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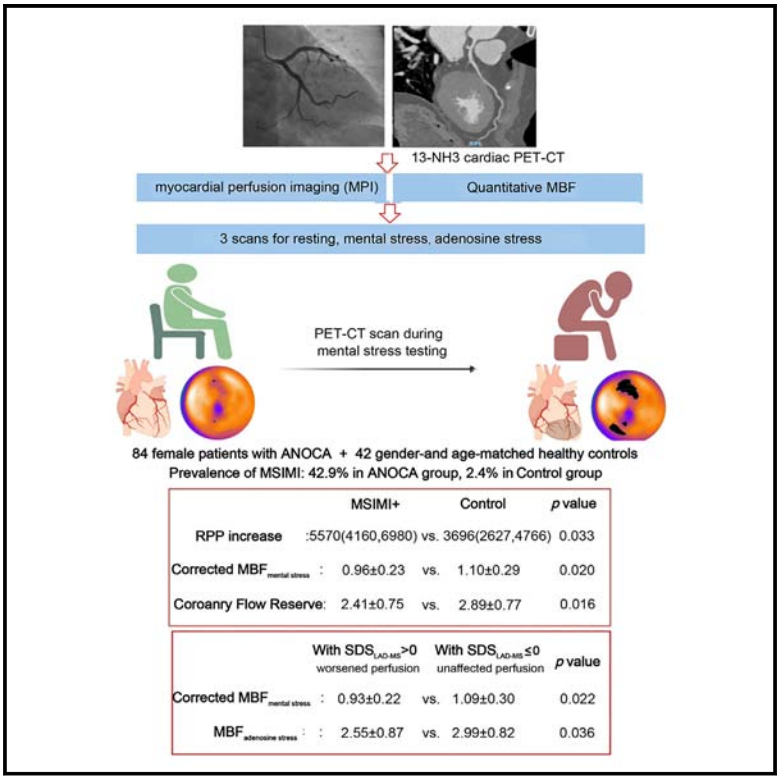
Myocardial blood flow mechanism of mental stress-induced myocardial ischemia in women with ANOCA

Graphical abstract



Highlights

- Around 40% of ANOCA women had MSIMI, vastly higher than that in healthy controls
- ANOCA&MSIMI+ patients had significantly higher CFR and corrected MBF_{MS} than controls
- MBF_{AS} and corrected MBF_{MS} were consistently lower in territories with increased SDS_{MS}
- Impaired coronary microvascular function and blood supply-demand mismatch drive MSIMI

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In brief

Health sciences; Cardiovascular medicine; Clinical finding; Mental state

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Myocardial blood flow mechanism of mental stress-induced myocardial ischemia in women with ANOCA

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SUMMARY

Mental stress-induced myocardial ischemia (MSIMI) is linked to a 2-fold increase in cardiovascular events, but its underlying myocardial blood flow (MBF) mechanisms remain underexplored. Using nitrogen-13-ammonia cardiac positron emission tomography-computed tomography (PET-CT) assessing myocardial perfusion defect and MBF under resting, mental stress (MS), adenosine stress (AS) conditions, angina with no obstructive coronary artery disease (ANOCA) women showed a significantly higher prevalence of MSIMI compared to age-matched healthy controls (36/84 vs. 1/42, $p < 0.001$). The MBF_{AS} and rate-pressure product-corrected MBF_{MS} were consistently lower, especially in the left anterior descending artery territory, in participants with increased perfusion defect scores under MS. The lowest values of restricted coronary flow reserve and corrected MBF_{MS} in participants of ANOCA&MSIMI+ group indicated that impaired coronary microvascular function and mismatch between myocardial blood supply and demand together constitute the pathogenic mechanism of MSIMI in ANOCA population. These findings deepen our understanding of the pathophysiological mechanisms of MSIMI and confirm the long-standing hypothesis of the involvement of impaired coronary microvascular function.

INTRODUCTION

Mental stress-induced myocardial ischemia (MSIMI) refers to the phenomenon where detectable imaging evidence of ischemia or functional changes indicative of ongoing ischemia occur in the heart under mental stress (MS).[1,2] Previous literature has found that approximately one-third of patients with obstructive coronary artery disease (CAD) experience MSIMI.[3,4] It is more common in women and is associated with nearly a 2-fold increased risk of adverse cardiovascular events.[5] However, due to the limitations of the detection methods employed in previous studies (such as echocardiography, single-photon emission computed tomography [SPECT], etc.), the mechanisms of myocardial blood flow (MBF) in MSIMI patients still remained unexplored.

Coronary microcirculation dysfunction (CMD) is proposed as an important pathological basis for the occurrence of MSIMI,[6,7] but this hypothesis has not been tested. A previous study found that blunt increase in MBF under MS in non-obstructive coronary vessels of CAD patients was related to microvascular dysfunction.[7] Another fact is that the phenomenon of MSIMI is observed not only in patients with CAD, but also in individuals with normal epicardial coronary arteries,[8] which also suggests the involvement of CMD.

Angina with no obstructive coronary artery disease (ANOCA) refers to a category of patients who exhibit angina symptoms but do not have obstructive CAD. It is an early symptomatic diagnosis that requires further examination, but routine tests may not adequately explain its causes.[9] ANOCA occurs in about 50% of

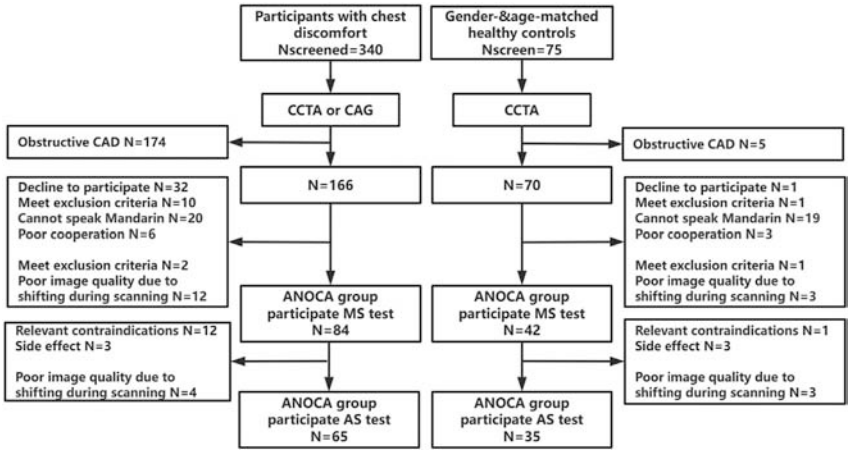

Figure 1. Flow chart for participants enrollment

Abbreviation: CCTA, coronary computed tomography angiography; CAG: coronary angiography; ANOCA, qngina with no obstructive coronary artery disease; CAD, coronary artery disease; AS: adenosine stress; MS: mental stress.

individuals undergoing coronary angiography.[10,11] It is also more common in women, associated with an increased risk of adverse cardiovascular outcomes,[11] and significantly impact quality of life.[12] Some studies also suggest that CMD may be associated with angina symptoms experienced by ANOCA patients.[10] However, it is still unknown whether MSIMI plays a role in the development of symptoms in ANOCA patients.

