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RESEARCH

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Dobutamine stress cardiac magnetic resonance-feature tracking in assessment of myocardial ischemia and viability

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Abstract

Background Cardiovascular magnetic resonance-feature tracking (CMR-FT) is a novel quantitative objective noninvasive technique in the assessment of myocardial deformation. The purpose of that study was to assess the capability of the CMR-FT in the detection of myocardial ischemia and viability. We investigated 30 patients ($n=480$ myocardial segments), with known or suspected coronary artery disease (CAD). Dobutamine stress cardiovascular magnetic resonance (DS-CMR) and late gadolinium enhancement (LGE) were used to identify the viable non-ischemic, ischemic, and non-viable myocardial segments. Cine images at rest were used to calculate the segmental radial (Err), circumferential (Ecc), and longitudinal (Ell) strain parameters by manual contouring of endocardial and epicardial borders using Segment Software.

Results Of the 480 myocardial segments and based on the DS-CMR and LGE results, 338 segments were defined as viable non-ischemic (remote), 101 segments were viable ischemic, and 41 segments were non-viable. Rest segmental Ecc, Err, and Ell values were significantly impaired in the non-viable (mean ± SD = − 3.94 ± 4.99%, 11.81 ± 12.55%, and − 7.50 ± 6.96%, respectively) compared to both viable groups, $p<0.001$. Ecc and Err significantly differentiated between the non-ischemic and ischemic groups (mean ± SD = − 19.14 ± 7.20% vs − 13.18 ± 8.57% and 44.03 ± 19.56% vs 32.79 ± 17.91% respectively), $p<0.001$. However, Ell showed no statistical significance between them (mean ± SD = − 16.44 ± 8.78% vs − 16.12 ± 10.00%, $p=0.945$).

Conclusions CMR-FT can differentiate between viable and non-viable as well as ischemic and non-ischemic myocardial segments. So, such a noninvasive technique has a promising additional objective diagnostic role in conjunction with CMR in ischemia and viability assessment or even may replace stress and LGE studies in the future.

Keywords Feature tracking, Strain, Dobutamine, Cardiac magnetic resonance, Coronary artery disease

Background

Cardiovascular diseases are responsible for about one-third of deaths worldwide. Ischemic heart disease (IHD) also referred to as coronary artery disease (CAD) ranks as the most prevalent illness. The primary pathological process that leads to IHD is atherosclerosis. Other risk factors include obesity, diabetes, metabolic syndrome, and population aging. Multiple hospitalizations, revascularization procedures, clinic visits, emergency visits, and prescribed drugs are all major financial sequels of IHD. So, prevention, early detection, and proper diagnosis have become essential [1].

Cardiovascular magnetic resonance (CMR) is an important noninvasive tool for the assessment of myocardial ischemia and viability in patients with known or suspected CAD. Regarding viability assessment, late

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gadolinium enhancement (LGE) has been established as the gold standard method for the detection of myocardial viability and scars [2, 3].

However, other non-contrast techniques were investigated. Visually detectable ventricular wall motion abnormality (WMA) after dobutamine infusion is considered a qualitative way for the assessment of myocardial viability and ischemia. However, this method is subjective and depends greatly on the observer's experience. Also, it has been found that visual WMA occurs relatively late in the ischemic process. So, quantitative methods for the assessment of myocardial wall deformation have been recently widely investigated for easier, safer, and more objective assessment [4, 5].

CMR myocardial tagging and feature tracking (FT) are novel techniques used for the quantitative assessment of myocardial deformation. However, myocardial tagging acquisition and its post-processing analysis are sophisticated, time-consuming, and laborious. Cardiovascular magnetic resonance-feature tracking (CMR-FT) overcomes such disadvantages by using balanced steady-state free-precession (bSSFP) sequences, with rapid acquisition and post-processing time [6].

CMR-FT can automatically calculate the degree of myocardial strain, which is defined as the ratio of initial and final myocardial dimensions during the cardiac cycle. Circumferential (Ecc), longitudinal (Ell), and radial (Err) strain are different myocardial strain parameters that can be used for both global and segmental functional assessment [7].

The study aimed to assess the capability of cardiac magnetic resonance-feature tracking (CMR-FT) in the objective quantitative assessment of the myocardial viability and ischemia in patients with coronary artery disease.

Methods

Patient population

Adult patients who were referred to the radiology department at our institution for myocardial viability and ischemia assessment **using Dobutamine Stress CMR** from the period March 2019 to June 2021 were prospectively enrolled in our study. Inclusion criteria included those suspected or known CAD diagnosed by either clinical symptoms, laboratory investigations, echocardiography, electrocardiogram, or history of previous revascularization or previous ischemic events.

Exclusion criteria included patients with contraindications for dobutamine injection (within 24 h of troponin-positive acute coronary syndrome (ACS) or 7 days of acute myocardial infarction (STEMI), decompensated heart failure, a recent history of life-threatening arrhythmia, severe left ventricular outflow tract obstruction, highly elevated Blood pressure ≥ 220/120, recent pulmonary embolism, active myocarditis, endocarditis, pericarditis, and hypokalemia), contraindications for MRI contrast agents (e.g., GFR below 30 mL/min/1.73 m^2 or a previous severe allergic reaction), general MRI contraindications (those with intraocular foreign bodies or implanted non-MRI compatible electronic devices, e.g., cardiac pacemakers, implanted hearing aids, or claustrophobic patients), and those with poor image quality due to either inadequate breath holding or arrhythmias.

