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Impaired coronary flow velocity reserve is associated with cardiovascular risk factors but not with angina symptoms

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ABSTRACT

Objectives Coronary microvascular dysfunction (CMD) is considered to cause angina pectoris in a large proportion of women with no obstructive coronary artery disease (CAD). However, data supporting a relation between angina pectoris and CMD are limited. We compared CMD in women with angina with asymptomatic women and evaluated the relation between presence of CMD, angina characteristics, cardiovascular risk factors and results of stress testing.

Methods In a cross-sectional study, we included 1684 women with angina and <50% coronary artery stenosis on invasive angiography. Asymptomatic women from the community-based Copenhagen City Heart Study served as reference group (n=102). Coronary microvascular function was determined by coronary flow velocity reserve (CFVR) assessed by transthoracic Doppler stress echocardiography. CFVR < 2 was defined as CMD. Symptoms were obtained from standardised angina questionnaires and results of stress testing from health records.

Results Median CFVR was 2.33 (IQR 2.00–2.75) in symptomatic women versus 2.60 (2.19–2.95) in asymptomatic (p=0.007). CFVR <2 was found in 25% of symptomatic and in 19% of asymptomatic women. Symptomatic women had a greater risk factor burden. After adjusting for age, hypertension, diabetes, smoking and heart rate the difference in CFVR between groups disappeared (p=0.213). We found no associations between CFVR and angina characteristics, symptom burden or results from stress testing.

Conclusions Impaired CFVR is more prevalent in symptomatic than in asymptomatic women and related to the cardiovascular risk factors hypertension, diabetes, smoking and increased heart rate. Neither a positive bicycle test, single photon emission CT stress test nor chest pain characteristics identify women with impaired CFVR among women with angina and no obstructive CAD. Results may question the concept of microvascular angina as currently defined.

Key questions

What is already known about this subject?

► Angina pectoris in the absence of obstructive coronary artery disease (CAD) is very common and more in women than in men. Coronary microvascular dysfunction (CMD) is thought to explain symptoms in many of these women. CMD has a poor prognosis; however, the link between angina pectoris and CMD is unclear. Further research is needed to increase our knowledge about CMD and its significance for angina pectoris.

What does this study add?

► We determined coronary microvascular function in 1684 women with angina and no obstructive CAD by Doppler echo. This method is highly feasible, reproducible and has shown high correlation with invasively measured coronary flow reserve. By comparison with 102 age-matched asymptomatic women, we were able to demonstrate that CMD is common in both symptomatic and asymptomatic women and seems to be explained by the prevalence of risk factors. CMD was not related to angina characteristics or angina severity and was not correlated to results from functional testing. To determine the causality of CMD in angina, intervention studies documenting effect on both CMD and angina burden are needed.

How might this impact on clinical practice?

► Contrary to current understanding, abnormal functional testing or particular angina characteristics in patients with no obstructive CAD does not identify those with coronary microvascular dysfunction.

INTRODUCTION

Coronary microvascular dysfunction (CMD) is believed to explain symptoms in a large proportion of patients with angina and no obstructive coronary artery disease (CAD).[1] Under normal circumstances, small resistance vessels dilate in response to increased oxygen demand. When vessels are dysfunctional, their ability to dilate is reduced or they may have a paradoxical vasoconstrictive reaction leading to inhibited blood flow to the coronary arteries and to ischemia and pain.[1 2] Several studies have shown that CMD is common among patients suspected

of ischemia with no obstructive CAD and particularly common in women.[3–6] While there is good evidence that CMD is associated with common cardiovascular risk factors[7] and increased cardiovascular morbidity and mortality,[5 8] the link between angina symptoms, demonstration of ischaemia and CMD is less well established.[9 10]

Angina attributed to CMD is termed microvascular angina (MVA). As CMD is a flow-limiting condition, expert consensus describes MVA as predominantly exercise induced and related to positive stress testing.[11 12] However, previous studies have found no association between positive exercise ECG or stress imaging and the demonstration of CMD.[10 13 14] Moreover, prior work has identified angina as a construct impacted of sensory, emotional, autonomic, motor and cognitive components.[15] Angina may thus occur unrelated to coronary flow.

The aims of this study were to determine whether CMD is more frequent among women with angina and no obstructive CAD than among a reference group of asymptomatic women. Furthermore, we wished to assess whether CMD is associated with typical exercise induced angina and with inducible ischemia by myocardial stress testing. Coronary microvascular function was determined non-invasively by transthoracic Doppler echocardiography (TTDE) under pharmacologically induced stress, measuring the coronary flow velocity reserve (CFVR).