Therefore, this study aims to utilize 13-NH3 (nitrogen-13-ammonia) cardiac positron emission tomography-computed tomography (PET-CT) to assess MBF and perfusion changes in ANOCA subjects and age-matched healthy controls under MS and adenosine stress (AS). Our objective is to investigate the prevalence of MSIMI in women with ANOCA and explore the underlying blood flow mechanisms of MSIMI, in order to provide new insights into the pathological mechanisms and treatment strategies for MSIMI.

RESULTS

Clinical characteristics of participants

A total of 84 women with ANOCA and 42 age-matched healthy women were included in analyses (Figure 1). All participants were hospitalized and underwent 3 PET scans for resting, MS and AS states (Figure 2). Detailed clinical characteristics of participants were presented in Table 1. The mean age was 53.5 ± 8.6 years in ANOCA group and 53.4 ± 8.5 years in control group. No differences were found between ANOCA and control group in the degree of coronary artery stenosis (p = 0.722). Angina discomfort was reported by 71.4% (n = 60) and 28.6% (n = 24) of ANOCA participants in Canadian Cardiovascular Society grade I and II, respectively.

As compared to the control group, ANOCA group demonstrated a heightened prevalence of hypertension (p = 0.002) and a notably poorer psychological state (with higher HADS-D, HADS-A, and PSS-14, all $p < 0.001$).

Occurrence of MSIMI

The prevalence of MSIMI was significantly higher in ANOCA group at 42.9% (36/84) compared to only 2.4% (1/42) in control group ($p < 0.001$). The increase in summed difference score (SDS_{MS}) was also supported by the changes of total perfusion defect (TPD) (Table S1). In the comparisons between ANOCA&MSIMI+ and ANOCA&MSIMI− participants, no difference was found in clinical features including psychological state, marital status, and menopausal rates (Table 1).

In fact, the MS induced perfusion defects occurred not only in ANOCA&MSIMI+ group (SDS 4.83[95% CI (4.10, 5.56)]), but also in ANOCA&MSIMI− (SDS 0.63[95% CI (0.40, 0.85)]) and control group (SDS 0.45[95% CI (0.18, 0.79)]). Similar results were found in the analyses of the SDS changes in different coronary territories (Table S1) and cardiac segments (Table S2; Figure S1), hinting an overall relative insufficient myocardial blood supply.

Specifically, the areas supplied by the left anterior descending artery (LAD) and right coronary artery (RCA) showed more pronounced perfusion defects compared to the area supplied by the left circumflex artery (LCX) (Table S1). From a spatial perspective, these myocardial perfusion reductions induced by MS occurred more frequently in the apical and mid-ventricular segments, rather than the basal segments (Table S3; Figure S2).

Subtle changes in cardiac function indicators in MSIMI

Overall, the changes in abnormal wall motion scores under MS were very mild. The cardiac wall motion, Wall Motion Score (WMS), remained statistically unchanged in MS test in each group (ANOCA&MSIMI+, ANOCA&MSIMI−, and control) (Table 2). However, the only 4 individuals with WMS increased by $\geq$ 2 point were all in ANOCA&MSIMI+ group.

Under MS, individuals in the ANOCA&MSIMI+, ANOCA&MSIMI−, and control groups displayed a graded reduction in hemodynamic reactivity (Tables 3 and S4). Possibly due to the non-obstructive coronary arteries and elevated hemodynamic reactivity, phenomena of MSIMI being associated with decreased left ventricular ejection fraction (LVEF) in CAD patients were not observed in our research. Instead, we noticed a slight increase in LVEF and a significant decrease in end-systolic volume (ESV) in the ANOCA&MSIMI+ group (Tables 2 and S4), suggesting a more powerful contraction of the heart and more intensive myocardial oxygen demand in MSIMI patients under MS.

During the MS testing, 8 out of 36 MSIMI patients exhibited dynamic ischemic changes on electrocardiograph (ECG) monitoring, while 1 case was observed in both the MSIMI− and control groups. Although the chest pain scores for the ANOCA&MSIMI+ and ANOCA&MSIMI− groups were significantly higher than those for the control group (both $p < 0.001$), the difference between the ANOCA&MSIMI+ and ANOCA&MSIMI− groups was not statistically significant (Tables 3 and S4).

