European Journal of Nuclear Medicine and Molecular Imaging (2019) 46:1257–1267
https://doi.org/10.1007/s00259-019-4265-7

ORIGINAL ARTICLE

Heart rate reserve during pharmacological stress is a significant negative predictor of impaired coronary flow reserve in women

Ahmed Haider[1,2] · Susan Bengs[1,2] · Monika Maredziak[1,2] · Michael Messerli[1] · Michael Fiechter[1,2] · Andreas A. Giannopoulos[1] · Valerie Treyer[1] · Moritz Schwyzer[1] · Christel Hermann Kamani[1] · Dimitri Patriki[1] · Elia von Felten[1] · Dominik C. Benz[1] · Tobias A. Fuchs[1] · Christoph Gräni[1] · Aju P. Pazhenkottil[1] · Philipp A. Kaufmann[1] · Ronny R. Buechel[1] · Catherine Gebhard[1,2]

Received: 14 September 2018 / Accepted: 4 January 2019 / Published online: 16 January 2019

Abstract

Purpose Evidence to date has failed to adequately explore determinants of cardiovascular risk in women with coronary microvascular dysfunction (CMVD). Heart rate responses to adenosine mirror autonomic activity and may carry important prognostic information for the diagnosis of CMVD.

Methods Hemodynamic changes during adenosine stress were analyzed in a propensity-matched cohort of 404 patients (202 women, mean age 65.9 ± 11.0) who underwent clinically indicated myocardial perfusion ^{13}N-ammonia Positron-Emission-Tomography (PET) at our institution between September 2013 and May 2017.

Results Baseline heart rate (HR) was significantly higher in patients with abnormal coronary flow reserve (CFR, $p < 0.001$ vs normal CFR). Accordingly, a blunted HR response to adenosine (=reduced heart rate reserve, %HRR) was seen in patients with abnormal CFR, with a most pronounced effect being observed in female patients free of myocardial ischemia (45.9 ± 34.9 vs 26.5 ± 18.0, $p < 0.001$ in women and 29.1 ± 16.9 vs 24.3 ± 21.7, $p = 0.15$ in men). Hence, a fully-adjusted multivariate logistic regression model identified HRR as the strongest negative predictor of reduced CFR in women free of myocardial ischemia, but not in men. Accordingly, receiver operating characteristics (ROC) curves for the presence of reduced CFR revealed that a %HRR <35 was a powerful predictor for abnormal CFR with a sensitivity of 81% and a specificity of 60% in women.

Conclusion A blunted HRR <35% is associated with abnormal CFR in women. Taking into account HR responses during stress test in women may help to risk stratify the heterogeneous female population of patients with non-obstructive coronary artery disease (CAD).

Keywords ^{13}N-ammonia PET · Coronary artery disease · Women · Adenosine · Heart rate reserve

Introduction

Despite remarkably reduced mortality rates over the last two decades, cardiovascular disease (CVD) remains the leading cause of death in Europe, currently responsible for more than four million deaths per year [1]. Notably, declines in cardiovascular mortality were primarily attributed to the improved survival of male patients, ultimately leading to a higher cardiovascular mortality in women as compared to men [2]. Limited data availability on female-specific characteristics of CVD as well as limitations of current diagnostic strategies in women with suspected CVD have been suggested to account for these sex-disparities in cardiovascular outcomes. Indeed, contemporary imaging modalities for the diagnosis of coronary artery disease (CAD) exhibit higher test accuracy in men than in women, rendering risk assessment and patient stratification in the latter sub-population a major challenge [3–5]. Key features complicating CAD assessment in women include, but are not limited to, technical artefacts related to breast tissue, reduced heart size and exercise capacity, concerns regarding radiation safety and the greater prevalence of non-obstructive CAD in women [3, 4, 6–8]. Indeed, symptomatic women are less likely to have obstructive CAD than

Ahmed Haider, Susan Bengs, Ronny R. Buechel and Catherine Gebhard contributed equally to this work.

✉ Catherine Gebhard
Catherine.gebhard@usz.ch

[1] Department of Nuclear Medicine, University Hospital Zurich, Raemistrasse 100, 8091 Zurich, Switzerland

[2] Center for Molecular Cardiology, University of Zurich, Zurich, Switzerland

men and tend to have a higher prevalence of coronary microvascular dysfunction (CMVD), also known as microvascular angina [9]. Emerging data document that CMVD is not a benign condition and that especially women with CMVD have severe symptoms and encounter a three times higher risk than men to experience adverse cardiovascular outcomes [10, 11]. However, despite the female propensity towards worse outcomes, recognition of CVMD is often delayed or deferred in this population [11, 12]. This is mainly due to symptom heterogeneity in women and the more complex invasive or non-invasive diagnostic work-up needed to provide evidence of CVMD. In fact, patients with suspected CMVD typically undergo coronary flow reserve (CFR) quantification by invasive coronary reactivity testing or noninvasive myocardial perfusion imaging (MPI), both technically demanding procedures that are not widely available [13].

Given (1) the higher prevalence and case-fatality of CMVD in women, (2) the current undertreatment of patients with CMVD, and (3) the frequent use of pharmacological stress testing in women due to the higher incidence of decreased exercise capacity in this population, we aimed to assess whether heart rate responses to adenosine may help to risk stratify patients with suspected CMVD.

Methods

Study population

Myocardial perfusion was assessed by ^{13}N-ammonia Positron-Emission-Tomography (PET)-myocardial perfusion imaging (MPI) in 669 consecutive patients (202, 30% women) who were referred to our institution between September 2013 and May 2017 for evaluation of known or suspected CAD. Patients were stratified by sex, and propensity-matched models were applied to adjust for baseline differences between women and men. Following propensity matching, 404 patients remained in the final analysis. Patients were assigned to either normal or abnormal myocardial perfusion and/or global CFR upon stratification by sex for the assessment of outcome. The patient's history including risk factors, medication use and key symptoms including chest pain and shortness of breath were recorded at the time of the imaging study by patient interview as well as by review of medical records. Moreover, prior diagnosis of CAD and a history of revascularization were documented. Our study was approved by the Cantonal Ethics Board in Zurich, Switzerland (BASEC No. 2017–01112). All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments. For this type of study formal consent is not required.

^{13}N-ammonia PET- myocardial perfusion imaging protocol

All 669 patients underwent ^{13}N-ammonia PET-MPI according to a one-day protocol at rest and during adenosine-induced stress conditions with a standard dose rate of 0.14 mg/min/kg over a time period of 7 min, as previously reported [14–17]. PET imaging was conducted on either a Discovery [LS/RX] PET/CT scanner or a GE Advance PET scanner (both GE Healthcare, Milwaukee, WI). Patients were devoid from caffeine intake during 12 h preceding the study. Medications (including beta-blockers) were withheld for 24 h prior to ^{13}N-ammonia PET-MPI according to recommendations made by current guidelines [18, 19]. For anatomical orientation and photon attenuation correction, PET acquisitions were preceded by a computed tomography (CT) transmission scan. Upon intravenous injection of ^{13}N-ammonia (700–900 MBq), standardized rest and pharmacological stress imaging protocols were applied according to the guidelines published by the American Society of Nuclear Cardiology [20].

