Impact of Homocysteine Level on Long-term Cardiovascular Outcomes in Patients after Coronary Artery Stenting

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Aim: The prognostic value of homocysteine (HCY) in patients with coronary artery diseases (CAD) is still controversial. The objective of this study was to investigate whether elevated HCY level at admission predict long-term outcomes in patients after percutaneous coronary interventions (PCI) with coronary artery stenting.

Methods: From the institutional registry of Cardiovascular Atherosclerosis and Percutaneous TrAns-luminal INterventions (CAPTAIN), we enrolled a total of 1,307 patients with documented CAD underwent PCI with bare metal stents from July 2003 to December 2014. They were divided into two groups according to the fasting plasma HCY levels before catheterization: group I (883 patients, <12 µmol/L) and group II (424 patients, ≥12 µmol/L). The primary endpoint was occurrence of major adverse cardiac events (MACE), including cardiac death, nonfatal myocardial infarction, stroke, target lesion revascularization, new lesion stenting, and requiring bypass surgery.

Results: After a mean follow-up period of 58 ± 41 months, the group II patients had a higher MACE rate (33.3% vs. 25.6%, p = 0.005). The main differences between two groups were cardiac death (8.0% vs. 3.4%, p = 0.001) and new lesion stenting (13.6% vs. 9.5%, p = 0.034). The risks of long-term MACE remained significantly higher in patients with elevated HCY level (≥12 µmol/L) after adjusting for clinical variables, with a hazard ratio of 1.29 (95% CI, 1.02 – 1.64, p = 0.036).

Conclusions: Elevated HCY level (≥12 µmol/L) was independently associated with increased risk of long-term cardiovascular events in patients after coronary artery bare metal stents implantations. Thus, hyperhomocysteinemia may remain a useful prognostic marker for the risk assessment in clinical care of CAD patients.

Key words: Homocysteine, Coronary artery stent, Clinical outcome

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Materials and Methods

Patient Population and Laboratory Analysis
The Cardiovascular Atherosclerosis and Percutaneous TrAnsluminal INterventions (CAPTAIN) registry, a prospective, physician-initiated, single-center database, which includes 6,300 patients undergoing elective or emergent PCI with stenting at Chang Gung Memorial Hospital. We enrolled consecutive patients in the CAPTAIN registry from July 2003 to December 2014, who had acute myocardial infarction or stable CAD with >70% stenosis in coronary arteries underwent stent implantations. To minimize the effects of stent property differences between drug eluting stents and bare metal stents (BMS) on long-term clinical outcomes, only patients with BMS implantations were included into analysis. We excluded patients with severe multivessel disease requiring bypass surgery, intolerable for dual antiplatelet therapy, or not follow the study protocol. Patients with renal insufficiency (serum creatinine level >1.5 mg/dL) or concurrent active infections, inflammatory disorders, or cancer were also excluded to minimize the influence of comorbidities on HCY levels. Stent implantation was performed through the femoral or radial artery according to standard techniques. The type of stent was based on the indication and available stent size. After initial stent deployment, high-pressure balloon inflation (≥14 atm) was applied in most patients. The creatine kinase myocardial-band (CK-MB) isoenzyme and troponin I were measured in all of the patients immediately and 6 h after the procedure to detect periprocedure myocardial infarction (MI). All of the patients received standard ischemia heart disease therapies after procedure, including dual antiplatelet therapy with aspirin and clopidogrel or
The correlation coefficient was 0.95 ($p < 0.01$).

**Definitions of the Endpoints**

The study endpoint was the occurrence of major adverse cardiac events (MACE), a composite of cardiac death, nonfatal MI, stroke, target lesion revascularization, new lesion stenting, and necessitation of coronary bypass surgery. Cardiac death was defined as any death due to immediate cardiac cause, such as MI, low-output pumping failure, or fatal arrhythmia. The MI was diagnosed if the patient experienced prolonged chest pain, ischemic ST-T changes shown on electrocardiography, and elevated CK-MB isoenzyme or troponin $I$ levels. Clinical follow-up visits at the outpatient clinics were scheduled at 1, 2, 3, 6, 9, and 12 months, and every 3 months thereafter. Information regarding clinical events was obtained from hospital medical records, the referring physician, and phone interviews with the patients or their relatives through December 30, 2015.

