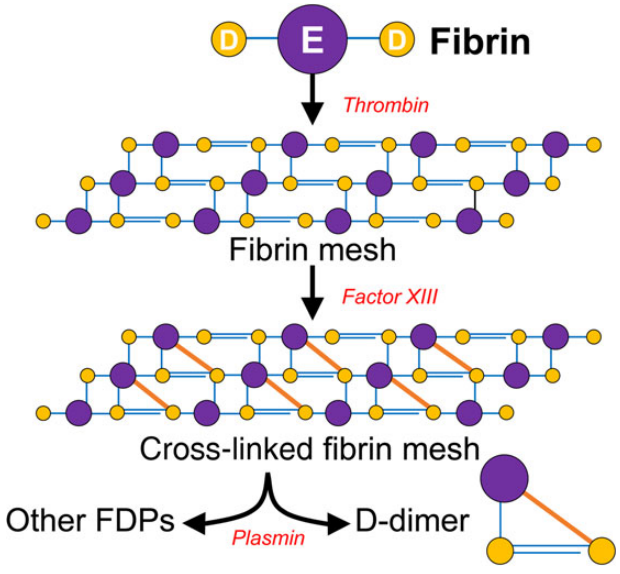

Figure 1 Fibrinogen to cross-linked fibrin mesh to fibrin degradation products and D-dimers.

Table 1 Validated clinical scoring: table comparing Wells criteria to the Geneva criteria

Items	Wells criteria		Geneva criteria	
	Original	Simplified version	Original	Simplified version
Clinical decision points				
Previous PE or DVT (+)	1.5	1	3	1
Surgery or immobilization within past 4 weeks	1.5	1	2	1
Haemoptysis	1	1	2	1
Active neoplasia	1	1	2	1
Clinical signs of DVT (+)	3	1		
Alternative diagnosis less likely than PE	3	1		
Heart rate >100 b.p.m.	1.5	1		
Heart rate >95 b.p.m.			5	2
Heart rate 75–94 b.p.m.			3	1
Unilateral LE pain			3	1
Pain on lower limb deep venous palpation with unilateral oedema			4	1
Age >65 years			1	1
Clinical probability				
Three-level score				
Low	0–1	n/a	0–3	0–1
Intermediate	2–6	n/a	4–10	2–4
High	≥	n/a	≥11	≥5
Two-level score				
PE unlikely	0–4	0–1	0–5	0–2
PE likely	≥5	≥2	≥6	≥3

modalities.[14] In the *PE likely* subpopulation or those with positive D-dimer result, proceed to imaging in order to rule out PE. If amongst imaging modalities V/Q scan is done and is non-diagnostic (i.e. either normal or high probability), it is advised to use results of lower extremity venous duplex for ruling out PE. Also in cases when V/Q scan is highly probable of PE; however, venous duplex is negative, it is imperative to use further imaging such computed tomographic pulmonary angiography (CTPA) to rule out PE (*Figure 2*).[15] D-dimer level may also be helpful in these ambiguous clinical situations.

Studies following the Wells clinical decision criteria to categorize patients as *PE likely* and *PE unlikely*, followed by a CTPA, which is negative have safely excluded PE.[16] However in patients with symptoms suggestive of DVT or PE, it is reasonable to use ultrasound results to aid in diagnosis.[9] Goldhaber *et al.*[17] reported that when serum D-dimer values were elevated >500 ng/mL, the sensitivity of the assay was >90% in diagnosing PE. In the case of PE, D-dimer values serve as both a diagnostic and a prognostic indicator.[18,19] Klok *et al.*[18] surveyed a patient population who had been diagnosed with PE. These patients were stratified with respect to their D-dimer values and were then followed over a period of 3 months. Patients in the higher D-dimer cohort had worse survival. These findings have been confirmed in a meta-analysis by Becattini *et al.*,[19] which observed that higher D-dimer values were associated with worse short-term and 3-month mortality rates.

