


# Relationship Between Plasma Total Homocysteine Levels and Mean Corrected TIMI Frame Count in Patients with Acute Myocardial Infarction

Feng Hu <sup>1</sup>Feng Lu<sup>2</sup>Xiao Huang <sup>1</sup>Xiaoshu Cheng<sup>1</sup>

<sup>1</sup>The Department of Cardiovascular Medicine, The Second Affiliated Hospital of Nanchang University, Nanchang, People's Republic of China; <sup>2</sup>The Department of Cardiothoracic Surgery, The Second Affiliated Hospital of Nanchang University, Nanchang, People's Republic of China

**Objective:** To explore the relationship between the plasma total homocysteine (tHcy) levels and slow coronary flow (SCF) measured by mean corrected TIMI frame count (CTFC) in patients with acute myocardial infarction (AMI).

**Methods:** Ninety-one patients with primary myocardial infarction were enrolled in this study. The quantitative measurement of coronary blood flow was performed using the mean CTFC method. Plasma tHcy levels were determined using enzymatic assay from venous blood samples. Multivariable linear regression models indicated were used to estimate the effect size ( $\beta$ ) of plasma tHcy levels on mean CTFC levels.

**Results:** Compared with patients with the mean CTFC  $\leq 23.61$  frames per second (FPS) group, there were increased plasma tHcy levels in patients of the second, third and highest mean CTFC quartiles ( $P < 0.001$ ). Linear regression models indicated that plasma tHcy levels were positively associated with mean CTFC levels (adjusted- $\beta$  per  $SD$  increase: 1.96, 95% CI 1.20 to 2.73,  $P < 0.001$ ). Compared to the tHcy  $\leq 12.30$   $\mu\text{mol/L}$  group, the third and highest tHcy-quartile groups had higher mean CTFC levels (adjusted- $\beta$ : 2.52, 95% CI 0.39 to 4.65,  $P = 0.023$ ; adjusted- $\beta$ : 5.07, 95% CI 2.98 to 7.16,  $P < 0.001$ , respectively;  $P$  for trend  $< 0.001$ ). Moreover, this positive relationship was modified by diabetes mellitus ( $P$ -value for interaction was 0.046).

**Conclusion:** We found a positive relationship between plasma tHcy levels and mean CTFC levels in patients with AMI. Moreover, diabetes mellitus played an interactive role in this positive association between the plasma tHcy levels and mean CTFC levels.

**Keywords:** hyperhomocysteinemia, mean corrected TIMI frame count, acute myocardial infarction, slow coronary flow, coronary microcirculation dysfunction

## Introduction

Slow coronary flow (SCF), a syndrome presenting with chest pain or tightness related to a delay in the progression of contrast injected into the distal coronary vasculature during coronary angiography, was first proposed in 1972.<sup>1</sup> Although its pathogenesis is still not fully elucidated, it has been reported that coronary microcirculation dysfunction (CMD) was responsible for the development of SCF phenomenon.<sup>1-3</sup>

In the setting of acute myocardial infarction (AMI) after primary percutaneous transluminal coronary intervention (PCI), CMD is an indicator of poor long-term clinical prognosis.<sup>4</sup> There are multiple founded techniques to assess microvascular injury or dysfunction in the catheterization room.<sup>5</sup> Corrected TIMI frame count

Correspondence: Xiao Huang; Xiaoshu Cheng  
Department of Cardiovascular Medicine, The Second Affiliated Hospital of Nanchang University, No. 1 Minde Road, Nanchang, 330006, Jiangxi, People's Republic of China  
Tel +8613607089128  
Fax +86-791-86262262  
Email drxiaohuang@163.com; xiaoshumenfan126@163.com



(CTFC) is an angiographic argument that can re-stratify the risk of patients with post-procedural thrombolysis in myocardial infarction (TIMI) grade III flow and reflect the existence of SCF and CMD.<sup>6,7</sup>

Homocysteine is a sulfur-containing amino acid that functions as a key intermediate in methionine metabolism and hyperhomocysteinemia (HHcy) is well established risk factor for cardiovascular disease.<sup>8</sup> Angiographic findings<sup>9–16</sup> found that patients with SCF had significantly higher plasma total homocysteine (tHcy) levels than those with normal coronary flow and HHcy was strongly correlated with SCF phenomenon in patients without organic coronary stenosis. In addition, HHcy was a potential predictor for SCF prognosis and related to cumulative cardiac events.<sup>14</sup> Moreover, Li et al<sup>17</sup> observed that increased plasma tHcy levels ( $\geq 17.55 \mu\text{mol/L}$ ) were independent negative predictor of spontaneous reperfusion (defined as achievement of TIMI grade III flow in the infarct-related artery (IRA) before primary PCI) in patients with AMI, implying the potential relationship between HHcy with less early IRA patency. However, to our knowledge, there is no detailed study evaluating the relationship between plasma tHcy levels and SCF measured by mean CTFC in patients with AMI. Therefore, the aim of this study was to investigate the relationship between plasma tHcy levels and mean CTFC in patients with AMI.

