Endothelial function assessment: flow-mediated dilation and constriction provide different and complementary information on the presence of coronary artery disease

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Aims

A number of risk factors for atherosclerosis have been identified, but it remains difficult, on an individual patient basis, to predict how these factors interact in determining the development of coronary artery disease (CAD). It also remains unclear whether the study of endothelial function provides information that is additive to that of traditional risk factors.

Methods and results

Flow-mediated dilation (FMD) and low-flow-mediated constriction (L-FMC) were measured in 451 consecutive patients before coronary angiography. Low-flow-mediated constriction ($P$ < 0.0001) and FMD ($P$ = 0.0005) progressively decreased with the number of diseased vessels, and L-FMC showed a significant linear correlation with the SYNTAX score ($R$ = 0.38; $P$ < 0.0001). Logistic regression analysis confirmed the association between endothelial function parameters and CAD ($P$ = 0.001 for L-FMC, $P$ = 0.02 for FMD). Receiver operating characteristic analysis demonstrated that the addition of L-FMC alone and of the combination of FMD and L-FMC improved the predictive power of a model based on traditional risk factors for CAD (area under the curve of the risk factor model = 0.716; risk factor model + FMD = 0.734, $P$ = 0.1 compared with risk factor model; risk factor model + L-FMC = 0.771, $P$ = 0.004; risk factor model + FMD + L-FMC = 0.779, $P$ = 0.002). Reclassification statistics showed that the introduction of FMD to the model based on the traditional risk factors correctly reclassified an additional 5% of patients, and that the introduction of L-FMC net correctly reclassified 19% of the patients. There was no correlation between different parameters of endothelial function.

Conclusion

Endothelial function assessment provides modest but statistically significant additional information in predicting the presence of CAD.

Keywords

Endothelial function and dysfunction • Coronary artery disease

Introduction

Coronary artery disease (CAD) is a lifelong process resulting from the interaction of many risk factors, environmental influences, and genetic predisposition. Although the collection of medical history and standard risk factors provides essential information, the existence of complex interactions among different risk factors, risk modifications by medical therapy, and inter-individual differences complicate these issues. In the light of these limitations, alternative approaches have been sought, and the non-invasive assessment of endothelial function

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has been proposed as a possible non-invasive and inexpensive end-
point that could reflect the cumulative cardiovascular burden and/or the responsiveness to therapies at the level of individual patients.\(^1\)\(^2\) It remains however unclear whether the data provided by these measures provide information that is additional (or simply repetitive) to that provided by traditional risk factors.

Of all methods that have been developed to assess endothelial function in human in vivo, the most commonly employed is flow-mediated vasodilation (FMD). Flow-mediated dilation is expressed as the vaso-
dilation induced in response to a sudden increase in shear stress and, as such, quantifies the capacity of the endothelium to cause smooth muscle cell relaxation and vasodilation when stimulated by a specific stimulus.\(^3\)\(^4\) Importantly, FMD measured in the forearm provides information which predicts the extent and severity of coronary atherosclerosis,\(^5\) correlates with coronary endothelial function,\(^6\)\(^7\) and has prognostic implications that are similar to those of endothelial function measured invasively in the coronary circulation.\(^8\) Despite these strengths, a limitation to the concept of FMD is that this method provides information on the ‘recruitability’ of endothelial function (i.e. the capacity of the endothelium to increase its biosynthetic responses in response to a specific stimulus) but it does not take into account (nor measures) resting endothelial activity (i.e. the endothelial pro-
duction of vasomotor substances in resting conditions). To address this issue, a new method, which, in analogy to FMD, was termed ‘low-flow-mediated constriction’ (L-FMC), was recently proposed and developed.\(^9\)\(^10\) Low-flow-mediated constriction quantifies the decrease in forearm conduit artery diameter that occurs in response to decreases in blood flow and shear stress. Recent studies empha-
sized the importance of this parameter of resting vascular/endothelial function in interpreting FMD data.\(^11\)\(^12\)

In the present study, we set out to address the relationship between FMD, L-FMC, and the degree of CAD in a cohort of patients undergoing diagnostic coronary angiography. As well, we tested whether the introduction of FMD and L-FMC, or their com-
bination, provides information that is additional to that of traditional risk factors in predicting the presence and extent of coronary atherosclerosis.