METHODS

Study design and population

In this cross-sectional study, we included 1684 women with angina pectoris but no significant obstructive CAD. The population constitutes the complete cohort of the iPOWER (ImProve diagnOsis and treatment of Women withangina pEctoris and micRovessel disease) study[16] which comprises consecutively included Danish women aged 18–80 with angina pectoris but no obstructive CAD, defined as <50% stenosis on invasive coronary angiography (ICA). Thus, ICA excluding obstructive CAD was performed on all participants within 6 months prior to inclusion in the iPOWER study. Women were excluded if they had a history of myocardial infarction, valvular or congenital heart disease, systolic heart failure (left ventricular ejection fraction (LVEF) <45%) or severe pulmonary disease (forced expiratory volume in the first second, FEV_1 <50% or moderate/severe asthma). Women were also excluded if a non-cardiac cause of chest discomfort was considered more likely than angina pectoris or if they no longer had symptoms. Symptoms were verified by telephone call prior to the appointment at the clinic and reconfirmed on the day of examination when interviewed by trained healthcare professionals. The iPOWER cohort is described in detail elsewhere.[10 16] As a reference group, we included 102 asymptomatic women invited from the community-based, prospective Copenhagen City Heart Study.[17] Participants from the reference group had no history of cardiovascular disease, as the same exclusion criteria applied to both groups. Age matching with the symptomatic group was attempted. All participants had a successful CFVR examination. Inclusion flow chart is shown in figure 1.

Clinical and demographic assessments

Standard assessments included physical examinations, symptom questionnaires and self-reported data collected from interviews, such as history of hypertension, dyslipidaemia, comorbidity and medication use. We collected data from the regional ICA database and information on diagnostic tests conducted within 6 months prior to the ICA, including non-invasive cardiac CT angiography, exercise ECG and myocardial perfusion scintigraphy (single photon emission CT (SPECT)). Blood pressure and heart rate were obtained at rest. Fasting blood samples were analysed for haemoglobin, creatinine, sodium, potassium, glycosylated haemoglobin (HbA1c),

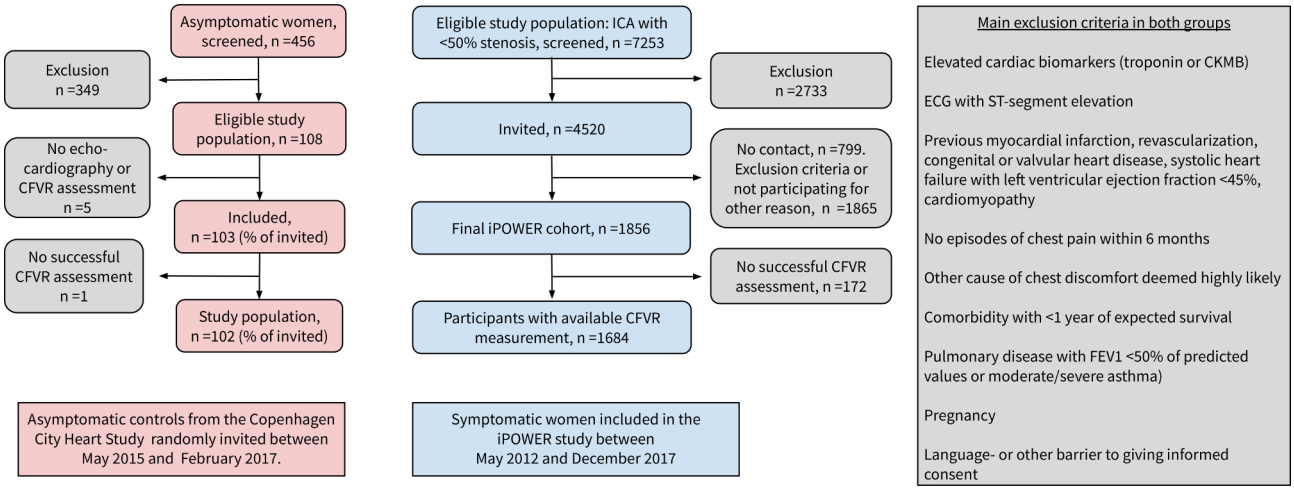

Figure 1 Inclusion process of the symptomatic women and the asymptomatic reference group. This figure shows the inclusion process of the iPOWER cohort and the asymptomatic reference women recruited from the Copenhagen City Heart Study. The grey box to the right lists the exclusion criteria which were similar for both groups. ICA, invasive coronary angiography; CFVR, coronary flow velocity reserve.

thyroid stimulating hormone (TSH), urate and cholesterol levels (total cholesterol, low-density lipoprotein, high-density lipoprotein and triglycerides).

Echocardiographic assessments

Coronary flow velocities (CFV) were measured with TTDE of the left anterior descending artery (LAD) at rest and during hyperaemia, induced by a 6 min high-dose dipyridamole infusion (0.84 mg/kg). CFVR is the ratio of CFV at peak hyperaemia to rest. Blood pressure and heart rate were measured every 3 min. After the examination, intravenous theophylline (maximum dose 220 mg) was administered. Every CFVR examination was analysed offline independently by two blinded experts. If estimates differed by more than 0.2, consensus reading was performed.

Before the examination, participants were instructed of 24 hours abstinence from caffeine, tobacco, food containing significant amount of methylxanthines (coffee, tea, chocolate, cola and banana) and anti-ischaemic agents, antihypertensive medication and diuretics. Short-lasting nitroglycerine was paused for 1 hour and medication containing dipyridamole for 48 hours before the examination.

A standard resting transthoracic echocardiography was conducted before the CFVR examination. Images were saved under hyperaemia for the calculation of LVEF and global longitudinal strain. We acquired 2-dimensional images of the left ventricle in apical long axis, 2-chamber and 4-chamber views at frame rates between 60 and 90 frames/s. LVEF was analysed as a semiautomated biplane calculation (Auto-EF tool, GE EchoPAC v112). All examinations were performed in a standardised setting by an experienced echocardiographer using GE Healthcare Vivid E9 cardiovascular ultrasound system (GE Healthcare, Horten, Norway). A 1.3–4.0 MHz transducer (GE Vivid 5S probe) was used for the transthoracic echocardiography and a 2.7–8 MHz transducer (GE Vivid 6S probe) for the CFVR examination.

