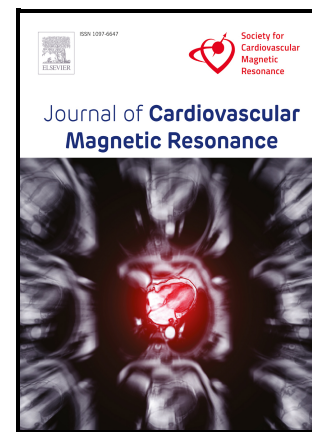


Splenic Switch-Off in 3D Adenosine Stress CMR Perfusion for Differentiating False-Negative from True-Negative Studies Identified by FFRShort title: Splenic Switch-Off for CMR Stress Validation

Mihály Károlyi, Maximilian Fuetterer, Márton Kolossváry, Verena C. Wilzeck, Sven Plein, Andrea Biondo, Alexander Gotschy, Michael Frick, Rolf Gebker, Hatem Alkadhi, Ingo Paetsch, Cosima Jahnke, Sebastian Kozerke, Robert Manka



PII: S1097-6647(25)00095-X

DOI: <https://doi.org/10.1016/j.jocmr.2025.101933>

Reference: JOCMR101933

To appear in: *Journal of Cardiovascular Magnetic Resonance*

Received date: 17 March 2025

Revised date: 30 June 2025

Accepted date: 15 July 2025

Please cite this article as: Mihály Károlyi, Maximilian Fuetterer, Márton Kolossváry, Verena C. Wilzeck, Sven Plein, Andrea Biondo, Alexander Gotschy, Michael Frick, Rolf Gebker, Hatem Alkadhi, Ingo Paetsch, Cosima Jahnke, Sebastian Kozerke and Robert Manka, Splenic Switch-Off in 3D Adenosine Stress CMR Perfusion for Differentiating False-Negative from True-Negative Studies Identified by FFRShort title: Splenic Switch-Off for CMR Stress Validation, *Journal of Cardiovascular Magnetic Resonance*, (2025) doi:<https://doi.org/10.1016/j.jocmr.2025.101933>

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Magnetic Resonance.

Splenic Switch-Off in 3D Adenosine Stress CMR Perfusion for Differentiating False-Negative from True-Negative Studies Identified by FFR

Mihály Károlyi, MD, PhD ^{a,b}, Maximilian Fuetterer, PhD ^c, Márton Kolossváry, MD, PhD ^{d,e},
Verena C. Wilzeck, MD ^{a,b,c}, Sven Plein, MD, PhD ^f, Andrea Biondo, MD ^a,
Alexander Gotschy, MD, PhD ^{a,b,c}, Michael Frick, MD ^g, Rolf Gebker, MD ^h, Hatem Alkadhi, MD, MPH ^a,
Ingo Paetsch, MD ⁱ, Cosima Jahnke, MD ⁱ, Sebastian Kozerke, PhD ^c, Robert Manka, MD ^{a,b,c}

^a Diagnostic and Interventional Radiology, University Hospital Zurich, Zurich, University of Zurich, Switzerland

^b Department of Cardiology, University Heart Center, University Hospital Zurich, University of Zurich, Zurich, Switzerland

^c Institute for Biomedical engineering, University and ETH Zurich, Switzerland

^d Gottsegen National Cardiovascular Center, Budapest, Hungary

^e Physiological Controls Research Center, University Research and Innovation Center,
Óbuda University, Budapest, Hungary

^f Multidisciplinary Cardiovascular Research Centre & the Department of Biomedical Imaging
Science, Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, Leeds, UK

^g Department of Internal Medicine I, Cardiology, Angiology and Internal Intensive Care Medicine, University hospital RWTH
Aachen University, Aachen, Germany

^h MVZ Diagnostikum Berlin 2020 GmbH, Berlin, Germany

ⁱ Department of Electrophysiology, HELIOS Heart Center Leipzig at University of Leipzig, Leipzig, Germany

Short title: Splenic Switch-Off for CMR Stress Validation

Manuscript type: Original research article

Word count: 3914

Correspondence: Robert Manka, MD

Department of Cardiology, University Heart Center & Department of Diagnostic and Interventional
Radiology, University Hospital Zurich, University of Zurich, Zurich, Switzerland

Raemistrasse 100,

8091 Zurich, Switzerland

Phone: +41442551111

Fax: +41442551819

E-mail: Robert.Manka@usz.ch

Abstract

Background: False-negative cardiac magnetic resonance (CMR) perfusion results may arise from inadequate stress responses, even when patients exhibit an adequate clinical or heart-rate response to adenosine. This study aimed to explore the ability of qualitative and quantitative splenic switch-off markers to differentiate false-negative from true-negative adenosine stress-perfusion CMR findings, in a cohort where fractional flow reserve (FFR) was used to adjudicate lesion significance.

Methods: Patients with known or suspected coronary artery disease (CAD) from five centers underwent 3D adenosine stress perfusion CMR and coronary angiography with FFR. Splenic switch-off was assessed qualitatively using both standard stress-to-rest (SSO) and a stress-only (SSO_{stress}) approach. In addition, quantitative signal intensity (SI) ratios were assessed, including the splenic stress-to-rest SI-ratio (SI_{stress/rest}) and the spleen-to-myocardium SI ratio at stress (SI_{spleen/myocardium}). The diagnostic accuracy of these measures was evaluated using cross-validated area under the curve (cvAUC) analysis.

Results: Among 179 patients (mean age 63 ± 10 years; 130 male), SSO prevalence was 73% and was significantly more frequent in true-negative than false-negative CMR cases (80.6% vs. 36.8%, $p < 0.001$). SSO_{stress} showed moderate agreement ($\kappa = 0.60$) and robust diagnostic performance (AUC 0.80), as compared to SSO. Splenic SI_{stress/rest} and SI_{spleen/myocardium} at stress demonstrated high predictive accuracy for visual SSO, with cvAUCs of 0.94 (95% CI: 0.90–0.96) and 0.90 (95% CI: 0.86–0.95), respectively. The positive likelihood ratio of SSO for true-negative CMR was 1.70, while the negative likelihood ratio was 0.24, indicating false-negative CMR when SSO was absent. Qualitative and quantitative splenic-switch off metrics classified 77–80% of negative CMR cases correctly as true- or false-negatives, with sensitivities ranging from 81.4% to 91.2%. Clinically applicable cut-offs for differentiating true- and false-negative studies with splenic SI_{stress/rest} and SI_{spleen/myocardium} at stress were identified as ≤ 0.32 and ≤ 0.38 , respectively.

Conclusion: In a multicenter cohort using FFR-adjudicated reference for lesion severity, qualitative SSO and quantitative signal intensity metrics were associated with myocardial stress adequacy and these markers may improve the interpretation of negative stress-perfusion CMR studies.

Keywords:

Coronary Artery Disease, Fractional Flow Reserve, Myocardial Perfusion Imaging, Cardiac magnetic resonance

Abbreviations

cvAUC	Cross-validated Area Under the Curve
CAD	Coronary artery disease
CMR	Cardiac magnetic resonance
FFR	Fractional flow reserve
HR	Heart rate
LV	Left ventricle
ROC	Receiver Operating Characteristic
ROI	Region of interest
RR	Riva Rocci / Blood pressure
SA	Short-axis
SI	Signal intensity
SSO	Splenic switch-off assessed visually between stress and rest
SSO _{stress}	Splenic switch-off assessed visually at stress only

1. Background

Over the past decade, the use of non-invasive imaging for coronary artery disease (CAD) assessment has significantly expanded, with cardiac magnetic resonance imaging (CMR), particularly with

adenosine stress-perfusion protocols, emerging as a cornerstone for myocardial ischemia assessment [1]. Contemporary guidelines now provide Class I recommendations for functional imaging as the first diagnostic step in suspected CAD, reinforcing the pivotal role of CMR in clinical decision-making [2–4].