**Methods**

**Subjects and study protocol**

Four hundred and fifty-one consecutive patients undergoing elective coronary angiography between November 2009 and May 2011 were studied. Patients had chest pain on effort according to the American College of Cardiology/American Heart Association 2007 Guidelines and/or a pathological exercise or dobutamine stress test. Patients undergoing catheterization for any reason other than stable (sus-
pected) CAD (e.g. for hypertensive crisis associated with troponin elevation, or acute coronary syndromes, valvular heart disease, conge-
nital heart disease, cardiomyopathy, etc.) were excluded. Patients with known chronic inflammatory diseases, dialysis, or decompensated/ severe heart failure were also excluded. All patients gave informed consent to participate in the study, which was approved by the local Ethics Committee. Blood samples were drawn from all patients after a fasting period of at least 12 h and were examined with the use of routine laboratory methods for blood counts, lipid parameters, C-reactive protein, renal and hepatic function. Coronary risk factors were defined as: obesity (body mass index > 30 kg/m\(^2\)); age; smoking (or previous smoking); hyperlipidaemia (total serum cholesterol > 220 mg/dL and/or serum triglycerides > 200 mg/dL); hypertension (systolic blood pressure > 140 mmHg or diastolic blood pressure > 90 mmHg on two consecutive seated measurements or therapy with antihypertensive medication); family history (first-degree relatives with cardiovascular disease); diabetes mellitus (fasting serum glucose levels > 126 mg/dL or therapy with oral hypoglycaemic agents or insulin). The characteristics of the study population are presented in Table 1. All patients were asked to refrain from smoking or drinking coffee or tea and from physical activity for 60 min and to discontinue medications for 12 h before endothelial function measurements.

**Assessment of endothelial function**

The methods employed for L-FMC/FMD analysis in our laboratory have been previously published\(^6\)\(^10\) and are detailed in the Supplemen-
tary material online. Briefly, patients were placed supine, the left arm immobilized, and L-FMC and FMD were measured using a Vivid 7 (General Electrics, Munich, Germany) ultrasound platform equipped with a 14 MHz matrix probe and a micrometric probe holder. Low-flow-mediated constriction corresponds to the constriction observed during a 4.5 min occlusion of a pneumatic cuff placed distal to the site of arterial diameter measurement. Flow-mediated dilation corresponds to the maximal dilation observed in the 5 min following deflation of the cuff, i.e. during reactive hyperaemia. Repeatability and reproducibility data of these methods have been recently reported (intra-class correlation coefficient = 0.68 and 0.80 for FMD and L-FMC, respectively).\(^9\) All data were acquired digitally and analysed in a randomized, blinded fashion prior to coronary angiography by an investigator not aware of the clinical status of the patient, using automatic dedicated software.\(^9\) Low-flow-mediated constriction could not be measured (images of insufficient quality for analysis) in 14 patients, and FMD could not be measured in another 14 patients.

**Coronary angiography**

Coronary angiography was performed in all patients 1–7 days after the assessment of L-FMC/FMD using a standard Judkins technique. Expert interventional cardiologists, who were blinded to the clinical data, interpreted all angiograms independently. For the purpose of the analysis presented in what follows, the presence of CAD was defined as angiographically detectable stenoses > 50% or a history of percutaneous coronary intervention. The SYNTAX score,\(^13\) a score developed for multivessel disease patients which takes into account the number, position, and anatomical characteristics of coronary lesions, was calculated in 124 patients with two- and three-vessel disease and no previous coronary artery by-pass surgery.

**Statistical methods**

Continuous variables are described by median, 25th and 75th percen-
tiles, if they had a skewed distribution (as defined by the Shapiro–Wilk test; skewness was < 1 for all variables) or by mean values and standard deviation if normally distributed. Discrete variables are described through relative and absolute frequencies. In order to detect differences across different stages of CAD, discrete variables were tested for trend with logistic regression; continuous variables were analysed for trend with the Jonckheere–Terpstra trend test. For descriptive purposes, patients were categorized based on the gender-specific median value of L-FMC and FMD; for this analysis, discrete variables were tested with \(\chi^2\)-test for contingency tables and continuous variables were ana-
lysed with t-test or the Mann–Whitney U test. Linear regression analysis was used to test for any relationship with the SYNTAX score.

