Elevated Homocysteine Levels Are Associated With Increased Ischemic Myocardial Injury in Acute Coronary Syndromes

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BACKGROUND
Elevated homocysteine levels are associated with increased thrombosis in patients presenting with ACS. It is not known whether this association is reflected in the degree of myocardial injury in those patients.

METHODS
We studied consecutive patients presenting with acute myocardial infarction (MI) (n = 205) and unstable angina pectoris (UAP) (n = 185). Plasma samples were collected on admission and prior to clinical intervention and were assayed for homocysteine by high performance liquid chromatography (HPLC). Myocardial necrosis was assessed by measurements of cardiac troponin T (cTnT) on admission and 12 h after admission (peak cTnT). The patients were studied by quintiles of homocysteine concentration.

RESULTS
There was a significant increase in peak cTnT in the 5th homocysteine quintile in MI (analysis of variance [ANOVA], p = 0.005), the levels being 4.10, 3.86, 4.13, 6.20 and 7.85 µg/liter for quintiles 1 to 5, respectively (p < 0.0001, for top vs. bottom quintile). Similarly, there was a step-up in peak cTnT levels in the top homocysteine quintile in UAP (ANOVA, p < 0.0001), the levels being 0.03, 0.03, 0.02, 0.04 and 0.15 µg/liter, (p < 0.0001 for top vs. bottom quintile). In a multivariate regression model, the association between peak cTnT and the top homocysteine quintile remained strong after adjustment of other confounders including age, gender, final diagnosis and thrombolysis treatment (odds ratio [OR]: 2.92 (1.75–4.87) p < 0.0001). The patients with UAP were further examined, according to peak cTnT levels below (cTnT negative) or above (cTnT positive) 0.1 µg/liter. Homocysteine levels were significantly higher in cTnT positive than cTnT negative patients; 13.8 (11.7–15.3) vs. 10.3 (9.4–11.3) µmol/liter, respectively, p = 0.002.

CONCLUSIONS
Elevated homocysteine levels are associated with a higher risk of ischemic myocardial injury in patients presenting with ACS.

Moderate elevation of plasma homocysteine concentrations has been reported to be an independent risk factor in the development of coronary artery disease (1,2). Many of the reported effects of plasma homocysteine are thought to be mediated by its atherogenic and prothrombotic properties (3,4). These were clearly evident in patients suffering from the congenital disorder of homocystinuria where marked elevation of plasma homocysteine was accompanied by widespread atherosclerosis and vascular thrombotic events at an early stage in life (5).

Patients with angiographically determined coronary artery disease have been reported to experience a concentration dependent increase in the risk of death with increasing homocysteine concentration (6). The mechanisms of this increase in mortality risk have not, however, been determined. It is known that atherosclerotic coronary artery disease may progress undetected with no serious threat to life. In contrast, the development of acute coronary syndromes (ACS) of myocardial infarction (MI) and unstable angina pectoris (UAP) mark a significant rise in the risk of death and long-term morbidity (7). We have recently reported an association between homocysteine concentrations and plasma markers of thrombosis activation in patients presenting with ACS (8). These results have led us to postulate that elevated homocysteine concentrations would lead to increased myocardial injury in this patient group.

In the present study we investigated the relationship between homocysteine concentrations determined on admission in patients presenting with acute MI or UAP and the degree of myocardial necrosis. The latter is estimated by measurement of admission and peak plasma release of the structural cardiac protein troponin T (cTnT).

METHODS
The study population consisted of patients presenting to a single coronary care unit with clinical signs of acute coro-
samples were obtained on admission from a cuffed antecubital vein before initiation of thrombolysis or anticoagulant treatment. Within 15 min of collection, platelet-poor plasma was obtained by centrifugation for 15 min at room temperature at 3,000g and flash-frozen on dry ice before transfer to a −80°C freezer pending batch analysis. A further sample was obtained after 12 h from admission, the time point when cardiac cTnT is most efficient for diagnosing myocardial damage in this population (10). All management decisions were made without knowledge of patients’ cTnT or homocysteine results. All samples were batch-assayed for cTnT and homocysteine by two different operators and were blinded from the final diagnostic classification.

**Homocysteine assay.** Plasma total homocysteine, which includes the sum of protein-bound and free homocysteine, was measured by high-performance liquid chromatography with fluorescence detection (11,12). The coefficients of variation within and between days for the assays were 5% or less. Plasma homocysteine was recorded in units of μmol/liter.

