Vascular Medicine
http://vmj.sagepub.com

Association of improvement of brachial artery flow-mediated vasodilation with cardiovascular events

Alois Suessenbacher, Matthias Frick, Hannes F Alber, Verena Barbieri, Otmar Pachinger and Franz Weidinger
Vasc Med 2006; 11; 239
DOI: 10.1177/1358863x06075006

The online version of this article can be found at:
http://vmj.sagepub.com/cgi/content/abstract/11/4/239

Published by:
SAGE Publications
http://www.sagepublications.com

Association of improvement of brachial artery flow-mediated vasodilation with cardiovascular events

Alois Suessenbacher[a], Matthias Frick[a], Hannes F Alber[a], Verena Barbieri[b], Otmar Pachinger[a] and Franz Weidinger[a]

Abstract: The aim of this pilot study was to test the prognostic value of serial measurements of peripheral endothelial function, assessed by brachial artery flow-mediated dilation (FMD), in patients with angiographically proven coronary artery disease. In 68 patients, FMD was measured on the day after coronary angiography and again after a mean of 14 ± 12 months. Patients were divided into two groups: absolute improvement in FMD ≥ 3% (FMD-improver = FMD-i) and <3% (FMD-non-improver = FMD-ni). After a mean follow-up of 44 ± 12 months, cardiovascular events were recorded. Baseline characteristics were similar between groups, except the number of risk factors which was smaller in FMD-i (1.6 ± 0.7 vs 2.1 ± 0.9, $p < 0.02$). Cardiovascular events were more frequent in FMD-ni (9 vs 1 event; $p < 0.05$). In Kaplan–Meier analysis, a trend towards a better outcome in patients with improved FMD was found using the log-rank test ($p = 0.08$). The single baseline FMD showed no relationship with late cardiovascular events. Thus, 'delta-FMD' may be more closely related to prognosis than a single FMD measurement.

Key words: coronary artery disease; flow-mediated dilation; prognostic value

Introduction

Flow-mediated dilation (FMD) of the brachial artery (BA) is a non-invasive test for measuring peripheral endothelial function.[1] It has been related to cardiovascular risk factors[1–5] and was shown to correlate with coronary endothelial function.[6] Recent studies investigating the prognostic value of this test yielded conflicting results.[7–11] An impaired FMD was associated with increased cardiovascular events, but trials were conducted in selected populations such as postmenopausal hypertensive women[8] or patients with symptomatic peripheral vascular disease.[9,10] In contrast, a more recent study by Fathi et al[12] in 444 patients showed no relation between an impaired FMD and cardiovascular events.

A major limitation for the use of FMD in clinical practice is its known inter-individual variability,[13] which is less important in serial FMD measurements. Several studies have demonstrated an improvement in FMD after various therapeutic interventions.[14–16] Whether this improvement translates into a clinical benefit is unclear.

The aim of this retrospective data analysis was to assess whether changes in FMD, rather than a single FMD measurement, are associated with outcome.

Methods

Patients

From 1999 to 2003, FMD was determined in more than 500 patients in the Cardiovascular Research Laboratory of the Division of Cardiology at Innsbruck Medical University. All patients underwent coronary angiography for the evaluation of chest pain. Coronary artery disease (CAD) was defined as visually estimated percent diameter stenosis ≥30% in one or more major vessel. A modified Gensini score[17] was used to grade the extent of CAD. From the original cohort, 68 patients with CAD (mean age 53.7 ± 9.1 years, range 33–71 years) underwent reassessment of FMD after 14.2 ± 11.9 months. Exclusion criteria were congestive heart failure, significant valvular disease, left ventricular ejection fraction <40%, severe hypertension, insulin-dependent diabetes mellitus, and pretreatment with statin or ACE inhibitor for more than 2 months prior to enrollment. Patients were retrospectively divided twice into two groups: 'FMD-improvers' (FMD-i) ($n = 22$), absolute improvement

[a]Division of Cardiology, Innsbruck Medical University, Innsbruck, Austria; [b]Department of Medical Statistics, Informatics and Health Economics, Innsbruck Medical University, Innsbruck, Austria