It is not clear whether following D-dimer levels over time is of any clinical value once it is known that they are elevated after an episode of VTE. Palareti *et al.*[20] assessed serum D-dimer levels in patients who had suffered a first time, unprovoked, proximal DVT or PE. Following diagnosis, these patients received a vitamin K antagonist for at least 3 months. D-dimer levels were measured 1 month after cessation of anticoagulation therapy. Patients with an abnormal D-dimer level 1 month after the discontinuation of anticoagulation therapy had a statistically significant incidence of recurrent VTE, suggesting that D-dimer assays could possibly be utilized to determine the optimal duration of anticoagulation therapy. D-dimer values in diagnosing recurrent vs. new onset VTE have concluded that D-dimer in combination with an appropriate clinical scoring system retains clinical value. However, the utility of the D-dimer assay in excluding PE or DVT without further radiological testing is reduced when compared with before.[10]

As D-dimers can serve as a means of assessing haemostasis and active fibrinolysis, this principle is put to use in order to diagnose disseminated intravascular coagulation (DIC). When used along with FDPs, the combination of the two serum markers serves as both, sensitive and specific means of diagnosing DIC,[21] a common coagulopathy triggered by a variety of disease states. Lattimer *et al.*[22] investigated D-dimer levels in varicose veins. The study included separate cohort of patients with varicose veins and matched controls. The study consisted of a total sample of 48 patients distributed evenly across both arms. Concurrent venous blood samples were taken from the arm and a varicosed lower extremity vein. The median D-dimer level was significantly elevated in the ankle than in the arm blood of the same patient. This was not observed in the venous samples of the control sample, suggesting there is more fibrinothrombolytic activity in varicose veins with superficial vein

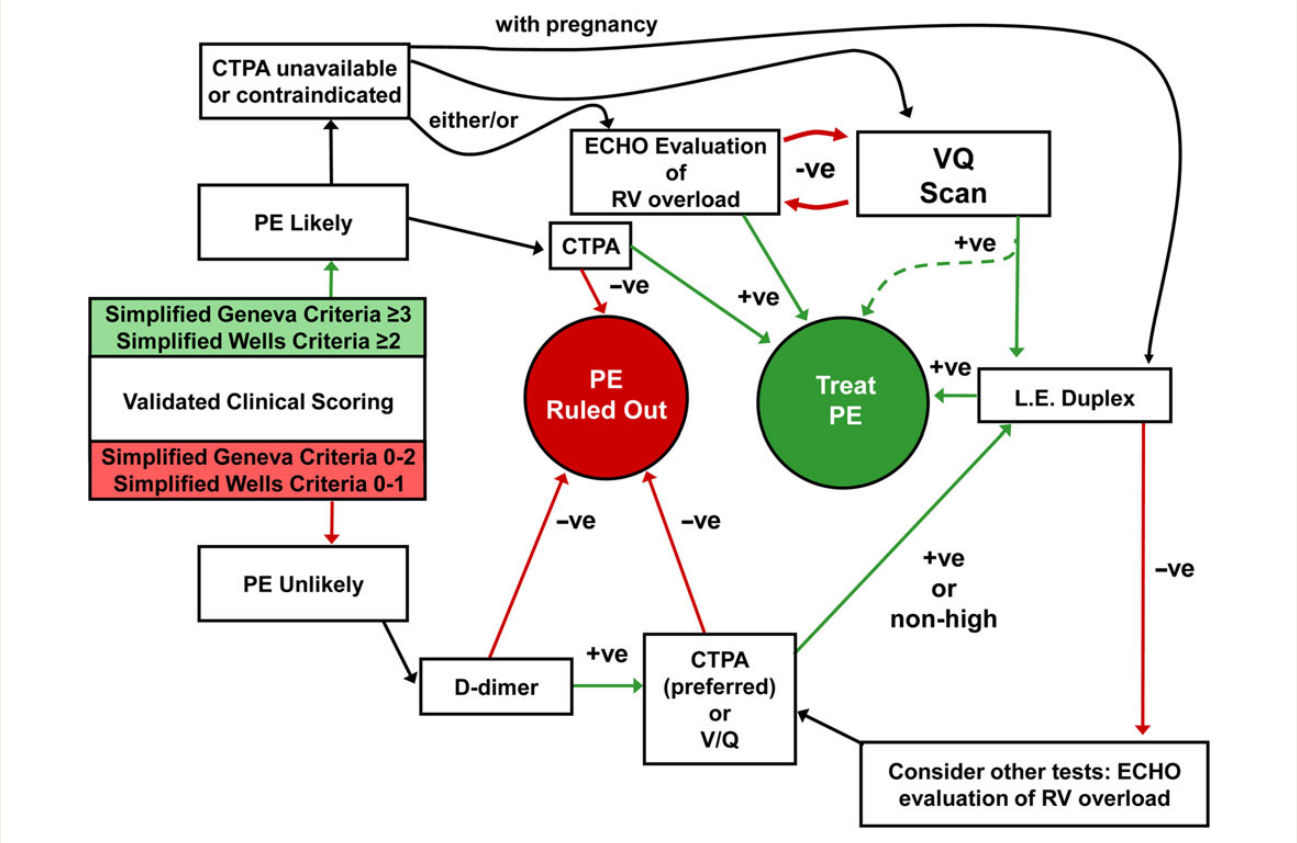

Figure 2 Clinical strategy algorithm for diagnosing pulmonary emboli following validated clinical scoring.

