Effect of Plasma Homocysteine Concentration on Early and Late Events in Patients With Acute Coronary Syndromes

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Background—Although a raised plasma homocysteine is a risk factor for vascular disease, it is not known whether it is associated with an adverse cardiac outcome in patients admitted with acute coronary syndromes. We evaluated the relationship between plasma homocysteine and short-term (28 days) and long-term (median 2.5 years) prognosis in acute coronary syndromes.

Methods and Results—We evaluated the relationship of quintiles of homocysteine to fatal and nonfatal coronary disease early (28 days) and late (29 days to a median of 2.5 years) after admission to a single unit of patients with unstable angina (n = 204) and myocardial infarction (n = 236). The end points studied were cardiac death (n = 67) and/or myocardial (re)infarction (n = 30). Cox regression and logistic regression were used to estimate the relationship of homocysteine to coronary events. The event rate within the first 28 days (22 cardiac deaths and 5 nonfatal infarctions) was not related to the admission homocysteine level. In the 203 unstable angina and 214 myocardial infarction survivors, an apparent threshold effect was seen on long-term follow-up, with a significant step-up in the frequency of events between the lowest 3 quintiles (14 cardiac deaths and 11 nonfatal infarctions) and the upper 2 quintiles (31 fatal and 12 nonfatal events). Patients in the upper 2 quintiles (>12.2 μmol/L) had a 2.6-fold increase in the risk of a cardiac event (95% CI, 1.5 to 4.3, P < 0.001).

Conclusions—Elevated total homocysteine levels on admission strongly predict late cardiac events in acute coronary syndromes. (Circulation. 2000;102:605-610.)

Key Words: homocysteine ■ coronary disease

Epidemiological studies have identified moderately raised concentrations of homocysteine as a potentially modifiable risk factor for coronary heart disease.1,2 This nonessential amino acid is produced by the demethylation of methionine, and very high levels are seen in genetically inherited enzyme defects of homocysteine metabolism, which are known to be associated with aggressive and premature vascular disease.3,4

The relationship between plasma homocysteine and prognosis has been less well studied. The reported effect of homocysteine as a prothrombotic factor5–9 might lead one to predict that high homocysteine might exacerbate intracoronary thrombosis during the acute phase of these syndromes. In addition, the known effect of high homocysteine on endothelium,9,10 seen most dramatically in homocystinuria,3,4 might cause a more aggressive course of ischemic heart disease after discharge, leading to more rapid reinfarction and death in the follow-up period.

The aim of the present study was to examine a possible relationship between admission plasma homocysteine and prognosis in subjects presenting with acute coronary syndromes.

Methods

Patients

Consecutive patients (n = 440) were recruited on admission to the coronary care unit. Myocardial infarction (MI) (n = 236) was diagnosed according to the World Health Organization criteria11; all were troponin T–positive. Unstable angina (n = 204) was retrospectively diagnosed if the WHO criteria for MI were not met11; all enzyme measurements (creatine kinase, aspartate aminotransferase, hydroxybutyrate dehydrogenase) were below twice the upper limit of the 95% reference range throughout the routine sampling period. Evidence of ischemic heart disease was demonstrated by either follow-up cardiac event, a positive coronary angiogram (≥5% stenosis in a major coronary segment), a positive exercise treadmill test (>0.1 mV ST-segment depression 80 ms after the L point), or a demonstration of ischemia on thallium scintigraphy. Troponin T was
positive (>0.1 µg/L) in 65 of 204 unstable angina patients (32%) at 12 to 24 hours after admission.

Previous MI was established by evidence of previous hospital admission and a previous discharge diagnosis of MI according to WHO criteria. Discharge medications were standardized in accordance with the recommendations of the Action on Secondary Prevention through Intervention to Reduce Events (ASPIRE) study. Follow-up data were determined by examination of hospital records, postmortem results when available, death certificates, general practitioner questionnaire, or patient or next-of-kin questionnaire, with follow-up telephone contact if necessary. The follow-up flow chart is illustrated in Figure 1. Survival status and cause of death were established for all patients. Cause of death was classified according to American Heart Association criteria.

