D-dimer as a marker for cardiovascular and arterial thrombotic events in patients with peripheral arterial disease

A systematic review

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Summary

Peripheral artery disease (PAD) is associated with an increased risk for cardiovascular events. D-dimers are a marker for hypercoagulability and are linked with thrombotic events in patients with venous as well as arterial thrombosis. The predictive value of plasma D-dimer levels in relation to cardiovascular events in patients with PAD is not unambiguously established. It was our objective to gather evidence evaluating the value of D-dimer as a predictor of arterial thrombotic events patients with PAD. The Pubmed, Embase, and Cochrane databases were searched (January 1980-November 2012), and 65 abstracts were found. The strategy was supplemented with manual review of reference lists. Case-control, cohort or prospective cohort studies that measured fibrin D-dimer in patients with PAD, were included. Studies were excluded if there was no follow-up for arterial thrombotic events or when no specific information on D-dimer was available. The search yielded 10 studies for our analysis, comprising 2,420 patients with PAD, with a total of 1,036 cardiovascular events in 10,599 patient-years. Two studies with a follow-up of one year showed that fibrin D-dimer predicts both deterioration of PAD and subsequent thrombotic events. Five out of six studies with a median follow-up of 2–4 years revealed that an increased D-dimer is predictive of various arterial thrombotic events including mortality. Two studies with a longer follow-up (over 6 years) did not show an independent association between increased D-dimer levels, arterial thrombotic events and CVD mortality. In conclusion, an increased D-dimer appeared to be independently associated with a two times increased risk of near-term cardiovascular events (relative risk 2.30, 95% confidence interval 1.43–3.68). However formal meta-analysis was only feasible for four out of 10 included studies. Due to the extended heterogeneity of the included studies cautious interpretation of these data is warranted.

Keywords

Peripheral arterial disease, D-dimer, fibrin degradation products, intermittent claudication, arterial thrombotic events

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Introduction

Peripheral arterial disease (PAD) is a manifestation of generalised atherosclerosis and is associated with coronary artery disease (CAD) and cerebrovascular disease (CBVD) (1). The mortality rate of patients with claudication is approximately 2.5-times higher than that of patients without claudication (2). Literature shows an annual overall major cardiovascular (CV) event rate (comprising myocardial infarction [MI], ischaemic stroke and vascular death) of approximately 5-7% (2). This high risk of death is only partially explained by the coexistence of ischaemic heart disease (3-5). Within 15 years after the initial diagnosis the all-cause morbidity and mortality rates increase up to 70%, and only 20-30% of these patients die of non-cardiovascular causes. Despite extensive research, there still is poor understanding of the natural history of PAD.

Conventional risk factors: e.g. age, smoking, male sex, and CAD have been associated with increased morbidity and mortality rates (3, 5, 6). More recently thrombogenic and inflammatory risk factors have also been implicated in the pathogenesis of PAD, as well as the acute cardiovascular complications (7-11). In selected populations, an association between plasma D-dimer level and an increased risk of venous and arterial thrombosis has been proposed (10). In cardiovascular disease (CVD), an increased fibrin turnover may represent not only a prothrombotic state, but also be a marker for the severity of atherosclerosis (10, 12, 13). Based on a variety of experimental data, it now seems evident that activated coagulation and atherogenesis are closely linked processes (14).

It is known that D-dimer is associated with an increased risk of arterial thrombotic events, irrespective of baseline vascular disease, even after adjusting for confounders such as age, smoking and diabetes, as has been shown in various large prospective

studies published over the last 20 years (15-29). In most of these studies a relative risk of around 2 was found for D-dimer in relation with future MI, ischaemic heart disease or stroke. In a number of the studies, the cohorts were screened for concordant PAD. However, as these PAD-patients were part of the total study-population and the associations were solely calculated for this total population, it was impossible to transpose the results to PAD-patients only (30, 31).

Although the risk factor profile for cardiovascular events is comparable for PAD and CAD/CBVD, the incidence of future events is higher in PAD than the latter two groups (32). Despite clear guidelines on risk factor management in the PAD-population, recent studies show that risk factor control in patients with PAD is reached less frequently in comparison with patients with CAD or CBVD (33, 34). This stresses the need for further optimisation of risk factor management in PAD patients, as it is known that with good risk factor control the rate of major CV events in PAD-patients is lower. Given the high risk of CV death in patients with PAD and the uncertainty regarding the individual risk profile, there is a need for simple markers that can help identify patients at risk. D-dimer is such a simple test, which is widely available and adds no extra burden as part of routine CV risk assessment. High-risk patients could thus be monitored and treated more intensely. Our objective was therefore to assess the utility of D-dimer as a marker of morbidity and death due to arterial thrombotic events in the PAD population.

Methods

A systematic literature search was performed including the Pubmed, Medbase and Cochrane databases to locate all prospective clinical studies on D-dimer as a marker for arterial thrombotic events. We searched the three databases for publications from January 1980-November 2012 using the subject headings ("fibrin degradation products OR D-dimer") AND ("peripheral arterial disease OR intermittent claudication OR claudicants") AND ("coronary artery disease OR myocardial infarction OR stroke OR amputation OR thrombotic events OR mortality"). The following search filters were applied: English language, studies using human adults of 19+ years of age.

In an effort to prevent a possible publication bias the authors searched a clinical trials registry (clinicaltrials.gov) for possible (unpublished) studies concerning this topic. Since no studies were found with this search, a publication bias cannot be completely excluded; however, it seems less apparent.