thrombosis in contrast with earlier studies reporting reduced fibrinolysis in venous disease.

Aortic dissection

Acute aortic dissection (AAD) is an uncommon, severe disease, and is associated with high morbidity and mortality rates. Unfortunately, there are no sensitive screening tests regarding the disease. Weber *et al.*[23] studied the association of D-dimer values in a cohort of patients with a definitive diagnosis of AAD. They reported that serum D-dimer assays were positive in all patients with AAD and assay levels increased in proportion to extent of dissection. The authors mentioned that delay in testing from onset of symptoms affected assay levels and that elevated levels did not return to baseline immediately. Thus, assay levels have a high negative predictive value; however, its clinical value might wane with time. Sodeck *et al.*[24] conducted a literature review and meta-analysis of 16 articles, further validating their findings in a prospective cohort of 65 patients, assessing the marker's sensitivity, specificity, and evaluating for a possible optimal cut-off value in cases of AAD. The meta-analysis data showed that D-dimers had a high sensitivity (0.97, 95% CI 0.94–0.98) and negative likelihood ratios (0.06, 95% CI 0.02–0.13), suggesting that the test is a cheap and effective way to rule out acute dissection. They also analysed the total data with different serum cut-off values in order to explore an 'ideal' cut-off value for this reason. At low cut-off values, the marker retained nearly 100% sensitivity. As the cut-off value was increased, sensitivity decreased and the lowest was ~86%.[24] At a cut-off level of 0.5 μg/mL, commonly used value for exclusion of pulmonary embolism, negative predictive value of 99% would be achieved. Sodeck *et al.*[24] were unsuccessful in posting an ideal cut-off value to exclude AAD, primarily because of the different types of quantitative assays that were used to measure D-dimers in the different studies. A similar message was reiterated in another meta-analysis to assess use of D-dimers in AAD.[25] Cui *et al.*[25] performed a meta-analysis of the relevant data available. They attained a pooled sensitivity of D-dimer levels in AAD patients of 94.5% [95% confidence interval (CI) 78.1–98.8%, $P < 0.001$] and concluded that D-dimer levels can be used to rule out AAD in low-risk patient, results similar to those stated by Sodeck *et al.* It must be mentioned that there was heterogeneity in results of the studies, which the authors adjusted for prior to meta-analysis of the data. Pathophysiologically, this correlation is believed to be secondary to tissue injury leading to release of tissue factor, which then activates the coagulation cascade and consequent fibrinolytic activity, eventually leading to increased D-dimer values.

These studies provide an interesting aspect to a disease, which requires timely, cost-effective and convenient means of diagnosis. Currently, AAD does not have any validated screening tests, which meet these criteria. As seen in these studies, serum D-dimer assays are a possible gateway to rule out disease. However, it is imperative

that patients first be clinically stratified, as current data suggests that serum D-dimer assays are only useful to rule out disease in low-risk patients. Also larger RCT trials are warranted to validate these results, prior to advocating usage of D-dimers to rule out disease in such patients.