Reconstruction protocol and image analysis

Image reconstruction was performed using a filtered back projection and the images were resliced into short-axis, vertical and horizontal long-axis orientations as previously described [16]. Applying the 17-segment scoring system, rest and stress images were visually scored and the summed stress score was further used to determine the percentage of abnormal myocardium during stress. Image interpretation was performed by two independent and experienced readers, blinded to the clinical data. If the summed stress score was smaller than four the scan was considered normal [15]. Image analysis was carried out using PMOD software package (version 2.1 to 2.8; PMOD Technologies Ltd., Zurich, Switzerland) and myocardial blood flow (MBF) was estimated by the model fitting of the blood pool as well as the myocardial time-activity curves corrected for partial volume effects and spillover, according to previous reports [15, 16]. We did not normalize baseline MBF to rate-pressure product (RPP) as this parameter was not retrieved in the patient sample. The global CFR was obtained from the ratio of stress to rest absolute MBF in the whole left ventricle. For stratification and statistical analysis, a CFR > 2.0 was considered normal [21].

Statistical analysis

The study population was stratified by sex, and propensity score matching was used to balance both groups for baseline differences. The propensity score for each patient was estimated using a multivariable logistic regression model in which sex was modelled using age, body mass index (BMI), diabetes, smoking, hypertension, dyslipidaemia, family history of CAD, prior myocardial infarction (MI), prior revascularisation, and medication as

covariates in the model. The propensity score was then used to match women and men with similar estimated propensity to two decimal places. Data are presented as mean ± standard deviation (SD) for continuous variables and frequency and percentage for categorical variables. Prior to analyses, basic assumptions were checked. Student's t test, Mann-Whitney test, analysis of variance (ANOVA) or Kruskal-Wallis test were used for group comparisons of continuous variables. For categorical variables, chi-square tests were used. For clinical convenience, CFR was displayed as a dichotomous variable using ≤2 as a cut point for an impaired ratio. As an effect measure modification by reversible perfusion defect was detected, patients with myocardial ischemia were excluded for the analysis of associations between heart rate reserve (HRR) and CFR/hyperemic MBF. The relationship between CFR and HRR was evaluated using Pearson's correlation coefficient. Using a multivariate logistic regression model, predictors of abnormal CFR were tested after stepwise selection in men and women (criterion: probability-of-F-to-enter <=0.05). A multivariate linear regression model was used to assess predictors of hyperaemic MBF in men and women. Variables included in both analyses were age, BMI, left ventricular ejection fraction (LVEF), rate pressure product (RPP), pathologic MPI findings, smoking status, hypertension, diabetes, dyslipidemia, as well as previous MI and/or revascularization. Statistical significance was set at $p < 0.05$. SPSS version 24.0 (SPSS Inc., Chicago, Illinois) was used for all statistical analyses.

Results

Patient characteristics

Following propensity score matching, a total of 404 subjects (202 [50.0%] women, mean age 65.9 ± 11.0 years) remained in the analysis. Before matching, 669 subjects (30% women, mean age 65.8 ± 11.0 years) were analyzed. In the unmatched cohort, mean age at presentation was 66.7 ± 11.3 years for women and 65.4 ± 10.8 years for men ($p = 0.17$, Table 1). Men were more often smokers (32.3% vs 21.3%, $p = 0.004$, Table 1) and were more often on medication than women ($p < 0.05$, Table 1). More men than women had previously undergone revascularization (38.1% for PCI and 21.6% for CABG vs 19.3% vs 12.9% in women, $p < 0.01$, Table 1) or had previously experienced an MI (24.6% vs 10.9% in women, $p < 0.001$, Table 1). Patient's characteristics of both unmatched and matched cohorts stratified by sex are depicted in Table 1.

Myocardial perfusion findings and hemodynamic changes during adenosine stress

Ninety-three percent of patients reported discomfort, dyspnoea or chest pain during adenosine infusion ($p = 0.5$ for men vs women, Table 2). On ^{13}N-Ammonia PET, reversible perfusion defects were found in 29.7% of patients (31.2% of men and 28.2% of women, p = 0.5), while 40.6% of patients showed an irreversible perfusion defect (Table 2). Men had a higher prevalence of fixed perfusion defects than women (47.5% vs 33.7% in women, $p = 0.01$, Table 2). CFR values were found to be abnormal (≤2.0) in 185 (46.0%) patients. Abnormal CFR ≤2.0 was detected in 43% of women and 49.0% of men ($p = 0.23$ men vs women). Both baseline and hyperemic myocardial blood flow (MBF) were higher in women as compared to men (0.98 ± 0.3 mL/min/g vs 0.74 ± 0.2 mL/min/g, $p < 0.001$, and 2.1 ± 0.8 mL/min/g vs 1.6 ± 0.6 mL/min/g, $p < 0.001$, Table 2). Accordingly, no significant sex differences were found for CFR (2.2 ± 0.8 in women vs 2.1 ± 0.8 in men, $p = 0.40$, Table 2). In patients without myocardial ischemia detected by ^{13}N-ammonia PET, abnormal CFR was observed in 35.9% ($n = 52$) of women and 43.9% ($n = 61$) of men (data not shown). Baseline LVEF, heart rate (HR) and systolic blood pressure (SBP) were significantly higher in women as compared to men ($p < 0.01$, Table 2). Pharmacological stress with adenosine caused a slight increase in diastolic and systolic blood pressure (DBP and SBP) in both men and women ($p < 0.05$ vs baseline), while a decrease in LVEF was noted in both sexes ($p < 0.05$ vs baseline). Similarly, a significant increase in HR ($p < 0.001$ vs baseline) was observed, which was most pronounced in women (99 bpm vs 86 bpm in men, $p < 0.001$, Table 2). Accordingly, HRR during adenosine stress was significantly higher in women as compared to men (40.2 ± 39.3 vs 27.5 ± 22.6, $p < 0.001$, Table 2). No difference between men and women was observed with regard to adenosine-induced LVEF changes (LVEF reserve, $p = 0.53$, Table 2).

Adenosine-induced hemodynamic changes and their association with coronary flow reserve

In the overall population, baseline HR was significantly higher in patients in whom an abnormal CFR ≤2 mL/min was detected by ^{13}N-ammonia PET ($p < 0.001$, data not shown). The association between an increased baseline HR and reduced CFR was observed in both men and women ($p = 0.013$ for women and $p = 0.001$ for men, Fig. 1a). No significant differences in peak HR during adenosine infusion were observed between patients with normal and reduced CFR (p = NS, Fig. 1b). In contrast, a profound decrease in HRR was seen in women with reduced CFR ($p = 0.002$, Fig. 1c), while a less pronounced effect was observed in men with reduced CFR ($p = 0.006$ vs normal CFR, Fig. 1c). In a subpopulation of patients without myocardial ischemia, the above differences were even more pronounced. In fact, in this subgroup of patients, a profoundly decreased %HRR was observed in women, but not in men with abnormal CFR (45.9 ± 34.9 vs 26.5 ± 18.0, $p < 0.001$ in women and 29.1 ± 16.9 vs 24.3 ± 21.7, $p = 0.15$ in men, Fig. 1f). This significant reduction in HRR in women with abnormal CFR was due to an increased baseline HR and a concomitantly reduced peak HR during pharmacological stress in this

