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Abnormal coronary reserve and left ventricular wall motion during cold pressor test in patients with previous left ventricular ballooning syndrome

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Aims To investigate whether and how cold pressor test (CPT) could affect myocardial perfusion and left ventricular (LV) function in patients with previous LV ballooning syndrome (LVBS).

Methods and results Cold pressor test (3 min hand immersion in ice-water) was performed in 17 women with previous LVBS and in 7 age- and risk factor-matched women with chest pain and normal coronary arteries. At baseline and peak CPT, global and regional LV function, and myocardial perfusion were quantitatively assessed by real-time three-dimensional echocardiography (RT3DE) and myocardial contrast (SonoVue, Bracco) 2D echocardiography (MCE), respectively (Philips iE33 machine, X3-1 and S5-1 probes). Data were analysed off-line (QLab 6.0 software). Peripheral venous catecholamines were assayed by high performance liquid chromatography with electrochemical detection. Cold pressor test induced similar haemodynamic changes and catecholamine increase in controls and LVBS patients. Left ventricular ejection fraction decreased and transient new mid-ventricular and apical motion abnormalities developed in LVBS patients only (quantitative RT3D analysis), without corresponding perfusion defects (MCE). At peak CPT, coronary blood flow and velocity increased (quantitative MCE analysis) in control subjects only.

Conclusion Cold pressor test induced LV wall motion abnormalities unmatched to regional coronary flow reduction in LVBS patients only. The reduced coronary reserve in response to CPT suggests microvascular dysfunction in LVBS patients.

Keywords Cold pressor test • Coronary flow • Echocardiography • Tako-Tsubo

Introduction

Transient left ventricular ballooning syndrome[1] (LVBS), also known as Tako-Tsubo[2] or stress-induced cardiomyopathy,[3] is a recently described clinical condition, largely confined to female gender, which may mimic acute myocardial infarction (AMI).[4] Initially described in Japan[2] and subsequently recognized in the USA[5] and in Europe,[6,7] LVBS incidence has increased to 12% since attention was focused on women with suspected anterior AMI.[8] In LVBS patients, a history of angina was reported in 30[9] to 43%[10] of patients. After the acute presentation, chest pain recurred in up to 30%;[11] however, a clear demonstration of recurrence of overt LVBS is uncommon (up to 11.4% over the first 4 years).[7,12]

Myocardial adrenergic hyperstimulation appears to be an important component of the pathogenesis of the disease due to the proximity to an acute emotional and/or physical stress. However, the pathogenesis of LVBS remains poorly understood. Several mechanisms have been proposed on the basis of the invasive and non-invasive findings gathered at or immediately after acute presentation. They include, among others, multivessel

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epicardial coronary spasm, catecholamine-associated myocardial dysfunction,[13,14] functionally impaired adrenergic neurotransmission, and microvascular spasm or dysfunction.[15,16]

Cold pressor test (CPT) is a standardized technique that physiologically submits the patient to an abnormal stress, the principle being a nociception-induced activation of the sympathoadrenomedullary system. In the normal heart, vasoconstriction mediated by the surge of catecholamines induced by CPT is overwhelmed by increased myocardium oxygen demand, so that the net effect of CPT is coronary vasodilation largely mediated by an endothelium-dependent flow-related mechanism, and increased coronary blood flow (CBF).[17,18]

In this study, we submitted to CPT patients who had completely recovered from a previous LVBS episode with the aim to investigate whether and how CPT could affect myocardial perfusion and LV function in patients with previous LVBS. For this purpose, we designed a study protocol comprising myocardial contrast echocardiography (MCE) and real-time three-dimensional echocardiography (RT3DE) performed one immediately after the other, in order to analyse almost simultaneously myocardial perfusion, LV wall motion, and global systolic function.