## Methods

### Study Population

This single-center study enrolled 91 angiographically identified patients with AMI, admitted to chest pain center at the Second Affiliated Hospital of Nanchang University between July 2019 and December 2020. There were 60 patients with ST-segment elevation myocardial infarction (STEMI), 31 cases with non-ST-segment elevation myocardial infarction (NSTEMI) in our analysis. Patients with diagnosed coronary artery disease, history of myocardial infarction, left ventricular dysfunction (left ventricular ejection fraction  $< 50\%$ ), severe heart valve disease, cardiomyopathy, arrhythmia, left ventricular hypertrophy, uncontrolled hypertension, diabetes mellitus, connective tissue disease and liver, kidney, thyroid dysfunction or vitamin intake were excluded from the study. Left ventricular ejection fraction (LVEF) and left ventricle end diastolic diameter (LVEDD) were collected and analyzed independently by a third part. Written approved informed consent was obtained from each participant. All

procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Ethical Review Committee of the Second Affiliated Hospital of Nanchang University (Number: 2018242).

### Coronary Angiography and Corrected TIMI Frame Count

All enrolled patients were accepted urgent coronary angiography and we used iohexol as contrast agent. All angiograms were performed using Standard Judkin's left and right 6 French diagnostic catheters. Acquisition positions were right anterior oblique  $30^\circ$  for the left anterior descending (LAD) and the left circumflex artery (LCX), and left anterior oblique  $50^\circ$  for the right coronary artery (RCA). The speed of imaging collection was 15 frames per second (FPS). The angiographic analysis was performed retrospectively by an independent core lab using software of iMedical 8.0. Coronary flow rates of these subjects were measured by CTFC according to the means described by Gibson et al.<sup>6</sup> In brief, the first frame was considered that where a column of dye extended across the entire width of the origin of the artery, touching both its borders with evidence of antegrade motion of the dye. The last frame was defined as the frame when the dye first entered a certain distal landmark branch of (but did not necessarily fill) each vessel.<sup>6</sup> Because the LAD artery TFC is approximately 1.7- times greater than that of the LCX and RCA, the TIMI frame count (TFC) of the LAD was corrected by dividing by 1.7.<sup>6</sup> We calculated mean CTFC =  $(\text{LAD TFC}/1.7 + \text{LCX TFC} + \text{RCA TFC})/3$ . The subjects with mean CTFC greater than 27 FPS were accepted as having SCF in the present study according to previous reports.<sup>18</sup> Furthermore, the risk of nosocomial death was assessed by Grace Score which stratified the risk of AMI patients and accurately predicted the mortality during hospitalization or in the long term.<sup>19</sup> The severity of coronary artery lesions was evaluated by the Gensini scoring system according to the narrowing degree and functional significance of each coronary vessel.<sup>20</sup>

### Laboratory Assay

Blood samples were collected utilizing venipuncture after an overnight fast of at least 12 hours. The levels of plasma tHcy were measured by enzymatic cycling assay analysis

using a commercially available “Immulite Homocysteine” kit (Product ID: YZB/JI 0163–2013, Hebei aioulu biotechnology company, CHINA). The levels of B-type natriuretic peptide (BNP), hyper-sensitivity cTnI, blood urea nitrogen (BUN), estimated glomerular filtration rate (eGFR); serum creatinine and uric acid, white blood cells, hemoglobin, platelets, plasma albumin, total cholesterol (TC), total triglyceride (TG), low density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL), and non-high-density lipoprotein cholesterol (NHDLC) were measured using automatic clinical analyzers (Beckman Coulter). The estimated glomerular filtration rate (eGFR) was calculated by the equation of Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI).

## Statistical Analysis

Continuous variables are presented as the means  $\pm$  standard deviation (*SD*) or the median (quartiles), as appropriate, and are compared using the Student's *t*-test, one-way analysis or the Mann Whitney *U*-test, depending on whether the quantitative data were consistent with a normal distribution. Categorical variables were presented as count (percentage), differences between groups were measured by chi-square test.

Linear regression models indicated were used to estimate the effect size ( $\beta$ ) of plasma tHcy levels on mean CTFC levels. The crude model was not adjusted for any confounder. The model I was only adjusted for age and sex. The model II was confounder model. The confounder model screened covariates including age, sex, systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), current smoking and drinking status, the type of AMI, Killip classification, hypertension, diabetes mellitus, TC, TG, HDL-C, LDL-C, NHDLC, serum uric acid, eGFR, LVEF, Grace risk scores and Gensini coronary lesion scores. We selected these confounders on the basis of their associations with the outcomes of interest more than 10%. We considered the confounder model to be the main model.

Furthermore, the generalized additive model and smooth curve fitting (penalized spline method) was used to visually show the relationship between plasma tHcy levels and mean CTFC levels.