### Table 1. Baseline clinical characteristics ($n = 1,307$)

| Homocysteine level | $< 12.0 \mu$mol/L ($n = 883$) | $\geq 12.0 \mu$mol/L ($n = 424$) | $P$ value |
|--------------------|-------------------------------|---------------------------------|-----------|
| Age $\pm$ SD (years) | 61.1 $\pm$ 12 | 65.2 $\pm$ 12 | $< 0.001$ |
| Woman, n (%) | 169 (19.1) | 73 (17.2) | 0.447 |
| Diabetes mellitus, n (%) | 246 (27.9) | 153 (36.1) | 0.003 |
| Hypertension, n (%) | 497 (56.3) | 270 (63.7) | 0.033 |
| Currently smoking, n (%) | 397 (45.0) | 170 (40.1) | 0.107 |
| Hyperlipidemia, n (%) | 462 (51.6) | 203 (46.1) | 0.063 |
| Hx of PCI, n (%) | 99 (11.2) | 60 (14.2) | 0.148 |
| Hx of stroke, n (%) | 42 (4.8) | 33 (7.8) | 0.031 |
| Hx of MI, n (%) | 468 (53.0) | 231 (54.5) | 0.636 |
| Homocysteine, mean $\pm$ SD, $\mu$mol/l | 8.7 $\pm$ 2.0 | 16.4 $\pm$ 8.5 | $< 0.001$ |
| Serum creatinine, mean $\pm$ SD, mg/dL | 1.01 $\pm$ 0.23 | 1.12 $\pm$ 0.24 | $< 0.001$ |
| eGFR, mean $\pm$ SD, ml/min/1.73 m$^2$ | 74.9 $\pm$ 26.8 | 65.8 $\pm$ 20.1 | $< 0.001$ |
| Stable angina, n (%) | 486 (55.0) | 242 (57.1) | 0.488 |
| ACS, n (%) | 397 (45.0) | 182 (42.9) | 0.521 |
| LVEF $< 40\%$, n (%) | 76 (9.3) | 70 (17.9) | $< 0.001$ |
| Cardiogenic shock, n (%) | 46 (5.2) | 27 (6.4) | 0.440 |
| Multivessel disease, n (%) | 578 (65.5) | 295 (69.6) | 0.149 |

ACS: acute coronary syndrome; eGFR: estimated glomerular filtration rate, calculated by the Modification of Diet in Renal Disease (MDRD) equation. Hx: history of occurrence 6 months or longer before the study; LVEF: left ventricular ejection fraction, measured by angiography; MI: myocardial infarction; PCI: percutaneous coronary intervention; SD: standard deviation.

Angiographic Evaluation

The minimal luminal diameter (MLD), reference vessel diameter (RVD), percent of diameter stenosis (DS%), and balloon diameter were measured from multiple projections using the automatic edge-detection method. Acute gain was defined as the difference in MLD between before and after stenting. The quantitative coronary angiographic analysis was performed by two experienced interventional cardiologists, and images were selected with an end-diastolic cine-frame of the most severe and nonforeshortened projection. A contrast-filled guiding catheter was used as a reference for calibration. The interobserver correlation coefficient was 0.93 ($p < 0.01$), and the intraobserver correlation coefficient was 0.95 ($p < 0.01$).

### Statistical Analysis

Data were prospectively collected and analyzed using SPSS statistical software package (version 22.0, IBM) for all statistical analyses. Continuous variables were represented as means $\pm$ standard deviation (SD) and compared between groups using a two-tailed $t$ test. Categorical variables were presented as numbers.
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Table 2. Angiographic and PCI-related characteristics (n = 1,604)