Plasma homocysteine measurement on admission in a separate group of patients with MI (n = 22) showed a median, in μmol/liter, with 25th to 75th interquartile range of 11.9 (10.7–12.6) on admission and of 11.5 (9.1–13.4) on day 2. Day 2 is the first measurement in published data on changes following an acute coronary syndrome (13). A letter to the *Lancet* shows a slight decrease on day 2 compared with admission, compatible with our results (14).

**Troponin-T assay.** Cardiac-specific troponin T was determined by an enzyme-linked immunosorbent assay (ELISA) using an ES-300 immunoassay analyzer (Boehringer Mannheim, Lewes, Sussex) as previously described (10). The detection limit for the assay was 0.01 μg/liter. The inter-assay coefficient of variation was 12.3%, 7.7% and 4.2% at mean cTnT levels of 0.13, 1.6 and 7.1 μg/liter, respectively. The intra-assay coefficient of variation was 2.0% and 1.9% at mean cTnT values of 1.7 and 7.0 μg/liter, respectively.

**Statistical analysis.** Homocysteine and cTnT values were logarithmically transformed; the latter followed a censored log-normal distribution with values less than 0.01 and greater than 40 μg/liter, representing the limits of measurements. Values for homocysteine and cTnT are expressed as geometric means (95% confidence interval [CI]). Qualitative values for cTnT were determined by categorizing the patients above (troponin-T positive) and below (troponin-T negative) the cTnT value of 0.1 μg/liter. This cutoff value has often been reported to signify future cardiac morbidity and mortality in ACS (15,16). Homocysteine values were categorized into quintiles (interquintile ranges 8.50, 10.20, 12.26, 15.8 μmol/liter in ACS; 8.40, 10.20, 12.20, 15.70 μmol/liter in UAP; and 8.50, 10.18, 12.60, 16.46 μmol/liter in MI). Differences between categorical variables were tested using the chi-square test. Group comparisons of the log-transformed values were made by independent sample *t* test for two groups and analysis of variance.

**Abbreviations and Acronyms**

ANOVA = analysis of variance  
ACS = acute coronary syndromes  
AST = aspartate transaminase  
CI = confidence interval  
CK = creatine kinase  
cTnT = cardiac troponin T  
ECG = electrocardiogram  
ELISA = enzyme-linked immunosorbent assay  
HBD = hydroxybutyrate dehydrogenase  
MI = myocardial infarction  
UAP = unstable angina pectoris  
WHO = World Health Organization
(ANOVA) for more than two groups. In the latter situation, differences between individual groups were then determined by a post hoc Dunnett test. Multivariate regression analysis of continuous variables was made using a linear regression model and of categorical variables using a logistical regression model. All reported probability values are two-sided. Analyses were performed using SPSS for Windows.

RESULTS

A total of 390 consecutive patients presenting with ACS (MI, n = 205; UAP, n = 185) were studied. Table 1 shows the basic characteristics of the patient groups. Patients presenting with MI were of older age, whereas patients with UAP had a more frequent history of prior MI. Otherwise, risk factor profiles were similar in both groups. Myocardial injury was significantly more prominent in patients with MI than UAP as expected and as evidenced by higher peak CK, AST, HBD levels and also by higher admission and peak cTnT levels. Homocysteine concentrations were not different between the two groups.

The relationship between homocysteine and cTnT was first examined by categorizing the patients according to quintiles of admission homocysteine. Figure 1 shows the relationship between cTnT concentrations on admission in relation to homocysteine quintiles. There was no significant difference in cTnT levels in MI (Fig. 1a) (0.08, 0.09, 0.10, 0.05 and 0.20 µg/liter for quintiles 1 to 5, respectively, ANOVA, p = NS) or in UAP (Fig. 1b) (0.02, 0.02, 0.02, 0.02 and 0.03 µg/liter for quintiles 1 to 5, respectively, ANOVA, p = NS). However, when the two groups were combined (Fig. 1c), there was an increase in cTnT levels in the upper homocysteine quintile (0.04, 0.05, 0.04, 0.03 and 0.08 µg/liter for quintiles 1 to 5, respectively, ANOVA, p = 0.02). The likelihood of a troponin-T positive status in patients within the top homocysteine quintile versus the bottom four quintiles was 58% and 40% in MI (p = 0.07), and 25% versus 12% in UAP (p < 0.05).