Methods

Patient population

Seventeen patients (all females, aged 73 ± 12 years) who had recovered from acute LVBS diagnosed according to the Mayo Clinic criteria[19] were recruited 688 (518–936) days (median and inter-quartile range) after the acute episode (LVBS group). A stressful situation had been the precipitating event of LVBS in 15 patients whose symptoms had been chest pain (13) or dyspnoea (2); prolonged epileptic crisis and cholecystectomy had been the triggering event in the other two cases. ST-segment elevation followed by T-wave inversion had been the most frequent ECG presentation (14 patients); LV ejection fraction (LVEF) had been 38 ± 10%; peak troponin I had reached 7 ± 5 μg/L and N-terminal pro-brain natriuretic peptide (NT-pro-BNP) (in 6 patients) 2887 ± 2759 ng/L. At angiography, epicardial coronary vessels were normal in all patients; no spasm and no muscle bridge were documented. Corrected Thrombolysis In Myocardial Infarction trial (TIMI) frame count was abnormal (i.e. >28)[20,21] in 88% of patients: one vessel was affected in seven patients, two vessels in two patients and three vessels in six patients. Corrected TIMI frame counts in LVBS patients compared with controls were: 29.9 ± 10.2 vs. 21.8 ± 1.8 frames in the left anterior descending coronary artery ($P = 0.05$), 31.9 ± 9.2 vs. 23.4 ± 4.2 frames in the left circumflex coronary artery ($P = 0.005$), and 31.6 ± 11.8 vs. 21.7 ± 4 frames in the right coronary artery ($P = 0.03$). The typical apical-ballooning-appearance LV dysfunction was documented both at angiography and at echocardiography, and had reverted completely in all LVBS patients at follow-up. As a control group, we recruited seven age- and risk factor-matched women (aged 70 ± 8 years) who were admitted to the Emergency Department because of acute chest pain 205 (83–379) days (median and inter-quartile range) before the study, and received on that occasion urgent diagnostic coronary angiography with documentation of normal epicardial coronary vessels. By study design, eligibility criteria included normal LV wall motion and systolic function (LVEF 63% ± 2), normal ECG, and no troponin I rise at the index hospitalization.

Table 1 Patient population characteristics

	Controls	LVBS patients
Post-menopausal females	7/7	16/17
Hypertension	2/7 (29%)	5/17 (29%)
Hyperlipidaemia	3/7 (43%)	8/17 (47%)
Diabetes	0	0
Family history of CAD	1/7 (14%)	3/17 (18%)
Current smoking	1/7 (14%)	3/17 (18%)
LVEF (%)	64 ± 5	65 ± 7
LV mass (g/m^2)	80 ± 8	83 ± 13

LVBS, left ventricular ballooning syndrome; CAD, coronary artery disease; LVEF, left ventricular ejection fraction; LV, left ventricular.

At the time of the present study, all LVBS patients and control subjects had normal baseline wall motion and systolic function, and were asymptomatic. Cardiovascular risk factor profile and baseline echocardiographic data of the two groups are detailed in *Table 1*.

Any vasodilator therapy was discontinued 24 h before the study; β-adrenergic blockers were discontinued 48 h before the study.

All subjects agreed to participate in the study and gave their informed written consent. The study protocol complies with the Declaration of Helsinki and was approved by the locally appointed Ethics Committee.

Study protocol

Baseline assessment included transthoracic two-dimensional (2D) colour Doppler echocardiography, myocardial perfusion echocardiography (MCE), contrast-enhanced RT3DE (LVO) [iE33—Philips Medical Systems, Andover, MA, USA; S5-1 (1–5 MHz) probe for 2D imaging, X3-1 (1–3 MHz) matrix transducer for RT3D], blood sampling for catecholamine and NT-pro-BNP dosage, blood pressure (Siemens Sirecust 888), and heart rate monitoring. Immediately after the baseline assessment, patients were asked to keep still in left lateral decubitus with their left arm extended; CPT was performed by right-hand immersion in ice-water for 180–220 s. During the third minute of CPT, MCE and RT3D LVO were performed, and blood samples were collected. At the end of CPT, patients were asked to grade the cold and pain sensations felt during the test.[22] Patients were then monitored for 1 h and 2D echocardiography was repeated before dismissal.

Myocardial contrast echocardiography

Low-mechanical index (0.19) imaging was used to perform MCE during SonoVue infusion (Bracco Research SA). Myocardial contrast echocardiography parametric quantification (Q-Lab, Version 6.0, Philips Medical Systems) was performed off-line by an investigator blinded to the medical history of the patient and the results of RT3D analysis (see Supplementary material online).