A critical aspect of adenosine stress CMR is ensuring an adequate vasodilatory response, as suboptimal stress may contribute to false-negative results. Conventional markers of pharmacologic stress adequacy—such as symptomatic responses and hemodynamic changes—are inconsistent predictors of adenosine-induced myocardial blood flow increase [5,6]. To address this limitation, splenic switch-off (SSO) has been proposed as a physiological marker of adenosine induced stress adequacy, reflecting the expected vasodilatory reduction in splanchnic perfusion during stress perfusion [7,8].

The clinical value of SSO lies primarily in cases where stress CMR yields a negative result. If stress CMR is positive, patients will regularly undergo further evaluation with coronary angiography regardless of SSO findings. However, in negative cases, an inadequate stress response could lead to false-negative results, potentially delaying appropriate management. Therefore, the ability of SSO to distinguish true-negative from false-negative studies is of particular clinical relevance.

Despite its potential, the clinical utility of SSO remains incompletely defined. Previous large-scale studies have primarily relied on qualitative visual assessment, and defined SSO as visual difference between the splenic signal intensity at stress compared to rest [7,8]. However, in routine clinical practice, SSO is often evaluated solely on stress CMR images by visually comparing splenic signal intensity to the myocardium during first-pass perfusion. Additionally, prior studies exploring the utility of SSO during CMR adenosine stress perfusion used quantitative coronary angiography (QCA) rather than fractional flow reserve (FFR) as the reference standard, limiting their ability to assess the physiological significance of coronary stenoses [7,8].

This study addresses these gaps in current evidence by (1) evaluating the ability of qualitative SSO to differentiate true-negative from false-negative CMR perfusion results using invasive FFR as the reference standard; (2) assessing the accuracy and clinical utility of a simplified, stress-only

qualitative assessment (SSO_{stress}); (3) testing quantitative splenic signal intensity (SI) biomarkers to enable objective, reader-independent assessment of stress adequacy.

Unlike prior studies, we utilized 3D CMR stress perfusion protocols that maximize splenic coverage, ensuring consistent splenic analysis across all cases. We hypothesized that both visual and quantitative SSO metrics could serve as reliable markers of myocardial stress adequacy during adenosine stress CMR perfusion, improving the interpretation of negative studies.

2. Methods

2.1. Study population

This study retrospectively analyzed participants from a previously published multicenter study [9,10], conducted between 2009 and 2013 at five centers. This post-hoc analysis comprised all patients from the original study. All participants underwent a 3D perfusion CMR with adenosine stress in addition to a clinically indicated invasive coronary angiography for suspected or known CAD, where FFR used to adjudicate the functional significance of angiographically intermediate lesions. Contraindications for adenosine-perfusion CMR included bronchial asthma, high-degree AV block, metallic foreign bodies, or claustrophobia. Exclusion criteria for the current study included non-retrievable or incomplete 3D CMR datasets, or insufficient splenic coverage on stress or rest imaging, preventing qualitative assessment of splenic perfusion or quantitative analysis due to the inability to place a region of interest (ROI) of at least 1 cm². Study protocol was approved by local ethics committee in each participating center and all patients provided written informed consent. As this was a post-hoc analysis of a predefined cohort, no separate a priori power calculation was performed; the sample size was determined by the number of eligible patients with complete imaging and invasive data. The study population is outlined in **Figure 1**.

2.2. Myocardial perfusion cardiac magnetic resonance imaging

CMR examinations were performed at either 1.5T or 3.0T MRI systems using vector ECG synchronization and multichannel torso coil arrays (5–32 channels). Imaging included cine sequences, whole-heart 3D first-pass perfusion sequences, and late gadolinium enhancement images. This analysis focused exclusively on 3D first-pass perfusion sequences, obtained at stress and rest, acquired in a short-axis orientation covering the entire left ventricle. Perfusion imaging used a saturation-recovery gradient-echo sequence, as previously described in the 3D perfusion imaging protocol [11]. Participants abstained from caffeine 24 hours prior to imaging. Adenosine was administered intravenously with a standard dose of 140 $\mu\text{g}/\text{kg}/\text{min}$ for at least 3 minutes to induce stress. A gadolinium-based contrast agent (0.1 mmol/kg) was injected, followed by a saline bolus. Rest and stress imaging were performed in a breath-hold with shallow expiration as needed, using identical acquisition protocols. Hemodynamic parameters, including heart rate (HR) and blood pressure (RR), were recorded during adenosine stress and at rest to monitor physiological responses to stress induction and ensure patient safety during the procedure.

2.3. Assessment of myocardial ischemia with perfusion CMR and invasive FFR

Perfusion CMR data were centrally analyzed for ischemia by experienced readers blinded to clinical and angiographic information. Myocardial ischemia was defined visually as a stress perfusion deficit with $\geq 25\%$ transmural persistence across ≥ 3 consecutive dynamics, without a corresponding rest perfusion defect or late gadolinium enhancement. For this study, ischemia was considered on a per-patient basis, classifying the entire CMR study as either positive or negative. This approach aimed to capture the overall myocardial response to adenosine, identifying ischemia in any myocardial segment to differentiate patients with CAD from those without.

Invasive coronary angiography was performed following standard protocols, with QCA conducted offline in a blinded core laboratory. FFR was selectively performed in vessels showing 50%–80% luminal stenosis in two orthogonal views and a diameter ≥ 2 mm. FFR < 0.8 was deemed functionally significant, while stenosis $> 80\%$ was considered hemodynamically significant without requiring FFR

testing. Similar to CMR ischemia evaluation, for the present post-hoc analysis, FFR results were classified on a per-patient basis, ensuring that ischemia was accounted for if present in any vessel. This approach aligns with the strategy of evaluating myocardial ischemia as a whole-organ response.

2.4. Assessment of splenic switch-off

Qualitative SSO and simple quantitative signal intensity ratios were analyzed using commercially available software (OsiriX MD, Version 12.0, Geneva, Switzerland). A single experienced observer (MK, Level 3 certification) conducted image analysis while blinded to clinical data. Qualitative splenic switch-off was visually assessed by comparing splenic enhancement between stress and rest CMR images, focusing on maximal splenic visibility. SSO was defined as a visual reduction in splenic SI during stress, observed at the time of maximum myocardial SI during first-pass perfusion, compared to rest [8]. Additionally, a simplified qualitative assessment technique was evaluated, in which splenic enhancement was visually judged relative to the myocardium on stress images alone (SSO_{stress}), at peak myocardial first-pass perfusion. SSO_{stress} assessment was performed by the same reader (MK), blinded to clinical data and the original SSO assessment, 8 weeks after the initial reading. Failed visual SSO was defined as either similar splenic enhancement at both stress and rest or splenic enhancement comparable to that of the myocardium. Simple quantitative ratios were assessed at the time of peak myocardial SI during first-pass perfusion. First, ROIs were placed on consecutive time frames of the perfusion scan in a remote myocardial segment without corresponding ischemia or late gadolinium enhancement, and the time frame with the highest SI was selected. Second, on the selected time frame, the slice with the largest splenic coverage within the 3D dataset was chosen, and ROIs were placed on the spleen and remote myocardium. These ROIs were copied between stress and rest images using the software's copy-paste function. The stress-to-rest SI ratio ($SI_{\text{stress/rest}}$) of the spleen was calculated as: $(SI_{\text{spleen stress}} - SI_{\text{spleen pre-contrast}}) / (SI_{\text{spleen rest}} - SI_{\text{spleen pre-contrast}})$. Similarly, the splenic-to-myocardial SI ratio ($SI_{\text{spleen/myocardium}}$) at stress was determined as: $(SI_{\text{spleen stress}} - SI_{\text{spleen pre-contrast}}) / (SI_{\text{myocardium stress}} - SI_{\text{myocardium pre-contrast}})$. SI values were normalized by

subtracting baseline (pre-contrast) SI measurements. All SI values were measured in arbitrary units (a.u.).