Address for correspondence: Franz Weidinger, Division of Cardiology, Innsbruck Medical University, Anichstrasse 35, A-6020 Innsbruck, Austria. Tel: +43 512 504 23406; Fax: +43 512 504 22767; E-mail: F.Weidinger@uibk.ac.at

10.1177/1358863x06075006

of FMD $\geq$ 3%; 'FMD-non-improvers' (FMD-ni) ($n = 46$), absolute improvement <3%. The cut-off value was chosen based on evaluation of the spontaneous course (repeatability) of 3% in our laboratory as shown in a recent study.[18] To assess the prognostic value of a single FMD value, patients were further divided according to the FMD-median (7.5%) at study entry.

Written informed consent was obtained from all patients and the study complied with the declaration of Helsinki.

Assessment of cardiovascular risk factors

Fasting blood samples were obtained from all patients for the measurement of plasma total cholesterol, triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol. Individuals with plasma LDL cholesterol levels >130 mg/dl or who were receiving cholesterol-lowering therapy were classified as hypercholesterolemic patients.[19] Smokers were defined as subjects who had smoked regularly during the previous 12 months.[19] Systemic hypertension was defined as systolic blood pressure $\geq$140 mmHg and/or diastolic blood pressure $\geq$90 mmHg[20] based on the average of two or more readings taken on each of two or more different days, or as current use of antihypertensive drugs. Patients were considered diabetic if they were receiving treatment with insulin or oral hypoglycemic agents or if fasting blood glucose was >140 mg/dl.[19] A family history of CAD and a history of diabetes were obtained.

Ultrasound examination of the BA

On the day after angiography, high-resolution ultrasound (13MHz; Acuson Sequoia, C 256, Mountain View, CA, USA) was used for the assessment of FMD. The examination was performed between 8 a.m. and 12 noon by an observer blinded to the patients' diagnoses. All vasoactive drugs were withdrawn 18–24 h before examination. Patients were instructed not to smoke and to remain fasting before the ultrasound examination. After a resting period of at least 10 minutes in the supine position, blood pressure was measured using a manual sphygmomanometer, and the right BA was scanned. Transducer position and gain settings were optimized, and ECG-triggered images were stored to the peak of the T-wave on the hard disk for off-line measurements. Changes in vessel diameter (intima to intima) after reactive hyperemia (FMD) and after 0.8mg sublingual nitroglycerin (nitroglycerin-mediated dilation; NMD) were examined according to previously described methods.18 In brief, after recording the resting diameter a cuff was placed on the upper arm and inflated to suprasystolic levels for 5 minutes. After deflation, serial post-hyperemia scans were stored on the hard disk. When the BA diameter had returned to baseline, 0.8mg sublingual nitroglycerin was administered and the diameters within the next 10 minutes were recorded. Vasodilation (FMD, NMD) was calculated as the percent change in diameter compared with baseline.

The second FMD was assessed during visits for studies investigating the pleiotropic effects of atorvastatin (55% of all included patients), or incidentally from patients visiting our outpatient clinic for routine control.

Reassessments of FMD and NMD were performed at the same time of the day as baseline FMD measurement and following the same protocol.

Assessment of follow-up data

After a mean follow-up of 44 $\pm$ 12 months, patients were contacted via telephone or by written questionnaire (the latter was to those not reached by telephone). All registered cardiovascular events were verified by review of hospital records. Cardiovascular events were defined as follows: (1) need for revascularization (percutaneous transluminal coronary angioplasty or coronary artery bypass grafting); (2) repeat coronary angiography (due to typical angina and/or positive stress test) with documented progression of CAD; (3) cardiac death; (4) hospitalization for exclusion of unstable angina pectoris; and (5) myocardial infarction (CK and troponin I/T above the upper limit of normal).

