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Evaluation of splenic switch off in a tertiary imaging centre: validation and assessment of utility

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Aims Adenosine can induce splenic vasoconstriction (splenic switch-off, SSO). In this study, we aim to evaluate the utility of identifying a lack of SSO for detecting false-negative adenosine stress perfusion cardiac magnetic resonance (CMR) scans.

Methods and results We visually analysed 492 adenosine stress perfusion CMR scans reported as negative in a cohort of patients with no previous history of coronary artery disease. A lack of SSO was identified in 11%. We quantified the phenomenon by drawing regions of interest on the spleen and comparing intensity between stress and rest scans, the spleen intensity ratio (SIR). Inter-rater agreement for qualitative determination of SSO was $\kappa = 0.81$ and inter-class correlation for quantitative determination of SSO was 0.94. The optimal threshold for SIR as an indicator of SSO was 0.40 (sensitivity = 82.5%, specificity = 92.3%, AUC = 0.91). 23 065 CMR scans and 9926 invasive coronary angiogram reports were retrospectively examined to identify patients with negative CMR scans who required coronary intervention in the subsequent 12 months (false negatives). We compared these scans with true positives who had positive adenosine stress perfusion CMR scans followed by coronary intervention. The rate of lack of SSO was 20.7% in the false-negative group versus 13.1% in true positives ($P = 0.37$).

Conclusion The lack of SSO is prevalent, easily measureable, and has potential to improve on haemodynamic criteria as a marker of adenosine understress in CMR perfusion scans.

Keywords Stress CMR • Splenic switch-off • Adenosine • Cardiac magnetic resonance

Introduction

The presence of myocardial ischaemia during functional imaging of the heart is independently associated with an increase in cardiac death and non-fatal complications.[1] In contrast, the absence of ischaemia appears to carry a very favourable prognosis for cardiovascular mortality and morbidity.[2] As such, functional testing for ischaemia forms a major part of current guidelines for both diagnosis and risk stratification in stable coronary artery disease (CAD).[3,4]

Stress perfusion cardiac magnetic resonance (CMR) provides a non-invasive means for the evaluation of ischaemia. Meta-analyses have consistently shown a sensitivity of 89% and a specificity of ~80% in detecting angiographically significant CAD.[5,6] Adenosine is the most prevalent stressor in stress perfusion CMR.[5] The use of CMR has increased in recent years and is now a valuable tool in routine clinical management.[7]

However, a false-negative rate of up to 10% has been reported for adenosine stress-perfusion CMR when compared with quantitative

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coronary angiography. More than a third of these false-negative results may be a result of inadequate pharmacological stressing with adenosine,[8] producing insufficient vasodilation to unmask functionally significant perfusion abnormalities.

The currently accepted protocol for adenosine studies relies on administration of a fixed dose of the stressor, with uptitration if there is failure to reach pre-specified physiological targets (heart rate increase by 10 bpm, drop in systolic blood pressure and/or the experience of physical symptoms associated with adenosine administration).[9] These targets carry a risk of misclassification of a study as adequately stressed for several reasons. First, reporting of symptoms is subjective and even with no adenosine present there can be non-specific changes in heart rate during testing. Second, a drop in blood pressure is not significantly associated with adenosine administration.[10] Third, changes in myocardial blood flow and coronary vascular resistance correlate poorly with changes to peripheral haemodynamics (heart rate and non-invasive blood pressure) in response to adenosine.[11] Finally, inadequate stressing may result from pharmacological interactions of adenosine with caffeine, methylxanthines, smoking, antidepressants and a host of related substances the patient may be taking.[12]

Manisty *et al.* have previously described adenosine-induced splenic vasoconstriction (splenic switch-off, SSO), manifest as decreased brightness of the spleen during adenosine stress perfusion CMR. They showed that rates of lack of SSO were significantly higher in subjects with proven false-negative studies than in those with true-negative studies, in the large CE-MARC study comparing SPECT and adenosine perfusion CMR for detection of angiographically significant coronary disease.[13]

In this retrospective observational study, we assessed the prevalence, ease of measurement and reproducibility of SSO in a real-world and diverse cohort of patients presenting to a tertiary referral centre in London, UK.