Advanced signal post-processing and perfusion quantification were performed using the Agora research platform (Gyrotools LLC) and MATLAB (MathWorks, Version 2023b) in order to identify further quantitative biomarkers to assess SSO. For this analysis, ROIs were placed on the slices where the spleen was most visible within the 3D dataset and copied across all frames of the first-pass perfusion sequence. Parameters such as time-to-peak (TTP) signal intensity and upslope during stress (defined as the change between minimum and maximum SI) were extracted. Inter-observer reliability was assessed in a randomly selected subset comprising 25% of the cohort for each technique (VCW, Level 3 certification), and intra-observer readings were performed after an 8-week interval (MK). Examples of patients with present and failed SSO from the study cohort are shown in **Figure 2**.

2.5. Classification of negative CMR findings based on splenic switch-off

Negative CMR cases (no stress perfusion deficits) were analyzed to assess SSO's role in differentiating true-negative from false-negative cases. SSO presence and absence were compared with true- and false-negative classifications, respectively. Positive and negative likelihood ratios were calculated to quantify SSO's diagnostic accuracy in negative CMR studies. Similarly, thresholds for $SI_{\text{stress/rest}}$ and $SI_{\text{spleen/myocardium}}$ were identified and tested for the differentiation of true-negative from false-negative results.

2.6. Statistical analysis

Statistical analyses were performed using SPSS (ver. 23; Armonk, NY) and R (ver. 3.6.1; <https://www.r-project.org>). Continuous data are reported as mean $\pm$ standard deviation and analyzed using Student's t-test or Mann-Whitney U test, as appropriate. Categorical data were analyzed using the Chi-squared test. Agreement between visual and simplified stress-only SSO methods was evaluated using Cohen's Kappa and ROC analysis. Diagnostic performance of

quantitative SSO parameters was evaluated using area under the curve (AUC) from ROC curves with 5-fold cross-validation (cvAUC package ver. 1.1.4 in R), and Youden's J statistic determined optimal cutoffs. Reliability metrics were assessed with intraclass correlation coefficients (ICC), categorized as poor (<0.5), moderate ($0.5-0.75$), good ($0.75-0.9$), or excellent (≥ 0.9)[12]. Statistical significance was set at a 2-sided $p < 0.05$.

3. Results

3.1. Patient demographics, CMR parameters and diagnostic findings

From the original cohort of 416 patients, 179 patients were included into this post-hoc analysis due to data availability constraints. Missing images were entirely random, with no patient characteristics associated with data unavailability, as shown in **Supplementary Table**. Adequate splenic coverage was available in all included cases and no patients were excluded. The study population and patient stratification based on the presence or absence of CAD (defined by FFR) and corresponding SSO status are outlined in Figure 1. Baseline parameters and diagnostic findings are summarized in **Tables 1-3**, respectively.

3.2. SSO as an indicator of true negative CMR results

Visual SSO was present in 73% (130/179) of the cohort, with a significantly higher prevalence among true-negative versus false-negative CMR studies (80.6% vs. 36.8%, $p < 0.001$). Among all negative CMR scans ($n=86$), visual SSO was observed in 78% (61/86), correctly identifying 63% (54/86) as true-negative. In contrast, absence of SSO was associated with a higher-negative rate (14%, 12/86). **Figure 3** illustrates this distribution.

Overall, 77% (66/86) of negative CMR studies were correctly classified using visual SSO, yielding a positive likelihood ratio of 1.70, and a negative likelihood ratio of 0.24 (**Table 4**). Inter- and intra-

observer reliability for SSO were good, with ICCs of 0.82 (95% CI: 0.66–0.90) and 0.87 (95% CI: 0.73–0.93), respectively.

3.3. Simplified qualitative SSO assessment using stress-only CMR images

SSO_{stress} demonstrated moderate agreement with the original stress-rest method (Cohen's kappa: 0.60, 95% CI: 0.59–0.85). Sensitivity and specificity for the simplified method were 72.6% and 70.0%, compared to SSO, respectively. ROC analysis showed good alignment between the two techniques, with an AUC of 0.80 (95% CI: 0.72–0.88). Intra- and inter-observer reliability for visual SSO_{stress} was good, with ICCs of 0.84 (95% CI: 0.70–0.91) and 0.78 (95% CI: 0.62–0.88), respectively. Diagnostic performance of SSO_{stress} is summarized in **Table 4**.

3.4. Quantitative biomarkers predicting visual SSO

Quantitative splenic perfusion parameters demonstrated high predictive accuracy for SSO, with the SI_{stress/rest} of the spleen yielding a cvAUC of 0.94 (95% CI: 0.90–0.96), while SI_{spleen/myocardium} at stress showed a cvAUC of 0.90 (95% CI: 0.86–0.95). Optimal thresholds for SSO-prediction were identified as ≤ 0.25 for the splenic SI_{stress/rest} (sensitivity: 93.2%, specificity: 80.8%) and ≤ 0.28 for the SI_{spleen/myocardium} at stress (sensitivity: 91.8%, specificity: 86.9%). Other assessed parameters, including upslope and TTP during stress, demonstrated poor diagnostic performance, with cvAUCs of 0.46 (95% CI: 0.35–0.57) and 0.44 (95% CI: 0.34–0.55), respectively. Intra- and inter-observer reliability for SI ratios between stress and rest imaging were good, with ICCs of 0.85 (95% CI: 0.70–0.92) and 0.83 (95% CI: 0.68–0.91).

3.5. Quantitative biomarkers for predicting true-negative stress perfusion

Among negative CMR studies, splenic SI_{stress/rest} demonstrated moderate accuracy for true-negative classification, with a cvAUC of 0.70 (95% CI: 0.57–0.84). At a threshold of ≤ 0.32 , it correctly classified

69.7% (60/86) as true-negative and identified 10.4% (9/86) as false-negative. The overall classification accuracy was 80.2% (69/86). $SI_{\text{spleen/myocardium}}$ at stress achieved a cvAUC of 0.72 (95% CI: 0.60–0.85), with a threshold of ≤ 0.38 correctly identifying 60% (52/86) as true-negative and 16% (14/86) as false-negative. In contrast, upslope and TTP showed limited predictive value, with cvAUCs of 0.42 (95% CI: 0.26–0.58) and 0.46 (95% CI: 0.29–0.62). Predictive performance of quantitative parameters for SSO is illustrated in **Figure 4**. **Figure 5** demonstrates cvROC curves for splenic SI ratios in predicting true-negative CMR studies.