Table 1 Characteristics of the study population by sex

Baseline characteristics	Unmatched cohort				PS Matched cohort			
	Total $n = 669$	Women $n = 202$	Men $n = 467$	p value	Total $n = 404$	Women $n = 202$	Men $n = 202$	p value
Age (years), mean ± SD	65.8 ± 11.0	66.7 ± 11.3	65.4 ± 10.8	0.17	65.9 ± 11.0	66.7 ± 11.3	65.1 ± 10.6	0.15
BMI, mean ± SD	28.7 ± 6.3	28.6 ± 7.7	28.7 ± 5.5	0.89	28.7 ± 6.8	28.6 ± 7.7	28.8 ± 5.8	0.78
Hypertension, *n*(%)	368 (55)	101 (50)	267 (57.2)	0.087	209 (51.7)	101 (50)	108 (53.5)	0.486
Smoking, *n*(%)	194 (29)	43 (21.3)	151 (32.3)	0.004	97 (24.0)	43 (21.3)	54 (26.7)	0.20
Diabetes, *n*(%)	170 (25.4)	48 (23.8)	122 (26.1)	0.52	85 (21.0)	48 (23.8)	37 (18.3)	0.179
Dyslipidemia, *n*(%)	332 (49.6)	91 (45)	241 (51.6)	0.12	193 (47.8)	91 (45)	102 (50.5)	0.273
Family history of CAD, *n*(%)	140 (20.9)	40 (19.8)	100 (21.4)	0.64	69 (17.1)	40 (19.8)	29 (14.4)	0.146
Previous MI, *n*(%)	137 (20.5)	22 (10.9)	115 (24.6)	<0.001	55 (13.6)	22 (10.9)	33 (16.3)	0.111
Previous PCI, *n*(%)	217 (32.4)	39 (19.3)	178 (38.1)	<0.001	89 (22.0)	39 (19.3)	50 (24.7)	0.08
Previous CABG, *n*(%)	127 (19)	26 (12.9)	101 (21.6)	0.008	63 (15.6)	26 (12.9)	37 (18.3)	0.13
Typical angina symptoms, *n*(%)	100 (14.9)	28 (13.9)	72 (15.4)	0.6	61 (15.1)	28 (13.9)	33 (16.3)	0.487
Atypical angina symptoms, *n*(%)	124 (18.5)	44 (21.8)	82 (17.6)	0.16	78 (19.3)	44 (21.8)	34 (16.8)	0.207
Dyspnea, *n*(%)	128 (19.1)	46 (22.8)	80 (17.1)	0.12	84 (20.8)	46 (22.8)	38 (18.8)	0.327
Medication								
Platelet inhibitors	327 (48.9)	82 (40.6)	245 (52.5)	0.005	168 (41.6)	82 (40.6)	86 (42.6)	0.686
Beta-blocker	233 (34.8)	57 (28.2)	176 (37.7)	0.018	122 (30.2)	57 (28.2)	65 (32.3)	0.386
ACEI/Aldosterone antagonist	310 (46.3)	79 (39.1)	231 (49.5)	0.014	173 (42.8)	79 (39.1)	94 (46.5)	0.132
Lipid lowering agents	333 (49.8)	70 (34.7)	263 (56.3)	<0.001	152 (37.6)	70 (34.7)	82 (40.6)	0.06

Unmatched (*left*) and matched (*right*) cohort following propensity score (PS) matching. Data are presented as mean ± SD or % of patients

PS propensity score, *BMI* body mass index, *CAD* coronary artery disease, *MI* myocardial infarction, *PCI* percutaneous coronary intervention, *CABG* coronary artery bypass grafting, *ACEI* angiotensin-converting-enzyme inhibitor

population (Fig. 1d and e). The latter was observed in women, but not in men (Fig. 1e). No significant changes in baseline mean arterial blood pressure (MAP) or peak MAP were observed, while a slight increase in peak SBP was seen in men with abnormal CFR (Fig. 2a–c). In contrast, a moderate decrease in peak DBP was observed in women CFR ($p = 0.03$ vs normal CFR), but not in men, with abnormal CFR (Fig. 2d). Both, baseline and peak LVEF were slightly reduced in patients with abnormal CFR ($p < 0.05$, Fig. 2e and f). Accordingly, in patients free of myocardial ischemia, a significant and positive association was observed between baseline and peak LVEF and CFR ($r = 0.19$, $p = 0.001$ for baseline LVEF and $r = 0.18$, $p = 0.002$ for peak LVEF, data not shown). Further, a strong and positive correlation between HRR and CFR was found in these patients (Pearson's $r = 0.33$, $p < 0.001$). However, a sex-specific analysis revealed that this association was present only in women, while no significant association between HRR and CFR was observed in men (Fig. 3a). Hence, a significant difference between regression lines for HRR and CFR in women and men was observed ($p_{interaction} = 0.001$, Fig. 3a). Finally, in men but not in women, a significant association between typical angina symptoms or dyspnea with reduced CFR was observed ($p = 0.7$ in women and $p = 0.04$ in men, Fig. 3b).

Predictors of abnormal CFR in women and men without myocardial ischemia

A multivariate logistic regression model taking into account age, BMI, RPP, pathologic MPI findings, known CAD, and cardiovascular risk factors including smoking status, hypertension, diabetes, and dyslipidemia, identified HRR (continuous variables) as the only significant negative predictor of reduced CFR in women (Table 3A). In contrast, diabetes mellitus and baseline LVEF, but not HRR, were selected as significant predictors of abnormal CFR in men (Table 3B). Accordingly, in patients without myocardial ischemia, receiver operating characteristics (ROC) curves for the presence of reduced CFR revealed that a %HRR < 35 was a powerful predictor for abnormal CFR with a sensitivity of 81% and a specificity of 60% in women (Fig. 3c), while the predictive value of HRR for the presence of abnormal CFR was significantly less in men (area under the curve, AUC = 0.61, $p = 0.03$, Fig. 3d). As some studies suggest that hyperemic MBF might be superior to CFR in the evaluation of cardiovascular risk [22], and given the potential underestimation of CFR in women, an additional regression analysis in patients without myocardial ischemia was performed, with hyperemic MBF being the dependent variable. This fully adjusted linear regression

Table 2 Patient hemodynamic and ^{13}N-ammonia PET parameters

13 N-Ammonia PET and hemodynamic variables	Total N = 404	Women N = 202	Men N = 202	*p* value
Symptoms (flush, chest pain, dyspnea, nausea) during adenosine infusion, *n*(%)	374 (92.6)	189 (93.6)	185 (91.6)	0.5
Perfusion defect				
None, *n*(%)	172 (42.6)	100 (49.5)	72 (35.6)	0.01
Fixed, *n*(%)	164 (40.6)	68 (33.7)	96 (47.5)	0.01
Reversible, *n*(%)	120 (29.7)	57 (28.2)	63 (31.2)	0.51
Baseline MBF (mL/min/g), mean ± SD	0.9 ± 0.3	0.98 ± 0.3	0.74 ± 0.2	<0.001
Hyperemic MBF (mL/min/g), mean ± SD	1.8 ± 0.8	2.1 ± 0.8	1.6 ± 0.6	<0.001
CFR, mean ± SD	2.2 ± 0.8	2.2 ± 0.8	2.1 ± 0.8	0.40
Abnormal CFR (<2), *n*(%)	185 (46.0)	86 (43.0)	99 (49.0)	0.23
Agatston calcium score > 75th percentile, *n*(%) (available in 200 patients)	62 (40.8)	37 (40.2)	25 (41.7)	0.86
LVEF				
Rest (%), mean ± SD	52.02 ± 12.9	55.6 ± 12.9	48.2 ± 12.2	<0.001
Hyperemic (%), mean ± SD	49.1 ± 12.2	52.5 ± 12.3	45.6 ± 11.4	<0.001
LVEF reserve (%), mean ± SD	−5.0 ± 10.1	−5.3 ± 8.1	−4.6 ± 11.8	0.53
Diastolic blood pressure (DBP)				
Baseline (mmHg), mean ± SD	76.8 ± 12.5	73.8 ± 12.9	75.0 ± 12.9	0.49
Peak effect (mmHg), mean ± SD	96.0 ± 14.9	76.8 ± 12.4	76.8 ± 12.8	0.99
Systolic blood pressure (SBP)				
Baseline (mmHg), mean ± SD	139.6 ± 24.5	144.0 ± 25.7	134.4 ± 22.0	0.003
Peak effect (mmHg), mean ± SD	142.0 ± 22.9	145.7 ± 23.4	138.2 ± 21.8	0.002
Mean arterial pressure (MAP)				
Baseline (mmHg), mean ± SD	96.0 ± 14.9	97.1 ± 15.2	94.8 ± 14.4	0.25
Peak effect (mmHg), mean ± SD	98.2 ± 14.1	99.5 ± 14.3	96.9 ± 13.8	0.09
Heart rate				
Baseline (bpm), mean ± SD	69.6 ± 12.1	71.2 ± 11.9	67.9 ± 12.2	0.007
Peak effect (bpm), mean ± SD	92.3 ± 24.8	98.7 ± 28.7	85.9 ± 18.0	<0.001
% HRR, mean ± SD	33.9 ± 32.6	40.2 ± 39.3	27.5 ± 22.6	<0.001
Peak heart rate * SBP (RPP) product, mean ± SD	13,106 ± 3917	14,417 ± 4269	11,796 ± 3017	<0.001