Real-time three-dimensional imaging

Real-time three-dimensional LVO was performed at baseline and peak CPT immediately after 2D MCE imaging (within 8 s, i.e. the time lapse necessary to switch from S5-1 to X3-1 probes and select the appropriate 3D preset). To accomplish volumetric analysis of global and regional LV function, RT3D data sets were analysed off-line (3DQ ADV, QLAB, Version 6.0, Philips) by an investigator experienced in RT3D image interpretation, who was blind to the medical history of

the patient and the results of perfusion analysis (see Supplementary material online). The minimum volume reached by each region at end-systole reported as percentage of the corresponding end-diastolic volume provided for quantification of regional LV function. Abnormal wall motion response was defined as rest-to-CPT variation of per cent regional end-systolic volume greater than rest normal standard deviation (SD), provided peak CPT per cent regional end-systolic volume was beyond the upper limit of normal range (mean ± 1 SD). In our laboratory, test–retest reproducibility relative to the diagnosis of ischaemia by means of such a criterion was blindly assessed in 35 randomly selected coronary artery disease patients in whom the diagnosis was validated by coronary angiography, and showed false-positive diagnosis in 2.2% of regions (1.0% for basal, 1.9% for middle, and 4.0% for apical regions).

Hormones

Plasma norepinephrine (NE) and epinephrine (E) levels were assayed by high performance liquid chromatography with electrochemical detection.[23]

N-terminal pro-brain natriuretic peptide plasma levels were assayed by means of proBNP II Cobas® diagnostic system.

Statistical analysis

Continuous variables were expressed as mean value ± SD. Statistical analysis was performed by means of SPSS 15.0.1. For repeated measure analysis, the general linear model was used. Homogeneity of variance was evaluated by Levene's test. Pillai's Trace F was used to test the response to CPT and the different behaviour of CPT in the two groups. The observed power of the test was computed using $\alpha = 0.05$. Baseline data and coronary reserve were compared by means of Student's *t*-test or χ^2 test as appropriate. A value of $P < 0.05$ was considered significant. All tests were two-sided.

Results

The score of cold and pain sensation during CPT did not differ between LVBS patients and controls (2.75 ± 0.50 vs. 2.00 ± 0.82; 2.75 ± 0.50 vs. 2.75 ± 1.26, respectively).

Baseline functional data and the haemodynamic effects of CPT are reported in *Table 2*. Rate-pressure product increased significantly in the two groups (CPT effect, $P = 0.013$) driven by a similar increase in heart rate (CPT effect, $P = 0.003$). Blood pressure increase induced by CPT was not statistically significant vs. baseline nor differed between LVBS patients and controls. Cold pressor test induced no changes of LV end-diastolic volume both in patients and controls; LVEF slightly increased in controls (+4.4 ± 7.7%), whereas it decreased in LVBS patients (−3.9 ± 5.5%, CPT test and group $P = 0.007$, power 0.813). No LV outflow tract obstruction was elicited by CPT.

Cold pressor test induced a significant increase in peripheral venous catecholamine levels of the two groups, as shown in *Figure 1* (CPT effect on E, $P = 0.006$; CPT effect on NE, $P = 0.002$).

N-terminal pro-brain natriuretic peptide levels were unchanged in control subjects (rest: 175 ± 73 ng/L; peak CPT: 180 ± 82 ng/L; +2 ± 6%) and in LVBS patients (rest: 219 ± 106 ng/L; peak CPT: 221 ± 103 ng/L; +3 ± 11%).

Three-dimensional wall motion analysis demonstrated in all but one LVBS patient that CPT induced transient impairment of wall motion of apical and mid-ventricular LV regions (mean 2.6 ± 1.8 LV regions, range 0–5) that fully recovered at the 2D echocardiographic examination repeated before dismissing the patient after the study. The per cent regional distribution of wall motion abnormalities is detailed in the bull's eye representation of the 17-segment model of the LV endorsed by the American Society of Echocardiography reported on the right side of *Figure 2*; CPT-induced regional systolic dysfunction comprised more than a single coronary bed as defined by the American Heart Association (*Figure 2*). Cold pressor test did not induce any wall motion impairment in control subjects. No significant ECG changes were noticed during the test.