Study selection

Studies were eligible for this review if:

- The study contained data on patients with either baseline stated intermittent claudication (IC), symptomatic or asymptomatic PAD of the lower extremities. PAD had to be confirmed with either an ankle brachial index (ABI) <0.9, other diagnostic (imaging) tests such as Doppler ultrasound or angiography showing arterial stenosis or patients had to be treated with stenting or revascularisation for flow limiting stenosis,
- Information on baseline levels of D-dimer could be extracted
- Information on CV events or (CV) mortality in the follow-up could be extracted.

Studies were excluded if:

- Patients had no baseline IC or PAD,
- Fibrin D-dimer measurement was not included as a possible predictor of CVD,
- There was no follow-up for arterial thrombotic events or (CV) mortality.

Manual search of the references of all studies meeting the inclusion criteria and abstracts was carried out. Two of the authors (MCK, AtC) reviewed all identified titles and abstracts and independently assessed eligibility (►Figure 1). Both authors reviewed all data and consensus was reached for all data described. In addition, the quality of all studies included in the review was assessed, using a validated generic checklist designed for quantitative studies (35) (►Table 1).

Data extraction

Data were extracted making use of a standard data extraction form, including:

- Information on the study including author, journal, and year of publication,
- Total number of patients per included study,
- Patient characteristics (patients with PAD alone or in conjunction with other atherosclerotic burden (coronary artery disease etc),
- Definition of PAD,
- Type of D-dimer assay,
- Outcome measures (total number of arterial thrombotic events and D-dimer values),
- Statistical analyses, results and adjustments.

Patients and event calculation

Calculation of the number of included patients and CV events during follow up was performed by creating a summary table. In this table the number of included patients, the mean follow-up time and the number and the type of events were registered. Per study, the number of included patients was multiplied with the mean follow-up time in years to calculate the total number of patient-years. The number and type of events (if stated in the article) were registered to assess whether they were due to CV causes or non-CV causes. Only the CV events were taken into account in the event calculation. Because we have included two publications that reported on early findings of the same study populations in two later reported studies with increased follow-up duration, we excluded these early papers from the calculation (with the exception of 10 patients in the Fowkes-study [40] with one year follow-up

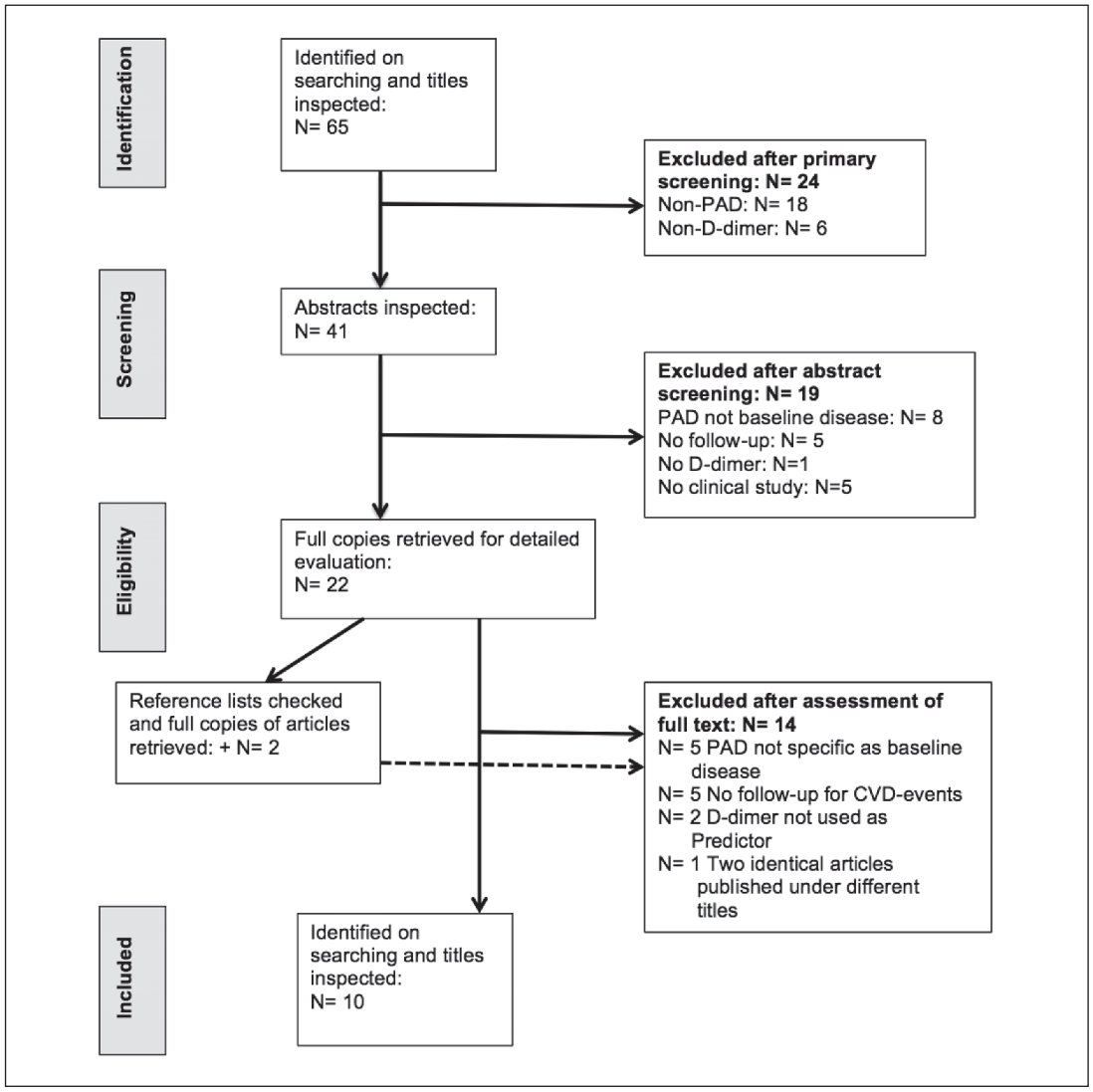

Figure 1: Study selection.

that were not included in the Smith-study [29] with 6 years follow-up).