Aortic aneurysm

Aortic aneurysm poses a significant burden to healthcare costs and patient quality of life. Owing to fear of aneurysmal rupture and ensuing complications, it is often serially imaged and surgically repaired when deemed most appropriate. Although there are no guidelines regarding the usage of biomarkers in this disease entity, there is substantial evidence associating haemostatic biomarkers and AA. Golledge *et al.*[26] assessed serum D-dimer levels in a cohort of 1260 patients. When the study data were analysed using the multiple logistic regression model, amongst all the risk factors assessed, plasma D-dimer had the most powerful association with AA with an odds ratio (OR) of 12.1 and 24.7 for plasma cut-offs of 400 and 900 ng/mL, respectively. The AAA diameter growth also correlated with serum D-dimer values: average yearly AAA growth was positively and significantly correlated with rank-transformed D-dimer. Most importantly, a higher plasma concentration of D-dimer (>900 ng/mL), correlated with a faster aneurysmal growth rate while lower D-dimer concentrations (≤150 ng/mL) correlated with slower growth rates. Sidloff *et al.*[27] published a review and meta-analysis of data from 22 studies assessing fibrinogen, D-dimers, and thrombin-antithrombin complex III (TAT) levels in cases of AAA. Upon pooled analysis of the data, it was observed that patients with AAA's had a significantly higher plasma concentration of D-dimer (mean difference, 325.82 ng/mL; 95% CI, 199.74–451.89 ng/mL; $P \leq 0.00001$). Meta-regression analysis of the studies found that D-dimer concentration and AAA diameter were highly significant having a strong, linearly, positive association ($r^2 = 0.94$; $P \leq 0.0001$).

A study of cadaveric dissection of AAA reported that nearly all AAA's consist of a non-occlusive mural thrombi.[28] The thrombi were further dissected and analysed for thrombolytic molecule release using RT-PCR, immunohistochemical staining as methods of measurement. It was observed that the thrombus was the epicentre of fibrinolytic activity, leading to the release of PAP's and D-dimers into the blood.[28]

Further research and trials are warranted to investigate disease management utilizing D-dimers assays. Assay levels would not only diagnose the condition but also, more importantly, predict rate of growth of the aneurysm alerting clinicians to higher risk patients requiring closer monitoring. Currently other than imaging modalities, there is no other way to follow this pathology. Patients assessed to have a faster than usual rate of growth, irrespective of their aneurysmal size would benefit from having a surgical evaluation and thus prioritize resources for patients who need them the most. Again because there is little research on the matter, more work needs to be conducted to assess the validity of this relationship. We feel that clinicians would benefit greatly if a simplified 'rate of growth' equation of sorts could eventually be established by serial monitoring of d-dimer assays helping clinicians easily siphon out high-risk patients from low risk for purposes of closer monitoring. Owing to limited data, there is no such imminent relationship postulated. Further research work needs to be carried out in order to evaluate whether the biomarker holds it strength in assessing disease as seen by authors in currently available data and to establish an integral role in management of AAA.

Peripheral artery disease

Although current guidelines establish PAD as CAD equivalent, medical management to prevent MACE in this cohort is not often aggressively addressed. Rehring *et al.*[29] studied 2839 patients with PAD and reported that 33.1% were taking a β-blocker, 32.5% were taking angiotensin-converting-enzyme inhibitor/angiotensin receptor blocker, and 31.3% were taking a statin. Although their PAD symptoms were being treated, physicians often did not address risk factors for MACE. They also reported that risk factor control proved to be more challenging than patients in PAD than those who suffer from CAD/CVD, a conclusion similar to that of the REACH registry investigators.[30] D-dimers assays, which assess PAD, in such cases, could be an objective measure to risk stratify PAD patients and consequently the intensity of medical management. The Edinburgh study is one of the earlier studies that assessed the association of D-dimers with PAD. Lee *et al.*[31] investigated a cohort of 1592 PAD patients and concluded that D-dimers linearly increased with severity of disease in both sexes. On multivariate analysis, fibrin D-dimer was independently related to the risk of intermittent claudication and amongst men, to the extent of arterial narrowing in the lower limb which corroborated with the ankle brachial pressure index.[31] Fowkes *et al.*[32] followed 617 patients diagnosed with PAD and concluded that D-dimer assays were predictive of both coronary events and progression of PAD. The investigators involved in these studies attributed their findings to fibrinothrombolysis, which leads to increased assays and corroborates with atherosclerotic burden. It is worth mentioning that D-dimers if measured in PAD patients just one time are of limited significance due to possible inter-subject variability.[33] Engelberger *et al.*[33] investigated the inter-subject variability of the biomarker because biomarker trends would be effective to monitor disease only when if the biomarker remained steady and did not have other factors affecting it. To assess inter-subject variability, they investigated a total of 80 patients, divided into two groups; one consisting of patients suffering from PAD and the other a control group consisting of healthy individuals. The research concluded that although variability of biomarkers did exist, D-dimers were one of the most reliable biomarker and least subject to inter-patient variation. To minimize for variability, the mean of approximately three readings could be used for purposes of serial trending. Although this study has several limitations namely small sample populations and existence of confounders, it emphasizes an undersighted yet important fact; serum biomarkers can be subject to variation and prior to engaging in studies which hinge on biomarker measurement, authors should be aware of its inherent variability within patients.[33]