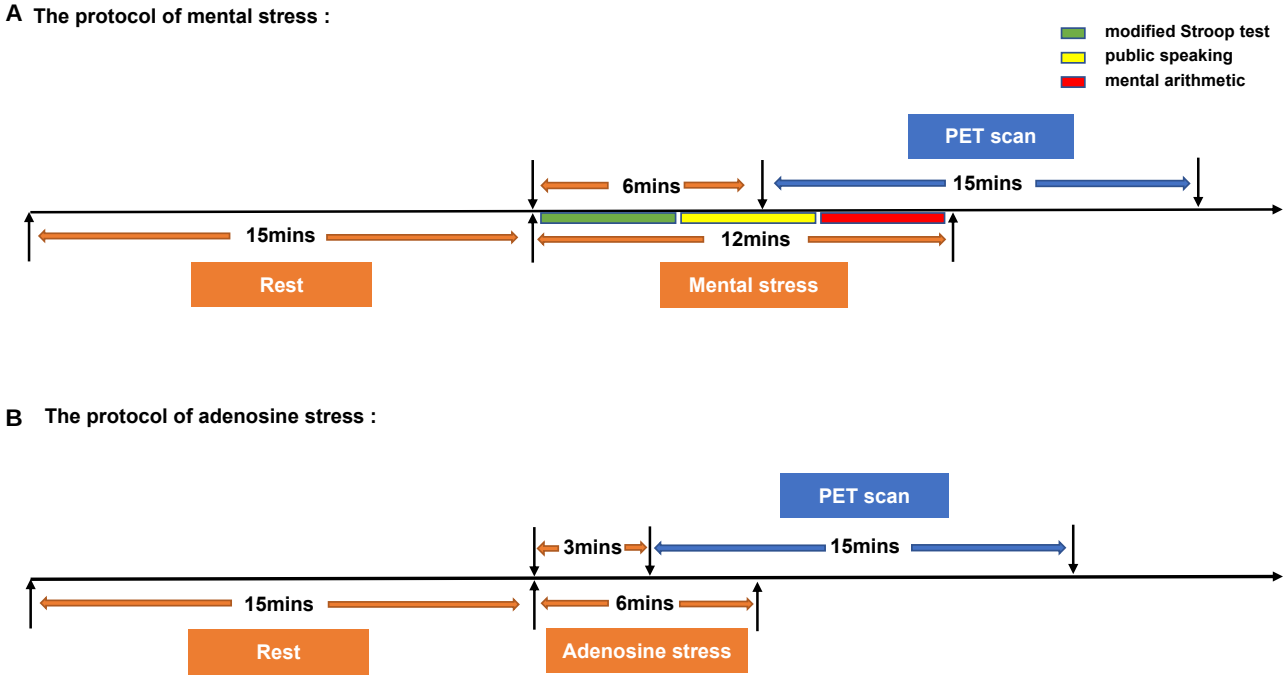

Figure 2. Protocol of mental stress and adenosine stress tests

(A) Mental stress test (n = 126).

(B) Adenosine stress test (n = 100).

Imbalance between MBF_{MS} and oxygen demand

Globally, a strong correlation between MBF_{MS} and rate pressure product (RPP_{MS}) was also observed across all three groups in both univariate and multivariate linear regression analyses (all $p < 0.05$) (Table S5), indicating a natural increase in MBF_{MS} occurs in response to the heightened demand for physiological oxygen consumption. However, there were no significant differences in the absolute MBF_{MS} and relative changes of MBF (myocardial flow reserve [MFR]) among ANOCA&MSIMI+, ANOCA&MSIMI−, and control groups (Table 2).

We attempted to use RPP_{MS} corrected MBF_{MS} as an indicator of the balance between myocardial blood supply and oxygen demand. It was revealed that all three groups experienced a significant decrease in MBF under equivalent cardiac workload (all $p_{\Delta corrected\ MBF\text{-}MS} < 0.001$), with the most pronounced reduction observed in the ANOCA&MSIMI+ group (Tables 3 and S4). A gradient increase in corrected MBF_{MS} from the ANOCA&MSIMI+ group to control group was observed (Tables 3 and S4; Figure S3). Participants in the ANOCA&MSIMI+ group exhibited significantly lower corrected MBF_{MS} than those in control group (ANOCA&MSIMI+: 0.96 ± 0.23 vs. control: 1.10 ± 0.29, $p = 0.020$).

In the comparisons focused on coronary territories regarding the increase in perfusion defect severity under MS in ANOCA patients, the corrected MBF_{MS} in participants with increased SDS were consistently lower than in those without an increase in defect severity (Figure S4). The difference was most pronounced in the LAD territory ($p = 0.022$). The LCX and RCA territories did not reach statistical significance, possibly due to the lower frequency of increased perfusion defects or relatively lower MBF_{MS} in these regions.

Impaired coronary flow reserve in MSIMI

Overall, ANOCA&MSIMI+ group showed a higher incidence of CMD (9/29, 31.0%) compared to ANOCA&MSIMI− (7/36, 19.4%) and control group (3/35, 8.6%) ($p_{for\ trend} = 0.023$). The global coronary flow reserve (CFR), indicative of microvascular function, demonstrated a gradient increasing trend across the ANOCA&MSIMI+, ANOCA&MSIMI−, and control groups. Participants in the ANOCA&MSIMI+ group exhibited a lower CFR compared to the control counterparts (ANOCA&MSIMI+: 2.41 ± 0.75 vs. control: 2.89 ± 0.77, $p = 0.016$) (Table 2). Similar findings were also noted in the analyses of CFR within different coronary artery territories (Table 4; Figure S5). Figure S6 depicted the linear fitting of MBF in individuals before and after AS. The results demonstrated a significant linear correlation between MBF_{rest} and MBF_{AS} exclusively in control group (r = 0.43, $p = 0.009$), and a marginally significant linear correlation in ANOCA&MSIMI− group (r = 0.29, $p = 0.084$). These findings remained consistent after adjusting for covariates including age, hypertension, and diabetes (Table S6).

Linear correlation between MBF_{MS} and MBF_{AS} was depicted in Figure S7. Taking adenosine-induced hyperemia as a benchmark for maximum MBF reserve capacity, only participants in ANOCA&MSIMI+ group failed to demonstrate a comparable increase in MBF_{MS} after adjusting for confounding factors (Table S7).

In comparing CFR between participants with and without increased coronary defect severity under MS in ANOCA patients, the mean CFR in territories with increased severity was lower, although these differences did not reach statistical significance (Table S8). However, when comparing MBF, while

Table 1. Clinical characteristics of participants

	ANOCA			Control	*p* value[b]
	MSIMI +	MSIMI −			
	n = 36	*n* = 48	*p* value[a]	*n* = 42	–
Age, year	54.0 ± 8.8	53.2 ± 8.5	0.654	53.4 ± 8.5	0.935
Canadian angina rating (CCS), *n* (%)	–	–	0.19	–	<0.001
I	23 (63.9)	37 (77.1)	–	0 (0)	–
II	13 (36.1)	11 (22.9)	–	0 (0)	–
Angiographic data, *n* (%)	–	–	0.248	–	0.722
Without visible stenosis	28 (77.8)	42(87.5)	–	36 (85.7)	–
Maximum stenosis ≤ 30%	5 (13.9)	4(8.3)	–	4 (9.5)	–
30% < Maximum stenosis < 50%	3 (8.3)	2(4.2)	–	2 (4.7)	–
Psychological profile	–	–	–	–	–
HADS-depression score	6.0 (3.0–9.0)	7.0(3.0,9.0)	0.885	2.5 (1.0–5.0)	<0.001
HADS-anxiety score	8.0 (6.0–10.0)	7.0(55.0,10.5)	0.496	3.0 (1.0–5.0)	<0.001
PSS-14 score	27.0 (20.5–33.0)	27.0(21.0,32.0)	0.957	18.0 (15.0–22.0)	<0.001
Educational level, *n* (%)	–	–	0.109	–	0.734
Primary school	4 (11.1)	9 (18.8)	–	7 (16.7)	–
Middle school	7 (19.4)	13 (27.1)	–	6 (14.3)	–
Senior middle school	9 (25.0)	12 (25.0)	–	14 (33.3)	–
Graduate and above	16 (44.4)	14 (29.2)	–	15 (35.7)	–
Income per month, *n* (%)	–	–	0.088	–	0.933
<3000¥	9 (25.0)	19 (39.6)	–	12 (28.6)	–
3000¥–7000¥	13 (36.1)	18 (37.5)	–	18 (42.9)	–
7000¥–20000¥	9 (25.0)	7 (14.6)	–	10 (23.8)	–
>20000¥	5 (13.9)	4 (8.3)	–	2 (4.8)	–
Married, *n* (%)	34 (94.4)	43 (89.6)	0.693	36 (85.7)	0.469
Menstrual history: menopausal, *n* (%)	23 (63.9)	31 (64.6)	0.948	28 (66.7)	0.792
Comorbid disorders, *n* (%)	–	–	–	–	–
Hypertension	10 (28.8)	13 (27.1)	0.824	1 (2.4)	0.002
Diabetes	3 (8.3)	4 (8.3)	1	1 (2.4)	0.196