To evaluate the contribution of FMD, L-FMC, and their combination in the prediction of CAD, the cut-off value associated with minimal
Assessment of vascular function

false-positive and false-negative results was determined for each variable by receiver operating characteristic (ROC) analysis. The area under the curve (AUC) in ROC analysis reflects the sensitivity and specificity at each cut-off level and therefore the overall accuracy of a model.\(^\text{14-16}\) To assess whether the addition of FMD, L-FMC, and/or the combination of both parameters to a logistic prediction model including all the above risk factors would improve the prediction of CAD, the AUC of the different ROC curves was compared using the DeLong test. The proportion of the variation in the diagnosis of CAD explained by different models (risk factors alone, risk factors + FMD, and L-FMC, risk factors + L-FMC + FMD, etc.) was evaluated using Nagelkerke’s pseudo-$R^2$ statistic. Data are presented with 95% confidence intervals (CIs). The increased discriminative value of L-FMC and FMD (over the model based on classical risk factors) was further examined using the net reclassification improvement (NRI) and the integrated discrimination improvement (IDI)

## Results

### Characteristics of the population

The 451 subjects included in this analysis had a mean age of 66 ± 10 years, and 306 (68%) were males. Ninety (20%) of the subjects had no angiographic evidence of CAD. Patient characteristics of the entire population and in each of the subgroups identified by the presence of one-, two-, and three-vessel CAD are presented in Table 1. In a logistic regression analysis, age ($P < 0.0001$), male gender ($P = 0.001$), hypertension ($P = 0.06$), and family history ($P = 0.045$) were associated with a diagnosis of CAD. Male gender, age >65 years, and hypertension were associated with lower L-FMC values ($P < 0.05$ for all); only male gender was associated with impaired FMD ($P < 0.05$). When patients were divided based on gender, the incidence of smoking (29.7 vs. 47.8%, $P = 0.001$) was higher in males, and a family history of CAD was more frequent in females (42.4 vs. 27.1, $P = 0.004$). Coronary artery disease was significantly more frequent in males (86.0 vs. 62.9%, $P < 0.0001$). Low-flow-mediated constriction and FMD were significantly lower in males (L-FMC: $-1.8\% (-3.4/1.0) \text{ vs. } -2.7 (-4.4/-0.9)$, $P = 0.007$; FMD: $3.3\% (1.7/6.1) \text{ vs. } 5.0\% (3.3/7.1)$, $P < 0.0001$).

Subjects with L-FMC values lower than the gender-specific median ($-2.7\%$ for women and $-1.8\%$ for men) were older, had higher prevalence of hypertension and smoking (all $P < 0.05$, Table 2). Patients with FMD values lower than the gender-specific median (5.0% for women and 3.3% for men) had higher prevalence of diabetes ($P < 0.05$, Table 2). There was no correlation between L-FMC or FMD ($P = 0.5$) and C-reactive protein level or other laboratory parameters, except for a negative correlation between total cholesterol levels and L-FMC ($R = -0.12$, $P = 0.02$).