Examples of rest and peak CPT RT3D LVO images relative to one representative LVBS patient are shown in *Figures 3* and *4*, respectively.

In all LVBS patients and control subjects, MCE analysis showed homogeneous LV perfusion at baseline and peak CPT. Visual and parametric analyses were concordant in all cases. Video intensity plateau value (the value *A*, which is proportional to blood volume and to the microvascular cross-sectional area), the rate constant of video intensity rise (the value β, which correlates with blood velocity), and their product ($A \times \beta$, which represents blood flow) are reported in *Table 3*, relative to the four-, two-, and three-chamber apical views. Repeated measure analysis showed: (i) no differences in *A* value in both groups; (ii) a significant increase in β value in the control group only (CPT and group effect four-chamber $P < 0.0001$, power 0.989; two-chamber $P = 0.021$, power 0.945; three-chamber $P = 0.002$, power 0.933); (iii) an

Table 2 Haemodynamic effects of cold pressor test

	Control group		LVBS patients	
	Baseline	CPT	Baseline	CPT
Systolic arterial pressure (mmHg)	136 ± 18	147 ± 19	138 ± 17	141 ± 28
Heart rate (b.p.m.)	70 ± 6	75 ± 9	73 ± 9	82 ± 12
Rate-pressure product	9535 ± 1598	11019 ± 1659	10124 ± 1936	11573 ± 3009
EDVI (mL/m^2)	59 ± 9	60 ± 9	58 ± 12	58 ± 12
LVEF (%)	64 ± 5	66 ± 7	65 ± 7	62 ± 7

EDVI, end-diastolic volume index; LVEF, left ventricular ejection fraction; CPT, cold pressor test; LVBS, left ventricular ballooning syndrome. Statistical significances are reported in the text.

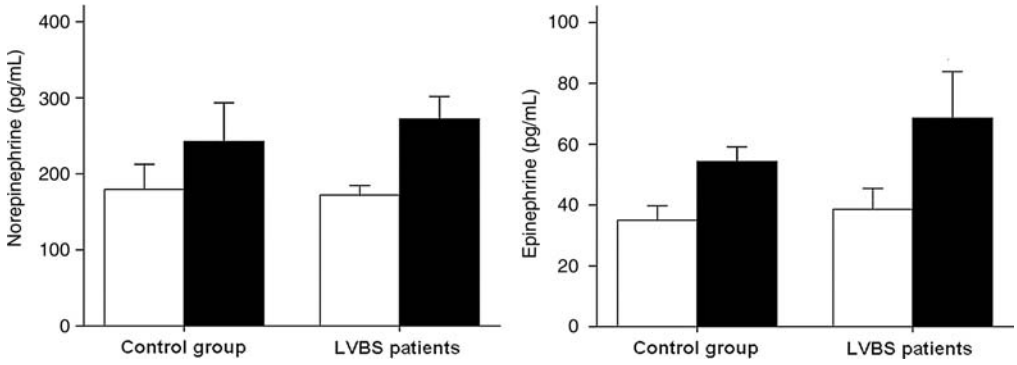

Figure 1 Plasma norepinephrine and epinephrine levels at baseline and at peak cold pressor test. Cold pressor test effect on epinephrine, $P = 0.006$, and on norepinephrine, $P = 0.002$.