A written informed consent was read and signed by the patient himself or his/her relative. The study was conducted after the approval of our institution's Ethical and Scientific Committee to ensure data confidentiality and the privacy of our participants.

Patient preparation

Patients were informed to fast 4–6 h before the test, patient's vital signs (e.g., blood pressure and heart rate) and a pre-test ECG were checked, followed by insertion of two wide-bore cannulas (one for dobutamine infusion and the other for contrast injection). A full explanation of the study's steps and the warning symptoms to terminate the study (e.g., severe chest pain or dyspnea) was performed. A crash cart with appropriate resuscitative medications, supplies, and an established location outside the CMR scanner room was available.

Image acquisition

The study was performed on *1.5 T* **Siemens MR machine** (Magnetom Aera, Siemens Medical Systems, Erlangen, Germany). All examinations were ECG and respiratory-gated using a dedicated phased array cardiac coil, under the supervision of a cardiologist, and with accurate observation of the patient's vital data.

Examinations were started with ECG-gated breath-hold **rest cine images** (balanced steady-state free-precession sequence; bSSFP) acquired in the three left ventricular (LV) long axis views (2-, 3-, and 4-chamber views) and three short-axis slices (basal, mid, and apical level), with the following parameters (repetition time (TR): 29.9 ms, echo time (TE): 1.34 ms, flip angle: 62°, slice thickness: 5 mm, field of view (FOV) read: 400 mm, and FOV phase: 82.4%). After that, dobutamine infusion was started with gradual dose titration from 5mic/kg/min up to 40 mic/kg /min ± atropine (with 3-min intervals) and acquisition of the same rest cine views with every dose until the target heart rate was reached or wall motion abnormality (WMA) ischemic changes were visualized. Afterward, injection of the gadolinium-based contrast agent (Dotarem, Paris, France, 0.2 mmol/kg) was done and 5–10 min later, late gadolinium enhancement images (LGE) were acquired after detecting the optimum inversion time using the following parameters (repetition

time (TR): 764 ms, echo time (TE): 1.12 ms, slice thickness: 8 mm, field of view (FOV) read: 430 mm and FOV phase: 100%). Once the heart rate declined below 100, recovery-phase images were also acquired.

Image analysis

All examinations were reviewed by two expert cardiac imagers, radiologists/cardiologists, with more than 10 years of experience. Analysis of the wall motion abnormalities and correlation with the scar extent in LGE images (using the standardized 16-AHA segmentation model) were performed using PaxeraUltima browser-based medical viewer. Myocardial segments were classified into three main groups: ischemic group including those segments with any new or worsening wall motion abnormalities > / = 1 grade during peak stress or recovery phase. Biphasic response (when there was improvement of WMA at low dose that became worse at high dose) was also used to identify ischemic segments. Non-viable segments included those showing akinesia at rest, low-dose, and high-dose dobutamine with transmural (TM) or subendocardial > 50% scars in LGE images. Other segments were considered as viable non-ischemic (remote).

Feature tracking processing was performed by a radiologist (with more than 5 years of experience) using Segment Software (Medviso, Lund, Sweden). Rest short-axis bSSFP cine images at the LV basal, mid, and apical levels for quantification of the peak segmental circumferential (Ecc) and radial strain (Err), while the longitudinal 2-, 3-, and 4 chamber views were used to measure the peak segmental longitudinal strain (Ell). Manual contouring of the endocardium and epicardium at the end-diastolic phase was performed for each view, while the software automatically calculated the peak segmental strain for each segment according to the 17-AHA segmentation model (Fig. 1). Strain segmental data were exported in an Excel sheet and correlated with the findings of dobutamine stress CMR and LGE. It is to be mentioned that radial strain values were represented in positive values while both circumferential and longitudinal strains were calculated in negative values.

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov–Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, and standard deviation. Significance of the obtained results was judged at the 5% level.

The used test was: **Paired *t* test**: For normally distributed quantitative variables, to compare between two periods.

Results

Out of 34 patients, 30 cases were enrolled in the current study (25 males and 5 females, mean age ± SD of 58.1 ± 9.8 years (35.0–75.0). We excluded four patients because of poor images' quality due to breathing artifacts or due to the development of arrhythmia, which