In a second retrospective cohort, we aimed to assess the prognostic value of SSO. We carried out a case–control study to assess whether patients with a negative stress perfusion CMR who required coronary angiography and/or percutaneous intervention within a year after the scan were more likely to have had an understressed study, as determined by SSO.

Methods

MRI protocol

All scans were performed in a single centre (London Chest Hospital, part of Barts Health) using a 1.5 T MR scanner (Achieva CV, Philips Healthcare, Best, the Netherlands). All patients had been asked to refrain from caffeine for 12 h prior to their scan. Intravenous adenosine was infused at 140 μg/kg/min for 3 min with continuous heart rate recording, with uptitration to 175 μg/kg/min if there was failure to reach prespecified physiological targets (heart rate increase by 10 bpm, drop in systolic blood pressure and/or the experience of physical symptoms associated with adenosine administration). For the purposes of this study, the patients' haemodynamic responses were defined as adequate if they demonstrated a heart rate increase $\geq$10 bpm. Following this, an intravenous bolus of 0.05 mmol/kg of gadoteric acid was administered. Three short-axis slices, each of 10 mm thickness, were acquired per cardiac cycle during free-breathing, at the basal, mid, and apical levels of the left ventricle. The same perfusion sequence parameters were used for stress then rest perfusion for all patients: single-shot balanced steady-state free precession sequence (TR 2.6 ms TE 1.3 ms, flip angle 50°), typical acquired voxel size of 2.8 × 2.9 mm^2 (readout × phase encoding) and typical matrix size of 108 × 117 (readout × phase encoding). A 90° saturation preparation RF pulse was applied prior to every acquired slice with a saturation delay time of 100 ms. A parallel imaging acceleration factor of 2.3 was also used (sensitivity encoding) to reduce slice acquisition time.

Validation cohort

We sought to investigate the prevalence of SSO and evaluate the demographic and haemodynamic predictors of SSO in a cohort of patients undergoing adenosine stress perfusion CMR for the investigation of chest pain of recent onset between 2008 and 2011.

As such, we retrospectively identified 503 consecutive patients with no previous history of CAD (diagnosis of angina, previous acute coronary syndrome or heart failure) or any imaging findings suggestive of underlying cardiac disease (scan reported as normal with ejection fraction >50%, no late gadolinium enhancement and no severe valvular defects) who had a stress perfusion scan which was reported as negative (i.e. no adenosine-induced perfusion defects detected).

Basic demographic data were extracted from electronic patient records and the cohort's heart rate response to the adenosine bolus was extracted from the DICOM meta-data. As per protocol, a heart rate increase of $\geq$10 bpm was considered a marker of adequate stressing.

Visual analysis of splenic switch-off

All scans were analysed by two independent observers for the presence of SSO (see *Figure 1*). In the rest image, the splanchnic circulation is vasodilated; following the administration of adenosine there is splanchnic vasoconstriction and therefore when gadolinium contrast is administered, the spleen has reduced signal intensity compared with resting perfusion imaging. Splenic switch-off is a graded and transitory response which can be assessed visually on comparison of the rest and stress perfusion images. Therefore, we defined the filling of the left ventricle as time zero to ensure synchronous comparison of rest and stress scans. Any disagreement in the visual analysis was resolved by an experienced third observer (>10 years of experience).

Quantitative analysis of splenic switch-off

In addition, a subgroup of 252 scans were analysed quantitatively by the two observers using cvi[42] (cvi42 v5.1, Circle Cardiovascular Imaging Inc., Calgary, Canada). A region of interest (ROI) was selected on the slice with the spleen most clearly visible and propagated through the whole scan for the first 30 s on both rest and stress images. The ratio between the brightest mean signal intensity of the ROI between stress and rest images, adjusted for baseline (pre-contrast) signal intensity, gave a single parameter—the spleen intensity ratio (SIR).