All statistical analyses were performed using the statistical package R (<http://www.R-project.org>, The R Foundation, version 3.4.3) and the Empower (R; [www.empowerstats.com](http://www.empowerstats.com); X&Y Solutions, Inc, Boston, MA,

USA). All P-values are two-tailed, and  $P < 0.05$  was considered to be statistically significant.

## Results

### Patient Characteristics

The present study included 91 AMI patients (age:  $65.34 \pm 11.12$  years, range 38–86 years; male, 75.82%). The clinical characteristics of the study participants were presented grouped by quartiles of mean CTFC. There were no significant differences between the groups in terms of clinical parameters other than the plasma tHcy levels (Table 1). Compared with patients with mean CTFC  $\leq 23.61$  FPS, there were increased plasma tHcy levels in patients of the second, third and highest mean CTFC quartiles ( $P < 0.001$ , Table 1). Furthermore, compared with patients with mean CTFC  $\leq 23.61$  FPS, there were increased numbers of slow flow arteries in patients of the third and highest mean CTFC quartiles ( $P < 0.001$ , Table 2). However, there were no significant differences between the groups in terms of Gensini score of epicardial coronary artery lesion and Grace score of nosocomial death risk ( $P > 0.05$ , Table 2).

### Association Between Plasma tHcy Levels and Mean CTFC Levels

Linear regression models indicated that plasma tHcy levels were positively associated with mean CTFC levels (adjusted- $\beta$  per *SD* increase: 1.96, 95% CI 1.20 to 2.73,  $P < 0.001$ ; Table 3). Compared without HHcy group (Hcy  $< 15 \mu\text{mol/L}$ ), patients with HHcy had higher mean CTFC levels (adjusted- $\beta$ : 2.89, 95% CI 1.35 to 4.43,  $P < 0.001$ ; Table 3). Compared to the tHcy  $\leq 12.30 \mu\text{mol/L}$  group, the third and highest tHcy-quartile groups had higher mean CTFC levels (adjusted- $\beta$ : 2.52, 95% CI 0.39 to 4.65,  $P = 0.023$ ; adjusted- $\beta$ : 5.07, 95% CI 2.98 to 7.16,  $P < 0.001$ , respectively;  $P$  for trend  $< 0.001$ ; Table 3). The smooth curve fitting further confirmed this positive relationship between plasma tHcy levels and mean CTFC levels (Figure 1).

### Subgroup Analyses by Potential Effect Modifiers

To explore whether this positive correlation between plasma tHcy levels and mean CTFC levels was still stable in different subgroups, we conducted stratified and interaction analyses. We found that this association between plasma tHcy levels and mean CTFC levels was modified by diabetes mellitus ( $P$ -value for interaction was 0.046, Figure 2). There were not

**Table 1** Clinical Characteristics of Participants Grouped by Quartiles of Mean CTFC

Characteristics	Total (n=91)	Mean CTFC Quartiles				P-value
		Q1 (n=23) [16.71, 23.61]	Q2 (n=21) [23.73, 25.29]	Q3 (n=24) [25.57, 27.41]	Q4 (n=23) [27.45, 35.69]	
Age (years)	65.34 ± 11.12	66.09 ± 10.50	63.38 ± 9.56	63.92 ± 11.71	67.87 ± 12.49	0.512
Aging (≥70 years), n (%)	34 (37.36%)	11 (47.83%)	5 (23.81%)	6 (25.00%)	12 (52.17%)	0.092
Male, n (%)	69 (75.82%)	13 (56.52%)	16 (76.19%)	20 (83.33%)	20 (86.96%)	0.073
SBP (mmHg)	124.03 ± 19.90	127.57 ± 21.44	124.29 ± 18.95	123.88 ± 20.01	120.43 ± 19.70	0.693
DBP (mmHg)	77.76 ± 14.09	77.30 ± 14.90	77.71 ± 14.15	76.46 ± 11.54	79.61 ± 16.22	0.894
HR (times/min)	78.98 ± 15.55	84.96 ± 17.10	80.10 ± 10.75	76.21 ± 17.89	74.87 ± 13.90	0.116
STEMI, n (%)	60 (65.93%)	18 (78.26%)	14 (66.67%)	11 (45.83%)	17 (73.91%)	0.088
Killip classification						
Class 1	36 (39.56%)	4 (17.39%)	8 (38.10%)	13 (54.17%)	11 (47.83%)	0.130
Class 2	54 (59.34%)	18 (78.26%)	13 (61.90%)	11 (45.83%)	12 (52.17%)	
Class 3	1 (1.10%)	1 (4.35%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	
Hypertension, n (%)						
No	53 (58.24%)	12 (52.17%)	10 (47.62%)	14 (58.33%)	17 (73.91%)	0.317
Grade 1	3 (3.30%)	1 (4.35%)	1 (4.76%)	1 (4.17%)	0 (0.00%)	
Grade 2	14 (15.38%)	3 (13.04%)	7 (33.33%)	2 (8.33%)	2 (8.70%)	
Grade 3	21 (23.08%)	7 (30.43%)	3 (14.29%)	7 (29.17%)	4 (17.39%)	
Diabetes mellitus, n (%)	25 (27.47%)	9 (39.13%)	7 (33.33%)	7 (29.17%)	2 (8.70%)	0.110
COPD, n (%)	5 (5.49%)	0 (0.00%)	1 (4.76%)	2 (8.33%)	2 (8.70%)	0.535
Current smoker, n (%)	27 (29.67%)	6 (26.09%)	5 (23.81%)	10 (41.67%)	6 (26.09%)	0.516
Current drinker, n (%)	17 (18.68%)	3 (13.04%)	3 (14.29%)	6 (25.00%)	5 (21.74%)	0.678
Plasma tHcy (μmol/L)	14.25 (12.37–17.45)	12.90 (9.61–14.07)	14.25 (13.03–17.02)	15.04 (12.54–17.45)	17.38 (14.29–19.95)	<0.001
BNP (pg/mL)	231.25 (91.96–449.26)	350.10 (184.56–562.86)	156.12 (65.16–368.80)	270.14 (96.86–537.77)	197.35 (123.98–315.84)	0.227
Hyper-sensitivity cTnl (pg/mL)	25.20 (7.92–50.00)	20.04 (12.90–34.56)	20.50 (5.61–50.00)	18.63 (3.05–50.00)	46.05 (16.75–50.00)	0.345
BUN (mmol/L)	6.36 (5.01–8.24)	6.89 (5.86–8.84)	7.14 (6.20–9.47)	5.64 (4.73–7.09)	6.09 (4.81–7.11)	0.132
Serum creatinine (μmol/L)	79.90 (68.48–103.47)	86.30 (69.28–103.82)	82.42 (69.28–109.73)	72.25 (65.30–90.59)	79.05 (70.82–97.41)	0.567