The association between homocysteine concentrations and cTnT was more prominent when the latter reached its maximal values. Figure 2 illustrates a significant elevation in peak cTnT in the 5th homocysteine quintile in MI (Fig. 2a, ANOVA, p = 0.005; 4.10, 3.86, 4.13, 6.20 and 7.85 µg/liter for quintiles 1 to 5, respectively [p < 0.0001], for top vs. bottom quintile). Similarly, there was a rise in peak cTnT levels in the top homocysteine quintile in UAP (Fig. 2b, ANOVA, p < 0.0001; 0.03, 0.03, 0.02, 0.04 and 0.15 µg/liter [p < 0.0001] for top vs. bottom quintile). Figure 2c shows the same analysis with both groups combined together (ANOVA, p < 0.0001).

Table 2 shows a multivariate regression analysis with peak cTnT as the dependent variable in the ACS patients. The cTnT levels were influenced by greater age, thrombolysis, a final diagnosis of MI and homocysteine levels in the 5th quintile.

![Figure 1](image.png)

**Figure 1.** Admission cTnT levels by quintiles of admission homocysteine in patients with (a) MI, (b) UAP, and (c) all ACS patients.
The number of UAP patients with a positive cTnT on admission (14%) more than doubled when cTnT was measured at the peak time point (29%), $p < 0.0001$. No differences were seen in homocysteine concentration between admission cTnT negative and cTnT positive UAP patients (11.2 [10.6–11.9] vs. 12.6 [10.8–14.7] μmol/liter, respectively, $p = 0.14$). In contrast, homocysteine concentrations were significantly higher in peak cTnT positive compared to peak cTnT negative patients (13.8 [11.7–15.3] vs. 10.3 [9.4–11.3] μmol/liter, respectively, $p = 0.002$).

**Figure 2.** Peak cTnT levels by quintiles of admission homocysteine in patients with (a) MI, (b) UAP, and (c) all ACS patients.

The likelihood of a peak troponin T positive status in UAP in the top homocysteine quintile versus bottom four quintiles was 62% versus 24%, $p < 0.0001$. In a multivariate logistic regression analysis, homocysteine concentrations in the top quintile were the strongest predictor of positive peak cTnT status in UAP (Table 3).

We carried out an analysis of other biochemical markers of myocardial injury by quintiles of homocysteine, which showed CK, AST and HBD are higher in the top homocysteine quintile than in the others; however, the $p$ value from the ANOVA was greater than 0.05. These less clear-cut results are presumably related to the nonspecificity of these markers.

**DISCUSSION**

The main outcome of the present study was a significant association demonstrated between elevated homocysteine and peak cTnT levels in UAP and MI patients. Threshold levels of 16.5 μmol/liter in MI and 15.7 μmol/liter in UAP were strong and independent predictors of ischemic myocardial necrosis in those patient groups.

We have used the WHO definition of MI (based on CK) because it is generally accepted (until it is replaced), but obviously cTnT is a specific biochemical marker while CK is not. However, we have shown that within the range of cTnT in unstable angina (below 0.2 μg/liter) there is a variable release of cTnT, with those patients who develop levels between 0.1 and 0.2 at higher risk (17,18). This is in a range of cTnT that approximately corresponds to negative CK. Although we believe these patients do not

**Table 3.** Multivariate Logistic Regression Model of Positive Peak cTnT Status (>0.1 μg/liter) With the Legend Variables Listed in UAP Patients

<table>
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<tr>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>$p$ Value</th>
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</thead>
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<td>Age</td>
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<tr>
<td>Male gender</td>
<td>1.03</td>
<td>0.47–2.27</td>
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<tr>
<td>Hypertension</td>
<td>1.29</td>
<td>0.59–2.81</td>
</tr>
<tr>
<td>Current smoking</td>
<td>1.29</td>
<td>0.59–2.80</td>
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<tr>
<td>Accelerated angina</td>
<td>0.90</td>
<td>0.35–2.34</td>
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<td>Homocysteine quintile (2)</td>
<td>2.04</td>
<td>0.67–6.22</td>
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<tr>
<td>Homocysteine quintile (3)</td>
<td>0.87</td>
<td>0.27–2.87</td>
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<tr>
<td>Homocysteine quintile (4)</td>
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<td>0.46–4.41</td>
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<tr>
<td>Homocysteine quintile (5)</td>
<td>7.32</td>
<td>2.26–23.71</td>
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</table>