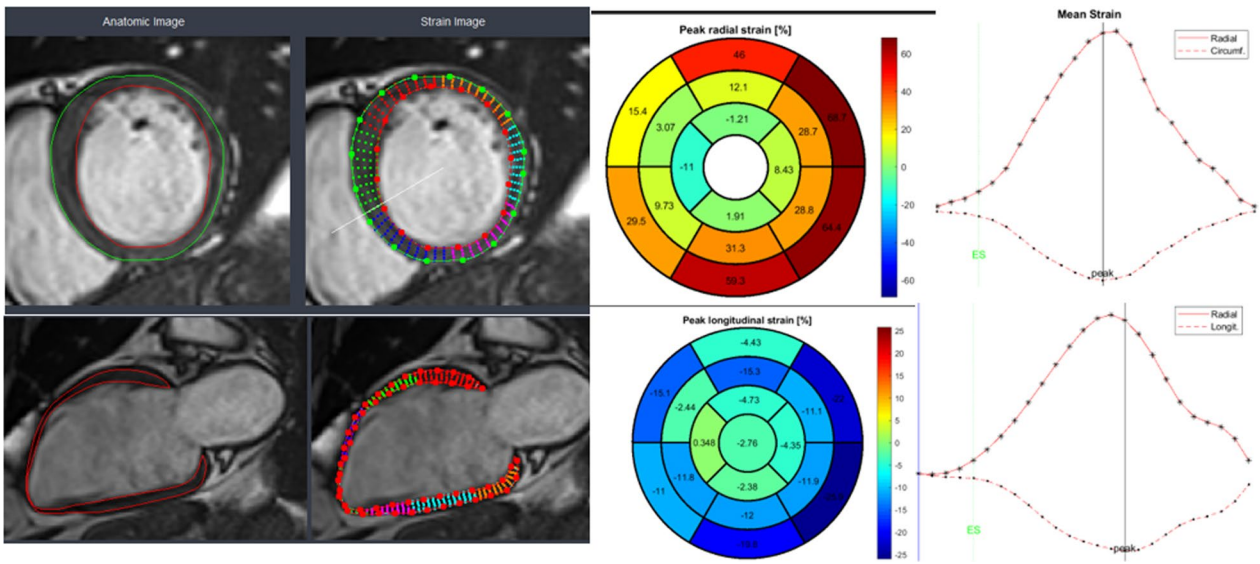

Fig. 1 Segment Software for strain analysis. First row shows the manual contouring of the epicardial and endocardial borders of the LV basal cine images, with subsequent feature tracking results, showing segmental radial strain values in bull's eye view and its corresponding graph of global radial and circumferential strain. Second row shows the LV two-chamber cine images, with its corresponding bull's eye showing the segmental longitudinal strain values and the graphical presentation of the global longitudinal strain

hinder proper manual contouring and tracking by the FT software.

Among the 30 patients, 14 had diabetes, 12 had hypertension, and 12 were smokers. Thirteen of the cases had a previous history of percutaneous coronary intervention (PCI) and 2 had coronary artery bypass grafts (CABG). One experienced recurrent ischemic ECG changes, 8 had a history of old STEMI, and 1 had previous NSTEMI. As regards the territorial coronary affection of our patients, 6 had LAD, 9 had non-LAD, and 15 had both LAD and non-LAD territorial ischemic changes. Demographic data are presented in (Table 1).

Dobutamine stress (DS-CMR) and LGE results

Of the 30 patients ($n=480$ segments), only 27 patients ($n=432$ segments) completed the study with LGE due to study termination in three of them because of the development of severe typical chest pain and ECG changes. As regards the LGE results: subendocardial < 50% or small focal transmural scars were identified in 47 segments (10.8%), transmural scars in 37 segments (8.5%), and no scars in 348 segments (80.5%). All non-viable segments showed transmural scars.

Regarding the combination of both visual WMA assessment of DS-CMR and the correlated LGE scar extent of the 480 segments: 338 segments were assigned as viable non-ischemic (remote), 101 segments were viable ischemic, and 41 segments were non-viable.

Table 1 Distribution of the studied cases according to demographic data ($n=30$)

	No	%
Sex		
Male	25	83.3
Female	5	16.6
Age (years)		
Min. – Max	35.0–75.0	
Mean ± SD	58.1 ± 9.8	
Hypertension	12	40.0
Diabetes	14	46.6
Smoking	12	40.0
IHD		
Previous CABG	2	6.0
Previous PCI	13	53.3
Previous STEMI	8	26.6
Previous NSTEMI	1	3.3
Recurrent ischemic ECG changes	1	3.3
LAD/non-LAD		
Non-LAD	9	30.0
LAD	6	20.0
Both	15	50.0

SD Standard deviation, *IHD* Ischemic heart disease, *CABG* Coronary artery bypass graft, *STEMI* ST-elevation myocardial infarction, *NSTEMI* Non-ST-elevation myocardial infarction, *LAD* Left anterior descending

Segmental strain analysis

At rest, peak segmental Ecc, Err, and Ell differentiated significantly between the three groups: viable non-ischemic (remote), viable ischemic, and non-viable (Table 2 and Figs. 2, 3, 4, 5, 6, 7). Comparing each two groups; both rest segmental Ecc and Err were significantly impaired in the non-viable (mean ± SD = − 3.94 ± 4.99%, and 11.81 ± 12.55%, respectively) compared to both viable groups, $p<0.001$. Also, Ecc and Err mean values were significantly lower in the ischemic group compared to the non-ischemic one (mean ± SD = − 19.14 ± 7.20% vs − 13.18 ± 8.57% and 44.03 ± 19.56% vs 32.79 ± 17.91% respectively), $p<0.001$.

Regarding the segmental Ell, there was a statistically significant difference between the mean strain values of the non-viable (mean ± SD = − 7.50 ± 6.96%) and the viable segments (non-ischemic and ischemic), $p<0.001$. However, it was weak in differentiating between non-ischemic and ischemic segments with no statistical significance, despite showing relatively lower mean strain value in the ischemic group (mean ± SD = − 16.44 ± 8.78% vs − 16.12 ± 10.00%, $p=0.945$).

Discussion

CMR-FT is a novel objective quantitative technique for the assessment of myocardial deformation. Studies showed a reasonable agreement with the myocardial tagging technique and an acceptable interobserver reproducibility [8, 9].