Data are stratified by sex

MBF myocardial blood flow, *CFR* coronary flow reserve, *LVEF* left ventricular ejection fraction, *DBP* diastolic blood pressure, *SBP* systolic blood pressure, *MAP* mean arterial pressure, *bpm* beats per minute, *HRR* heart rate reserve, *RPP* rate pressure product

analysis identified HRR as a strong and independent predictor of hyperemic MBF only in women (beta coefficient 0.331, p = 0.001), but not in men, while BMI and baseline LVEF were selected as significant predictors of hyperemic MBF in both sexes (Table 4).

Discussion

Our study is the first reporting that a blunted %HRR <35 during coronary vasodilator stress is a powerful independent predictor of abnormal CFR in women free of myocardial ischemia on ^{13}N-ammonia PET-MPI. Our data further illustrate that predictor variables of abnormal CFR differ substantially between men and women, with traditional cardiovascular risk factors such as diabetes being associated with abnormal CFR only in men. Similarly, although women with non-obstructive CAD and suspected CMVD more often report severe chest pain, angina symptoms were not associated with abnormal CFR in women in our study. Given the high prevalence of non-obstructive CAD in women and their augmented clinical event rates, our data emphasize the need to better define the female cardiovascular phenotype associated with CMVD and suggest that taking into account hemodynamic variables routinely obtained during coronary stress testing might help to identify female patients that should undergo further testing.

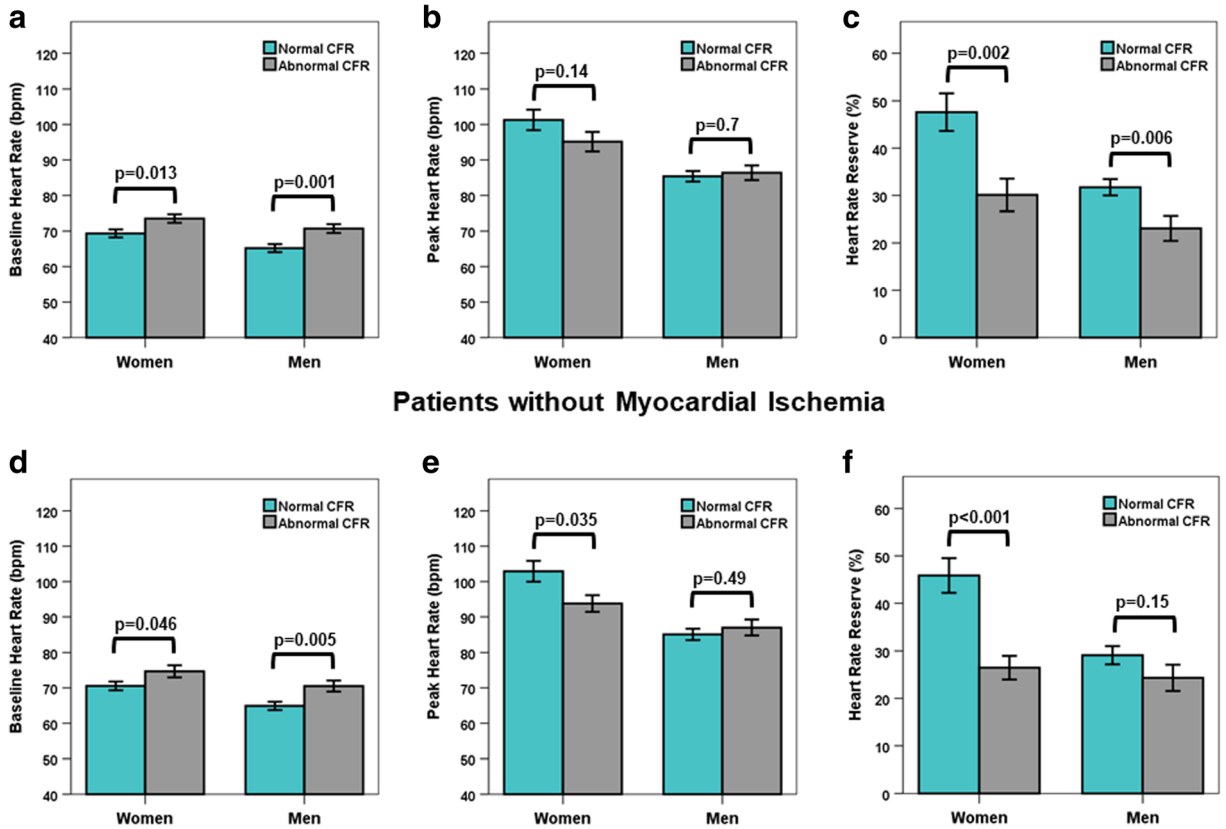

Fig. 1 Heart rate changes before and after pharmacological stress with adenosine stratified by sex and the presence/absence of abnormal coronary flow reserve. **a**–**c** Analysis performed in total population. **a** Baseline heart rate. Values are reported in beats per minute (bpm). **b** Peak heart rate during adenosine infusion. Values are reported in beats per minute (bpm). **c** Heart rate change during adenosine stress. Values are reported as percent heart rate reserve [(peak HR minus baseline HR)/baseline HR]*100). **d**–**e** Analysis performed in patients without myocardial ischemia. **d** Baseline heart rate. Values are reported in beats per minute (bpm). **e** Peak heart rate during adenosine infusion. Values are reported in beats per minute (bpm). **f** Heart rate change during adenosine stress. Values are reported as percent heart rate reserve [(peak HR minus baseline HR)/baseline HR]*100). **d** Baseline heart rate. Values are reported in beats per minute (bpm). Data are presented as mean ± SEM. Two-sided *p* values are indicated. *CFR*, coronary flow reserve

