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Traditional and Color M-Mode Parameters of Left Ventricular Diastolic Function During Low-dose Dobutamine Stress Echocardiography: Relations to Contractility Reserve

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Background: Cross-sectional studies reported that left ventricular (LV) systolic and diastolic functions are correlated. However, whether changes in wall-motion score index (WMSI) or 2-dimensional ejection fraction (EF) predict changes of Doppler parameters of LV diastolic function is unclear.

Methods: Patients with known or suspected history of coronary artery disease underwent assessment of LV systolic function (WMSI, EF) and diastolic function at baseline and during stress echocardiography by low-dose dobutamine (LDD) (peak infusion 10 µg/kg/min). Peak velocities of early (E) and late (A) LV filling waves and E wave deceleration time were measured according to standard protocol. E wave propagation rate (EVp) was assessed by color Doppler M-mode across the mitral valve. Tei index was calculated as: (A wave to E wave time – ejection time)/ejection time. Changes at LDD were calculated as: $100 \times (\text{value at LDD} - \text{value at baseline})/\text{baseline}$.

Results: The study group comprised 66 patients, mean age 61 ± 10 years, 80% men. Worse LV systolic function was associated with more severely impaired LV diastolic function both at baseline and at LDD. However, percent change of WMSI and change in EF did not correlate with percent change of EVp and E/E wave propagation rate, but with percent change of Tei index. At LDD, patients with myocardial viability did not show greater percent change of LV diastolic function parameters but significantly lower Tei index.

Conclusions: In patients with suspected or known coronary artery disease, assessment of diastolic function reserve by LDD stress echocardiography using traditional and color M-mode Doppler may add quantitative information on myocardial function beyond traditional assessment of contractility reserve by WMSI or EF. (J Am Soc Echocardiogr 2006; 19:483-490.)

Congestive heart failure is a common sequel of coronary heart disease (CHD).¹ Chronic myocardial ischemia affects left ventricular (LV) diastolic function as well,² and diastolic dysfunction is pathophysiologically related to heart failure even in the presence of normal LV ejection fraction (EF).³⁻⁵ Cross-sectional studies in patients with stable hemodynamic conditions have demonstrated that LV

systolic and diastolic function are correlated.^{6,7} There is evidence that LV diastolic function is directly correlated with maximal exercise capability more strongly than LV systolic function in physiologic^{8,9} and pathologic^{10,11} conditions. Moreover, LV diastolic dysfunction predicts cardiac prognosis independent to abnormal EF.¹² However, the extent to which myocardial systolic function reserve correlates with parameters of LV diastolic function remains substantially unclear.

In patients with CHD, resting and dobutamine stress echocardiography allows risk stratification by assessing relevant prognostic factors such as EF,¹³ myocardial ischemia,¹⁴ and myocardial viability.^{13,15,16} In a recent study in patients with CHD, LV contractility reserve correlated with an indicator of LV systolic and diastolic function during dobutamine stress echo.¹⁷ However, the extent to which LV contractility reserve impacts on preload-insensitive parameters of LV diastolic function remains to be clarified. Therefore, we evaluated a group of pa-

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tients with a wide range of LV systolic and diastolic functions to assess the impact of change in LV systolic performance on traditional and color M-mode Doppler parameters of LV diastolic function.

METHODS

Population

We selected consecutive patients who underwent dobutamine stress echocardiography for functional assessment in recent or previous myocardial infarction and/or to rule out the diagnosis of coronary artery disease. Atrial fibrillation, pacemaker-dependent cardiac rhythm, and unstable angina were pretest exclusion criteria. After exclusion criteria, the study sample comprised 68 patients, 82% with recent (within 3 months) or nonrecent myocardial infarction assessed by medical chart review; 81% were on low-dose β - or α/β -blocker, which were discontinued 24 hours before dobutamine stress echocardiography.