Statistical analysis

All analyses were conducted with the use of statistical software (SPSS® for Windows, version 10.1). Data are expressed as means $\pm$ standard deviations or as frequencies (percentages). A p-value <0.05 was considered statistically significant. Normal distribution of the variables was assessed using the Kolmogorov–Smirnov test. Patient characteristics between groups were compared using Student's t-test for normal distributed variables and the Mann–Whitney U-test for non-normal distributed continuous variables. The chi-squared test or Fisher's exact test were used for comparing categorical variables. Changes between baseline and follow-up in FMD-i and FMD-ni, respectively, were compared using Student's t-test or the Mann–Whitney U-test for continuous variables and the McNemar test for categorical variables. Cumulative event rates were calculated according to the Kaplan–Meier method, and the differences between groups were evaluated with the log-rank test. Linear correlations were determined using Pearson's or Spearman's correlation coefficient as appropriate. To investigate possible relationships between changes in FMD or baseline risk factors and outcome, Cox regression analysis was performed.

Results

Patient characteristics

Clinical characteristics are shown in Table 1. At study entry, when the baseline FMD measurement was

Table 1 Patient characteristics.

	FMD-i ($n = 22$)			FMD-ni ($n = 46$)		
	Baseline	Follow-up	p	Baseline	Follow-up	p
Age (years)	55.6 ± 7.9			52.8 ± 9.7		0.24
Number of risk factors	1.6 ± 0.7*	2.1 ± 0.6	<0.001	2.1 ± 0.9*	2.4 ± 1.0	0.058
Hypertension	10 (45.5%)	14 (63.6%)	NS	22 (47.8%)	34 (73.9%)	0.002
Smokers	6 (27.3%)	4 (18.2%)	NS	19 (41.3%)	10 (21.7%)	0.012
Diabetes mellitus	0**	1 (4.5%)	–	7 (15.2%)**	7 (15.2%)	NS
Positive family history (CAD)	4 (18.2%)	4 (18.2%)	NS	13 (28.3%)	13 (28.3%)	NS
Hypercholesterolemia	15 (68.2%)	17 (77.3%)	NS	40 (86.9%)	40 (87.0%)	NS
Total cholesterol (mg/dl)	211.2 ± 52.9	211.5 ± 45.5	NS	237.8 ± 71.7	211.5 ± 53.5	<0.01
LDL cholesterol (mg/dl)	137.6 ± 49.7	139.0 ± 4 4.6	NS	155.8 ± 49.7	141.6 ± 45.5	<0.05
HDL cholesterol (mg/dl)	49.2 ± 10.2*	51.0 ± 18.2	<0.05	42.8 ± 9.4*	47.2 ± 10.5	<0.05
Triglycerides (mg/dl)	138.9 ± 56.4*	143.9 ± 44.5	NS	212.2 ± 173.5*	163.4 ± 72.2	0.09
BMI (kg/m^2)	26.8 ± 4.3	26.8 ± 4.0	NS	27.2 ± 2.8	27.6 ± 3.0	NS
Baseline diameter (mm)	4.21 ± 0.58	3.99 ± 0.51†	0.014	4.18 ± 0.42	4.33 ± 0.44†	0.011
FMD (%)	7.3 ± 4.0	13.3 ± 4.3‡	<0.001	8.81 ± 3.9	7.71 ± 2.9‡	0.038
NMD (%)	17.9 ± 6.0	23.8 ± 6.2†	<0.001	18.6 ± 6.1	18.4 ± 7.2†	NS
Ratio FMD/NMD	0.40 ± 0.19	0.57 ± 0.15†	<0.001	0.48 ± 0.16	0.46 ± 0.20†	NS

Significant difference in baseline parameters: *$p < 0.02$; **$p = 0.053$.
Significant difference in follow-up parameters: $^\dagger p < 0.01$; $^\ddagger p < 0.001$.
CAD, coronary artery disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein; BMI, body mass index; FMD, flow-mediated dilation; NMD, nitroglycerin-mediated dilation.

performed, no significant differences in clinical characteristics between study groups were found except for the mean number of risk factors, which was smaller in FMD-i (1.6 ± 0.7 vs 2.1 ± 0.9; $p < 0.02$), and the proportion of diabetic patients, which was greater in FMD-ni (0 vs 15%; $p = 0.05$). At the end of the follow-up period no significant difference in the number of risk factors was found anymore (2.1 ± 0.6 vs 2.4 ± 1.0; p = NS).