Indeed, despite a growing awareness of sex-specific differences in the diagnosis of CAD, contemporary diagnostic strategies have failed to provide effective risk correlates for women with non-obstructive CAD and persisting symptoms of myocardial ischemia. This lack of recognition is primarily related to the fact that a considerable proportion of women does not present obvious perfusion defects by routinely applied imaging techniques but, instead, tend to suffer from visually non-detectable CMVD [13]. CMVD can be assessed using PET-MPI derived CFR due to its distinct advantage of excellent temporal and spatial resolution and absolute MBF quantification across coronary vessels [23, 24]. In women, PET-MPI has tackled several standing issues by means of the excellent breast tissue attenuation correction, and the favorable safety profile with effective doses ranging from 2 to 3 mSv [25, 26]. However, despite its excellent diagnostic performance, the use of PET-MPI is currently limited by technical and logistic challenges as well as high costs associated with radiotracer production [25]. Consequently, the vast majority of functional non-invasive diagnostic imaging approaches rely on stress echocardiography, stress magnetic resonance imaging (MRI) or stress single-photon emission computed tomography (SPECT)-MPI, which all provide information on epicardial coronary lesions at a relatively low cost. These imaging modalities, however, are not suitable for accurate CFR quantification, resulting in a false sense of reassurance in women without evident perfusion defect or wall motion abnormality. Our study findings suggest that the analysis of HRR during vasodilator stress might be a potential gatekeeper-test for further assessment of CMVD in symptomatic women without evidence of myocardial ischemia. In addition, our study emphasizes the value of examining and reporting hemodynamic changes that occur with adenosine stress and point out that the reliance on traditional risk markers might not be an accurate risk estimate for the presence of CMVD in women.

Hemodynamic changes during pharmacologic stress testing have been reported previously. Consistent with earlier studies in patients undergoing SPECT-MPI, we observed a significant increase in HR during adenosine infusion, resulting in an average %HRR of 34 in our total study population [27]. In previous SPECT-MPI studies, a diminished HR response to adenosine

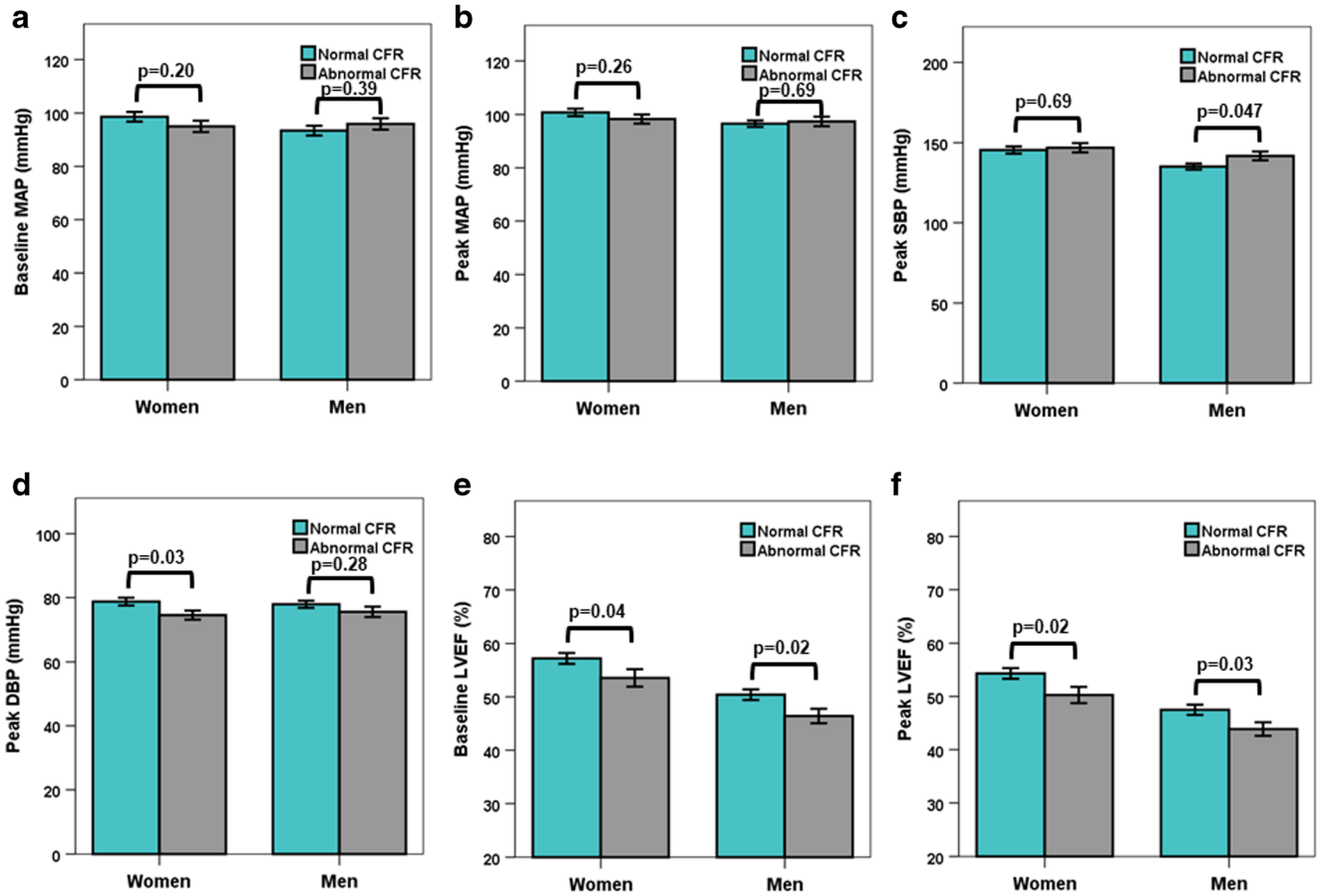

Fig. 2 Hemodynamic changes in patients without myocardial ischemia during pharmacological stress with adenosine stratified by sex and the presence/absence of abnormal coronary flow reserve. **a** Baseline mean arterial pressure (MAP). **b** Peak mean arterial pressure (MAP). **c** Peak systolic blood pressure (SBP) during adenosine infusion. **d** Peak diastolic blood pressure (DBP) during adenosine infusion. **e** Left ventricular ejection fraction (LVEF) quantified by gated ^{13}N-ammonia PET at baseline. **f** Left ventricular ejection fraction (LVEF) quantified by gated ^{13}N-ammonia PET following pharmacological stress with adenosine. Data are presented as mean ± SEM. Two-sided *p* values are indicated. CFR, coronar flow reserve

was observed in patients with myocardial ischemia [27, 28]. However, while the former was associated with an increased risk of cardiac death in two studies [29, 30], an investigation conducted in a female population did not detect any correlation between chronotropic responses to adenosine and cardiovascular outcomes [31]. Previous work suggests that an increased sympathetic activity might have accounted for the adverse outcomes seen in patients with a blunted HR response [29, 30]. Thus, taking into account the effect of age, sex, and comorbidities such as diabetes or heart failure on cardiac autonomous function, different study populations and exclusion criteria might have accounted for the conflicting findings [30, 32–34].

Our study now extends these previous data by demonstrating that not only epicardial CAD but also CMVD is associated with changes in chronotropic responses to adenosine and that these changes differ by sex. To the best of our knowledge, only one previous study has assessed the association between HR responses to adenosine and MBF in a heterogeneous population of 31 patients. Although these authors did not report sex-specific findings, they observed a blunted HRR in patients with reduced CFR, in accordance with our data [35]. Notably, while the majority of published articles describe a diminished HR response to vasodilator stress in patients with pathologic findings on MPI, we recently observed a strong increase in adenosine related HRR in younger patients <55 years in whom myocardial ischemia was detected by SPECT-MPI. The strongest increase in HR during adenosine stress was seen in young women with myocardial ischemia [17]. As hemodynamic responses to adenosine have been shown to depend on baseline cardiac autonomic tone, and considering the known age-dependent changes of cardiac autonomic control [32–34, 36, 37], a stronger, sympathetic-driven hemodynamic response to adenosine in premenopausal women with ischemic cardiac processes may account for these seemingly divergent findings.