Therefore, echocardiography is a simple and inexpensive technique that can measure myocardial strain (e.g., by speckle tracking technique). However, it could not perform accurate short-axis views, for strain analysis in all patients, because of wide variability in the echo window and cardiac position among different patients. Also, it is more subjective and operator-dependent than the CMR which has greater consistency and reproducibility [10].

In the current study, we found that segmental Ecc and Ell mean values of the remote viable segments were lower than the already published normal FT-derived strain values of healthy subjects [4, 11, 12]. However, the mean radial strain values were slightly higher than the previously mentioned published normal values and lower than those in other studies [13, 14]. This may be due to the wider variability in the range of the normal radial strain and due to lower intraobserver and interobserver reproducibility compared to longitudinal and circumferential strain [15, 16].

Table 2 Comparison between viable (non-ischemic and ischemic) and non-viable segments according to segmental Ecc, Err and Ell (at rest)

Rest	Viable		Non-viable ($n=41$)	p
	Non-ischemic ($n=338$)	Ischemic ($n=101$)		
Ecc				
Max.– Min	− 46.60–1.10	− 38.71–11.55	− 12.99–5.28	< 0.001*
Mean ± SD	− 19.14 ± 7.20	− 13.18 ± 8.57	− 3.94 ± 4.99	
Sig.bet.Grps	$p_1 < 0.001^*, p_2 < 0.001^*, p_3 < 0.001^*$			
Err				
Min. – Max	− 23.10–106.4	− 19.02–74.94	− 10.66–47.41	< 0.001*
Mean ± SD	44.03 ± 19.56	32.79 ± 17.91	11.81 ± 12.55	
Sig.bet.Grps	$p_1 < 0.001^*, p_2 < 0.001^*, p_3 < 0.001^*$			
Ell				
Max.– Min	-44.97–5.04	− 46.49–10.32	− 26.01–3.46	< 0.001*
Mean ± SD	− 16.44 ± 8.78	− 16.12 ± 10.00	− 7.50 ± 6.96	
Sig.bet.Grps	$p_1 = 0.945, p_2 < 0.001^*, p_3 < 0.001^*$			

Pairwise comparison between each two groups was done using **Post Hoc Test (Tukey)**

p: p value for comparing between the studied groups

p_1: p value for comparing between **Non-ischemic and Ischemic Viable**

p_2: p value for comparing between **Non-ischemic and non-viable**

p_3: p value for comparing between **ischemic and non-viable**

*: Statistically significant at $p \leq 0.05$

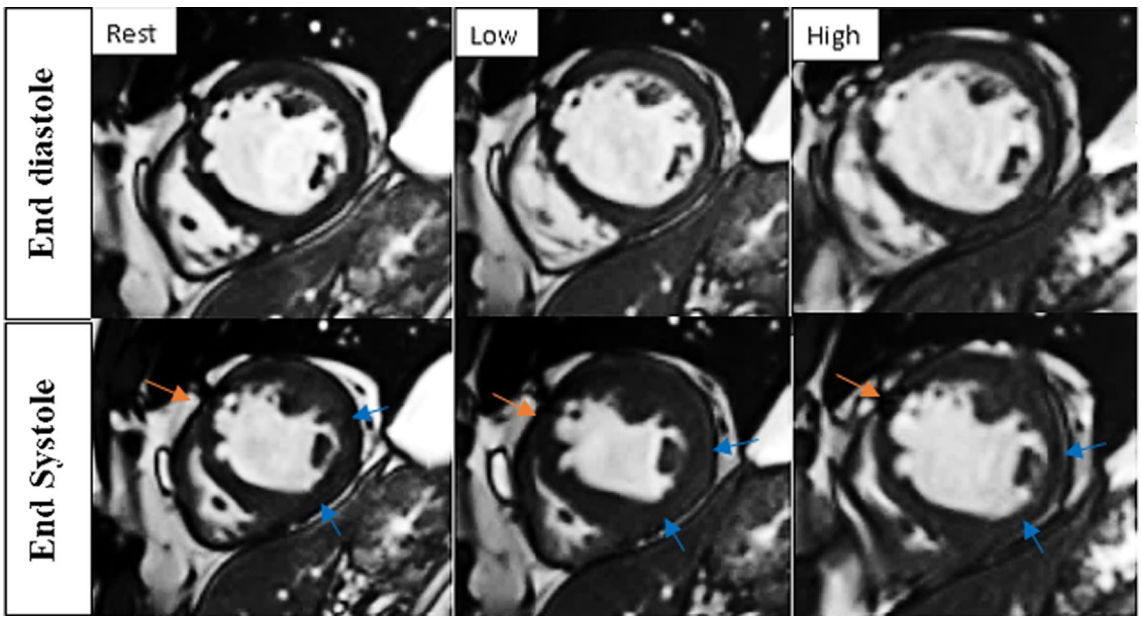

Fig. 2 DS-CMR of a 71-year-old male patient with history of previous PCI to LAD, showing LV short-axis cine views at the mid ventricular level at rest, low-dose, and peak/high-dose dobutamine stages, during both end-diastolic and end-systolic phases. The akinetic thinned out anterior and anteroseptal walls showed no endocardial inward movement or systolic wall thickening during the three phases, indicative of non-viable segments (orange arrows). Moreover, the inferoseptal, inferior, and lateral walls that were hypokinetic at rest, developed pronounced increase of the endocardial inward movement and wall thickening at low-dose dobutamine (denoting viability). However, at peak stress, the latter segments turned hypokinetic again (biphasic response) indicating stress-induced ischemia (blue arrows)