### Table 1 Clinical characteristics of the patient population divided by coronary artery disease status

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No CAD ($n = 90, 21%$)</th>
<th>One-vessel CAD ($n = 67, 16%$)</th>
<th>Two-vessel CAD ($n = 100, 23%$)</th>
<th>Three-vessel CAD ($n = 173, 40%$)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>64 ± 10</td>
<td>65 ± 11</td>
<td>66 ± 11</td>
<td>68 ± 9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male gender</td>
<td>43 (48%)</td>
<td>44 (66%)</td>
<td>79 (79%)</td>
<td>141 (82%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>16 (18%)</td>
<td>12 (18%)</td>
<td>23 (23%)</td>
<td>62 (36%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>63 (70%)</td>
<td>56 (84%)</td>
<td>82 (95%)</td>
<td>138 (80%)</td>
<td>0.12</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>48 (53%)</td>
<td>39 (58%)</td>
<td>64 (64%)</td>
<td>113 (65%)</td>
<td>&lt;0.029</td>
</tr>
<tr>
<td>Family history</td>
<td>25 (28%)</td>
<td>29 (43%)</td>
<td>29 (33%)</td>
<td>49 (33%)</td>
<td>0.11</td>
</tr>
<tr>
<td>Obesity</td>
<td>31 (32%)</td>
<td>25 (39%)</td>
<td>32 (32%)</td>
<td>73 (42%)</td>
<td>0.35</td>
</tr>
<tr>
<td>Smokers</td>
<td>35 (39%)</td>
<td>36 (54%)</td>
<td>44 (44%)</td>
<td>71 (41%)</td>
<td>0.25</td>
</tr>
<tr>
<td>PCI</td>
<td>0</td>
<td>35 (36%)</td>
<td>74 (54%)</td>
<td>130 (52%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CABG</td>
<td>0</td>
<td>2 (4%)</td>
<td>7 (7%)</td>
<td>55 (16%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MI</td>
<td>0</td>
<td>19 (28%)</td>
<td>46 (46%)</td>
<td>84 (49%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>5.6 ± 1.1</td>
<td>4.2 ± 0.7</td>
<td>3.3 ± 0.4</td>
<td>4.0 ± 0.5</td>
<td>0.17</td>
</tr>
<tr>
<td>Blood flow at rest, mL/min</td>
<td>41 ± 22</td>
<td>41 ± 23</td>
<td>43 ± 23</td>
<td>44 ± 23</td>
<td>0.66</td>
</tr>
<tr>
<td>During cuff inflation</td>
<td>9 ± 5</td>
<td>10 ± 7</td>
<td>10 ± 7</td>
<td>10 ± 6</td>
<td>0.33</td>
</tr>
<tr>
<td>After cuff deflation</td>
<td>148 ± 80</td>
<td>173 ± 108</td>
<td>158 ± 67</td>
<td>151 ± 76</td>
<td>0.67</td>
</tr>
<tr>
<td>Therapy with</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>24 (27%)</td>
<td>40 (60%)</td>
<td>75 (75%)</td>
<td>138 (80%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ACE-inhibitors</td>
<td>50 (56%)</td>
<td>45 (50%)</td>
<td>86 (86%)</td>
<td>135 (78%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nitrates</td>
<td>4 (0.4%)</td>
<td>10 (11%)</td>
<td>15 (15%)</td>
<td>41 (24%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aspirin</td>
<td>37 (41%)</td>
<td>56 (83%)</td>
<td>90 (90%)</td>
<td>151 (87%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>43 (47%)</td>
<td>46 (51%)</td>
<td>77 (77%)</td>
<td>139 (80%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as number (%) or mean ± SD.
Confirming previous observations, there was a highly significant negative correlation between resting arterial diameter and FMD ($R = -0.37; P < 0.0001$). In contrast, there was no significant correlation between resting arterial diameter and FMC ($R = 0.08; P = 0.09$). Importantly, no correlation was found between L-FMC and FMD ($R = 0.05; P = 0.30$). These data are presented in Figure 1.

**Correlation of endothelial function parameters with coronary artery disease**

Baseline radial artery diameter was not influenced by the presence of CAD ($P = 0.4$). In contrast, both L-FMC and FMD were significantly blunted in patients with CAD, and a clear dose–effect relationship was observed between these variables and the number of diseased vessels ($P$-value for trend $< 0.0001$ for L-FMC; $0.00026$ for FMD, Figure 2). In addition, a significant correlation was found between L-FMC and the SYNTAX score ($R = 0.37; P < 0.0001$; Figure 1). There was however no significant correlation between resting diameter and the SYNTAX score ($R = 0.06; P = 0.5$) or between FMD and the SYNTAX score ($R = 0.15; P = 0.14$).

The logistic regression analysis (Table 3) showed that resting diameter, L-FMC, FMD, as well as male gender and family history of CAD, were independently associated with the presence of CAD. When only subjects without previous history of CAD were considered, the $P$-value expressing the association between FMD and the presence of CAD was of threshold significance ($0.052$), and that of L-FMC remained highly significant ($0.0014$). There was no difference between genders in these observations.

**Receiver operating characteristics analysis**

The AUC for a univariate model of FMD was 0.62 (95% CI 0.58–0.67, $P < 0.0001$, Figure 3A); the AUC for L-FMC was 0.71 (0.66–0.75, $P < 0.0001$) and that for the combination of FMD and L-FMC was 0.70 (0.65–0.74, $P < 0.0001$, $P < 0.001$ compared with FMD, $P = 0.83$ compared with L-FMC). The AUC for a model including only the resting diameter was 0.55 (0.50–0.59, $P = NS$).

There was no difference across genders in this analysis, and similar results were obtained when only those patients without previously known CAD were included. The cut-off associated with the best combination of sensitivity and specificity for each variable is presented in Table 4.