4. Discussion

This post-hoc analysis of a multicenter cohort reinforces the role of splenic switch-off as a robust marker of myocardial stress adequacy during adenosine stress perfusion CMR. In this multicenter study, which used FFR to determine the functional relevance of angiographically intermediate lesions, SSO was associated with true-negative CMR results, supporting its potential as a diagnostic adjunct. Additionally, we identified quantitative splenic signal intensity biomarkers that offer standardized assessment of stress adequacy. A simplified, stress-only SSO assessment technique demonstrated moderate agreement with conventional stress-rest comparisons in our study, which may enhance clinical workflow efficiency. Notably, spleen was assessable in 100% of our cohort due to the expanded 3D coverage of the applied perfusion sequence. In contrast, previous studies reported splenic visibility rates of 96–99% [8,13], justifying the suitability of this imaging approach for our study objectives.

SSO reflects a reduction in splenic perfusion during adenosine stress, which is attributed to adenosine-induced vasoconstriction within the splanchnic circulation, leading to a redistribution of blood flow away from the splanchnic circulation, including the spleen. Prior studies using Doppler ultrasound and nuclear techniques have reported reductions in splanchnic organ perfusion during pharmacologic stress, supporting this mechanism [13,14]. While SSO is not a direct measure of splanchnic flow, the visibly decreased splenic signal intensity on stress perfusion images in CMR

images reflects this physiological response. The role of SSO as a marker of myocardial stress adequacy in adenosine perfusion CMR was first highlighted by Manisty et al. in 2015 within the CE-MARC cohort, using QCA as the reference standard [8]. Subsequent studies have further elucidated its clinical utility in real word cohorts and explored variations in SSO prevalence across specific patient subgroups [15–17]. Our work builds on this foundation by evaluating the performance of SSO in a cohort where lesion significance was defined by invasive FFR.

The prevalence of SSO in our cohort (73%) was similar to that reported in a previous study (72%), where adenosine was administered at the standard dose of 140 $\mu\text{g/kg/min}$, as in our study [13]. In studies using higher adenosine doses (140–210 $\mu\text{g/kg/min}$), SSO was observed in up to 89–93% of cases [7,8,18]. While the lower prevalence in our cohort may reflect a relatively lower stress burden, we still confirmed that SSO was significantly more frequent in true-negative than in false-negative cases, emphasizing its role in confirming adequate myocardial stress. A higher adenosine dose might have further strengthened our findings.

We identified $SI_{\text{stress/rest}}$ of the spleen as a highly accurate predictor of qualitative SSO, with a cross-validated AUC of 0.94 and an optimal cut-off of ≤ 0.25 (sensitivity: 93.2%, specificity: 80.8%). This closely aligns with Hosking et al., who reported an AUC of 0.91 (sensitivity: 82.5%, specificity: 92.3%), though their optimal splenic $SI_{\text{stress/rest}}$ was slightly higher (0.40) [7]. A more recent study by Patriki et al., using hybrid ^{13}N -ammonia PET and 3-Tesla CMR during adenosine-induced stress reported even higher ratio of 0.71 for SSO prediction (sensitivity: 94%, specificity: 94%, AUC = 0.947) [13]. While the authors attributed this discrepancy more likely to different adenosine dosing, the difference to our result may stem from different splenic $SI_{\text{stress/rest}}$ definitions: while we assessed splenic stress and rest signal intensities at the time of maximal myocardial first-pass perfusion, the other two studies compared maximal splenic signal intensities across stress and rest [7,13].

Importantly, as splenic-switch-off can be assessed on stress-only images alone, it theoretically enabling real-time evaluation of stress adequacy during routine CMR. Its primary advantage lies in its alignment with current clinical protocols, as most institutions now omit rest perfusion imaging to streamline workflows and reduce scan duration [19], in accordance with guidelines advocating for

stress-only protocols whenever feasible [6]. This is especially relevant as the number of non-invasive imaging studies for coronary artery disease continues to grow[1], driving efforts to improve patient throughput and comfort. Advances in acceleration techniques, including those enabled by deep learning, further facilitate shorter acquisition protocols. In this evolving context, stress-only SSO assessment may offer a practical adjunct for real-time evaluation of vasodilator response[20].

Building on this, beyond the original definition of visual splenic switch-off, which compares splenic perfusion between stress and rest, our study also evaluated stress-only approaches for SSO assessment. A simplified qualitative method, SSO_{stress} demonstrated moderate agreement with the conventional stress-rest method (Cohen's kappa: 0.60) in our study. While slightly less sensitive and specific, SSO_{stress} correlated well with SSO and represents a clinically viable alternative. Incorporating SSO_{stress} into CMR workflows could enhance efficiency, particularly in high-volume settings. Similarly, $SI_{\text{spleen/myocardium}}$ at stress, a quantitative metric of splenic switch-off derived from stress-only images, demonstrated high accuracy in predicting qualitative SSO, with a cross-validated AUC of 0.90 (95% CI: 0.86–0.95) and an optimal cut-off of ≤ 0.28 , achieving a sensitivity of 91.8% and a specificity of 86.9%.

However, stress-only assessment may also have limitations. The absence of rest imaging could reduce diagnostic certainty in borderline cases, and visual interpretation may be less reliable in low-contrast situations. Moreover, although our results stem from a multi-center cohort, broader validation across institutions and scanner platforms is warranted before wide adoption. Interestingly, Patriki et al. reported a markedly different $SI_{\text{spleen/myocardium}}$ threshold at stress to predict visual SSO (1.53, sensitivity: 61%, specificity: 85%, AUC = 0.76) [13]. However, this threshold appears counterintuitive, as a splenic-to-myocardial signal intensity ratio above 1 contradicts the expected perfusion pattern in SSO, where splenic SI should be lower than myocardial SI [13]. Nevertheless, similar to $SI_{\text{spleen/myocardium}}$ at stress, the primary reason for the discrepancies of their finding and ours likely lies in differences in perfusion metric definitions. While Patriki et al. derived maximal splenic and myocardial SIs at stress from perfusion curves, potentially from different time frames [13], we assessed SI ratios at the time of maximal myocardial SI during first-pass perfusion, ensuring both values were derived from the same time frame. This approach is more practical for routine clinical

use, as it eliminates the need for perfusion curve analysis. Other contributing factors may include variations in imaging modalities, scanner settings, perfusion protocols (e.g., stress-rest order and timing), and patient populations.

In our study, quantitative splenic perfusion ratios deemed also valuable for predicting a true-negative CMR study. Splenic $SI_{\text{stress/rest}}$ classified negative CMR cases with an accuracy of 80.2% as either true- or false negative, while $SI_{\text{spleen/myocardium}}$ at stress demonstrated comparable performance. Our findings suggest that these metrics may provide practical cut-offs to aid the clinical interpretation of negative CMR studies. $SI_{\text{stress/rest}}$ of the spleen, in particular, may offer several potential advantages over conventional visual SSO assessment, including increased objectivity, reduced inter-observer variability, and the potential for automation and standardization of stress adequacy evaluation. It may also serve as a helpful adjunct in borderline or ambiguous cases where visual SSO is inconclusive. However, similar to the thresholds for predicting SSO, these cut-offs may vary depending on the study cohort, imaging technique used and biomarker definitions. Moreover, the use of SIR requires both stress and rest perfusion images, which may limit its utility in centers using stress-only protocols. Therefore, relying solely on absolute thresholds for SSO assessment needs further validation in larger, diverse cohorts to establish universally applicable cut-off values. Advanced quantitative parameters, such as TTP and upslope had limited predictive value in our study, emphasizing the need for further refinement of myocardial and splenic perfusion quantification techniques.