Study quality assessment

Assessment of the methodological quality of the included studies was performed using a checklist for quality assessment for evaluating primary research papers from a variety of fields (35). This checklist, with a scoring system based on fourteen standardised questions, allows appraising the methodological quality of quantitative studies. This checklist includes criteria which are consistent with the recommendations from the Centre for Reviews and Dissemination (CRD) (36). The quality of the included studies was appraised independently by two authors (MK and AtC); inter-rater agreement was reached on all criteria (▶Table 1).

Analysis

The 10 selected studies presented different effect size estimates. Some studies indicated the mean difference of levels of D-dimer between patients who experienced CV events and patients who did not (37, 38). Other studies used relative risks (RR) (3, 39-42) or hazard ratios (HR) as estimates of relative risk for CV events. To determine sources of heterogeneity, sensitivity analyses were performed in case of considerable heterogeneity (defined as $I^2 \geq 50\%$). Fixed-effect models or random-effects models were incorporated in the generic variance method of data pooling in Review Manager 5.1 (The Cochrane Collaboration).

Results

Study identification

Our search identified 65 papers. Of these, 24 were excluded based on the title not meeting the inclusion criteria. Of the 41 potentially

Table 1: Methodological quality assessment of the included studies.

	Fowkes 1993 (40)	Smith 1997 (29)	Cortellaro 1994 (37)	Boneu 1998 (42)	Komarov 2002 (41)	Bosevski 2005 (39)	Musicant 2006 (45)	Vidula 2008 (9)	Vidula 2010 (38)	Criqui 2010 (44)
1. Clearly defined objective	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
2. Appropriate study design	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
3. Patient selection appropriate	Y	Y	Y	Y	Y	Y	PA	Y	Y	PA
4. Subject characteristics sufficiently described	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
5. Interventional and random allocation described	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
6. Interventional and blinding investigators reported	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
7. Interventional and blinding subjects reported	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
8. Outcome well defined	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
9. Sample size appropriate	Y	Y	N	Y	Y	PA	PA	PA	PA	PA
10. Analytic measures described appropriately	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
11. Estimate of variance reported	Y	Y	Y	Y	Y	PA	Y	Y	Y	Y
12. Controlled for confounding	Y	Y	N	Y	Y	Y	Y	Y	Y	Y
13. Results in sufficient detail	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
14. Results support conclusions	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y

Abbreviations: Y; Yes, N/A; Not Applicable, PA; Partially, N; No.

eligible studies using D-dimer on patients with either claudication, or diagnosed PAD, 19 had to be excluded based on the abstract, since the patients had no PAD (or claudication) at baseline, or because there was no follow-up for arterial thrombotic events. Hence, of 22 studies the full text was analysed. One article appeared to be published under two different titles, but the content of the full article was identical for the two publications (39, 43). After manual screening of the reference lists two extra studies that met the primary inclusion criteria based on title and abstract were found (6, 42). After assessment of the full text, 14 studies were excluded because they did not meet the inclusion criteria. Finally, ten prospective, observational studies combined measurements of D-dimer at baseline with follow-up for arterial thrombotic events in patients with either diagnosed peripheral arterial disease or claudication (►Figure 1).

After a second full text assessment, two publications that reported on early findings of the same study population as reported in later studies, were identified (9, 40); we decided to report the findings of both studies in the narrative part of this systematic review. Therefore in total 10 studies were included (►Figure 1). The 10 identified studies represented a total number of 2,420 patients (corrected for the overlap of the two extra studies) with either diagnosed PAD (ABI ≤0.9) or (intermittent) claudication with a total of 1036 CV events in 10,599 patient-years, which translates to 9.77 CV events per 100 patient-years.

The majority of the studies were prospective, observational cohort-studies where D-dimer was used as a laboratory parameter to predict arterial thrombotic events. We created a summary table of study characteristics. The primary outcome was either short-term (1-2 years), or long-term (3-6 years) incidence of all arterial thrombotic events in patients with either claudication or objectively confirmed PAD in relation to a D-dimer measurement at baseline (►Table 2).

General characteristics of the included studies

Studies identified for this review were published between 1993 and 2011. Three studies contained a patient population where PAD was confirmed with an ABI <0.9 (9, 38, 42), in one other study an ABI of either <1.00, or > 1.40 was deemed indicative of PAD (44). Two studies included a target population of subjects with symptoms of intermittent claudication, and in most of these patients an index ABI confirmed the diagnosis of PAD (29, 40). In two studies PAD had to be confirmed with either angiography, or ultrasound Doppler and duplex scanning of the lower limb arteries (37, 41). One study included only patients with coexisting PAD and CAD (39), and one other study only included patients with symptomatic disease and at least one lower extremity vascular surgical procedure (45).

Table 2: Characteristics of the included studies.