eGFR (mL/min)	84.83 (64.76–103.82)	76.09 (63.85–104.17)	76.89 (62.12–98.52)	92.87 (73.68–105.16)	91.28 (69.29–103.72)	0.423
Serum uric acid ( $\mu\text{mol/L}$ )	387.62 $\pm$ 114.72	384.95 $\pm$ 134.24	422.00 $\pm$ 105.43	369.22 $\pm$ 103.45	378.12 $\pm$ 113.69	0.451
White blood cells ( $10^9/\text{L}$ )	9.42 $\pm$ 3.35	9.00 $\pm$ 3.31	9.11 $\pm$ 2.99	10.28 $\pm$ 3.42	9.23 $\pm$ 3.69	0.538
Hemoglobin (g/L)	128.85 $\pm$ 20.72	119.65 $\pm$ 26.68	126.71 $\pm$ 18.24	135.79 $\pm$ 19.48	132.74 $\pm$ 13.56	0.072
Platelets ( $10^9/\text{L}$ )	200.98 $\pm$ 73.82	188.04 $\pm$ 76.31	234.24 $\pm$ 73.91	194.75 $\pm$ 64.48	190.04 $\pm$ 75.90	0.054
Plasma albumin (g/L)	36.17 $\pm$ 5.37	35.16 $\pm$ 4.47	36.50 $\pm$ 3.51	37.67 $\pm$ 8.22	35.33 $\pm$ 3.43	0.348
TC (mmol/L)	4.72 $\pm$ 1.19	4.61 $\pm$ 1.15	5.09 $\pm$ 1.51	4.65 $\pm$ 1.28	4.58 $\pm$ 0.72	0.772
TG (mmol/L)	1.81 $\pm$ 1.59	1.79 $\pm$ 2.24	2.11 $\pm$ 2.03	1.86 $\pm$ 0.99	1.51 $\pm$ 0.67	0.14
LDL-C (mmol/L)	2.85 $\pm$ 0.94	2.72 $\pm$ 1.06	2.92 $\pm$ 1.07	2.94 $\pm$ 0.97	2.81 $\pm$ 0.65	0.794
HDL-C (mmol/L)	1.04 $\pm$ 0.25	1.07 $\pm$ 0.26	1.06 $\pm$ 0.31	1.00 $\pm$ 0.20	1.04 $\pm$ 0.25	0.719
NHDL-C (mmol/L)	3.67 $\pm$ 1.10	3.58 $\pm$ 1.24	3.88 $\pm$ 1.21	3.70 $\pm$ 1.20	3.53 $\pm$ 0.70	0.712
LVEDD (mm)	46.55 $\pm$ 5.23	47.05 $\pm$ 3.98	46.85 $\pm$ 5.45	44.58 $\pm$ 4.74	48.00 $\pm$ 6.24	0.152
LVEF (%)	53.49 $\pm$ 9.78	51.14 $\pm$ 11.38	56.25 $\pm$ 11.37	53.75 $\pm$ 8.04	52.90 $\pm$ 8.12	0.414

**Abbreviations:** CTFC, corrected thrombolysis in myocardial infarction frame count; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; STEM1, ST-segment elevated myocardial infarction; COPD, chronic obstructive pulmonary disease; tHcy, total homocysteine; BNP, B-type natriuretic peptide; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; TC, total cholesterol; TG, total triglyceride; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; NHDL-C, non-high-density lipoprotein-cholesterol; LVEDD, left ventricle end diastolic diameter; LVEF, left ventricular ejection fraction.