Validation of the TTDE method

The gold standard method for the assessment of coronary microvascular function is invasive and can be performed during ICA after obstructive CAD has been ruled out: by intracoronary administration of vasoactive substances and/or by thermodilution technique, CFVR can be determined as a measure of microvascular function.[12 18] From ethical and economic concerns, non-invasive methods are preferable when an invasive procedure is not indicated for other reason.[11 19 20] CFVR assessed by TTDE is free from radiation, highly feasible, reproducible and correlates well with invasive methods.[21–25]

We have previously reported feasibility of 95%[10] and good reproducibility and repeatability of CFVR measurements in women from the iPOWER cohort (Bland-Altman limits of agreement (CI)=0.48 (0.22 to 0.74), and correlation coefficient r=0.90 (p<0.01)) and in healthy volunteers (limits of agreement (CI)=0.44 (0.21 to 0.68), r=0.96 (p<0.01)).[23]

Moreover, the TTDE method has shown to be a strong predictor of cardiovascular prognosis.[6 26 27] Although the relationship between CMD and prognosis is graded,[28] a cut-off of 2.0, or 2.5 is often used to define CMD.[1 6 11] We also grouped CFVR into three categories: CFVR<2.0 indicating CMD,[6 11] CFVR>2.0 and<2.5 indicating borderline CMD, and CFVR>2.5 indicating normal coronary microvascular function.

Symptoms

The symptomatic women were included if symptoms were susceptive of stable angina pectoris or if women were hospitalised due to suspected unstable angina before the including ICA, since this might have been the first manifestation of stable angina.[10] We collected a detailed symptom description with respect to location, duration, character, radiation, frequency and provoking and alleviating factors.[10] Afterwards, we divided chest pain symptoms into categories of typical or atypical angina pectoris, or non-cardiac chest pain according to the European guideline classification.[11] Symptom burden and characteristics were further explored by the WHO's Rose's Angina Questionnaire (online supplemental reference 1) and the Seattle Angina Questionnaire (SAQ) (online supplemental reference 2). Rose's angina Questionnaire was developed decades ago to detect symptoms of ischaemic heart disease and has been widely used and validated. Answers are evaluated as definite angina pectoris or not, further subdivided as 'severe' or 'non-severe'. SAQ is a 19-item health-related quality of life measure for patients with CAD. The answers calculate scores on five scales evaluating different dimensions of functional status: physical limitation, angina stability, angina frequency, treatment satisfaction and quality of life. Scores are measured on a 0–100 scale; higher scores indicate less symptom burden. Definitions of chest pain classifications can be found online supplemental figure 1 and questionnaire items online supplemental table 1.

Statistical analysis

Continuous normally distributed data are presented as mean (SD) and non-normally distributed data as median (IQR). Comparison across groups were performed by age-adjusted linear or logistic regression analysis for continuous and categorical outcome variables, respectively. To explore predictors of reduced CFVR, age-adjusted multivariable linear regression analyses were performed with logarithmically transformed CFVR as continuous outcome variable. Variables with significantly different distribution across groups were tested as potential determinants of CFVR and discarded if p>0.05. CI refers to 95% CIs. To evaluate the distribution of independent variables across CMD groups, age-adjusted trend tests by logistic or linear regression analysis were performed.

Missing values in the SAQ were imputed according to a validated scoring system described in detail by Kimble *et*

Table 1 Baseline characteristics across groups

	Symptomatic women n=1684	Asymptomatic women n=102	P value*
Self-reported data			
Age, years	62.8 (9.6)	60.9 (10.2)	0.059†
Hypertension	922 (55)	17 (19)	<0.001
DM I	20 (1)	0 (0)	0.02
DM II	182 (11)	3 (3)	
Smoking	270 (16)	13 (14)	0.38
Dyslipidaemia	1042 (62)	19 (21)	<0.001
Family history	882 (54)	29 (32)	<0.001
Cerebrovascular disease	132 (8)	1 (1)	0.04
Peripheral artery disease	100 (6)	4 (4)	0.58
Clinical assessments			
Body mass index, kg/m^2	27.1 (5.4)	24.9 (3.5)	<0.001
Heart rate, beats/min	70.6 (10.9)	69.4 (11.5)	0.24
Systolic BP, mm Hg	131.3 (21.0)	115.9 (17.0)	<0.001
Diastolic BP, mm Hg	70.4 (16.1)	60.3 (8.2)	<0.001
Total cholesterol, mmol/L	5.0 (1.1)	5.4 (0.9)	<0.001
LDL, mmol/L	2.8 (1.0)	3.1 (0.8)	0.004
HDL, mmol/L	1.6 (0.5)	1.9 (0.5)	<0.001
Triglycerides, mmol/L	1.3 (0.7)	1.0 (0.5)	<0.001
HbA1c, non-diabetics, IFCC	38.1 (4.7)	37.4 (4.1)	0.568
Medication, n (%)			
Acetyl salicylic acid	675 (41)	3 (3)	<0.001
Beta-blocker	469 (28)	3 (3)	<0.001
Calcium antagonist	378 (23)	7 (7)	0.001
ACE-I/ARB	561 (34)	11 (11)	<0.001
Statin	842 (51)	9 (9)	<0.001