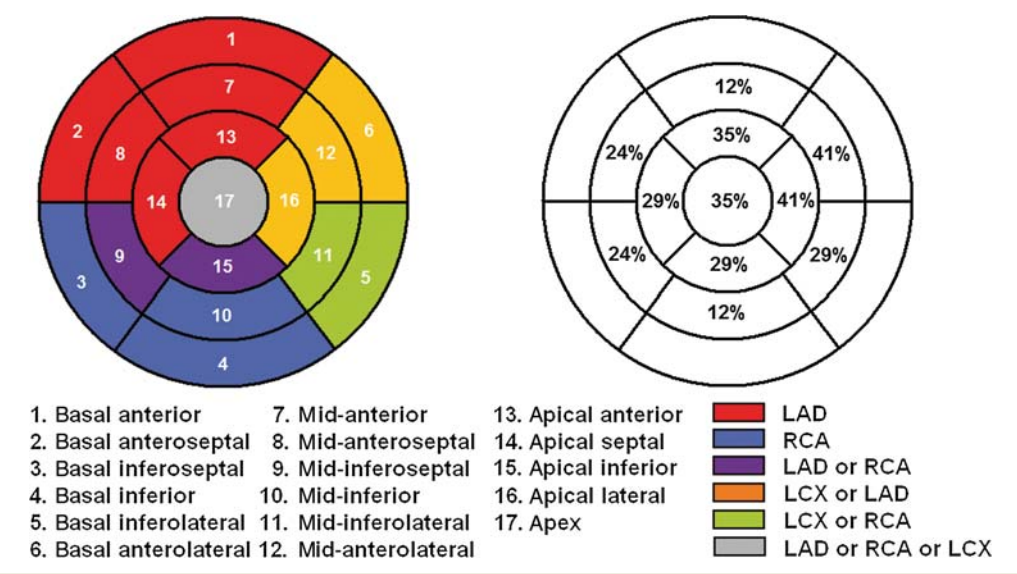

Figure 2 Incidence and coronary distribution of wall motion abnormalities at peak cold pressor test in left ventricular ballooning syndrome patients. In the bull's eye representation of the 17-segment model of the left ventricle on the right, numbers indicate the percentage of cold pressor test-induced dysfunction per region. Each of the 17 regions of the model is identified by the numbers reported in the bull's eye representation of the model on the left, and the corresponding label; each epicardial coronary vessel distribution is colour coded on the same bull's eye. LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; RCA, right coronary artery.

increase in $A \times \beta$ value in the control group only (CPT and group effect four-chamber $P = 0.008$, power 0.795; two-chamber $P = 0.030$, power 0.603; three-chamber $P = 0.001$, power 0.935). The mean ($\pm$SE) coronary flow reserve was 2.77 ± 0.70 in control subjects and 1.14 ± 0.19 in LVBS patients ($P = 0.006$).

Discussion

The novelty of the present study is multifarious. First, the patient population consisted of subjects who had completely recovered from a previously acute LVBS episode and who were studied at a long time distance since the index episode (1–3 years); secondly, in these patients, we demonstrated an abnormal response of the heart and coronary circulation to the catecholaminic hyperstimulation induced by the CPT stimulus; and thirdly, the study protocol combined RT3DE and MCE allowing the almost simultaneous evaluation of LV wall motion and myocardial perfusion at peak of the 3 min provocative test. The possibilities for reaching accurate quantitative evaluation of global[24] and regional[25] LV function simultaneous with MCE in our experimental setting characterized by patient's discomfort and rigid time-constraint was uniquely offered by RT3DE. The fast and almost instantaneous imaging of the entire heart from a single acoustic window, the short acquisition time, and the opportunity to better align and compare