The findings on coronary angiography were as follows: overall, 19 patients (27.9%) had 1-vessel disease (VD), 13 patients (19.1%) 2-VD and 36 patients (52.9%) 3-VD; in FMD-i, eight patients (36.4%) had 1-VD, four patients (18.2%) 2-VD and 10 patients (45.5%) 3-VD. In FMD-ni, 11 patients (23.9%) had 1-VD, nine patients (19.6%) 2-VD and 26 patients (56.5%) 3-VD. No significant differences in the extent of CAD were present between groups using a modified Gensini score.[17]

At enrollment, the use of statins (18% vs 17%, p = NS), calcium-channel antagonists (18% vs 24%, p = NS), angiotensin-II antagonists (9% vs 2%, $p = 0.19$) and acetylsalicylic acid (82% vs 80%, p = NS) was comparable between FMD-i and FMD-ni, respectively, whereas angiotensin-converting enzyme inhibitors tended to be used more often in FMD-ni (5% vs 22%, $p = 0.071$). At the end of the follow-up period no difference in the use of medication was found anymore.

At baseline, total and LDL cholesterol were not different between FMD-i and FMD-ni, whereas triglycerides were lower in FMD-i (139 ± 56 vs 212 ± 174 mg/dl; $p < 0.02$) and HDL cholesterol was lower in FMD-ni (49 ± 10 vs 43 ± 9 mg/dl; $p < 0.02$).

In FMD-i, total cholesterol, LDL cholesterol, and triglycerides did not change significantly between baseline and second FMD, whereas HDL cholesterol slightly increased from 49 ± 10 to 51 ± 18 mg/dl ($p < 0.05$). In FMD-ni, total cholesterol (238 ± 72 to 212 ± 54 mg/dl; $p < 0.01$) and LDL cholesterol (156 ± 50 to 142 ± 46 mg/dl, $p < 0.05$) decreased and HDL cholesterol increased (43 ± 9 to 47 ± 11 mg/dl; $p < 0.05$). Triglycerides tended to decrease (212 ± 174 to 163 ± 72 mg/dl; $p = 0.09$). Furthermore, in FMD-ni the prevalence of hypertension increased (48% to 74%, $p = 0.002$) and the proportion of smokers decreased from the beginning to the end of the follow-up period (41% to 22%, $p = 0.01$). However, none of these parameters was different between FMD-i and FMD-ni at the time the second FMD measurement was performed.

Brachial artery ultrasound results (Figures 1 and 2)

No difference in FMD and NMD were found between groups at baseline. After a mean of 14 ± 12 months, FMD improved from 7.3 ± 4.0% to 13.3 ± 4.3% ($p < 0.001$) in FMD-i; whereas in FMD-ni, FMD showed a small decrease (8.8 ± 3.9% to 7.7 ± 2.9%; $p = 0.038$). NMD increased from 17.9 ± 6.0% to 23.8 ± 6.2% ($p < 0.001$) in FMD-i, but remained unchanged in FMD-ni (18.6 ± 6.1% to 18.4 ± 7.2%).

No linear correlations were found between the change in FMD and the change in total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides and blood pressure.