**Table 2** The Severity of Coronary Artery Disease Grouped by Quartiles of Mean CTFC

Characteristics	Total (n=91)	Quartiles of Mean CTFC				P-value
		Q1 (n=23) [16.71, 23.61]	Q2 (n=21) [23.73, 25.29]	Q3 (n=24) [25.57, 27.41]	Q4 (n=23) [27.45, 35.69]	
Gensini coronary lesion scores						
LAD	40.00 (10.00–45.00)	40.00 (27.00–49.00)	32.00 (12.00–40.00)	36.00 (9.50–42.50)	40.00 (9.00–42.00)	0.530
LCX	4.00 (0.00–20.00)	2.00 (1.00–20.50)	3.50 (1.00–10.00)	8.00 (1.50–17.00)	2.50 (0.00–22.00)	0.825
RCA	8.00 (2.00–18.00)	5.00 (2.00–16.00)	16.00 (4.00–26.00)	11.00 (2.00–18.50)	4.00 (1.50–18.00)	0.231
Total	50.00 (41.00–72.50)	50.00 (41.00–82.00)	46.00 (40.00–60.00)	46.00 (40.00–60.00)	46.00 (41.00–71.25)	0.933
Grace risk scores	162.59 ± 31.82	171.91 ± 32.82	162.67 ± 31.77	150.12 ± 26.31	166.22 ± 33.90	0.112
TIMI frame count (FPS)						
LAD	37.71 ± 7.19	33.30 ± 7.45	37.14 ± 5.28	38.67 ± 6.20	41.65 ± 7.25	0.002
LCX	25.58 ± 5.71	19.65 ± 3.98	23.71 ± 3.48	28.42 ± 3.23	30.26 ± 4.87	<0.001
RCA	28.09 ± 6.89	21.48 ± 3.68	28.29 ± 5.52	28.58 ± 2.98	34.00 ± 7.82	<0.001
Mean CTFC (FPS)	25.29 ± 3.78	20.24 ± 1.96	24.62 ± 0.51	26.58 ± 0.62	29.59 ± 2.42	<0.001
Numbers of slow flow arteries						
One vessel, n (%)	27 (29.67%)	3 (13.04%)	11 (52.38%)	9 (37.50%)	4 (17.39%)	<0.001
Two vessel, n (%)	27 (29.67%)	0 (0.00%)	1 (4.76%)	13 (54.17%)	13 (56.52%)	
Three vessel, n (%)	5 (5.49%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	5 (21.74%)	

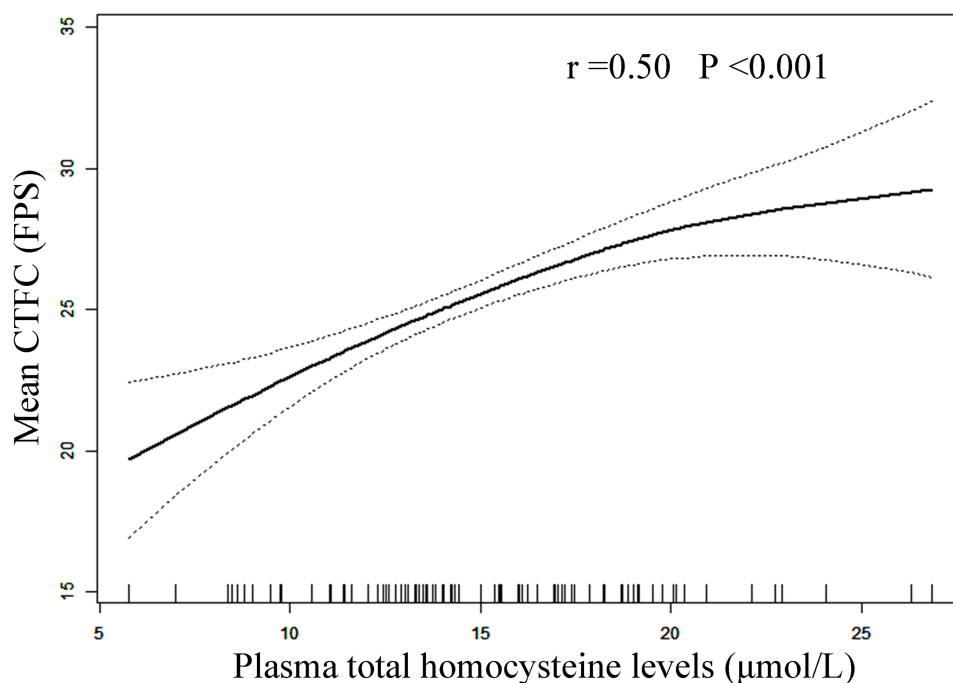
**Abbreviations:** CTFC, corrected thrombolysis in myocardial infarction frame count; LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery; FPS, frames per second; TIMI, thrombolysis in myocardial infarction.