Echocardiography

Echocardiography was performed by standard procedures according to current recommendations.¹⁸ EF was estimated by bidimensional approach in apical views¹⁹; wall-motion score index (WMSI) was assessed based on the 16-segment standard, dividing the LV in 3 slices on its longitudinal axis (base at mitral valve; midcavity at papillary muscles; apical below the papillary muscles). Basal and midcavity slices were subdivided each in 6 segments (anterior and posterior septum, anterior, lateral posterior, and inferior walls) whereas the apical slice was divided in 4 segments (septum, anterior, lateral, and inferior).²⁰ Evaluation of WMSI relied on systolic endocardial displacement and, therefore, wall thickening. By the score system, 1 identified normal contractility, 2 was assigned to hypcontractility, 3 identified akinesis, 4 identified dyskinesis, and 5 was assigned to aneurysm; WMSI was computed as:

$$\frac{\sum_{i=1}^{16} \text{IndividualSegmentScore}}{16}$$

The contractility of each segment was judged in two orthogonal views, using the parasternal long- and short-axis views, and apical 4- and 2-chamber views. To the score system, patients with higher WMSI had lower contractility and EF, and those who had an increase in contractility at low-dose dobutamine (LDD) compared with baseline had a negative delta percent of WMSI, whereas those who had new wall-motion abnormalities or showed worsen motion at LDD of a segment, had a positive delta percent of WMSI. Because hypercontractility was not accounted for in the score system, segments that showed increase in contractility during dobutamine infusion received a score of 1 and did not contribute to delta percent change in WMSI. Within the group of patients with previous myocardial infarction, those who

Table 1 Clinical characteristics of the study population

	N = 66
Age, y	61 $\pm$ 10
Male sex (%)	53 (80)
Body mass index, kg/m ²	26.0 $\pm$ 3.9
Hypertension, n (%)	36 (55%)
Diabetes, n (%)	16 (25%)
Dyslipidemia, n (%)	36 (55%)
Previous or recent myocardial infarction, n (%)	54 (82%)
Previous PTCA, n (%)	25 (38%)
Previous CABG, n (%)	4 (6%)
Use of β - or α/β -blocker 24 h before echocardiography stress n (%)	54 (81%)

PTCA, percutaneous transluminal coronary angioplasty; CABG, coronary artery bypass graft.

Data are mean $\pm$ SD or percent.

had an absolute difference between baseline and LDD WMSI greater than 0.22 because of biphasic response of LV segments akinetic or severely hypokinetic at baseline were identified as having myocardial viability.¹⁵ With regard to diastolic parameters, a standard echocardiographic protocol was used as previously described.^{21,22} Diastolic parameters used for the current study were previously tested for test-retest reproducibility²²: peak velocities of the early (E) and late (A) transmitral waveforms assessed by pulsed Doppler with the sample volume placed at the tips of the mitral valve leaflets, and their ratio (E/A); E deceleration time (EDT); and E wave propagation rate (EVp) by color Doppler M-mode across the mitral valve, and the ratio of E/EVp, as indicator of LV filling pressure.^{23,24} In addition, we computed the Tei index according to standard procedures.²⁵ Because we previously reported suboptimal test-retest reproducibility for assessment of isovolumic relaxation time,²² such a measure was not assessed separately.

Statistical Analysis

Data in tables are mean $\pm$ SD or absolute numbers and percent. Student *t* test for paired data was used to compare continuous variables at baseline versus LDD. Pearson's correlation matrix was used to assess bivariate correlations. Separate multiple regression analyses were used to assess multivariate correlations between delta percent WMSI, or delta percent EF, with each parameter of LV diastolic function. Student *t* test for independent groups was used to compare continuous variables between patients with myocardial viability and those without. A 2-tailed *P* less than .05 indicated statistical significance.

RESULTS

General characteristics of the study sample are reported in Table 1. As can be seen in Table 2, at LDD, on average, the R-R intervals slightly shortened, systolic blood pressure slightly increased,

Table 2 Baseline and low-dose dobutamine stress echocardiographic features

	Baseline	P	Low-dose dobutamine	Delta %
R-R, s	0.97 ± 0.14	<.01	0.90 ± 0.14	-7 ± 12
Systolic BP, mm Hg	127 ± 14	.06	131 ± 22	+3 ± 13
Diastolic BP, mm Hg	77 ± 8	<.01	65 ± 9	-15 ± 14
WMSI	1.88 ± 0.46	<.01	1.77 ± 0.47	-8.4 ± 8.1
EF, %	42 ± 11	<.01	53 ± 13	+29 ± 18
LV ejection time, ms	310 ± 28	<.01	282 ± 36	-10 ± 9
E, m/s	0.63 ± 0.18	<.01	0.74 ± 0.21	+20 ± 14
A, m/s	0.61 ± 0.16	<.01	0.68 ± 0.20	+11 ± 14
E/A	1.1 ± 0.5	<.05	1.3 ± 0.7	+15 ± 39
EDT, ms	187 ± 48	<.05	197 ± 48	+8 ± 22
EVp, cm/s	41.9 ± 11.58	<.01	59.2 ± 19.6	+38 ± 27
E/EVp	1.52 ± 0.35	<.01	1.27 ± 0.35	-14 ± 19
Tei index	0.37 ± 0.15	<.01	0.31 ± 0.17	-22 ± 35