Values are mean (SD) or number (%) unless otherwise indicated.
*P values from age-adjusted linear or logistic regression analysis.
†P value unadjusted.
ACE-I, ACE inhibitor; ARB, angiotensin-II receptor blocker; BP, blood pressure; DM, diabetes mellitus; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.;

al, 2012 (online supplemental reference 3). In a subanalysis, we excluded participants aged more than 62 years to detect a possible association between CFVR and angina symptoms in younger women who often have more characteristic symptoms.

All statistical analyses were performed with STATA/IC V.13.1 (StataCorp LP).

RESULTS

Study population

Mean age (SD) was 62.8 (9.6) years in symptomatic women and 60.9 (10.2) years in the reference group (p=0.059). Overall, cardiovascular risk factor burden was highest in the symptomatic women (table 1). Cholesterol levels, blood pressure and heart rate were slightly higher in the symptomatic group but in the normal range in both groups indicating a well-treated population. Antihypertensive medication was used in 23%–34% of the symptomatic women and in 3%–11% of asymptomatic women. Fifty-one per cent of symptomatic and 9% of asymptomatic women were treated with statins (table 1). Body mass index was significantly higher in symptomatic women, who also had more diabetes. Levels of haemoglobin, sodium, potassium, creatinine, TSH, urate and urine albumin/creatinine ratio did not differ between groups (results not shown).

Coronary microvascular function in symptomatic women and reference group

Median (IQR) CFVR was 2.33 (2.00–2.75) in symptomatic women and 2.60 (2.19–2.95) in the reference group (age-adjusted p value=0.007): 25% of symptomatic women had CMD defined as CFVR<2 versus 19% in asymptomatic references (p=0.009). After adjustment for age, history

Table 2 Echocardiography derived parameters across groups

	Symptomatic women n=1684	Asymptomatic women n=102	P value*
Coronary microvascular function, continuous variable, median (IQR)			
CFVR	2.33 (2.00–2.75)	2.60 (2.19–2.95)	0.007
CFV at rest, m/s	0.23 (0.19–0.29)	0.21 (0.19–0.25)	<0.001
CFV at hyperaemia m/s	0.56 (0.46–0.68)	0.54 (0.48–0.62)	0.086
Coronary microvascular function category, n (%)			
CFVR≤2.0	425 (25.2)	19 (18.6)	0.009
CFVR>2 and≤2.5	593 (35.2)	27 (26.5)	
CFVR>2.5	666 (39.6)	56 (54.9)	
Systolic heart function, mean (SD)			
LVEF at rest, %	58.3 (5.9)	55.6 (4.7)	<0.001
LVEF at hyperaemia, %	62.1 (6.3)	59.6 (4.9)	0.001

*P value from age-adjusted linear regression analysis with logarithmically transformed CFVR/CFV. χ^2 test is performed when CFVR is grouped into three categories
CFV(R), coronary flow velocity (reserve); LVEF, left ventricular ejection fraction.

of hypertension, diabetes, smoking and heart rate at rest in the multivariable regression analysis the difference in CFVR between groups was not significant (p=0.213). The model explained only little of the variation in CFVR (R^2=0.07). We constructed a Receiver Operating Curve (ROC) to determine what CFVR diagnostic threshold separate between the symptomatic women versus the asymptomatic women. AUC was 0.726 when including CFVR and the risk factors hypertension, diabetes, age and smoking as predictor variables. Area Under the Curve (AUC) was 0.596 when including CFVR as the sole predictor variable. CFVR=1.625 defined the threshold (the Youden index) that best distinguished the symptomatic and the asymptomatic women. This supports that CFVR is not great at distinguishing between symptomatic and asymptomatic women.

A subanalysis excluding 81 symptomatic women and 4 women from the reference group with lower quality of the CFVR examination gave similar results (not shown).

CFV at rest was higher in symptomatic women than in asymptomatic women, with a median CFV (IQR) of 0.23 m/s (0.19–0.29) versus 0.21 m/s (0.19–0.25) (age-adjusted p value<0.001) (table 2). LVEF was also significantly higher in symptomatic women (mean (SD)=58.3 (5.9)) than in the reference group (mean (SD)=55.6 (4.7), p<0.001) and similarly under hyperaemia. Heart rate did not differ between groups (table 1). The difference between groups in CFV at rest remained significant after adjustment for relevant independent variables including LVEF.

Coronary microvascular function, risk factors and results from stress testing

Twenty-five per cent of the symptomatic women had a CFVR ≤2.0, 35.2% had a CFVR between 2.0 and 2.5, and 39.6% had a CFVR >2.5 (table 2). Lower CFVR was associated with higher age, history of hypertension, diabetes, active smoking and heart rate at rest.