Continuous variables are represented as mean ± SD or median (IQR).
CCS, Canadian Cardiovascular Society; SD, standard deviation; IQR, interquartile range.
[a]*p* value for comparisons between participants with and without MSIMI in ANOCA group.
[b]*p* value for comparisons between ANOCA group and control group.

MBF_{rest} and MBF_{MS} showed no significant differences between participants with and without increased coronary defect severity, MBF_{AS} demonstrated significant differences, particularly in the LAD territory ($p = 0.036$) (Figure 3), suggesting the involvement of a reduction in flow reserve.

Redistribution of MBF in coronary territories

We calculated the extent of hyperemia for different coronary artery territories in each subject with the maximum MBF under AS as the reference (Tables 4 and S9). The ANOCA&MSIMI+, ANOCA&MSIMI−, and control groups all exhibited an increase in the MBF ratio (MBF/MBF_{AS}) from resting state to MS state in the left coronary artery territories (both LAD and LCX). However, there was a more significant increase of hyperemia extent in LCX territory than LAD territory in ANOCA&MSIMI+ group. This finding was consistent with that myocardial perfusion defects more frequently occurred in the LAD, rather than LCX territory. Considering that both LCX and LAD are branches originating from the left main coronary artery, the higher hyperemia extent in the LCX territory under MS in ANOCA&MSIMI+ group may indicate increased vascular resistance in the LAD territory, and blood flow redistribution is involved in the pathogenesis of MSIMI.

DISCUSSION

This study employs 13-NH3 cardiac PET-CT, the gold standard for non-invasive MBF measurement, to examine the prevalence of MSIMI and its underlying MBF mechanism in a population of women with ANOCA. It unveils that the mismatch between myocardial blood supply and demand, along with limited CFR, collectively constitutes the occurrence of MSIMI. These findings deepen our understanding of the pathophysiological mechanisms of MSIMI, confirm the long-standing speculation about

Table 2. Comparison of myocardial perfusion, myocardial blood flow, and cardiac function parameters

	ANOCA MSIMI+		ANOCA MSIMM-		Control	
	resting	MS state	resting	MS state	resting	MS state
Myocardial Perfusion imaging						
Perfusion defect score	0.58 ± 1.34	5.42 ± 2.35	0.42 ± 0.68	1.04 ± 1.05	0.24 ± 0.66	0.69 ± 0.90
SDS (ΔPerfusion defect score)	4.83(4.10, 5.56)***		0.63(0.40, 0.85)***		0.45(0.18, 0.73)**	
Myocardial MBF quantification						
MBF, ml/g/min	1.03 ± 0.21	1.25 ± 0.39	1.09 ± 0.32	1.29 ± 0.48	1.06 ± 0.26	1.26 ± 0.51
ΔMBF, ml/g/min	0.23(0.10, 0.35)***		0.21(0.09,0.32)***		0.20(0.05, 0.35)**	
corrected MBF, ml/g/mmHg	1.30 ± 0.29	0.96 ± 0.23	1.37 ± 0.34	1.05 ± 0.29	1.37 ± 0.36	1.10 ± 0.29
Δcorrected MBF, ml/g/mmHg	−0.34(-0.45, −0.24)***		−0.31(-0.42, −0.21)***		−0.26(-0.40, −0.13)***	
Myocardial flow reserve (MFR)[a]	1.24 ± 0.36		1.20 ± 0.32		1.21 ± 0.43	
Coronay flow reserve (CFR)[b]	2.41 ± 0.75		2.66 ± 0.81		2.89 ± 0.77	
Coronary microvascular dysfunction (CMD)	9/29(31.0)		7/36(19.4)		3/35(8.6)	
Adenosine induced myocardial ischemia (ASIMI)	3/29 (10.3)		0/36 (0)		0/35 (0)	
Cardiac function assesment						
Wall motion score	0.69 ± 1.43	0.83 ± 1.48	0.20 ± 0.40	0.23 ± 0.47	0.22 ± 0.53	0.10 ± 0.37
ΔWall motion score	0.14(-0.50,0.78)		0.04(-0.10 , 0.18)		−0.13(-0.32,0.07)	
ΔEDV, ml	−3.69(-5.92,1.47)**		0.09(-1.25,1.42)		0.88(-1.51,3.26)	
ΔESV, ml	−2.92(-4.26,-1.58)***		−1.07(-1.72,-0.42)**		0.95(-0.60,2.50)	
ΔSV, ml	−0.89(-2.67,0.89)		1.29(0.03,2.55)*		−0.05(-2.80,2.70)	
ΔLVEF, %	2.50(1.10, 3.90)***		1.80(1.01, 2.59)***		−1.28(-4.31, 1.76)	

Continuous variables are represented as mean ± SD. Changes in mental stress test are represented as mean(95% CI).
Note: Corrected MBF is calculated as MBF/RPP for resting and mental stress conditions.
*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, paired t test.
MS: mental stress; SDS, summed difference score; MBF, myocardial blood flow; EDV, end-diastolic volume; ESV, end-systolic volume; SV, stroke volume; LVEF, left ventricular ejection fraction; SD, standard deviation; CI, confidence interval.
[a]MFR refers to MBF_{MS}/MBF_{rest}.
[b]Coronay flow reserve (CFR) refers to MBF_{AS}/MBF_{rest}.

the involvement of impaired coronary microcirculation function, and provide insights for the prevention and treatment of MSIMI.

