Two-Dimensional Speckle Tracking Echocardiography Identifies Coronary Artery Disease in 690 Patients: A Retrospective Study from a Single Center

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Background: Two-dimensional speckle tracking echocardiography (2D-STE) is a novel and non-invasive technique for the diagnosis of coronary artery disease (CAD). This retrospective study from a single center aimed to identify myocardial ischemia using 2D-STE in CAD patients identified by angiography.

Material/Methods: From March 1 to November 30, 2019, 690 patients in Beijing Hospital were enrolled. After angiography, 346 patients were diagnosed with CAD. Reduction in vessel diameter of ≥50% by stenosis in at least 1 major coronary artery or its main branch was considered CAD. Analysis of 2D-STE was performed using EchoPAC version 201.

Results: The global strain was significantly impaired in CAD patients (P<0.01). Global longitudinal peak strain (GLPS) was analyzed in layers. For GLPS of the epicardium, the odds ratio (OR) was 1.297 (1.217-1.382; P=0.002), the area under the curve (AUC) was 0.727, and the cut-off value was -16.95; sensitivity and specificity were 73.7% and 63.0%, respectively. For GLPS of the middle layer, the OR was 1.260 (1.192-1.333; P<0.001), the AUC was 0.732, and the cut-off value was -20.95; sensitivity and specificity were 82.4% and 56.2%, respectively. For GLPS of the endocardium, the OR was 1.193 (1.137-1.251; P<0.001), the AUC was 0.708, and the cut-off value was -22.95; sensitivity and specificity were 82.9% and 52.9%, respectively.

Conclusions: The findings from this study support the clinical application of 2D-STE in patient populations with suspected myocardial ischemia due to CAD. Therefore, 2D-STE combined with ECG monitoring may have a future role for early screening of CAD patients.

Keywords: Coronary Vessels • Echocardiography • Myocardial Ischemia

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Background

Non-invasive modalities that are convenient and efficient, especially for early screening, can play a key role in the control of coronary artery disease (CAD). Conventional echocardiography mainly relies on detecting decreased ventricular wall motion by the echocardiologist using the naked eyes, and only provides reliable results in some patients with CAD and myocardial infarction [1]. Thus, it is difficult to apply this procedure in all kinds of CAD. Two-dimensional speckle tracking echocardiography (2D-STE) is a novel technique and the results are based on the deformation of the myocardium. Recently, studies have found that 2D-STE can be used in clinical practice to diagnose coronary heart disease in a specific population with acute myocardial infarction, angina pectoris, and chronic coronary heart disease, especially in outpatient departments [2-4]. With 2D-STE, cardiologists can implement timely interventional strategies in patients with non-ST-segment elevation myocardial infarction [5]. 2D-STE has an extensive application value in clinical outpatient management, whether for screening patients or for evaluating postoperative rehabilitation. Therefore, this retrospective study from a single center aimed to identify myocardial ischemia using 2D-STE in patients with CAD identified by coronary artery angiography.

Nevertheless, in a multicenter prospective study in Israel, the longitudinal strain of 2D-STE was not sufficient to rule out acute coronary syndrome (ACS) in the emergency department [6]. The diagnostic efficacy of 2D-STE for patients with CAD varies between studies, as some studies reported high specificity and sensitivity, while others reported low values [7-9]. Routine application of 2D-STE in patients with coronary heart disease is still controversial.

2D-STE is convenient, cost effective, and readily available compared with exercise electrocardiograph (ECG), cardiovascular magnetic resonance (CMR), and angiography. The latest guideline on non–ST-segment elevation acute coronary syndromes (NSTE-ACS) suggests that using GLPS might improve the diagnostic value of conventional echocardiography [8]. This guideline does not have sufficient standardizations to recommend the routine use of 2D-STE in all patients with CAD [10]. Thus, this study aimed to investigate the diagnostic value of 2D-STE for coronary heart disease in a large population of patients who underwent angiography. Our focus was to provide more evidence of the clinical application of 2D-STE in patients suspected of having coronary artery disease.

Material and Methods

Population

This is a cross-sectional study. We enrolled all consecutive patients referred for coronary angiography for the first time from March to November 2019 in Beijing Hospital. All patients provided informed written consent to participate in this study. All procedures complied with the principles of the Declaration of Helsinki and were approved by the Beijing Hospital Ethics Committee. The study was registered in ClinicalTrials (NCT03905200).