Source	Type of study	Study population	Outcomes of interest	Confounders	Results	Conclusion
1993 Edinburgh Fowkes, Housley et al. (40)	Longitudinal study Mean FU: 1 year	n=617 Consecutive patients with intermittent claudication Mean ABI: 0.58 ± 0.2	- Deterioration of PAD - Risk of coronary events: death, coronary heart disease	- Age - Sex - Smoking - Other coagulation, fibrinolytic and rheological factors	N=36 CHD-events Coronary events RR: 4.4 (1.3–19.0) between top and bottom quintiles (DD < 65 ng/ml vs DD > 179 ng/ml)	Increased concentration XLFDP predict coronary events and progression of PAD
1994 Milan Cortellaro, Turri et al. (37)	Case-control study Mean FU: 1 year	n=50 Patients with ath. lesions in ≥ one of 10 leg arteries Doppler confirmed or a documented history of revascularisation. Mean ABI: 0.74 ± 0.2	- (non)fatal MI - Sudden cardiac death - (non)fatal stroke - TIA - Acute peripheral ischaemia - Peripheral bypass occlusion	- Age - Fibrinogen - tPA antigen - delta tPA antigen - fibrinol. activity - delta fib. activity - PAI-1 - total cholesterol - HDL cholesterol - triglycerides	N= 18 events DD cases: 182.55 ng/ml (± 21.89) DD controls: 136.44 ng/ml (± 16.41)	D-dimer was significantly higher in subgroup with subsequent thrombotic events
1997 Edinburgh Smith, Lowe et al. (3)	Cohort study Mean FU: 6 years	n=607 Consecutive patients with IC Mean ABI: Not stated	Combined: - (non)fatal stroke - MI - AP - coronary events – death - coronary and vascular procedures	- Age - Sex - PY - Systemic blood pressure - Glucose - Baseline evidence IHD (angina and/or MI)	N= 160 IHD and stroke Total RR: 1.26 (0.97–1.65) S RR: 1.27 (0.87–1.85) NF IHD RR: 1.37 (0.97–1.92)	Haemostatic factors may contribute to prediction of CVD risk, but no longer significant when adjusted for confounders
1998 Royat Boneu, Leger et al. (42)	Prospective study Mean FU: 2 years	n=324 Patients with moderately severe forms of PAD Mean ABI: 0.64 ± 0.17	- MI - angina - cerebral ischaemic event - clinical progression of PAD: amputation, surgery	- History of smoking - Arterial hypertension - Hyper-cholesterolaemia - Other haemostatic factors	N=51 events D-dimer R: 1.7 (95% CI 0.86–3.47), p = 0.12	Levels of D-dimer not predictive of vascular event when adjusted for confounders
2002 Moscow Komarov, Markova et al. (41)	Longitudinal study Mean FU: 4.2 years	n=121 Patients with PAOD and stable claudication (FS II-III), confirmed by angiography/Doppler and duplex scanning of the lower limb arteries Mean ABI: Not stated Fontaine stage: IIA 7.5% IIB 85% III 7.5%	Fatal and non-fatal thrombotic events in any main arterial area: – sudden cardiac death - definite MI - ischaemic stroke - deaths from non-vascular causes	- Conventional cardiovascular risk factors - Baseline clinical manifestations of cardiac, cerebral, and peripheral atherosclerosis	N=38 thrombotic events DD > 861 ng/ml: RR 5.99 (1.3–27.2)	Increased DD is independent haemostatic predictor of thrombotic events in PAD patients
2005 Skopje Boševski, Borozanov et al. (39)	Observational study Mean FU: 3 years	n=90 Patients with PAD and CAD (previous MI/ myocardial revascularisation / clinical symptoms) Mean ABI: 0.58 ± 0.23	- Mortality	- Smoking - Age - Dislipidaemia - Arterial hypertension	N=126 events (acute MI, heart failure, peripheral thromboembolism Hazard ratio: 2.55 for onset cardiovascular events	Patients stratified for elevated DD (>300 ng/ml) higher mortality than other patients

Table 2: continued

Source	Type of study	Study population	Outcomes of interest	Confounders	Results	Conclusion
2006 Oregon Musicant, Moneta et al. (45)	Prospective trial Mean FU 3.2 years	n=332 Patients with symptomatic PAD (and/or cerebral vascular disease) in at least one lower extremity and only when ≥ 1 leg had undergone surgery Mean ABI: 0.75 ± 0.24	Composite of: - ABI progression – stroke - MI - amputation - death from CV disease - death from any cause	- Age - Gender - Diabetes mellitus - Hypertension - Log-cholesterol - Log-homocysteine level - Smoking	N=19 CVD-death N=161 primary composite Hazard ratio: 2.31 (1.12–4.76)	D-dimer significantly associated with time to subsequent MI (not with stroke, amputation, CV death)
2008 Chicago San Diego, Boston Vidula, McDermott et al. (9)	Prospective cohort study Mean FU: 3.4 years	n=377 Consecutive patients with lower extremity testing with ABI <0.9. Mean ABI: 0.659 ± 0.008	- (Cardiovascular) death including deaths due to: - coronary heart disease - stroke - PAD - other CV disease	- Age - Sex - Race - Diabetes - Number of CV diseases - Smoking - ABI	N=76 all cause death, not specified for CVD Hazard ratio 1 year: 1.2 (1.08–1.33) Hazard ratio 2 years: 1.14 (1.02–1.27)	Increased DD and inflammatory biomarkers independently associated with higher mortality
2010 Chicago Vidula et al. (38)	Observational prospective study Mean FU: 3.7 years	n=569 Consecutive patients with PAD (ABI ≤ 0.9). Mean ABI: 0.64 ± 0.15	- (CV) death	- Age, gender, race - ABI and BMI, PY - Stroke - Angina, MI and heart failure - Diabetes - Hypertension - Pulmonary disease - Cancer - TC and HDL	N=43 CV-death	Not stated in the text: DD is associated with CVD events
2010 Chicago Boston Chapel Hill Criqui, McDermott et al. (44)	Cohort study Average FU: 6.6 years	n=379 Patients seen in previous 10 years for non-invasive arterial testing with ABI <1.00 or >1.40, or PPTF-velocity of <11 cm/sec, or previous leg vascularisation Mean ABI: Not stated	- All cause mortality - CVD mortality - non-CVD mortality	- Age - Gender	After 6.6 years: N= 150 CVD-death. Comparison survivors vs CVD-mortality: DD: 920 ng/ml vs DD 1020 ng/ml, respectively p-value 0.4	DD did not meet p <0.1 inclusion criterion for the multivariable survival models: DD did not significantly predict mortality