**Protocol**

Venous blood samples were obtained on admission before initiation of thrombolysis or anticoagulant treatment and transferred into heparin-coated tubes (Becton Dickinson). Platelet-poor plasma was immediately obtained by centrifugation at room temperature for 15 minutes at 3000g. Aliquots of plasma were then transferred to a −80°C freezer within 1 hour of sampling and stored for batch analysis.

The mortality within the first 28 days was analyzed separately to provide evidence on short-term mortality. We followed the remaining 417 patients from day 28 thereafter, for a median of 944 days (lower quartile 845, upper quartile 1185, maximum of 1607 days), by quintiles of admission homocysteine.

**Homocysteine Measurements**

Plasma total homocysteine, which includes the sum of protein-bound and free homocysteine, was measured by high-performance liquid chromatography with fluorescence detection by a laboratory with a published validation. The coefficients of variation within and between days for the assay were ≤5%.

**Troponin T Assay**

Troponin T was determined by an ELISA using an ES-300 immunoassay analyzer (Boehringer Mannheim) as previously described.

**Cholesterol**

Admission cholesterol concentration (reference interval 3.5 to 6.5 mmol/L) was measured by a cholesterol oxidase method on a Technicon Axon (Bayer Technicon) by the manufacturer’s recommended procedure (within-run coefficient of variation 2.2%; between-run, 5%).

**Statistical Methods**

Data were analyzed with Stata Release 5 software.

Homocysteine closely followed a log-normal distribution (correlation 0.991 with expected log-normal values) and was log-transformed before use of linear models. Because of the log-normal distribution, geometric means and their associated 95% CIs are reported.

The plasma homocysteine concentration values were divided into quintiles to examine its relationship with end points adjusted for factors known to influence homocysteine concentrations and to determine the usefulness of regression-based analysis.

Logistic regression was used to examine the relationship of homocysteine to 28-day mortality.

Long-term prognosis was analyzed with an event history approach. This is an extension of Cox regression to handle the occurrence of multiple end points per patient (in this case, nonfatal reinfarction and cardiac death). The analysis also handles a change in risk factor status over the course of follow-up (in this case, a patient who was admitted to the study with unstable angina and then suffered a nonfatal infarction would be reclassified in the subsequent follow-up as a postinfarction patient). Data were checked graphically for departure from the proportional-hazards assumption by use of log-log plots.

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**Table 1. Baseline Demographics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>No.</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>61.5 (53, 74)</td>
<td></td>
</tr>
<tr>
<td>Male sex</td>
<td>74.7% (329/440)</td>
<td></td>
</tr>
<tr>
<td>Previous MI</td>
<td>25.7% (113/440)</td>
<td></td>
</tr>
<tr>
<td>Previous revascularization</td>
<td>4.8% (21/440)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>26.4% (116/440)</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>13.6% (60/440)</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>35.2% (155/440)</td>
<td></td>
</tr>
<tr>
<td>CCSC 3/4</td>
<td>12.3% (53/440)</td>
<td></td>
</tr>
<tr>
<td>Rest pain on admission</td>
<td>37.7% (166/440)</td>
<td></td>
</tr>
<tr>
<td>ST elevation</td>
<td>31.4% (138/440)</td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>140 (120, 160)</td>
<td></td>
</tr>
<tr>
<td>Pulse rate, bpm</td>
<td>80 (68, 95)</td>
<td></td>
</tr>
<tr>
<td>Time from pain to sample, h</td>
<td>5.0 (3, 8)</td>
<td></td>
</tr>
<tr>
<td>Admission homocysteine, µmol/L</td>
<td>11.0 (9, 15)</td>
<td></td>
</tr>
<tr>
<td>Admission cholesterol, mmol/L</td>
<td>6.0 (5, 7)</td>
<td></td>
</tr>
<tr>
<td>Final diagnosis of MI, WHO criteria</td>
<td>53.6% (236/440)</td>
<td></td>
</tr>
<tr>
<td>Final diagnosis of UAP</td>
<td>46.4% (204/440)</td>
<td></td>
</tr>
<tr>
<td>Hospital stay, d</td>
<td>9.0 (5, 13)</td>
<td></td>
</tr>
<tr>
<td>Aspirin therapy</td>
<td>74.3% (327/440)</td>
<td></td>
</tr>
<tr>
<td>β-Adrenergic antagonist therapy</td>
<td>24.1% (106/440)</td>
<td></td>
</tr>
<tr>
<td>Calcium antagonist therapy</td>
<td>27% (119/440)</td>
<td></td>
</tr>
<tr>
<td>Follow-up, d</td>
<td>944 (845, 1185)</td>
<td></td>
</tr>
<tr>
<td>Revascularization after ACS</td>
<td>26.4% (116/440)</td>
<td></td>
</tr>
</tbody>
</table>

