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Non-invasive endothelial function testing and the risk of adverse outcomes: a systematic review and meta-analysis

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Objectives We performed a systematic review and meta-analysis to understand the role of flow-mediated dilatation (FMD) of the brachial artery (BA) and peripheral arterial tonometry (PAT) in predicting adverse events, including cardiovascular (CV) events and all-cause mortality.

Background FMD of the BA and PAT are non-invasive measures of endothelial function. Impairment of endothelial function is associated with increased CV events. While FMD is the more widely used and studied technique, PAT offers several advantages. The purpose of this systematic review and meta-analysis is to determine whether brachial FMD and PAT are independent risk factors for future CV events and mortality.

Methods Multiple electronic databases were searched for articles relating FMD or PAT to CV events. Data were extracted on study characteristics, study quality, and study outcomes. Relative risks (RRs) from individual studies were combined and a pooled multivariate RR was calculated.

Results Thirty-six studies for FMD were included in the systematic review, of which 32 studies consisting of 15, 191 individuals were meta-analysed. The pooled RR of CV events and all-cause mortality per 1% increase in brachial FMD, adjusting for potential confounders, was 0.90 (0.88–0.92). In contrast, only three studies evaluated the prognostic value of PAT for CV events, and the pooled RR per 0.1 increase in reactive hyperaemia index was 0.85 (0.78–0.93).

Conclusion Brachial FMD and PAT are independent predictors of CV events and all-cause mortality. Further research to evaluate the prognostic utility of PAT is necessary to compare it with FMD as a non-invasive endothelial function test in clinical practice.

Keywords Endothelial function • Cardiovascular outcomes

Introduction

Endothelial function is a major contributor to vascular health. The endothelium regulates vasomotor tone, smooth muscle cell proliferation, platelet aggregation, monocyte and leucocyte adhesion and thrombosis. Decreased nitric oxide (NO) bioavailability leading to vasodilator dysfunction is the initial step to atherosclerosis and has been shown to predict cardiovascular (CV) events, even in patients with angiographically normal coronary vessels.[1] Therefore, endothelial dysfunction is reflective of atherosclerotic risk[2] and measurement of endothelial function may serve as a prognostic marker for future CV events.[1]

Since endothelial dysfunction is a systemic process, it can be assessed in both the coronary and peripheral circulation.[2] Intracoronary or intrabrachial infusions of vasoactive agents offer direct quantification of vascular response to NO and are considered the gold standard for endothelial function testing.[3] However, these methods are invasive and not suitable for bedside evaluation. Thus,

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non-invasive techniques to assess endothelial function, such as brachial artery (BA) flow-mediated dilatation (FMD) and peripheral arterial tonometry (PAT), have been developed. Although these techniques do not directly assess coronary endothelial function, they have been shown to correlate well with more invasive measures.[2]

Brachial FMD indirectly assesses vascular endothelium dilation in response to shear stress forces.[3,4] During the FMD test, an acute decrease in blood flow is induced by inflating an arm cuff to suprasystolic pressure for 5 min. During this period NO (and other vasoactive molecules) is released from the endothelial cells and result dilatation of the BA. Upon release, there is a characteristic increase in flow which can then be assessed by Doppler ultrasound. Numerous patient, environmental, and procedural factors can influence FMD and thus, adequate subject preparation and a standardized approach are necessary for accurate FMD measurements.[5]

PAT is a novel method of measuring endothelial function by using finger plethysmography to assess pulse wave amplitude (PWA) at rest and during shear stress. Reactive hyperaemia-PAT (RH-PAT) index is calculated as a ratio of PWA signal after cuff release compared with baseline as calculated through a computer algorithm. The advantages of PAT are that the subject's contralateral arm serves as an internal control and it requires minimal training with low intraobserver variability.[6] PAT has been shown to correlate with endothelial function in several populations and predict CV events.[7]

The prognostic value of brachial FMD for CV events has been demonstrated in two previous meta-analyses.[8,9] Since then, several prospective studies have been published which further add evidence to the role of FMD. Although the prognostic value of PAT has been demonstrated in a few studies, no overall quantitative estimate of risk exists. Therefore, we performed a systematic review and meta-analysis to examine the prognostic impact of brachial FMD and PAT on CV outcomes in all populations. We hypothesized that endothelial dysfunction as measured by brachial FMD and PAT is independently associated with future CV events and all-cause mortality.

Methods

Design and study selection

Studies were deemed eligible if they: (i) were prospective observational studies with follow-up time of ≥6 months, (ii) evaluated brachial FMD or PAT, (iii) reported CV events or mortality, (iv) calculated a hazards ratio or relative risk (RR), (v) included human adults, and (vi) available in English.

Data sources and search strategy

We searched the following electronic databases: PubMed MEDLINE, Embase, the Cochrane Central Register of Controlled Trials, the Health Technology Assessment database (Cochrane Library), Scopus, and Science Citation Index (Web of Science). The database search was supplemented by searching the trials registry ClinicalTrials.gov. Peer review of the PubMed MEDLINE search was conducted in accordance to the Peer Review of Electronic Search Strategies checklist.[10] An experienced librarian conducted the searches 24 July–2 August 2012 and updated 27 February 2013. Search terms included: endothelial function, ultrasound, flow-mediated dilation (FMD-BA), brachial artery reactivity test (BART), PAT, CV events, reactive hyperaemia, myocardial infarction, death, prediction, risk, and prognosis. The final PubMed strategy and complete list of search terms are available at the authors' institutional repository.[11]

Article eligibility criteria

Abstracts were independently evaluated by two reviewers (B.L. and Y.X). Any article deemed potentially relevant by either reviewer was retrieved for full-text review. The full-text articles were then independently assessed for eligibility. Disagreements were resolved by consensus after discussion with a third reviewer (N.T).