5. Limitations

Despite its insights, our study has limitations. While FFR was not used to assess stress adequacy directly, it served to adjudicate the significance of intermediate lesions, allowing classification of CMR results as true- or false-negative. Importantly, no outcome data (e.g., major adverse cardiovascular events) were available for this post-hoc analysis, as follow-up was not part of the original study design. Although prognostic data would offer valuable complementary insights, the primary aim was to evaluate diagnostic performance and assess splenic switch-off as a marker of

stress adequacy. Therefore, the lack of follow-up does not compromise the study objectives. Although we cross-validated our results to provide a better unbiased estimate of real-world performance, as a post-hoc analysis, the identified SSO prevalence and quantitative cut-offs may reflect the specific CAD prevalence and imaging protocols of this cohort. Also, the use of a standardized 140 µg/kg/min adenosine dose may have influenced SSO specificity. Previous studies suggest higher specificity with increasing doses [18], warranting future investigations into optimized adenosine titration strategies. Additionally, while we did not specifically analyze atrial fibrillation as a confounder, prior studies suggest that SSO is less prevalent in patients with AF compared to those in sinus rhythm [15]. This may impact the generalizability of our findings, particularly in populations with a high prevalence of atrial fibrillation. While derived from data between 2009 and 2013, our findings of visual SSO and simple ratios likely remain applicable with modern CMR technology, though advanced acquisition and reconstruction techniques might enhance results for parameters like upslope and TTP, possibly enabling simultaneous quantification of myocardial and splenic blood flow. Advances in imaging, such as T1 mapping and quantitative perfusion techniques, may further enhance the evaluation of myocardial and splenic stress adequacy, enabling simultaneous measurement of myocardial and splenic blood flow [21]. Lastly, SSO does not occur with alternative vasodilators such as regadenoson, restricting the applicability of our results to adenosine-based stress CMR protocols.

6. Conclusion

In conclusion, this study provides evidence supporting the use of splenic switch-off as an indicator of myocardial stress in adenosine stress-perfusion CMR. Quantitative splenic perfusion metrics provide objective tools for assessing splenic switch-off, which may enhance the reproducibility and efficiency of CMR interpretation. Furthermore, the simplified stress-only SSO assessment appears promising for clinical implementation, underscoring the potential value of SSO-based evaluation in contemporary CMR workflows.

Acknowledgements

Márton Kolossváry was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences and by the 2024-2.1.1 University Research Scholarship Program of the Ministry of Culture and Innovation from the National Research, Development, and Innovation Fund of Hungary. Project number PD 147269 and Excellence 151118 has been implemented with the support provided by the Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund, financed under the PD_23 and Excellence_24 funding scheme.

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Figure legends

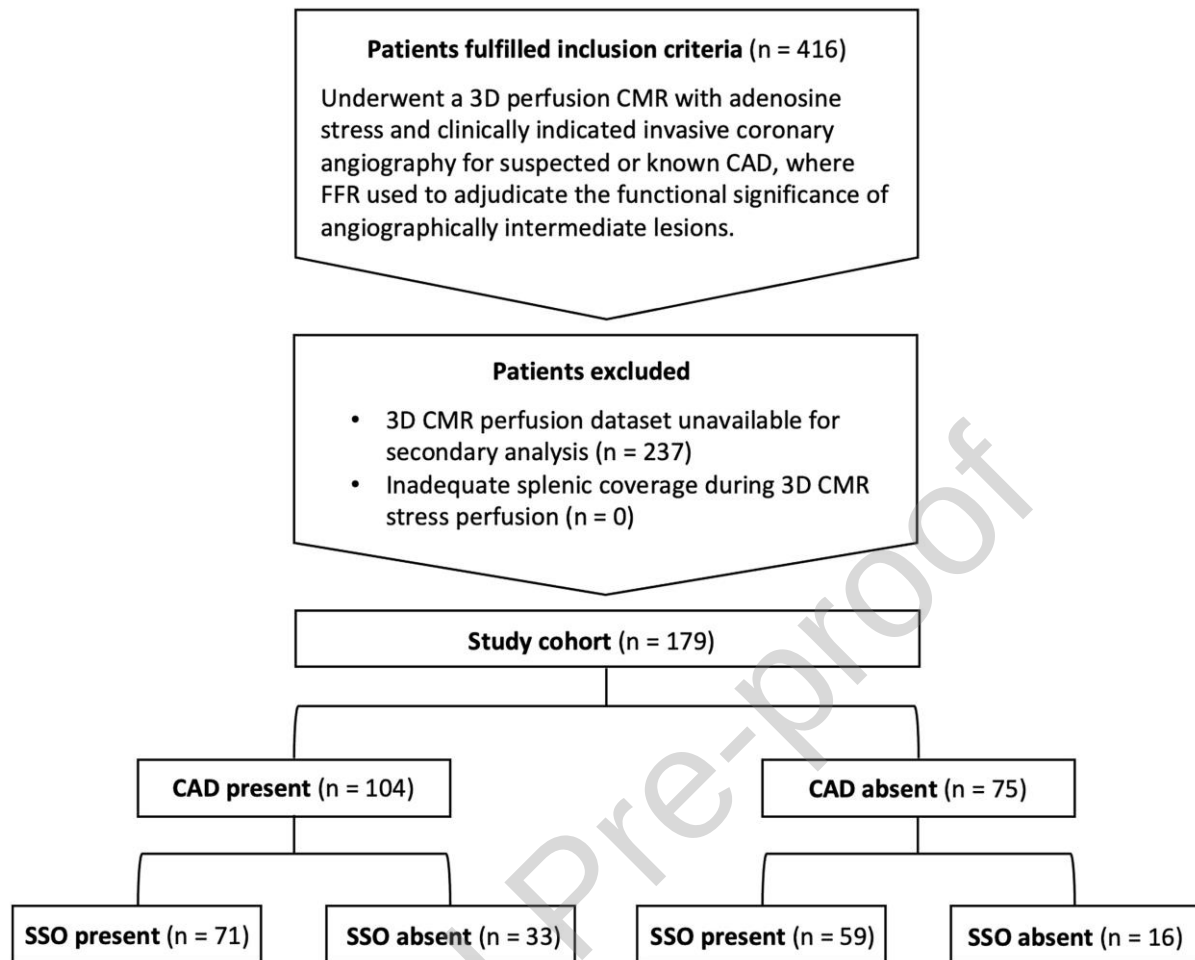


Figure 1 Study flowchart

Patient inclusion flowchart showing initial enrollment, exclusions, and final study population. The number of patients with and without CAD (based on $\text{FFR} \leq 0.80$) and their respective SSO status (present or absent) are also presented.