**Table 3** Relationship Between Plasma tHcy Levels and Mean CTFC Levels in Different Models

Variables	Crude Model		Model I		Model II	
	$\beta$ (95% CI)	P-value	$\beta$ (95% CI)	P-value	$\beta$ (95% CI)	P-value
tHcy ( $\mu\text{mol/L}$ )						
Per 1 increase	0.42 (0.25, 0.59)	<0.001	0.37 (0.20, 0.54)	<0.001	0.45 (0.29, 0.65)	<0.001
Per SD increase	1.75 (1.05, 2.44)	<0.001	1.54 (0.85, 2.24)	<0.001	1.96 (1.20, 2.73)	<0.001
tHcy ( $\mu\text{mol/L}$ )						
<10	Ref		Ref		Ref	
$\geq 10$	3.60 (1.41, 5.78)	0.002	3.14 (1.03, 5.25)	0.005	3.35 (1.01, 5.69)	0.006
tHcy ( $\mu\text{mol/L}$ )						
<15	Ref		Ref		Ref	
$\geq 15$	2.68 (1.22, 4.14)	<0.001	2.22 (0.76, 3.68)	0.004	2.89 (1.35, 4.43)	<0.001
tHcy tertiles ( $\mu\text{mol/L}$ )						
T1 [5.76, 13.04]	Ref		Ref		Ref	
T2 [13.09, 16.50]	1.46 (-0.25, 3.18)	0.098	1.05 (-0.66, 2.75)	0.233	1.92 (0.03, 3.80)	0.050
T3 [16.92, 26.85]	4.18 (2.48, 5.88)	<0.001	3.54 (1.79, 5.29)	<0.001	4.20 (2.37, 6.02)	<0.001
P for trend	<0.001		<0.001		<0.001	
tHcy quartiles ( $\mu\text{mol/L}$ )						
Q1 [5.76, 12.30]	Ref		Ref		Ref	
Q2 [12.44, 14.24]	1.61 (-0.40, 3.61)	0.120	1.24 (-0.72, 3.20)	0.218	1.40 (-0.61, 3.41)	0.176
Q3 [14.25, 17.38]	2.14 (0.14, 4.15)	0.039	1.65 (-0.32, 3.62)	0.104	2.52 (0.39, 4.65)	0.023
Q4 [17.45, 26.85]	4.66 (2.70, 6.62)	<0.001	4.07 (2.10, 6.04)	<0.001	5.07 (2.98, 7.16)	<0.001
P for trend	<0.001		<0.001		<0.001	

**Notes:** Model I adjusted for age and sex. Model II adjusted for sex, HR, SBP, Killip classification, hypertension, diabetes mellitus and eGFR.

**Abbreviations:** tHcy, total homocysteine; CTFC, corrected thrombolysis in myocardial infarction frame count; Ref, reference;  $\beta$ , effect size; CI, confidence interval; SD, standard deviation.



**Figure 1** Smooth curve of correlation between plasma tHcy levels and mean CTFC levels. Smooth curve and  $r$  adjusted for sex, HR, SBP, Killip classification, hypertension, diabetes mellitus and eGFR.

**Abbreviations:** CTFC, corrected thrombolysis in myocardial infarction frame count; FPS, frames per second;  $r$ , coefficient of partial correlation.

significant interactions in any of the following subgroups, including aging, sex (male vs female), SBP (<140 vs  $\geq$ 140 mmHg), HR dichotomy ( $\leq$ 75 vs  $\geq$ 76 times/min), AMI (NSTEMI vs STEMI), current smoker (no vs yes), eGFR dichotomy ( $\leq$ 82.3 vs  $\geq$ 84.8 mL/min/1.73m<sup>2</sup>) and Grace risk scores dichotomy ( $\leq$ 164 vs  $\geq$ 165) (all  $P$ -values for interaction respectively were greater than 0.05, Figure 2).