| Homocysteine level | <12.0 µmol/L (n=1082) | ≥12.0 µmol/L (n=522) | P value |
|--------------------|------------------------|-----------------------|--------|
| Complex lesion (type B2 or C*), n (%) | 832 (76.9) | 421 (80.7) | 0.094 |
| Target vessel site | | | 0.924 |
| Left main, n (%) | 24 (2.2) | 14 (2.7) | |
| LAD, n (%) | 442 (40.9) | 221 (42.3) | |
| LCA, n (%) | 207 (19.1) | 94 (18.0) | |
| RCA, n (%) | 402 (37.2) | 189 (36.2) | |
| Graft, n (%) | 7 (0.6) | 4 (0.8) | |
| Ostial lesion, n (%) | 78 (7.2) | 39 (7.5) | 0.838 |
| Bifurcation, n (%) | 76 (7.0) | 31 (5.9) | 0.456 |
| Lesion length, mean ± SD (mm) | 20.2 ± 10 | 20.5 ± 11 | 0.629 |
| Stent size, mean ± SD (mm) | 3.3 ± 0.7 | 3.3 ± 0.6 | 0.470 |
| Calcified, n (%) | 126 (11.6) | 89 (17.0) | 0.004 |
| Thrombus containing, n (%) | 163 (15.1) | 58 (11.1) | 0.037 |
| Number of stents per lesion | 1.10 | 1.12 | 0.178 |
| Pre-procedure target lesion | | | |
| % diameter stenosis, mean ± SD | 82.5 ± 13 | 82.9 ± 13 | 0.547 |
| MLD (mm), mean ± SD | 0.58 ± 0.5 | 0.57 ± 0.5 | 0.627 |
| RVD (mm), mean ± SD | 3.32 ± 0.6 | 3.33 ± 0.6 | 0.884 |
| Post-procedure target lesion | | | |
| % diameter stenosis, mean ± SD | 6.4 ± 6 | 6.6 ± 6 | 0.523 |
| MLD (mm), mean ± SD | 3.36 ± 0.5 | 3.10 ± 0.5 | 0.479 |
| RVD (mm), mean ± SD | 3.32 ± 0.6 | 3.36 ± 0.6 | 0.894 |
| Acute gain (mm), mean ± SD | 2.52 ± 0.6 | 2.53 ± 0.6 | 0.812 |

LAD: left anterior descending artery; LCx: left circumflex artery; LIMA: left internal mammary artery; OM: obtuse margin; MLD: minimal luminal diameter; PCI: percutaneous coronary intervention; PDA: posterior descending artery; PL: posterolateral artery; RCA: right coronary artery; RVD: reference vessel diameter; SD: standard deviation.

A complex lesion was defined as a lesion of type B2 and C according to the classification of the American College of Cardiology and the American Heart Association.

Results

Baseline Characteristics

A total of 1,340 patients were in accordance with eligibility criteria for study participants in the CAPTAIN registry. There were 33 patients excluded from relevant analyses for missing data. Finally, 1,307 patients (mean age 62 ± 12 years, range 24–93 years; 81.5% male) with 1,604 lesions and 1,800 BMS implantations were consecutively enrolled for analyses (Fig. 1). The patients were divided into two groups according to baseline plasma total HCY level with a cutoff value of 12 µmol/L: group I (883 patients, HCY <12 µmol/L), group II (424 patients, ≥12 µmol/L). Comparisons of the baseline characteristics are shown in Table 1. The patients in group II were significantly older (65.2 ± 12 vs. 61.1 ± 12 years, p < 0.001), and there were more patients with diabetes (36.1% vs. 27.9%, p = 0.003), hypertension (63.7% vs. 56.3%, p = 0.033), history of stroke (7.8% vs. 4.8%, p = 0.031), and left ventricular systolic dysfunction (ejection fraction <40%) at the initial event (17.9% vs. 9.3%, p < 0.001). The mean plasma total HCY levels in group I and II were 8.7 µmol/L and 16.4 µmol/L, respectively (Table 1). The mean serum creatinine level in group II was higher than that in group I (1.12 ± 0.24 vs. 1.01 ± 0.24 mg/dL, p < 0.001) and the mean estimated glomerular filtration rates (eGFR),
the target lesions. Also, there was no significant difference in the sizes and lengths of the implanted coronary artery stents between the two groups (Table 2).