Continuous variables are expressed as medians (lower quartile, upper quartile). Nominal variables are expressed as percentages (number/total). CCSC indicates Canadian Cardiac Society Angina grade in the week before admission; BP, blood pressure; UAP, unstable angina pectoris; and ACS, acute coronary syndrome.
Results

The baseline demographics for the cohort are shown in Table 1. Of these, 53.6% (236/440) had a final diagnosis of MI and 46.4% (204/440) a final diagnosis of unstable angina as defined above. A flow diagram with patient numbers, including the numbers of patients in each group who had suffered a previous MI, is depicted in Figure 1. The geometric mean value of plasma homocysteine for the whole group was 11.7 μmol/L (95% CIs, 11.3 to 12.2). These CIs do not overlap with those for normal subjects obtained by the European Concerted Action Study Group of 9.50 to 9.96 μmol/L (geometric mean, 9.73 μmol/L). Patients received secondary prevention therapy in line with a recently published United Kingdom survey. 12

Table 2 shows the study group characteristics known to influence homocysteine concentration presented as geometric means and their 95% CIs. Multiple regression confirmed independent relationships between homocysteine and sex (higher in men, \( p<0.001 \)), smoking (higher in current smokers than ex-smokers or nonsmokers, \( p=0.047 \)), and age (higher in older patients, \( p<0.001 \)). Corrected for these characteristics, there was no relationship between homocysteine and cholesterol, systolic blood pressure, triglycerides, history of angina, history of diabetes, or presenting diagnosis (MI or unstable angina). There was a relationship between plasma homocysteine concentration and blood urea (Spearman rank correlation coefficient, 0.39; \( p<0.0001 \), even though most urea values were in the normal range (mean, 6.75 mmol/L; 95% CI, 6.44 to 7.06). The distribution of important risk factors is presented in Tables 2 and 3.

Outcome at 28 Days

Among the 236 patients admitted with MI, there were 22 cardiac deaths (9.3%), 1 noncardiac death (a cerebrovascular accident), and 3 nonfatal reinfarctions. There were 1 cardiac death and 2 nonfatal infarctions among the 204 patients admitted with unstable angina. Homocysteine was examined visually for association with mortality among the MI patients with a kernel-density smoother and numerically, by examination of mortality in successive quintiles of homocysteine concentration. There was no evidence of increased risk of death within 28 days associated with homocysteine level, with mortality rates of 6.1%, 10.6%, 9.1%, 8.7%, and 11.8% in the first to the fifth quintiles of homocysteine. Logistic regression confirmed that there was neither a linear trend of increasing risk of cardiac death with homocysteine (\( z=-0.151, p=0.880 \)), nor did comparison of death rates by quintiles of homocysteine reveal any evidence of a risk threshold. Inclusion of nonfatal cardiac events produced substantially similar results.

Long-Term Outcome

The values obtained for plasma homocysteine in the patients surviving 28 days and their event rates for cardiac death plus nonfatal MI are presented for each quintile in Table 4. There were 67 follow-up cardiac events, comprising 45 cardiac deaths and 22 nonfatal reinfarctions.