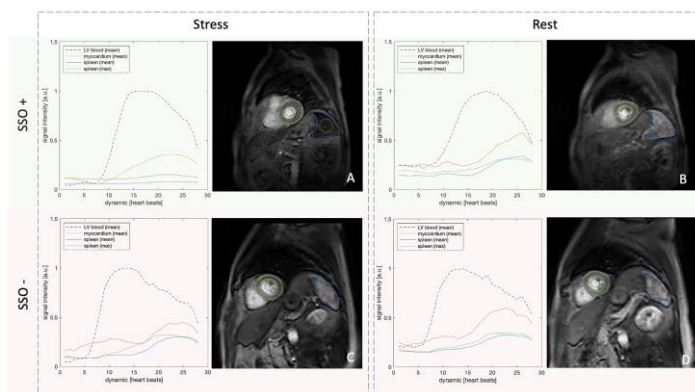


Figure 2. Example of splenic SSO and signal intensity assessment

Representative first-pass perfusion images at peak myocardial signal intensity from a patient with splenic switch-off (SSO+) during 3D adenosine stress perfusion (A), and the corresponding rest perfusion image (B). Images (C) and (D) show another patient with absent splenic switch-off (SSO-) during adenosine stress and rest, respectively. Corresponding signal intensity-time curves display: splenic mean (blue solid line) and maximum (orange solid line) signal intensities, left ventricular (LV) blood pool mean signal intensity (purple dotted line), and myocardial mean signal intensity (green solid line). Signal intensity values are normalized to the peak LV blood pool signal and are given in arbitrary units (a.u.).

While the anterolateral wall in panel C appears relatively hypointense, the patient had no perfusion defect on clinical read and no flow-limiting lesion on FFR. Furthermore, the green signal intensity curve demonstrates similar myocardial signal enhancement between stress and rest, indicating no quantitative evidence of ischemia.

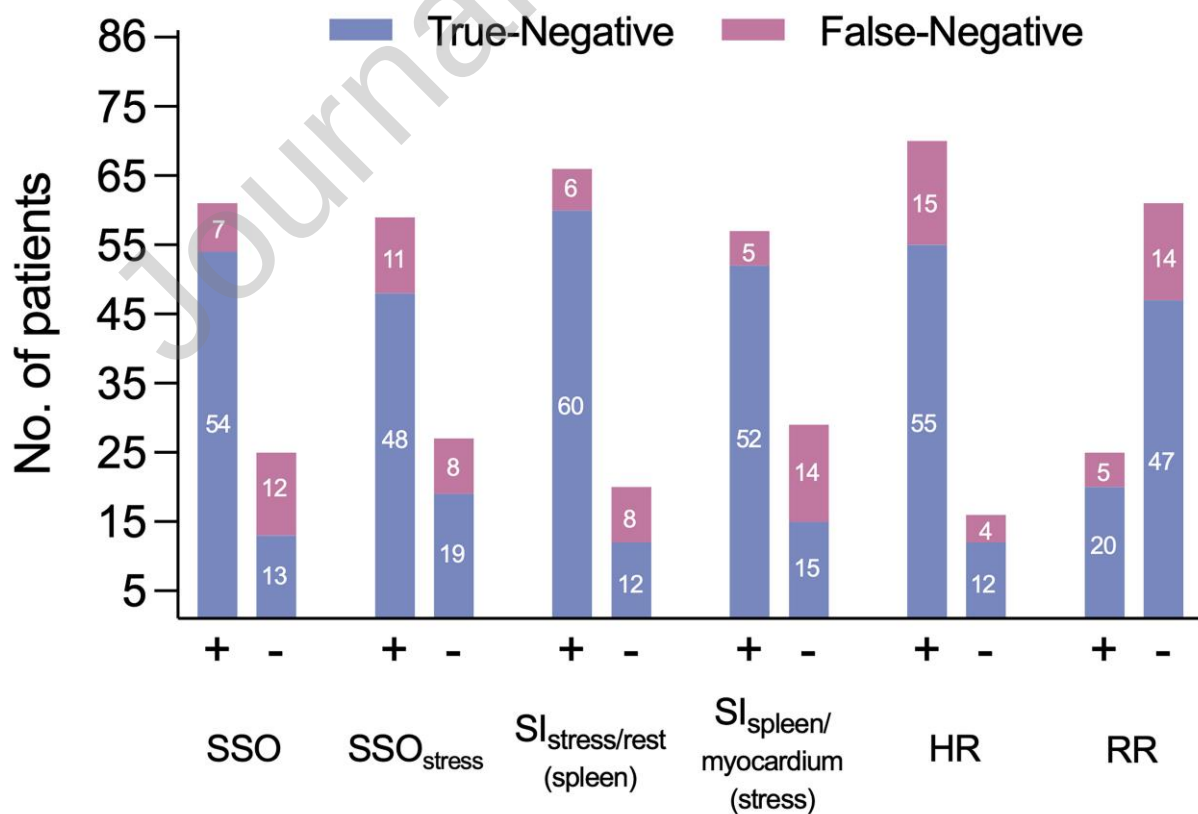


Figure 3. Markers of stress adequacy in CMR: distribution of true and false-negatives

This diagram depicts the distribution of true-negative and false-negative CMR perfusion results among negative CMR studies, stratified by the presence or absence of different stress adequacy markers, with invasive FFR serving as the reference standard. The following thresholds were used to define an adequate stress response: SI ratio stress-to-rest of spleen ≤ 0.32 , SI ratio spleen-to-myocardium at stress ≤ 0.38 , heart rate increase ≥ 10 bpm, and blood pressure decrease ≥ 10 mmHg.