WMSI, wall-motion score index; E, peak velocity of the early left ventricular filling wave; A, peak velocity of the atrial-dependent late left ventricular filling wave; EDT, E wave deceleration time; BP, blood pressure; EF, ejection fraction; EVp, E wave propagation rate; LV, left ventricular; Tei index, left ventricular isovolumic time/left ventricular ejection time.

Data in table are mean ± SD.

diastolic blood pressure and WMSI decreased, and EF increased significantly. Only one patient showed a change in WMSI at LDD suggestive of ischemic myocardial response; LV ejection time (ET) was shorter at LDD after adjustment for heart rate (data not shown, $P < .05$). E, A, and E/A ratio increased, and EDT was slightly longer at LDD compared with baseline; however, EVp increased, and E/EVp and Tei index decreased significantly.

At baseline, WMSI correlated with EF ($r = -0.76$, $P < .01$); higher WMSI correlated with lower EVp, and higher E/EVp and Tei index (Figure 1); WMSI did not show significant correlations with A, E/A, and EDT (Table 3). Similar results were obtained using baseline EF for WMSI (r values vs EVp = 0.68, $P < .01$; E/EVp = -0.53, $P < .01$; Tei index = -0.30, $P < .05$); in addition, higher baseline WMSI was associated with shorter LVET. Higher baseline systolic blood pressure correlated with higher EVp ($r = 0.46$) and lower E/EVp ($r = -0.35$, both $P < .01$), but not with other parameters of LV diastolic function (data not shown, all $P > .1$); diastolic blood pressure did not correlate with parameters of LV diastolic function (data not shown, all $P > .1$). No correlations were found between baseline blood pressures and baseline WMSI or EF (data not shown, all $P > .1$).

At LDD, WMSI correlated with EF ($r = -0.69$, $P < .01$). Correlations of higher WMSI with lower EVp and higher E/EVp and Tei index at LDD (Figure 2) were consistent with baseline data; in addition lower WMSI at LDD correlated with higher E. The lack of significant correlations of WMSI with A, E/A, and EDT at LDD were also consistent with baseline data; EF at LDD showed significant correlations with EVp ($r = 0.72$, $P < .01$), with E/EVp ($r = -0.48$, $P < .01$), and with Tei index ($r = -0.25$, $P < .05$) consistent with findings at baseline; the correlation

Figure 1 Relation between wall-motion score index (WMSI) at baseline (natural log transformed) on horizontal (X) axis with baseline Tei index (natural logarithm transformed) on vertical (Y) axis. Mean regression line with individual 95% confidence interval are shown.

of higher WMSI with shorter LVET at baseline was consistent but fell short of statistical significance. At LDD, higher systolic blood pressure correlated with higher E ($r = 0.39$), E/A ($r = 0.28$), and EVp ($r = 0.54$), and with lower Tei index ($r = -0.27$, all $P < .05$), whereas no correlations were found with other parameters of LV diastolic function (data not shown, all $P > .1$). Diastolic blood pressure at LDD did not show correlations with parameters of LV diastolic function (data not shown, all $P > .1$). Higher systolic blood pressure correlated with lower WMSI ($r = -0.26$) and higher EF ($r = 0.33$, both $P < .05$).

In bivariate analyses, greater reduction in delta percent change of WMSI showed a tendency toward a correlation with higher delta percent change in EVp and E/EVp, although the correlations did not

Table 3 Correlations of wall-motion score index with diastolic parameters at baseline and low-dose dobutamine

	WMSI	
	Baseline	Low-dose dobutamine
	<i>r</i>	<i>r</i>
E	0.03	-0.25
A	-0.18	-0.21
E/A	-0.01	0.03
EDT	0.10	-0.03
EVp	-0.63†	-0.61†
E/EVp	0.29*	0.38*
Tei index	0.25*	0.33*
LV ejection time	-0.28*	-0.14

E, peak velocity of the early left ventricular filling wave; A, peak velocity of the atrial-dependent late left ventricular filling wave; EDT, E wave deceleration time; EVp, E wave propagation rate; LV, left ventricular; Tei index, left ventricular isovolumic time/left ventricular ejection time.