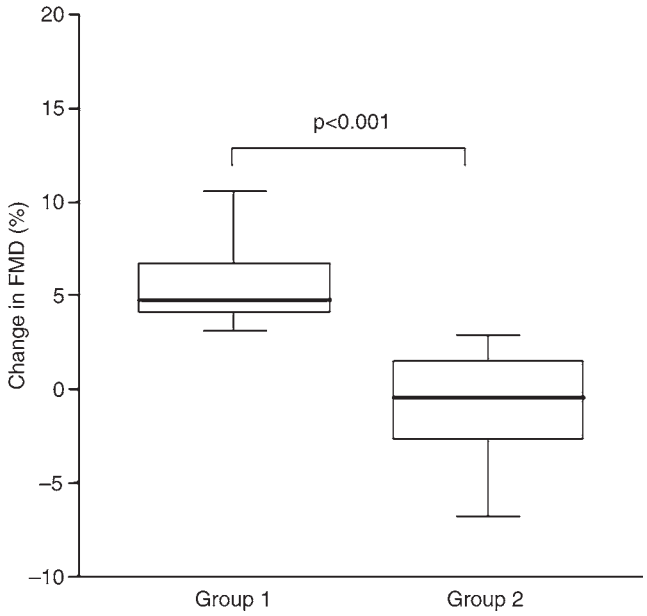

Figure 1 Box plots for differences in the change of FMD between groups. The *p*-value is calculated using the *t*-test.

Outcome

After a mean follow-up period of 44 ± 12 months (range 27–78 months), 10 cardiovascular events were documented: cardiac death ($n = 1$), myocardial infarction ($n = 1$), coronary angiography that revealed progression of CAD ($n = 1$), the need for percutaneous coronary intervention ($n = 6$), and hospitalization for the exclusion of instability with positive exercise test ($n = 1$). FMD-i had significantly fewer events compared to FMD-ni (one vs nine events; $p < 0.05$). In Kaplan–Meier analysis, this difference was borderline significant using the log-rank test ($p = 0.08$, Figure 3).

When patients were divided according to the FMD median, no difference in outcome between the two groups was observed (six vs four events) (Figure 4).

Cox regression analyses did not reveal any significant relation between changes in FMD or baseline risk factors and outcome.

Discussion

In this retrospective data analysis of 68 patients undergoing coronary angiography, an absolute improvement in FMD ≥ 3% appears to be related to a lower risk of future cardiovascular events, whereas a single FMD measurement was not associated with clinical outcome during a mean follow-up period of up to 4 years.

In recent years, FMD has been evaluated as a surrogate endpoint parameter by testing its prognostic value. Most studies so far have examined a single FMD measurement in relation to cardiovascular events and have shown controversial results: two studies demonstrated a correlation of a single FMD value with outcome in patients with symptomatic peripheral artery disease.[9,10] In contrast, Fathi et al[12] did not find