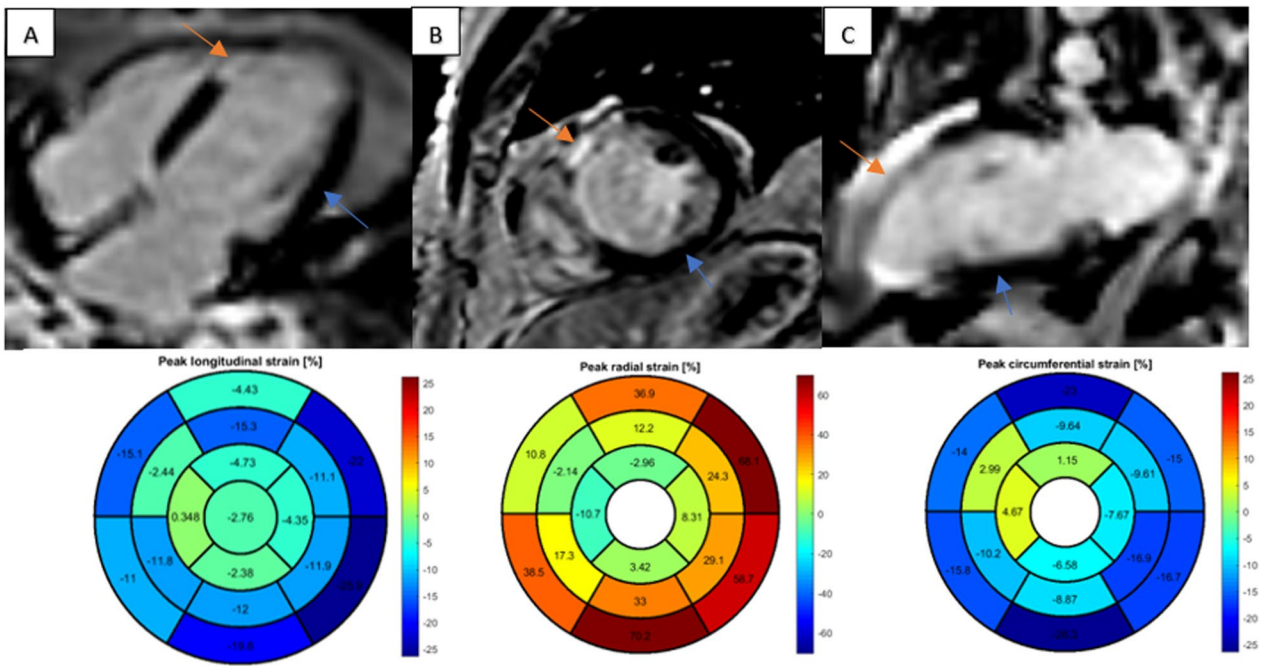

Fig. 3 First row: CMR LGE images of the same patient showing **A** 4-chamber, **B** short axis, and **C** 2-chamber views, respectively. Transmural infarctions are noted at the mid anterior and anteroseptal as well as apical walls with focal involvement of the apical lateral wall (orange arrows). Rest of LV wall showed no LGE scars denoting viability (blue arrows). Second row shows the corresponding **Segment Software** feature tracking displaying bull's eye color-coded map of the segmental longitudinal, radial, and circumferential strain at rest (from left to right side), showing markedly reduced strain values of the mid anterior, anteroseptal walls, and apical walls (LAD perfusion territory). However, the ischemic viable mid inferoseptal, inferior, and lateral walls show higher strain values, yet they are lower than the mean values of the viable non-ischemic basal walls

Some studies suggested a generalized change and remodeling in the whole myocardium of ischemic patients, involving the neighboring apparently normal remote segments. This could explain why strain values of the remote viable segments were lower than the normal healthy subjects [17, 18]. Also, Zhao et al. [12] interlinked such findings with the effect of other comorbidities including microvascular ischemic disorders, like diabetes and hypertension, that could affect myocyte metabolism and contractility.

Many studies have previously investigated the ability of CMR-FT to discriminate between viable and non-viable myocardial segments [4, 19]. However, few studies have investigated the ability of CM-FT-derived strain to quantitatively differentiate between ischemic, viable, and non-viable myocardial segments in CAD patients using high-dose dobutamine stress CMR and LGE [17]. Our study showed differences in the mean values of the segmental Ecc, Err, and Ell among the viable non-ischemic, ischemic, and non-viable myocardial segments. That was proved using only rest bSSFP cine images. Non-ischemic segments had the highest strain mean values followed by ischemic segments and then the non-viable segments.

More precisely, Ecc and Err were able to discriminate significantly between viable (either ischemic or non-ischemic) and non-viable segments and between non-ischemic and ischemic segments, $p < 0.001$. Ell also showed a statistically significant difference between viable and non-viable segments, $p < 0.001$. However, it could not achieve a statistically significant difference between non-ischemic and ischemic segments, despite showing lower mean values in the ischemic group compared to the non-ischemic one. Oyama-Manabe et al. [20] proposed an explanation for such a finding; since ischemic myocardial changes begin from the subendocardial layer outwards, it seems logical that longitudinal strain would be affected before the circumferential strain. Thus, a significant differentiation between ischemic and non-ischemic segments was not feasible by Ell.