* $P < .05$; † $P < .01$.

Data in table are mean $\pm$ SD. Data in table are Pearson's correlation coefficients.

Figure 2 Relation between wall-motion score index (WMSI) at low-dose dobutamine (LDD) (natural log transformed) on horizontal (X) axis with Tei index at LDD (natural logarithm transformed) on vertical (Y) axis. Mean regression line with individual 95% confidence interval are shown.

achieve the statistical significance, whereas increase in delta percent change of WMSI correlated with significant increase in delta percent change of Tei index (Table 4, Figure 3). Delta percent of WMSI was not significantly associated with age, sex, baseline systolic blood pressure, baseline LV end-diastolic volume, baseline heart rate, or previous myocardial infarction (all $P =$ not significant).

Correlations between delta percent change of EF and delta percent changes of diastolic function parameters were consistent with those shown previously using delta percent change of WMSI, with exception

Table 4 Correlation of delta percent change of wall-motion score index and ejection fraction with delta percent change of diastolic parameters during low-dose dobutamine

	Δ WMSI		Δ EF	
	<i>r</i>	β †	<i>r</i>	β †
Δ E	-0.23	-0.08	-0.07	-0.08
Δ A	-0.04	-0.03	-0.37*	-0.36*
Δ E/A	-0.13	-0.04	0.20	0.18
Δ EDT	0.12	0.04	-0.16	-0.16
Δ EVp	-0.22	-0.17	-0.05	-0.07
Δ E/EVp	-0.17	-0.11	-0.06	-0.02
Δ TEI index	0.32*	0.11	-0.29*	-0.28*
Δ LV ejection time	0.01	0.004	0.15	0.17

Δ , Delta percent change at low-dose dobutamine from baseline.

* $P < .05$.

†betas: adjusted correlation coefficients from multiple regression analyses using delta percent change in systolic blood pressure and heart rate as standard covariates when testing multivariate correlations between delta percentage change of WMSI (or EF) and each parameters of LV diastolic function.

Figure 3 Relation between percent delta change of wall-motion score index (WMSI) (natural log transformed) on horizontal (X) axis with percent change of Tei index between baseline and low-dose dobutamine (natural logarithm transformed) on vertical (Y) axis. Mean regression line with individual 95% confidence interval are shown.

of a significant correlation between greater increase in EF and lower A at LDD.

Higher delta percent change of systolic blood pressure correlated with higher delta percent change of E ($r = 0.35$) and E/A ($r = 0.44$), and with lower delta percent change of A ($r = -0.33$) (all $P < .01$); delta percent change of diastolic blood pressure did not show significant correlations with parameters of LV diastolic function (data not shown, all $P > .1$). Delta percent changes of systolic and diastolic blood pressure did not correlate with delta percent change in WMSI and EF.

Table 5 Diastolic parameters and their delta percent changes according to the presence of myocardial viability within the group of patients with previous myocardial infarction

	Viability		P
	Yes	No	
N	13	41	
E, m/s	0.58 ± 0.14	0.65 ± 0.19	NS
A, m/s	0.64 ± 0.17	0.59 ± 0.16	NS
E/A	0.94 ± 0.24	1.22 ± 0.60	<.01
EDT, ms	215 ± 66	180 ± 47	<.05
EVp, cm/s	41 ± 9	40 ± 10	NS
E/EVp	1.50 ± 0.58	1.70 ± 0.50	NS
Tei index	0.39 ± 0.16	0.37 ± 0.14	NS
LV ejection time, ms	317 ± 35	309 ± 25	NS
Δ % E	38 ± 26	19 ± 24	NS
Δ % A	8 ± 16	12 ± 29	NS
Δ % E/A	26 ± 36	14 ± 40	NS
Δ % EDT	1 ± 22	8 ± 22	NS
Δ % EVp	42 ± 29	40 ± 25	NS
Δ % E/EVp	-7 ± 24	-16 ± 18	NS
Δ % Tei index	-43 ± 32	-6 ± 41	<.001
Δ % LV ejection time	-8 ± 14	-10 ± 6	NS

Δ, Delta percent change from baseline.
Data in table are mean ± SD.