SI: signal; SSO, splenic switch-off between stress and rest

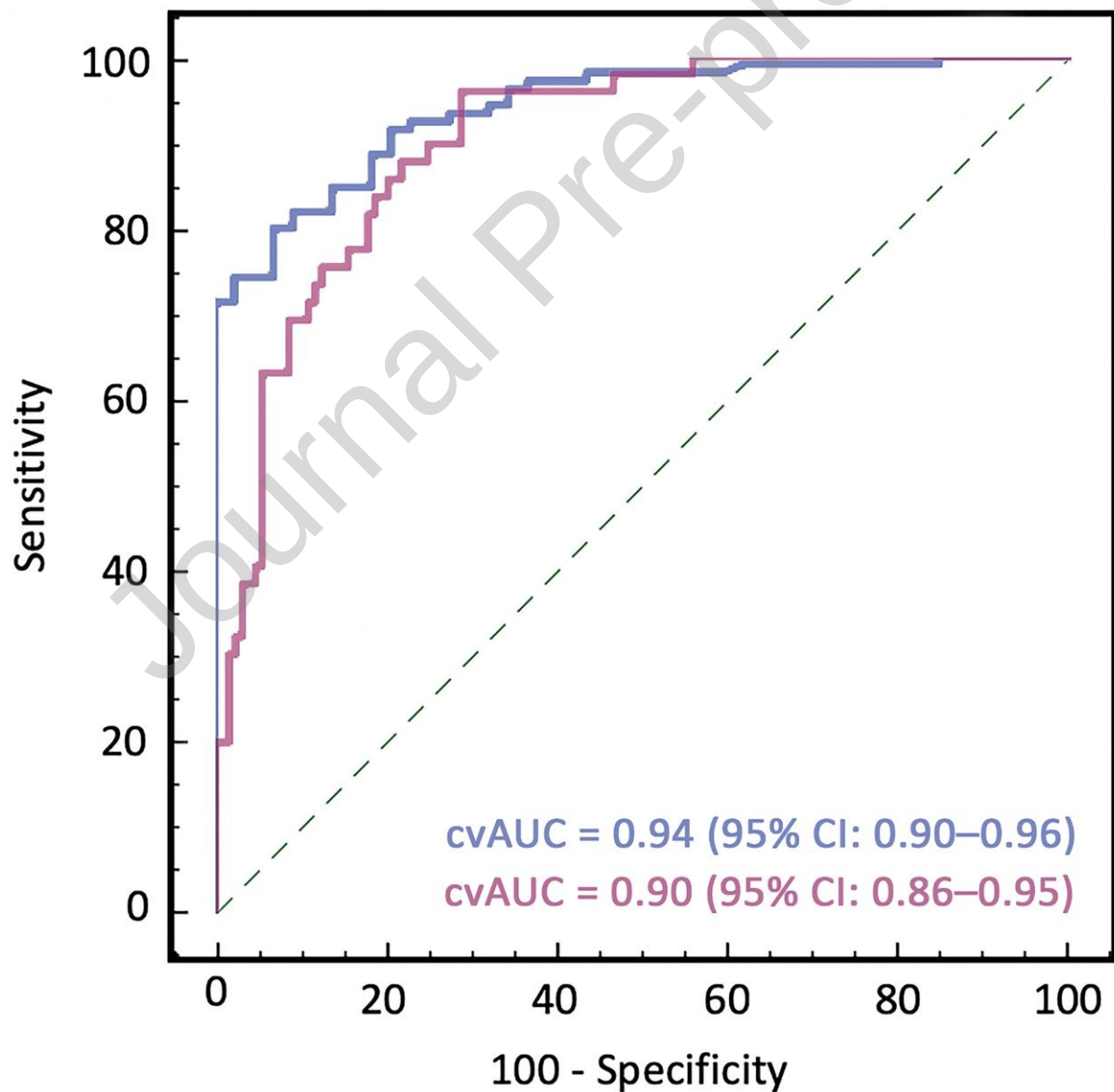


Figure 4. Predictive performance of signal intensity ratios for visual splenic switch-off

Receiver operating characteristic (ROC) curves demonstrating the ability of quantitative splenic signal intensity ratios to predict visually assessed splenic switch-off (SSO). Both the stress-to-rest signal intensity ratio of the spleen (blue) and the splenic-to-myocardial signal intensity ratio under stress (purple) showed excellent agreement with visual SSO status. These findings support the use of objective markers to complement or potentially replace visual evaluation in the absence of rest images.

cvAUC, cross-validated area under the curve

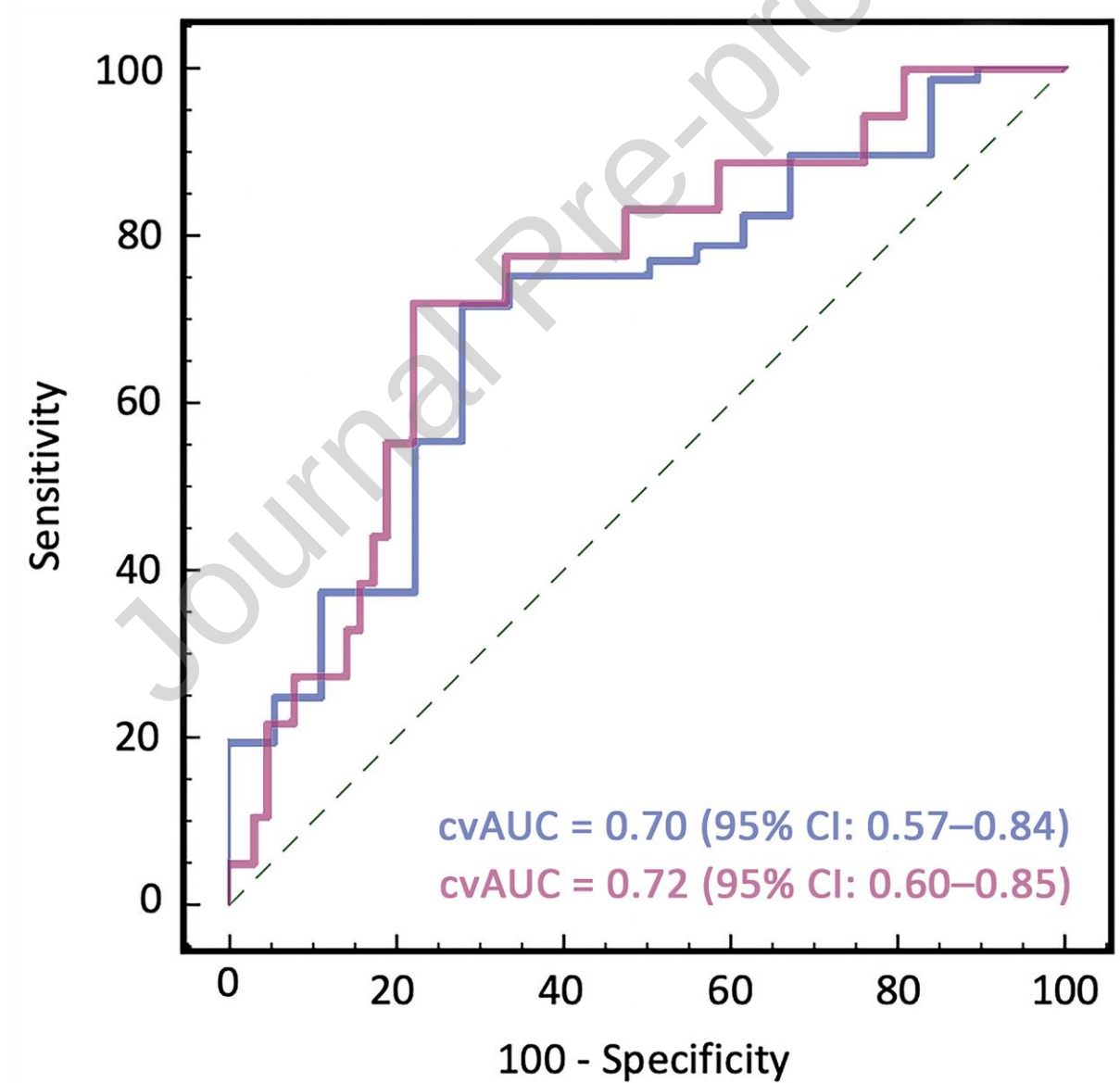
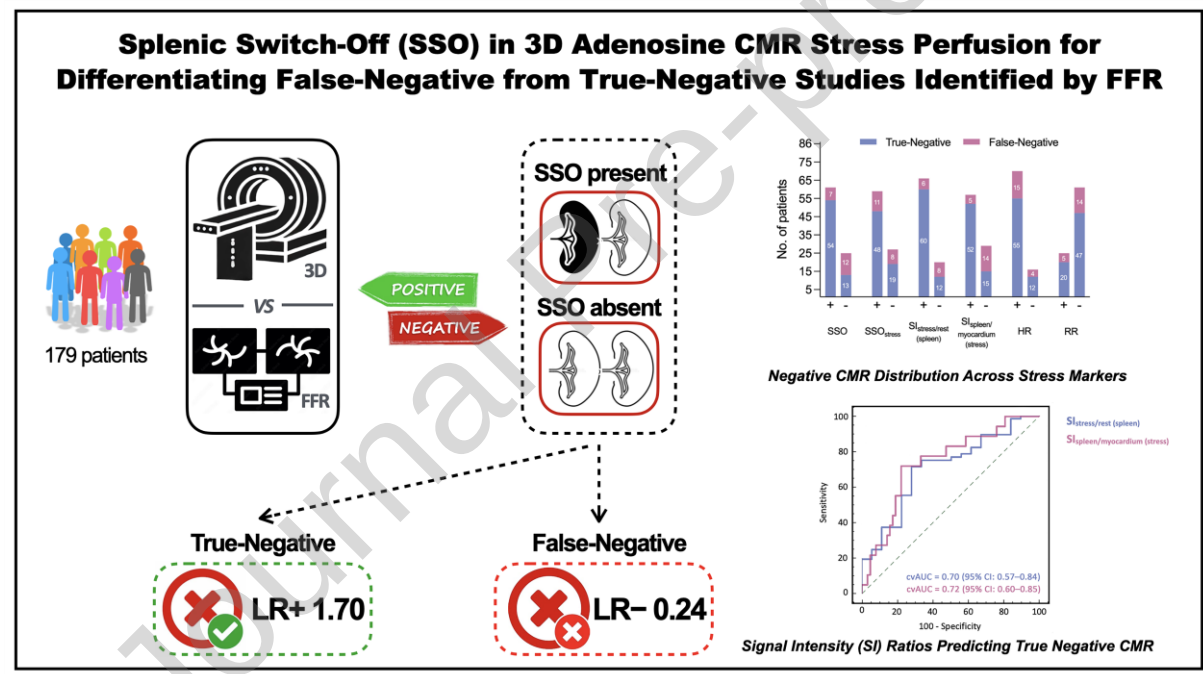