Results given with 95% confidence intervals. Abbreviations: XLFDP; cross-linked fibrin degradation products, IC; intermittent claudication, PAD; peripheral arterial disease, MI; myocardial infarction, CHD; coronary heart disease, F; fatal, NF; non-fatal, TIA; transient ischaemic attack, AP; angina pectoris, IHD; ischaemic heart disease; PAOD; peripheral obstructive arterial disease, FS; Fontaine stage, ABI; ankle-brachial index, CVD; cardiovascular disease, CA; coronary angiography, CV; cardiovascular disease, CBVD; cerebral vascular disease, PPTF; peak posterior tibial flow velocity, RR; risk ratio, HZ; hazard ratio, DD; D-dimer, PY; pack-years, TC; total cholesterol.

There was variability in the set exclusion criteria concerning previous procedures, disease severity, CV morbidity and medication for the studies included in this review. Also, the follow-up duration differed; three studies had a follow-up of 1-2 years, five studies had an average follow-up of 3-4 years while two studies had a mean follow-up of >6 years. Four studies were performed in the USA (Chicago and Oregon), the other six studies were centered in Europe (Royat, Milan, Edinburgh, Moscow and Skopje). A point of practical interest is the type of D-dimer assay used. Nine of the 10 studies included in this review made use of a quantitative D-dimer assay; only in one study a semi-quantitative assay was used (▶Table 3).

Study quality

Overall the studies were of acceptable to good quality: the overall reporting of the studies, the study design and selection of study population and statistical analysis were adequate. Since all studies

were observational, no randomisation and blinding reports were applicable. All studies reported the results in sufficient detail and drew conclusions based on the stated results. The main weakness of some of the studies was the number of included patients, which was often too small to yield an adequate number of outcomes (►Table 1).

Analysis

The data representation of five out of 10 of the included studies did not allow us to make 2x2 tables or use the generic inverse variance method. The mean estimated risk for an increased D-dimer for the prediction of arterial thrombotic events could therefore only be calculated for a subgroup of studies. We pooled the data of four studies (38, 40-42), based on the fact that they were similar for duration of follow-up (short to intermediate), patient population (ABI 0.58-0.64 or Fontaine IIB), all analyses were adjusted for confounders, and the outcome measures could be considered similar. All studies reported on (combined) fatal and non-fatal CV events except for the study by Vidula et al. (38) that solely reported CV mortality. However, in excluding the data from this study, the heterogeneity increased, resulting in an I^2 of 40%, p=0.19. Therefore we decided against excluding this study. The meta-analysis now includes data of four studies. Pooling the RRs resulted in an overall relative risk of 2.30 (95% CI 1.43-3.68) with an I^2 of 16% (p=0.31) (►Figure 2).

Study results

The first study that assessed the value of D-dimer as an independent predictor for arterial thrombotic events was performed in 1993 by Fowkes et al. (40), followed by a report on the extended follow-up of six years for the same study population in 1997 by Smith et al. (3). In the first report, results of 617 consecutive patients diagnosed with PAD were published. In accordance with findings of the previous Northwick Park study (6), plasma fibrinogen concentration and age were the most important independent predictors of coronary events. In addition, the plasma concentrations of cross-linked fibrin degradation products (XLFDP) were independently associated with coronary heart disease (CHD) events (combined fatal and non-fatal), as well as progression of PAD, the latter being measured by a decrease in ABI. Relative risks for combined CHD events were shown as quintiles of baseline-XLFDP expressed relative to the lowest quintile. An increased baseline-XLFDP was independently associated with a fall in ABI (indicating further progression of PAD) as well, even after adjustment for baseline ABI.

In 1997, the long-term outcomes for the remaining PAD patients (n=607) were presented (3). The prospective cohort showed that baseline levels of fibrin D-dimer were higher in claudicants who subsequently developed ischaemic heart disease or stroke during this longer follow-up when compared to the group that did not develop such events. However, after adjusting for confounders (as stated in ►Table 2) none of the haemostatic factors were significant predictors anymore. The authors concluded that haemostatic factors, one of which being D-dimer, might have a possible contributory role in the prediction of CVD.

Table 3: D-dimer tests used in the assessed studies.

Study	Product	Supplier
Fowkes et al. (40)	ELISA	Agen
Smith et al. (3)	ELISA	Agen
Cortellaro et al. (3)	Dimertest®	Ortho
Boneu et al. (42)	Asserachrom® D-dimer	Stago/Roche
Komarov et al. (41)	ELISA	Boehringer-Mannheim
Bosevski et al.(39)	Turbiquant® D-dimer	Dade-Behring
Musicant et al.(45)	Electro immunoassay	Trinity Biotech
Vidula et al. (9)	Asserachrom® D-dimer	Stago/Roche
Vidula et al. (38)	Asserachrom® D-dimer	Stago/Roche
Criqui et al. (44)	K-assay D-dimer kit	Kamiya

In 1994 the Italian group of Cortellaro et al. found in a cohort of 50 patients with leg atherosclerosis, combined in 40% of the cases with coronary and/or cerebral atherothrombotic disease, that the subset of patients that experienced subsequent thrombotic events had higher D-dimer level at baseline than the patients remaining free of thrombotic events during the follow-up of one year (37). A major weakness of this study was, however, an insufficient sample size and therefore the data could not be subjected to multiple regression analysis (37).