Our study revealed that the prevalence of MSIMI in ANOCA reached 42.9%. This is higher than the 16% reported by Vaccarino et al.[4] in patients with myocardial infarction, identified by SPECT, and slightly lower than the 43.5% reported by Jiang et al.[3] using trans-thoracic echocardiography (TTE) and ECG in patients with stable CAD.

Regarding the location of MS-induced perfusion defects, they occurred more frequently in the apical and mid-ventricular segments, which was consistent with the outcomes of REMIT study[13] and the common site of wall motion abnormalities in stress-induced cardiomyopathy.[14] However, it should be noted that changes in wall motion abnormality index in our research were mild and not significant in all groups during MS testing. Besides, the phenomenon of decreased LVEF reported in CAD&MSIMI+ patients[3] was not observed.

Previous studies have indicated that in MSIMI patients, relatively elevated troponin levels within the normal range at rest,[15] along with abnormal myocardial systolic and late diastolic velocities, may point to a potential underlying pathology.[6] An intriguing discovery of our research was the worse perfusion defect score and TPD in ANOCA&MSIMI+ participants at rest compared to the control counterparts, which supported the existence of an objective pathological basis for the occurrence of MSIMI.

A linear correlation between hemodynamic reactivity (as measured by rate-pressure product [RPP]) under MS and MBF has been reported by previous researches.[16] Similarly, we also observed a strong correlation between MBF_{MS} and RPP_{MS} across all three groups after adjusting for covariates. The phenomenon likely indicated a natural increase in MBF_{MS} in response to the heightened demand for physiological oxygen consumption.[17] In our study, MSIMI+ patients have exhibited greater heart rate and blood pressure responses under MS. Based on our observation, this may be attributed to their higher proportion of experiencing more traumatic events,[18,19] which could result in a more pronounced emotional response to the stress task. Under heightened myocardial workload, corrected MBF became significantly lower in the ANOCA&MSIMI+ group and in regions of ANOCA participants with increased perfusion defect. These findings suggest that the mismatch between myocardial oxygen demand and blood supply is a crucial mechanism driving the development of MSIMI.[20] This was also in line with findings of Hammadah et al.[21]

Coronary microvascular dysfunction has long been recognized as an important pathological basis for MSIMI.[22] This

Table 3. Hemodynamic response to mental stress, accompanied symptoms, and other objective evidence of ischemia

	ANOCA MSIMI+		ANOCA MSIMM-		Control	
	resting	MS state	resting	MS state	resting	MS state
Hemodynamic measuements						
Heart rate, beat/min	69 ± 10	91 ± 22	70 ± 12	94 ± 22	69 ± 9	87 ± 19
Systolic blood pressure, mmHg	115 ± 12	142 ± 23	115 ± 13	140 ± 20	116 ± 17	131 ± 24
Diastolic blood pressure, mmHg	72 ± 9	87 ± 20	71 ± 9	86 ± 14	71 ± 9	79 ± 15
Rate-pressure Product (RPP), mmHg/min	7978 ± 1179	13548 ± 4591	8026 ± 1788	12704 ± 3807	7953 ± 1720	11649 ± 4221
ΔRPP, mmHg/min	5570(4160, 6980)***		4679(3904, 5454)***		3696(2627, 4766)***	
Accompanied symptoms						
Chest pain score	2.40 ± 2.06		1.94 ± 1.75		(1.08 ± 0.47	
Ischemic ECG monitoring	8/36		1/48		1/42	
Objective ischemia tests						
Ischemia during holter, n (%)	11/31(35.5)		13/42(31.0)		5/41(12.2)	
ESIMI, n (%)	5/34(14.7)		9/47(19.1)		3/42(7.1)	
ESIMI + holter, n (%)	13/29(44.8)		14/42(33.3)		7/41 (17.1)	
ESIMI + MSIMI, n (%)	34/34(100)		9/47(19.1)		4/42 (9.5)	
ESIMI + holter + MSIMI, n (%)	29/29(100)		14/42(33.3)		8/41 (19.5)	

Continuous variables are represented as mean ± SD. Changes in mental stress test are represented as mean(95% CI).
Note: *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, Paired t-test.
MS: mental stress; MBF, myocardial blood flow; ESIMI: exercise (conventional stress) induced myocardial ischemia; SD, standard deviation; CI, confidence interval; ECG: electrocardiograph.

hypothesis is based on the observation that MSIMI can also occur in patients with non-obstructive coronary arteries or in regions supplied by non-obstructive coronary arteries. Additionally, J A Arrighi et al.[7] found that in CAD patients, blunted MBF under MS was associated with reduced CFR. Mads Ersbøl et al.[6] demonstrated that CAD&MSIM+ patients have impaired annular velocities even at rest. Since this subclinical myocardial dysfunction has been reported to be associated with abnormal CFR, their findings indicate microvascular dysfunction to be involved in the pathogenesis of MSIMI.

Our study attempted to investigate CFR in MSIMI by using the adenosine test. Regarding adenosine-induced perfusion defects, ASIMI was observed in only three cases (A03, A19, and A44) (Figure S8). All three participants had ANOCA and MSIMI, but the perfusion defect locations associated with MSIMI and ASIMI did not consistently overlap. By measuring MBF and CFR, our study revealed that ANOCA patients with MSIMI exhibit relatively lower CFR and a poorer correlation between MBF_{rest} and MBF_{AS} as compared to the other 2 groups. More importantly, within the ANOCA group, analyses showed a significant decline of MBF_{AS} in regions with increased perfusion defects induced by MS. Our study is indirectly supported by the findings of Viola Vaccarino and colleagues,[23] who discovered that posttraumatic stress disorder (PTSD) is associated with reduced coronary microcirculatory function and greater deterioration over time. Meanwhile, PTSD is also a significant risk factor for MSIMI.[18,24]

However, it is crucial to point out that although the incidence of CMD in the ANOCA group was marginally higher than in the control group, the difference in CFR between the ANOCA&MSIMI+ and ANOCA&MSIMI− groups was not particularly pronounced. This could be because perfusion defects occur locally, whereas CFR is a global measure. In the comparison of territory MBF based on the presence or absence of new-onset ischemia, we indeed observed a more significant decline in MBF_{AS} within the ischemic regions.