Coronary atherosclerosis

The value of D-dimers in the diagnosis and risk stratification of patients with CAD remains controversial. There is evidence that

D-dimers could potentially have a clinical role much greater than once envisioned and may be a helpful in identifying the severity of CAD.[34] In the Edinburgh artery study, Fowkes *et al.*[32] followed 617 patients diagnosed with PAD to assess this relationship. They concluded that patients with intermittent claudication have increased risk of cardiovascular events, and D-dimer assays were predictive of future coronary events. This studies' patient cohort was later prospectively followed by Smith *et al.* to analyse new ischaemic heart disease (IHD) and stroke events. D-dimer assays were predictive of myocardial infarction on univariate, but not multivariate analyses.

Another nested case–control, prospective study assessed the association of patient's baseline D-dimer values and future coronary artery events.[35] Investigators concluded that patients who had baseline plasma concentrations of D-dimers exceeding the 95th percentile, had twice the risk of future myocardial infarction's than those with lower levels.[35] These findings held true in multivariate analysis when adjusted for non-lipid confounders, however, were attenuated when controlled for total and high-density lipoprotein cholesterol. The plausible operational hypothesis is that the underlying coronary atherosclerotic activity may lead to fibrinolysis, and a resulting increase in serum D-dimer levels.[36] As coronary thrombosis precedes myocardial necrosis, it is possible that coagulation markers such as D-dimer may be sensitive markers of ACS, rising earlier than markers of myocardial necrosis (e.g. troponins). Although D-dimers have been associated with VTE and AA; however, these are much longer sized vessels and it remains questionable if the detection of fibrinothrombolytic activity in small-sized arteries such as the coronaries can be reliably detected with the biomarker.

The Caerphilly study cohort was re-examined to assess 1998 men, only to conclude that graded increases in plasma D-dimer values were independently associated with major IHD events.[37] This relationship was also studied in another nested prospective cohort in which 5661 men were monitored for ~16 years for outcomes of all-cause mortality and cardiovascular morbidity.[38] Patients were categorized in order of increasing D-dimer. Those in the upper third of D-dimer values (tertile cut-offs, >94 vs. <49 ng/mL) had OR for coronary heart disease (CHD) was 1.67 (95% CI, 1.31–2.13; $P < 0.0001$) after adjustments for age. The OR increased slightly after further adjustment for smoking, and other cardiac risk factors, and indicators of socioeconomic status (1.79; 95% CI, 1.36–2.36).[38] These results were consistent with a meta-analysis of D-dimer levels in IHD and/or PAD.[32] The clinical utility of these observations has not yet been determined.

Acute myocardial infarction

Acute myocardial infarction is probably the most famed complication of atherosclerotic disease and its timely diagnosis is one of the fundamental principles of Emergency Medicine. Although the classic biomarkers involved in routine diagnosis of AMI are troponin and creatine kinase MB, there have been studies assessing the role of D-dimers in this clinical scenario. Shitrit *et al.*[39] conducted a prospective observational study assessing a cohort of 124 patients who presented to the Emergency Department (ED) with a diagnosis of ACS and had normal cardiac biomarkers. They observed that D-dimer levels positively correlated not only with sex, hypertension, and smoking but also with the clinical, laboratory, and ischaemic electrocardiographic findings, catheterization findings and with hospital length of stay. In another study by Shitrit *et al.*, D-dimer levels were prospectively followed in a cohort of 81 patients who presented to the ED with unstable angina.[40] By multivariate analysis, D-dimer level, age, and sex were predictors of hospital length of stay ($P = 0.018$), suggesting that D-dimer levels at admission to the ED may serve as an additional prognostic tool in patients with unstable angina pectoris.[40] Prisco *et al.*[41] conducted a trial and saw that increased D-dimer levels post-percutaneous coronary intervention (PCI) in patients with AMI was associated with subsequent restenosis. This relationship did not hold true in patients who underwent elective angioplasty. Although this study had a small sample, it points out interesting results which if remain valid in a larger RCT, could help identify high-risk patients who would warrant longer duration anti-platelet treatment.