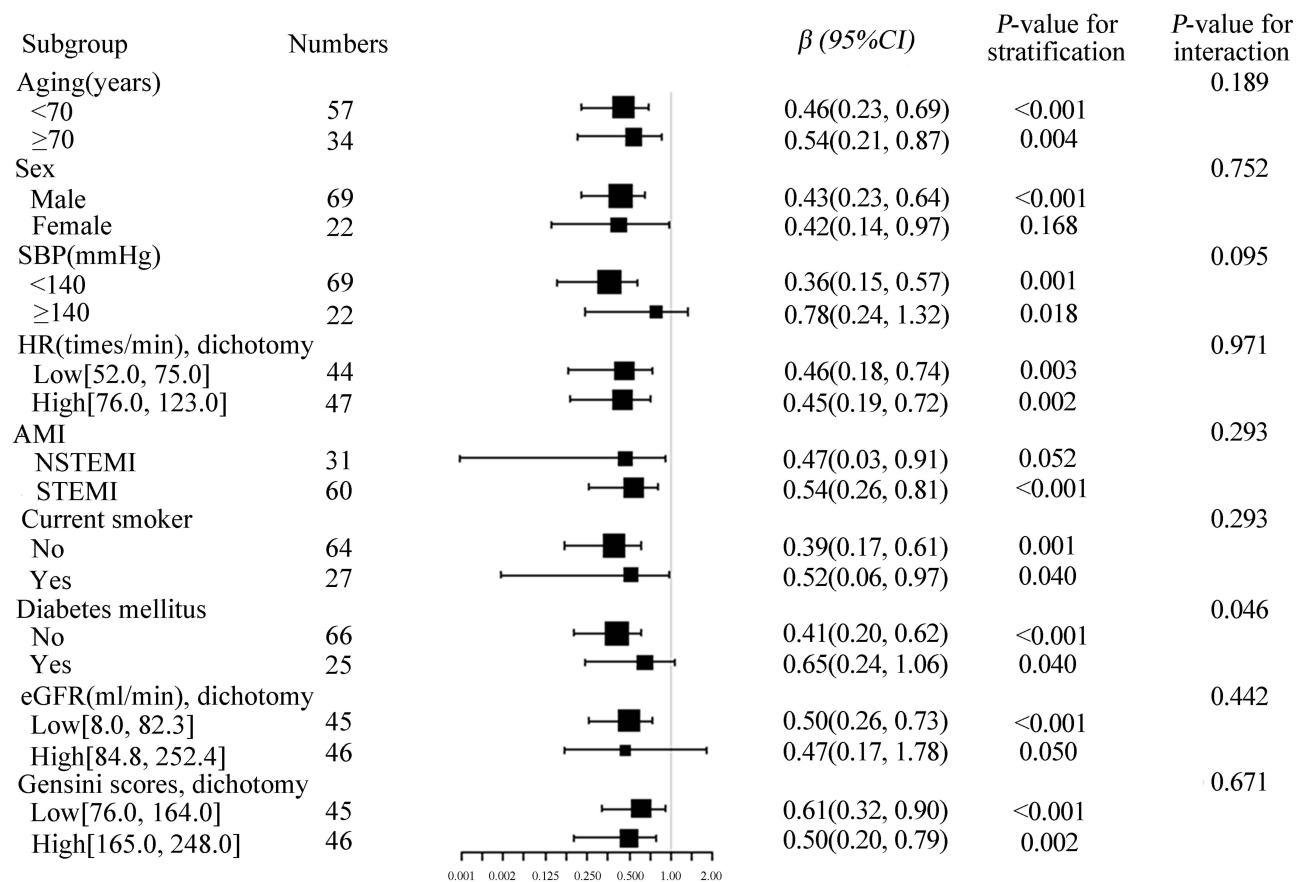
## Discussion

The present study showed that plasma tHcy levels were significantly positively associated with mean CTFC levels, a metric of reflecting SCF and CMD, in patients with AMI. Moreover, diabetes mellitus played an interactive role in this positive association between the plasma tHcy levels and mean CTFC levels.

The risk factors and pathophysiology for the appearance of SCF are still unclear, and different ethnic populations have different risk factors on account of the different prevalence rates of comorbidities and genetic variation regarding predisposition to SCF. In a study performed by Beltrame et al<sup>21</sup> in an Australian population, male sex and smoking were found to be independent risk factors for SCF. In two studies done in North Indian subjects, body mass index<sup>18</sup> or hypertension, dyslipidemia, smoking and tobacco chewing were independently positively associated

with SCF phenomenon.<sup>22</sup> A study done on Iranian population identified smoking, total cholesterol, hematocrit, fasting blood glucose and red cell distribution to be independent risk factors associated with SCF.<sup>23</sup> Demirci et al<sup>16</sup> showed that patients with SCF had significantly higher plasma tHcy levels than those with normal coronary flow. Li et al<sup>14</sup> found HHcy and mean platelet volume to be independent risk factors for SCF in a Chinese Han population. The coronary microvasculature is the dominant gatekeeper for myocardial blood flow below the visible epicardial arteries. The CMD can lead to angina pectoris which is prolonged or poorly responsive to nitrates and may persist despite successful revascularization, affect ventricular function and remodeling in the long term.<sup>24</sup> The evidence suggests that CMD in AMI patients is associated with adverse long-term survival.<sup>4</sup>

The development of CMD in AMI patients involved multiple pathophysiological processes, especially for endothelial dysfunction (ED).<sup>5</sup> Homocysteine is a possibly cytotoxic amino acid synthesized during methionine metabolic cycle. Celermajer et al<sup>25</sup> found that HHcy was associated with impaired endothelial function of systemic arteries and decreased nitric oxide bioavailability in hyperhomocystinuria children. Tanriverdi et al<sup>13</sup> showed that HHcy impaired endothelial function via



**Figure 2** Effect size of plasma tHcy levels on mean CTFC in prespecified and exploratory subgroups. Each stratification adjusted for age, sex, HR, SBP, Killip classification, hypertension, diabetes mellitus and eGFR except the subgroup variable.