Treatment with aspirin and betablockers was more prevalent with lower CFVR, presumably related to a higher proportion of women with atherosclerosis on ICA. There was no association between impaired CFVR and dyslipidaemia, statin treatment, cerebrovascular disease, peripheral artery disease, body mass index or blood pressure at rest (table 3). The proportion with diffuse atherosclerosis on their ICA was inversely associated with CFVR. Three hundred twenty-four (19%) had undergone CCTA, 183 (11%) SPECT and 481 (29%) bicycle test with more than one test performed in 85 (5%). There was no association between CFVR and calcium score on CCTA, positive SPECT or bicycle stress test, including after multivariable adjustment for risk factor burden. (table 3).

Coronary microvascular function and chest pain symptoms

In symptomatic women, 18% had exercise related angina, 35% had angina at rest and 42% had angina both at rest and during exercise. In 56%, chest pain occurred at least weekly. Chest pain was triggered by dipyridamole infusion in 29% of symptomatic women and resembled habitual pain experience in 23% of symptomatic women (table 4). In reference group women, 5.9% experienced chest pain during dipyridamole infusion (results not shown). Symptom characteristics and burden of symptoms according to the classic classification of angina pectoris and Rose's angina questionnaire did not differ across groups of CFVR. No association was found between impaired CFVR and angina frequency, stability, treatment satisfaction or quality of life assessed by the SAQ. However, participants with low CFVR had significantly higher degree of physical limitation. Results were similar when including only women younger than 62 years. (Results not shown.)

Table 3 Risk factors across level of coronary flow velocity reserve (CFVR) in symptomatic women

	CFVR≤2.0 n=425	2<CFVR≤ 2.5 n=593	CFVR>2.5 n=666	P value*
Self-reported data				
Age, years	65.6 (9.2)	63.0 (9.7)	60.8 (9.3)	<0.001†
Hypertension	273 (64.7)	324 (54.7)	325 (49.3)	0.002
Diabetes mellitus I+II	69 (16.3)	73 (12.4)	60 (9.1)	0.001
Smoking	76 (18.0)	100 (17.0)	94 (14.3)	0.001
Heart rate, beats/min	72.3 (11.6)	71.1 (10.7)	69.0 (10.3)	<0.001
Dyslipidaemia	283 (67.1)	357 (60.5)	402 (61.2)	0.649
Family history	201 (48.9)	311 (53.7)	370 (57.9)	0.074
Cerebrovascular disease	43 (10.2)	43 (7.3)	46 (7.0)	0.257
Peripheral artery disease	36 (8.6)	32 (5.4)	32 (4.9)	0.136
Clinical assessments				
Body mass index, kg/m^2	27.4 (5.7)	26.9 (5.2)	27.1 (5.2)	0.186
Abdominal circumference, cm	97.7 (14.3)	96.2 (14.1)	96.0 (14.4)	0.070
Systolic BP at rest, mm Hg	132.7 (21.1)	130.6 (21.0)	130.9 (20.9)	0.441
LVEF at rest	54.5 (15.3)	56.3 (13.6)	55.8 (12.7)	0.105
Medication				
ASA	199 (47.0)	240 (41.0)	236 (35.9)	0.013
Beta-blocker	143 (33.8)	175 (29.9)	151 (22.9)	0.006
Calcium antagonist	113 (26.7)	124 (21.2)	141 (21.4)	0.417
ACE-I/ARB	156 (39.2)	204 (34.9)	192 (29.4)	0.068
Statin	242 (57.2)	282 (48.2)	318 (48.3)	0.269
Results of diagnostic tests‡				
Atherosclerosis at ICA (n=1577)	191 (47.4)	219 (39.8)	190 (30.4)	<0.001
CCTA (n=324)	70 (16.8)	124 (21.2)	130 (19.7)	0.303
CT calcium score, HU, mean (SD)	408.1 (805.4)	280.3 (457.7)	259.5 (391.8)	0.434
Positive exercise bicycle test (n=481)	85 (71.4)	132 (74.6)	131 (70.8)	0.767
Positive SPECT (n=183)	52 (86.7)	49 (86.0)	52 (78.8)	0.254
Positive stress test (n=579)	116 (76.3)	166 (79.0)	162 (74.7)	0.604

Values are mean (SD) or number (%) across level of CFVR, unless otherwise indicated.
*P value from age-adjusted logistic or linear regression analyses.
†Unadjusted p value.
‡(n =): number of participants who had the given test performed. Stress test, n=participants who had ≥1 test (bicycle and/or single photon emission CT (SPECT)) performed.
ACE-I, ACE inhibitor; ARB, angiotensin-II receptor blocker; BP, blood pressure; CCTA, coronary CT angiography; HU, Hounsfield units.;

DISCUSSION

We hypothesised that CMD is a frequent cause of angina pectoris due to myocardial ischaemia, and that angina caused by CMD would be predominantly exercise induced and associated with pathological cardiac stress testing. We found a higher prevalence of impaired CFVR in women with angina than in asymptomatic women. Impaired CFVR was related to a higher prevalence of risk factors but not to positive bicycle or SPECT stress tests nor to exercise induced angina.