Although the studies by Shitrit *et al.* show statistical significance between D-dimer levels and inherent risk factors in AMI patients, they do not offer insight if the biomarker has any clinical impact on patient management. In order to assess the diagnostic value of D-dimers in AMI cases, Lippi *et al.*[42] conducted a trial in which they measured serum Troponin T (TnT) levels and D-dimer levels in patients who presented to the ED for symptoms suggestive of AMI in order to assess if the two biomarker corroborated with each other in case of AMI and if D-dimers could reduce the window of ER lab workup to diagnosis. Eligible samples were those collected prior to medical intervention and within 12–24 h of arrival to ED. In the final data analysis, 741 patients were included, out of which 252 had TnT >0.03 μg/L. Although the D-dimer value distribution (median and 95% CI) was significantly different in patients with increased cTnT values, linear regression analysis revealed no significant association between D-dimer and cTnT in patients with cTnT levels exceeding the decisional threshold of the assay. Accordingly, these authors deduced that D-dimer assays in the clinical setting of AMI, did not provide additional information to TnT assays alone. Although ample data are available on the prognostic role of D-dimers predicting cardiovascular events in patients with CAD, to our knowledge limited studies have been done to assess this role in the cohort of AMI patients treated with PCI and followed on dual anti-platelet therapy. The HORIZONS-AMI[43] study analysed a subset of patients with STEMI undergoing PCI for assessment of the prognostic role of D-dimer assays and risk of subsequent cardiovascular events. Patients with higher D-dimer levels at time of discharge had a two-fold increase in their risk of an MACE, which is a composite of all-cause death, recurrent MI, stroke, or target vessel revascularization for ischaemia at 3-year follow-up.[44]

A similar study was done to evaluate the prognostic role of D-dimers in ACS patient population. Researchers stratified STEMI patients undergoing PCI into tertiles with respect to increasing serum D-dimer levels upon hospital admission and concluded that high admission D-dimer levels were associated with increased in-hospital cardiovascular mortality and 6-month all-cause mortality.[45]

In a subset of the ESTEEM study,[46] 518 patients who had AMI were followed after D-Dimer was measured. Higher D-dimer levels were not prognostically relevant. A recently published study assessed the role of serum D-dimers and angiographic reflow rate,

as well as long-term prognosis.[47] An increased D-dimer level was associated with increased mortality at long-term follow-up. However, this finding was related to no-reflow phenomenon in patients who had higher D-dimer levels, which was independently predictive of MACE.[47] When removing this confounding factor, elevated D-dimer was predictive of MACE but not of mortality.

These studies indicate that although D-dimer assays do not offer superior diagnostic insight than currently available tests, it is a consistent prognosticator of cardiovascular disease. It is not very well understood how this biomarker can be used to clinically monitor these patients and thus further workup is required to validate the extent of the biomarker's efficacy. Current limited data suggest that the biomarker would be best suited to stratify patients who have higher risk of future MACE. Unfortunately due to lack of specificity, the biomarker is unlikely to accurately indicate what particular event would occur and instead its value would lie in serving a 'red flag' lab value, which would help clinicians to recognize patients who need more intensive therapy than others.

Cerebrovascular events

There have been many studies assessing the biomarker's association with cerebrovascular accidents (CVAs). Ageno *et al.*[48] reported on D-dimer levels in predicting stroke subtype. The study included patients who were hospitalized secondary to a possible acute CVA. The study consisted of 126 cases and 63 appropriately matched controls. D-dimer levels were measured at multiple intervals during hospitalization. Patients with transient ischaemic attack (TIA) had significantly higher D-dimer levels than controls at all measurement intervals. No statistical difference was found between the patients with stroke and TIA. D-dimer levels did not correlate with stroke severity. When D-dimer assays were analysed with respect to stroke subtype, it was observed that D-dimer levels were significantly higher in the groups of patients with a cardioembolic stroke or a TIA (2.96 ± 0.51 μg/mL) than in the groups with an atherothrombotic (1.34 ± 0.21 μg/mL; $P < 0.05$) or a lacunar (0.67 ± 0.08 μg/mL; $P < 0.01$) event. There was also a statistically significant difference in D-dimer values between the atherothrombotic and lacunar subgroups; however, no difference was noted in the lacunar group and control population. Serial measurement of D-dimer measurements showed that after ~12 days, there was an increase in serum assays in the cardioembolic and atherothrombotic groups, but no change was seen in the lacunar group.[48] The recently published EPICOR study investigated this clinical relationship. A nested case–control design studied a sub-cohort of 832 patients who were compared with 289 control patients, over a mean follow-up of 9 years. Their data analysis revealed that individuals with elevated D-dimer levels had a higher incidence of both haemorrhagic and ischaemic stroke.[49] This was independent of common confounders such as sex, blood pressure, and CRP. After stratification for stroke type, the hazard ratio for every standard deviation increase was similarly significant both for ischaemic (1.21; 95% CI: 1.01–1.45) and haemorrhagic (1.24; 95% CI: 1.00–1.65) strokes. Authors concluded that low D-dimer levels in the first few hours are a sensitive biomarker test to rule out cardioembolic stroke and may be useful to guide further investigations.[50] Owing to abundant imaging done in cases of stroke, not much work has been done to assess these findings further. Clinical scenarios where D-dimers would add further to diagnosing stroke subtype would be few as imaging is near essential as clinical workup of stroke. To date, there is inconclusive data assessing if D-dimers could be used to rule out stroke.