Schneeweis et al. [17] conducted a study using DS-CMR on 25 patients with known or suspected CAD with no rest WMA, impaired EF, or LGE scars. They measured the **Ecc** strain using the feature tracking technique (**TomTec Imaging** Systems, Munich, Germany). They found no significant differences in the **rest** Ecc between normal, stenotic, and remote segments. However, high statistically significant differences were found at high dose between normal and stenotic segments and between remote and stenotic segments as well as between normal and remote

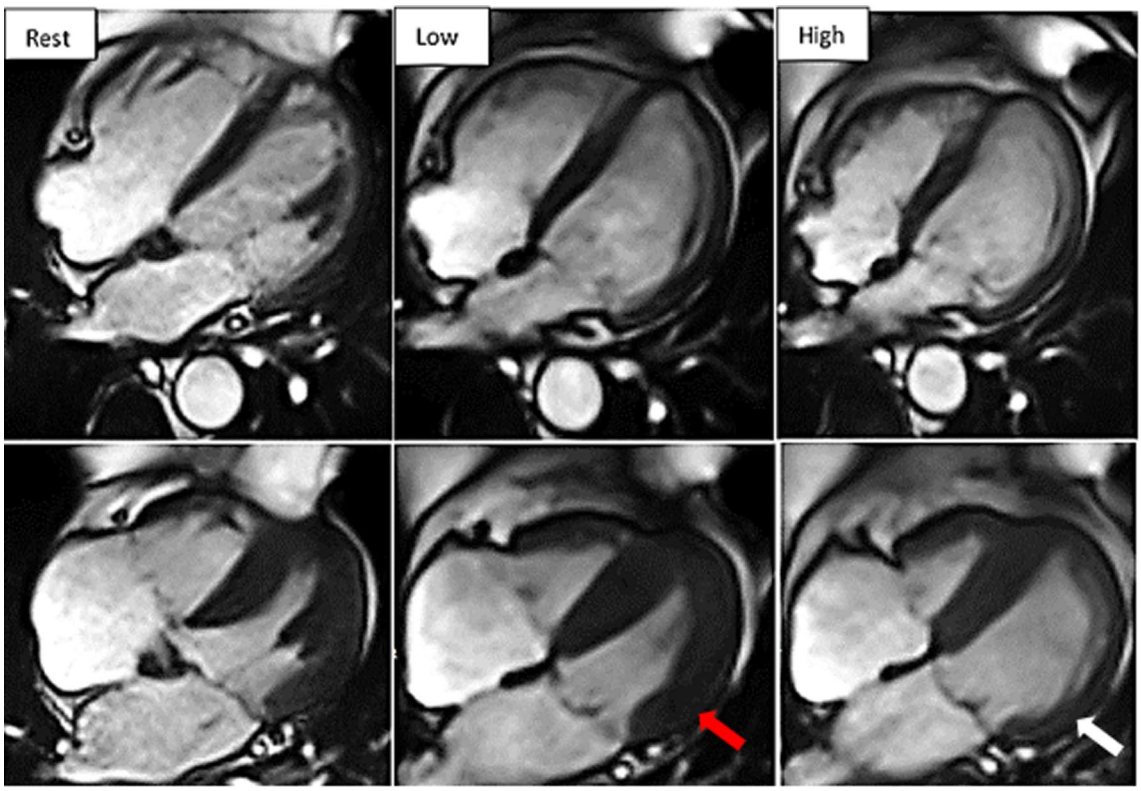

Fig. 4 Four-chamber view of DS-CMR study, of a 60-year-old male patient, known hypertensive and diabetic with history of PCI, during the end-diastole (1st row) and end-systole (2nd row) at rest, low-dose, and high-dose phases. The whole LV lateral wall showed end-systolic increase in the wall thickening at LDD compared to rest (red arrow) indicating viable myocardium; however, the patient then developed lateral wall severe hypokinesia (white arrow) at high-dose phase (induced ischemia)

segments. That was also found during the intermediate dose phase. Also, the recent study by Zhao et al. [12] investigated 109 CAD patients, but they used stress perfusion MR to diagnose ischemic segments and the **CVI42 FT** module to measure the strain. They statistically proved a significant difference in **rest** segmental longitudinal and circumferential strain between ischemic and negative remote segments.

Since the limitation of coronary flow within the ischemic myocardium affects its contractility, probably due to changes in glucose transporters activity, and based on the previously mentioned studies, we propose that FT has the ability to detect changes in the strain of such ischemic myocardium, even at rest [18]. We also suggest that Schneeweis et al. [17] did not find a statistical significance between normal and stenotic segments due to their limitations in the inclusion criteria (no rest WMAs or impaired left ventricular function or scar tissue) that could suggest an early stage of ischemic process that did not change the strain values at rest and did appear during peak stress.

Study limitations and recommendations

Some study limitations must be acknowledged. The study included data from a relatively small sample size (30 CAD patients). A larger sample is needed to establish reliable cutoff segmental strain values for ischemic and non-viable myocardium. Also, most of the study's patients were male, and since previous studies have shown a difference in the global strain values between males and females, we recommend further investigation of an equal number of both genders.

Additionally, we depended on the LGE and DS-CMR as a reference standard in ischemia and viability diagnosis. However, we think that combination with additional modalities, e.g., coronary angiography or FFR, will increase the diagnostic accuracy of the results. Furthermore, the intraobserver variability and interobserver variability were not assessed in our study and better to be investigated in future studies. We only calculated the myocardial strain; however, it will be more beneficial to include an assessment of additional indices such as strain rate and displacement and measure their diagnostic values.

