The inclusion criteria remained substantially homogeneous over time. Included patients had (1) typical chest pain, exclusively or mainly related to effort, suggesting obstructive CAD; (2) positive EST, as diagnosed according to exercise-induced ST-segment depression on electrocardiogram (ECG); (3) normal epicardial coronary arteries at angiography; and (4) absence of any evidence of coronary artery spasm (based on clinical history, non-invasive tests and, if necessary, i.c. or i.v. ergonovine test), other heart diseases (e.g., valvular disease, cardiomyopathy), and clinically relevant systemic diseases. Patients with significant left ventricular (LV) hypertrophy (LV wall thickness >12 mm) and/or even mild impairment of LV function (LV ejection fraction <50%) on echocardiography, were also always excluded.

All patients had carefully been characterized at enrollment with regard to cardiovascular risk factors (hypertension, smoking, diabetes, obesity, family history of CAD), symptom status and diagnostic work-up.

Patients were contacted by telephone and invited to undergo a follow-up clinical visit, standard ECG and
treadmill EST. Patients who underwent new coronary angiography during follow-up and were found to have obstructive CAD (≥50% diameter stenosis in one or more epicardial coronary arteries), whether or not treated with coronary revascularization, were excluded.

Symptom status was assessed on weekly frequency of angina episodes and nitroglycerin tablet consumption as reported by the patients. Patients were also asked to report whether their symptoms had worsened, improved, or remained unchanged over time.

All patients were informed of the purpose and nature of the study and gave informed consent to participate. The study was approved by the Institutional Review Board.

Patients were excluded in the case of predicted poor effort tolerance or presence at baseline of ECG abnormalities that could interfere with a correct assessment of ischemic changes. Enrolled patients underwent a symptom-sign-limited treadmill EST, using the Bruce protocol, after 48 h of anti-ischemic medication wash-out.

EST at the present institution are always conducted following the same standard methods, which were also applied for this study. Patients were continuously monitored with 3 ECG leads (DII, V2 and V5) throughout the test, and a 12-lead ECG was printed at the end of each stage; at peak exercise; when clinically indicated; and at 1-min intervals during the recovery period. Blood pressure was taken at rest; at peak exercise; during the last minute of each stage; and at 1-min intervals during recovery. The test was stopped in the case of (1) physical exhaustion; (2) worsening angina (Borg scale >6); (3) potentially harmful clinical events (e.g., dyspnea, hypotension, arrhythmias); and (4) ST-segment depression >4 mm. The ST-segment depression was considered diagnostic of myocardial ischemia when horizontal or downsloping and ≥1 mm, at 0.08 s from the J point.

Patients were divided into 2 groups according to follow-up EST. Group 1 included patients who developed EST-induced myocardial ischemia, as defined (positive EST), whereas group 2 included patients who did not develop any significant ST-segment abnormality at follow-up EST (negative EST).

Table 1. Reference EST: Clinical Data

|                      | Group 1 (n=41) | Group 2 (n=30) | P-value |
|----------------------|---------------|---------------|---------|
| Age (years)          | 50.4±8        | 49.5±11       | 0.69    |
| Follow-up (years)    | 15.2±5        | 17.9±8        | 0.075   |
| Sex (F)              | 29 (71)       | 21 (70)       | 1.00    |
| Cardiovascular risk factors |   |               |         |
| Family history of CAD| 12 (29)       | 10 (33)       | 1.00    |
| Hypertension         | 27 (66)       | 16 (53)       | 0.33    |
| Hypercholesterolemia | 28 (68)       | 17 (57)       | 0.33    |
| Obesity              | 10 (24)       | 9 (30)        | 0.60    |
| Diabetes             | 11 (27)       | 4 (13)        | 0.24    |
| Smoking              | 3 (7)         | 5 (17)        | 0.27    |
| Rest angina          | 7 (17)        | 12 (40)       | 0.056   |
| Dyspnea on effort    | 23 (56)       | 10 (33)       | 0.09    |
| Positive stress scintigraphy | 33 (80) | 24 (80) | 1.00    |
| CBF response to adenosine† | 1.80±0.70 | 2.60±1.35 | 0.039   |
| CBF response to CPT‡  | 1.24±0.44     | 1.78±0.67     | 0.019   |

Data given as mean±SD or n (%). †Data obtained in 20 and 10 patients with positive and negative EST at follow-up, respectively. δData obtained in 17 and 9 patients with positive and negative EST at follow-up, respectively. CAD, coronary artery disease; CBF, coronary blood flow; CPT, cold pressor test; EST, exercise stress test.