Barber *et al.*[51] studied a pool of ischaemic stroke patients. Patients whose neurological functioning deteriorated as a result of progression of stroke were seen to have significantly increased D-dimer levels. They postulated that these differential levels found, could be secondary to ongoing thrombus formation within cerebral vessels, suggesting that serial measurement in already diagnosed stroke patients would be of more value when compared with using the biomarker solely for diagnostic purposes. To date limited data exist which assess D-dimer's role in the prediction of future stroke events. The Edinburgh study noted to not only the relation of D-dimer assays to incidence to CAD, but also the incidence of stroke. This was one of the first studies which reported that D-dimers were independently related to the risk of subsequent stroke.[52] The Caerphilly study also investigated the relation of haematological biomarkers and their relationship to the risk of cardiovascular diseases and ischaemic stroke. They concluded D-dimer was strongly predictive of both CHD events and prospective stroke.[53] Wannamethee *et al.*[54] also examined abnormalities in the fibrinolytic system and its association with the risk of stroke. They prospectively studied 3358 men with no previous diagnosis of myocardial infarction or stroke, and without atrial fibrillation, followed-up for an average of 9 years, during which there were 187 stroke events. Amongst the biomarkers investigated, D-dimer levels were most strongly associated with the risk of stroke especially amongst hypertensive individuals. Even after adjusting for traditional stroke risk factors, the risk of stroke was observed to be strongly co-related to increasing serum D-dimer assays. Similar to other atherosclerotic diseases discussed earlier, serially trending D-dimer assays could offer a means of stratifying patients who have increased risk of future stroke events and consequently require closer clinical monitoring.

Wu *et al.*[55] conducted a meta-analysis comprising of 22 000 patients to assess if increased biomarkers are predictive of increased risk of stroke in patients suffering from atrial fibrillation. They concluded that elevated circulating PAI-1 and TAT was significantly associated with increased risk of stroke in patients with AF; higher levels of D-dimer were associated with increased subsequent thrombo-embolic events risk. The authors proposed that D-dimers being a by-product of fibrinolysis are predictive of level of hypercoagulability in atrial fibrillation. Based on available data, adding serum assay levels such as D-dimers in scoring schemes such as $CHADS_2$ or CHA_2DS2-VASC would improve its stroke predictive accuracy.[56]

Other potential uses

In another study inflammatory markers, including D-dimers were assessed in HIV patients on anti-retroviral therapy. D-dimer levels were measured prior to treatment and then at different serial intervals. D-dimer levels were seen to be significantly lower in treated cases compared with controls and upon stopping treatment, D-dimer levels increased back up to pre-treatment levels.[57] Keeping in mind that anti-retroviral treatment has mortality benefits for HIV

patients, measuring these serum biomarker levels can serve as a means to assess patient compliance to treatment.

Santulli *et al.*[58] conducted an experimental study in which they aimed to use microRNA-based technology to selectively modulate cell growth aiming to allow stent re-endothelization while minimizing smooth muscle proliferation and consequent stent thrombosis. In order to assess extent of hypercoagulability, the authors used D-dimer as a surrogate marker. In cases where microRNA-based vectors successfully decreased neointimal growth, D-dimer assays were low, implying that there was reduced thrombotic activity in the stents microenvironment.[58] Being an animal model experimental study, it would be premature to project results to existing clinical scenarios. However, this strongly suggests that D-dimers being a marker of hypercoagulability, if assessed in the appropriate circumstances, can give an idea to underlying thrombotic activity, which may manifest as atherosclerotic disease complications. Going forward, as clinical experiments advance in human studies, D-dimers can possibly pose to be a biomarker that gives researchers an idea of experimental modifications in extent of coagulation and thrombosis.