The inclusion criteria were: 1) patients with ischemic symptoms (chest pain or discomfort (angina) suspected to be caused by myocardial ischemia) or positive examination results; 2) patients aged > 18 years; and 3) patients with sinus rhythm.

The exclusion criteria were: 1) Patients with prior history of CAD; 2) patients with ST-elevation myocardial infarction, 3) patients with moderate to severe valve disease, malignant arrhythmia, hypertrophic cardiomyopathy, or dilated cardiomyopathy; 4) patients with other end-stage diseases (a serious disease of organs and systems other than the cardiovascular system, with a life expectancy of less than one year); or 5) patients with poor echocardiographic images.

Conventional Echocardiography

All echocardiograms were obtained using Vivid E9 (GE Healthcare; Horten, Norway) with a M5SC transducer. Conventional echocardiography was performed by a doctor blinded to the results of other tests (ECG, creatine kinase, and troponin). The left ventricular (LV) end-diastolic diameter, septal wall thickness, and LV posterior wall thickness were obtained from the parasternal long-axis view using 2D echocardiography. Early (E) peak velocity, atrial (A) diastolic filling velocity, and deceleration time of the E wave were measured using pulsed-wave Doppler. The E/A ratio was calculated. The left ventricular ejection fraction was determined using the biplane Simpson method [31].

STE

Patients underwent echocardiography before coronary angiography, which was performed by the same experienced sonographer, using GE Vivid E9. Images of the left ventricle (short-axis views at basal, middle and apical levels; apical two-, three-, and four-chamber views) were obtained at end-expiration at 50-70 frames/s. Offline analysis was performed by an independent analyst, using EchoPAC version 201 (GE Medical Systems, Chicago, IL).

At end-systole, the endocardial border of each view was manually traced. The region of interest (ROI) was readjusted if tracking was unsatisfactory. Poor-quality images were excluded from further analysis. The global longitudinal peak strain (GLPS) was analyzed. GLPS in 3 layers – GLPS (epicardium, epi), GLPS average (GLPSavg), and GLPS (endocardium, endo)
were analyzed separately in the regression model. GLPSavg indicates the middle layer. In most studies, it also represents the whole GLPS of the left ventricle [2,11,29]. Global circumferential strain (GCS) was obtained from basal, middle, and apical short views. Peak strain deviation (PSD) and other parameters were also obtained (Figures 1, 2).

**Coronary Angiography**

Patients were sent to the catheter laboratory after undergoing echocardiography. Coronary angiography was performed by an experienced interventional cardiologist. Angiograms were assessed by 2 experienced senior interventional cardiologists. Reduction $\geq 50\%$ in luminal diameter due to stenosis in at least 1 major coronary artery or its main branch was considered CAD. The procedure was based on 2015 European Society of Cardiology (ESC) Guidelines [8].

**Statistical Analysis**

Proportions were compared between CAD and non-CAD groups using the chi-square test. Continuous variables were compared using the $t$ test. Univariate analysis was performed on baseline characteristics: age, sex, hypertension, diabetes mellitus (DM), hypercholesterolemia, smoking, CAD family history, heart rate, diastolic blood pressure, body mass index (BMI), GLPS (epi), GLPSavg, GLPS (endo), and PSD. Logistic regression analysis was performed on specific characteristics that were significantly different between the 2 groups: sex, DM, smoking, diastolic blood pressure, GLPS (epi), GLPSavg, GLPS (endo), and PSD. Logistic regression analysis was performed on specific characteristics that were significantly different between the 2 groups: sex, DM, smoking, diastolic blood pressure, GLPS (epi), GLPSavg, GLPS (endo), and PSD. Receiver operating characteristic (ROC) curves were constructed, and the area under the curve (AUC) was calculated. GLPSavg indicates the middle layer of the myocardium and is derived using a special algorithm involving the whole myocardium. From the ROC constructed for GLPS (epi), GLPSavg, and GLPS...
we obtained the optimal cut-off value with the highest sensitivity and specificity. Intra-observer and inter-observer reproducibility was assessed in 20 random patients using the Bland-Altman method. A P value of <0.05 was considered statistically significant. Analyses were performed using SPSS Statistics version 22.0 software (IBM Corp., Armonk, NY, USA).