For coronary vasomotion, the functional state of the vascular endothelium,[21,25] the release of neurotransmitters[26] are likely key determinants. In patients with coronary atherosclerosis, reduced nitric oxide (NO) release capacity in narrowed vessels may result in vasoconstriction outweighing vasodilation, leading to a decrease in coronary blood flow in response to the neurotransmitters.[27] The Psychophysiological Investigations of Myocardial Ischemia Study (PIMI) study reveals that the rise in catecholamine levels during MS is linked to increases in heart rate, blood pressure, and systemic vascular resistance.[28] Therefore, under increased cardiac workload and overall reduced relative MBF supply, the stronger catecholamine receptor activation in the ANOCA&MSIMI+ group not only produces positive inotropic effects but, combined with endothelial damage from coronary atherosclerosis, triggers localized myocardial ischemia. During this process, the elevation of resistance, particularly in the LAD, leads to a synchronous increase in MBF supply by redistributing blood flow toward the LCX.

One point that needs to be clarified is that previous studies have clearly established that both the brain and heart are involved in the pathophysiological mechanisms of MSIMI. For example, the notable work by Viola Vaccarino's team show that peripheral vascular reactivity under stress[26] and brain function factors (e.g., cognitive function,[29] post-traumatic stress disorder[23]) are associated with MSIMI. In this study, we have gone beyond our primary objectives by including several secondary endpoints that explore inflammatory and immune markers, sex hormone levels, neuroendocrine indicators,

Table 4. Comparison of MBF, CFR and MBF redistribution in coronary territories in mental stress test

	ANOCA MSIMI+		ANOCA MSIMM-		Control	
	resting	MS state	resting	MS state	resting	MS state
LAD						
Perfusion defect score	0.31 ± 0.89	2.53 ± 1.87	0.19 ± 0.53	0.40 ± 0.82	0.19 ± 0.59	0.38 ± 0.58
MBF_{LAD}, ml/g/min	1.01 ± 0.22	1.25 ± 0.40	1.09 ± 0.33	1.32 ± 0.50	1.06 ± 0.26	1.27 ± 0.51
ΔMBF_{LAD}, ml/g/min	0.24(0.11, 0.36)***		0.24(0.12, 0.36)***		0.21(0.06, 0.36)**	
MFR_{LAD}	1.25 ± 0.36		1.24 ± 0.35		1.22 ± 0.44	
CFR_{LAD}	2.53 ± 0.83		2.77 ± 0.88		2.99 ± 0.83	
$MBF/MBF_{LAD\text{-}as}$,%	44 ± 20	54 ± 27	40 ± 17	49 ± 24	36 ± 11	43 ± 16
$\Delta MBF/MBF_{LAD\text{-}as}$,%	9(4, 15)**		8(2, 14)**		7(2, 12)**	
LCX						
Perfusion defect score	0.03 ± 0.17	0.67 ± 1.22	0.02 ± 0.14	0.10 ± 0.31	0	0.07 ± 0.34
MBF_{LCX}, ml/g/min	1.11 ± 0.22	1.41 ± 0.44	1.13 ± 0.32	1.38 ± 0.49	1.11 ± 0.28	1.38 ± 0.52
ΔMBF_{LCX}, ml/g/min	0.31(0.17, 0.44)***		0.26(0.14, 0.38)***		0.26(0.11, 0.41)**	
MFR_{LCX}	1.29 ± 0.37		1.26 ± 0.34		1.26 ± 0.43	
CFR_{LCX}	2.27 ± 0.68		2.54 ± 0.78		2.79 ± 0.73	
$MBF/MBF_{LCX\text{-}AS}$,%	48 ± 18	60 ± 28	43 ± 17	52 ± 23	39 ± 12	48 ± 17
$\Delta MBF/MBF_{LCX\text{-}AS}$,%	12(6, 18)***		9 (3, 15)**		9(4, 13)***	
RCA						
Perfusion defect score	0.31 ± 0.92	2.22 ± 1.33	0.21 ± 0.46	0.54 ± 0.82	0.05 ± 0.22	0.24 ± 0.58
MBF_{RCA}, ml/g/min	0.93 ± 0.19	1.04 ± 0.35	1.01 ± 0.30	1.06 ± 0.45	0.97 ± 0.27	1.07 ± 0.50
ΔMBF_{RCA}, ml/g/min	0.12(0, 0.23)*		0.06(-0.04,0.16)		0.10(-0.05, 0.25)	
MFR_{RCA}	1.15 ± 0.37		1.06 ± 0.29		1.12 ± 0.46	
CFR_{RCA}	2.30 ± 0.70		2.52 ± 0.72		2.72 ± 0.72	
$MBF/MBF_{RCA\text{-}AS}$,%	48 ± 21	53 ± 29	43 ± 16	45 ± 19	40 ± 13	44 ± 18
$\Delta MBF/MBF_{RCA\text{-}AS}$,%	5(−2, 12)		2(−2, 6)		4(−1, 9)	

Continuous variables are represented as mean ± SD. Changes in mental stress test are represented as mean (95% CI).
Note: *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, paired t test
MS: mental stress; AS: adenosine stress; MBF, myocardial blood flow; MFR, myocardial flow reserve; CFR, coronary flow reserve; LAD, left anterior descending; LCX, left circumflex; RCA, right coronary artery; SD, standard deviation; CI, confidence interval.

proteome expression, and the consistency of novel echocardiographic measurements with and PET-CT parameters.[30] While some individual parameters demonstrated statistically significant differences, they did not appear to have a direct theoretical link to the MBF mechanism and were therefore excluded from the main body of the manuscript. Nonetheless, we have included some of the data in the supplementary material **(**Table S10**)**.

In addition, our study may suggest that MSIMI is probably one of the crucial mechanisms underlying angina occurrence in ANOCA. Although there was no significant difference in self-rated chest pain scores between MSIMI+ and MSIMI− subjects during MS testing, MSIMI identified objective ischemic evidence in 42.9% of ANOCA individuals. In contrast, conventional stress tests combined with Holter monitoring detected ischemic evidence in only 38.0% of ANOCA individuals. Previous studies have revealed that MSIMI is associated with a retrospectively high frequency of angina,[31] and anginal symptoms in women may be a marker of vulnerability toward ischemia induced by psychologic stress.[32]

The strengths of our study lie in the utilization of 13-NH3 cardiac PET-CT for assessing myocardial perfusion and MBF, as well as the implementation of virtual reality (VR) devices to ensure that each participant received identical objective stimuli. Our findings have a number of implications. Firstly, our findings may imply that beta-blockers and drugs that improve coronary microcirculation function have great potential in the treatment for MSIMI. Secondly, only a portion of the ANOCA&MSIMI+ group met the diagnostic criteria for coronary microvascular disease, which may indicate the necessity to emphasize MSIMI-related pathogenic mechanisms in the treatment of angina. Thirdly, due to the cumulative impact of MSIMI on the cardiovascular system and its important predictive value for adverse cardiovascular prognosis,[5,33] the VR-based standardized MS testing may be a promising new diagnostic tool worth promoting.