Case–control study

To ascertain whether the lack of SSO is associated with the need for invasive coronary angiography, we identified as our cases all patients who presented between 2008 and 2014 with a negative adenosine stress perfusion CMR scan who later required coronary intervention within 1 year of their scan. The control inclusion criterion was patients who had a positive adenosine stress perfusion scan followed by coronary angiography with intervention within a year of their scan). From this control group, we age- and gender-matched controls in a ratio of two controls per one case, using nearest neighbour matching without

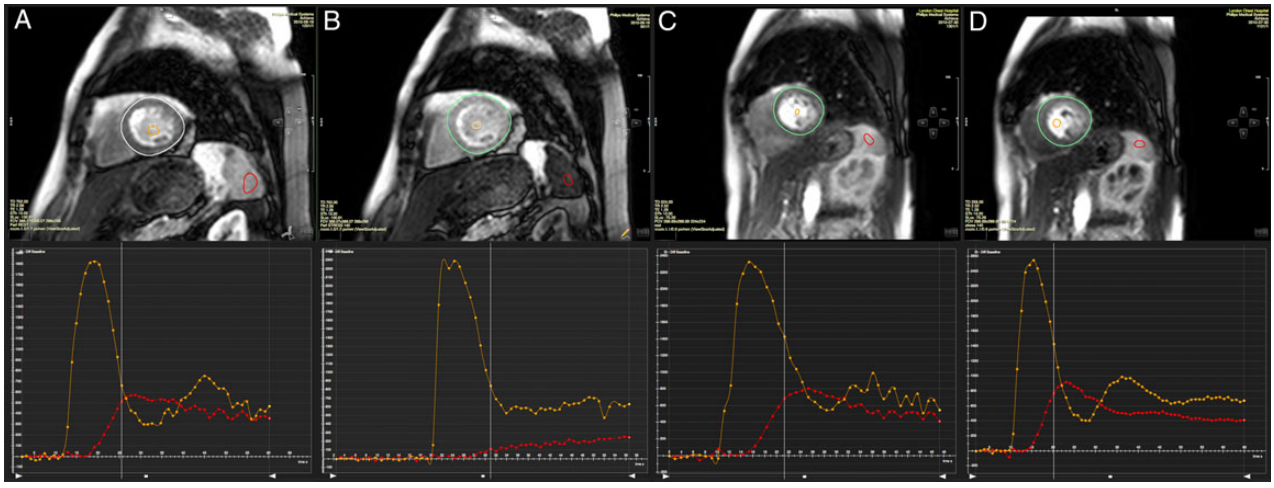

Figure 1 Example of SSO. (*A* and *B*) Rest and stress scans, respectively, from a patient with SSO. (*C* and *D*) Rest and stress scans from a patient with no SSO. The red contour shows signal intensity in the spleen, and the orange contour shows signal intensity in the blood pool, used for baseline adjustment.

replacement. Cases and controls with missing image data were excluded. *Figure 4* shows the flowchart of selection of cases and controls from the 23 065 CMR scans and 9926 coronary angiographies

Blinded visual assessment for the presence of SSO in cases and controls was performed by two independent assessors, with disagreements again resolved by the experienced third observer.

Statistical analysis

All continuous data are expressed as mean ($\pm$SD). All continuous baseline characteristics were assessed using Student's *t*-test and categorical characteristics were compared using Fisher's exact test.

Inter- and intra-observer agreement for the visual identification of SSO was analysed using Cohen's kappa with repeat analysis after 3 weeks ($n = 50$).

Inter-observer agreement for quantitative measurements was assessed by the inter-class correlation coefficient. Bland–Altman plots were constructed to assess for the presence of systematic bias in quantitative measurements of SSO using a random sample of cases. To find out the SIR that best correlated to visual discrimination of SSO, we constructed a receiver–operator curve (ROC).

To investigate the association of SSO with haemodynamic and demographic parameters, a logistic regression model using a generalized linear model with a logit link function was used. Age was expressed as a discretized continuous variable (<60 vs. 60 or greater years old), and ethnic groups were categorized as white, Asian, black, and others/not stated. Haemodynamic response was defined as above. A logistic regression model was also used to examine the relationship of pharmacological understressing and need for coronary intervention at 1 year. Basic demographic variables as well as clinically important covariates were examined for inclusion in the model using a stepwise selection method (history of hypertension, hypercholesterolaemia, diabetes mellitus, smoking, systolic dysfunction, previous percutaneous coronary intervention, previous myocardial infarction, previous CABG, and family history of CAD). Differences were deemed significant at the $P < 0.05$ level.

All statistical analyses were performed using R (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/).