Along this line of reasoning, our results agree with the concept of sexual dimorphism in cardiovascular autonomous activity. In fact, we did not observe a significant BP drop during adenosine administration in our study indicating that the adenosine-related

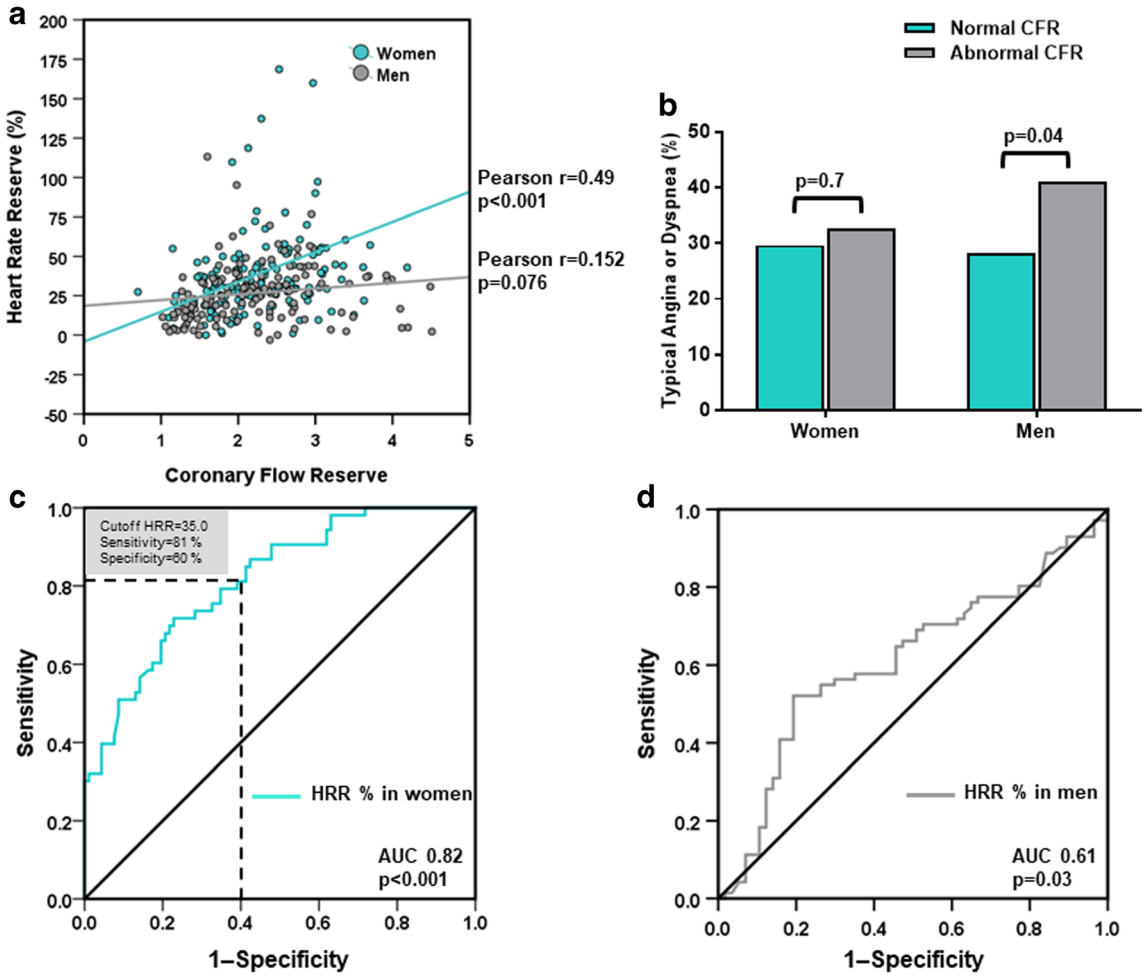

Fig. 3 Association between heart rate reserve, symptoms and abnormal coronary flow reserve in patients without myocardial ischemia. **a** Correlation of heart rate reserve and coronary flow reserve (CFR) in women and men. Pearson's r and *p* values are indicated. **b** Typical angina symptoms and dyspnea in patients with normal/abnormal coronary flow reserve stratified by sex. Two-sided *p* values are indicated. **c** Receiver operating characteristics (ROC) curves for the presence of reduced coronary flow reserve (CFR) in women. **d** Receiver operating characteristics (ROC) curves for the presence of reduced coronary flow reserve (CFR) in men. Area under the ROC curve (AUC) and *p* value for heart rate reserve (HRR) following pharmacological stress with adenosine

increase in HR occurs due to direct sympathetic stimulation rather than due to a reflex to the decrease in BP. Overall, women showed a stronger HR increase during adenosine infusion as compared to men in our study, independent of CFR. As augmented plasma norepinephrine levels along with an increase in HR following adenosine administration have been reported [36], our observations suggest a stronger sympathetic response to vasodilator stress in women. The fact that this response was blunted in the presence of abnormal CFR or reduced hyperaemic MBF in women, but not in men, might point towards a higher baseline sympathetic activity in women with CMVD. A higher resting HR in women with abnormal CFR in our study as well previous evidence indicating that chronotropic responses to adenosine depend on baseline sympathetic tone further support this hypothesis [32–34, 36]. Notably, individuals with CMVD are considered a high risk subset of patients with cardiac syndrome X, a condition that is more common in women and characterized by chest pain, repolarization abnormalities during exercise, and exclusion of significant epicardial CAD [38–40]. While a direct association between autonomic dysregulation and CMVD has never been demonstrated, an increasing body of evidence suggests that cardiac syndrome X is associated with cardiac sympathetic hyperactivity and parasympathetic impairment [41–43].

As with any study, certain design limitations are inherent. First, the present study is a retrospective analysis conducted at a single center, which confines its generalizability. Second, due to the limited sample size, adjustment was only feasible for a limited number of confounders. Thus, residual confounding factors may remain after accounting for clinically pertinent variables. Also, unmeasured variables not incorporated into the propensity-matched models may have affected the results. Third, we did not normalize baseline MBF to RPP, a measure that may reduce variability within and between individuals [21]. However, as in our study HRR was not only associated

Table 3 Predictors of abnormal coronary flow reserve at multivariate logistic regression analysis in women (A) and men (B) without myocardial ischemia

A			
Stepwise logistic regression model for abnormal coronary flow reserve (CFR) in women without myocardial ischemia as assessed by ^{13}N-Ammonia PET (N = 145)			
Independent variables	OR	OR (95% CI)	*p* value
Heart rate reserve	0.91	0.87–0.94	0.002
B			
Stepwise logistic regression model for abnormal coronary flow reserve (CFR) in men without myocardial ischemia as assessed by ^{13}N-Ammonia PET (N = 139)			
Independent variables	OR	95% CI	*p* value
Diabetes mellitus	1.94	1.46–3.62	0.019
Baseline LVEF	0.96	0.94–0.99	0.03

Regression analysis was performed among age, body mass index (BMI), rate pressure product (RPP), % heart rate reserve (%HRR, continuous variable), baseline left ventricular ejection fraction (LVEF, %) and abnormal findings on ^{13}N-Ammonia PET, known coronary artery disease, and cardiovascular risk factors including smoking status, hypertension, diabetes, and dyslipidemia. Only variables staying in the final model are presented

CI confidence interval, *OR* odds ratio, *LVEF* left ventricular ejection fraction

with CFR but also with hyperemic MBF, a parameter that is independent of RPP, our data suggest that the observed sex differences occur independent of baseline MBF. The latter is consistent with a previous study reporting that sex differences in baseline MBF persisted after correction for RPP, thereby indicating that other variables account for the higher baseline MBF observed in women [44]. Finally, although patients were asked to suspend their regular medications 24 h prior to the scan, we cannot exclude an effect of individual treatment regimen, in particular of β-blockers, on hemodynamic responses to adenosine infusion. Nevertheless, no difference in %HRR was observed in our study between patients who were on long-term beta-blocker therapy as compared to those without (33.9 ± 40.0 vs 32.5 ± 26.7, $p = 0.68$). These observations are consistent with previous reports showing that β-blocker treatment did not interfere with the expected HR increment during adenosine infusion [17, 36].