Data extraction

The following information was extracted from each study: (i) study characteristics, such as year of publication, study design, study population, and sample size; (ii) method of endothelial function assessment (iii) CV outcomes measured (non-fatal CV outcomes and death) and number of events that occurred during follow-up; (iv) duration of follow-up; (v) method of statistical analysis and univariate hazard ratios (HRs); and (vi) multivariate HR and covariates in the multivariable analyses. The studies were divided into cardiovascular disease (CVD) and non-CVD groups based on patient recruitment criteria. Patients who had established CVD, including coronary artery disease, cerebrovascular disease, congestive heart failure, and peripheral vascular disease (PVD) at the start of the study were categorized into the CVD group, whereas patients without established CVD or PVD formed the non-CVD group.

Statistical analysis

The risk estimates of each study were reported as HR, RR, or odds ratio. We treated HRs as RRs. In the studies reviewed, FMD was treated as either a continuous or categorical variable. If FMD was reported as a categorical variable, we converted it into one continuous RR using Greenland and Longnecker's[10] covariance-corrected generalized least-square trend (GLST) estimation method. In this meta-analysis, RR represents the increase in risk per 1% increase in brachial FMD. To assess the robustness of our meta-analysis, we examined the following study characteristics in subgroup analyses: study population (CVD vs. non-CVD, age, sample size, mean FMD, duration of follow-up), FMD technique (forearm vs. upper arm occlusion), risk of bias, and study outcome (CV mortality vs. all-cause mortality). In order to evaluate the effect of baseline BA diameter on the association between FMD and outcomes, we also performed a meta-regression of studies that reported the BA diameter.

Two of the three studies relating to PAT described HR as per 0.1 increase in the natural logarithmic scaled reactive hyperaemia index (RHI). One study treated logarithmic RHI as a categorical variable and was converted into a continuous RR using GLST estimation method. Owing to the limited number of studies, no subgroup analyses were performed.

Risk of bias assessment

The risk of potential bias was examined in the included studies using a modified Newcastle Ottawa Scale (NOS). This scale evaluates cohort studies for bias in selection, comparability, and outcome. There are eight NOS items: representativeness of the exposed cohort, selection of the non-exposed cohort, ascertainment of exposure, demonstration that outcome of interest was not present at start of study, comparability of cohorts on the basis of the design or analysis, assessment of outcome, length of follow-up and adequacy of follow-up of cohort.

Publication bias was also assessed by visual inspection for funnel plot asymmetry.

Results

Search results and study selection

The initial search strategy retrieved 6092 individual citations for screening. After the initial screen, 121 studies were selected for full-text review. We then excluded 82 articles that did not meet the study selection criteria on full-text review. The final set consisted of 39 studies,[7,11–48] 36 of which examined FMD and 3 studies regarding PAT. Thirty-two of the FMD studies were included in the meta-analysis. (*Figure 1*) The remaining four studies[43–46] involved special populations and were not meta-analysed due to clinical heterogeneity. The details of included studies are summarized in *Tables 1* and *2*.

PAT study characteristics

Three studies[7,47,48] involving 865 subjects were included in the PAT meta-analysis. All the studies examined patients with established CVD, including patients with non-ST elevation myocardial infarction (NSTEMI) and heart failure. The sample sizes were similar between studies, ranging from 215 to 329 participants and mean follow-up ranged from 10 to 70 months. The pooled univariate RR per 0.1 increase in L_RHI was 0.82 (0.76–0.89) and the pooled multivariate RR per 0.1 increase in L_RHI was 0.85 (0.78–0.93; *Figure 2*).

FMD study characteristics

The 36 included studies examining the prognostic value of FMD for CV events and mortality encompassed a total of 15 544 participants. The studies were divided into categories based on whether the study cohort consisted of patients with absence of established CVD or PVD, presence of established CVD/PVD, and special populations. The pooled univariate RR per 1% increase in FMD was 0.90 (0.88–0.93) and the multivariate RR per 1% increase in FMD was 0.90 (0.88–0.92; *Figure 3*).

Established CVD/PVD

Nineteen studies[24–42] included patients with established CVD. The mean follow-up time ranged from 6 months to 11 years and sample sizes ranged from 73 to 444 individuals. Ten studies reported a univariate RR. The pooled univariate estimate of the RR was 0.90 (0.87–0.94).