**Patient and Public Involvement**

Patients and the public were not involved in the design, recruitment, or implementation of the study.

**Results**

In our study, a total of 690 patients were enrolled. Overall, 68 patients were excluded due to poor image quality or absence of angiography. Of the remaining 622 patients, 346 had CAD and 276 did not have CAD.

|Table 1| presents the baseline characteristics of the patients. GLPS (epi), GLPSavg, and GLPS (endo) were significantly more impaired in the CAD group than in the non-CAD group (P<0.01). PSD was not significantly different between the 2 groups. Absolute values of GCS apical and GCS basal decreased significantly in the CAD group compared with the non-CAD group (P<0.01), whereas the absolute value of the GCS in the middle planes showed no significant difference between the 2 groups.

**Diagnostic Performance of GLPS (epi)**

In **Table 2**, multifactor regression analysis results showed that DM [odds ratio (OR) 1.819 (1.242-2.664; P=0.002), diastolic blood pressure, and GLPS (epi) [OR 1.297 (1.217-1.382; P=0.002) per 1% decrease] were independent risk factors for CAD. Diastolic blood pressure was an independent protective factor, while diabetes and GLPS (epi) were independent risk factors. In the regression model, smoking, GCS apical, and GCS basal were not independent predictors of CAD. In **Figure 3 and Table 3**, the predictive value analysis showed that the AUC was 0.727, indicating a good predictive value, and the cut-off value

**Figure 2.** Layer longitudinal peak strain assessed by 2D-STE in a patient with CAD. The first-row panels indicate the global and segment strain from apical long-axis view in endocardium, middle layer, and epicardium. The second-row panels indicate the corresponding segmental strain traces. The lower panels indicate the corresponding qualitative color M-mode strain referring to the 6 consecutive myocardial segments. Dark red, normal strain; light red, decreased strain; and pink color, strongly reduced strain. ENDO – endocardium; MID – middle layer; EPI – epicardium; GS – global strain.

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Diagnostic Performance of GLPSavg

Multifactor regression analysis results showed that DM [OR 1.825 (1.246-2.675; P=0.002)], diastolic blood pressure, and GLPSavg (OR 1.260 (1.192-1.333; P=0.000) per 1% decrease) were independent factors of CAD (Table 4). Diastolic blood pressure was an independent protective factor, while DM and

Table 1. Baseline characteristics of the population.

|                         | Non-CAD (n=276) | CAD (n=346) | χ²/t | P       |
|-------------------------|-----------------|-------------|------|---------|
| Age, y                  | 64.22±9.22      | 64.19±9.88  | 0.048| 0.962   |
| Male (%)                | 135 (48.9)      | 251 (72.5)  | 36.41| <0.001  |
| Hypertension (%)        | 183 (66.3)      | 230 (66.5)  | 0.002| 0.965   |
| DM (%)                  | 81 (29.3)       | 151 (43.6)  | 13.41| <0.001  |
| Hypercholesterolemia (%)| 200 (72.5)      | 262 (75.7)  | 0.853| 0.356   |
| Smoke (%)               | 94 (34.1)       | 193 (55.8)  | 29.15| <0.001  |
| CAD family history      | 85 (30.8)       | 111 (32.1)  | 0.117| 0.732   |
| Heart rate (bpm)        | 75.38±13.36     | 74.5±11.21  | 0.890| 0.374   |
| Systolic blood pressure | 134.9±16.67     | 135.08±17.15| -0.136| 0.892   |
| Diastolic blood pressure| 79.33±11.31     | 77.38±10.96 | 2.169| 0.03    |
| BMI                     | 25.73±3.96      | 26.03±6.16  | -0.721| 0.471   |
| GLPS (epi)              | -18.57±3.22     | -15.74±3.32 | -10.72| <0.001  |
| GLPSavg                 | -21.79±3.78     | -21.23±4.27 | -9.654| <0.001  |
| PSD                     | 51.25±22.77     | 54.08±26.31 | -1.414| 0.158   |
| GS apical               | -30.45±12.28    | -28.20±12.36| -2.263| 0.024   |
| GS mid                  | -23.23±9.08     | -22.37±8.96 | -1.18| 0.238   |
| GS basal                | -16.77±7.30     | -14.89±7.14 | -3.225| 0.001   |

Continuous data are presented as mean±SD. Categorical data are presented as percentage n (%). DM – diabetes mellitus; CAD – coronary artery disease; BMI – body mass index; GLPS (epi) – global longitudinal peak strain of epi-myocardium; GLPSavg – average global longitudinal peak strain; GLPS (endo) – global longitudinal peak strain of endo-myocardium; PSD – peak strain dispersion; GS – global strain.