A total of 1,800 stents were implanted including 40 Bx Velocity (Johnson and Johnson, Miami Lakes, Florida, USA), 427 Driver (Medtronic, Santa Rosa, CA, USA), 122 Express (Boston Scientific, Natick, Massachusetts, USA), 45 Integrity (Medtronic), 88 Liberte (Boston Scientific), 14 Multilink (Guidant, Santa Clara, CA, USA), 1 Multi-Link TETRA (Guidant), 312 Multi-Link PENTA (Guidant), 76 Multi-Link PIXEL (Guidant), 469 Multi-Link VISION (Abbott Vascular, Santa Clara, CA, USA), 53 Multi-Link ZETA (Abbott), 27 Omega (Boston Scientific), 61 R (Orbus-Neich, Hong Kong), and 65 S7 (Medtronic) stents.

### In-Hospital Cardiac Events

The in-hospital cardiac events in the two groups calculated by the Modification of Diet in Renal Disease (MDRD) equation, in group I and II were 74.9 ± 26.8 and 65.8 ± 20.1 mL/min/1.73 m², respectively (p < 0.001).

**Angiographic Characteristics**

There were total 1,082 lesions in group I and 522 lesions in group II, of which 1,253 (78.1%) were complex lesions (type B2 and C), 107 (6.7%) were bifurcation lesions, and 117 (7.3%) were ostial lesions. The mean lesion length was 20 ± 10 mm. There was no significant difference in lesion location, length, or complexity between the two groups. However, patients in groups II had more calcified lesions (17.0% vs. 11.6%, p = 0.004) and fewer thrombus-containing lesions (11.1% vs. 15.1%, p = 0.037). For PCI-related characteristics, there was no significant difference in the pre- or post-procedure DS%, MLD, or RVD of the target lesions. Also, there was no significant difference in the sizes and lengths of the implanted coronary artery stents between the two groups (Table 2).

### Table 4. Clinical events during long-term follow-up (n = 1,289, 58 ± 41 months)

| Homocysteine level |  <12.0 µmol/L | ≥12.0 µmol/L | P value |
|--------------------|---------------|--------------|---------|
| Number of patients | 876           | 413          |         |
| Overall mortality, n (%) |               |              |         |
| Cardiac, n (%)      | 56 (6.4)      | 50 (12.1)    | 0.001   |
| Non-cardiac, n (%)  | 30 (3.4)      | 33 (8.0)     | 0.001   |
| Non-fatal MI, n (%) | 26 (3.0)      | 17 (4.1)     | 0.318   |
| Target lesion revascularization, n (%) | 87 (9.9) | 46 (11.2) | 0.494 |
| New lesion stenting, n (%) | 83 (9.5) | 56 (13.6) | 0.034 |
| Coronary bypass surgery, n (%) | 15 (1.7) | 9 (2.2) | 0.659 |
| Non-fatal stroke, n (%) | 19 (2.2) | 9 (2.2) | 1.00   |
| MACE, n (%)         | 224 (25.6)    | 137 (33.3)   | 0.005   |

MACE: major adverse cardiac events; MI: myocardial infarction; NSTEMI: non ST-segment elevation myocardial infarction; STEMI: ST-segment elevation myocardial infarction

### Table 3. In-hospital cardiac events

| Homocysteine level |  <12.0 µmol/L | ≥12.0 µmol/L | P value |
|--------------------|---------------|--------------|---------|
| Number of patients | 883           | 424          |         |
| Death, n (%)       | 6 (0.7)       | 12 (2.9)     | 0.004   |
| Cardiac            | 6 (0.7)       | 10 (2.4)     | 0.014   |
| Non-Cardiac        | 0 (0)         | 2 (0.5)      | 0.105   |
| Non-fatal MI, n (%)|               |              |         |
| NSTEMI             | 18 (2.0)      | 8 (1.9)      | 1.00    |
| STEMI              | 2 (0.2)       | 0            | 1.00    |
| Acute stent thrombosis, n (%) | 3 (0.3) | 2 (0.5) | 0.662 |
| Subacute stent thrombosis, n (%) | 5 (0.6) | 4 (0.9) | 0.483 |
| Emergent bypass surgery, n (%) | 2 (0.2) | 1 (0.2) | 1.000 |
| Non-fatal stroke, n (%) | 2 (0.2) | 0          | 1.00    |
| MACE, n (%)        | 33 (3.7)      | 19 (4.5)     | 0.546   |