Sixteen studies reported a multivariate RR, of which only four studies adjusted for all the traditional Framingham CV risk factors such as age, sex, lipids, smoking, and blood pressure. The remaining studies adjusted for numerous other variables, including medications and comorbidities depending on the study population. The pooled

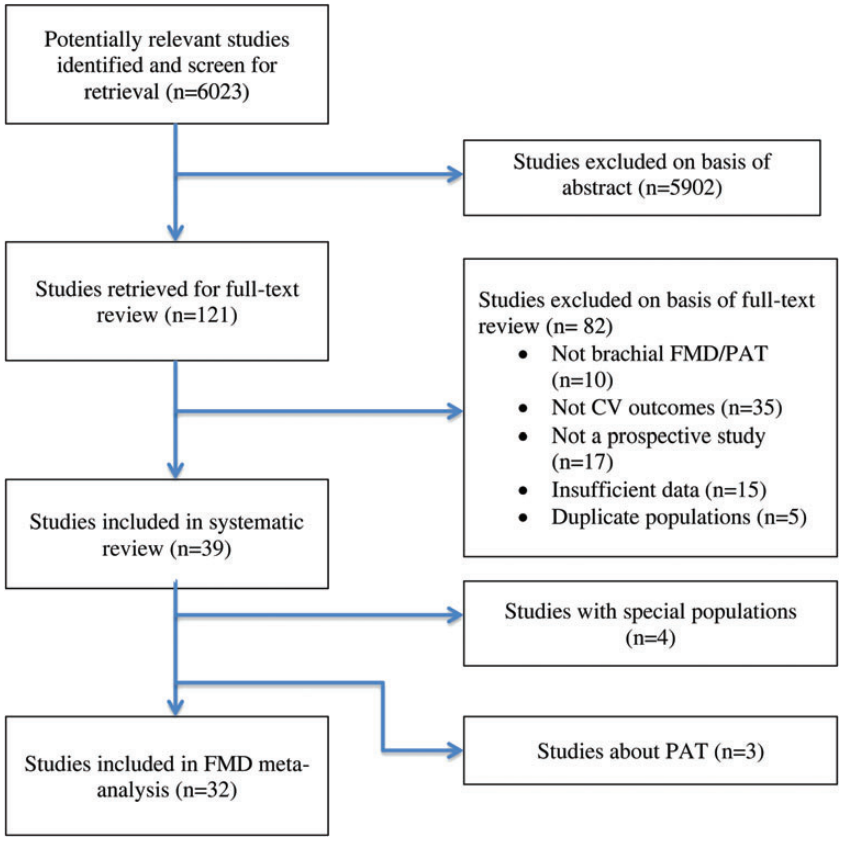

Figure 1 Flow chart for study selection.

Table 1 FMD study characteristics

Study	Sample size (*n*)	Gender (% male)	Age (years)	Follow-up (months)	Number of events	Population	Study outcomes
No Established CVD							
Akishita *et al.*[11]	171	100	47 ± 13	77 ± 46	20	Males with coronary risk factors	CV death, CVE
Anderson *et al.*[12]	1574	100	49 ± 10	86 ± 20	71	Male fire-fighters	CV death, CVE
Corrado *et al.*[13]	84	77	62 ± 12	24	21	Asymptomatic subjects	CV death, CVE
Hirsch *et al.*[14]	268	63	53 ± 11	45 ± 21	19	Asymptomatic subjects	All-cause mortality, CVE
Huang *et al.*[15]	205	69	63 ± 14	24	29	Patients with chest pain symptoms	CV death, CVE
Kanbay *et al.*[16]	283	51	53 (imputed)	38	111	Subjects with chronic kidney disease	CV death, CVE
Lind *et al.*[17]	1016	48	70	60	101	Subjects 70 years old	All-cause mortality, CVE
Muiesan *et al.*[18]	172	59	56 ± 8	95 ± 37	32	Subjects with hypertension	CV death, CVE
Rossi *et al.*[19]	2264	0	54 ± 6	45 ± 13	90	Post-menopausal women	CV death, CVE
Suzuki *et al.*[20]	819	43	66.5 ± 8.8	81 ± 21	84	Subjects ≥40 years old	CVE death, CVE
Yilmaz *et al.*[21]	304	52	46 ± 12	41	89	Chronic kidney disease patients	CV death, CVE
Yeboah *et al.*[23]	1330	67	63.8 ± 9.5	91	94	Asymptomatic subjects	CV death, CVE
Yeboah *et al.*[22]	3026	50	61 ± 10	60	182	Asymptomatic subjects	CV death, CVE
Established CVD							
Akamatsu *et al.*[24]	93	88	71 ± 7	47 ± 13	18	Subjects with atherosclerosis	CV death, CVE
Brevetti *et al.*[25]	131	90	64 ± 10	23 ± 10	39	Subjects with peripheral arterial disease	CV death, CVE
Careri *et al.*[26]	60	73	62 ± 8	32	14	Subjects with NSTEMI	CV death, CVE
Chan *et al.*[27]	127	69	67 ± 11	30	12	Subjects with ischaemic/ haemorrhagic stroke	CV death, CVE
Fathi *et al.*[28]	444	60	58 ± 14	24	70	Subjects with CAD	All-cause mortality, CVE
Frick *et al.*[29]	398	100	54 ± 9	39 ± 12	44	Subjects undergoing coronary angiography	CV death, CVE
Hu *et al.*[30]	279	58	62 ± 12	16	36	Subjects undergoing angiography	CV death, CVE
Huang *et al.*[31]	267	71	65 ± 10	10	50	Subjects with peripheral arterial disease	CV death, CVE
Karatzis *et al.*[32]	98	100	63 ± 11	24.8 ± 5.9	20	Men with NSTEMI	CV death, CVE
Neunteufl *et al.*[34]	73	52	51 ± 11	60	27	Subjects with chest pain evaluated by coronary angiography	All-cause mortality, CVE
Katz *et al.*[33]	259	84	54 ± 1	28	17	Subjects with class 2-3 CHF	All-cause mortality, CVE
Patti *et al.*[35]	136	82	63 ± 8	6	23	Subjects with CAD undergoing stenting	CVE
Santos-Garcia *et al.*[36]	120	58	73 ± 12.37	48	32	Subjects with ischaemic stroke	CV death, CVE
Shechter *et al.*[37]	82	91%	64 ± 12	14 ± 2	30	Subjects with ischaemic cardiomyopathy NYHA class IV	All-cause mortality, CVE
Suessenbacher *et al.*[38]	396	100%	54 ± 9	141 ± 12	145	Males undergoing coronary angiography	CV death, CVE
Takase *et al.*[39]	103	77	62 ± 9	50 ± 15	15	Subjects with suspected CAD	CV death, CVE
Takishima *et al.*[40]	245	68	66 ± 12	33 ± 9	33	Subjects with stable chronic ischaemic HF and impaired FMD < 5.5%	CV death, CVE
Ulriksen *et al.*[41]	223	76	54 ± 12.3	50	90	Subjects with acute chest pain	CV death, CVE
Wang *et al.*[42]	101	67	62 ± 9	12 ± 3	29	Subjects with STEMI	CV death, CVE