Table 2. Regression model (A) including global longitudinal peak strain epicardium (GLPSepi).

|                | B     | S.E.  | Wald  | P     | OR   | OR 95% CI |
|----------------|-------|-------|-------|-------|------|-----------|
|               |       |       |       |       | Upper| Lower     |
| DM            | 0.598 | 0.195 | 9.449 | 0.002 | 1.819| 1.242     |
| Smoke         | 0.278 | 0.232 | 1.435 | 0.231 | 1.320| 0.838     |
| Diastolic blood pressure | -0.023 | 0.008 | 7.390 | 0.007 | 0.977| 0.961     |
| GLPS (epi)    | 0.020 | 0.032 | 64.33 | <0.001| 1.297| 1.217     |
| GS apical     | -0.004| 0.008 | 0.227 | 0.634 | 0.996| 0.981     |
| GS basal      | 0.007 | 0.013 | 0.286 | 0.593 | 1.007| 0.981     |

DM – diabetes mellitus; GLPSavg (epi) – global longitudinal peak strain of epi-myocardium; GS – global circumferential strain.

was -16.95, with sensitivity and specificity of 73.7% and 63%, respectively. In addition, the AUC of the regression model was 0.777, indicating that the regression model had a good effect.

Diagnostic Performance of GLPSavg

Multifactor regression analysis results showed that DM [OR 1.825 (1.246-2.675; P=0.002)], diastolic blood pressure, and GLPSavg [OR 1.260 (1.192-1.333; P=0.000) per 1% decrease] were independent factors of CAD (Table 4). Diastolic blood pressure was an independent protective factor, while DM and
GLPSavg were independent risk factors. In the regression model, smoking, GCS apical, and GCS basal were not independent predictors of CAD. In Figure 4 and Table 3, the predictive value analysis showed that the AUC was 0.727, indicating a high predictive value, and the cut-off value was -20.95, with sensitivity and specificity of 82.4% and 56.2%, respectively. The AUC of the regression model was 0.732, indicating that the regression model had a good effect.

**Table 3.** Predictive value analysis of GLPS.

|                  | AUC       | 95% CI     | P        | Sensitivity (%) | Specificity (%) | Cutoff value |
|------------------|-----------|------------|---------|----------------|-----------------|--------------|
| **Regression model (A)** |           |            |         |                |                 |              |
| GLPS (epi)       | 0.727     | 0.688-0.767| <0.001  | 73.7           | 63.0            | -17.95       |
| Regression model | 0.777     | 0.741-0.814| <0.001  | 74.6           | 67.8            | –            |
| **Regression model (B)** |           |            |         |                |                 |              |
| GLPSavg          | 0.732     | 0.693-0.772| <0.001  | 82.4           | 56.2            | -21.95       |
| Regression model | 0.780     | 0.744-0.817| <0.001  | 81.5           | 61.2            | –            |
| **Regression model (C)** |           |            |         |                |                 |              |
| GLPS (endo)      | 0.708     | 0.668-0.749| <0.001  | 82.9           | 52.9            | -24.95       |
| Regression model | 0.767     | 0.729-0.804| <0.001  | 53.5           | 85.5            | –            |

GLPS (epi) – global longitudinal peak strain of epi-myocardium; GLPSavg – average global longitudinal peak strain; GLPS (endo) – global longitudinal peak strain of endo-myocardium.

GLPSavg were independent risk factors. In the regression model, smoking, GCS apical, and GCS basal were not independent predictors of CAD. In Figure 4 and Table 3, the predictive value analysis showed that the AUC was 0.727, indicating a high predictive value, and the cut-off value was -20.95, with sensitivity and specificity of 82.4% and 56.2%, respectively. The AUC of the regression model was 0.732, indicating that the regression model had a good effect.