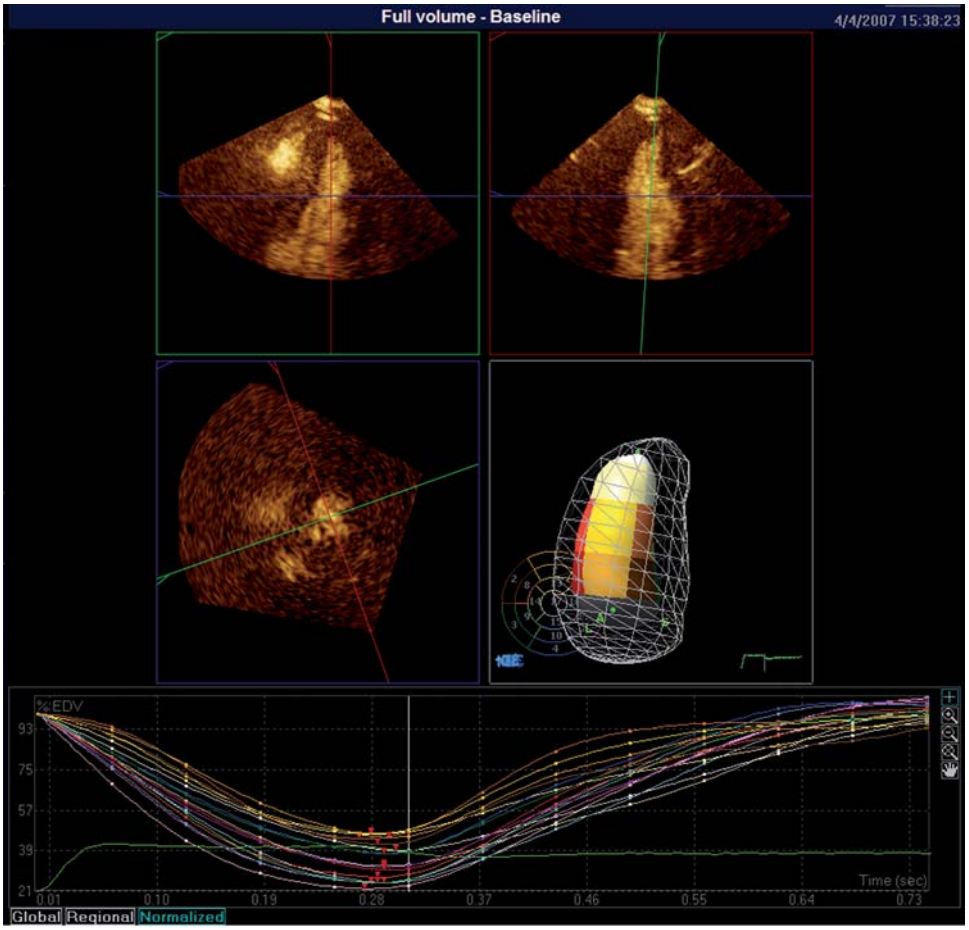

Figure 3 Real-time three-dimensional full volume analysis of a representative left ventricular ballooning syndrome patient at baseline. Images represent the reconstructed four-chamber (top left), two-chamber (top right), and short-axis (middle left) end-systolic frames, and diastolic and systolic three-dimensional casts (middle right). Regional volumetric curves over the cardiac cycle are shown at the bottom. Good contraction of each region is evident: minimum end-systolic volume [plotted as percentage of the corresponding end-diastolic volume (% EDV)] $<50\%$ for all the 17 left ventricular regions. Red squares indicate the time to minimum systolic volume.

baseline and CPT volumetric images were indeed essential to our protocol.

The main result of our study is that in LVBS patients, CPT induced new LV wall motion abnormalities similar to those of the acute phase of the syndrome, which were associated with a reduction in coronary vasodilation reserve.

This is the first systematic prospective study to show that adrenergic stress induces the onset of new LV wall motion abnormalities in previous LVBS patients: to date, in fact, only case reports described the development of LV ballooning in the setting of dobutamine stress echocardiography,[26–29] treadmill exercise test,[30] or dobutamine magnetic resonance imaging.[31]

We evaluated CBF by means of MCE and myocardial parametric quantification. Myocardial contrast echocardiography has been proposed as a powerful tool for the non-invasive measurement of CBF in humans: β value indicates myocardial vascular resistance and β value changes provide a robust and accurate estimate of CBF reserve.[32] In our study, CBF increased in response to CPT in control subjects, whereas it did not in LVBS patients. These data suggest the presence of a chronic impairment of coronary vasodilation reserve in LVBS patients, while the dissociation between CPT-induced wall motion abnormalities and regional CBF reduction argues against a pathogenetic role of vasospasm of epicardial coronary vessels.

Altogether our findings concur to indicate a persistent myocardial microvascular dysfunction in LVBS patients,[33] possibly together with a coronary microvascular spasm.[34]

In the setting of acute LVBS presentation, microvascular dysfunction has been suggested by using different techniques including angiography (TIMI frame count[19,35,36] and TIMI perfusion grade),[16] MCE,[37] and coronary perfusion/flow reserve as assessed by nuclear imaging,[35,38] transthoracic,[39] and intracoronary Doppler techniques;[15] in two other small series of patients, however, it could not be demonstrated.[40,41] TIMI frame count in our LVBS patients is in keeping with the literature.