Continued

Table 1 *Continued*

Study	Sample size (*n*)	Gender (% male)	Age (years)	Follow-up (months)	Number of events	Population	Study outcomes
Special populations							
Becker *et al.*[43]	42	38	51 ± 19	8 days	14	Patients with sepsis	Hospital mortality
Dalton *et al.*[44]	17	76	60 (23–78)	18	16	Haemodialysis patients	All-cause mortality
Morimoto *et al.*[45]	199	56	61 ± 13	43 ± 10	24	Haemodialysis patients	All-cause mortality
Wexler *et al.*[46]	95	52	62 (49–74)	Discharge or death	17	Patients with severe sepsis or septic shock	Severe sepsis and hospital mortality

CAD, indicates coronary artery disease; CHF, congestive heart failure; CV, cardiovascular; CVE, cardiovascular event; NSTEMI, non-ST elevation myocardial infarction; NYHA; New York Heart Association.

Table 2 **PAT study characteristics and pooled RR**

PAT study	Sample size (*n*)	Gender (% male)	Age (year)	Follow-up (months)	Number of events	Population	Outcomes	Univariate RR	Multivariate RR
Rubinshtein *et al.*[7]	329	52	54 ± 12	70	98	Subjects with chest pain	All-cause mortality, CVE	L_RHI < 0.4 (*n* = 130) RR = 1.82 (1.18–2.81)	L_RHI < 0.4 RR = 1.68 (1.02–2.78)
Matsue *et al.*[47]	215	44	75 ± 11	10	32	Subjects with HF and preserved EF	CV deaths, CVE	L_RHI per 0.1 increase HR = 0.59 (0.43–0.81)	L_RHI per 0.1 increase HR = 0.56 (0.39–0.80)
Akiyama *et al.*[48]	321	50	72 ± 10	20	59	Subjects with heart failure and normal EF	CV death, CVE	L_RHI per 0.1 increase HR = 0.72 (0.61–0.85)	L_RHI per 0.1 increase HR = 0.82 (0.69–0.97)
Pooled RR per 0.1 increase in L_RHI								0.82 (0.76–0.89)	0.85 (0.78–0.93)

CV, cardiovascular; CVE cardiovascular event; EF, ejection fraction; L_RHI, reactive hyperaemia index; RR, relative risk.

multivariate RR was 0.86 (0.82–0.89). A detailed list of all variables adjusted for in the multivariable models is available in Supplementary material online, Appendix 1.

No established CVD/PVD

There were 13 studies[11–23] that involved patients without established CVD. These studies included healthy patients, patients with CV risk factors, hypertension, metabolic syndrome, chronic kidney disease, and post-menopausal women. The mean duration of follow-up ranged from 2 to 7.2 years and sample sizes ranged from 84 to 3026 individuals. Eight studies reported a univariate RR. The pooled univariate RR was 0.90 (0.87–0.94).

Eleven reported a multivariate RR, of which four adjusted for traditional CV risk factors. Almost all studies adjusted for age and gender but other variables differed based on study cohort. The multivariate RR for this group was 0.93 (0.90–0.96).

Special populations

Four studies[43–46] were designated as special populations. These studies included patients with end-stage renal disease and sepsis, involved smaller sample sizes ranging from 17 to 199 individuals and had shorter duration of follow-up. Only one study[45] reported a HR but all the studies found that FMD was not significantly associated with death. None of the studies adjusted for any of the traditional Framingham CV risk factors. These studies were not meta-analysed.