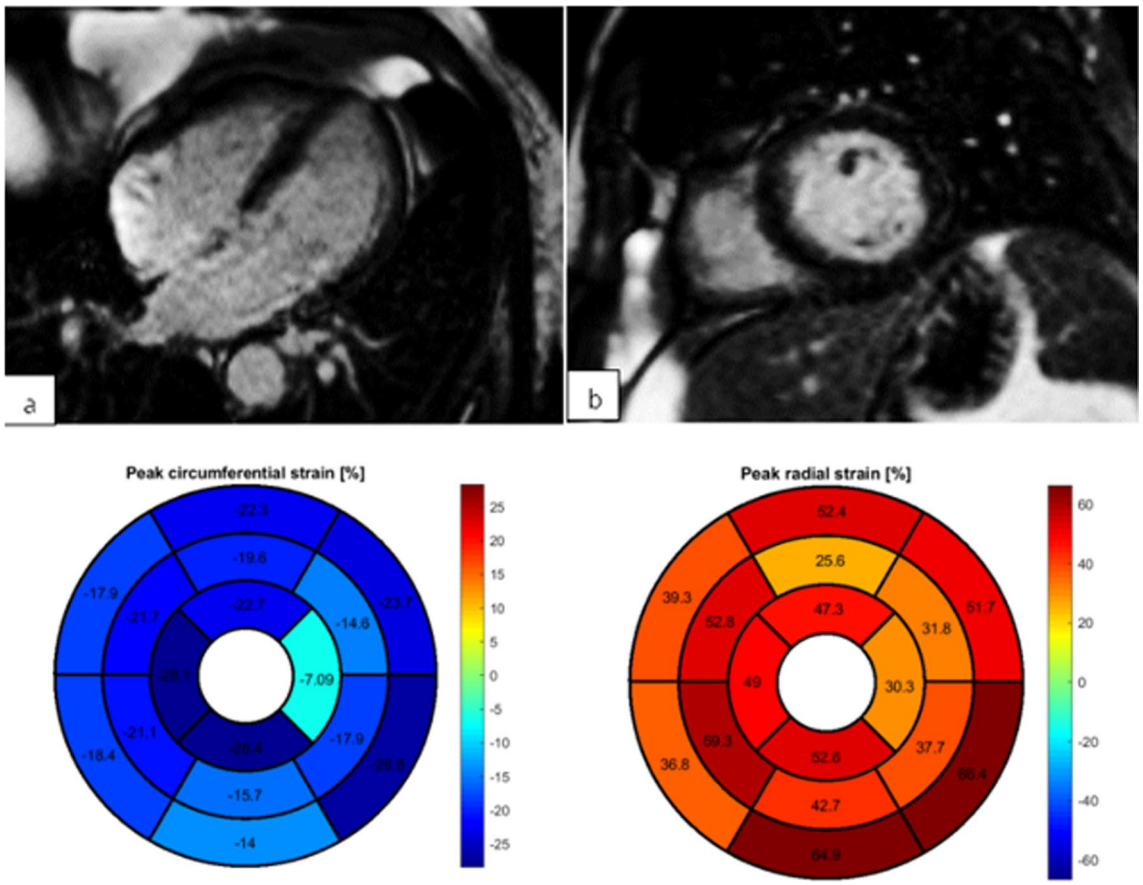

Fig. 5 LGE images (1st row) of the previous case in **a** 4-chamber and **b** short-axis views with no visible LGE scars (viable myocardium). Corresponding color-coded bull's eye (2nd row) displays the segmental circumferential and radial strain at rest showing relatively reduced strain values at the lateral wall more obvious at the mid and apical levels (ischemic myocardium)

Conclusions

CMR-FT is a new evolving promising objective technique for viability and ischemia detection. By using routinely acquired rest cine images and with no need for additional sequence acquisition, CMR-FT was able to differentiate between non-ischemic, ischemic, and non-viable myocardial segments. So, we think that FT can have an effective additional diagnostic role in conjunction with DS-CMR and LGE or even may replace them in the future, eliminating the need for contrast administration or injection of high-risk chronotropic agents.