Coronary microvascular function in symptomatic and asymptomatic women

The prevalence of CMD in this study was similar to that reported in other studies of patients with angina, regardless of whether the threshold for CMD is set at 2 or 2.5.[3 6 13] Although CFVR was reduced in symptomatic women compared with asymptomatic women, this difference between groups was rather small and seemed to be explained by the presence of hypertension, diabetes and smoking as well as age and heart rate at rest. It is noteworthy that, as has been shown in other studies,[26 27] impaired CFVR is common among asymptomatic individuals and seems more related to risk factors than to symptoms. This does not rule out that CMD is a cause of symptoms in a proportion of the women but indicates that the relation between CMD and symptomatic ischaemia is not straightforward.

CMD and obstructive CAD have risk factors in common,[29] and among the symptomatic women atherosclerosis on

Table 4 Symptoms according to level of coronary flow velocity reserve (CFVR) in symptomatic women

	CFVR≤2.0 n=425	2<CFVR ≤2.5 n=593	CFVR>2.5 n=666	P value*
Classic chest pain classification, n (%)				
Typical AP	84 (19.8)	118 (19.9)	154 (23.1)	0.104
Atypical AP	208 (48.9)	288 (48.6)	279 (41.9)	
Non-cardiac chest pain	133 (31.3)	187 (31.5)	233 (35.0)	
Rose's Angina Questionnaire, n (%)				
Severe definite AP	80 (19.9)	98 (17.8)	126 (20.0)	0.850
Non-severe definite AP	102 (25.4)	138 (25.0)	159 (25.3)	
Non-definite AP	220 (54.7)	316 (57.2)	344 (54.7)	
Seattle Angina Questionnaire, mean (SD)				
Physical limitation	70.2 (22.5)	74.0 (23.0)	76.2 (22.7)	<0.001
Angina stability	62.3 (28.3)	63.0 (28.4)	63.9 (27.8)	0.133
Angina frequency	75.1 (27.1)	76.2 (28.4)	76.4 (26.1)	0.177
Treatment satisfaction	67.6 (38.7)	66.2 (39.3)	66.5 (25.5)	0.656
Perception/quality of life	53.7 (21.6)	54.6 (21.1)	54.0 (22.1)	0.230
Further chest pain classification, n (%)				
Chest discomfort at exertion	84 (20.8)	109 (19.2)	103 (16.3)	0.285
Chest discomfort at rest	139 (34.5)	204 (35.9)	250 (39.6)	
Chest discomfort at exertion and rest	180 (44.7)	255 (44.9)	279 (44.1)	
Chest discomfort during dipyridamole intravenous	118 (29.4)	179 (31.1)	194 (30.0)	0.580
Reproduced symptoms during dipyridamole	98 (24.9)	145 (25.8)	152 (23.9)	0.401
Weekly chest discomfort	252 (59.3)	311 (52.4)	380 (57.1)	0.614

*P value from age-adjusted trend test (logistic or linear regression analysis) or χ^2 test when the independent variable is divided into three categories.
AP, angina pectoris.

angiography was associated with impaired CFVR. Risk factors affect CMD beyond propagating atherosclerosis in epicardial vessels: hypertension is thought to induce CMD by capillary rarefaction[1] and remodelling of small arteries leading to arteriolar constriction and reduced microvascular density.[30] Ageing induces arterial wall stiffening, thickening of the media layer and lumen enlargement which may result in increased pulse pressure, hypertrophy of arteries and finally in endothelial dysfunction, dysregulation of ventricular-aortic coupling and subendocardial hypoperfusion—all contributing to CMD and in some cases to also to ischaemic angina.[31] Chronic hyperglycaemia may lead to CMD by reducing coronary vasodilator capacity.[32] Thus, risk factors clearly play a detrimental role in CMD but explained only a small part of the variation in CFVR.

Coronary microvascular function and angina typicality

We have previously reported that impaired CFVR was not associated with angina symptoms.[10] We now confirm this finding in a larger population. The symptom burden in the iPOWER population was similar to that reported in studies of patients with obstructive CAD. In the ORBITA (Percutaneous coronary intervention in stable angina) and COURAGE (Clinical Outcomes Utilizing Revascularization and Aggressive Drug Evaluation) trials, patients with CAD receiving optimal medical treatment and no percutaneous coronary intervention had similar scores on the SAQ physical limitation scale and angina stability scale[33] and comparable scores on the frequency and disease perception scales.[34]

In a situation with increased demand, such as during physical exercise, the microvasculature is pivotal in meeting this demand by increasing flow up to 3–5 times.[2] When the coronary microvasculature is dysfunctional and the increase in flow limited, regional or global ischemia can result, similar to flow limitations in epicardial vessels.[35] MVA is therefore described as a condition with typical chest pain[11] and we expected women with impaired CFVR to be more likely to have symptoms during exercise than at rest.

However, angina symptoms have shown to be a poor predictor of ischaemia. Data from the CONFIRM (COronary CT Angiography EvaluatioN For Clinical Outcomes: An InteRnational Multicenter) of more than 10.000 patients (both sexes) revealed that among patients with calcium score of 0 (who were predominantly younger women with low cardiovascular risk factor burden) angina characteristics could not predict significant obstructive CAD by CCTA.[36] The ORBITA trial showed that even though PCI improved objective signs of ischaemia, improvement of angina symptom burden was equal in

the control group participants receiving sham PCI, which demonstrates the importance of placebo in pain perception.[33] Our results in this present study support this, as we found no association between angina typicality and impaired CFVR. Nor did the burden of symptoms vary with degree of microvascular impairment, including in a subgroup of younger women.