In conclusion, our study reveals that the high prevalence of MSIMI in the ANOCA population is attributed to a decrease in CFR and regional coronary blood flow supply and demand mismatch, accompanied by the redistribution of blood flow under MS. These findings confirm the long-standing hypothesis that coronary microvascular dysfunction serves as the pathological basis for MSIMI. Further research is necessary to validate the generalizability of our findings and investigate the brain-heart interaction mechanism in the pathogenesis of MSIMI.

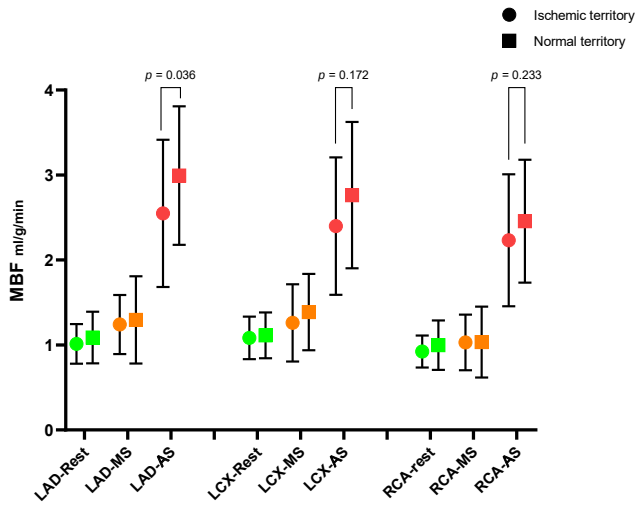

Figure 3. Comparison of MBF between participants with and without increased perfusion defect under mental stress

In the comparison of MBF among participants with ANOCA ($n = 65$), there were no significant differences in MBF_{rest} and MBF_{MS}. However, MBF_{AS} showed pronounced differences between participants with and without increased coronary defect severity (defined by SDS >0) especially in the LAD territory. Data are represented as mean ± SD. p values are calculated with independent t test. Abbreviation: MBF: myocardial blood flow; AS: adenosine stress; MS: mental stress; SDS: summed difference score; LAD: left anterior descending; LCX: left circumflex artery; RCA: right coronary artery; SD: standard deviation.

Limitations of the study

There were several limitations to our study. Firstly, the limited sample size constrained the statistical power of the experiment, leading to the possibility that certain differences, which might have been meaningful, failed to achieve statistical significance in this study. Secondly, In MSIMI patients, the significantly reduced corrected MBF and CFR were predominantly observed in the LAD territory, whereas the differences in the LCX and RCA territories were not as pronounced. This could be attributed to the fact that, in PET/CT myocardial perfusion imaging (MPI), the RCA and LCX territories—especially the RCA—are more susceptible to artifacts or errors. Compared to routine scans, individuals under MS testing are more likely to exhibit unconscious movement. Thirdly, the diagnosis of ANOCA was mainly based on patients' perception of chest discomfort, previous medical history and the judgment of experienced cardiologists, which cannot completely exclude chest discomfort caused by digestive system or mental illnesses. However, the clinical characteristics of the overall sample were consistent with previous literature.[10] Fourthly, since we did not perform the intracoronary acetylcholine provocation test, the association of MSIMI with coronary artery spasm could not be eliminated. Fifthly, theoretically, the MBF in our study was calculated based on data fitting within 2 min before and after injection of 13-NH3, while myocardial perfusion imaging was based on the accumulation of images collected over 15 min. The period quantitating MBF did not strictly match that assessing perfusion defects, a considerable number of participants did not undergo the AS test. Finally, this is single-center clinical research conducted only in ANOCA women, so the generalizability to other populations needs further investigation.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Qingshan Geng (gengqingshan@gdph.org.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data generated or analyzed during this study are included in the manuscript and supplementary tables and figures.
- The VR-integrated program designed to simulate MS stimuli (GDGHhospital.apk) and the mobile-compatible app for VR viewer monitoring (GDGH.apk) can be downloaded from the following link: https://github.com/yinhanii/new/blob/master/GDGHospital.apk.1.1.zip.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.
- All data reported in this paper will be shared by the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Designed the study: H.Y., Y.L., H.M.,W.J., and Q.G.; wrote the manuscript: H.Y., F.L., and B.B.; performed the study: H.Y., F.L., B.B., Q.L., Y.L., H.W., Y.W., Y.Y.L., A.L., X.Y., C.J., C.W., B.K., J.L., L.G., S.W., and H.M.; analyzed and interpreted data: H.Y., F.L., and B.B.; revised and edited the manuscript: S.W., W.J., H.M., and Q.G.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
R Project for Statistical Computing	https://www.r-project.org/	RRID:SCR_001905
Statistical Analysis System	http://www.sas.com	RRID:SCR_008567
VR device based mental stress test program	https://github.com/yinhanii/new/blob/master/GDGHospital.apk.1.1.zip	RRID:SCR_025961

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Participants

In this study, we recruited 84 ANOCA women who met the inclusion criteria and 42 age and gender matched healthy controls at the Guangdong Provincial People's Hospital between June 2019 and April 2021. All of them were of east asian descent. ANOCA was defined as the presence of typical or atypical angina, and without obstructive coronary artery (luminal stenosis $\geq$50%) confirmed by either CAG or coronary computed tomography angiography ,CCTA within the previous year. The main inclusion criteria were female gender, aged between 18 and 75 years old, and confirmed ANOCA. Age-matched healthy female controls (without angina and obstructive coronary artery confirmed by CCTA) were also enrolled in a ratio of 1:2 with the ANOCA group. Key exclusion criteria included chest pain due to non-cardiac circulatory system-related causes, and the use of anti-depressants or antipsychotics in the past month.