Subgroup and sensitivity analyses

We performed several a priori defined subgroup and sensitivity analyses. The pooled multivariate RR for CV mortality and all-cause mortality were 0.90 (0.88–0.92) and 0.91 (0.86–0.96), respectively. Studies deemed to be of low risk of bias based on the NOS had a pooled multivariate RR of 0.90 (0.88–0.93), whereas the RR for

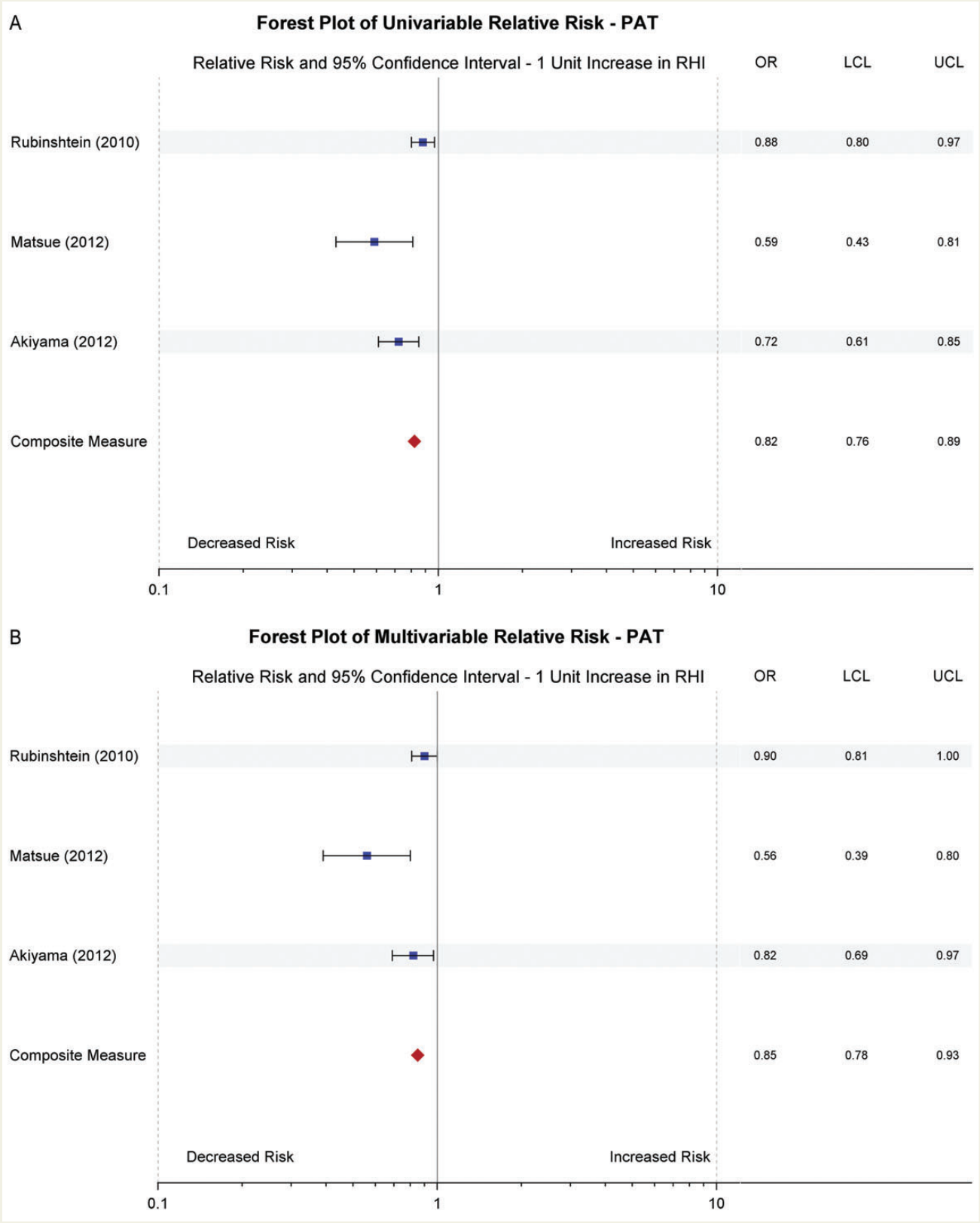

Figure 2 Forest plots for PAT RR. (*A*) Forest plot of univariate risk. (*B*) Forest plot of multivariate risk.