**Diagnostic Performance of GLPS (endo)**

The multifactor regression analysis results showed that DM [OR 1.846 (1.267-2.689; P<0.001)], diastolic blood pressure, and GLPS (endo) [OR 1.193 (1.137-1.251; P<0.001) per 1% decrease] were independent factors for CAD (Table 5). Diastolic blood pressure was an independent protective factor, while diabetes and GLPS (endo) were independent risk factors. Predictive value analysis showed that the AUC was 0.708, indicating a good predictive value, and the cut-off value was -22.95 with sensitivity and specificity of 82.9% and 52.9%, respectively (Figure 5, Table 3). The AUC of the regression model was 0.767, indicating that the regression model had a good effect.
Reproducibility

The mean difference±standard deviations (SDs) for the intra-observer agreement was -0.6±3.1% and was not significantly different (Figure 6A). The mean difference±SDs for the inter-observer agreement was -1.8±4.6% which was significantly different (Figure 6B).

Discussion

In our study, GLPS was significantly impaired in all layers in the CAD patients. GCS in the basal and apical planes was reduced in the CAD group, whereas GCS in the middle plane was not different between the CAD and the non-CAD groups. In the regression model, GCS had no predictive value, while GLPS in all 3 layers had a good predictive value. Furthermore, the value was greater when modeled in conjunction with other parameters. Previous studies have reported impairment of strain beginning from the endocardium in CAD patients [2,11]. However, in this study, epicardial wall deformation was more sensitive to coronary stenosis. However, based on pathophysiological

Table 4. Regression model (B) including global longitudinal peak strain average (GLPSavg).

| B       | S.E.   | Wald  | P       | OR   | OR 95% CI |
|---------|--------|-------|---------|------|-----------|
| Gender  | -0.976 | 0.245 | 15.865  | <0.001 | 0.377     | 0.233     | 0.609     |
| DM      | 0.602  | 0.195 | 9.528   | 0.002 | 1.825     | 1.246     | 2.675     |
| Smoke   | 0.287  | 0.233 | 1.155   | 0.218 | 1.333     | 0.844     | 2.105     |
| Diastolic BP | -0.023 | 0.008 | 7.587   | 0.006 | 0.977     | 0.961     | 0.993     |
| GLPSavg | 0.231  | 0.029 | 65.772  | <0.001 | 1.260     | 1.192     | 1.333     |
| GS apical | -0.006 | 0.008 | 0.505   | 0.477 | 0.994     | 0.979     | 1.010     |
| GS basal | 0.007  | 0.013 | 0.255   | 0.613 | 1.007     | 0.981     | 1.034     |

DM – diabetes mellitus; BP – blood pressure; GLPSavg – average global longitudinal peak strain; GS – global circumferential strain.

Table 5. Regression model (C) including global longitudinal peak strain endocardium (GLPSendo).

| B       | S.E.   | Wald  | P       | OR   | OR 95% CI |
|---------|--------|-------|---------|------|-----------|
| Gender  | -0.981 | 0.241 | 16.552  | <0.001 | 0.375     | 0.234     | 0.601     |
| DM      | 0.613  | 0.192 | 10.199  | 0.001 | 1.846     | 1.267     | 2.689     |
| Smoke   | 0.284  | 0.230 | 1.528   | 0.216 | 1.328     | 0.847     | 2.083     |
| Diastolic BP | -0.023 | 0.008 | 7.347   | 0.007 | 0.978     | 0.962     | 0.994     |
| GLPS (endo) | 0.176  | 0.024 | 52.863  | <0.001 | 1.193     | 1.137     | 1.251     |
| GS apical | -0.004 | 0.008 | 0.227   | 0.633 | 0.996     | 0.981     | 1.011     |
| GS basal | 0.011  | 0.013 | 0.733   | 0.392 | 1.011     | 0.986     | 1.038     |

DM – diabetes mellitus; BP – blood pressure; GLPS(endo) – global longitudinal peak strain of endocardium; GS – global circumferential strain.

Reproducibility

The mean difference±standard deviations (SDs) for the intra-observer agreement was -0.6±3.1% and was not significantly different (Figure 6A). The mean difference±SDs for the inter-observer agreement was -1.8±4.6% which was significantly different (Figure 6B).