Statistical Analysis
Continuous variables are reported as mean±SD, while discrete variables are reported as proportions. Between-group comparisons were done using independent t-test and Fisher exact test for continuous and discrete variables, respectively. Comparisons of follow-up EST data with those of the reference EST were done separately for the 2 groups using paired t-test and McNemar test for continuous and discrete variables, respectively. Two-sided P<0.05 was required for statistical significance. SPSS 21.0 (SPSS Italy, Florence, Italy) was used for statistical analysis.

Results
Overall, 250 patients formed the original group that underwent long-term clinical follow-up, the results of which have been reported elsewhere.* Overall, 26 patients died (10.8%) and 43 (17.2%) could not be contacted directly.

Of 181 patients who were directly contacted, 87 (48%) agreed to participate in the study. Of these patients, 11 were excluded because they had developed obstructive CAD, and 5 because of basal ECG abnormalities that could interfere with ST-segment analysis (atrial fibrillation in 2 and left bundle branch block in 3).

Thus, the final subject group consisted of 71 patients (39%). EST was performed at an average follow-up of 16.2 years (range, 5–25 years) from the reference EST. Forty-one patients (58%) developed significant ST-segment depression at the ECG during EST (group 1), whereas 30 (42%) had negative EST (group 2). The main clinical characteristics of the 2 groups at the time of the reference EST are listed in Table 1.

Compared with group 2, group 1 patients less frequently had a history of rest angina, but more frequently reported exercise-induced dyspnea, although the differences were of borderline statistical significance. No relationship was found between cardiovascular risk factors and persistence of a positive EST.
On perfusion stress scintigraphy in 28 group 1 patients (68%) and in 20 group 2 patients (67%), reversible perfusion defects were noted in 82% and 80%, respectively (P=1.0). Coronary blood flow (CBF) dilator response to adenosine and cold pressor test, assessed in the left anterior descending coronary artery on transthoracic Doppler echocardiography, was available for 30 (73%) and for 26 patients (87%) patients in the 2 groups, respectively, and was lower in group 1 compared with group 2 (P<0.039 and P<0.019, respectively).

Baseline EST results are compared in Table 2. Overall, group 1, compared with group 2, had a slightly higher blood pressure, a higher maximum ST-segment depression and a tendency to a lower time to 1-mm ST-segment depression but longer duration of exercise. The proportion of patients who developed angina during EST was similar.
57% vs. 29% of group 2 and group 1 patients, respectively (P=0.028).

The main clinical findings at follow-up are summarized in Table 3. No significant differences in outcome of angina status were observed between the 2 groups, which had also similar frequency of angina episodes and nitrate consumption. Medication also did not differ significantly between the 2 groups. Group 2 patients, however, had more frequent use of multiple anti-ischemic treatment. In particular, the addition to basal β-blocker and/or calcium-antagonist treatment of one or more of long-acting nitrates, ivabradine, ranolazine and spinal cord stimulation was noted in the 2 groups.

Tables 4 and 5 list results of follow-up EST. Follow-up EST in group 1 showed a tendency to worsening of ischemic threshold, compared with the basal test, as indicated by shorter time to 1 mm ST-segment depression. In group 2 patients, the duration of exercise at follow-up was significantly shorter than that of the reference EST (P=0.001). Exercise duration at follow-up, however, was similar to time at 1 mm ST-segment depression in the refer-
ence test; moreover, heart rate and rate-pressure product at peak exercise were significantly higher compared with those at 1-mm ST-segment depression on the reference test (P<0.01 for both).

Angina during the follow-up EST was induced in a similar proportion of patients in the 2 groups (P=1.0), which was lower in each group compared with the baseline EST, although the difference did not achieve statistical significance (P=0.18 for both groups), likely due to the low number of patients who developed the symptom.