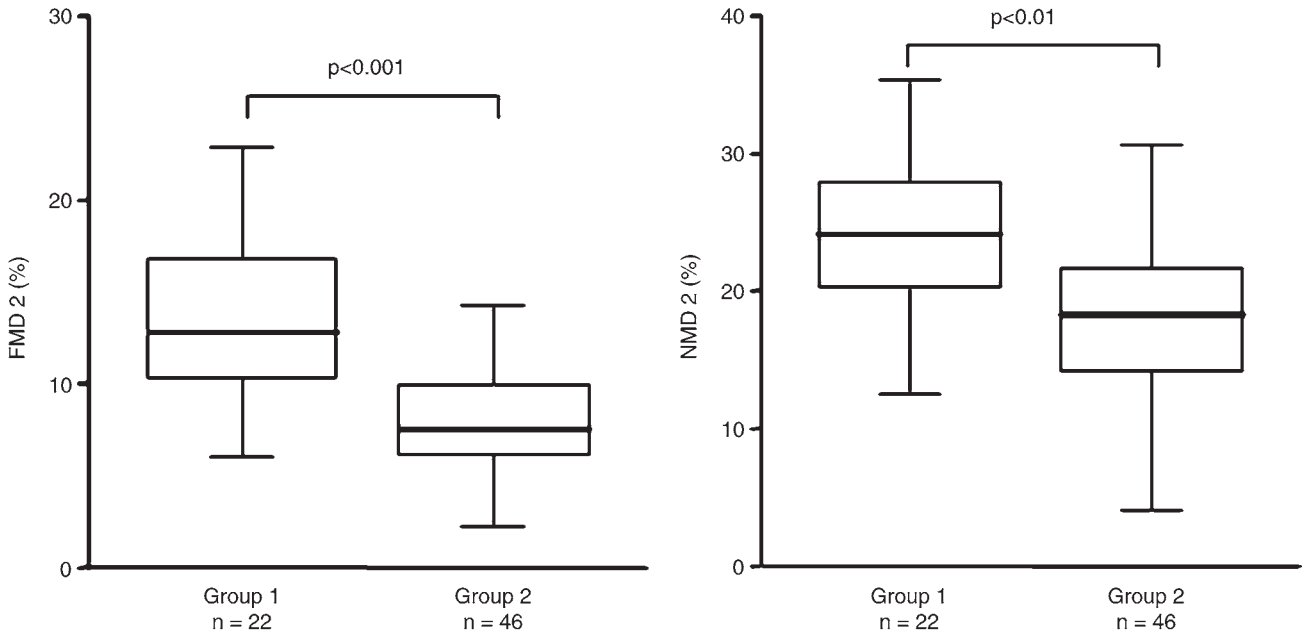

Figure 2 Box plots showing differences in FMD 2 (left panel) and NMD 2 (right panel) between groups at follow-up. The *p*-value is calculated using the *t*-test.

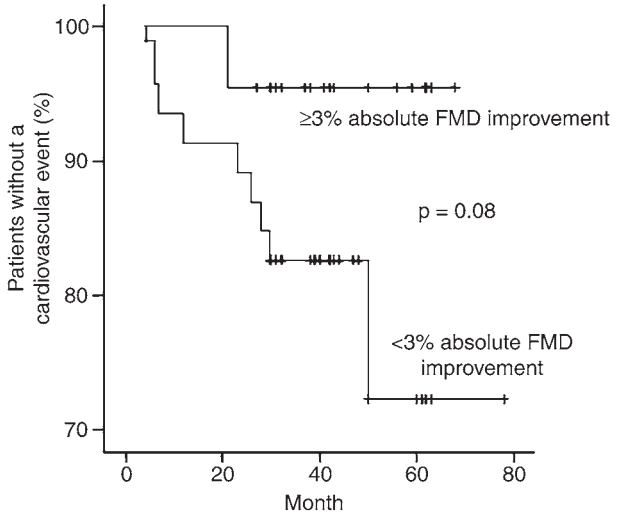

Figure 3 Kaplan–Meier curves for FMD improvement. Patients with absolute improvement ≥3% show fewer events. The *p*-value is calculated using the log-rank test.

an association between FMD and cardiovascular events in a high-risk population. Possible explanations for this discrepancy include differences in the study population and inter-individual variation of FMD. The latter problem might be overcome when serial measurements are performed.

We and others have demonstrated that the mean values of FMD often do not change in the control group but increase with therapeutic interventions.[16,18,21,22] Therefore, an improvement in FMD could be interpreted as vascular response to medical treatment which may translate into better clinical outcome. In the present study, a difference in clinical outcome was indeed found between patients showing an improvement in FMD and patients without improvement, although the treatment was not different between groups. This finding suggests that patients with a poorer response to treatment and subsequently worse clinical outcome may be identified by serial assessments of FMD.

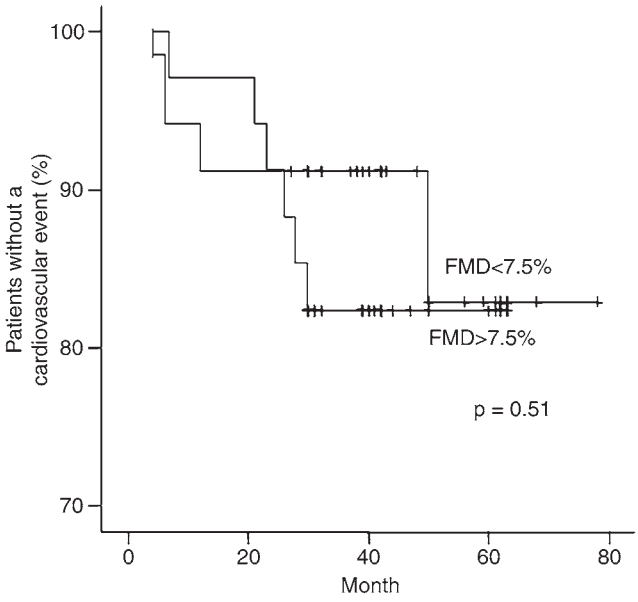

Figure 4 Kaplan–Meier curves for FMD median. No difference occurs between groups. The *p*-value is calculated using the log-rank test.