In our experimental setting, microvascular spasm can be hypothesized because of evidence of myocardial ischemia, i.e. the onset of wall motion abnormalities at peak CPT. Identification of the

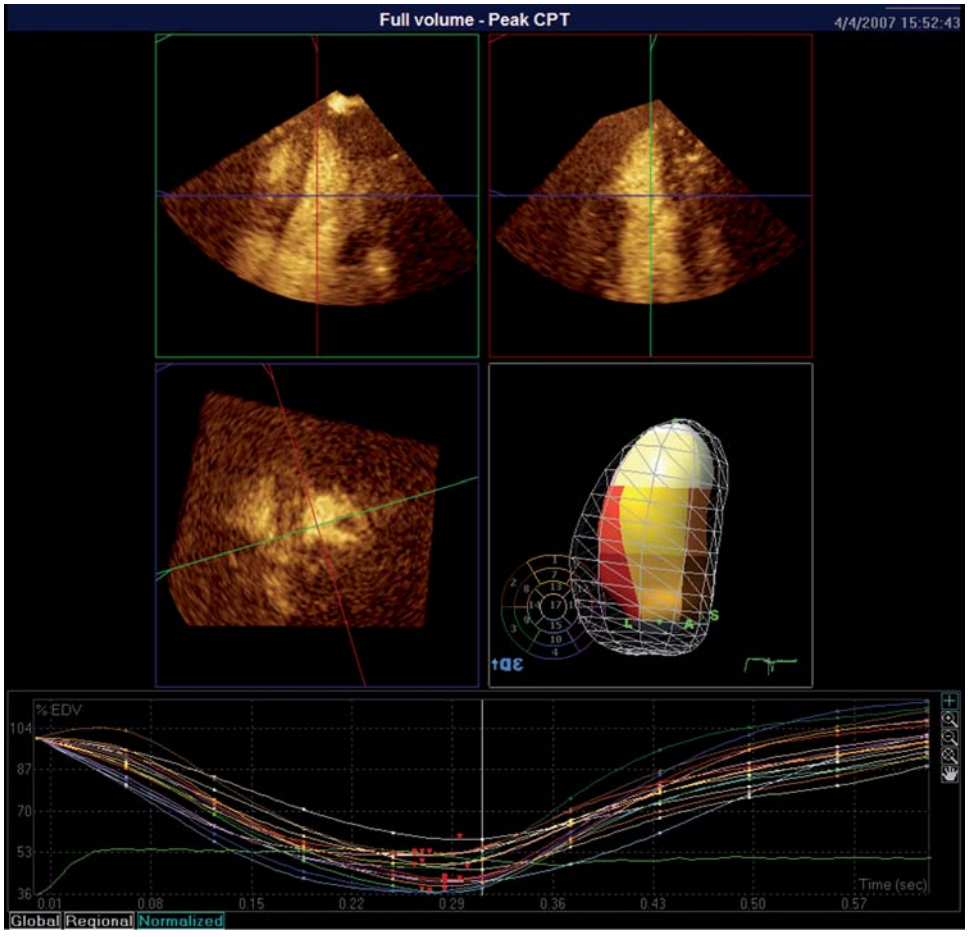

Figure 4 Real-time three-dimensional full volume analysis of a representative left ventricular ballooning syndrome patient at peak cold pressor test. Images represent the reconstructed four-chamber (top left), two-chamber (top right), and short-axis (middle left) end-systolic frames, and diastolic and systolic three-dimensional casts (middle right). Regional volumetric curves over the cardiac cycle are shown at the bottom. Asynergy of the antero-apical region is evident both on the left ventricular cast and the regional curve diagram: minimum end-systolic volume [plotted as percentage of the corresponding end-diastolic volume (% EDV)] >50%.