MACE: major adverse cardiac event; MI: myocardial infarction; NSTEMI: non ST-segment elevation myocardial infarction; STEMI: ST-segment elevation myocardial infarction
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Fig. 2. The MACEs-free survival by Kaplan–Meier analysis between groups
The event-free survival rate was significantly higher in the group I patients with lower homocysteine levels at admission (<12 µmol/L) (Log-rank test, p=0.001).
MACE: major adverse cardiac event.

are shown in Table 3. The overall and cardiac-related mortality rates were higher in group II than in group I (2.9% vs. 0.7%, p=0.004; 2.4% vs. 0.7%, p=0.014, respectively). However, there were no significant differences in the incidence of MI, acute, and subacute stent thrombosis, emergency bypass surgery, nonfatal stroke, or in-hospital MACE between the two groups.

Long-Term Clinical Outcomes
A total of 1,289 patients, who survived and discharged from hospital after PCI, were followed up for a mean of 58±41 months. The patients in group II had a significantly higher mortality rate than those in group I (12.1% vs. 6.4%, p=0.001) and majority were from cardiac death (8.0% vs. 3.4%, p=0.001). In addition, the patients in group II had a higher percentage of new coronary artery lesions requiring stenting than patients in group I (13.6% vs. 9.5%, p=0.034). Overall, the group II patients had a higher MACE rate than group I patients (33.3% vs. 25.6%, p=0.005) (Table 4). In Kaplan–Meier analysis, there was a significant difference in the MACE-free survival rate between the two groups (log-rank test; p=0.001) (Fig. 2). The univariate Cox proportional hazards analysis showed that the MACE rates were significantly associated with diabetes mellitus, LVEF <40%, multivessel disease, complex type B2 or C lesion, post-procedure MLD, serum creatinine level, and plasma HCY level ≥12 µmol/L. After adjusting for factors of age, gender, hypertension, diabetes mellitus, hyperlipidemia, serum creatinine, HCY level >12 µmol/L, multivessel disease, type B2 or C lesions, post-procedure MLD, and LVEF <40% in multivariable analysis, elevated plasma HCY levels (≥12 µmol/L) remained a statistically significant predictor for long-term cardiovascular outcomes, with a HR of 1.29 (95% CI: 1.02–1.64, p=0.036). Other significant predictors were LVEF <40% (HR: 1.52, 95% CI: 1.11–2.09, p=0.010), multivessel disease (HR: 1.51, 95% CI: 1.17–1.95, p=0.002), post-procedure MLD (HR: 0.66, 95% CI: 0.53–0.83, p<0.001), and serum creatinine (HR: 1.69, 95% CI: 1.06–2.71, p=0.027) (Table 5). Moreover, the plasma HCY values were divided into quintiles to examine their relationship with the long-term MACEs (Table 6). There was a graded association between HCY levels and MACE rates, which compared with quintiles 1–3, the adjusted HRs in quintile 4 (HCY range 11.3–13.4 µmol/L) and quintile 5 (HCY range >13.4 µmol/L) were 1.06 (95% CI: 0.78–1.44) and 1.37 (95% CI: 1.03–1.82), respectively.
miologic studies have shown a strong association between plasma HCY levels and the risks of atherosclerotic diseases. As observational studies have shown, even mildly elevated plasma HCY levels (between 11.4 and 14.3 µmol/L) increase the risk of cardiovascular events. However, the pathophysiological mechanisms were not fully understood. As implied from basic research on the mechanism of HCY associated with vascular injury, HCY produced reactive oxygen species and caused lipid peroxidation, platelets and leukocytes activation, and increased prothrombotic factors, which resulted in vascular inflammation, thrombus formation. Furthermore, HCY causes endothelial dysfunction with impaired release of nitric oxide and vasodilatation in response to shear stress in vascular wall, which predisposes to vulnerable plaques rupture, contributes to atheroma progression, and ultimately gives rise to vascular events. Case control studies showed HCY level was significantly higher in patients with acute coronary syndrome (ACS).