In order to study whether the addition of any of the endothelial function measures would improve the prediction of CAD, an ROC curve for a basic model including the coronary risk factors (age, male gender, obesity, hypertension, smoking, hyperlipidaemia, diabetes, family history of CAD) was developed (Table 3). The AUC of this curve was 0.716. Addition of FMD to this basic model including the risk factors increased the AUC statistic to 0.734 ($P = 0.13$). Addition of L-FMC to the basic model increased the AUC statistic to 0.771 ($P = 0.004$). Finally, addition of both L-FMC and FMD increased the AUC of the basic model to 0.779 ($P = 0.002$ compared with basic model alone, $P = 0.007$ compared with basic model + FMD, $P = 0.37$ compared with basic model + L-FMC). When only patients without previously known CAD were included in the analysis, the introduction of FMD or L-FMC alone did not change significantly the AUC of the ROC curve ($P = 0.42$ for FMD; 0.37 for L-FMC), but the addition of both variables on top of the basic model increased this AUC from 0.686 to 0.766 ($P = 0.013$). Model calibration with the Hosmer–Lemeshow statistic did not show any significant deviation between predicted and observed risk ($P > 0.5$ for all models).

**Reclassification properties**

The NRI and IDI statistics were used to assess to what extent adding one of the endothelial function measures to the risk factors model would (correctly or incorrectly) assign an individual to a different risk category. The Nagelkerke-$R^2$ of the model based including the eight risk factors alone was 0.177, i.e. this model could explain $\sim 18\%$ of the variability in the diagnosis of CAD. When FMD was added to this basic model, the NRI was 0.06.

### Table 2  Prevalence of each coronary artery disease risk factor when the population is divided based on endothelial function measurements

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Gender-Specific Median</th>
<th>Above Gender-Specific Median</th>
<th>Below Gender-Specific Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>65 ± 10</td>
<td>68 ± 10*</td>
<td>65 ± 11</td>
</tr>
<tr>
<td>Male gender</td>
<td>149 (71%)</td>
<td>150 (70%)</td>
<td>150 (71%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>52 (25%)</td>
<td>58 (28%)</td>
<td>50 (24%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>153 (73%)</td>
<td>176 (84%)*</td>
<td>159 (75%)</td>
</tr>
<tr>
<td>Hyperlipidaemia</td>
<td>130 (62%)</td>
<td>129 (61%)</td>
<td>126 (59%)</td>
</tr>
<tr>
<td>Family history</td>
<td>69 (33%)</td>
<td>62 (29%)</td>
<td>69 (32%)</td>
</tr>
<tr>
<td>Obesity</td>
<td>75 (36%)</td>
<td>85 (40%)</td>
<td>77 (36%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>80 (38%)</td>
<td>100 (48%)*</td>
<td>90 (42%)</td>
</tr>
</tbody>
</table>

An L-FMC lower than gender-specific median was associated with higher prevalence of hypertension and smoking and with older age. An FMD lower than median was associated with higher prevalence of diabetes. Data are expressed as number (%) or mean ± SD.

*P < 0.05.
(CI 0.06 to 0.18), with a Nagelkerke-$R^2$ \(= 0.199\), i.e. addition of FMD to the risk factors model net correctly reclassified \(\approx 6\% (P = 0.31)\) of the subjects by either attributing a higher probability of having CAD to a patient later diagnosed with CAD or by attributing a lower probability to a patient without CAD. The IDI was 0.02 (CI 0–0.03), with a \(P = 0.04\). After adding L-FMC to the eight risk factors model, the NRI was 0.19 (CI 0.06–0.33, \(P = 0.005\)), i.e. L-FMC net correctly reclassified 19% of the subjects; the IDI was 0.05 (CI 0.03–0.08, \(P < 0.0001\)), with a Nagelkerke-$R^2$ of 0.248. After adding both L-FMC and FMD to the risk factors model, the NRI was increased to 0.18 (CI 0.05–0.32, \(P = 0.009\)), i.e. an NRI of 18%; the IDI was 0.07 (0.04–0.10, \(P < 0.0001\)). The Nagelkerke-$R^2$ of this model was as high as 0.266. Thus, reclassification analysis showed that the introduction of endothelial function measures to the classical risk factors model improves the prediction of CAD, with a significant improvement in both reclassification parameters after the addition of L-FMC and a borderline improvement in IDI (non-significant improvement in NRI) after the addition of FMD (see Supplementary material online for the reclassification tables). Finally, when L-FMC was added to a model containing the eight risk factors and FMD, the IDI was 0.05 (\(P = 0.0001\)), the INR 0.17 (\(P = 0.01\)). These observations were unchanged when only patients without previously known CAD were included in the analysis.