**Table 2.** Statistical Association Between Peak Troponin T Levels and Legend Variables Listed in ACS Patients

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>95% Confidence Interval</th>
<th>$Z$</th>
<th>$p$ Value</th>
</tr>
</thead>
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<td>Age</td>
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<td>Male gender</td>
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<tr>
<td>Thrombolysis</td>
<td>1.135</td>
<td>0.658–1.614</td>
<td>4.656</td>
</tr>
<tr>
<td>Final diagnosis</td>
<td>5.155</td>
<td>4.664–5.646</td>
<td>20.582</td>
</tr>
<tr>
<td>Homocysteine quintile (5)</td>
<td>1.072</td>
<td>0.561–1.583</td>
<td>4.113</td>
</tr>
</tbody>
</table>
have thrombotic coronary occlusion, we do believe they have intracoronary thrombosis leading to peripheral embolization, which causes cTnT release. We believe these patients need aggressive nonthrombolytic treatment (e.g., IIb/IIIa antagonism). Our main excitement from the present study is that in this range, in UAP, myocardial damage could be determined, at least partly, by homocysteine concentration, homocysteine being prothrombotic (8).

It is assumed above that homocysteine is the independent variable and that cTnT is dependent upon homocysteine. At a single time point, one might argue the opposite, namely that the increased cTnT is causing the elevated homocysteine. This question is resolved by examining the time courses of change of the two variables. All the published data deal with changes from a first sample on the day after patient admission to later levels. There are no published data on changes from admission day to the day after admission. We do not have such data in the present study, only the values for the admission day.

However, we have performed another study (19) on a different cohort of patients that clearly shows homocysteine levels do not change from admission day to the following day. They do not change from the time of basal cTnT measurement and the time of measurement of peak troponin. The only other data are found in a letter to the Lancet (14), which are entirely consistent with our own. Thus, we can state that the rise of cTnT, consequent upon admission conditions, occurs at a constant level of one of those conditions, namely homocysteine; we are describing in the present article a situation in which troponin rises to a higher peak when background homocysteine remains at a steady higher level throughout the period of study.

The rise in cTnT levels was most notably observed in the top homocysteine quintile in both MI and UAP. This suggests the relationship between homocysteine and peak cTnT to be a threshold rather than a concentration-related association. This contrasts with our previous finding of a concentration-related association between homocysteine and activation of thrombosis in similar (different) patient group (8). Although thrombosis plays an important pathophysiological role in acute coronary artery occlusion, the degree of myocardial injury culminates from the interaction of several factors in addition to thrombus formation, including the duration of ischemia, physical characteristics of the plaque, coronary vascular spasm and the extent of collateral circulation (20). Homocysteine, in contrast, may enhance myocardial tissue injury by more than its prothrombotic effects. Both experimental (21) and recent clinical (22,23) evidence have shown that homocysteine adversely affects endothelial-dependent vasodilation, thereby possibly further limiting coronary flow reserve during acute coronary occlusion.

In the present study we used both qualitative and quantitative cTnT values on admission and at the peak time point to study the relationship with homocysteine. Despite a trend toward higher admission cTnT values in the top homocysteine quintile, the relationship with homocysteine was significantly more prominent when cTnT levels were taken at the peak time point, reflecting a stronger association between homocysteine and the final myocardial injury/infarct size. Levels of cTnT begin to rise 3 to 6 h after the onset of ischemic injury, and they reach a peak by 12 to 24 h, with levels remaining elevated for four to five days thereafter (24,25). Admission levels are therefore useful for diagnostic purposes in confirming the presence of myocardial injury, but they are not useful for estimating the degree of myocardial damage. The latter is more reliably related to the maximal rise in cTnT level (10,26).

The utilization of cTnT as a marker of myocardial injury has significantly improved the diagnostic accuracy of acute ischemic events, especially in the settings of equivocal clinical presentation and/or nonspecific ECG changes (15). However, because of the release-kinetic characteristics described earlier, qualitative (values above or below 0.1 to 0.2 μg/liter) rather than quantitative assays have been advocated more in the clinical setting for both diagnostic and predictive purposes. Therefore, using a defined cut of value, both early (17,18) and peak detection (16) of cTnT (troponin-T positive) in patients with or without ST segment elevation have been shown to predict a worst short- and long-term outcome in patients presenting with acute coronary events. In our study, homocysteine levels were clearly elevated in cTnT positive patients, especially in UAP. This result is in agreement with the previous finding of a relationship between homocysteine and mortality in patients with angiographically determined coronary artery disease (6) and in patients presenting with ACS (27).

Homocysteine concentrations can readily be lowered with either folic acid or vitamin B6 supplementation or a combination of both. Current trials are underway to assess the effect of vitamin supplementation on the development/progression of coronary artery disease. Our results suggest further studies are required to assess the effect of homocysteine lowering treatment during acute coronary events on the degree of myocardial injury.

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