In the study by Boneu et al. patients with moderately severe PAD (n=324) were included while on annual stay in the thermal resort of Royat (42). Blood samples were taken during the first 10 days of the patients stay with follow-up examinations performed at one and two years during subsequent visits. D-dimer levels were associated with a slight, progressive increase in risk of a vascular event across successive higher quartiles (quartile 4 vs quartile 1, p=0.02); however, these results became non-significant when major vascular events (vascular death, cerebral ischaemia, cardiac events, acute peripheral ischaemia) were considered separately. When the relative risks were adjusted for potential confounders, D-dimer levels were no longer predictive of a vascular event (RR=1.7, with 95% CI 0.86-3.47: P= 0.12). As potential explanations for these findings the authors suggested that the included patients only presented with PAD of moderate severity, while subsequent life style modification may have further attenuated the risk of thrombotic events.

In 2002, Komarov et al. intended to evaluate the frequency of thrombotic events with peripheral arterial occlusive disease during a follow-up period of 3-5 years and determine whether the baseline levels of haemostatic factors, including D-dimer, were related to the risk of future thrombotic events (41). During the mean observation period, from entry to final evaluation (average of 4.2 years) there were 54 validated outcome events. The main findings were that PAD patients who had D-dimer levels that exceeded the upper limit of normal were at increased risk of developing thrombotic events, it was shown that a baseline D-dimer level in the

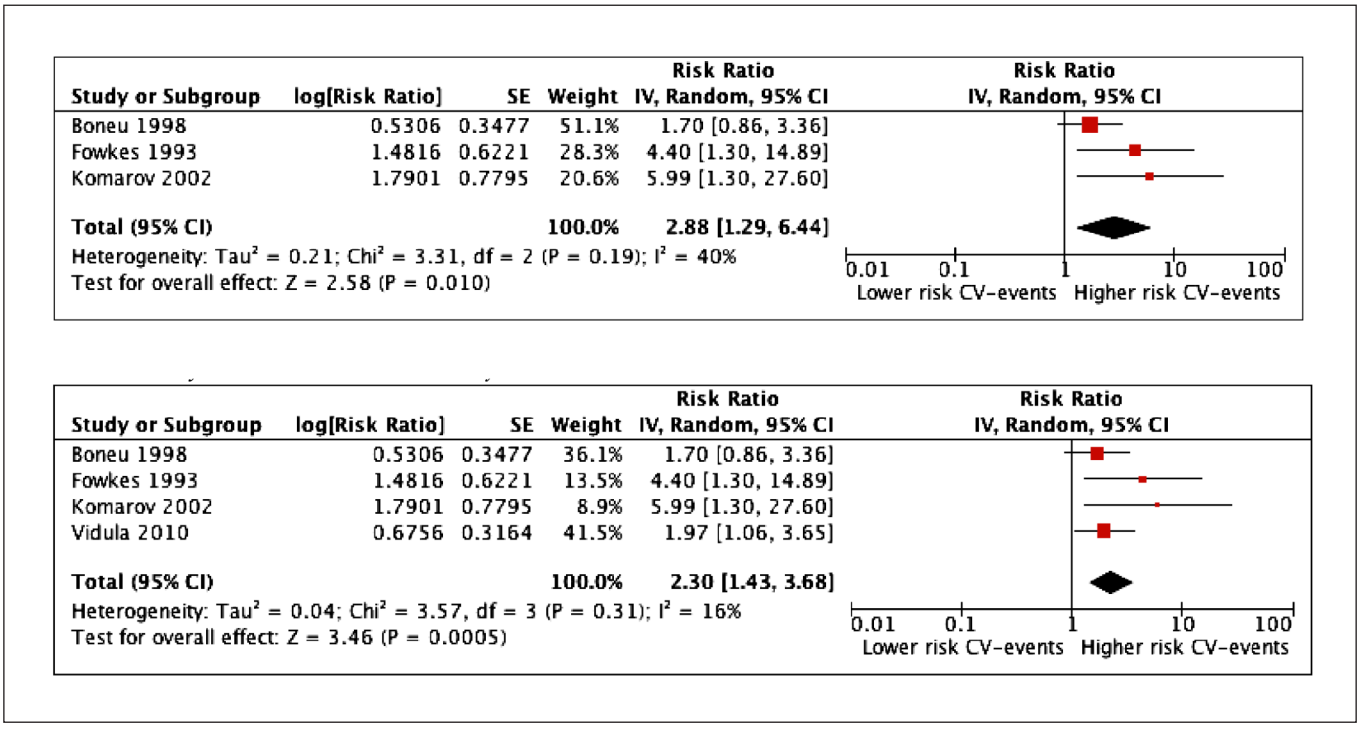

Figure 2: Meta-analysis. A) 2010 Vidula study (38) excluded. B) 2010 Vidula study (38) included.

highest quintile (>861 ng/ml) was associated with arterial thrombotic events compared to a baseline D-dimer level of the lowest quintile (<280 ng/ml). This finding was independent of conventional CV risk factors and baseline clinical manifestations of cardiac, cerebral and peripheral atherosclerosis. The authors emphasised the relevance of fibrinolytic activity in relation to the D-dimer increase, in showing an inverse correlation between D-dimer and PAI-1 activity.

Bosevski et al. (2005) examined whether the value of D-dimer in plasma could predict mortality in patients with both PAD and CAD. In the 90 patients with coexistent PAD and CAD, the subset stratified for an elevated plasma level of D-dimer >300 ng/ml showed a higher mortality. The authors concluded from this, that D-dimers are a strong predictor of mortality in patients with PAD and CAD, supporting the concept that activated thrombogenesis dictates the clinical outcome (39).