Discussion

In our study, GLPS was significantly impaired in all layers in the CAD patients. GCS in the basal and apical planes was reduced in the CAD group, whereas GCS in the middle plane was not different between the CAD and the non-CAD groups. In the regression model, GCS had no predictive value, while GLPS in all 3 layers had a good predictive value. Furthermore, the value was greater when modeled in conjunction with other parameters. Previous studies have reported impairment of strain beginning from the endocardium in CAD patients [2,11]. However, in this study, epicardial wall deformation was more sensitive to coronary stenosis. However, based on pathophysiological
considerations, the endocardial myocardium is more vulnerable to myocardial ischemia. The discrepancy may be due to geometric causes of wall deformation.

Myocardial function is damaged at the cellular level when ischemia starts. Different myocardial layers respond differently to ischemia [11]. Ever since Heimdal et al applied STE to analyze the deformation of myocardial tissue in 1998, the technique has proved to be useful in various heart diseases [12]. Longitudinal strain is one of the sensitive markers of CAD [13]. Strain measured using tissue Doppler imaging (TDI) are angle-dependent and depend greatly on the experience of the doctors using the machine [14]. Introduction of the novel 2D-STE can resolve the problems associated with TDI and conventional echocardiography; therefore, the strain value assessed by 2D-STE is more accurate and reliable.

In a recent study, the strain attained a sensitivity of 77% and a specificity of 93%, which predicted 70% of CAD cases [7]. Another study showed that GLPS had a high sensitivity (83%) and specificity (77%) in identifying patients with obstructive CAD [15]. A study on 150 NSTE-ACS patients showed a high performance of GLPS in predicting CAD (AUC=0.92) [16]. In our study, the performance of GLPS was not as satisfactory as in previous studies, yet it was acceptable. This might be due to the heterogeneity of the population. GLPS decreases differently according to the levels of myocardial ischemia in different CAD patients.

Several studies have proven that speckle tracking echocardiography has great value in the diagnosis and prognosis of coronary heart disease [11,16-22]. Biering-Sorensen et al found that the absolute value of GLPS was significantly lower in patients with CAD and was an independent predictor of CAD [7]. In a study of patients with acute myocardial infarction, GCS was able to predict acute coronary occlusion [5]. In our study, GCS in the basal and apical planes was reduced in patients with CAD, but it could not predict CAD. Furthermore, STE could even predict the final infarct size [23]. Haugaa et al found that peak strain dispersion could predict ventricular arrhythmias after infarction [18, 24]. Gjesdal et al [25] discovered that GLS was valuable in the identification of small- and medium-sized infarcts in patients with chronic ischemic heart disease. Hagemann et al [11] found that layer-specific GLS was significantly impaired in patients with significant CAD. The results of these studies are consistent with the findings of our study.

As a novel echocardiography technology, 2D-STE can detect ischemic region quantitatively, while conventional echocardiography assesses myocardial ischemia based on the operator’s naked eyes. Compared with exercise ECG and CMR, 2D-STE is much more available and requires a shorter procedure and post-processing time [26]. It also has more diagnostic value in disabled patients who cannot undergo exercise ECG, coronary computed tomographic angiography, or CMR. As a non-invasive modality, 2D-STE has the advantages of reduced economic and biological costs [27,28].

However, echocardiography interpretations are subjective. In our study, echocardiography showed good intra-observer reproducibility, but there were inconsistencies between different observers. The reason might be that different researchers have different understandings of the definition of the endocardial border. The operator must have sufficient experience to make an accurate measurement. We chose several STE parameters according to the reference and analysis. There are still relevant parameters of territory strain that have not been analyzed in the article; thus, more detailed information is needed. We did not analyze non-ischemic factors, such as diabetes and hypertension, which may reduce strain values.
We defined coronary artery disease as at least 50% stenosis of coronary vessels. However, the results may have been different if the criteria were 75% or 90% stenosis, or in different types of CAD patients. Research with larger samples and more detailed analysis may produce more information on the diagnostic value of STE. In this study, we used only 1 method (EchoPAC version 201) and vendor (GE). The results might have been different using different vendors. Finally, STE is a developing technique that is still limited by image quality, patient condition, and software issues. It is a semi-automated and semi-quantitative technique. Automation is the future of 2D-STE.

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Conclusions

The findings from this study support the clinical application of 2D-ST in patient populations with suspected myocardial ischemia due to CAD. Therefore, 2D-ST combined with ECG monitoring may have a future role for early screening of patients with coronary heart disease.

Conflict of Interests

None.