**Discussion**

The main findings of the present study are as follows. First, EST-induced myocardial ischemia improves over time in a sizeable proportion of MVA patients. Although several previous studies reported on outcome of typical MVA, surprisingly no previous study investigated the trend of EST-induced myocardial ischemia at long-term follow-up in these patients. Interestingly, we noted a varying outcome of ischemic changes induced by EST in these patients. The test showed persistence of exercise-induced ST-segment depression in 58% of patients, but was negative for myocardial ischemia in 42%.

The reduced EST duration at follow-up might suggest a possible pseudo-improvement of EST in the latter group, related to missed achievement of ischemic threshold. The duration of the test, however, was similar to time 1-mm ST-segment depression on the reference EST, and both heart rate and rate-pressure product at peak exercise were higher than those observed at ischemic threshold on the reference test, thus suggesting true improvement of EST-induced myocardial ischemia.

In patients with persistent positive EST, in contrast, the tendency to a lower time and rate-pressure product at 1-mm ST-segment depression at follow-up compared with the reference EST, together with a similar maximum ST depression despite significant lower duration and rate-pressure product at peak exercise, suggests some worsening, although mild, of EST results, possibly related to a mild worsening of CMVD.

The reasons for the improvement of EST results in a group of patients and worsening (or lack of improvement) in another group cannot be established from the present data. Differences in the control of cardiovascular risk factors via both adequate lifestyle changes and appropriate medical therapy, might have significantly contributed to the observed different control of CMVD.

The second relevant finding is that there was no significant association between changes in EST results and symptom status. Indeed, variations in angina were similar in the 2 groups, with most patients reporting reduction of angina episodes, confirmed by the lower (albeit non-significant) and similar prevalence in the 2 groups of angina during follow-up EST compared with basal EST.

The discrepancy between ECG signs of myocardial ischemia and angina symptoms might be related to some different role of cardiac nociception, as compared with myocardial ischemia, in determining symptom outcome in individual cases of MVA. Increased cardiac nociception has indeed been shown to be present in a sizeable proportion of MVA patients and is suggested to play a significant role in determining symptom status. A greater symptom burden in group 2 patients, in fact, is suggested by the greater use of additional anti-ischemic medications, compared with group 1, despite a similar number of angina episodes. Of note, the present results are in agreement with those of our previous study in which MVA symptom status was significantly predicted by abnormalities in cardiac adrenergic nerve fibers, but not by EST results nor severity of CMVD.

A prevalent coronary microvascular constrictor mechanism (rather than impaired dilatation) in group 2 patients might also contribute to explain the normal follow-up EST results, despite the similar symptom burden, in at least some patients. A coronary microvascular spasm triggered by sympathetic activation related to exercise might indeed be responsible for exercise-induced myocardial ischemia, but this mechanism might be less reproducible than the inadequate increase of CBF during exercise related to impaired microvascular dilatation. The role of coronary microvascular spasm/constriction in at least a number of group 2 patients is, in fact, suggested by the fact that rest angina had a greater prevalence in this group compared with group 1 and, in the subset of patients who underwent assessment of CMVD, CBF responses to adenosine and CPT were within the normal range in group 2.

The last interesting finding is that a history of exercise-induced dyspnea, together with angina, tended to be associated with persistence of positive EST at follow-up. This suggests that microvascular ischemia in some patients might induce a form of heart failure with preserved LV function, possibly related to the development of CMVD-related diastolic dysfunction. This hypothesis, however, needs to be investigated in appropriately designed studies.

Some limitations of the present study should be considered. First, patients who were able or agreed to participate in this study comprised only 39% of those who could potentially be recruited; thus, we cannot exclude some selection bias.

Coronary angiography was not performed during follow-up in most of the patients; thus, although unlikely, we cannot completely exclude the possibility that some patients might have developed obstructive CAD.

Basal coronary microvascular function was available for a subgroup of patients only and it was not reassessed at follow-up. Thus, the association of EST and angina outcomes to possible changes of coronary microvascular function cannot be established from the present data.

**Conclusions**

This is the first study to report data on long-term follow-up of EST results in patients diagnosed with primary coronary MVA. ECG signs of microvascular ischemia improved in a sizeable proportion of patients, suggesting improvement of coronary microvascular function. Of note, persistence of positive EST showed no significant association with symptom outcome.

**Disclosures**

The authors declare no conflicts of interest.

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