A study performed around the same time by Musicant et al. assessed the relationship between elevated baseline levels of D-dimer (and biomarkers of inflammation) and the progression of PAD in patients with symptomatic PAD (45). Remarkably, patients were only included in this study if they had undergone vascular surgery in at least one leg or carotid artery. The hypothesis that D-dimer was associated with progression of PAD and/or CAD was not confirmed, as only a significant association between baseline D-dimer and MI was found (p=0.04). Explanations for the fact that no other associations were found were the limited sample size as well as the length of follow-up [average of 38.4 months (± 20.4 months)]. The authors drew the overall conclusion that, even though the D-dimer levels are significantly associated with time to subsequent MI, there is little role for the use of D-dimer as initial screening test for the presence of PAD or for the risk of progression of symptomatic PAD.

Vidula et al. published two studies on D-dimer and PAD (9, 38). The objective of the first study, published in 2008, was to determine whether elevated levels of D-dimer were more closely associated with short-term rather than long-term mortality in patients with PAD (n=377) and whether greater increases in biomarker levels were associated with higher mortality rates during the first year after the increase than during the later years. It was found that higher levels of D-dimer were associated with higher all-cause mortality among patients who died within one year, and in the time span between one to two years after biomarker measurement. Elevated levels of D-dimer were not associated with greater all-cause mortality two to three years after the measurement. Among the deceased, it was found that D-dimer levels were highest at the last visit prior to death. Higher D-dimer levels during each follow-up interval were statistically significantly associated with CV mortality. The results did not substantially vary when analyses were repeated and adjusted for covariates including age, sex, race, diabetes, number of CV diseases, smoking and the ABI. A greater increase in D-dimer level between baseline measurement and one-year follow-up measurement was associated with a significantly increased risk for all-cause mortality and CVD-mortality during the first year after the first annual follow-up visit. Increase in D-dimer level between baseline and two-year follow-up measurement was only associated with a higher all-cause mortality, but not CVD-mortality, during the following year after the increase. According to the authors short-term increases in D-dimer levels may

reflect temporary increases in atherosclerotic plaque remodelling. A limitation of the study was that especially for CVD death sample sizes were too small to confidently predict mortality. Comparing their study to previous ones, the authors mentioned that CV disease accounted for fewer than 50% of the deaths, something that possibly could be attributed to improved treatment of atherosclerotic risk factors.

In the study performed by Vidula et al. in 2010 (38) the primary objective was different to that of the 2008 study: to determine whether statin use was associated with CV mortality in PAD patients according to presence or absence of increased D-dimer levels. The included patient population of this study was, according to the authors (Dr. McDermott, personal communication), partially identical to that of the 2008 study (9). The median level of D-dimer was 650 ng/ml (measured in baseline samples of 587 patients). During the mean follow-up of 3.7 years, 43 of the in total 129 deaths were attributable to CVD. Of the patients with a D-dimer level above 650 ng/ml 28 died due to CV causes, of the patients with a D-dimer below 650 ng/ml only 14 died due to CVD. Statin users had a significantly lower all-cause and CVD-related mortality compared to statin nonusers. Since no statistically significant interaction was found between the high versus low D-dimer level and statin use in the analyses of statin use with mortality it was concluded that participants with baseline D-dimer values >650 ng/ml did not have significantly greater benefit from statin use than those with baseline D-dimer values <650 ng/ml.

The main objective of Criqui et al. (2010) was to determine whether novel biomarkers, including D-dimer, could improve risk prediction of (CVD) mortality beyond the standard risk factors in PAD. Noteworthy in this study is the definition of PAD, which was set as a peak posterior tibial flow velocity of <11 cm/second, a previous leg revascularisation (angioplasty or surgery) or an ABI of <1.00, or >1.40. Cox-proportional hazard models were fitted for the mortality endpoints, being all-cause mortality, CVD mortality and non-CVD mortality for two years and at long-term follow-up. Beside CRP, none of the other biomarkers, including D-dimer, significantly predicted any kind of mortality. According to the authors, however, at a full follow-up, the already known standard risk factors dominated the c-statistic. This led to the conclusion that the morbidity and mortality in PAD patients may depend on the baseline risk composition of the cohort and the length of the risk period.

Discussion

We conducted this systematic review of the literature to identify the value of D-dimer as a predictor of adverse CV events in PAD patients. Ten studies from six different Western countries were included in the review. To the best of our knowledge this is the first systematic review featuring all available evidence on D-dimer and the follow-up of patients with PAD exclusively.

We found that increased D-dimer was independently associated with a two times increased risk of near-term CV events (RR 2.30, 95% CI 1.43-3.68). However, formal meta-analysis was only feasible for four out of 10 included studies. We were able to pool data for these studies (38, 40-42) because they were similar for duration of follow-up (short to intermediate), patient population (ABI 0.58-0.64 or Fontaine IIB) and outcome measures.

Overall, we encountered several sources of heterogeneity, making it difficult to perform a meta-analysis for most study data. In some studies patients were included directly upon diagnosis of PAD, while in other studies patients that had already been diagnosed with PAD during the past decade could be included. In the majority of the studies, the duration of PAD was not clearly described making it difficult to draw proper conclusions. While diagnostic criteria upon inclusion in general will adjust for major differences in severity of PAD, the use of slightly deviant criteria in some studies may have had some impact on the study results, but also on the baseline D-dimer levels. Extent of the disease was difficult to assess as study populations differed in apparent duration of disease at baseline.

Concomitant vascular disease, another possible indicator of the extent of the disease, was quite variable in the different studies. In the study performed by Bosevski, for example, only patients with concomitant coronary and PAD were included, whereas in the Boneu study all patients with concomitant vascular disease were excluded from participation. Comorbidity, such as cancer or other (inflammatory) diseases could also have influenced baseline D-dimer levels, as well as study outcomes. Several studies therefore excluded patients with cancer or evidence of diseases with a possible fatal prognosis in the following two years. In the Vidula studies (9, 38), however, the percentage of included patients with cancer and pulmonary disease was quite high (up to 23% and up to 38%, respectively). The average baseline D-dimer level found in these populations was therefore probably also relatively high (>950 ng/ml), clearly higher in comparison to the baseline levels of the other studies. Unfortunately, in most of the included studies cancer or inflammatory disease rates were not even assessed and made it therefore unclear what the influence was on the baseline D-dimer levels.