**Discussion**

Atherosclerosis is an extremely complex phenomenon, and although many of the factors that predispose to this condition are well known on a population level, their interactions and their impact on each individual patient are very complex, if not impossible, to predict. Although easily measured in the peripheral circulation, endothelial function is a systemic phenomenon, and it has been proposed that FMD might provide indirect but relevant information on clinically more valuable vascular beds. Flow-mediated dilation is strongly influenced by the presence of risk factors and their interaction. However, it remains to be clarified whether the assessment of endothelial function provides information that is additive to, or whether it simply recapitulates, that of traditional risk factors. Further, the role of L-FMC (and whether this parameter is altered in different stages of the disease) remains unexplored.

Findings concerning the existence of a correlation between peripheral FMD and the extent of CAD are somewhat controversial.
Initially, Corretti et al. reported no significant difference in FMD between patients with known CAD and a control group of healthy individuals. Later, Rohani et al. proposed that morphological (i.e. brachial intima-media thickness) rather than functional (FMD) parameters provide information on the extent of CAD. Similarly, Frick et al. reported an association between CAD and brachial artery intima-media thickness, but no difference in FMD between patients with and without CAD. In contrast, several other authors have reported the existence of a strong correlation between FMD and CAD severity. In line with this, Matsushima et al. showed that FMD and intima-media thickness correlate to a similar degree to the extent of CAD.

Beyond differences in study populations and methods (for instance, the use of the proximal cuff method in the paper by Corretti, the use of brachial vs. radial artery), it needs to be acknowledged that the complex pathophysiology of endothelial (dys)function cannot be explained by a single parameter. Our incomplete understanding of endothelium’s complex mechanisms might explain the heterogeneity of the above findings, and in this sense the development of additional tools seems essential in an effort to improve our understanding of vascular homeostasis seems essential. In a recent paper, Spiro et al. provided evidence in support of the concept that the vasoconstriction observed during induction of a low-flow state (L-FMC) helps interpret FMD data, and might represent another clinically relevant important parameter of vascular (endothelial) function. Evidence suggests that L-FMC, whose existence has been confirmed using magnetic resonance imaging, is blunted in the presence of cardiovascular risk factors and cardiovascular disease, and that this low-shear-stress-induced vasoconstriction is mediated by the release (or the inhibition of the release) of endothelial autacoids such as prostaglandins, endothelin-1, and cyclooxygenase products.

The present study was designed to investigate, in a larger cohort of patients, whether L-FMC correlates with FMD and with the extent of CAD. Further, we addressed whether the introduction of these measures of endothelial function measures provides information that is additional to that of traditional risk factors.

Summary of the findings

In line with previous findings with FMD, we demonstrate an association between traditional risk factors and impaired L-FMC. Further, both L-FMC and FMD showed a dose–effect relationship with the severity of CAD (i.e. a progressive decline in both parameters was observed across levels of CAD). In particular, a statistically significant correlation was demonstrated between L-FMC and the SYNTAX score, a morphological score that has been shown to predict clinical outcome in cardiovascular patients. Importantly, confirming the hypothesis that endothelial function measures provide information that goes beyond that of the traditional risk factors, logistic regression analysis showed that the association between FMD, L-FMC, and CAD remained significant when coronary risk factors and resting arterial diameter were accounted for.

Data from ROC and reclassification analyses provide further insight on the role of endothelial function measures in the detection of CAD. In the present study, the predictive accuracy of a basic model including eight ‘traditional’ risk factors for CAD was quite good; the addition of L-FMC or the combination of FMD + L-FMC (modestly but significantly) increased the model discrimination. Confirming that the two measures provide different
Figure 3 Receiver operating characteristic analysis for the presence of coronary artery disease. (A) Comparison of the receiver operating characteristic curves for low-flow-mediated constriction (L-FMC), flow-mediated dilation (FMD), and their combination. The area of the receiver operating characteristic curve of low-flow-mediated constriction + flow-mediated dilation was significantly larger than that of flow-mediated dilation. There was no significant difference between flow-mediated dilation and low-flow-mediated constriction. (B–D) Comparison of the area under the curve (AUC) of a model based on traditional risk factors (in red) with that of a model based on the same risk factors plus low-flow-mediated constriction (B), flow-mediated dilation (C). (D) The impact of adding low-flow-mediated constriction measurements to a model containing the eight traditional risk factors and flow-mediated dilation.