A recent study demonstrated that an impairment of FMD is associated with cardiovascular events: Chan et al[11] measured FMD in 152 patients with CAD. After a mean follow-up time of 34 months, 22 vascular events were observed (myocardial infarction, unstable angina, transient ischemic attack, cerebrovascular accident, revascularization procedure). Multivariate analysis identified FMD/NMD ratio, as well as carotid plaque area and the use of long-acting nitroglycerin as independent predictors of events. As in our study, a single FMD measurement was not correlated with cardiovascular events. In a subgroup of 106 patients who had at least one reassessment follow-up versus baseline, FMD was significantly associated with subsequent cardiovascular events. However, the FMD value that was assumed to represent an impairment in endothelial function was not specified.[11]

In our pilot observation, the difference in cardiovascular events between groups was only borderline significant due to the small number of patients included in this retrospective analysis. Thus, our findings are hypothesis-generating and underscore the need for larger studies investigating the prognostic value of a change in FMD in patients with CAD. To the best of our knowledge, only one study has explicitly investigated the prognostic value of a change in FMD, but was performed in a highly selected patient group: Modena et al[8] tested the prognostic role of reversible endothelial dysfunction in 400 postmenopausal mild to moderate hypertensive women. FMD was measured at baseline and after 6 months of therapy. After a mean follow-up time of 67 months, 47 cardiovascular events were recorded. In 250 (62.5%) patients, FMD had significantly improved to >10% (mean FMD in this group: 13.9 ± 2.6%) after 6 months of treatment, which was associated with fewer events compared to patients with no change in FMD (5.9 vs 21.3%). The mean FMD value in the group without improvement was 7.1 ± 2.5%. Interestingly, FMD values in both groups are comparable to the values measured in our study population: 13.3 ± 4.3% in patients with improvement in FMD and 7.7 ± 2.9% in patients without improvement and more cardiovascular events.

The findings of Modena et al[8] and our findings suggest that FMD may be used to individualize risk factor management. However, because the FMD test is still not standardized, the lack of 'normal' cut-off values remains the main limitation for the use of FMD in daily clinical practice.

As described by Celermajer et al,[1] the magnitude of FMD is inversely proportional to the arterial diameter. Accordingly, one could argue that the improvement in

FMD in group 1 relates to a smaller baseline arterial diameter. Although differences in diameter is statistically significant, the absolute differences (3.99 ± 0.51 vs 4.33 ± 0.44) are quite small and therefore unlikely to have influenced the results importantly. To fully exclude the influence of the arterial diameter, a greater sample size is needed.

Limitations

Baseline diameter decreased in FMD-i but increased in FMD-ni. Therefore, the difference in baseline diameter between groups at visit 2 is significant and could partially explain our results. Although this small study was not powered for multivariate calculation, Cox regression analyses did not reveal any significant relation between changes in FMD, risk factors and outcome. Consequently, a greater sample size is needed to confirm the hypothesis that an improvement in FMD is associated with better outcome. Furthermore, FMD-i also had a significant increase in NMD. This may be due to changes in baseline brachial artery diameter or even smooth muscle cell effect. To rule out these two reasons, a greater sample size would also be needed.

In conclusion, 'delta-FMD', i.e. the change in FMD between baseline and follow-up, was correlated with long-term clinical outcome in our retrospective pilot observation. In contrast, the prognostic role of a single FMD measurement seems to be limited. To confirm superiority of serial FMD measurements over single assessments, further studies in larger patient populations are required.

References

1 Celermajer DS, Sorensen KE, Gooch VM et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 1992; **340**: 1111–15.

2 Celermajer DS, Sorensen KE, Bull C, Robinson J, Deanfield JE. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. *J Am Coll Cardiol* 1994; **24**: 1468–74.