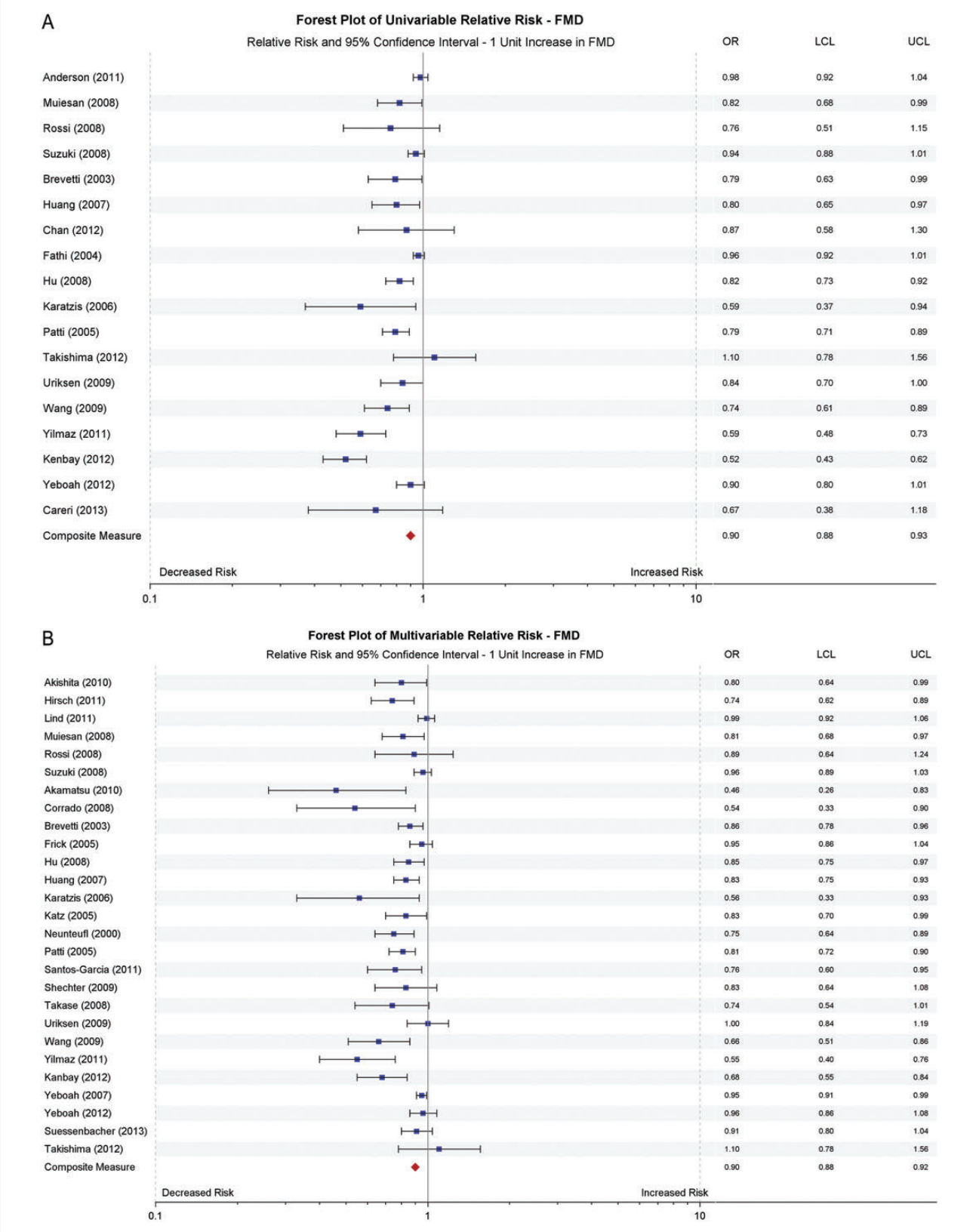

Figure 3 Forest plots for FMD RRs. (*A*) Forest plot of univariate risk. (*B*) Forest plot of multivariate risk.

Table 3 FMD subgroup analysis

	Univariate RR	Multivariate RR
All studies	0.90 (0.88–0.93)	0.90 (0.88–0.92)
Non-CVD	0.90 (0.87–0.94)	0.93 (0.90–0.96)
CVD	0.90 (0.87–0.94)	0.86 (0.82–0.89)
CV mortality	0.87 (0.84–0.90)	0.90 (0.88–0.92)
All-cause mortality	0.96 (0.916–1.01)	0.91 (0.86–0.96)
Forearm occlusion	0.87 (0.84–0.90)	0.91 (0.88–0.93)
Upper arm occlusion	0.96 (0.92–1.01)	0.89 (0.86–0.93)
Low risk of bias	0.89 (0.84–0.93)	0.91 (0.88–0.94)
Mod. high risk of bias	0.91 (0.88–0.93)	0.90 (0.86–0.93)
Age > 62	0.89 (0.85–0.93)	0.93 (0.90–0.95)
Age < 62	0.91 (0.88–0.94)	0.84 (0.80–0.88)
FMD > 5.2	0.88 (0.85–0.92)	0.86 (0.83–0.89)
FMD ≤ 5.2	0.92 (0.88–0.95)	0.94 (0.91–0.97)
Follow-up >41 months	0.93 (0.89–0.96)	0.93 (0.90–0.95)
Follow-up <41 months	0.88 (0.85–0.91)	0.85 (0.81–0.88)
Sample size <223	0.79 (0.74–0.85)	0.81 (0.77–0.85)
Sample size ≥223	0.92 (0.90–0.95)	0.93 (0.90–0.95)

CVD, cardiovascular disease; RR, relative risk.

studies of moderate and high risk of bias was 0.90 (0.86–0.93). The association between FMD and CV events remained significant across different age groups, sample sizes, duration of follow-up, and mean FMD (*Table 3*). In addition, baseline BA diameter did not affect the association between FMD and CV outcomes.

Risk of bias

The majority of the studies included in the review represented patients referred for CV investigation or to subspecialty clinics, as such, these studies may not be entirely representative of community dwelling adults. Most studies described a systematic approach to measuring FMD. Nine studies used upper arm occlusion, while the remainder of the studies used forearm occlusion.

Comparability of cohorts was evaluated based on whether studies controlled for traditional CV risk factors: age, gender, lipids, blood pressure, and smoking. Eight studies adjusted for all the traditional CV risk factors. Owing to the lack of consistent multivariate adjustment, we could not rule out the role of confounders.

The majority of studies used a combination of record linkage or self-report to assess for outcomes. Only two studies[31,35] had follow-up of <1 year. Although many studies lacked a clear description of follow-up, all the studies had <10% loss to follow-up. We also evaluated publication bias for both FMD and PAT (*Figure 4*). The evaluation was limited for PAT as there were only three articles included. For FMD, we observed significant asymmetry of the funnel plot suggesting publication bias may be present.