**Abbreviations:** SBP, systolic blood pressure; HR, heart rate; AMI, acute myocardial infarction; NSTEMI, non-ST-segment elevation myocardial infarction; STEMI, ST-segment elevation myocardial infarction; eGFR, estimated glomerular filtration rate;  $\beta$ , effect size; CI, confidence interval.

oxidative stress in SCF patients. Cheng et al<sup>26</sup> found that HHcy impaired endothelium-dependent micro-vasculature relaxation to acetylcholine in mouse small mesenteric artery via oxidation of SK<sub>Ca</sub>/IK<sub>Ca</sub>. Intermediate HHcy potentiated diabetes impaired endothelium-derived hyperpolarizing factor-induced microvasculature relaxation via H<sub>2</sub>S-downregulation and IK<sub>Ca</sub> tyrosine nitration in T2DM/HHcy mice.<sup>27</sup> Otherwise, recent research showed HHcy could cause mitochondrial dysfunction and tissue damage mostly by inhibiting mitochondrial complex I gene expression which leads to impaired electron transfer, complex assembly and redox homeostasis dysregulation in human disease and experimental models.<sup>28</sup> Therefore, we speculated that HHcy might cause endothelial dysfunction or injury, which further result in CMD and SCF in AMI patients.

Moreover, previous studies have proved that plasma tHcy levels were relevant with coronary slow flow in patients without occlusive coronary arteries.<sup>9–15</sup> It's worth noting that partially differed from that of stable

coronary artery disease, microvascular embolism or spasm might play a vital role due to severe thrombus burden on the onset of acute coronary events.

We found diabetes mellitus might play an interactive role in the association between the plasma tHcy levels and mean CTFC levels after adjusted all possible covariates. It is widely recognized that diabetes mellitus was associated with ED and CMD<sup>29</sup> in animal models<sup>27,30,31</sup> and clinical studies.<sup>32–36</sup> Verma et al<sup>31</sup> showed that diabetic microcirculatory disturbances were provoked by deposition of amyloid-forming amylin in red blood cells (RBC) and micro vasculatures, which thus altering RBC-capillary interaction leading to activation of hypoxia signaling pathways and pathologic erythropoiesis. Di et al<sup>32</sup> found that markedly reduced and similar endothelium-dependent and -independent coronary vasodilator function in subjects with both type I and type II diabetes mellitus, by measuring myocardial blood flow measured in a different state using Positron emission tomography imaging. Von et al<sup>34</sup>

showed that type II diabetic patients had a reduced coronary flow reserve, especially with concomitant albuminuria, suggesting a common microvascular impairment occurring in multiple microvascular beds. But the *P*-value for interaction was 0.046, belonging to marginal significance. Therefore, more researches with larger sample size is needed to prove this correlation.

In addition to the improvement of pharmacological and interventional therapy, the integrated management of CMD in AMI patients should incorporate lifestyle modification and the control of numerous risk factors, such as tHcy-lowering therapy, hypoglycaemic therapy, and cardiac rehabilitation therapy. Treatment combining drug therapy and cardiac rehabilitation could reduce the plasma levels of high-sensitivity C-reactive protein, homocysteine and arginine, significantly improve the symptoms of SCF phenomenon and increase coronary blood flow velocity.<sup>37</sup>

## Limitations

Our study has some limitations. First, mean CTFC is not that stability because of the difference between physiological status, operator technique, the force of injection and catheter engagement. Second, the angiographic definition of SCF relies on axial contrast angiograms of the vessel lumen. Hence, future studies call for establish a diagnostic criteria for CMD using precise verified techniques, such as coronary flow velocity reserve (CFVR), cardiovascular magnetic resonance myocardial perfusion imaging and Index of microvascular resistance (IMR).<sup>7,38,39</sup> Moreover, another restriction is that plasma levels of the folic acid, vitamin B12 and helicobacter pylori infection<sup>40</sup> as well as methylenetetrahydrofolate reductase gene polymorphism were not able to be surveyed to determine the possible disturbance of methionine metabolism.<sup>15</sup> Finally, in consideration of the small sample size in our study, further studies with larger samples are needed to confirm our results and to evaluate the effectiveness of homocysteine reducing treatment.

## Conclusions

In conclusion, we found a positive relationship between plasma tHcy levels and mean CTFC levels in patients with AMI. HHcy may play an important role in the pathogenesis of SCF in patients with AMI. Moreover, diabetes mellitus played an interactive role in this positive association between the plasma tHcy levels and mean CTFC levels.

## Data Sharing Statement

The data used to support the findings of this study are available from the corresponding author on request.

## Ethics Approval

Written approved informed consent was obtained from each participant. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Ethical Review Committee of the Second Affiliated Hospital of Nanchang University (Number: 2018242).

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## Disclosure

The authors have no conflicts of interest regarding this work or the publication of this paper.

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