The percentage of patients with diabetes and arterial hypertension varied from 8% to 55.5% and from 20% to 91%, respectively. Previous studies have not observed consistent significant correlations of D-dimer with diabetes mellitus (type 2) and systolic blood pressure (46, 47). However, it is known that diabetes and hypertension can contribute to CV mortality. The difference in included patient populations, although probably not contributing to differences in baseline D-dimer levels, might have significantly influenced the study results. As most studies were performed around the end of the 20th century, the treatment of PAD for all patients of the studies included in the review can be considered rather well comparable with similar attention for lifestyle changes, antiplatelet therapy and lipid control. Nevertheless, in the majority of studies the statin and antiplatelet administration was not described making it difficult to estimate the influence on outcome and D-dimer levels.

Comparing the 10 studies, a division can be made into three different groups: studies with a short-term follow-up of one year, studies with an intermediate follow-up of 2-4 years and studies

with a long follow-up duration of over six years. The studies performed by Fowkes and Cortellaro, both with a follow-up of one year showed that fibrin D-dimer predicted both deterioration of PAD and subsequent thrombotic events (37, 40). When the duration of the follow-up of the Fowkes-cohort increased to over six years, D-dimer levels lost their independent significant association after adjustment for confounders. Also in the study by Criqui 2010, D-dimer was not able to predict neither all-cause-, nor CVD-mortality. Nevertheless, the three studies with an intermediate follow-up that could be pooled do show a significant positive association with increased D-dimer (38, 41, 42). The study performed by Boneu et al. with a two-year follow-up, showed no predictive value of D-dimer in arterial events when adjusted for confounders such as smoking etc. The average onset of disease however was 9.3 years before inclusion in the study (42).

A pathophysiological explanation for the association between high plasma D-dimer levels and the increased risk of arterial thrombotic events in the near-term period following the measurement can possibly be found in the fact that D-dimer is part of the so-called inflammation-coagulation-axis. The role of inflammation in atherosclerosis progression and plaque destabilisation has been clearly established (increase in C-reactive protein [CRP] and other pro-inflammatory mediators like interleukin [IL]-1 and IL-6), while the role of coagulation in this process is not yet fully clarified (14). In previous studies it has been found that an increased CRP and atherosclerotic progression are positively associated with an increase in fibrinogen and a procoagulant response causing an increase in the formation of cross-linked fibrin (29). It has been suggested that the generation of several haemostatic enzymes (e.g. thrombin) and the increase in fibrin contribute to atherosclerosis progression and modulation of the plaque phenotype. Indeed, cross-linked fibrin is a detectable component of the atherosclerotic plaque (16). It may be assumed that a greater vascular burden of fibrin generates a larger amount of fibrin cleavage fragments, due to ongoing fibrinolysis, in patients with extensive atherosclerosis (16). Supporting this model is the fact that with an increased severity and larger extent of PAD, and subsequently a higher fibrin level, also higher D-dimer levels are found (11). In a general population with presumed lower disease severity a recent meta-analysis showed that D-dimer was indeed more modestly, but still significantly associated with first CHD events (47).

An important question that remains is whether D-dimer is: a. causally related to thrombogenesis through temporary increase in plaque remodeling leaving a plaque more prone to rupture or b. solely an indicator of increased fibrinolysis. However, in both cases it seems likely that the fibrinolytic system is a fast acting system that responds to the acute situation (as needed in the normal situation to preserve haemostasis) and therefore a good predictor of near-term events. The traditional atherosclerotic risk factors (smoking, diabetes, etc.) predict long-term CV events and mortality better than D-dimer levels (9).

Variation in D-dimer tests has been an issue of debate because of the potential discrepancies between test outcomes due to differences between calibrators and different manufacturer cut-off values (48). However, Boeer et al. showed that even though there was no standardisation among the commercially available D-dimer assays, most assays showed similar characteristics concerning the detection of D-dimer fragments (49). The largest factor influencing the D-dimer outcome was suggested to be the cut-off values used by the manufacturers in relation to the study population. In the assessed studies self-set cut-off values for high vs low levels of D-dimer based on the baseline measurements of the study population and not on the manufacturers cut-off values were used. Manufacturer influences on cut-off values are therefore not to be expected.

Just as with follow-up duration, comparing the 10 studies for D-dimer levels, a division can be made into three different groups: studies with a low D-dimer cut-off (<200 ng/ml), studies with an intermediate D-dimer cut-off value (200 to <900 ng/ml) and the studies with a high D-dimer cutoff value (>900 ng/ml). These variations might be due to concomitant diseases in the different study populations, influencing the height of the mean baseline D-dimer levels. Both in studies with a high mean baseline D-dimer level and in studies with a lower mean baseline D-dimer level significant associations were found between D-dimer and arterial thrombotic events.

This review supports the hypothesis that plasma D-dimer is a potential risk predictor for CV events. However, it remains unclear whether D-dimer, or the formation of the precursor product, cross-linked fibrin, plays a causal role in the pathophysiology of these adverse events, or whether D-dimer is simply a marker of the extent of the disease and therefore related to the event (50).

Conclusion

Elevated levels of D-dimer are associated with a two times increased risk of arterial thrombotic events and CV mortality in the time period following this measurement in patients with PAD and could therefore be applied as a simple tool to identify patients at increased risk. Elevated D-dimer seems to be a better predictor for the short-term (<4 years) rather than the long-term risk of arterial thrombotic events and CV mortality.

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Conflicts of interest

None declared.

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