Table 1 Baseline characteristics of the validation cohort

N	503
Able to assess SSO	492
Age (SD)	59.1 (13.6)
Male	50.4% (248)
Ethnicity	
White (English, Scottish, Welsh, Irish, other)	27.8% (137)
Asian (Pakistani, Indian, Bangladeshi, other, Chinese)	23.3% (115)
Black (African, Caribbean, other)	6.30% (31)
Other	6.71% (33)
Not stated	35.8% (176)

SSO, splenic switch-off.

Results

Baseline characteristics

The baseline characteristics of both cohorts are presented in *Tables 1 and 2*. Our validation cohort represents a realistic sample of the population this tertiary centre serves, with the two most prevalent ethnicities being White and Asian. 50.1% of patients were male. Our case–control cohort is well matched for age and gender. Cases and controls differed significantly for prevalence of previous CABG and family history of CAD. Indication for angiography was management of ACS in 20.4%, and the remainder was for stable angina. All proceeded to angioplasty $\pm$ stenting according to clinical indication.

Validation cohort

For the validation cohort, six scans (1.2%) were excluded due to poor quality and 5 (1%) had no visible spleen. A lack of SSO

Table 2 Baseline characteristics of case control cohort

	Negative scan ($n = 31$)	Positive scan ($n = 62$)	*P*-value
Age	66	66	0.97
CABG	6.45%	21.0%	0.08
Hypercholesterolaemia	63.3%	63.3%	1
DM	25.8%	35.5%	0.35
EF <50%	22.6%	21.0%	0.85
FHx	45.2%	16.7%	0.003
HTN	76.7%	70.7%	0.73
Male	71.0%	72.6%	0.87
Previous MI	38.7%	40.3%	0.88
Previous PCI	41.9%	33.9%	0.45
Smoker	48.4%	46.8%	0.88

CABG, coronary artery by-pass grafting; DM, diabetes mellitus; EF, ejection fraction; FHx, family history; HTN, hypertension; MI, myocardial infarction; PCI, percutaneous coronary intervention.

occurred in 11.0% of scans (53/492) when assessed qualitatively. Inter-rater agreement for visual, qualitative assessment of SSO was excellent with $\kappa = 0.81$; intra-rater agreement was also good, with $\kappa = 0.70$.

For quantitative measurements, the mean SIR was 0.31 and the interquartile range 0.15–0.40. The inter-class correlation coefficient between observers was 0.94, and the Bland–Altman plot (*Figure 2*) shows good agreement across a range of SIRs without systematic bias for a random sample of cases ($n = 50$, bias = 0.00248, limits of agreement = −0.162–0.157, arbitrary units, a.u.).

Using visual assessment as the reference standard, ROC analysis showed that the optimal threshold for the SIR as an indicator of SSO, giving equal weight to specificity and sensitivity (the point closest to the upper left corner) was 0.40 (sensitivity = 82.5%, specificity = 92.3%, AUC = 0.91). (*Figure 3*)

People with a lack of SSO were more likely to be white ($P < 0.001$). Age and gender did not appear to influence SSO rates significantly (*Table 3*). The presence of a haemodynamic response more than halved the odds of observing a lack SSO (OR = 0.45), even after adjusting for age, gender, and ethnicity in our multivariate model. However, a significant proportion of people had a lack of SSO, despite having a haemodynamic response (66.0%). Similarly, many people who had SSO did not have haemodynamic response (18.0%) (*Table 4*).

Case–control study

To ascertain whether the lack of SSO is associated with need for invasive coronary angiography, we obtained a database of all 23 065 CMR scans and 9926 coronary angiographies performed during the study period. The identification of cases and controls is shown in *Figure 4*. One thousand six hundred and twenty-two patients had both a CMR study and an angiogram. Of those, 231 had an adenosine stress perfusion followed by an angiogram within 12 months. Thirty-one of the above patients had a negative study followed by angiography (29 scans available). These cases were age and gender matched with 62 control patients (positive CMR