Table 4 Predictors of increased hyperaemic myocardial blood flow (continuous variable) at multivariate linear regression analysis

Linear regression analysis for hyperaemic myocardial blood flow in patients without myocardial ischemia as assessed by ^{13}N-Ammonia PET (N = 284)			
Independent variables	Standardized coefficient β	t	*p* value
Women (N = 145)			
Heart rate reserve	0.331	3.10	0.001
BMI	−0.375	−3.341	0.003
Baseline LVEF	0.324	2.94	0.005
Men (N = 139)			
BMI	−0.449	−2.77	0.008
Baseline LVEF	0.367	2.47	0.017

Regression analysis was performed among age, body mass index (BMI), rate pressure product (RPP), % heart rate reserve (%HRR, continuous variable), baseline left ventricular ejection fraction (LVEF, %) and abnormal findings on ^{13}N-Ammonia PET, known coronary artery disease, and cardiovascular risk factors including smoking status, hypertension, diabetes, and dyslipidemia. In women (*upper panel*) and men (*lower panel*)

LVEF left ventricular ejection fraction, *BMI* body mass index

In summary, our study demonstrates that a blunted %HRR <35 is associated with abnormal CFR in women undergoing stress testing. Taking this variable into account may help to risk stratify the heterogeneous population of patients with non-obstructive CAD and to tailor diagnostic strategies to the female cardiovascular phenotype. Further research is warranted to determine the role of the sympathetic nervous system in the development and clinical course of CMVD, and larger-scale investigations will have to assess whether HRR will add incremental prognostic value to PET-MPI in addition to image-related parameters.

Funding CG was supported by grants from the Swiss National Science Foundation (SNSF); the Olga Mayenfisch Foundation, Switzerland; the OPO Foundation, Switzerland; the Novartis Foundation, Switzerland; the Swissheart Foundation; and the Helmut Horten Foundation, Switzerland. MM was supported by the Iten-Kohaut Foundation, Switzerland.

Compliance with ethical standards

Conflict of interest All authors have the following to disclose: The University Hospital of Zurich holds a research contract with GE Healthcare. CG has received research grants from the Novartis Foundation, Switzerland.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Our study was approved by the Cantonal Ethics Board in Zurich, Switzerland (BASEC No. 2017–01112). The need to obtain informed consent was waived by the ethics committee due to the retrospective nature of the study.

References

1. Townsend N, Wilson L, Bhatnagar P, Wickramasinghe K, Rayner M, Nichols M. Cardiovascular disease in Europe: epidemiological update 2016. Eur Heart J. 2016;37(42):3232–45.
2. Wilmot KA, O'Flaherty M, Capewell S, Ford ES, Vaccarino V. Coronary heart disease mortality declines in the United States from 1979 through 2011: evidence for stagnation in young adults, especially women. Circulation. 2015;132(11):997–1002.