3 Johnstone MT, Creager SJ, Scales KM, Cusco JA, Lee BK, Creager MA. Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation* 1993; **88**: 2510–16.

4 Cockcroft JR, Chowienczyk PJ, Benjamin N, Ritter JM. Preserved endothelium-dependent vasodilatation in patients with essential hypertension. *N Engl J Med* 1994; **330**: 1036–40.

5 Frick M, Alber HF, Weidinger F. Endothelial function in a large community. *Circulation* 2004; **110**: e24; author reply e24.

6 Anderson TJ, Uehata A, Gerhard MD et al. Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol* 1995; **26**: 1235–41.

7 Neunteufl T, Heher S, Katzenschlager R et al. Late prognostic value of flow-mediated dilation in the brachial artery of patients with chest pain. *Am J Cardiol* 2000; **86**: 207–10.

8 Modena MG, Bonetti L, Coppi F, Bursi F, Rossi R. Prognostic role of reversible endothelial dysfunction in hypertensive postmenopausal women. *J Am Coll Cardiol* 2002; **40**: 505–10.

9 Gokce N, Keaney JF Jr, Hunter LM et al. Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. *J Am Coll Cardiol* 2003; **41**: 1769–75.

10 Brevetti G, Silvestro A, Schiano V, Chiariello M. Endothelial dysfunction and cardiovascular risk prediction in peripheral arterial disease: additive value of flow-mediated dilation to ankle–brachial pressure index. *Circulation* 2003; **108**: 2093–98.

11 Chan SY, Mancini GB, Kuramoto L, Schulzer M, Frohlich J, Ignaszewski A. The prognostic importance of endothelial dysfunction and carotid atheroma burden in patients with coronary artery disease. *J Am Coll Cardiol* 2003; **42**: 1037–43.

12 Fathi R, Haluska B, Isbel N, Short L, Marwick TH. The relative importance of vascular structure and function in predicting cardiovascular events. *J Am Coll Cardiol* 2004; **43**: 616–23.

13 De Roos NM, Bots ML, Schouten EG, Katan MB. Within-subject variability of flow-mediated vasodilation of the brachial artery in healthy men and women: implications for experimental studies. *Ultrasound Med Biol* 2003; **29**: 401–406.

14 Celermajer DS, Sorensen KE, Georgakopoulos D et al. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation* 1993; **88**: 2149–55.

15 O'Driscoll G, Green D, Rankin J, Stanton K, Taylor R. Improvement in endothelial function by angiotensin converting enzyme inhibition in insulin-dependent diabetes mellitus. *J Clin Invest* 1997; **100**: 678–84.

16 Dupuis J, Tardif JC, Cernacek P, Theroux P. Cholesterol reduction rapidly improves endothelial function after acute coronary syndromes. The RECIFE (reduction of cholesterol in ischemia and function of the endothelium) trial. *Circulation* 1999; **99**: 3227–33.

17 Adams MR, Nakagomi A, Keech A et al. Carotid intima-media thickness is only weakly correlated with the extent and severity of coronary artery disease. *Circulation* 1995; **92**: 2127–34.

18 Frick M, Alber HF, Hugel H, Schwarzacher SP, Pachinger O, Weidinger F. Short- and long-term changes of flow-mediated vasodilation in patients under statin therapy. *Clin Cardiol* 2002; **25**: 291–94.

19 Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. *Circulation* 1998; **97**: 1837–47.

20 Chobanian AV, Bakris GL, Black HR et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA* 2003; **289**: 2560–72.

21 Gokce N, Keaney JF Jr, Frei B et al. Long-term ascorbic acid administration reverses endothelial vasomotor dysfunction in patients with coronary artery disease. *Circulation* 1999; **99**: 3234–40.

22 Anderson TJ, Elstein E, Haber H, Charbonneau F. Comparative study of ACE-inhibition, angiotensin II antagonism, and calcium channel blockade on flow-mediated vasodilation in patients with coronary disease (BANFF study). *J Am Coll Cardiol* 2000; **35**: 60–66.