Discussion

Brachial FMD and PAT represent non-invasive measures of evaluating endothelial function and their association with CVD has been studied with varying results. In our meta-analysis, consisting of a pooled analysis of 32 studies evaluating 15 191 subjects using brachial FMD and three studies evaluating 865 participants using endoPAT, we found that both an increased brachial FMD and an increased RHI were independent predictors of CV events and death. These associations were similar in magnitude for both tests, and were consistent across a broad range of subgroups and patient populations that evaluated FMD.

Our findings of brachial FMD independently predicting CV events and death are consistent with work of previous investigators. A meta-analysis in 2010 showed that brachial FMD was significantly associated with future CV events, with a pooled multivariate RR of 0.872 for 1% increase in FMD. These investigators included 14 studies (5547 patients) and examined the multivariate RRs.[8] In 2012, another meta-analysis was published which included 23 studies. This meta-analysis separated articles based on reporting of categorical or continuous risk estimates, and estimated a pooled overall CVD risk of 0.92 (0.88–0.95) per 1% increase in FMD.[9] Our study, found a similar risk estimate 0.90 (0.88–0.92), but included 32 studies, and over 15 000 patients.

We found consistent associations between FMD and outcomes in our subgroup and sensitivity analyses. The predictive effect of brachial FMD was more substantial in studies with CV mortality as an endpoint compared with all-cause mortality, suggesting that impaired endothelial function is predominantly a CV risk factor. Furthermore, our results demonstrate that the risk associated with a lower brachial FMD (worse endothelial function) is larger in patients with existing CVD compared with patients without established CVD. Patients with established peripheral arterial disease, coronary artery disease, cerebrovascular disease, and congestive heart failure are associated with decreased FMD and increased risk of CV events and death. The association between impaired FMD and CV outcomes is independent of baseline BA diameters suggesting that FMD is equally useful as a prognostic marker across a range of BA sizes (3.6 ± 0.6 to 5.7 ± 1.0 cm). In addition, sensitivity analyses showed a significant association in studies with low risk and high risk of bias.

In addition to brachial FMD, we also conducted a meta-analysis of the prognostic value of PAT, which to our knowledge, has not been previously published. Since PAT is a relatively newer method compared with FMD, only a limited number of studies could be included in the analysis, and none compared PAT directly with FMD in the same study population. We analysed three studies including 865 patients and found that PAT is an individual predictor of CV events and death [OR 0.85(0.78, 0.93)]. Two studies involved patients with heart failure with normal ejection fraction. In one of these studies, an increased RHI was found to be an independent predictor of CV events, and was also shown to improve discrimination beyond traditional risk factors. A similar finding was reported in a long-term follow-up of patients presenting with chest pain, where RHI was again found to independently associate with adverse outcomes beyond Framingham risk factors. Together, these results suggest that PAT is a promising technique for evaluating endothelial function in patients across a range of pre-existing CVD (chest pain and heart failure), as it offers the advantage of ease of use, and is relatively operator independent.

In order for endothelial function testing to be a clinically useful test, it must provide prognostic value as well as be reliable, reproducible,