Figure 5. Ability of signal intensity ratios for identifying true-negative CMR studies

ROC curves for evaluating the ability of quantitative splenic perfusion parameters to discriminate between true-negative and false-negative CMR perfusion studies (using invasive FFR as the reference standard). Both stress-to-rest signal intensity ratio of the spleen (blue) and splenic-to-myocardial ratio at stress (purple) showed good predictive ability in identifying true-negative studies, indicating their potential role as objective markers of stress adequacy among negative CMR exams.

cvAUC, cross-validated area under the curve

Graphical abstract



Declaration of interests

- ☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
- ☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Mihaly Karolyi, Verena C. Wilzeck, Andrea Biondo, Alexander Gotschy, Hatem Alkadhi, Robert Manka reports a relationship with Bayer AG that includes: funding grants. Mihaly Karolyi, Verena C. Wilzeck, Andrea Biondo, Alexander Gotschy, Hatem Alkadhi, Robert Manka reports a

relationship with Canon Medical Systems Corporation that includes: funding grants. Mihaly Karolyi, Verena C. Wilzeck, Andrea Biondo, Alexander Gotschy, Hatem Alkadhi, Robert Manka reports a relationship with Guerbet that includes: funding grants. Mihaly Karolyi, Verena C. Wilzeck, Andrea Biondo, Alexander Gotschy, Hatem Alkadhi, Robert Manka reports a relationship with Siemens AG that includes: funding grants. Robert Manka reports a relationship with Swiss Heart Foundation that includes: funding grants. Robert Manka reports a relationship with University Hospital Zurich that includes: funding grants. Robert Manka reports a relationship with Philips that includes: speaking and lecture fees. Robert Manka reports a relationship with Bristol Myers Squibb that includes: speaking and lecture fees. Hatem Alkadhi, Robert Manka reports a relationship with Siemens AG that includes: speaking and lecture fees. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 1. Clinical characteristics of study population.

	<i>n=179</i>
Baseline characteristics	
Age (years)	63 ± 10
Male, n (%)	130 (73)
BMI (kg/m ²), mean ± SD	28 ± 4
Cardiovascular risk factors	
Hypertension, n (%)	130 (73)
Diabetes, n (%)	31 (17)
Dyslipidemia, n (%)	116 (65)
Smoker, n (%)	74 (41)
Family risk of CAD, n (%)	61 (34)
Regular medication	
ACE-inhibitor, n (%)	84 (47)
ARB, n (%)	30 (17)
Beta-blocker, n (%)	92 (51)
Calcium-antagonist, n (%)	34 (19)
Diuretic, n (%)	33 (18)
Nitrate, n (%)	14 (8)
Statin, n (%)	114 (64)

ACE angiotensin-converting, ARB angiotensin receptor blocker, BMI body mass index, CAD coronary artery disease, SD standard deviation.

Table 2. Invasive coronary angiography diagnostic findings

	<i>n=179</i>
CAD (>50% luminal stenosis), n (%)	114 (64)
Single-vessel disease, n (%)	53 (30)
Multi-vessel disease, n (%)	61 (34)

Pathological FFR (<0.8), n (%)

104 (58)

CAD, coronary artery disease; FFR, fractional flow reserve.

Table 3. CMR hemodynamic parameters and diagnostic findings

	n=179
CMR perfusion parameters	
<i>Stress:</i>	
Heart-rate (bpm), mean $\pm$ SD	82 $\pm$ 15
Systolic blood-pressure (mmHg), mean $\pm$ SD	127 $\pm$ 21
Diastolic blood-pressure (mmHg), mean $\pm$ SD	71 $\pm$ 10
<i>Rest:</i>	
Heart-rate (bpm), mean $\pm$ SD	66 $\pm$ 12
Systolic blood-pressure (mmHg), mean $\pm$ SD	129 $\pm$ 21
Diastolic blood-pressure (mmHg), mean $\pm$ SD	73 $\pm$ 10
CMR stress perfusion diagnostic findings (FFR as reference)	
Positive, n (%)	93 (52)
True positive, n (%)	85 (47)
False positive, n (%)	8 (5)
Negative, n (%)	86 (48)
True negative, n (%)	67 (37)
False negative, n (%)	19 (11)

SD, standard deviation.

Table 4. Predictive performance of stress adequacy indicators for identifying true-negative CMR diagnoses (vs. FFR).

	SSO	SSO _{stress}	SI _{stress/rest} of spleen	SI _{spleen/myocardium} at rest	HR	RR
Sensitivity (%)	88.5	81.4	91.2	91.2	78.6	80.0
Specificity (%)	48.0	29.6	41.2	48.3	25.0	23.0
Accuracy (%)	76.7	65.1	74.3	76.7	59.3	39.5
LR+	1.70	1.16	1.55	1.76	1.05	1.03
LR-	0.24	0.63	0.21	0.18	0.85	0.87

This table summarizes the predictive performance of stress adequacy markers for classifying negative CMR perfusion studies as true-negative or false-negative, using FFR as the reference standard for CAD. The metrics were derived within the subgroup of patients with negative stress perfusion imaging. Higher LR+ values reflect a greater probability of a true-negative classification when the

marker indicates adequate stress; conversely, lower LR- values suggest that the absence of the marker is associated with a higher probability of a false-negative result. Thresholds for stress adequacy were $SI_{\text{stress/rest of spleen}} \leq 0.32$, $SI_{\text{spleen/myocardium at rest}} \leq 0.38$, HR increase ≥ 10 bpm, and RR decrease ≥ 10 mmHg.

LR+, positive likelihood ratio; LR-, negative likelihood ratio; SSO, splenic switch-off between stress and rest; SSO_{stress} , splenic switch-off at stress only; $SI_{\text{stress/rest of spleen}}$, splenic stress-to-rest ratio; $SI_{\text{spleen/myocardium at rest}}$, splenic-to-myocardial ratio at rest; HR, heart rate response; RR, blood pressure response.