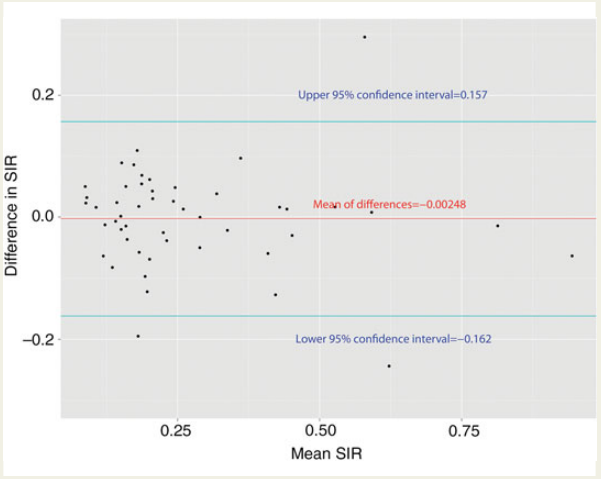

Figure 2 Bland–Altman plot of inter-observer agreement for a random sample of 50 cases. Bland–Altman plot for a random sample of 50 cases (for clarity of graphical representation) from the validation cohort shows good inter-observer agreement (intra-class correlation coefficient = 0.94). There was no evidence of random or systematic bias (mean of differences = 0.00248, 95% CI (+0.157, −0.162)).

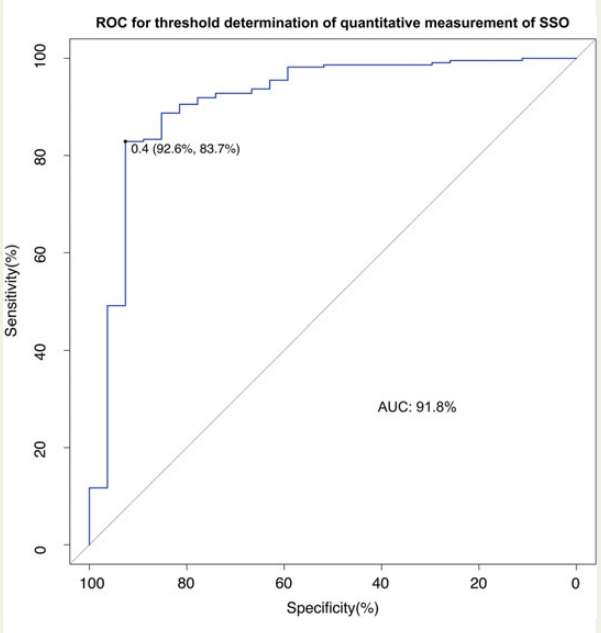

Figure 3 Receiver-operator characteristic curve for threshold determination of quantitative measurement of SSO. Using qualitative evaluation as the gold standard allows cut-off value determination for SIR in detecting SSO. For cut-off of 0.4, AUC = 91.8%, sensitivity = 82.5%, and specificity = 92.3%.

Table 3 Univariate and multivariate regression analyses for predicting SSO in the validation cohort

Predictor	Univariate regression		Multivariate regression	
	Odds ratio	*P*-value	Odds ratio	*P*-value
Male gender	0.62 (0.34—1.09)	0.10	0.60 (0.32–1.10)	0.10
Adequate haemodynamic response	2.61 (1.39—4.76)	<0.01	2.43 (1.27–4.57)	<0.01
White ethnicity	0.27 (0.15—0.48)	<0.001	0.25 (0.14–0.46)	<0.001
Asian ethnicity	1.92 (0.89—4.76)	0.12		
Age (over 60 vs. under 60 years old)	0.78 (0.44—1.38)	0.40		

Table 4 Comparison of SSO and haemodynamic response rates in the validation cohort ($n = 492$, $P < 0.01$).

	Haemodynamic response	No haemodynamic response
SSO	360 (82.0%)	79 (18.0%)
No SSO	35 (66.0%)	18 (34.0%)

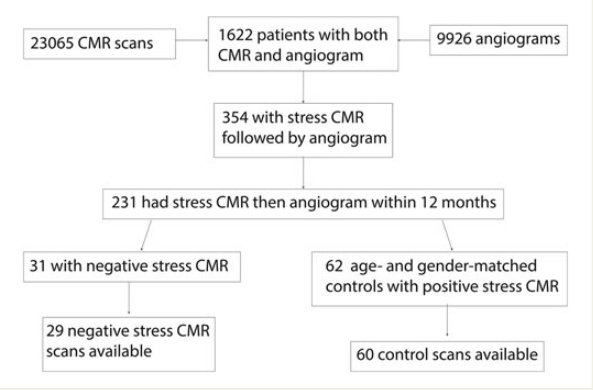

Figure 4 Flow chart showing identification of cases and controls.

scans followed by angiography, 60 scans available). All 93 angiographies proceeded to angioplasty.