Discussion
In our retrospective analysis of a single center registry, 1,307 patients with coronary artery diseases after bare metal stent implantation were included for nearly 5 years follow-up. The patients with hyperhomocysteinemia (≥12 µmol/L) had a higher long-term MACE rate, mainly from cardiac mortality and new lesion stenting. After adjusting for multiple clinical, angiographic, and PCI-related variables, the association remained significant (HR, 1.29; 95% CI: 1.02–1.64). There is a dose dependent association between HCY levels and risks for long-term MACE, where the adjusted HRs in quintile 4 (HCY range 11.3–13.4 µmol/L) and quintile 5 (HCY range >13.4 µmol/L) were 1.06 (95% CI: 0.78–1.44) and 1.37 (95% CI: 1.03–1.82), respectively.

Since McCully et al. described vascular diseases in patients with severe hyperhomocysteinemia (>100 µmol/L) in 1969, subsequent case control and epidemiologic studies have shown a strong association between plasma HCY levels and the risks of atherosclerotic diseases. As observational studies have shown, even mildly elevated plasma HCY levels (between 11.4 and 14.3 µmol/L) increase the risk of cardiovascular events. However, the pathophysiological mechanisms were not fully understood. As implied from basic research on the mechanism of HCY associated with vascular injury, HCY produced reactive oxygen species and caused lipid peroxidation, platelets and leukocytes activation, and increased prothrombotic factors, which resulted in vascular inflammation, thrombus formation. Furthermore, HCY causes endothelial dysfunction with impaired release of nitric oxide and vasodilatation in response to shear stress in vascular wall, which predisposes to vulnerable plaques rupture, contributes to atheroma progression, and ultimately gives rise to vascular events. Case control studies showed HCY level was significantly higher in patients with acute coronary syndrome (ACS).
In regard to the role of HCY in PCI, researchers also reported that patients with coronary slow flow phenomenon had higher HCY levels, increased oxidative stress markers and impaired endothelial cell function. Although hyperhomocysteinemia was considered to be associated with more ACS and periprocedural acute thrombotic events in previous reports, there was no significant difference in incidence of initial ACS presentation and in-hospital acute events between two groups in our study. We thought it could be explained by two main reasons. First, insufficient numbers of in-hospital thrombotic events and relatively mild to moderate elevated HCY levels in both groups are not enough to detect the difference in the prespecified clinical outcomes. Second, the thrombotic events in CAD caused from a complex interaction between numerous factors from patient-related, pharmacologic-related, procedural-related, or device-related related factors that might be unequal distribution between two groups in a nonrandomized trial.

With respect of the issue of increased cardiac mortality in patients with hyperhomocysteinemia, some relevant clinical observation results might explain this background issue. First, HCY levels correlated to the severity and extent of coronary atherosclerosis and associated with vascular calcification and unstable atherosclerotic plaques. These anatomic factors increased the difficulties of coronary intervention and associated with acute complications and worse long-term clinical outcomes. Second, the prothrombotic and proinflammatory effects of HCY are associated with coronary slow flow during intervention and may impair angiogenesis and collateral vessels development, resulting in inadequate myocardial reperfusion, increased extent of myocardial necrosis, and finally ventricular pumping failure and worse long-term outcomes. However, we did not prospectively include the prespecified variables, such as vulnerability of atherosclerotic plaques or quantification of coronary slow flow during PCI in our initial study protocol and thus, there was no complete clinical data to analysis this association. Nevertheless, this issue is sufficiently noteworthy to merit additional well-designed studies.

In human studies, Kosokabe et al. observed a positive correlation between plasma HCY levels and atherosclerotic plaque areas in reference segments of coronary arteries, which were measured by intravascular ultrasound 6 months after the intervention. This evidence supports the atherogenic propensity of HCY and also could explain our study results, which more numbers of new lesions stenting and worse long-term outcomes in our patients with hyperhomocysteinemia.