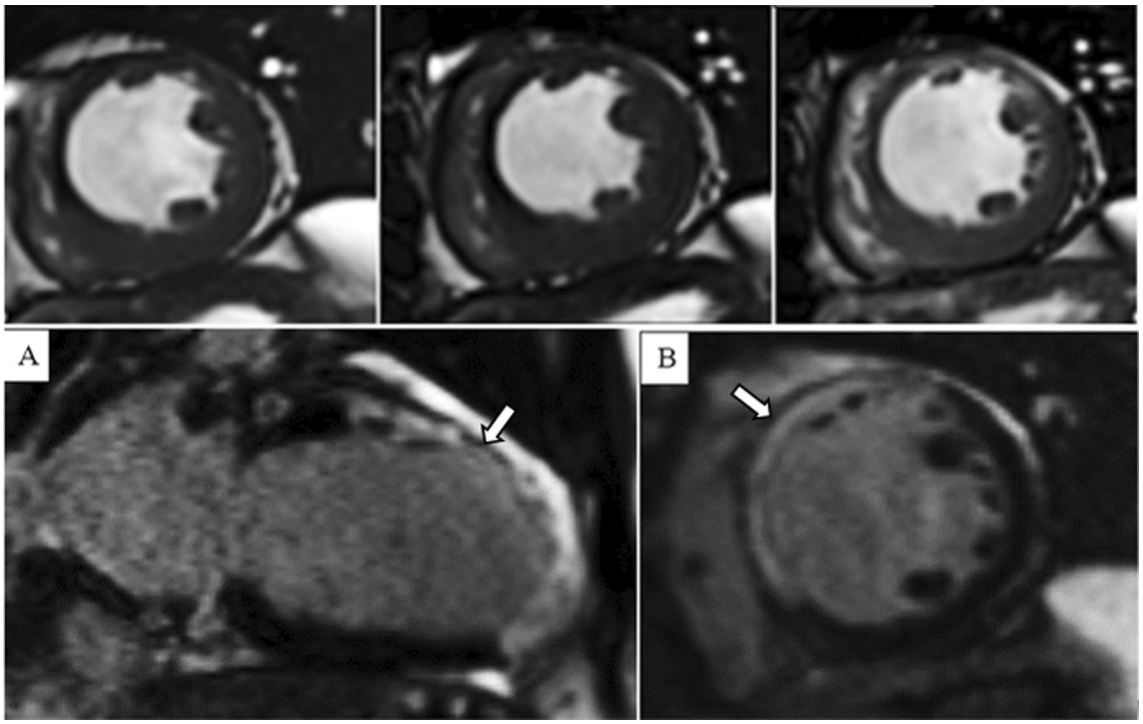

Fig. 6 DS-CMR cine images of a 45-year-old male, known IHD, with a previous history of PCI to LAD and RCA, showing thinned out akinetic mid anterior and anteroseptal walls (1st row) at the end-systolic phase during rest, low dose, and recovery phases (from left to right) with the same findings at the apical segments (not shown). Furthermore, the hypokinetic mid inferior and lateral walls show improved systolic wall thickening at low dose that become worse at the recovery phase, indicating ischemic biphasic response to dobutamine. LGE images of the same patient (2nd row) in **A** 2-chamber and **B** mid short-axis views showing scarred mid and apical anterior and mid anteroseptal walls (**white arrows**) with viable non-scarred mid lateral and inferior walls

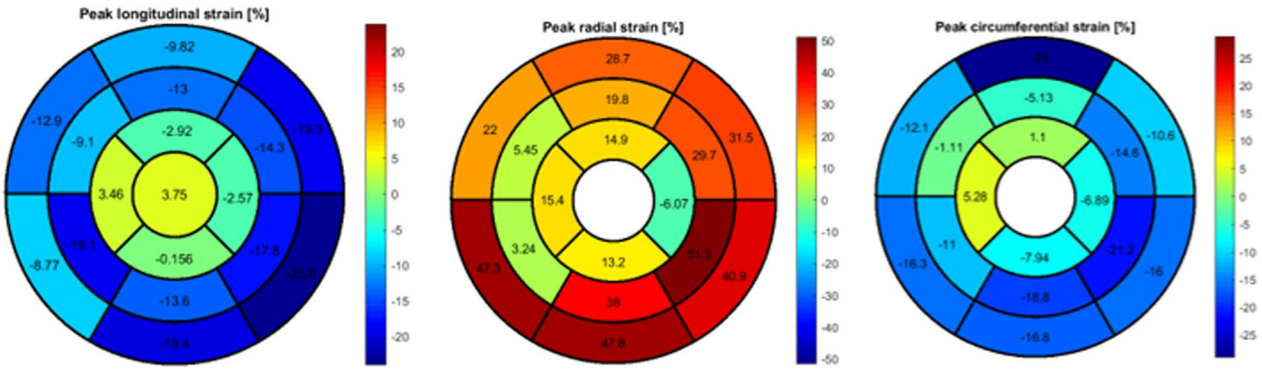

Fig. 7 Corresponding **Segment** Software FT-derived strain bull's eye color-coded map showing markedly reduced rest longitudinal, radial, and circumferential strain values of the mid and apical LAD perfusion territory (corresponding to non-viable segments). Yet, the ischemic mid inferior and lateral segments showed higher strain values

Abbreviations

CMR-FT	Cardiovascular magnetic resonance-feature tracking
CAD	Coronary artery disease
DS-CMR	Dobutamine stress cardiovascular magnetic resonance
LGE	Late gadolinium enhancement sequences
Err	Radial strain
Ecc	Circumferential strain
Ell	Longitudinal strain
IHD	Ischemic heart disease
WMA	Wall motion abnormality
bSSFP	Balanced steady-state free-precession
ACS	Acute coronary syndrome

STEMI	ST-elevation myocardial infarction
NSTEMI	Non-ST-elevation myocardial infarction
PCI	Percutaneous coronary intervention
CABG	Coronary artery bypass grafts
FFR	Fractional flow reserve

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Author contributions
AAMK collected and analyzed the data, wrote the manuscript, prepared the cases, performed required interval procedure, measurements and statistical analysis, and prepared figures and tables. AHS suggested the research idea, shared in data collection and analysis, reviewed literature, statistical analysis, and manuscript editing. SAG reviewed the manuscript and statistical analysis. AMAF reviewed the manuscript and statistical analysis. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was approved by FMASU REC (Faculty Of Medicine Ain Shams University Research Ethics Committee) approval of our study protocol with Assurance number FWA 000017585.

Consent for publication
All patients included in this research gave written informed consent to publish the data contained within this study.

Competing interests
The authors declare that they have no competing interests.

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