Table 4 Sensitivity and specificity of the cut-off values of each parameter (i.e. the values associated with the best combination of sensitivity and specificity) for the presence of coronary artery disease

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cut-off</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive likelihood ratio</th>
<th>Negative likelihood ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-FMC</td>
<td>-1.8%</td>
<td>53 (48–59)%</td>
<td>80 (70–88)%</td>
<td>2.69</td>
<td>0.59</td>
</tr>
<tr>
<td>FMD</td>
<td>3.7%</td>
<td>53 (48–59)%</td>
<td>69 (58–69)%</td>
<td>1.72</td>
<td>0.68</td>
</tr>
<tr>
<td>FMD + L-FMC</td>
<td>4.9%</td>
<td>44 (39–50)%</td>
<td>85 (75–92)%</td>
<td>2.87</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Data are expressed as % (specified in the table) or number.
information, there was no correlation between FMD and L-FMC (discussed in detail in Gori et al.15).

Taken together, these data demonstrate that the assessment of endothelial function provides information that is additive and complementary to that of traditional risk factors on the presence and extent of CAD, and that the combination of FMD and L-FMC is more informative than ‘traditional’ FMD alone.

**Limitations**

Limitations need to be acknowledged. Our study enrolled patients undergoing CAD diagnostics based on a clinical indication. Therefore, our observations should not be generalized to the general population. Larger studies will be required to fully explore the role of L-FMC, particularly in terms of its prognostic value and in other less high-risk populations; the impact of changes in L-FMC in response to therapies also needs to be evaluated. Nevertheless, the significant increases in AUC observed in the present study demonstrate that the introduction of endothelial function measures adds information on top of that provided by the interaction of eight ‘standard’ risk factors. The possible confounding effect of cardiovascular medications (unavoidable in a clinical study), and their interaction with risk factors, also needs to be acknowledged. Although these medications may have impacted the results, the larger use of statins, ACE-inhibitors and other protective medications in patients with CAD would likely rather reduce the differences across groups, leading to an underestimation of the role of FMD and L-FMC. Further, responses to nitroglycerin, an endothelium-independent vasodilator, were not tested, and no endothelium-independent vasoconstrictor has ever been tested to control for L-FMC. Finally, differences across vascular beds should also be considered: L-FMC is more consistently demonstrated in the radial as in the brachial artery (which is more commonly used for FMD studies), and very little data exist comparing the two vascular beds. Collectively, despite the above limitations, our data show that endothelial function measures provide information on the presence and extent of CAD also in real-life clinical settings. Although it is clear that the accurate collection of medical history remains of central importance, assessment of FMD and L-FMC might facilitate the interpretation of the cardiovascular risk at an individual level.

**Conclusions and perspectives**

The use of endothelial function measures as a clinical diagnostic method has several conceptual and practical limitations, and it is clear that these data are not meant to replace clinically accepted diagnostic tools used in the diagnosis of CAD (for instance, stress testing). On the other hand, the information provided by risk factors is sometimes hard to translate from population studies to individual patient care, and the vascular endothelium may in theory serve as a useful indicator for the global status of the cardiovascular system. However, the interpretation of complex phenomena cannot rely on one single measure taken at one single endpoint. There is a need for further definition of endothelial function employing methods that allow a more sophisticated, but still technically simple and non-invasive methodology. Future studies will have to test the time-course of the combination of L-FMC and FMD measurements over time, in different stages of the disease, in response to drugs, and in terms of their prognostic implications, as well as their relationship with measures on endothelial function in resistance vessels.13 Introduction of newer and more accurate methods that provide prognostic information might have several applications: for instance, in the detection of (risk of) progression of CAD, or in monitoring the impact of anti-atherosclerotic therapies on an individual basis. Before this possibility is tested, it is important to acknowledge that the pathophysiology of endothelial dysfunction is an extremely complex phenomenon and that its interpretation should not be reduced to a single parameter.

**Supplementary material**

Supplementary material is available at European Heart Journal online.

**Funding**

This study was funded, in part, by a grant from the Heart and Stroke Foundation of Canada and by a grant from the Schwerpunkt Vaskuläre Prevention of the University Medical Center Mainz. J.D.P. holds a Career Investigator Award from the Heart and Stroke Foundation of Ontario, Canada. The work is part of the doctoral thesis of S.M.

**Conflict of interest:** none declared.

**References**