Overall, 15.7% of patients had a lack of SSO. There were fewer cases of SSO in those with false-negative stress scans compared with positive stress scans, but this difference was not statistically significant (20.7 vs. 13.1% of positive scans, $P = 0.37$). In our multivariate model, no covariates reached statistical significance.

Discussion

We have validated the phenomenon of SSO in a real-world cohort of patients. Visual and quantitative assessment of SSO is consistent and reproducible. Splenic switch-off is only weakly associated with haemodynamic response, which corroborates with other studies showing that the hyperaemic and haemodynamic response to adenosine correlate poorly.[11] We believe SSO is a better indicator of adequate adenosine stress than heart rate response, based on previous observations and experimental data that show a good correlation of the myocardial hyperaemic response to adenosine with the splanchnic vasoconstrictor response.[14] Up to 23% of patients could have a misclassified response by current haemodynamic methods; SSO may reduce this rate.

Our case-–control study allowed us to compare rates of SSO in those with positive CMR perfusion scans and negative CMR perfusion scans, in patients who required revascularization within a year. Of 23 065 scans, we found only 231 patients (1%) who required an angiogram within 1 year of a CMR stress scan.

In the case–control cohort, we observed a more frequent lack of adenosine response (no SSO) in patients with no perfusion defect on CMR, compared with those with a perfusion defect on CMR (absolute difference of 7.6%) but this was not statistically significant likely due to small sample size and resultant lack of statistical power. If this observation held true in a larger cohort, SSO could be considered a good marker for false-negative scans. Furthermore, identifying a larger cohort of cases would allow us to have the statistical power in assessing whether the use of lack of SSO has any incremental value over the assessment of haemodynamic responses in predicting the need for coronary intervention as well as examining whether the combination of both SSO and haemodynamic response assessment can yield a reduction in false-negative scans.

Our validation cohort comprised a retrospective sample drawn from a socioeconomically and ethnically diverse area, presenting for the evaluation of suspected ischaemic heart disease. Compared with the case–control cohort our validation sample was younger, had a higher proportion of female patients, and had no previous adverse cardiovascular events. The case–control cohort represents a group of patients with significant cardiovascular risk factor burden, who all required coronary intervention within a year. Eighty-eight percent had at least one of hypertension, hypercholesterolaemia, diabetes mellitus or had ever smoked; 68% had at least two of these risk factors. Thus, we noted with interest the higher rate of a lack of SSO in the case–control cohort compared with the validation cohort (15.7 vs. 11%).

The case–control cohort may have been on treatments that would modify the response to adenosine. However, their risk factors may themselves affect haemodynamic response to adenosine.

The A1 adenosine receptor responsible for splenic vasoconstriction is also complicit in insulin resistance and the downregulation of adenosine receptors has been implicated in insulin insensitivity.[15] Hypercholesterolaemia, chronic hypertension, and family history have also been linked to attenuated vascular response to adenosine, both in cellular and in vivo studies.[16,17] This could also explain why our false-negative rate is higher than that in CE-MARC[7]. Our cohort includes patients with previous coronary artery bypass grafts, previous myocardial infarction, and patients presenting with acute coronary syndrome that were excluded from CE-MARC.

Our study results have to be interpreted in the context of the study design. The validation and case–control cohorts are from very different populations and thus limit the extent to which our data can be generalized. Although we had relatively complete risk factor profile in the case–control cohort, we did not have a complete medication history. In particular, aminophylline and caffeine attenuate the effect of adenosine and may account for some false negatives.[18,19] Patients were asked to refrain from caffeine for 12 h before their appointment, but compliance rates are unknown. Patients may not be entirely at fault with non-compliance, as drinks advertised as decaffeinated can contain significant quantities of caffeine.[20] Other vasodilators, such as nitrates and calcium-channel blockers, could interfere with haemodynamic response to adenosine.

In summary, we have shown that the lack of SSO is prevalent, easily measureable and has potential to improve on haemodynamic criteria as a marker of adenosine under-stress in CMR perfusion scans. The radiographer and clinician should keep the marker in mind when acquiring and reporting scans. Further work could combine SSO with haemodynamic response to further minimize false-negative rates.

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Conflict of interest: Consultancy Circle Cardiovascular Imaging Inc., Calgary, Canada (S.E.P.).

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