Table 3 Quantification of myocardial perfusion

	A (VI plateau, dB)		*β* (rate constant of VI rise, s^{-1})		*A* × *β* (dB/s)	
	Baseline	**CPT**	**Baseline**	**CPT**	**Baseline**	**CPT**
Four-chamber						
Control group	3.4 ± 0.2	3.4 ± 0.3	0.35 ± 0.09	0.53 ± 0.15*	2.5 ± 1.8	5.2 ± 2.8*
LVBS patients	3.1 ± 0.6	3.1 ± 0.7	0.46 ± 0.11	0.44 ± 0.12	3.2 ± 1.7	3.1 ± 1.6
Two-chamber						
Control group	3.7 ± 0.3	3.5 ± 0.3	0.37 ± 0.14	0.56 ± 0.18*	3.3 ± 1.8	5.5 ± 3.6*
LVBS patients	2.9 ± 0.6	3.0 ± 0.8	0.44 ± 0.16	0.47 ± 0.13	2.9 ± 2.1	3.5 ± 2.4
Three-chamber						
Control group	3.5 ± 0.2	3.4 ± 0.2	0.53 ± 0.09	0.73 ± 0.22*	5.8 ± 2.7	10.9 ± 5.6*
LVBS patients	3.1 ± 0.5	3.0 ± 0.6	0.57 ± 0.21	0.44 ± 0.12	4.4 ± 3.6	2.7 ± 2.3

VI, video intensity, *A* × *β*, product of VI plateau times rate constant of VI rise; CPT, cold pressor test; LVBS, left ventricular ballooning syndrome. *Statistical significances are reported in the text.

mechanism underlying microvascular spasm was beyond the scope of this study. However, with microvascular dysfunction in the background, pathological hyperconstriction of coronary microvessels in response to neuro-hormonal stimuli is one possible explanation.

Catecholamine stimulation is thought to be an important component of the pathophysiology of LVBS. Wittstein *et al.*[13] reported a two- to three-fold increase in catecholamine levels and their metabolites in a large LVBS patient population compared with AMI patients with a similar degree of heart failure; moreover, Ueyama *et al.*[14] demonstrated that α- and β-adrenergic blockage reduced the development of the LV dysfunction induced by the immobilization test in the rat, which represents an animal model of stress-induced cardiomyopathy.

In our study, CPT-induced peripheral catecholamine increase was similar in LVBS patients and controls, proving the comparable efficacy of the test in the two populations. However, peripheral catecholamines do not provide any information on the cardiac sympathetic system, whose activity is independent of peripheral sympathetic activity as previously demonstrated by our group,[42] and necessitates coronary sinus catheterization to be investigated. Very recently increased local release of cardiac catecholamines was reported in few LVBS patients in the acute phase.[43] The study of cardiac sympathetic system is becoming central to the understanding of the pathogenesis of LVBS in view of the association between LVBS and post-menopausal age, a time of female life characterized by a transition from parasympathetic to sympathetic dominance in the autonomic control of the cardiovascular system. Non-uniformity of the sympathetic nervous system in the LV has been demonstrated in terms of distribution of both sympathetic nerve endings and the β-adrenergic receptors:[44] changes in sensitivity or density of the cardiac adrenergic receptors and the reduction of oestrogen levels following menopause with its indirect and direct myocardial effects (unopposed stress-induced hypothalamo-sympathoadrenal activation and down-regulation of cardioprotective substances) may predispose women to develop LVBS. This can explain why CPT stimulus in our study was effective in replicating the LV wall motion abnormalities of LVBS at such a long time since the acute episode. Interestingly enough, the only patient in our series in whom CPT could not induce wall motion abnormalities was the 35-year-old regularly menstruating lady.

Limitations

The small number of LVBS patients is a limitation of our study and even more so the small number of control subjects, which was due to the necessity to identify women submitted to urgent coronary angiography because of acute chest pain with normal ECG, negative myocardial biomarkers, normal coronary arteries, and with the same risk factor profile as LVBS patients. However, the coronary response to CPT of our control subjects is in keeping with the literature.[17,18] and the power of statistical analysis supports our results.

Catheterization of coronary sinus that would have been required to assess cardiac catecholamine levels could not be performed in our experimental setting.

Multivessel vasospasm cannot be strictly ruled out in our experimental setting, because the use of relative scaling of myocardial parametric quantification to analyse myocardial perfusion data may have led to a potentially misinterpretation of perfusion abnormalities like in situations of diffuse or balanced reduction of blood flow.[45]

In conclusion, our results obtained in a small series of patients with previous LVBS demonstrate that a physiological adrenergic stimulus like CPT is able to elicit a pathological cardiac response both in terms of LV function and coronary vasodilation reserve in the absence of symptoms. These data explain the possibility of subclinical and even clinical recurrences of LVBS.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Conflict of interest: none declared.

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