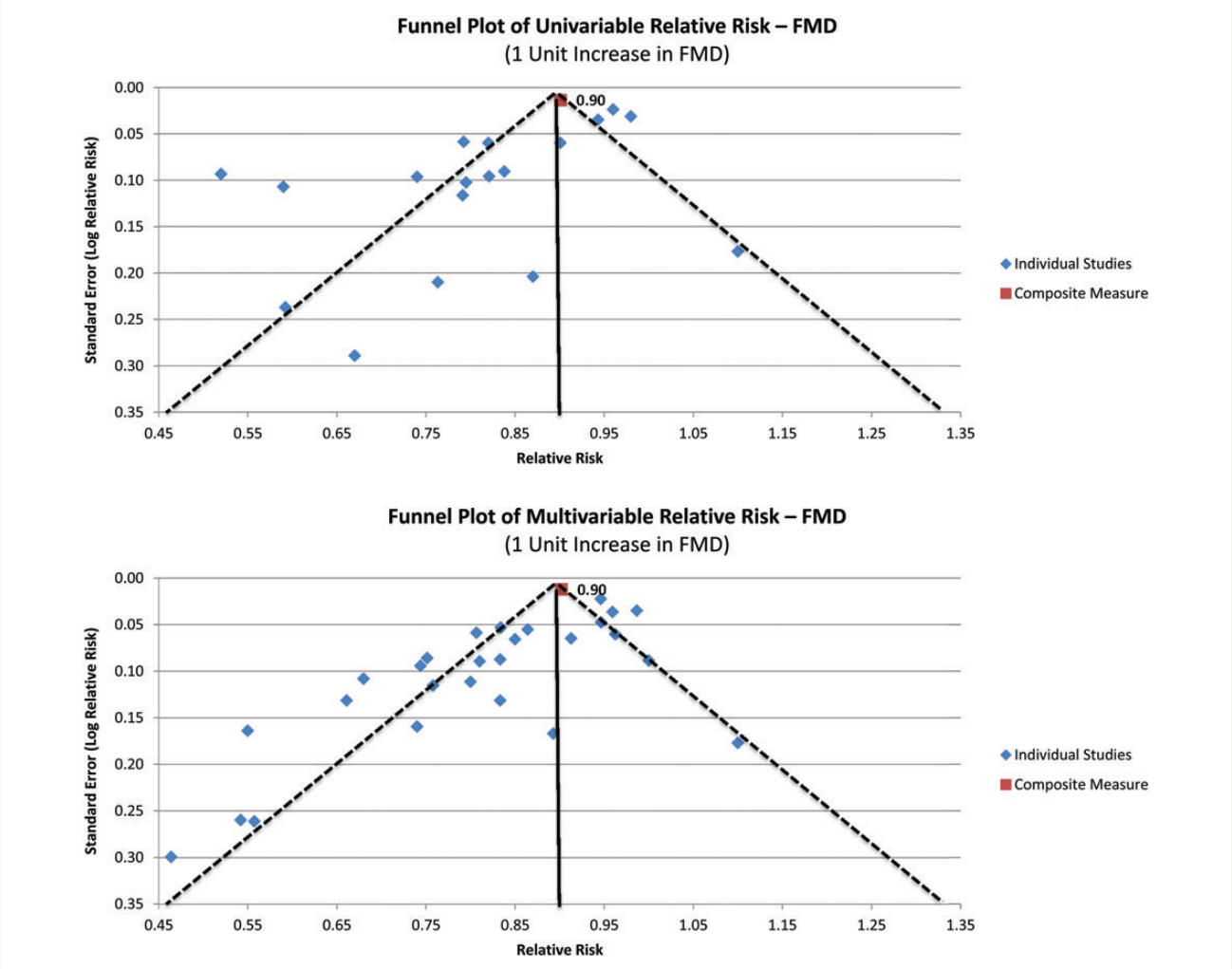

Figure 4 Funnel plot of multivariate RR of FMD studies.

and cost-effective. Our meta-analysis, consistent with previous work, has demonstrated that FMD is independently associated with CV events. However, several barriers must be overcome before it can be translated into clinical practice. Studies have shown that there is a wide range of mean FMD among populations, which hampers the determination of FMD reference values. Furthermore, technical aspects of brachial FMD testing such as cuff location and occlusion time can lead to intra-patient variability and thus guidelines outlining uniform methodology will facilitate standardization. The accuracy of brachial FMD depends on patient adherence to protocol, environmental factors, and equipment and measurement technique and thus, can be limited by cost and accessibility. Finally, it is possible that other measures such as hyperaemic BA velocity, and not FMD itself, are associated with CV events.[14] In contrast, PAT offers a non-invasive and reproducible method of measuring endothelial function, which appears to be similarly associated with CV events. Several studies have used PAT to assess the efficacy of clinical interventions and demonstrated the utility of PAT in assessing the effect of risk factor modification on vascular function.[6,49] Furthermore, PAT appears to be feasible in the ambulatory setting and not limited to the controlled research environment.[50]

It is important to note, however, that studies describing the correlation between FMD and PAT are contradictory and suggest that the two methods measure different aspects of vascular function. FMD measures the response to shear stress in larger vessels, which is largely NO dependent, whereas, PAT measures microvascular dilation to shear stress, which involves other vascular mediators in addition to NO.[6,49] Finally, to our knowledge, no studies thus far have examined the role of both FMD or PAT as competing additional data elements in prognostic models for CV outcomes, and compared their prognostic utility or cost-effectiveness.

We found several sources of potential bias in the existing literature on PAT and FMD. Completeness of follow-up was unclear in several studies and a few studies relied solely on patient self-reporting for assessment of outcomes. In addition, there was significant heterogeneity in the variables chosen for the multivariate models. Although most studies adjusted for age, only eight studies adjusted for traditional CV risk factors. Nonetheless, the point estimates for FMD

were consistent in studies with lower and higher risk of methodological bias, suggesting that bias alone could not explain the findings.

There are several strengths to our review. Our search strategy included multiple electronic databases in an attempt to ensure that all the published literature examining the predictive value of brachial FMD was captured. We also performed subgroup analysis to compare the predictive value of FMD in different populations (CVD vs. non-CVD patients) to evaluate the generalizability and robustness of our results. Finally, we used a random effects model to meta-analyse our findings, thus appropriately accounting for the clinical heterogeneity in our study population and patient outcomes. In addition, we also reviewed and meta-analysed the literature on PAT, thereby completing a more comprehensive review of non-invasive endothelial function tests rather than focusing on FMD alone.

There are some limitations to our study. Since we used aggregate data as reported by the studies rather than data for individual patients, we could not account for any methodological shortcomings in the original studies. Nonetheless, we performed a detailed assessment of bias using a validated tool, and our subgroup analysis showed that our findings were still positive in studies with a low risk of bias. We should note, however, that several studies that reported a non-significant association did not report a multivariate HR and thus could not be included in the pooled multivariate estimate. Since these results were excluded from our meta-analysis, our results may reflect a bias towards positive studies, and as such the true prognostic effect of FMD may not be as strong as reported in our meta-analysis. Although we found a similar association between PAT, FMD, and CV events, the lack of studies and events for PAT led to a less precise point estimate of the strength of association.

Conclusion

Our meta-analysis confirms that brachial FMD and PAT are independent predictors of future CV events and all-cause mortality, beyond traditional CV risk factors. The strength of association of FMD and CV events is higher in patients with already established CVD, suggesting that FMD may be more useful in screening for recurrent CVD events in patients at high risk, rather screening than in a healthier general population cohort. Studies examining the role of FMD and PAT in clinical risk prediction and medical decision-making are needed.

Supplementary data

Supplementary data are available at *European Heart Journal – Cardiovascular Imaging* online.

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