In multivariate analyses adjusting for delta percent change in systolic blood pressure and heart rate, delta percent change of WMSI did not show significant correlations with parameters of LV diastolic function (Table 4). Higher delta percent change of EF correlated with lower delta percent change of A and with lower delta percent change of Tei index independent of delta percent change of systolic blood pressure and heart rate.

Subgroup Analyses by the Presence of Myocardial Viability

Within the group of patients who had recent or previous myocardial infarction, 13 had viable myocardium (Table 5). Of the diastolic parameters at baseline, E/A was higher and EDT was shorter in those without viable myocardium, whereas E, A, EVp, E/EVp, Tei index, and LVET did not differ significantly between the two groups. With regard to delta percent change at LDD, patients with myocardial viability did not differ significantly from those who did not show significant viability in terms of parameters of diastolic function and LVET, but showed greater decrease of Tei index at LDD.

DISCUSSION

In this study, we evaluated a relatively large group of patients with known or suspected coronary artery disease, the majority of whom had overt CHD, and found that LV systolic chamber function, assessed

as WMSI or EF, correlated with preload-insensitive indices of LV diastolic function^{23,26} both at baseline and during LDD. However, delta percent change of WMSI, a widely used semiquantitative measure of LV contractility reserve, did not correlate with the delta percent change of Doppler parameters of LV diastolic function, but with delta percent change of Tei index, a mixed indicator of LV systolic and diastolic function.

In our study, LDD induced increase in E suggesting increased peak gradient between left atrium and LV during early filling; moreover, LDD induced a significant increase in EVp, a Doppler color M-mode parameter strongly correlated with the time constant of the isovolumic relaxation (tau).²⁷ Therefore, in our study sample, LV relaxation and diastolic suction properties of myocardium improved with LDD; in fact, a close correlate of pulmonary capillary wedge pressure and, therefore, of LV filling pressure, ie, E/EVp,²³ decreased significantly during LDD. This may have been possible by LDD-induced improvement of the LV elastic recoil energy, closely related LV relaxation, and major determinant of LV suction capability⁵; this is consistent with described lusitropic effect of LDD,²⁸ and is consistent with changes induced by LDD in patients with dilated cardiomyopathy.²⁹ Changes in LV diastolic properties occurred in parallel with a small increase in heart rate and systolic blood pressure, and with a significant increase in WMSI induced by LDD, suggestive of an overall increase in LV systolic function in our patient.^{4,30} The idea that stimulation of LV contractility may enhance relaxation and suction capacity is consistent with the notion that, to generate elastic potential energy, LV end systolic volume (ESV) has to decrease to value lower than an ideal LV volume at zero pressure while fully relaxed³¹; smaller ESV at LDD may, therefore, be associated with greater elastic potential energy.

Higher LDD-induced delta percent change of EF was associated with greater percent reduction of A and Tei index, but not with greater delta percent increase of EVp and decrease of E/EVp, the latter two indices being recognized measures of LV relaxation and filling pressure.^{23,26,27} Notably, delta percent change accounts for baseline values. Delta percent change of WMSI did not correlate with delta percent change of the Doppler parameters of LV diastolic function, but with delta percent change of Tei index. The lack of significant differences in pure LV diastolic function parameters between patients with viable myocardium versus those without, while Tei index improved more in patients with myocardial viability, is consistent with data in Table 4, because definition of myocardial viability in our study is based on difference between baseline and LDD WMSI.¹⁵ To reduce the potential confounding impact of tracking artifacts, wall motion was judged

based on radial endocardial shift and wall thickening. Therefore, assessment of WMSI or EF, although clinically conceivable, may be limited when applied to explore sophisticated physiology of the LV mechanics. Nevertheless, delta percent change of EF showed a correlation with a net decrease in LV filling pressure during atrial contraction, which is the potential result of improved LV filling dynamic during early diastole (ie, correlation between delta percent changes of EF and A). WMSI is a semiquantitative measure, which did not account for contribution of normally functioning segments because of the score system, because a normally contracting segment received a score of 1 at baseline and at LDD; therefore, delta percent change of WMSI may be less physiologically sensitive measure of LV chamber systolic function than EF. Nevertheless, because neither delta percent change in WMSI nor delta percent change of EF correlated with delta percent changes of EVp and E/EVp, both considered global parameters of LV diastolic function minimally affected by changes in loading conditions,²⁶ our data suggest that assessment of LV diastolic function by traditional Doppler echocardiography and color M-mode during LDD may be additive to assessment of LV systolic function reserve for reclassification of patients with known or suspected CHD.