There were 31 cardiac deaths and 12 nonfatal MIs in the upper 2 quintiles (>12.2 μmol/L) recorded during 409
patient-years of follow-up, an event rate of 10.5% (95% CIs, 7.8% to 14.2%). The events in the lower 3 quintiles of admission homocysteine concentration were 14 cardiac deaths and 11 nonfatal MIs in 691 patient-years of follow-up, an event rate of 3.6% (95% CIs, 2.4% to 5.4%). The events for cardiac death plus nonfatal MI for each individual quintile are presented in Table 4.

Examination of log-log plots suggested that the shape of the survival function differed between the sexes (the survival curves of men and women crossed), violating the proportional hazards assumption; sex was therefore entered into a model as a stratifying variable. Table 5 shows the relationship of quintiles of homocysteine to risk of a cardiac event. The hazard ratios represent the increase in risk of a cardiac event relative to the risk in the lowest quintile of homocysteine. The second and third quintiles had hazard ratios of 1.1 and 1.3, respectively, and the fourth and fifth had hazard ratios of 3.0 and 3.7, respectively. Post hoc Wald tests of the coefficients showed that the hazard ratios associated with the second and third quintiles did not differ significantly from each other ($\chi^2=0.04$, $P=0.851$). Likewise, the hazard ratios for the fourth and fifth quintiles did not differ from one another ($\chi^2=0.33$, $P=0.566$). However, a Wald test of the hypothesis that the coefficients for quintiles 2 and 3 jointly differed from those for quintiles 4 and 5 was highly significant ($\chi^2=12.1$, $P<0.001$). Similar results for the dependence of outcome on homocysteine by quintile were obtained when adjustment was made for age and smoking (Table 5).

### Hazard Ratios Associated With Other Risk Factors

In univariate analysis, the rate of cardiac events over the follow-up was significantly associated with age, with a hazard ratio of 1.9 (95% CI, 1.4 to 2.4) for a 10-year increase in age. The event rate was also significantly associated with systolic blood pressure, with a hazard ratio of 1.09 for a 10 mm Hg increase in pressure (95% CI, 1.002 to 1.18). Diabetes was associated with a hazard ratio of 1.9 (95% CI, 1.04 to 3.3). Those patients with a previous MI were at increased risk over the follow-up period (hazard ratio, 2.0; 95% CI, 1.3 to 3.3). Risk was not associated with current smoking on admission (hazard ratio, 0.97; 95% CI, 0.59 to 1.6) or with admission cholesterol (hazard ratio, 1.005; 95% CI, 0.80 to 1.3). Male sex was associated with a hazard ratio of 0.80 (95% CI, 0.47 to 1.3).

In multivariate analysis, 2 independent predictors emerged: age, with a hazard ratio of 1.8 associated with a 10-year increment (95% CI, 1.4 to 2.3), and previous MI (hazard ratio, 2.0; 95% CI, 1.2 to 3.4), with a third, current smoking, on the threshold of statistical significance, with a hazard ratio of 1.7 but with a 95% CI of 1.0 to 3.0. Diabetes also had a hazard ratio of 1.7 in multivariate analysis, but with a 95% CI of 0.93 to 3.1.

The data in Figure 2 confirm the sharp difference in prognosis in the upper 2 quintiles compared with the lower 3. A comparison of the Kaplan-Meier curves in Figure 2 with those predicted by the Cox regression revealed no detectable departure of the observed survival from that predicted by the Cox model.

### Discussion

This study shows that moderately raised plasma total homocysteine concentration measured on admission to hospital is a strong predictor of late adverse cardiac events in patients admitted with acute coronary syndromes. That homocysteine is such a predictor for patients with stable coronary artery disease undergoing coronary angiography has been shown previously, but the present study is the first to show that this is the case for patients in whom homocysteine is measured on admission with an acute MI or unstable angina. Follow-up events were not extended to include soft end points. We looked at revascularization and realized that it was not a disease-related end point, but rather an index of health service availability to the patients. No other end points than fatal and nonfatal MI were used.