A recent smaller study, found that among women with CMD, 39% had several episodes of objective, asymptomatic ischaemia on 24 hours ECG, compared with no episodes in the control group of healthy female peers. Moreover, symptom recordings in the women with CMD had no relation to episodes of objective ischemia.[37] This supports that silent ischaemia is a very prevalent condition in CMD and may contribute to the lack of relation between symptoms and impaired CFVR in symptomatic women in our present study. Also, since many consider a CFVR <3.0 as reduced, silent ischaemia may be present in a proportion of reference group women although they did not have diabetes. However, in the symptomatic group, a CFVR <2, positive stress test and few symptoms indicating silent ischaemia in CMD, was not over-represented among women with diabetes. We had expected so, because diabetes is highly associated with silent ischaemia.[38]

CMD and results of stress testing

Recently, it was suggested that a uniform definition of MVA in addition to symptoms, verified CMD and absence of obstructive CAD, should include objective evidence of myocardial ischaemia from ECG or imaging during stress.[12] We would therefore expect a greater proportion of women with angina and CMD to also have a pathological stress test compared with women with angina and normal coronary microvascular function. However, we found no relation between impaired CFVR and signs of ischaemia on functional testing. In patients with chest pain and non-obstructive CAD, non-invasive stress tests were poor predictors of CMD.[13 39] In CMD, ischaemia may be distributed throughout the myocardium or may affect only the subendocardium and therefore be difficult to visualise by functional stress testing requiring different regional distribution of blood flow.[1]

In the present study, symptomatic women had significantly more dipyridamole-induced chest pain than references. Literature supports that adenosine/dipyridamole infusions cause angina-like-symptoms in some patients, even with normal myocardial perfusion, because adenosine stimulates P1 receptors in the nociceptor pathway. Although not caused by ischaemia, a study has demonstrated less severe adenosine-induced chest pain in patients with silent ischaemia than in patients with stable angina pectoris.[40] This suggests a possible synergistic effect of ischaemic angina and non-ischaemic chest pain induced by adenosine stimulation.

Strengths and limitations

We recruited participants consecutively among all women with angina referred for invasive assessment in a large well-defined area of Denmark and applied uniform inclusion and exclusion criteria. This resulted in a study population less subject to selection bias and makes results more applicable to the general angina population. Similarly, the reference group was randomly selected with risk factor distribution reflecting the background population. Matching asymptomatic women with symptomatic women on cardiovascular risk factors was not possible in this study but should be considered in a future study.

As argued in the discussion, angina is multicausal and susceptible to placebo. A prior study found that treatment with ranolazine modestly but significantly improved objective signs of myocardial ischaemia and angina symptoms.[41] This study design was elegant; double-blinded, placebo-controlled, cross-over study. Such a study design is needed to draw conclusions about a causal relation between angina and CMD.

CMD may be overestimated and underestimated in our population due to methodological limitations. Adenosine and dipyridamole are commonly used vasodilators for inducing hyperaemia. We infused high-dose dipyridamole, 0.140 mg/kg/min for 6 min, corresponding to a total administration of 0.84 mg/kg. This dose has shown to be effective for inducing maximal hyperaemia in stress testing and equally potent as the equivalent dose of adenosine.[42] We measured CFV continuously throughout the 6 min infusion. Maximal hyperaemia was obtained and several Doppler flow curves at maximal hyperaemia without further increase was documented before the end of infusion. We thoroughly checked that participants were abstinent from coffee or other sources of caffeine before the examination. Therefore, we find it unlikely, that false-positive cases of CMD due to inadequate stress response was a problem of concern in this study.

Intravenously administered dipyridamole induces primarily non-endothelium dependent vasodilation. Both epicardial and microvascular spasms in response to acetylcholine provocation have been found to be related to chest pain characteristics.[4 14] We did not assess endothelial dependent vasoreactivity by acetylcholine stimulation. Thus, our results apply only to non-endothelial dependent CMD and does not include patients with vasospastic angina due to epicardial or microvascular endothelial dysfunction. Moreover, CFVR is a surrogate marker of total myocardial blood flow and measured in the LAD territory only, possibly overlooking impaired CFVR caused by ischaemia in non-LAD territories.

By excluding women with obstructive CAD, we might have underestimated associations with CMD because obstructive CAD and CMD often coexist. Thus, findings apply to the population referred for invasive assessment in whom obstructive CAD has been ruled out. In these women, a positive stress test and typical angina do not identify those with CMD.

CONCLUSION

Impaired CFVR was more frequent in symptomatic women compared with a reference group of asymptomatic women

and related to the presence of cardiovascular risk factors. We found no association between degree of impaired CFVR and symptom characteristics, symptom burden or results from diagnostic stress testing. To determine the causality of angina in CMD, randomised, placebo-controlled intervention trials are needed, documenting effect on both angina burden and CMD, determined by the measurement of both endothelial dependent and non-endothelial dependent vasodilation.

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Contributors KBB participated in data collection and echocardiographic analyses, performed the analysis and interpretation of data and drafted this manuscript. EP designed the iPOWER study, participated in drafting this manuscript and revised it critically. HES, DFB, MMM, NDM, AA and JS participated in data collection and echocardiographic analyses and revised this manuscript critically. JK and IG participated in designing the iPOWER study and revised this manuscript critically.