Our finding of a correlation between LV contractility reserve and change in Tei index is consistent with previous data in patients with CHD.¹⁷ Assessment of Tei index is relatively simple and not significantly time-consuming, although not completely immune from technical challenges in case of fast heart rate. However, we significantly add to the current literature by showing that the correlation between Tei index and LV contractility reserve is highly likely mediated by intrinsic correlation of Tei index with LV systolic more than diastolic functions; in fact, Tei index correlated with LV contractility reserve in the absence of a concomitant correlations between LV contractility reserve with pure indices of LV diastolic function. Such a result should be considered when interpreting change in Tei index during stress echocardiography. Nevertheless, because Tei index includes isovolumic relaxation and contraction times, such a parameter may be particularly helpful for assessment of LV function reserve under LDD in daily practice because it comprises factors intrinsically related to LV suction capability.³²⁻³⁴

LDD induced mild increase of systolic blood pressure. From a physiologic point of view, systolic blood pressure may be considered as the result of interaction between LV output (ie, stroke volume) and arterial compliance³⁵; higher systolic blood pressure may result in higher afterload and lower EF.³⁶ In our study, at LDD, higher systolic blood pressure correlated with higher EF and lower WMSI. Delta percent change of systolic blood pressure did

not correlate with delta percent changes of WMSI and EF. Those findings may be a result of the fact that in our dynamic setting, by Laplace's law, wall thickening may offset the small increase in systolic blood pressure and, therefore, systolic blood pressure may increase whereas myocardial afterload actually decreases at LDD. Our findings are consistent with data shown in patients with cardiomyopathy and healthy volunteers.²⁹ Moreover, EF is more closely related to afterload than to systolic blood pressure (data from a different population source³⁷; EF vs systolic blood pressure, $r = 0.12$, $P = .04$; EF vs meridional end-systolic stress, $r = -0.63$, $P < .01$; unpublished data). Therefore, increase in systolic blood pressure at LDD parallels increase in LV systolic chamber performance. Accordingly, correlations of higher systolic blood pressure with a few parameters of LV diastolic function were consistent with correlations of WMSI and EF with EVp and E/EVp. In any event, change in systolic blood pressure did not impact on the lack of relationships of delta percent changes of WMSI and EF with delta percent changes of parameters of LV diastolic function.

In the current study, we used traditional indices and color M-mode parameters of LV systolic and diastolic function, which can be derived using widely available echocardiographic machines. Therefore, our research setting is reproducible in standard laboratories of echocardiography. We did not use Doppler tissue imaging, which was not available in our laboratory at the time of our study. However, with regard to diastolic function, EVp was proven to be better than early diastolic excursion of the mitral annulus by Doppler tissue as measure of global LV diastolic function.²⁶ Regional systolic wall deformation index strain and deformation velocity strain rate by color Doppler tissue analyses has potential to provide further contribution to evaluation of correlations between LV systolic and diastolic function.³⁸ For instance, Hoffmann et al³⁹ studied correlations between myocardial viability, by positron emission tomography, and LV systolic reserve and diastolic function change using strain rate, and found that segments which did not change systolic strain rate by LDD also showed no significant change in diastolic strain rate; however, global assessment of LV systolic and diastolic function by color Doppler tissue modality to measure systolic and diastolic strain rates may be very time-consuming and requires, at this time, advanced technology not available widely. Therefore, our findings obtained using standard LV systolic function measurements and combining traditional and color Doppler M-mode, are likely to have broad applicability and more practical implications.

Conclusions

Our study, in patients with known or suspected coronary artery disease, showed that myocardial response to the lusitropic effect of LDD may be evaluated by traditional and color M-mode Doppler, allowing risk stratification of patients in addition to the traditional assessment of LV chamber contractility reserve by WMSI and EF.

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