The apparent threshold effect in the present study of $\approx 12.0 \mu\text{mol/L}$ corresponds to the top fifth of the distribution in samples taken from 800 European disease-free normal subjects. In this case-control study, this level was associated with a doubling of the risk for a first cardiac event. Similar primary epidemiological case-control and prospective studies have produced mixed results with regard to whether homocysteine exhibits a "dose response", or a threshold relationship with the risk of a first cardiovascular event. The Multiple Risk Factor Intervention Trial (MRFIT) also suggested that homocysteine may be a stronger risk factor for the recurrence of events than for a first cardiovascular event. Some support for this last statement has come from the recently published study by Nygard et al, who examined the prognostic significance for cardiac death of homocysteine concentrations in a selected group of patients referred for
coronary angiography. Most of this cohort subsequently underwent revascularization, but homocysteine was shown to be a strong predictor of subsequent cardiac death. Although reported as showing a graded response between homocysteine concentration and subsequent death, the upper quartile (>20.0 μmol/L) appeared to show a considerable step-up in hazard, although this was not significant, possibly because of the sample size and event rate.

It is interesting that both the study by Nygard et al and this study show that revascularization procedures were not related to homocysteine level. This may reflect variations in the thresholds and criteria for invasive investigation among individual physicians. The question also arises as to whether those with moderately raised homocysteine concentrations are adequately protected from future cardiac events with this strategy, because both studies show that revascularization did not affect subsequent outcome in this group.

The pathophysiological mechanism by which risk increases is not clearly understood but includes such aspects as a toxic effect on the vascular endothelium, impaired endothelium-dependent relaxation, a hypercoagulable state resulting from downregulation of thrombomodulin expression, activation of factor V, inhibition of protein C activation, and perhaps increased platelet aggregation. Whether some or all of these findings can explain the adverse outcome of patients with established coronary disease awaits study.

A critical question is whether the relation of homocysteine and mortality is confounded by an association of total homocysteine levels with other strong predictors of mortality, such as age, sex, and current smoking, and by a negative association with predictors of survival, such as thrombolysis, β-blockade, and revascularization. After adjustment for these factors, the predictive power of total homocysteine levels remained strong and significant. The frequency of revascularization (Table 1) is a relatively soft end point, the need being determined by individual physician practice and the timing being determined by health-care resources. The magnitude of the increase in risk associated with elevated homocysteine can be better appreciated by comparison with the risks of these conventional factors. Previous MI, the strongest of the other predictors of coronary prognosis, was associated with a hazard ratio of 2.0, whereas elevated homocysteine (fourth or fifth quintile, compared with the first 3 quintiles) was associated with a hazard ratio of 2.6.

We examined the effect of adding more predictors to the multivariate model of the effect of homocysteine on prognosis and ran a model that included age, current smoking, previous MI, diabetes, and sex (the latter as a stratification variable). The hazard ratios for quintiles of homocysteine were virtually unchanged: with the first quintile as the baseline, the subsequent quintile hazard ratios were 0.88, 0.96, 2.74, and 2.27. We have retained the estimates of effect from the simpler multivariate model presented in this article, because the associated CIs are narrower. The more complex model, although it did not change the point estimates of effect, is inevitably associated with a loss of precision.

The observation of an association between a raised plasma homocysteine level and the occurrence and recurrence of cardiovascular disease may imply the need for greater efforts to correct the nutritional factors that control homocysteine metabolism. Evidence from patients with homocystinuria shows that homocysteine-lowering therapy does reduce the risk of cardiovascular disease. Whether such therapy reduces risk in patients with moderately raised homocysteine

Figure 2. Kaplan-Meier survival curves according to admission plasma homocysteine concentration quintile for (A) cardiac death and (B) death or nonfatal myocardial infarction.
awaits the results of several randomized controlled trials currently under way. Our results suggest that any future study of folic acid in acute coronary syndrome patients would need to be designed to study the effect on long-term mortality rather than hospital mortality.

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References
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