Conclusions

Elevated serums D-dimers are a direct consequence of fibrinolysis and thus direct indicators of disorders of increased thrombotic activity such as DIC and VTE. However, studies indicate that potential uses of this biomarker could also be applied to atherosclerotic disorders, myocardial infarctions, stroke, AAs, and aortic dissections. In clinically risk-stratified patients, its role as a gateway diagnostic test for purposes of detecting PE is well established. If used in appropriate patient cohort, not only is it sensitive but retains sufficiently high specificity as well. Effective usage of serum D-dimers can clarify when and which type of imaging study is needed for patients with respect to diagnostic and therapeutic purposes. Although much is known about D-dimers diagnostic role, studies have shown that increased D-dimer levels at time of diagnosis indicate worse short-term mortality outcomes and prognosis. Also during anticoagulation treatment, studies reveal that abnormal D-dimer levels could be indicative of recurrent PE. Although this is not currently validated clinically, these serum assays may be used to determine ideal anticoagulant treatment duration for individual patients.

Studies have seen association of the biomarker with various other disease pathologies. In cases of AAs increased serum D-dimers have been strongly suggestive of the presence of AA, suggesting a role as a possible screening tool to this end. Even more interestingly higher serum assays have been associated with faster annual rate of aneurysmal growth, indicating that serum levels could be possibly used to stratify patients into high-risk groups who would warrant closer medical monitoring. Aortic dissection, which has no screening biomarker test available currently, was assessed to have elevated D-dimer assays, and if the test is done shortly from onset of symptoms, it was very effective to rule out acute dissections due to high negative predictive values. These results have been reproduced consistently in multiple studies and could be an effective tool to exclude acute dissection in low–moderate risk patients. Owing to different lab testing kits to measure assays, it is a challenge to propose a standard cut-off value; however, further studies need to be done to possibly establish reference cut-off values with each lab testing kit.

The biomarker's potentially most valuable clinical role yet to be explored is its potential ability to assess atherosclerotic burden and determine future MACE. In recent years, several studies have seen that patients with known atherosclerosis (both PAD and CAD) had increased baseline D-dimer levels and these levels were predictive of increased future MACE. In PAD patients D-dimer levels corroborated closely with severity of PAD. When this relationship was studied in patients suffering from AMI, the biomarker was not only high at time of ED admission but also predictive of future MACE in these patients. These results held significance even after adjustment for traditional cardiac risk factors, suggesting a prognostic role in cases of cardiovascular diseases. Interestingly enough, post-cardiac stenting, elevated assays were seen to be associated with future stent restenosis.

Studies have also observed elevated serum D-dimers in patients diagnosed of acute stroke/TIA and interestingly enough levels were noted to be higher in certain pathological subtypes than others. Also similar to CAD, studies have also assessed whether baseline levels could be predictive of future events of stroke. Although there is limited data, studies have observed increased levels to be associated with increased future stroke events especially in hypertensive patients. D-dimers are not only predictive of stroke subtype but also predictive of future stroke events, similar to results with PAD and CAD patients.

Studies strongly indicate that D-dimers, due to being a measure of thrombo-embolic activity, corroborate with atherosclerotic burden. Although current data suggest that greater the atherosclerosis, the greater the baseline biomarker assays and consequent stroke events and MACE, further studies need to be done to better understand these findings. Owing to these findings being of great importance to patients, it is imperative larger controlled prospective trials be conducted in order to further understand this prognostic role of the biomarker and patients in whom these findings are most relevant. These studies need to characterize which patients can be followed with serum D-dimer assays, serum cut-off levels and their practical management, and prognosis with respect to serum levels amongst other features. Owing to low specificity, it will be a challenge in knowing exactly when further testing is warranted and when can serial monitoring of the lab be appropriate. Also clinicians will have to be truly sure of disease in question as there are bound to be high burden of falsely positive results if testing is not done cautiously.

There have also been trials which have used D-dimer levels to monitor HAART treatment compliance. It is seen that medically compliant patients have decreased D-dimer levels and when treatment is stopped, levels return back to baseline. HAART treatment has been proved to improve mortality outcomes in HIV patients and thus a biomarker measuring compliance could be greatly beneficial for clinical purposes.

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