However, the results in past studies were inconsistent. In the late 1990s, Nygard et al. reported a strong and graded relationship between plasma HCY levels and the overall mortality in 587 patients with angiographically confirmed CAD after a median follow-up of 4.6 years. In their study, patients with a homocysteine level >15 mmol/L had a 24.7% total mortality rate, compared with 3.8% for those with a homocysteine level <9 mmol/L (p=0.02). Later, Stubbs et al. also stated the similar association of plasma HCY levels and the long-term prognosis (median 2.5 years) in 440 patients with acute coronary syndrome, in which patients with a HCY level (>12.2 µmol/L) had a 2.6-fold higher risk of cardiac death and myocardial re-infarction. In contrast, a prospective observational study by Zairis et al., including 483 patients with either stable angina or acute coronary syndrome after successful coronary stenting, found no significant association between plasma HCY levels and the composite endpoint of cardiac death, MI, or re-hospitalization for angina after a median follow-up of 22 months. The disparate results may be attributed to the shorter follow-up duration and different enrolled patient population and study endpoints in later study. In Zairis et al. study, patients with CAD and LVEF <35%, implicating severe and extensive disease may have a higher plasma HCY level and poor prognosis, were excluded. Patients with ST segment elevation myocardial infarction (STEMI) or non-ST segment elevation myocardial infarction (NSTEMI) presented more than 6 h or 24 h of index pain, respectively, were excluded. The endpoint of re-hospitalization for angina may not be objectively and difficult to differentiate in some clinical situation. These enrolled criteria and study endpoints were also different from our study.

Even though evidence has shown an association between elevated plasma HCY levels and increased risks of late cardiac events and poor prognosis in patients with CAD, in randomized controlled trials, secondary prevention management with folic acid and vitamins failed to prevent or decrease cardiovascular events, even decreasing homocysteine levels. However, it seems too early to make a conclusion before further well-designed clinical trials with different dosages or agents were performed. A longer follow-up period and specific patient population with moderately elevated homocysteine levels may be necessary to observe impacts of HCY on progression of coronary atherosclerosis and cause late stent failure and clinical events. In fact, a recent meta-analysis on patients with chronic kidney disease found significant benefits of reducing risks of cardiovascular disease from folate treatment, especially in those with a higher baseline homocysteine level (mean >25 µmol/L, relative risk 0.87, p=0.049). The further research to investigate the pathogenesis of
HCY in atherosclerotic disease is important to contribute a newly potential therapeutic target to reduce homocysteine levels and improve outcomes.

**Limitations**

There were several limitations in this study. First, we used a cutoff value of HCY levels 12 µmol/L make uneventful distribution of the study population. Second, the optimal timing for blood collection and testing methods for plasma HCY levels have not been well defined. We sample blood in early morning after fasting for more than 12 h and within 24 h after admission. HCY levels may influence by numerous environmental factors such as dietary contents, which we could not estimate and adjust. Third, the methylene tetrahydrofolate reductase (MTHFR) enzyme, folic acid, vitamin B6, and vitamin B12 are required for metabolizing homocysteine to methionine or cysteine. Gene polymorphism of MTHFR C677T and dietary deficiency of folic acid, vitamin B6, or vitamin B12 are associated with hyperhomocysteinemia. In our study protocol, we did not routinely measure serum folate, vitamin B12, and gene polymorphism. Thus, we could not analyze the interaction between folic acid, vitamin B12, gene polymorphism, and risks of cardiovascular events. However, the association of MTHFR genotype and cardiovascular events is still controversial, and it could be modified by the folate status, where high folate levels reduced the effect of MTHFR C677T on HCY levels. Thus, we suggested that HCY levels reflect the influence of the gene—environment interactions and should be a more relevant factor to predict cardiovascular outcomes. Fourth, this is a single center prospective study and the results may not be applicable to other racial and ethnic groups.

**Conclusions**

An elevated HCY level (≥12 µmol/L) increased the risk of long-term cardiovascular events in patients who undergo intracoronary BMS implantations. After adjusting for clinical, angiographic, and PCI-related variables, an elevated homocysteine level remained a robust independent predictor for long-term clinical outcomes. Even randomized trials failed to demonstrate clinical benefits of HCY reducing therapies, HCY levels remain a useful clinical predictive marker for early identification of high-risk patients with worse long-term outcomes after coronary artery stenting.

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