Individuals who had contraindications to adenosine, were unable to tolerate adenosine, or had serious concerns about potential side effects were exempted from the AS test. Due to our stringent eligibility criteria for the adenosine test, out of the 126 participants who completed the MS test, only 100 completed the AS test. The 26 participants were excluded due to contraindications (N = 13), adverse side effects (N = 6), or suboptimal image quality resulting from patient movement during the scan (N = 7).

Ethics approval

This study was conducted according to the guidelines of the Declaration of Helsinki. Ethical approval was given by the medical ethics committee of Guangdong Provincial People's Hospital with the following reference number: No. 2019-298H-5.

Informed consent

Informed consent was obtained from all individual participants included in the study.

METHOD DETAILS

Study design

The aims of the study were: 1) to determine the prevalence of MSIMI in women with ANOCA, and 2) to investigate whether changes in MBF during a MS test were associated with the occurrence of MSIMI. A detailed methodology has been previously published.[34]

All participants were hospitalized and underwent 3 PET scans for resting, MS and AS states. Standardized MS and AS tests were conducted in the morning of separate days within a week. The resting-state PET scan could be performed before the stress-state PET scans, but there needed to be a minimum 30-min interval between the two. Before these tests, participants were instructed to discontinue the use of beta-blockers and calcium channel blockers for a period of 3–5 half-lives, and abstain from tobacco and coffee for at least 12 h.

Sociodemographic and psychosocial data were collected from all participants before the tests. Samples of venous blood were drawn in the morning on the first day of admission to establish baseline clinical characteristics. During the PET scans, the electrocardiogram and heart rate were continuously monitored, while blood pressure was recorded once per minute. All study participants provided written informed consent before participating in the study.

Myocardial perfusion imaging and quantitative myocardial blood flow

Cardiac PET imaging was conducted using a Siemens Biograph-16 TruePoint PET-CT scanner (Siemens Healthcare, Knoxville, Tennessee, USA) equipped with the TrueV option, which allowed for a 21.6 cm axial field of view. The PET images were analyzed by two experienced nuclear cardiologists who were blinded to the conditions. Prior to interpretation, the images were checked for errors, such as artifacts or low count density.

The severity of myocardial ischemia (perfusion defects) was evaluated in each state using a 17-segment AHA-defined left ventricular model and a semi-quantitative scoring system for each segment. The scores ranged from 0 (normal) to 4 (no perfusion). The Summed Stress Score (SSS), Summed Resting Score (SRS), and Summed Difference Score (SDS, SDS = SSS - SRS) were calculated globally and in each coronary artery territory. TPD, which indicates ischemic burden, SMS, which indicates the extent of abnormal wall motion, and cardiac function measurements were also automatically calculated. MSIMI was defined as an $SDS_{MS} \geq 3$.[20] Adenosine induced myocardial ischemia (ASIMI) was defined as an $SDS_{AS} \geq 4$.[35]

Global MBF and regional MBF for each coronary artery territory were calculated using Cedars-Sinai software and a 2-compartment model based on the time-activity curve of left ventricle input and myocardial uptake obtained within the first 120 s after the injection of 13-NH3. Coronary flow reserve (CFR) was determined as the ratio of MBF_{AS} to MBF_{rest}. MFR for MS (MFR) was calculated as the ratio of MBF_{MS} to MBF_{rest}.

Mental stress testing

After a 15-min rest in a quiet and dim room, participants underwent MS testing wearing a VR device that presented three successive tasks in a fixed sequence. These tasks included the modified Stroop test, public speaking, and a mental arithmetic test. During the modified Stroop and mental arithmetic tests, the VR device's built-in sound prompted the subjects every 20 s. Researchers also monitored the participants' real-time view through external equipment and issued instructions to urge them on when mistakes were made (Figure S9). The public speaking test required participants to give a speech about the difficult, sad, or angry experience they had ever gone through. The whole stress period lasted for 12 min, and 13-NH3 was injected 5–8 min after the initiation of the MS testing.

A self-rated visual analog scale, ranging from 1 to 10, was used after the MS test to assess the severity of chest pain experienced by each participant during the testing.

Adenosine stress testing

The AS protocol followed the recommendations of the American Society of Nuclear Cardiology, with adenosine being infused at a rate of 140 μg/kg/min (100-120 μg/kg/min for those at risk of complications) for 6 min. 13-NH3 was injected at the beginning of the 4th minute after the delivery of adenosine. Coronary microvascular disease was defined as CFR <2.0.[36]

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis

The descriptive statistics were presented as follows: means ± standard deviation (SD) for continuous variables, median with interquartile range (IQR) for non-normally distributed variables, and frequency (percentage) for categorical variables. The prevalence of MSIMI and CMD in the ANOCA (n = 84) and Control (n = 42) groups was compared using Pearson's chi-square test. Corrected MBF was calculated as MBF divided by RPP for the resting state and MS state. Coronary vascular resistance was defined as mean arterial pressure divided by MBF. The proportion of MBF_{MS} to MBF_{AS} was also calculated to assess the extent of hyperemia. Changes in hemodynamic measurements and PET-CT indices (SDS, TPD, MBF, corrected MBF_{MS} and cardiac function parameters) from resting state to MS or adenosine stress conditions within each group (ANOCA&MSIMI+ group: n = 36, ANOCA&MSIMI- group: n = 48, Control group: n = 42) were compared using paired t-tests. The difference in hemodynamic measurements and PET-CT indices between the ANOCA&MSIMI+ group and the Control group, and MBF and CFR between individuals with ischemic and non-ischemic changes in different coronary artery territories were analyzed using Student's t-test or Kruskal-Wallis test, depending on the distribution pattern. Linear regression models were fitted for MBF_{rest} and MBF_{MS}, MBF_{rest} and MBF_{AS}, as well as MBF_{MS} and MBF_{AS} in each group. Multivariate linear regression models were adjusted for age, history of hypertension and diabetes, and/or RPP. All tests were two-sided with a significance level set at $\alpha = 0.05$. Statistical significance was indicated by asterisks: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{*}p < 0.001$.

Hardware and software for model development

Statistical analysis and plotting were performed for the linear regression fitting of MBFrest vs. MBFMS, MBFrest vs. MBFAS, and MBFMS vs. MBFAS across the MSIM+ & ANOCA, MSIM- & ANOCA, and Control groups using R software (version 4.1.3). All other statistical analyses were conducted using SAS 9.4 (TS1M6).

ADDITIONAL RESOURCES

The study has been registered at ClinicalTrials.gov with the Identifier NCT03982901 (https://clinicaltrials.gov/ct2/show/NCT03982901), and the protocol for this study has been previously published.[34]