3. Mieres JH, Shaw LJ, Arai A, Budoff MJ, Flamm SD, Hundley WG, et al. Role of noninvasive testing in the clinical evaluation of women with suspected coronary artery disease: consensus statement from the cardiac imaging committee, council on clinical cardiology, and the cardiovascular imaging and intervention committee, council on cardiovascular radiology and intervention, American Heart Association. Circulation. 2005;111(5):682–96.
4. Baldassarre LA, Raman SV, Min JK, Mieres JH, Gulati M, Wenger NK, et al. Noninvasive imaging to evaluate women with stable ischemic heart disease. J Am Coll Cardiol Img. 2016;9(4):421–35.
5. Gianrossi R, Detrano R, Mulvihill D, Lehmann K, Dubach P, Colombo A, et al. Exercise-induced ST depression in the diagnosis of coronary artery disease. A meta-analysis. Circulation. 1989;80(1):87–98.
6. Botvinick EH. Breast attenuation artifacts in Tl-201 scintigraphy. Radiology. 1988;168(3):878–9.
7. Hansen CL, Crabbe D, Rubin S. Lower diagnostic accuracy of thallium-201 SPECT myocardial perfusion imaging in women: an effect of smaller chamber size. J Am Coll Cardiol. 1996;28(5):1214–9.
8. Sanders GD, Patel MR, Chatterjee R, Ross AK, Bastian LA, Coeytaux RR, et al. AHRQ future research needs papers. Noninvasive technologies for the diagnosis of coronary artery disease in women: future research needs: identification of future research needs from comparative effectiveness review no 58. Rockville: Agency for Healthcare Research and Quality (US); 2013.
9. Pepine CJ, Ferdinand KC, Shaw LJ, Light-McGroary KA, Shah RU, Gulati M, et al. Emergence of nonobstructive coronary artery disease: a woman's problem and need for change in definition on angiography. J Am Coll Cardiol. 2015;66(17):1918–33.
10. Murthy VL, Naya M, Taqueti VR, Foster CR, Gaber M, Hainer J, et al. Effects of sex on coronary microvascular dysfunction and cardiac outcomes. Circulation. 2014;129(24):2518–27.
11. Pepine CJ, Anderson RD, Sharaf BL, Reis SE, Smith KM, Handberg EM, et al. Coronary microvascular reactivity to adenosine predicts adverse outcome in women evaluated for suspected ischemia results from the national heart, lung and blood institute WISE (women's ischemia syndrome evaluation) study. J Am Coll Cardiol. 2010;55(25):2825–32.
12. Sedlak TL, Lee M, Izadnegahdar M, Merz CN, Gao M, Humphries KH. Sex differences in clinical outcomes in patients with stable angina and no obstructive coronary artery disease. Am Heart J. 2013;166(1):38–44.
13. Dean J, Cruz SD, Mehta PK, Merz CN. Coronary microvascular dysfunction: sex-specific risk, diagnosis, and therapy. Nat Rev Cardiol. 2015;12(7):406–14.
14. Fiechter M, Gebhard C, Ghadri JR, Fuchs TA, Pazhenkottil AP, Nkoulou RN, et al. Myocardial perfusion imaging with 13N-ammonia PET is a strong predictor for outcome. Int J Cardiol. 2013;167(3):1023–6.
15. Herzog BA, Husmann L, Valenta I, Gaemperli O, Siegrist PT, Tay FM, et al. Long-term prognostic value of 13N-ammonia myocardial perfusion positron emission tomography added value of coronary flow reserve. J Am Coll Cardiol. 2009;54(2):150–6.
16. Gebhard C, Fiechter M, Herzog BA, Lohmann C, Bengs S, Treyer V, et al. Sex differences in the long-term prognostic value of (13)N-ammonia myocardial perfusion positron emission tomography. Eur J Nucl Med Mol Imaging. 2018;45(11):1964–1974.
17. Gebhard C, Messerli M, Lohmann C, Treyer V, Bengs S, Benz DC, et al. Sex and age differences in the association of heart rate responses to adenosine and myocardial ischemia in patients undergoing myocardial perfusion imaging. J Nucl Cardiol. 2018 Apr 23. https://doi.org/10.1007/s12350-018-1276-x.
18. Dorbala S, Di Carli MF, Delbeke D, Abbara S, DePuey EG, Dilsizian V, et al. SNMMI/ASNC/SCCT guideline for cardiac SPECT/CT and PET/CT 1.0. J Nucl Med. 2013;54(8):1485–507.
19. Reyes E, Stirrup J, Roughton M, D'Souza S, Underwood SR, Anagnostopoulos C. Attenuation of adenosine-induced myocardial perfusion heterogeneity by atenolol and other cardioselective beta-adrenoceptor blockers: a crossover myocardial perfusion imaging study. J Nucl Med. 2010;51(7):1036–43.
20. Hendel RC, Berman DS, Di Carli MF, Heidenreich PA, Henkin RE, Pellikka PA, et al. ACCF/ASNC/ACR/AHA/ASE/SCCT/SCMR/SNM 2009 appropriate use criteria for cardiac radionuclide imaging: a report of the American College of Cardiology Foundation appropriate use criteria task force, the American Society of Nuclear Cardiology, the American College of Radiology, the American Heart Association, the American Society of Echocardiography, the Society of Cardiovascular Computed Tomography, the Society for Cardiovascular Magnetic Resonance, and the Society of Nuclear Medicine. Circulation. 2009;119(22):e561–87.
21. Murthy VL, Naya M, Foster CR, Hainer J, Gaber M, Di Carli G, et al. Improved cardiac risk assessment with noninvasive measures of coronary flow reserve. Circulation. 2011;124(20):2215–24.
22. Joutsiniemi E, Saraste A, Pietila M, Maki M, Kajander S, Ukkonen H, et al. Absolute flow or myocardial flow reserve for the detection of significant coronary artery disease? Eur Heart J Cardiovasc Imaging. 2014;15(6):659–65.
23. Phelps ME. Positron emission tomography provides molecular imaging of biological processes. Proc Natl Acad Sci USA. 2000;97(16):9226–33.
24. Nakazato R, Berman DS, Alexanderson E, Slomka P. Myocardial perfusion imaging with PET. Imaging Med. 2013;5(1):35–46.
25. Nandalur KR, Dwamena BA, Choudhri AF, Nandalur SR, Reddy P, Carlos RC. Diagnostic performance of positron emission tomography in the detection of coronary artery disease: a meta-analysis. Acad Radiol. 2008;15(4):444–51.
26. Jaarsma C, Leiner T, Bekkers SC, Crijns HJ, Wildberger JE, Nagel E, et al. Diagnostic performance of noninvasive myocardial perfusion imaging using single-photon emission computed tomography, cardiac magnetic resonance, and positron emission tomography imaging for the detection of obstructive coronary artery disease: a meta-analysis. J Am Coll Cardiol. 2012;59(19):1719–28.
27. Vashist A, Heller EN, Blum S, Brown EJ, Bhalodkar NC. Association of heart rate response with scan and left ventricular function on adenosine myocardial perfusion imaging. Am J Cardiol. 2002;89(2):174–7.
28. Amanullah AM, Berman DS, Hachamovitch R, Kiat H, Kang X, Friedman JD. Identification of severe or extensive coronary artery disease in women by adenosine technetium-99m sestamibi SPECT. Am J Cardiol. 1997;80(2):132–7.
29. Abidov A, Hachamovitch R, Hayes SW, Ng CK, Cohen I, Friedman JD, et al. Prognostic impact of hemodynamic response to adenosine in patients older than age 55 years undergoing vasodilator stress myocardial perfusion study. Circulation. 2003;107(23):2894–9.
30. Hage FG, Dean P, Iqbal F, Heo J, Iskandrian AE. A blunted heart rate response to regadenoson is an independent prognostic indicator in patients undergoing myocardial perfusion imaging. J Nucl Cardiol. 2011;18(6):1086–94.
31. Amanullah AM, Berman DS, Erel J, Kiat H, Cohen I, Germano G, et al. Incremental prognostic value of adenosine myocardial perfusion single-photon emission computed tomography in women with suspected coronary artery disease. Am J Cardiol. 1998;82(6):725–30.
32. Hage FG, Heo J, Franks B, Belardinelli L, Blackburn B, Wang W, et al. Differences in heart rate response to adenosine and regadenoson in patients with and without diabetes mellitus. Am Heart J. 2009;157(4):771–6.
33. Hage FG, Perry G, Heo J, Iskandrian AE. Blunting of the heart rate response to adenosine and regadenoson in relation to hyperglycemia and the metabolic syndrome. Am J Cardiol. 2010;105(6):839–43.

34. Bravo PE, Hage FG, Woodham RM, Heo J, Iskandrian AE. Heart rate response to adenosine in patients with diabetes mellitus and normal myocardial perfusion imaging. Am J Cardiol. 2008;102(8):1103–6.
35. Tomiyama T, Kumita S, Ishihara K, Suda M, Sakurai M, Hakozaki K, et al. Patients with reduced heart rate response to adenosine infusion have low myocardial flow reserve in (13)N-ammonia PET studies. Int J Card Imaging. 2015;31(5):1089–95.
36. Conradson TB, Clarke B, Dixon CM, Dalton RN, Barnes PJ. Effects of adenosine on autonomic control of heart rate in man. Acta Physiol Scand. 1987;131(4):525–31.
37. Burger IA, Lohmann C, Messerli M, Bengs S, Becker A, Maredziak M, et al. Age- and sex-dependent changes in sympathetic activity of the left ventricular apex assessed by 18F-DOPA PET imaging. PLoS One. 2018;13(8):e0202302.
38. Agrawal S, Mehta PK, Bairey Merz CN. Cardiac syndrome X: update. Heart Fail Clin. 2016;12(1):141–56.
39. Camici PG, Marraccini P, Lorenzoni R, Buzzigoli G, Pecori N, Perissinotto A, et al. Coronary hemodynamics and myocardial metabolism in patients with syndrome X: response to pacing stress. J Am Coll Cardiol. 1991;17(7):1461–70.
40. Tousoulis D, Crake T, Lefroy DC, Galassi AR, Maseri A. Left ventricular hypercontractility and ST segment depression in patients with syndrome X. J Am Coll Cardiol. 1993;22(6):1607–13.
41. Kaski JC. Cardiac syndrome X in women: the role of oestrogen deficiency. Heart. 2006;92(Suppl 3):iii5–9.
42. Montorsi P, Fabbiocchi F, Loaldi A, Annoni L, Polese A, De Cesare N, et al. Coronary adrenergic hyperreactivity in patients with syndrome X and abnormal electrocardiogram at rest. Am J Cardiol. 1991;68(17):1698–703.
43. Gulli G, Cemin R, Pancera P, Menegatti G, Vassanelli C, Cevese A. Evidence of parasympathetic impairment in some patients with cardiac syndrome X. Cardiovasc Res. 2001;52(2):208–16.
44. Chareonthaitawee P, Kaufmann PA, Rimoldi O, Camici PG. Heterogeneity of resting and hyperemic myocardial blood flow in healthy humans. Cardiovasc Res. 2001;50(1):151–61.