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Competing interests None declared.

Patient consent for publication Not required.

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Provenance and peer review Not commissioned; externally peer reviewed.

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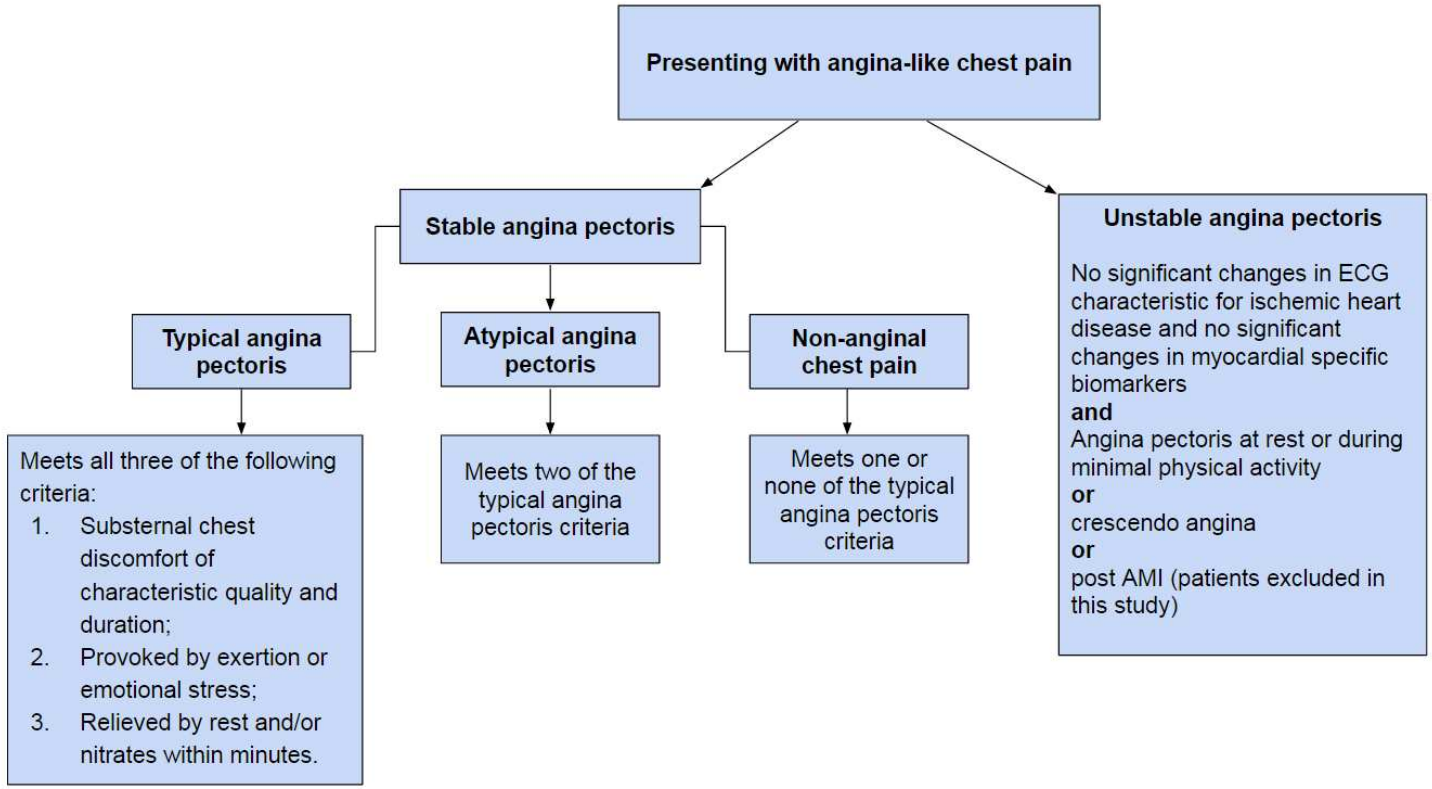
Online Figure 1. Definitions of chest pain characteristics



Online Table 1. Explanation of questionnaire categories.

Rose's Angina Questionnaire	
Category	**Answer to question**
Severe definite angina / Angina Grade II	Participants in this category have quoted the following answers: "yes" to a. Pain location stated as sternum or left anterior chest and left arm. "yes" to b, c and e. "Stop" or "slow down" to d. "10 minutes or less" to f. <u>Question:</u> a: "have you ever had any pain or discomfort in your chest?" b: "do you get this pain or discomfort when you walk uphill or hurry?" c: "do you get it when you walk at an ordinary pace on the level?" d: "When you get any pain or discomfort in your chest what do you do?" e: "does it go away when you stand still?" f: "how soon?"
Non-severe definite AP / Angina Grade I	"No" to c instead of "yes"
Non-definite AP	Participant answers do not fulfill the definite angina criteria
Seattle Angina Questionnaire	
Category	**Question**
Physical limitation	How much a patient's condition hampers her physical abilities
Angina stability	Whether a patient's symptoms are changing over time
Angina frequency	Present frequency of angina symptoms
Treatment satisfaction	How well a patient understands the treatment she is offered and what she thinks of it
Perception/Quality of life	Overall impact of a patient´s condition on her interpersonal relationships and state of mind